

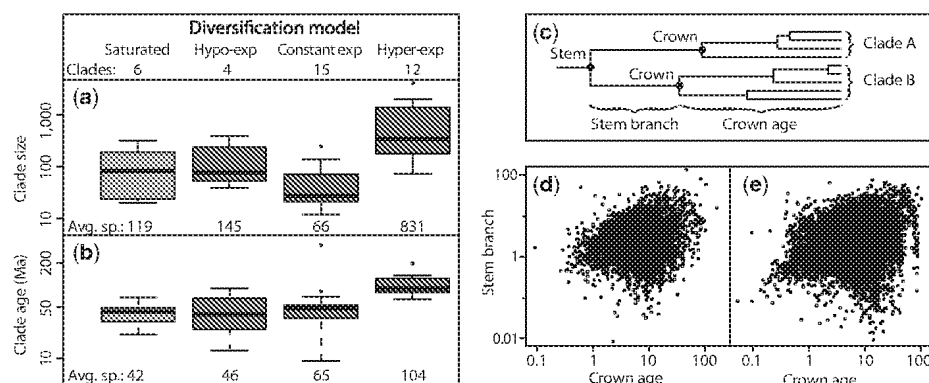


FIG. 5. (1 column) Diversification analyses of major Linnaean clades in the timetree of life. (a–b) Results of coalescent analyses testing models of diversification. Of 48 tetrapod clades, 37 showed significant model selected and they were used in these analyses. (a) Effect of clade size (number of described species). (b) Effect of clade age. (c) Diagram illustrating difference between stem and crown age. (d–e) Relationship of stem branch and crown age in mammals (d; $r^2 = 0.07$) and birds (e; $r^2 = 0.04$).

includes the time elapsed on the branch leading to the crown. We examined 1,990 clades in two separate analyses: Families (153 and 113 nonnested clades for birds and mammals, respectively) and genera (1,115 and 609 nonnested clades for birds and mammals, respectively). In each case, the relationship was highly significant for crown age ($r = 0.43$ – 0.47 , $P < 0.001$) but nonsignificant or weakly significant ($r = 0.02$ – 0.09 , $P = 0.01$ – 0.82) using stem age (see Materials and Methods). These results demonstrate a significant relationship between clade age and clade size in major groups of vertebrates, and provide an explanation why this was not observed in past studies, as they used stem ages (crown age + stem branch time).

Our results show that it is best to avoid stem branch time, because the length of any (stem) branch in the tree should not be related to the time depth of the descendant node (crown age; fig. 5d and e). Therefore, the use of stem branch time will introduce large statistical noise and make the test extremely conservative. For example, when considering every node in a timetree of species, the coefficient of variation of stem branch length relative to crown age is over 200% in the best sampled groups, mammals (208%) and birds (224%). That noise is further weighted by the pull of the present (Nee et al. 1994), which, we determined, adds 40% time (median) to crown age at any given node (if stem age is used instead of crown age), in separate analyses of birds, mammals, and all eukaryotes. This is because the pull of the present creates longer internal branches deeper in a tree, as more lineages are pruned by extinction. Therefore, the use of stem branches in diversification analyses adds noise (variance) and gives increased weight to that noise. We believe that the stronger signal of constant expansion in our results, compared with earlier studies that have supported hypo-expansion and saturation, is in part because we have identified and avoided some biases (e.g., sampling effort, clade size, and stem age) that can impact diversification analyses.

Speciation

The rate of formation of new species is the primary input into the diversification of life over time. This process starts as two

or more lineages split from an existing species at a node in the tree. To learn more about the timing of speciation, we assembled a separate data set of studies containing timetrees that included populations and species of eukaryotes (supplementary Materials and Methods, Supplementary Material online). Considering a standard model of speciation based on geographic isolation, in the absence of gene flow (Sousa and Hey 2013), we estimated the time required for speciation to occur. For a given species, the time-to-speciation (TTS), after isolation, must fall between the crown and the stem age of the species (fig. 6a). All lineages younger than the crown age of a species lead to populations that are still capable of interbreeding and, therefore, must be younger than the TTS. On the other hand, the stem age is the time when a species joins its closest relative and, therefore, it must be older than the TTS. A sampling omission of either a population or species could lower the crown age estimate or raise the stem age estimate, but the resulting interval would still contain the TTS. Although coalescence of alleles may lead to overestimates of time (Nei and Kumar 2000), those times were interpreted as population and species splits, not allelic splits, in the published studies reporting them. Also, calibrations can ameliorate the effect of allelic coalescence because they are usually based on population and species divergences, not coalescence events. We conducted a simulation to test this approach (supplementary Materials and Methods, Supplementary Material online) finding that the true TTS is estimated precisely with a mode because of skewness of the statistical distribution and it is robust to undersampling of lineages, either younger (populations) or older (species). We also found that the true TTS was not affected by the addition of 10% noise (intervals that do not include the TTS) and only weakly affected (2.5%) by adding a large amount (50%) of noise.

Analyses of three disparate taxonomic groups (vertebrates, arthropods, and plants) with greatly varying generation times and life histories produced similar TTS of ~ 2 My (fig. 6b). For comparison, divergence times among closely related species of prokaryotes, which are defined in practical terms (Cohan 2002), are about 50–100 times older (supplementary Materials and Methods, Supplementary Material online).

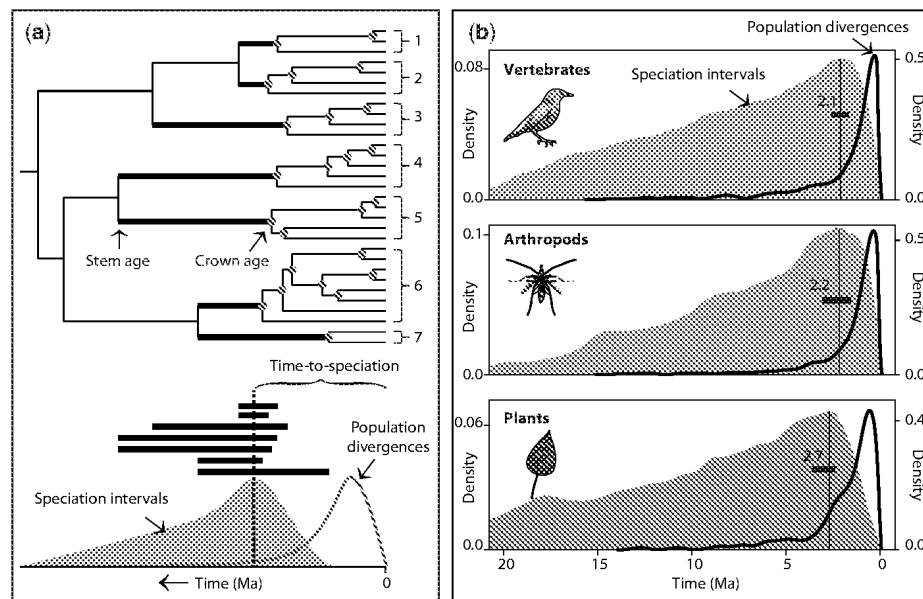


FIG. 6. (2 columns) Estimation of time-to-speciation. (a) Analytical design showing expected results. Intervals between stem age and crown age of seven species contain time-to-speciation (dashed line represents the modal time-to-speciation). Divergences among populations, and corresponding histogram, are in red. (b) Colored histograms of observed time-to-speciation, showing modes (vertical lines) and confidence intervals (bars) in vertebrates ($N = 213$, 2.1 Ma, 1.74–2.55 Ma), arthropods ($N = 85$, 2.2 Ma, 1.57–3.07 Ma), and plants ($N = 55$, 2.7 Ma, 2.37–3.63 Ma). Black curves to the right are histograms of population divergences.

The observed similarity of TTS in diverse groups of multicellular eukaryotes suggests a model of speciation that places importance on the time that two populations are isolated (fig. 7). If speciation is an outcome of the buildup of genic incompatibilities (GIs) between isolated populations (Coyne and Orr 2004; Matute et al. 2010), then the continuous fixation of selectively neutral mutations in two populations (Zuckermandl and Pauling 1965; Kimura 1968) will accumulate with time and eventually lead to a number, or fraction, of GIs (here, “S-value”) that will cause postzygotic reproductive isolation (fig. 7c). In essence, speciation—in the strictest sense—can be defined as this specific moment in time, a “point of no return,” because reproductive incompatibility and isolation are inevitable at that point. The diverging lineages will remain independent forever or until they become extinct. If they do come back into contact (fig. 7a), they might hybridize briefly but would then undergo reinforcement, leading to prezygotic reproductive isolation. We focus here on the major model, geographic isolation, but time constraints should be similar with ecological speciation, and other models exist (Coyne and Orr 2004).

A speciation clock has been suggested previously (Coyne and Orr 1989), where GIs buildup linearly over time in postzygotic reproductive isolation. However, current evidence indicates a faster-than-linear (“snowball”) rate of buildup of GIs in postzygotic reproductive isolation (Matute et al. 2010). The snowball pattern of increase in GIs is not a problem for a speciation clock as long as the time of attainment of the S-value is similar in different species. Our evidence from the TTS analysis (fig. 7b) suggests this to be the case.

Relatively few populations will remain isolated until the point of no return because environments often change

rapidly in the short term, raising and lowering barriers to gene flow (fig. 7a). This is amplified by a preponderance of low incline landscapes (Strahler 1952), resulting in a greater impact of climate and sea level change. Therefore, the buildup of GIs will be reset many times before a successful speciation event occurs (fig. 7c). This resetting, which results in failed isolates, may explain “barcode gaps” (Puillandre et al. 2011), which we interpret as prespeciation loss of potential lineages. We estimate that branches between nodes in well-sampled ($> 20\%$ coverage) groups of the TTOL are, on average, 4–5 My long using a slope method (4.92 ± 0.32 My) and a coalescent method (4.21 ± 0.76 My) (supplementary Materials and Methods, Supplementary Material online). This indicates that branch time is longer than TTS, and includes a significant “lag time,” referring to any time along a branch that is not part of a successful speciation event, such as the resetting of the speciation clock by reversal of the buildup of GIs. The high variance of branch length in trees (fig. 5d and e) may reflect the stochastic nature of environmental change and isolation of populations, contributing to a “diversification clock” (fig. 4). This is consistent with the absence of a strong correlation between reproductive isolation and diversification rate in some taxa (Rabosky and Matute 2013).

In relating this speciation model to a phylogeny (fig. 7d), it is evident that the split of two lineages in a tree is not the speciation event but rather the moment of isolation of two or more populations that will remain isolated until the point of no return. For example, if we assume a TTS of 2 My, then two populations that only recently became species would have split ~ 2 Ma (fig. 7d, species 1–2). Yet, much deeper in a tree, where branches may be tens of millions of years long, the TTS is a relatively small fraction of total branch time, which makes

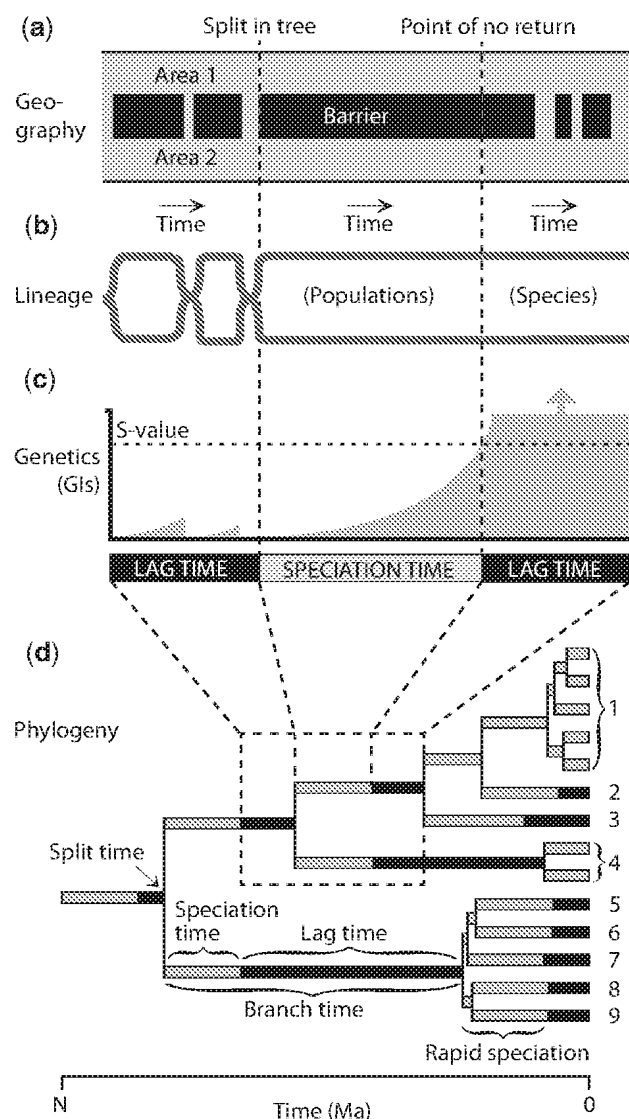


FIG. 7. (1 column) Summary model of speciation. (a) Biogeographic history showing the contact and isolation of areas occupied by the two populations. (b) Phylogenetic lineages showing times of independence (two lineages) and times of interbreeding (one lineage). (c) Genic incompatibilities between the two populations, showing how they accrue at a time-dependent rate during geographic isolation, reset to zero during contact (interbreeding), increase to the S-value (the number of GIs that will cause speciation, the point of no return), and continue increasing beyond the S-value despite later contact of the newly formed species. (d) Hypothetical phylogeny, with numbered species, illustrating parameters of speciation in (a–c) to splits and branches in a tree.

a splitting event more-or-less equivalent to the speciation event. Furthermore, branch splitting leading to eventual species formation can happen simultaneously, in many lineages, as long as the resulting populations remain isolated until the point of no return (fig. 7d, species 5–9).

Discussion

These results have implications for patterns of species diversification and the interpretation of timetrees. If adaptation is largely decoupled from speciation, we should not expect it to be a driver of speciation as is frequently assumed. Also, we should not expect to see major diversification rate increases

following mass extinction events, even though large adaptive changes took place at those times. That expectation is realized in our analyses (fig. 4) where we see constant splitting through time across the two major Phanerozoic extinction events (251 and 66 Ma). Likewise, our diversification analyses of smaller groups, such as birds and mammals (supplementary fig. S4, Supplementary Material online), and past studies of those groups (Bininda-Emonds et al. 2007; Meredith et al. 2011; Jetz et al. 2012) have not found rate increases immediately following the end-Cretaceous mass extinction event (66 Ma). Rate decreases from the extinction events themselves are not expected because of the inability to detect, in trees, a large proportion of species extinctions that occurred (Nee and May 1997).

The consistency in TTS among groups found here suggests that the time-based acquisition of GIs, and not adaptive change, is driving reproductive isolation, almost in a neutral process. By implication, geographically isolated populations, even if morphologically different and diagnosable, are not expected to be species until they have reached the point of no return. This is because some differentiation and adaptive change should occur in isolation, and many isolates will be ephemeral (Rosenblum et al. 2012), merging with other isolates or disappearing and never becoming species that enter the tree of life. More population data are needed before it is possible to identify the point of no return with precision. Nonetheless, these data suggest that, in most cases, described species separated by only tens of thousands of years are not real species. The Linnaean rank of subspecies, which has declined in use for decades, might be appropriate for such diagnosable isolates that have not yet reached the point of no return. Oversplitting of species by taxonomists may explain the sharp peak in diversification (hyper-expansion) in the last 10 My of eukaryote history (fig. 4b). On the other hand, it could also result from statistical (small sample) artifacts that were published in many different studies (summarized in the TTOL). Therefore, further analysis of that unusual rate spike is warranted.

In summary, the diversification of life as a whole is expanding at a constant rate and only in some small clades (<500 species) is there evidence of decline or saturation in diversification rate. We believe these results can be explained as many different groups of organisms undergoing expansion and contraction through time, with those patterns captured in different stages (expanding, slowing, or in equilibrium). If true, the predominant pattern of expansion in large clades is expected from the law of large numbers, where such smaller, random events would average to a constant rate. Constant expansion also follows from random environmental changes leading to isolation and speciation. Rate constancy is consistent with the fossil record (Benton 2009) and does not deny the importance of biotic factors in evolution (Ricklefs 2007), but it suggests an uncoupling of speciation and adaptation. Cases where the phenotype has changed little (e.g., cryptic species) or greatly during the TTS are interpreted here as evidence of uncoupling. The lineage splitting seen in trees probably reflects, in most instances, random environmental events leading to isolation of populations,

and potentially many in a short time. However, the relatively long TTS (~2 My), a process resulting from random genetic events, will limit the number of isolates that eventually become species. Under this model, diversification is the product of those two random processes, abiotic and genetic, and rate increases (bursts of speciation) are more likely to be associated with long-term changes in the physical environment (e.g., climate, sea level) causing extended isolation rather than with short-term ecological interactions. However, reductions in extinction rate could also explain increases in diversification rate. Adaptive change that characterizes the phenotypic diversity of life would appear to be a separate process from speciation. Although a full understanding of these processes remains a challenge, determining how speciation and adaptation are temporally related would be an important “next step.”

Materials and Methods

Detailed methods are described in supplementary Materials and Methods, Supplementary Material online.

TTOL Data Collection

We synthesized the corpus of scientific literature where the primary research on the TTOL is published. We first identified and collected all peer-reviewed publications in molecular evolution and phylogenetics that reported estimates of time of divergence among species. These included phylogenetic trees scaled to time (timetrees) and occasionally tables of time estimates and regular text. We assembled timetree data from 2,274 studies (http://www.timetree.org/reference_list.php) that have been published between 1987 and April 2013, as well as two timetrees estimated herein (supplementary table S1, Supplementary Material online). Most (96%) of nodal times used were published in the last decade.

TTOL Analytics and Synthesis

We used a hierarchical average linkage method of estimating divergence times (T_s) of clade pairs to build a Super Timetree, along with a procedure for testing and updating topological partitions to ensure the highest degree of consistency with individual timetrees in every study. For the TTOL, uncertainty derived from individual studies is available for each node (supplementary table S2, Supplementary Material online). Branch time modes of different Linnaean categories were estimated (supplementary table S3, Supplementary Material online).

Diversification Analyses

All diversification analyses were performed in R (<http://www.r-project.org/>) with the APE package (Paradis et al. 2013). The gamma statistic was employed to detect decrease in speciation rate over the history of the tree. To test if diversification is saturated or expanding, we used a coalescent method as well as the relationship between the ages of different clades and their sizes (numbers of species). The number and location of shifts in diversification rates were

also tested. We also estimated among-study uncertainty in both the lineages-through-time (LTT) curve and rate test by producing 500 replicates of the TTOL, sampling actual study times at each node randomly. These replicates were then used in the analysis of rate shifts to estimate and test rate change. Results of all diversification analyses were summarized (supplementary tables S4–S10, Supplementary Material online).

Gamma Test

The gamma statistic (Pybus and Harvey 2000) was employed on 59 clades (all eukaryotes plus 58 clades listed in supplementary table S5, Supplementary Material online) to detect decrease in speciation rate over the history of the tree (LASER package; Rabosky and Schliep 2013). These 58 groups were chosen to have an overview of the diversification processes on a diversity of clades across life, in terms of size, sampling effort (above 5%; median 66%, with 84% of the clades having more than 40% coverage), and clade age. Included in the 58 clades are 10 nonnested groups over the major Linnaean groups, which allowed us to draw conclusions about high-level and low-level clades. We further selected 10 nonnested clades from within each of the four well-sampled groups of tetrapods (amphibians, birds, mammals, and squamates). We also analyzed separately the Eukaryote clade because all the other groups are nested within this one.

A negative gamma value reveals a concentration of branching times near the root, meaning a decelerated diversification. Incomplete lineage sampling can affect the result of this test because some tips will be pruned, thus lengthening the branch length in the more recent past (Nee et al. 1994). An incomplete sampling could therefore lead to an erroneous detection of slowdown. To avoid this bias, the Monte Carlo constant rate test (MCCR test) (Pybus and Harvey 2000) was also performed on clades with a significant negative gamma value (LASER package). Here, the critical value for rejecting a constant rate ($\alpha = 0.05$) was calculated by examining the distribution of gamma for simulated trees with the same incomplete lineage sampling proportion as the observed tree. We conducted 5,000 Monte Carlo simulations for each MCCR test. If the MCCR test finds that the detection of slowdown is erroneous, then there is no support for decline in speciation rate for this clade.

Models of Diversification

To test if diversification is saturated or expanding we used a coalescent method (Morlon et al. 2010) on 58 clades (supplementary table S5, Supplementary Material online). We defined two sets of models: Saturated diversity (models 1 and 2) and expanded diversity (models 3, 4a, 4b, 4c, 4d, 5, and 6). As recommended by the author of the method, we first selected the model with the lowest second-order Akaike's Information Criterion in each subset (saturated vs. expanding models). We then evaluated the relative probability of these two models based of their Akaike weights. The relative probabilities of two models (l and k) were calculated as $\frac{w_l}{w_l + w_k}$ and $\frac{w_k}{w_l + w_k}$ with w_l (the model's l Akaike weight) as

$w_l = \frac{\exp(-\Delta l/2)}{\sum_{r=1}^R \exp(-\Delta r/2)}$ and R the number of candidate models.

This method can account for missing species. We therefore used the number of described species (supplementary table S5, Supplementary Material online) as the number of tips of the phylogenies (N_0). When neither of the two relative probabilities obtained exceeded 0.6, we did not draw a conclusion on this specific clade (referred as “nonsignificant” in supplementary table S5, Supplementary Material online) and the mean λ (between the best expanding and saturating model) was used (supplementary table S4, Supplementary Material online). Statistical tests were performed (supplementary table S6, Supplementary Material online) to compare the four models, saturated (sat), hyper-expanding (exp+), expanding (exp), and hypo-expanding (exp-), at the same time (Kruskal–Wallis rank-sum test) and by pair (Wilcoxon rank-sum test). Another test was performed (t -test) to compare the means (described number of species and crown age) between the clades under hyper-expansion and the others (saturated, hypo-expanding, and expanding). We confirmed for all clades that the BD polytomy resolution did not change the results, although a different model was selected for 3 of the 58 clades (5%). This method was computationally unfeasible with the entire TTOL.

Clade Age and Clade Size Analyses

To test, in another way, for expansion (hyper-, constant-, and hypo-) versus saturation of the diversification curve, we evaluated the relationship between the ages of different clades and their sizes (numbers of species) (Cornell 2013). Under the saturated diversity model, no relationship should be observed, in contrast to a positive relationship under models of expanding diversity. For this test, we chose the two best sampled groups, birds and mammals, and performed comparative analyses on clades (all nonnested) of genera (1,115 and 609 for birds and mammals respectively) and families (153 and 113 for birds and mammals, respectively), accounting for phylogeny (phylogenetic generalized linear models) with the CAPER package (Orme et al. 2013). In one set of analyses, we used stem age and in another set, crown age (supplementary table S7, Supplementary Material online) was used.

Detection of Shifts in Diversification Rates

The TREEPAR package (Stadler 2013) was used to estimate the number of changes in diversification rate under a BD model and to obtain estimates of the corresponding diversification rate ($\lambda - \mu$) and the extinction fraction (μ/λ). To analyze our eukaryote timetree, as well as the individual component timetrees of birds and mammals, we used the greedy approach, as described in Stadler (2011). We estimated rates in 0.1 My steps between 2 and 70 Ma and in 10 My steps between 2 and 3,500 My for eukaryotes. The BD shift model (without mass extinction) was used and we obtained maximum-likelihood rate estimates for the different data sets for zero, one, two, three, four, five, six, and seven rate shifts.

The likelihood ratio test was used to select the best model in each case at the 99% level.

For the TTOL, we estimated node time uncertainty in both the LTT curve and rate test by producing 500 replicates of the TTOL, sampling a time between the confidence intervals at each node under a uniform distribution. These replicates were then used in the TREEPAR analysis to estimate and test rate change, with the uncertainty shown in figure 4 and the significant shifts by time interval (20 My) shown in supplementary table S8, Supplementary Material online.

Our diversification results for birds are similar to an earlier analysis (Jetz et al. 2012), with a strong increase in diversification from ~45 Ma to the present. The mammal results are also generally similar to past mammal analyses in showing the absence of any rate increase immediately after the end-Cretaceous extinctions (Bininda-Emonds et al. 2007; Meredith et al. 2011). We did not find a sharp mid-Cenozoic increase in rate that was found in one other analysis (Stadler 2011), although we determined that the cause of that rate increase was from a polytomy in the mammal timetree used in that study (Stadler 2011); otherwise our results are comparable.

For the bird tree (supplementary fig. S4a, Supplementary Material online), the models with zero, one, two, three, four, and five rate shifts are rejected in favor of a model with six rate shifts ($P < 0.05$) (supplementary table S9, Supplementary Material online). A model with six rate shifts is not rejected in favor of a model with seven rate shifts ($P = 0.091$). The 6 shifts are detected at 1, 3.4, 14.4, 48.2, 73.3, and 84.4 Ma (supplementary table S10, Supplementary Material online). The parameters obtained for the 6 shifts model between 73.3 and 48.2 Ma ($\lambda - \mu = 0.04058$ and $\mu/\lambda = 0.0395$) were used to plot the confidence interval of the null distribution (supplementary fig. S4a, Supplementary Material online).

For the mammal tree (supplementary fig. S4b, Supplementary Material online), the models with zero, one, two, or three rate shifts are rejected in favor of a model with four rate shifts ($P < 0.05$) (supplementary table S9, Supplementary Material online). A model with four rate shifts is not rejected in favor of a model with five rate shifts ($P = 0.123$). The 4 shifts are detected, at 2.1, 9.6, 42.4 and 105 Ma (supplementary table S10, Supplementary Material online). The parameters obtained for the 4 shifts model between 105 and 42.4 Ma ($\lambda - \mu = 0.05186$ and $\mu/\lambda = 0.3528$) were used to plot the confidence interval of the null distribution (supplementary fig. S4b, Supplementary Material online).

For the eukaryote tree (fig. 4), the models with zero, one, two, three, four, and five rate shifts are rejected in favor of a model with six rate shifts ($P < 0.05$) (supplementary table S9, Supplementary Material online). A model with six rate shifts is not rejected in favor of a model with seven rate shifts ($P = 0.055$). Six shifts are thus detected at 1, 11, 21, 121, 141 and 151 Ma (supplementary table S10, Supplementary Material online). The parameters obtained for the 6 shifts model between 2,100 and 151 Ma ($\lambda - \mu = 0.00307$ and $\mu/\lambda = 0.9581$) were used to plot the confidence interval of the null distribution (fig. 4).

In addition, we used the BAMMtools package (Rabosky 2014) in order to detect the diversification rate differences across lineages. The function “setBAMMpriors” was used to generate a prior block that matched the “scale” (e.g., depth of the tree) of our data. Both the speciation and the extinction rate were allowed to vary through time and across lineages, and 50,000,000 generations of MCMC simulation were performed. The sampling fraction (0.02635422) was specified by setting the “globalSamplingFraction” parameter. A burnin of 0.5 was applied and a diversification rate plot was obtained with the function “plotRateThroughTime” (supplementary fig. S3, Supplementary Material online).

Time-to-Speciation Analyses

In addition to our species-level TTOL data collection described above, we collected a separate data set on TTS from published molecular timetrees that included timed nodes among populations and closely related species of three major groups: Vertebrates, arthropods, and plants (supplementary tables S11–S13, Supplementary Material online). To test the robustness of our approach for estimating TTS, we used simulations. A BD tree was simulated using the function “sim.bd.taxa” (TreeSim in R).

Supplementary Material

Supplementary figures S1–S4, tables S1–S13, and Materials and Methods are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

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EXHIBIT A

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M.D., 1980
State University of New York at Buffalo

Internship (Internal Medicine), 1981
University of Arizona at Tucson

Maryland State License
National Board of Medical Examiners Part III, 1981

Professional Appointments

June 2017 - Present
Chief Scientist, Impossible Foods

March 1989 – May 2017
Director, National Center for Biotechnology Information,
National Library of Medicine, NIH

April 1984 – December 2003
Senior Surgeon, Commissioned Corps, Public Health Service

July 1981 – 1989
Medical Staff Fellow, Mathematical Research Branch, NIDDK, NIH

Honors and Awards

- Elected Fellow, International Society of Computational Biology, 2009
- Elected Fellow, American Academy of Arts and Sciences, 2008

- National Institutes of Health NIH Director's Award (Public Access), 2008
- National Institutes of Health NIH Director's Award (Genome-Wide Association Studies), 2008
- Finalist, Service to America Medals, 2008
- American Society for Microbiology Promega Biotechnology Research Award, 2008
- National Institute of Allergy and Infectious Diseases NIH Merit Award, 2006
- U.S. Dept. of Health & Human Services Secretary's Award for Distinguished Service, 2004
- International Society for Computational Biology Senior Scientist Accomplishment Award, 2004
- Elected Member, National Academy of Sciences, 2003
- Priscilla M. Mayden Lecturer, University of Utah, 2002
- Elected Fellow, American College of Medical Informatics, 2001
- Elected Member, Institute of Medicine, 2000
- National Institutes of Health NIH Director's Award, Summer 1998
- Public Health Service Meritorious Service Medal, Summer 1997
- Association of Biomolecular Resource Facilities' Award, 1996
- NLM Director's Honor Award, 1996
- Public Health Service Meritorious Service Medal, Spring 1993
- Public Health Service Outstanding Service Medal, Spring 1991

Memberships

- American Academy of Arts and Sciences, Elected Fellow, April 2008 – Present
- National Academy of Sciences, Elected Member, April 2003 – Present
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Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms

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When many protein sequences are available for estimating the time of divergence between two species, it is customary to estimate the time for each protein separately and then use the average for all proteins as the final estimate. However, it can be shown that this estimate generally has an upward bias, and that an unbiased estimate is obtained by using distances based on concatenated sequences. We have shown that two concatenation-based distances, i.e., average gamma distance weighted with sequence length (d_2) and multiprotein gamma distance (d_3), generally give more satisfactory results than other concatenation-based distances. Using these two distance measures for 104 protein sequences, we estimated the time of divergence between mice and rats to be approximately 33 million years ago. Similarly, the time of divergence between humans and rodents was estimated to be approximately 96 million years ago. We also investigated the dependency of time estimates on statistical methods and various assumptions made by using sequence data from eubacteria, protists, plants, fungi, and animals. Our best estimates of the times of divergence between eubacteria and eukaryotes, between protists and other eukaryotes, and between plants, fungi, and animals were 3, 1.7, and 1.3 billion years ago, respectively. However, estimates of ancient divergence times are subject to a substantial amount of error caused by uncertainty of the molecular clock, horizontal gene transfer, errors in sequence alignments, etc.

The molecular clock hypothesis asserts that the number of amino acid substitutions in a protein is roughly proportional to the time since divergence of the two species compared (1, 2). Strictly speaking, no gene or protein would evolve at a constant rate for a long evolutionary time, because gene function is likely to change over time, and mutational and DNA repair mechanisms appear to vary among different groups of organisms (3). For this reason, the molecular clock has been controversial for several decades (3–6). However, even if the substitution rate is not strictly constant, it is still possible to obtain rough estimates of divergence times, and these estimates are very useful when there is no reliable fossil record (6, 7). Furthermore, if a gene evolves excessively fast or slow in a few evolutionary lineages, one can eliminate these lineages and then estimate divergence times for the rest of the species (8). The accuracy of time estimates is expected to increase as the number of genes or proteins used increases, and in recent years, many authors have used multiple genes or proteins for this purpose (9–11).

There are several statistical methods for estimating divergence times, but the theoretical basis of the methods is not well understood when multiple genes are used. For this reason, different authors obtained widely different estimates for the same set of species by using different methods (10, 12–16). We have therefore examined the reliability of different methods for estimating divergence times and have developed new methods that are likely to give more reasonable estimates than previous ones. The purpose of this paper is to discuss statistical problems related to this subject and to present new methods. These new methods will then be used to estimate the divergence times between mice and rats and between humans and rodents, which have been controversial for the last few

decades. We also consider the divergence times of animals, plants, fungi, protists, and bacteria to show the dependency of time estimates on the assumptions made and the statistical methods used. In this paper, we consider only protein sequences, because they usually give more satisfactory results than DNA sequences when long-term evolution is considered. We also consider only distance methods of time estimation, because most recent time estimates have been obtained by these methods.

Theoretical Basis of Estimation of Divergence Times

Individual Protein (IP) Approach. In the past, most investigators have used a method that may be called the IP approach (11, 13, 14, 17, 18). In this approach, the estimate of divergence time is computed for each protein, and the average of the estimates over all proteins is used as the final estimate. Consider Fig. 1*A*, in which a phylogenetic tree for five species is given. Here, a – f stand for the least-squares estimates of branch lengths (numbers of amino acid substitutions) obtained from a pairwise distance matrix for a protein. Species 5 is used as an outgroup to determine the root of the tree for the remaining sequences, and therefore the branch length estimate for this branch is not given. Here we assume that the topology of the tree for the five species has been established from other information. To estimate divergence times between species, it is convenient to construct a linearized tree (8), in which the branch lengths are reestimated under the assumption of a constant-rate evolution (Fig. 1*B*). When this linearized tree is constructed, a timescale for the tree is produced to estimate divergence times (t_1 and t_2). This timescale can be obtained by computing the rate of amino acid substitution per year (r) by using the known divergence time and the corresponding branch-length estimate for a pair of species or species clusters.

For example, if T is the calibration point in Fig. 1*B*, the rate of amino acid substitution can be estimated by $\hat{r} = \hat{b}_3/T$, where $\hat{b}_3$ is the branch-length estimate for species 4 after divergence from species 1, 2, and 3 in the linearized tree (Fig. 1*B*). When this rate is obtained, we can estimate t_1 by

$$\hat{t}_1 = \hat{b}_1/\hat{r} = (\hat{b}_1/\hat{b}_3)T. \quad [1]$$

Here, the estimates $\hat{b}_1$, $\hat{b}_2$, and $\hat{b}_3$ can be obtained from pairwise distances by Takezaki *et al.*'s (8) method. Similarly, the estimate of t_2 is given by $\hat{t}_2 = \hat{b}_2/\hat{r} = (\hat{b}_2/\hat{b}_3)T$. The variances of $\hat{t}_1$ or $\hat{t}_2$ can be obtained by Takezaki *et al.*'s method or by the bootstrap method (19). For certain data sets (18, 20), it is possible to use several calibration points (paleontological dates for different pairs of species). In this case, r may be estimated by fitting a regression line to the relationship between branch length estimates and paleontological dates.

Before the construction of a linearized tree, it is customary to conduct a statistical test of the molecular clock and eliminate

Abbreviations: IP, individual protein approach; CD, concatenation-based distance; MY, million years; PC, Poisson correction; NJ, neighbor joining.

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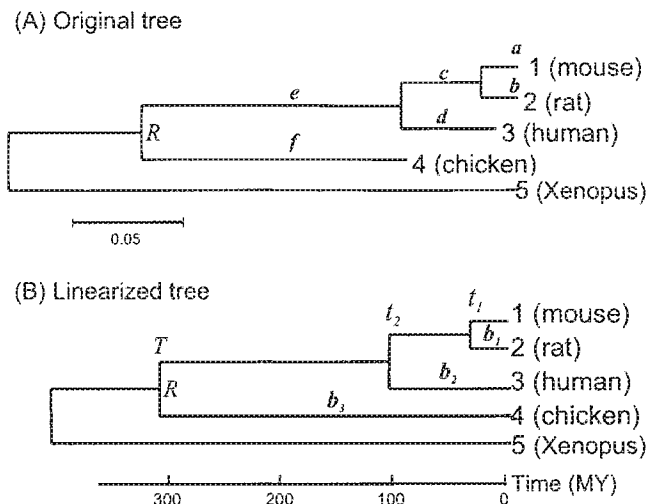


Fig. 1. Phylogeny of the five species used. (A) NJ tree constructed by using distance d_2 for 104 protein sequences. (B) Linearized tree of the above NJ tree. R, root of the four species under consideration.

species that evolved excessively fast or slow, although this is not always necessary (see below). A number of authors (7, 11) have used the relative rate tests (21–23) for this purpose. In these tests, three sequences (or three sequence clusters) are used, and the equality of evolutionary rate for two evolutionary lineages is tested by using a third sequence as an outgroup. For example, the equality of the expectations of branch lengths a (for mice) and b (for rats) in Fig. 1A can be tested by using the chicken sequence as the outgroup. Theoretically, however, this test is not appropriate for estimating the divergence time t_1 , because it does not test the equal rates for sequences 1, 2, and 3. For this purpose, we should test the null hypotheses $E(a) = E(b)$ and $E(a/2 + b/2 + c + e) = E(f)$, where E is the expectation operator. These null hypotheses can be tested by Takezaki *et al.*'s (8) U statistic, which approximately follows the χ^2 distribution and tests the equality of the branch lengths from the root (R) to tips (1, 2, 3, and 4) for all species. In this test, at least four sequences are necessary.

When there are data from many different proteins, the divergence time t_1 is often estimated by the simple average of $\hat{t}_{1i}$ for all the proteins used, as mentioned above. That is,

$$\hat{t}_1 = \sum_{i=1}^k \hat{t}_{1i} / k, \quad [2]$$

where $\hat{t}_{1i}$ is the estimate of t_1 obtained by the i th protein, and k is the total number of proteins used. Theoretically, however, $\hat{t}_1$ obtained in this way is not an unbiased estimator of t_1 , even if the branch length estimates (numbers of amino acid substitutions) for each protein are unbiased. Let $\hat{b}_{1i}$ and $\hat{b}_{3i}$ be unbiased estimates of b_1 and b_3 for the i th protein, so that $\hat{t}_{1i} = (\hat{b}_{1i}/\hat{b}_{3i})T$. In this case, an unbiased estimator of t_1 is given by $[(\sum_i \hat{b}_{1i})/(\sum_i \hat{b}_{3i})]T$ rather than by $\hat{t}_1 = \sum_i (\hat{b}_{1i}/\hat{b}_{3i})T/k$. If we assume that b_1 and b_3 are random variables (subscript i dropped), the expectation of $\hat{b}_1/\hat{b}_3$ is approximately given by

$$E(\hat{b}_1/\hat{b}_3) = \frac{E(\hat{b}_1)}{E(\hat{b}_3)} - \frac{\text{Cov}(\hat{b}_1, \hat{b}_3)}{E^2(\hat{b}_3)} + \frac{E(\hat{b}_1)V(\hat{b}_3)}{E^3(\hat{b}_3)}, \quad [3]$$

where $V(\hat{b}_3)$ is the variance of $\hat{b}_3$, and $\text{Cov}(\hat{b}_1, \hat{b}_3)$ is the covariance of $\hat{b}_1$ and $\hat{b}_3$ (24). Numerical evaluation of the second and third terms in Eq. 3 suggests that $\hat{t}_1$ in Eq. 2 often gives overestimates of

divergence times. This is particularly so when the calibration point is smaller than the divergence time to be estimated. Suppose we know t_1 instead of T in Fig. 1B and want to estimate T by $\hat{T} = \sum_i (\hat{b}_{3i}/\hat{b}_{1i})t_1/k$. In this case, $\hat{T} \equiv (\hat{b}_{3i}/\hat{b}_{1i})t_1$ may become very large if $\hat{b}_{1i}$ happens to be close to 0 by chance (the upper limit being infinity). If $\hat{b}_{1i}$ happens to be large relative to $\hat{b}_{3i}$, $\hat{T}$ becomes small but never smaller than t_1 . Therefore, $\hat{T}$ tends to be an overestimate when $\hat{b}_{1i}$ varies extensively. To avoid this overestimation, we should use concatenation-based distances (CDs) mentioned below.

Distance Measures to Be Used. When all protein sequences used are closely related, the Poisson correction (PC) distance appears to give sufficiently accurate estimates of divergence times (25). This distance is given by $d = -\ln(1 - p)$, where p is the proportion of sites at which the amino acids of the two sequences compared are different. However, the PC distance is obtained under the assumption that the rate of amino acid substitution per year (r) is the same for all amino acid sites. In practice, this assumption rarely holds, and empirical data have suggested that the rate varies from site to site approximately following the gamma distribution (26). In this case, the evolutionary distance between two sequences can be measured by the following PC gamma distance

$$d = a[(1 - p)^{-1/a} - 1], \quad [4]$$

where a is the shape parameter of the gamma distribution (gamma parameter) and decreases as the variation of r among sites increases (27, 28).

If we assume that a is a constant, the variance of d is given by $V_1(d) = p[(1 - p)^{-(1+2/a)}]/n$ (28), but if we take into account the sampling variance $[V(\hat{a})]$ of the estimate ($\hat{a}$) of a , the total variance is approximately given by

$$V(d) = V_1(d) + V(\hat{a}) \left[(1 - p)^{-1/a} \left\{ 1 + \frac{1}{a} \ln(1 - p) \right\} - 1 \right]^2, \quad [5]$$

where $V(\hat{a}) = [2a(a + 1)(p + a)^2]/(np^2)$, and n is the number of amino acids used. This equation was obtained by the delta method by using Anscombe's (29) formula for $V(\hat{a})$.

One might question the applicability of Eq. 4 to actual data, because it does not take into account higher rates of substitution between similar amino acids than between dissimilar amino acids (30). Grishin (31) developed a complex distance measure by taking into account variation in substitution rate among different amino acid sites as well as among different pairs of amino acids. However, this distance can also be approximated very well by a PC gamma distance with $a = 0.65$ (32). Therefore, for most practical purposes, we may use PC gamma distance.

CD Approach. Previously, we mentioned that to obtain an unbiased estimate of t_1 , pairwise CDs for all proteins should be computed and b_1 and b_3 be estimated from these distances. There are several ways of concatenating pairwise distances (d_s) for different proteins to obtain unbiased estimates of b_1 and b_3 .

(i) **Simple average distance (d_1).** In this method, a PC or PC gamma distance is computed for each protein, and the simple average of the distances for all proteins is used.

(ii) **Average distance weighted by sequence length (d_2).** One disadvantage of distance d_1 is that the average of d_s over loci is computed without regard to the number of amino acids (sequence length). Because a protein distance based on many amino acids would be more reliable than d_1 , it would be better to use the average distance weighted with sequence length.

(iii) **Multiprotein gamma distance (d_3).** As mentioned earlier, PC gamma distance is very flexible and can be applied to most amino acid sequence data (32). However, the gamma parameter a is expected to vary from protein to protein, and it has been shown that the rate of amino acid substitution per protein

roughly follows the gamma distribution (25). This suggests that if we consider many protein sequences simultaneously, the rate of amino acid substitution per site approximately follows the gamma distribution when the entire set of amino acids for all proteins is considered. We can therefore estimate the gamma parameter a for the entire set of amino acids and compute the gamma distance using Eq. 4. We call this distance the *multiprotein gamma distance* in this paper. The standard errors of the estimates ($\hat{a}_1$, $\hat{a}_2$, and $\hat{a}_3$) of the above three distances may be computed by the bootstrap or the jackknife method by using individual proteins as units of resampling. For distance estimate $\hat{a}_3$, the variance can also be computed by Eq. 5, but the jackknife variance appears to be more appropriate, because the unit of evolution is a gene or protein rather than an amino acid.

A standard way of concatenating different statistical estimators is to use the inverse of variance as the weight. Lynch (15) suggested that the average of the variances of all pairwise distances for each protein be used as the weight. Although the CD obtained in this way does not give unbiased estimates of b_1 and b_2 , it may be useful for the estimation of divergence times. The CD obtained by this method will be denoted by d_L . We used Eqs. 1 and 2 in his paper to compute the (gamma) distance and its variance (with $a = 0.5$) for each protein, following his suggestion.

Divergence Times Between Mice and Rats and Between Humans and Rodents

A large number of authors have estimated the times of divergence between different groups of mammals by using molecular data (11, 33, 34), but the results obtained are conflicting and controversial (35–37). Of special interest in this regard are the divergence times between mice and rats and between humans and rodents. Molecular estimates of these divergence times have been controversial, because the fossil record is poor (38, 39), and rodent genes appear to have evolved faster than primate genes (22). In this paper, we therefore focus our attention primarily on these divergence times. We use five vertebrate species, i.e., mice, rats, humans, chickens, and *Xenopus laevis*, of which the evolutionary relationships are well established and for which many shared protein sequences are available. *Xenopus* is used as an outgroup species (Fig. 1).

Protein Sequence Data. We obtained protein sequence data from the December 1999 edition of the HOVERGEN database (40) and used only sequences that are available for all five species. In this database, orthologous and paralogous genes are not always distinguished, and we attempted to exclude paralogous genes as much as possible by eliminating multigene families such as major histocompatibility complex and immunoglobulin genes. We also constructed a neighbor-joining (NJ) tree using p distance (41) for each gene and eliminated all genes that produced a topology different from the known tree for the five species.

Using the above procedure, we obtained 104 putative orthologous proteins (see Table 3, which is published as supplemental data at www.pnas.org). We used both PC and PC gamma distances in this paper. The gamma parameter a was estimated by Gu and Zhang's (42) method (the computer program available from the web site <http://mep.bio.psu.edu>) for each protein separately. Fig. 2 shows the estimates ($\hat{a}$) of a for the 104 proteins in relation to the extents of sequence divergence (average root-to-tip branch length; b_R). The $\hat{a}$ value varies extensively from protein to protein, and it is positively correlated with b_R (43) or the proportion (p_v) of variable sites among the five sequences. Because PC gamma distance is disproportionately large compared with PC distance when $\hat{a}$ is small, the relationships in Fig. 2 suggest that the extent of sequence divergence as measured by PC gamma is less heterogeneous among proteins than that obtained by PC. As mentioned earlier, the multiprotein gamma distance is computed by using the $\hat{a}$ value obtained from

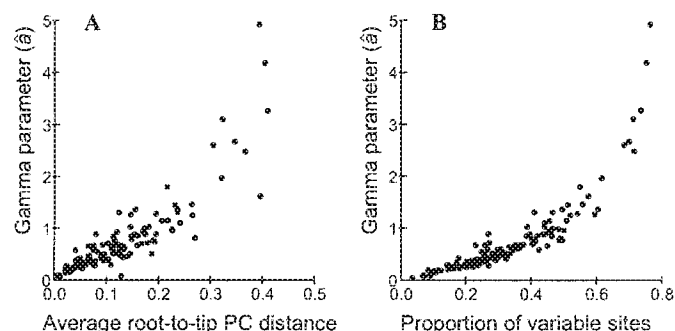
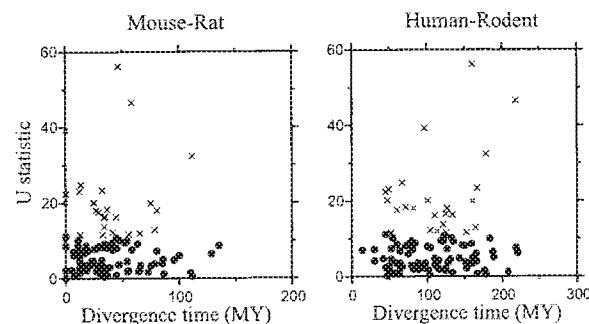


Fig. 2. (A) Relationship between estimated gamma parameter ($\hat{a}$) and the average root-to-tip distance (b_R) for 104 nuclear proteins from the five species used. (B) Relationships between $\hat{a}$ and the proportion of variable sites (p_v) among the five sequences.

the entire set of amino acids. This value was 0.57, whereas the average ($\bar{a}$) of $\hat{a}$ s for all proteins was 0.76.

Time Estimation. Theoretically, it is better to eliminate sequence data that do not pass the molecular clock test. In practice, however, proteins that violate the molecular clock hypothesis do not necessarily give unreasonable estimates of divergence times (11). We therefore examined the relationships between U statistic values and time estimates for individual proteins (Fig. 3). In the case of PC distance, 25 proteins did not pass the molecular clock test and showed a U value of 11.3 (1% significance level of χ^2 with 3 degrees of freedom) or higher. However, the estimates of the mouse-rat and the human-rodent divergence times were nearly the same whether these 25 deviant proteins were included or not. In the case

(A) PC distance



(B) PC gamma distance

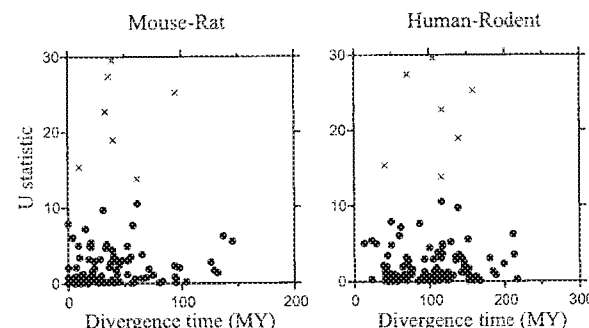


Fig. 3. Relationships between estimated divergence times and U statistic values for each of 104 proteins. The molecular clock hypothesis was rejected for the proteins indicated with the $\times$ symbol.

Table 1. Estimates ($\pm$ standard errors) of divergence times (MY) between mice and rats and between humans and rodents

Method	Mouse-rat (t_1)		Human-rodent (t_2)	
	PC	PC Gamma	PC	PC Gamma
IP approach				
	44.0 $\pm$ 3.4	38.5 $\pm$ 3.2	113.4 $\pm$ 5.0	102.9 $\pm$ 5.0
CD approach				
d_1 ($\bar{a} = 0.76$)	40.7 $\pm$ 3.0	34.4 $\pm$ 2.8	112.3 $\pm$ 5.4	99.9 $\pm$ 5.3
d_2 ($\bar{a} = 0.76$)	39.1 $\pm$ 2.3	33.0 $\pm$ 2.0	110.0 $\pm$ 4.4	97.6 $\pm$ 4.4
d_3 ($a = 0.57$)		32.9 $\pm$ 2.3		95.5 $\pm$ 4.2
d_3 ($a = 0.28$)		(25.2 $\pm$ 2.0)		(82.0 $\pm$ 4.0)
d_L ($a = 0.5$)		32.0 $\pm$ 5.0		90.0 $\pm$ 10.0

d_1 , unweighted average distance; d_2 , average distance weighted with sequence length; d_3 , multiprotein gamma distance; d_L , Lynch's distance. The standard errors for the CD approach were computed by the jackknife method. The standard errors of $\hat{t}_1$ and $\hat{t}_2$ obtained by using Eq. 5 for d_3 with $a = 0.57$ were 2.0 and 4.0, respectively.

of PC gamma distance, only 7 proteins did not pass the molecular clock test, and the mean estimates of divergence times were again virtually unaffected by inclusion or exclusion of these proteins. For this reason, we used the average time estimates for all proteins as the final estimates disregarding the U values in the independent protein (IP) approach. However, note that the extent of variation in t_1 and t_2 is so enormous that the average estimates based on a few proteins are quite unreliable. In this paper, we used $T = 310$ million year (MY) (divergence time between birds and mammals) as the calibration point (11, 38).

In the CD approach, CDs with a large number of amino acids (48,092 in the present case) are used, so that even small differences in evolutionary rate among species become statistically significant. For example, the tree in Fig. 1A shows the branch length estimates obtained when distance d_2 was used. The average branch length (0.053) for the mouse and rat lineages after their separation from the human lineage is about 1.2 times longer than that (0.043) of the human lineage, and the difference is highly significant (at the 0.01% level). Similarly, the branch length (0.17) for the rodent lineage after separation from the chicken lineage is 1.3 times greater than that (0.12) of the chicken lineage, and the difference is again significant at the 0.01% level.

However, this extent of variation in evolutionary rate among lineages does not seem to affect time estimates seriously (32). In Fig. 1A, it is unclear whether the evolutionary rate was accelerated in the rodent lineage compared with the chicken lineage or was decelerated in the chicken lineage. In either case, however, it is possible to estimate the divergence times t_1 and t_2 by considering the times of separation of chicken and humans from the rodent lineage. For example, t_1 and t_2 can be estimated by $[(a + b)/(a + b + 2c + 2e)]T$ and $[(a + b + 2c)/(a + b + 2c + 2e)]T$, respectively, where $T = 310$ MY. The estimates of t_1 and t_2 obtained in this way are 31 MY and 97 MY, respectively. These estimates are close to those obtained by the linearized tree method (Table 1). For this reason, we decided to use all 104 protein data in all the methods of the CD approach.

Estimates of divergence times between mice and rats and between humans and rodents (t_1 and t_2 in Fig. 1B) obtained by all methods are presented in Table 1. When the IP approach is used, the estimates of t_1 and t_2 obtained with PC distance are 44 and 113 MY, respectively. When PC gamma distances are used, the estimates of t_1 and t_2 are both considerably smaller than those obtained by using PC distances.

In the case of the CD approach, PC distances d_1 and d_2 with $a = \infty$ give similar estimates of t_1 (39~41 MY) and t_2 (110~112 MY). When PC gamma is used, the time estimates are somewhat smaller than those obtained by the IP approach for both t_1 and t_2 . In general, distance d_2 with $\bar{a} = 0.76$ and d_3 with $a = 0.57$

give very close estimates. Distance d_L gives $\hat{t}_1 = 32$ MY, which is similar to $\hat{t}_1$ obtained by d_3 , but $\hat{t}_2$ obtained by d_L is considerably smaller than the values obtained by the other methods. Note also that the standard errors of the estimates obtained by d_L are greater than those obtained by other methods.

Previously we mentioned that the IP approach tends to give overestimates of divergence times, particularly when the times are estimated from recent calibration points. Assuming that t_1 is known to be 33 MY but T is unknown, we can estimate T for the present data set. In this case, we obtain $\hat{T} = \infty$, because there are four proteins for which b_{1i} is 0. If we eliminate these four proteins, the average estimate is still 450 MY. By contrast, if we use d_3 with $a = 0.57$, we obtain $\hat{T} = 310$ MY, as expected. If we use t_2 (=96 MY) as the calibration point, the IP approach gives $\hat{T} = 353$ MY, but the CD with d_3 gives $\hat{T} = 310$ MY.

Estimates of Gamma Parameter a and Divergence Times. In the above computation of divergence times for humans and rodents, we estimated gamma parameter a from the entire set of species. In practice, the estimate of a tends to be smaller when closely related species are used than when distantly related species are used (43). This occurs because multiple substitutions at the same amino acid sites can be detected more easily in the former group of species than in the latter. Because the estimate obtained from closely related species should be closer to the true a value in the absence of sampling bias (43), one might argue that this estimate should be used for computing pairwise distances for all species. We therefore estimated the a value for d_3 using the mammalian sequences only and obtained $\hat{a} = 0.28$. When this estimate was used, we obtained $\hat{t}_1 = 25$ MY and $\hat{t}_2 = 82$ MY, respectively (Table 1).

However, our computer simulation (G.G. and M.N., unpublished data) has shown that when the a value estimated from closely related species is used for computing pairwise distances, divergence times smaller than the calibration point tend to be underestimated, whereas divergence times greater than the calibration point tend to be overestimated. This happens because for distantly related species the amino acid sites that evolve very fast have little effect on overall sequence divergence and further divergence is primarily determined by slowly evolving sites, which show a rather large a value. Therefore, if we use a small a value obtained from closely related species, it will give unduly large pairwise distances for distantly related species and consequently give overestimates of divergence times for them but may give underestimates for closely related species. Therefore, time estimates of 82 and 25 MY for the human-rodent and the mouse-rat divergence time appear to be underestimates. In practice, this problem is rather complex, and detailed aspects will be discussed elsewhere.

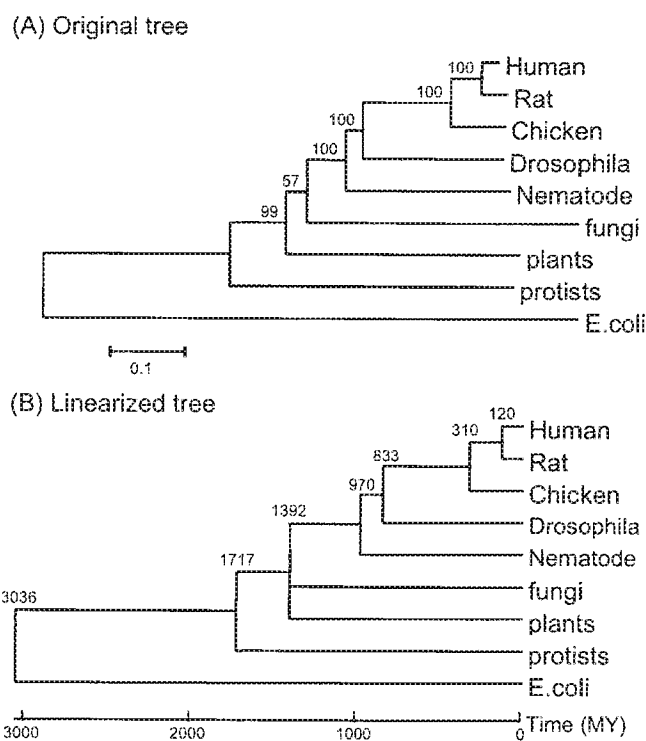


Fig. 4. (A) NJ tree constructed by using distance d_3 with $a = 1.24$ for 11 protein sequences. The numbers given for this tree stand for the bootstrap values (500 replications). (B) Linearized tree of the above NJ tree. The numbers given for this tree represent the estimates of divergent times.

Estimation of Ancient Divergence Times

Estimation of early divergence times such as those between animals, fungi, and plants is much more difficult than that of divergence times of mammalian species, because the timescale for a linearized tree for these species has to be produced from the fossil record for vertebrates (16, 18, 33), and there is no assurance of constant-rate evolution for a long evolutionary time (12, 44). Furthermore, different statistical methods often give different time estimates even if the same calibration time is used. Here we would like to examine only statistical problems considering protein sequence data from eubacteria (mostly *Escherichia coli*), protists (mostly *Plasmodium*), plants (*Arabidopsis*), fungi (yeast), and five species of animals (nematode, *Drosophila*, chicken, rat, and human). Although a large number of genes have been sequenced in some of these organisms, we could find only 11 orthologous genes that are shared by the above nine species

and show relatively few alignment gaps (see supplemental Table 4 at www.pnas.org). All of these proteins were considered to be of eubacterial rather than archeobacterial origin (45).

We tested the molecular clock hypothesis for each protein using PC gamma distance, but none of the proteins except one violated the clock hypothesis. This hypothesis was not rejected even when the multiprotein gamma distance for all proteins (3,310 amino acids) was used. The a value obtained for the latter set of proteins was 1.24, and the NJ and the linearized trees constructed from the multiprotein gamma distances are presented in Fig. 4. The timescale for this tree was obtained by using the calibration point of 310 MY between chicken and mammals. The time estimates obtained by this and other methods are presented in Table 2. The divergence time between the *E. coli* genes and their homologues from the eukaryotes used here was obtained under the assumption of a molecular clock, because there was no outgroup for this species group.

CDs d_2 with $\bar{a} = 1.53$ and d_3 with $a = 1.24$ gave essentially the same estimates for all the divergence times considered here (Table 2). Distance d_1 gave slightly smaller estimates than those obtained by d_2 and d_3 . Table 2 includes the estimates obtained by d_3 with $a = 0.54$, which was obtained by using only animal sequences (five species). This distance again gives a smaller estimate (115 MY) for the human-rat divergence, which is below the calibration point (310 MY). However, it gives rather high estimates for divergence times earlier than the calibration point. In particular, the estimate of the *E. coli*-eukaryote divergence is unrealistic, because it is older than the age of Earth (ca. 4,500 MY).

Table 2 also includes the time estimates obtained by the IP approach. These estimates are similar to those obtained by Wang *et al.* (16) with a similar method. However, they are considerably higher than the estimates obtained by d_2 and d_3 with $a = 1.24$. Because the IP approach is expected to give overestimates, the values obtained by d_2 and d_3 with $a = 1.24$ appear to be more reliable than those obtained by the IP approach. Unlike the case of mammalian data, Lynch's distance (d_L) gives the smallest time estimates (62 MY) for the human-rat divergence but give large estimates for ancient divergence times. However, the standard errors of these estimates are very large.

Our estimate of divergence time (about 3,000 MY) between eubacteria and eukaryotes based on d_2 and d_3 with $a = 1.24$ is younger than the age (ca. 3,500 MY) of some old microfossils reported (46). If these microfossils are genuine and if the molecular clock hypothesis holds up to ancient bacterial evolution, the difference can be explained by (i) the large standard error of our estimate, (ii) horizontal gene transfer that might have occurred between the ancestors of current eubacteria and eukaryotes, and/or (iii) the possibility that the ancient microfossils reported do not represent the ancestors of current eubacteria and/or eukaryotes.

Table 2. Estimates ($\pm$ standard errors) of divergence times (MY) of various organisms from the human lineage

Method	Rats	Chicken	<i>Drosophila</i>	Nematodes	Fungi	Plants	Protists	Eubacteria
IP approach								
PC gamma	124 $\pm$ 28	310	962 $\pm$ 132	1,225 $\pm$ 211	1,768 $\pm$ 311	1,715 $\pm$ 257	2,282 $\pm$ 557	3,557 $\pm$ 649
CD approach								
d_1 ($\bar{a} = 1.53$)	113 $\pm$ 38	310	745 $\pm$ 196	930 $\pm$ 274	1,229 $\pm$ 402	1,343 $\pm$ 394	1,578 $\pm$ 485	2,600 $\pm$ 568
d_2 ($\bar{a} = 1.53$)	128 $\pm$ 38	310	798 $\pm$ 121	951 $\pm$ 168	1,372 $\pm$ 275	1,372 $\pm$ 272	1,707 $\pm$ 379	3,000 $\pm$ 476
d_3 ($a = 1.24$)	120 $\pm$ 36	310	833 $\pm$ 114	970 $\pm$ 160	1,392 $\pm$ 256	1,392 $\pm$ 252	1,717 $\pm$ 349	3,036 $\pm$ 470
d_3 ($a = 0.54$)	115 $\pm$ 35	310	931 $\pm$ 153	1,115 $\pm$ 229	1,740 $\pm$ 422	1,740 $\pm$ 424	2,276 $\pm$ 667	5,010 $\pm$ 1,060
d_L ($a = 0.50$)	62 $\pm$ 10	310	798 $\pm$ 274	881 $\pm$ 354	1,779 $\pm$ 651	1,557 $\pm$ 970	1,834 $\pm$ 1,034	6,468 $\pm$ 5045

d_1 , unweighted average PC gamma; d_2 , average PC gamma weighted with sequence length; d_3 , multiprotein gamma; d_L , Lynch's distance. The divergence time (310 MY) between mammals and birds was used as the calibration point. Evolutionary relationships among animals, fungi, and plants varied with distance measure.

Discussion

We have examined various methods of estimating divergence times and have shown that the IP approach is expected to give biased estimates, which are usually greater than those obtained by the CD approach. In the latter approach, distances d_2 and d_3 are expected to give more reliable estimates than distance d_1 , although the difference is usually small unless ancient divergence times are considered. Distances d_2 and d_3 usually give similar estimates, but it is easier to compute d_3 than d_2 .

We have seen that molecular estimates of divergence times depend on a number of assumptions, and they are generally very crude. Nevertheless, if we use a large number of protein sequences, the estimates appear to be reasonably good (11, 17, 18). Our estimate (96 MY) of the time of human-rodent divergence from d_3 is somewhat smaller than a recent estimate (112 MY) obtained by Kumar and Hedges (11). This difference occurred primarily because we used the CD approach with multiprotein gamma distance, whereas Kumar and Hedges used the IP approach with PC distance. In the case of the mouse-rat divergence, the difference between our estimate (33 MY) and Kumar and Hedges' (41 MY) is substantial.

In the present paper, we did not consider the uncertainty of the calibration point used. In general, the degree of this uncertainty is quite high (12), so that we should always keep in mind that molecular time estimates are very crude, and that the standard errors attached to them merely represent the statistical error associated with molecular data under the substitution model used. Therefore, small standard errors do not necessarily mean a high accuracy of estimates. If we consider uncertainty of the calibration point, the reliability of time estimates is reduced considerably. For example, Lee (12) states that the divergence between birds and mammals probably occurred 288–310 MY ago. In our study, we used $T = 310$ MY, because the true divergence time is likely to be at the higher end of paleontological estimates. However, if we use 288 MY, all the time estimates in Tables 1 and 2 will be lowered by 7.6%.

Molecular time estimates are usually greater than paleontological estimates. Molecular evolutionists tend to argue that this is mainly caused by incomplete fossil records and that molecular estimates are more accurate (4, 39). By contrast, paleontologists and other critics (12, 36, 37) often ascribe this difference to the inaccuracy of the molecular approach of dating. It is not easy to settle this controversy at this stage. Fortunately, molecular data

are now rapidly increasing thanks to the recent genome-sequencing projects for many different organisms, and when more data become available, we will be able to make more reliable phylogenetic trees and more reliable estimates of divergence times. If we can construct consistent phylogenetic trees with time estimates for many species and for many genes, we should be able to reconstruct a reasonably good evolutionary history of different organisms at the molecular level. This history can then be compared with paleontological data to develop a unified view of the tree of life. At the present time, the amount of molecular data used for phylogenetic inference and time estimation are often too small to give reliable results.

Of course, it is important to as much as possible use genes or proteins whose evolution follows the molecular clock hypothesis. In recent years, a number of authors have used mitochondrial genes or proteins for estimating divergence times (47, 48). However, these data appear to be inappropriate for time estimation when different orders or classes of vertebrates are considered, because the evolutionary rate varies extensively from species group to species group. For example, the evolutionary rate appears to be more than two times lower in fish than in mammals (49) and more than two times lower in artiodactyls than in primates (47, 50). In these cases, the linearized tree method would not give reliable time estimates.

Another problem is the absence of reliable fossil records to calibrate ancient divergence times. At present, it is customary to use vertebrate fossil records to infer ancient divergence times such as early metazoan divergence and the divergence between animals, fungi, and plants (e.g., refs. 14, 16, 18). Estimation of ancient divergence times by using recent calibration dates is more error prone than that of recent divergence times. In this case, erroneous sequence alignment often causes a serious problem, and small differences in the gamma parameter value influence the estimates substantially. In the case of bacterial evolution, horizontal gene transfer also plays an important role (51), and this would introduce another source of errors in inferring phylogenies and divergence times. Great caution is necessary in the estimation of ancient evolutionary times.

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Supplementary material for Nei *et al.* (February 20, 2001) *Proc. Natl. Acad. Sci. USA*,
10.1073/pnas.051611498

Table 3. 104 protein sequence data from five vertebrate species (data from Hovergen)

	Protein	Estimate (α) of gamma parameter	Number of amino acids used	Accession number*
1	Insulin-like growth factor I	0.588	112	FAM000007
2	Prepro-insulin	0.811	106	FAM000008
3	Heparin cofactor II	1.139	453	FAM000037
4	Muscarinic acetylcholine receptor M1	0.454	459	FAM000059d
5	Angiotensin II receptor type I	0.994	354	FAM000084
6	Alcohol dehydrogenase-1 (Adh-1)	0.938	228	FAM000111
7	Gastric H ⁺ , K ⁺ ATPase beta-subunit	1.144	281	FAM000133
8	Na ⁺ , K ⁺ ATPase beta-subunit	0.718	301	FAM000135
9	Acetylcholine receptor delta-subunit	0.886	508	FAM000152a
10	Acetylcholine gamma-subunit	1.307	500	FAM000152b
11	Acetylcholine receptor alpha-subunit	0.409	456	FAM000152e
12	Serum albumin	4.916	596	FAM000192
13	Gap junction protein connexin-4	0.23	377	FAM000242
14	Alpha-A-crystallin	0.887	163	FAM000248a
15	Cu-Zn superoxide dismutase (SOD1)	0.853	150	FAM000256
16	Neural cadherin (N-cadherin)	0.355	900	FAM000258
17	Basic fibroblast growth factor (bFGF)	0.369	152	FAM000274
18	Preproglucagon	1.358	131	FAM000286
19	Bone morphogenetic protein-4 (BMP-4)	0.392	394	FAM000306a
20	Bone morphogenetic protein-2 (BMP-2)	0.581	351	FAM000306b
21	Tissue inhibitor of metalloproteinase-3 (TIMP-3)	0.512	209	FAM000330b
22	Aldehyde dehydrogenase I (ALDH-1)	0.595	499	FAM000337
23	Ornithine decarboxylase (ODC)	0.489	448	FAM000348
24	Osteonectin (Sparc)	0.367	292	FAM000350
25	Poly (ADP-ribose) synthetase	0.634	990	FAM000356
26	Phosphatase 2A-beta (PP2A-beta)	0.039	309	FAM000362
27	Preprosomatostatin	0.398	115	FAM000371
28	ABC transporter (TAP2)	2.661	656	FAM000385
29	Tryptophan hydroxylase (Tph)	0.668	437	FAM000388b
30	Iron binding protein (ferretin) heavy chain	0.403	176	FAM000533
31	Glutamine synthetase	0.46	368	FAM000534
32	Retinol-binding protein (RBP)	0.924	189	FAM000555
33	Type IIA activin receptor	0.286	513	FAM000561a
34	Alpha-enolase (2-phospho-D-glycerate hydrolase)	0.252	434	FAM000627
35	Cyclin D1	0.711	290	FAM000646a
36	Complement component C	1.96	1556	FAM000664

37	Zona pellucida glycoprotein-3 (ZP3)	3.254	397	FAM000667
38	Helix-loop-helix protein (Id-2)	0.243	133	FAM000769
39	Cardiac troponin	0.74	162	FAM000868a
40	Hepatic nuclear factor 1-alpha unit	0.608	585	FAM001004b
41	Retinal degeneration slow (RDS) protein	0.602	343	FAM001091
42	Hepatocyte growth factor (HGF)	0.883	706	FAM001247a
43	Hepatocyte growth factor-like protein	1.243	692	FAM001247c
44	Vimentin (intermediate filament)	0.365	455	FAM001327a
45	Middle weight neurofilament (NF-M)	0.838	748	FAM001328b
46	Cardiac calsequestrin	1.301	380	FAM001355
47	Steroidogenic factor-1 SF-1	0.505	451	FAM001365a
48	Thyroid hormone receptor alpha (c-erbA)	0.288	408	FAM001369a
49	Thyroid hormone receptor beta subunit	0.192	368	FAM001369b
50	Very low density lipoprotein receptor (VLDLR)	0.398	830	FAM001390b
51	Domain transcription factor LIM-1	0.187	401	FAM001396
52	Cyclin-dependent kinase (CDC2)	0.422	296	FAM001432b
53	Neural cell-adhesion molecule (N-CAM)	0.576	716	FAM001444
54	Neural cell surface protein F	0.665	991	FAM001446a
55	Focal adhesion kinase (FAK)	0.182	1051	FAM001463
56	Protein-tyrosine (src) kinase (CSK)	0.224	450	FAM001467
57	Neural receptor protein-tyrosine kinase (trkB)	0.508	797	FAM001471b
58	Insulin-like growth factor I receptor	0.434	1301	FAM001471e
59	c-met oncogene	0.881	1366	FAM001472
60	c-kit receptor tyrosine kinase	0.957	924	FAM001478b
61	Oocyte maturation factor Mos (c-mos)	1.456	324	FAM001493
62	Fibronectin (FNI)	0.839	1069	FAM001519
63	Zinc finger protein slug (SLUG)	0.665	148	FAM001601
64	Wilms tumor protein	0.35	375	FAM001607
65	Integrin beta-1 subunit	0.376	789	FAM001621
66	Muscle regulatory factor (MRF4)	0.38	239	FAM001622c
67	Achaete-scute homologue	0.293	189	FAM001624
68	Collagenase-3 (matrix metalloproteinase-13)	1.02	378	FAM001631c
69	Fos-related antigen-2 (fra-2)	0.336	313	FAM001679a
70	Follistatin (activin antagonist)	0.354	315	FAM001730
71	Structure-specific recognition protein 1 (SSRP1)	0.305	529	FAM001987
72	Gonadotrophin-releasing hormone (GnRH)	3.093	87	FAM002205
73	Alpha 2,8-sialyltransferase-1 (pst-1)	0.577	353	FAM002490
74	Syndecan-3 (Syn-3)	2.598	317	FAM002852
75	Distal-less 3 (Dlx-5) homeobox	0.331	278	FAM002888a
76	Homeobox protein Nkx-2.5 subunit	1.097	285	FAM002890b
77	Corticotrophin-releasing factor type1 receptor	0.375	410	FAM003058b

78	T-cell leukemia (LERK-2)	0.439	326	FAM003670
79	Delta-1 protein	0.584	705	FAM003932a
80	Conductin/axin (AXIN)	0.667	818	FAM006148
81	Transforming growth factor – beta3	0.653	389	FAM000027 [†]
82	Na, K-ATPase beta-2 subunit (ATP1B2)	1.61	271	FAM000134 [†]
83	Embryonic beta-type globin	1.364	137	FAM000214 [†]
84	Alpha globin	1.277	142	FAM000215 [†]
85	Heat shock protein	0.138	650	FAM000300 [†]
86	Lactate dehydrogenase-A	0.616	330	FAM000364a [†]
87	Histone H2A	0.149	129	FAM000427 [†]
88	bcl-x regulator of cell death	0.764	200	FAM000506 [†]
89	c-ets-1 (p54) protein	0.202	437	FAM000514 [†]
90	Glutamine synthetase	0.471	368	FAM000534a [†]
91	Bone morphogenetic receptor	0.28	522	FAM000306d [†]
92	Cyclin (D2, D3)	0.291	288	FAM000646 [†]
93	Furin	0.522	768	FAM000747 [†]
94	Hepatocyte growth factor (HGF)	1.261	686	FAM001247 [†]
95	Liver glyceraldehyde-3-phosphate dehydrogenase	0.243	332	FAM001297 [†]
96	Nucleolin	1.793	605	FAM001318 [†]
97	Vimentin	0.361	454	FAM001327 [†]
98	Lamin B1	0.675	578	FAM001331 [†]
99	Nucleolar phosphoprotein B23 (NPM1)	1.022	285	FAM001359 [†]
100	N-myc protein	0.698	398	FAM001383 [†]
101	CDC2 involved in cell cycle control	0.332	296	FAM001432 [†]
102	Protein tyrosine phosphatase (PTPase-alpha)	0.280	778	FAM001447 [†]
103	Phosphotyrosil protein phosphatase	0.084	591	FAM001456 [†]
104	M-sam fibroblast growth factor	0.314	807	FAM001479 [†]

*Gene family names from the Hovergen database (pbil.univ-lyon1.fr/search/query.html).

[†]Entries 81--104 from Hovergen 1996 Release.

Attorney Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor :	Rachel Fraser	Art Unit :	Unknown
Serial No. :	15/398,479	Examiner :	Unknown
Filed :	January 4, 2017	Conf. No. :	2358
Title :	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES		

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the references listed on the enclosed PTO-1449 Form. Foreign patent documents and non-patent literature are enclosed; cited U.S. patents and patent application publications will be provided on request. Copies of a communication from a foreign patent office in counterpart applications are also enclosed.

This statement is being filed after a first action on the merits, but before receipt of a final action or a notice of allowance. A late submission fee in the amount of \$180, specified by 37 CFR §1.17(p), is being paid with this statement. Apply any necessary charges or credits to deposit account 06-1050, referencing the above attorney docket number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: July 14, 2017_____

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Sheet 1 of 2

Substitute Disclosure Form U.S. Department of Commerce Patent and Trademark Office Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))	Attorney Docket No. 38767-0015002	Application No. 15/398,479
	Applicant Impossible Foods Inc.	
	Filing Date January 4, 2017	Group Art Unit

U.S. Patent Documents							
Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	1.	2002/0034570	3/21/2002	Krammer et al.			
	2.	2003/0212281	11/13/2003	Sinha et al.			
	3.	2006/0204644	9/14/2006	Cavallini et al.			
	4.	2008/0254199	10/16/2008	Orcutt et al.			
	5.	2010/0196575	8/5/2010	Sanchez			
	6.	2010/0310738	12/9/2010	Ludwig			
	7.	2015/0296834	10/22/2015	Geistlinger et al.			
	8.	2015/0296835	10/22/2015	Anderson et al.			
	9.	5,597,594	1/28/1997	Matsuura et al.			

Foreign Patent Documents or Published Foreign Patent Applications								
Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation	
							Yes	No
	10.	WO 2013/013292	1/31/2013	WIPO				
	11.	WO 2014/110540	7/17/2014	WIPO				
	12.	WO 2015/127388	8/27/2015	WIPO				

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	13.	Australian Patent Examination Report No. 1 in Australian Application No. 2012281064, dated January 25, 2016, 5 pages
	14.	Australian Patent Examination Report No. 1 in Australian Application No. 2012281069, dated September 25, 2015, 5 pages
	15.	International Preliminary Report on Patentability in International Application No. PCT/US2014/011361, dated July 14, 2015, 13 pages
	16.	International Preliminary Report on Patentability in International Application No. PCT/US 2012/046560, mailed 1/23/2014, 12 pages
	17.	International Preliminary Report on Patentability in International Application No. PCT/US 2012/46552, mailed 1/23/2014, 9 pages
	18.	International Preliminary Report on Patentability in International Application No. PCT/US14/11362, dated July 23, 2015, 10 pages

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form

Sheet 2 of 2

Substitute Disclosure Form Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0015002	Application No. 15/398,479
		Applicant Impossible Foods Inc.	
		Filing Date January 4, 2017	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	19.	International Search Report and Written Opinion in International Application No. PCT/US 2012/046560, mailed 12/14/2012, 11 pages
	20.	International Search Report and Written Opinion in International Application No. PCT/US 2012/46552, mailed 11/19/2012, 12 pages
	21.	International Search Report and Written Opinion in International Application No. PCT/US14/11361, dated June 16, 2014, 26 pages
	22.	International Search Report and Written Opinion in International Application No. PCT/US14/11362, dated June 13, 2014, 19 pages
	23.	International Search Report and Written Opinion in International Application No. PCT/US2015/017147, dated May 01, 2015, 15 pages
	24.	International Search Report and Written Opinion in International Application No. PCT/US2015/023679, dated August 28, 2015, 26 pages
	25.	Invitation to Pay Fees in International Application No. PCT/US14/11361, dated April 10, 2014, 4 pages

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 387670016WO1	FOR FURTHER ACTION	See item 4 below
International application No. PCT/US2014/011362	International filing date (<i>day/month/year</i>) 13 January 2014 (13.01.2014)	Priority date (<i>day/month/year</i>) 11 January 2013 (11.01.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant IMPOSSIBLE FOODS INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).
2. This REPORT consists of a total of 9 sheets, including this cover sheet.

In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).

	Date of issuance of this report 14 July 2015 (14.07.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Mineko Mohri e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

13 JUN 2014

Applicant's or agent's file reference
387670016WO1

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US14/11362

International filing date (day/month/year)

13 January 2014 (13.01.2014)

Priority date (day/month/year)

11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)
USPC - 426/615, 634; 530/378

Applicant **MARAXI, INC.**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No: 571-273-3201

Date of completion of this opinion

14 May 2014 (14.05.2014)

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43 *bis*.1(a)).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11362

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-99 are so unclear that no meaningful opinion could be formed (*specify*):

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

Case No.: IPR2023-00206
PCT/US2014/011362 13-06-2014
International application No. 14/0943,096

PCT/US14/11362

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

☐ complied with

☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.

Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.

Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.

Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.

Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

---Continued Within Supplemental Box---

4. Consequently, this opinion has been established in respect of the following parts of the international application:

☐ all parts

☒ the parts relating to claims Nos. 1-4, 8/1-4, 73-77, 78/73-77, and 91-93

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

PCT/US14/11362

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	<u>8/1-4, 73-77, 78/73-77</u>	YES
	Claims	<u>1-4, 91-93</u>	NO
Inventive step (IS)	Claims	<u>NONE</u>	YES
	Claims	<u>1-4, 8/1-4, 73-77, 78/73-77, 91-93</u>	NO
Industrial applicability (IA)	Claims	<u>1-4, 8/1-4, 73-77, 78/73-77, 91-93</u>	YES
	Claims	<u>NONE</u>	NO

2. Citations and explanations:

Claims 1-4 and 91-93 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0151778 A1 to Richard, et al. (hereafter "Richard").

As per claim 1, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated and purified proteins from a non-animal source (solubilization of at least one plant protein in an aqueous medium; the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used, are extracted from plants (extraction comprise process for isolation of pure proteins from the mixture); paragraphs [0022], [0025], [0028]; [0039], [0040]).

As per claim 2, Richard discloses the non-dairy cheese replica of claim 1, and Richard further discloses wherein said one or more isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 3, Richard discloses the non-dairy cheese replica of claim 2, and Richard further discloses wherein said one or more isolated and purified plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*); paragraph [0040]).

As per claim 4, Richard discloses the non-dairy cheese replica of claim 3, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claim 91, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated proteins from a non-animal source (the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used in the context of the invention are extracted from plants; paragraphs [0022], [0025], [0039], [0040]).

As per claim 92, Richard discloses the non-dairy cheese replica of claim 91, and Richard further discloses wherein said one or more isolated proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 93, Richard discloses the non-dairy cheese replica of claim 92, and Richard further discloses wherein said one or more isolated plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*); paragraph [0040]).

-Continued Within the Next Supplemental Box-

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

****-Continued from Box V: Citations and Explanations-****

Claims 8/1-4 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of WO 2008/030089 A1 to Brandsma, et al. (hereafter 'Brandsma').

As per claims 8/1-4, Richard discloses the non-dairy cheese replica of any one of claims 1-4, but Richard does not disclose further comprising one or more microbes selected from bacteria, molds, and yeast. However, Brandsma discloses comprising one or more microbes selected from bacteria and yeast (adding at least two live food grade micro-organisms, said two micro-organisms are at least one a yeast strain selected from the genus Kluyveromyces and at least one bacterial strain selected from the genus Propionibacterium; to form a cheese; page 5, lines 26-32 to page 6, lines 1-3; claim 1). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide microbes such as bacteria or yeast, as disclosed by Brandsma, in order to have flavor formation in manufacture of fermented products, in order to provide non-dairy consumables.

Claims 73-76 and 78/73-76 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of US 4,435,438 A to Lehnhardt, et al. (hereafter 'Lehnhardt').

As per claim 73, Richard discloses a method of making a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; paragraph [0025]), said method comprising acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5 (method comprises, solubilization of at least one plant protein in an aqueous medium at a pH of between 2 and 7, and below the isoelectric pH of the protein; the pH of the medium is decreased, by adding IN hydrochloric acid; plant proteins used in the context of the invention are extracted from plants; paragraphs [0028],[0040], [0048]) and isolating said coacervate from said solution (encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; the wall formed is hardened by cooling the medium and crosslinked by the action of a crosslinking agent; paragraph [0011]). Richard does not disclose wherein said solution comprises 100 mM or less of a salt. However, Lehnhardt discloses wherein said solution comprises 100 mM or less of a salt (salt (sodium chloride); 2 parts by weight (2 pbw corresponds to 0.66 mM of sodium chloride); column 4, lines 1-5; column 16, lines 20-30). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein said solution comprises 100 mM or less of a salt, as disclosed by Lehnhardt, in order to provide plant protein coacervates having low salt content, in order to provide non-dairy protein consumables.

As per claim 74, Richard and Lehnhardt, in combination, disclose the method of claim 73, and Richard further discloses wherein said isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*) and cereals such as wheat, maize, barley, malt or oats; paragraph [0040]).

As per claim 75, Richard and Lehnhardt, in combination, disclose the method of claim 74, and Richard further discloses wherein said plant proteins are seed storage proteins, chickpea proteins, lentil proteins, pea proteins or Lupine proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*) and cereals such as wheat, maize, barley, malt or oats; plant proteins SWP100 and SWP50 and those marketed under the name Supro 670 and PISANE; paragraph [0040]).

As per claim 76, Richard and Lehnhardt, in combination, disclose the method of claim 75, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claims 78/73-76, Richard and Lehnhardt, in combination, disclose the method of claims 73-76, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the pH of the medium is decreased, by adding in hydrochloric acid; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

****-Continued Within the Next Supplemental Box-****

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

-Continued from Previous Supplemental Box-

Claims 77 and 78/77 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of Lehnhardt further in view of US 2008/0226810 A1 to Passe, et al (hereafter 'Passe').

As per claim 77, Richard and Lehnhardt, in combination, disclose the method of claim 76, but Richard does not disclose wherein said pea vicilins comprises convicilins. However, Passe discloses wherein said pea proteins comprise pea vicilins and/or pea legumins (pea proteins, like all proteins of leguminous plants, are constituted of three classes of proteins: globulins, albumins and insoluble proteins, the globulins (legumins, vicilins and convicilins) are the main storage proteins of the plant; legumins are hexameric proteins of 360 to 410 kDa composed of 6 double-stranded subunits of 60 kDa; vicilins are trimeric proteins whose mass ranges from 150 to 200 kDa; paragraphs [0005], [0007], [0014], [0015]). It would be obvious to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein pea proteins comprise pea vicilins and/or pea legumins, as disclosed by Passe, in order to provide other legumes, in order provide other suitable plant based proteins, in order to manufacture non-animal based imitation cheese.

As per claim 78/77, Richard, Lehnhardt, and Passe, in combination, disclose the method of claim 77, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

-Continued from Box No. IV: Lack of unity of invention-

The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non-animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 41778-701601	FOR FURTHER ACTION		See item 4 below
International application No. PCT/US2012/046552	International filing date (<i>day/month/year</i>) 12 July 2012 (12.07.2012)	Priority date (<i>day/month/year</i>) 12 July 2011 (12.07.2011)	
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237			
Applicant LYRICAL FOODS, INC.			

1.	This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).		
2.	This REPORT consists of a total of 9 sheets, including this cover sheet.		
In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.			
3.	This report contains indications relating to the following items:		
☒	Box No. I	Basis of the report	
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☐	Box No. VI	Certain documents cited	
☐	Box No. VII	Certain defects in the international application	
☐	Box No. VIII	Certain observations on the international application	
4.	The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).		

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70		Date of issuance of this report 14 January 2014 (14.01.2014) Authorized officer Yukari Nakamura e-mail: pt07.pct@wipo.int
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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITYTo: MICHAEL WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

19 NOV 2012

Applicant's or agent's file reference
41778-701601

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 12/46552

International filing date (day/month/year)

12 July 2012 (12.07.2012)

Priority date (day/month/year)

12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)

USPC - 426/573, 426/602, 426/632

Applicant LYRICAL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

31 October 2012 (31.10.2012)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43 *bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46552

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

☐ complied with

☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-33 and 89, directed to a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources; (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins; and (c) producing a cheese-replica from the gel.

Group II: claims 34, 38, 40-43 and 52, directed to a method comprising decomposing nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.

Group III: claims 35-37, directed to a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

Group IV: claim 39, directed to a composition comprising a non-dairy milk with less than 30% polysaccharides

Group V: claims 44-51 and 53-59 directed to a composition comprising a non-dairy milk and a cross-linking enzyme.

Group VI: claims 60-66, and 82-85, directed to a composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.

Group VII: claims 67-72, directed to a cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

Group VIII: claims 73-79, directed to a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

Group IX: claim 80, directed to a cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and/or aroma and /or texture of the cheese replica.

Group X: claim 81, directed to a cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and/or aroma and/or texture of the cheese replica.

The inventions listed as Groups I - X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-X are indicated above in the Group descriptions.

- Please see first supplemental box for continuation -

4. Consequently, this opinion has been established in respect of the following parts of the international application:

☐ all parts

☒ the parts relating to claims Nos. 1-33 and 89

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46552

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-33 and 89	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-33 and 89	NO
Industrial applicability (IA)	Claims	1-33 and 89	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-7, 17-19, 22-24, and 28-32 lack an inventive step under PCT Article 33(3) as being obvious over US 5,055,310 A to Nonaka et al. (hereinafter 'Nonaka') in view of US 4,678,676 A to Ishizuka et al. (hereinafter 'Ishizuka').

Regarding claim 1, Nonaka teaches a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources (col 2, In 9-43: 'soy milk'); and (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins (col 2, In 44-59). Nonaka fails to expressly teach wherein the method further comprises: (c) producing a cheese-replica from the gel. However, in a related disclosure, Ishizuka teaches a method of forming a similar soy-milk-based gel by coagulating soybean solids (col 1, In 60 to col 2, In 14) and using a gelling agent to form moldable soybean-milk-based gels (col 2, In 50 to col 3, In 2); and the specific formation of 'cheese-like emulsified foods' from the soybean-milk-based gels (col 3, In 45-56). In light of the soybean-milk-based cheese-like emulsified foods taught in Ishizuka, one with ordinary skill in the art could confirm without undue experimentation the effectiveness of forming the soybean-milk-based gel taught in Nonaka into a cheese-replica product, as taught in Ishizuka.

Regarding claims 2 and 3, claim 1 is obvious as above. Nonaka further teaches wherein the emulsion contains no animal products (col 1, In 35: soy-milk was the source of proteins and fats in the product).

Regarding claims 4, 5 and 6, claim 1 is obvious as above. Nonaka also teaches wherein the inducing step comprises adding an enzyme (col 1, In 36); more specifically a transglutaminase from *Streptovorticillium mobaraense* (col 4, In 15).

Regarding claim 7, claim 1 is obvious as above. Nonaka additionally teaches wherein the emulsion is a non-dairy milk (col 1, In 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claims 17, 18 and 19, claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a sugar to the emulsion; more specifically a monosaccharide or a disaccharide. However, Nonaka does teach the use of various monosaccharides and disaccharides as carbon sources for the incubation of the transglutaminase-forming microorganisms (col 4, In 19-46). Ishizuka also teaches the inclusion of a 'sweetener' component within the cheese-like composition (col 3, In 60), wherein the use of monosaccharides and disaccharides as sweetener components in a food product is known and routine to those with ordinary skill in the art. A person of ordinary skill in the art would have desired to produce a flavor similar to dairy-based cheese, and would have known to use sweeteners, particularly sugars, based on the combined teachings of Nonaka and Ishizuka.

Regarding claim 22, claim 1 is obvious as above. Nonaka also discloses wherein the method further comprises cutting the gel (col 8, In 48-49).

Regarding claim 23, claim 22 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises draining and shaping the cut gel. However, the processing of draining and shaping a food product, especially gel-based food products, was known and routine to those with ordinary skill in the art as an effective way of optimizing the texture, palatability, and marketability of a gel-based food product, such as a cheese-like product; such that one with ordinary skill could have devised, without undue experimentation, a method of producing a gel-based food product comprising a step of draining and shaping the cut gel product, such as a product taught in Nonaka, such as to achieve a desired likeness to a cheese product.

Regarding claim 24, Ishizuka teaches the method further comprising adding a flavoring component (claim 6).

Regarding claim 28, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein at least one of the plant sources is a legume (col 1, In 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claim 29, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein the proteins are concentrated prior to preparing the emulsion (col 2, In 61-62).

----- continued in Second Supplemental Box -----

WRITTEN OPINION OF THE
 INTERNATIONAL SEARCHING AUTHORITY

International application No.

 PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
 Continuation of:
 Box IV: Lack of Unity of Invention

There is no common technical element shared by all of the above groups. Groups I and VII-X are related to a cheese replica. Groups II-V are related to a non-dairy milk, which, in Groups II and III may be a nut milk. Groups I-III, V, and VI share the common technical element of a crosslinking enzyme, which, in Groups I-III and VI may be a transglutaminase. These common technical elements do not represent an improvement over the combined prior art of US 5,055,310 A to Nonaka et al. (hereinafter "Nonaka") and US 2005/0220937 A1 to Ito et al. (hereinafter "Ito"), as follows: Nonaka teaches a shelf-stable soybean curd product which is prepared by reacting a non-dairy milk (soy milk) with a solidifying agent and a transglutaminase (abstract); wherein the milk is produced by separating (filtering) plant solids from the liquid (col 9, ln 24-35). Although Nonaka does not specifically recite wherein the milk may also be a nut milk, it would have been obvious to a person of ordinary skill in the art to substitute a nut milk in place of the soy milk, as both were plant milk products in common use at the time of the invention. Further, Ito teaches producing a cheese-like food product (para [0058], [0071], [0072]) by using a transglutaminase (para [0059]) to enable crosslinking (shaping) of a soybean paste foodstuff (para [0059]), wherein the foodstuff comprises a non-dairy milk product (soy milk; para [0071]). It would have been obvious to a person skilled in the art to combine the teaching of Nonaka regarding the formation of a solid non-dairy milk product directly from the liquid with the teaching of Ito regarding the formation of a cheese replica made from plant material in order to make a cheese replica directly from the non-dairy milk without undue experimentation. A person of ordinary skill in the art would have desired to form a non-dairy cheese replica in a manner similar to dairy cheese making, while avoiding the use of allergenic dairy products to provide a cheese replica to users unable to consume dairy products.

Therefore, the inventions of Groups I-X lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITYInternational application No.
PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V.2. Citations and explanations:

Regarding claim 30, claim 29 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are each essentially homogeneous proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art could have produced without undue experimentation a food product using a mixture as taught by Nonaka using homogeneous proteins.

Regarding claim 31, claim 1 is obvious as above. Nonaka further discloses wherein the method further comprises concentrating, isolating, or purifying the proteins and fats from one or more plant sources (col 2, ln 9-43).

Regarding claim 32, claim 30 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are all the same essentially homogeneous plant-derived proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art would have been able, without undue experimentation, to produce the food product mixture taught by Nonaka using a homogeneous mixture of proteins.

Claims 20-21 and 25-26 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0299254 A1 to Kim et al. (hereinafter 'Kim').

Regarding claim 20, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises inoculating with lactic acid bacteria. However, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Regarding claim 21, claim 20 is obvious as above. Kim teaches wherein the method further comprises incubating to allow growth of the bacterial cultures (para [0013]: 'aging the resulting composition for a period sufficient for fermentation by the microorganism').

Regarding claims 25 and 26, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a lactic acid bacteria such as Lactobacillus species, however, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Claim 27 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0292749 A1 to Goodwins et al. (hereinafter "Goodwins").

Regarding claim 27; claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a halophile bacterium or achaea. However, in a related disclosure, Goodwins teaches the production of cheeses (para [0034]) using at least one bacterium producing bacteriocin (para [0029]), wherein the bacterium may be a halophilic bacterium (pediococcus halophiles; para [0021]). It would have been obvious to a person of ordinary skill in the art to apply a method of enhancing cheese production, as taught by Goodwin, to the cheese substitute taught by the combination of Nonaka and Ishizuka without undue experimentation, in order to improve the cheese substitute product and make it more cheese-like to a consumer.

Claim 89 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2003/0224476 A1 (Chou).

Regarding claim 89; claim 4 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the enzyme is lysyl oxidase, in a related disclosure, Chou teaches transglutaminase-reactive compounds (abstract), including the use of cheesegelling of proteins (para [0003]); wherein the transglutaminase-reactive compound is produced by denaturing a compound and refolding the compound (para [0008]). The compound may also be cross-linked with a biological agent (para [0042]), wherein the biological agent may be a lysyl oxidase (para [0050]), and may be used in the gelling of cheese (para [0176]). It would have been obvious to a person of ordinary skill in the art to substitute lysyl oxidase for transglutaminase to gel a proteinaceous product, as taught by Chou, wherein the product was a substitute cheese product, as taught by the combination of Nonaka and Ishizuka, in order to provide a cheese substitute with a flavor that was not affected by the presence of the transglutaminase enzyme.

----- continued in next Supplemental Box -----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITYInternational application No.
PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Previous Supplemental Box

Claims 8-16 and 33 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2011/0064847 A1 to McCready et al. (hereinafter 'McCready'), and further in view of US 5,807,601 A to Carpenter et al. (hereinafter "Carpenter").

Regarding claim 8; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the utility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies.

Regarding claim 9; claim 8 is obvious as above. Further, McCready teaches wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]).

Regarding claim 10; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the utility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies. Neither Nonaka, Ishizuka, nor Carpenter teaches wherein the method further comprises the steps of:

(a) obtaining nuts or seeds, and
(b) surface sterilizing the nuts or seeds, wherein the nuts or seeds comprise the plant sources. However, McCready further teaches wherein preparation further includes the steps of: (a) obtaining nuts (para [0016]), and (b) surface sterilizing the nuts (para [0016]: 'blanching'). It would have been obvious to one with ordinary skill in the art to obtain and prepare the nuts for a nut milk, as taught by McCready, in order to minimize contamination of the nuts with known pathogens, such as salmonella, or fungi that produced known carcinogens, such as aflatoxin. A person of skill in the art would have desired to appropriately sterilize the raw materials used in the process as a first step.

Regarding claim 11; claim 10 is obvious as above. Furthermore, McCready teaches wherein the surface sterilization is blanching (para [0016]).

Regarding claim 12; claim 8 is obvious as above. Furthermore, McCready teaches wherein the method further comprises washing the nuts (para [0016]: 'blanching').

Regarding claims 13 and 14; claim 10 is obvious as above. Furthermore, McCready teaches wherein the preparation further comprising decomposing the nuts; more specifically wherein the decomposing is blending (para [0017]).

Regarding claim 15 and 16; claim 10 is obvious as above. Ishizuka further teaches centrifuging the soy materials (col 4, ln 38-44). Although Ishizuka does not explicitly recite wherein the centrifuging removes at least 85% of the suspended solids, it would have been obvious to a person of ordinary skill in the art to determine an appropriate centrifugation speed and duration to separate or remove a desired amount of solids that varied in density from the solvent from any solution without undue experimentation.

----- continued in next Supplemental Box -----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Previous Supplemental Box

Regarding claim 33; claim 30 is obvious as above, Neither Nonaka nor Ishizuka explicitly teaches wherein each concentrated protein is from a separate plant species. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA". TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use concentrated proteins from a variety of separate plant species, particularly different nut species in combination with soy, based on combining the disclosure of Carpenter regarding using a nut product to produce a cheese substitute, wherein the nut product could have been any of a number of nut milks, based on the disclosure of McCready, processed according to the method disclosed by the combination of Nonaka and Ishizuka. A person of skill in the art would have recognized the potential of the different nut proteins to alter and contribute to the flavor and texture of the product, and would have desired to produce different combinations thereof, either to identify a composition most resembling a known cheese product, or to create a series of imitation cheese products with varying flavors.

Claims 1-33 and 89 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used by industry.

PATENT COOPERATION TREATY

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
PRELIMINARY REPORT ON PATENTABILITY
(CHAPTER I OF THE PATENT COOPERATION
TREATY)
(PCT Rule 44bis.1(c))

To:

McCORMICK GRAHAM, Monica
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440-1022
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
23 January 2014 (23.01.2014)

Applicant's or agent's file reference
38767-0012WO1

IMPORTANT NOTICE

International application No.
PCT/US2012/046560

International filing date (day/month/year)
12 July 2012 (12.07.2012)

Priority date (day/month/year)
12 July 2011 (12.07.2011)

Applicant
MARAXI, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Mineko Mohri

Facsimile No. +41 22 338 82 70

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 38767-0012WO1	FOR FURTHER ACTION		See item 4 below
International application No. PCT/US2012/046560	International filing date (<i>day/month/year</i>) 12 July 2012 (12.07.2012)	Priority date (<i>day/month/year</i>) 12 July 2011 (12.07.2011)	
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237			
Applicant MARAXI, INC.			

1.	This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).		
2.	This REPORT consists of a total of 8 sheets, including this cover sheet.		
In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.			
3.	This report contains indications relating to the following items:		
☒	Box No. I	Basis of the report	
☐	Box No. II	Priority	
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	
☒	Box No. IV	Lack of unity of invention	
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	
☐	Box No. VI	Certain documents cited	
☐	Box No. VII	Certain defects in the international application	
☐	Box No. VIII	Certain observations on the international application	
4.	The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).		

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland		Date of issuance of this report 14 January 2014 (14.01.2014)
Facsimile No. +41 22 338 82 70		Authorized officer Mineko Mohri
		e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITYTo: MICHAEL A. WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

14 DEC 2012

Applicant's or agent's file reference
41751-701601

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/US 12/46560International filing date (day/month/year)
12 July 2012 (12.07.2012)Priority date (day/month/year)
12 July 2011 (12.07.2011)International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23J 1/00 (2012.01)
USPC - 426/656

Applicant SAND HILL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
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- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

05 December 2012 (05.12.2012)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/46560

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 21-26, 47-63, 70-71, and 88-104

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 21-26, 47-63, 70-71, 88-104 are so unclear that no meaningful opinion could be formed (*specify*):

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 21-26, 47-63, 70-71, and 88-104

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit
- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46560

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I claims 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

----- (Continued in Supplemental Box) -----

4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts
 - ☒ the parts relating to claims Nos. 1-20

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46560

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5-6, 11, 16-17, 19, 20	YES
	Claims	1-4, 7-10, 12-15, 18	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, 7-10, 12-15, and 18 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0035003 A1 to McMIndes et al. (hereinafter "McMIndes").

As to claims 1, 3-4 and 12, McMIndes teaches a meat substitute composition comprising a protein content (para [0033]), wherein one or more isolated and purified proteins accounts for 50% or more of said protein content by weight (para [0054] - 10-50% gluten), wherein said meat substitute accurately mimics the taste, texture, or color of a meat product derived from animal sources (para [0004] - "a need exists for structured meat products which closely simulate natural steaks and roasts in appearance, taste, texture and nutritional value, but which cost less. One object of the present invention is to provide such structured meat products."; para [0033]) wherein said one or more isolated and purified proteins is gluten (para [0054] - 10-50% gluten).

As to claim 2, McMIndes teaches that said composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking (para [0033]).

As to claim 7, McMIndes teaches that gluten does not account for 10% or more of said protein content by weight (para [0052]).

As to claim 8, McMIndes teaches that each of said isolated, purified proteins is separately isolated and purified (para [0039], [0047]).

As to claim 9, McMIndes teaches that said composition comprises 1-7 isolated and purified proteins (para [0039], [0047], [0054]).

As to claim 10, McMIndes teaches that said 1-7 isolated and purified proteins are each isolated from different plant species (para [0039], [0047], [0054] - soy, wheat).

As to claim 13, McMIndes teaches that said one or more isolated and purified proteins are not isolated from an animal (para [0039], [0047], [0054]).

As to claim 14, McMIndes teaches that said one or more isolated and purified proteins are isolated from a single plant source (para [0053]).

As to claim 15, McMIndes teaches that said one or more isolated and purified proteins are isolated from multiple plant sources (para [0039], [0047], [0054]).

As to claim 18, McMIndes teaches that the isolated, purified protein has been formed into fibers (para [0050], [0058], [0065] - formation of protein fibers)

Claims 5-6, 11, 16-17, 19, and 20 lack an inventive step under PCT Article 33(3) as being obvious over McMIndes.

As to claims 5-6, McMIndes teaches a meat substitute composition, wherein said one or more isolated and purified proteins accounts for 50% of said protein content by weight (see explanation above for claim 4). McMIndes does not teach that said one or more isolated and purified proteins accounts for 75% or 90% or more of said protein content by weight. However, methods for further optimizing the protein content of the disclosed composition were routine in the art, and one of ordinary skill in the art would have arrived at the claimed percentages via routine experimentation.

As to claim 11, McMIndes teaches a meat substitute composition (see explanation above for claim 10). McMIndes does not expressly teach that said protein content comprises no more than trace amounts of any other proteins derived from different plant species. However, it would have been obvious to one of ordinary skill in the art that, absent any express teachings of other added proteins, it could be assumed that said protein content comprises no more than trace amounts of any other proteins derived from different plant species.

------(Continued in Second Supplemental Box)-----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46560

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box IV.3. Lack of Unity of Invention:

The inventions listed as Groups I-XI do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflawored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat ("In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

+++++

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/46560

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box V.2. Citations and Explanations:

As to claim 16, McMindes teaches a meat substitute composition (see explanation above for claim 13). McMindes further teaches that a component of the composition may be isolated from a genetically modified organism (para [0110], [0118]). Although McMindes does not specifically teach that the one or more isolated, purified proteins are isolated from a genetically modified organism, McMindes does teach that said proteins may be soy or wheat (para [0039], [0047], [0054]), and it would have been obvious to one of ordinary skill in the art that genetically modified soy and wheat plants were well-known in the art and routinely used to provide the materials (e.g., protein) for food production. Accordingly, it would have been obvious to isolate and purify the disclosed proteins from a genetically modified plant.

As to claim 17, McMindes does not teach that said genetically modified organism is a genetically modified bacteria or yeast organism. However, genetically modified bacteria and yeast were well-known and routinely used to inexpensively produce proteins in large volumes for a wide variety of uses, and it thus would have been obvious to one of ordinary skill in the art that the isolated and purified proteins taught by McMindes could be substituted by or supplemented with isolated, purified proteins isolated from a genetically modified bacteria or yeast organism.

As to claims 19-20, McMindes teaches a meat substitute composition, wherein said isolated, purified protein has been formed into fibers (see explanation above for claim 18). McMindes does not teach that said fibers resemble skeletal muscle fibers, or that said fibers are asymmetric fibers. However, McMindes does teach that the disclosed composition simulates the texture of natural meat products comprising skeletal muscle (para [0004]). In order to achieve such simulation of natural meat products, it would have been obvious to one of ordinary skill in the art that the fibers comprising the isolated, purified protein would need to resemble natural skeletal muscle fibers, including asymmetric fibers.

Claims 1-20 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 387670010WO1	FOR FURTHER ACTION	See item 4 below
International application No. PCT/US2014/011361	International filing date (<i>day/month/year</i>) 13 January 2014 (13.01.2014)	Priority date (<i>day/month/year</i>) 11 January 2013 (11.01.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant IMPOSSIBLE FOODS INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).
2. This REPORT consists of a total of 12 sheets, including this cover sheet.

In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).

	Date of issuance of this report 14 July 2015 (14.07.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Mineko Mohri e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P. O. Box 1022
Minneapolis, MN 55440-1022
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

16 JUN 2014

Applicant's or agent's file reference
387670010WO1

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/US14/11361

International filing date (day/month/year)
13 January 2014 (13.01.2014)

Priority date (day/month/year)
11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/212 (2014.01)
USPC - 424/478, 479

Applicant
Maraxi, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

28 May 2014 (28.05.2014)

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11361

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☒ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☒ subsequently to this Authority for the purposes of search
4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11361

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. ***-Please See Below-*** are so unclear that no meaningful opinion could be formed (*specify*):

Claims 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80 and 84-90 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. ***-Please See Above-***

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13^{ter}.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11361

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:

-Please See Supplemental Page-

4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts
 - ☒ the parts relating to claims Nos. 1-9, 17-22, 52-54

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

PCT/US14/11361

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	3-7, 9/3-9/7, 18-21, 22/20, 22/21, 52, 53, 54/52, 54/53	YES
	Claims	1, 2, 8, 9, 9/1, 9/2, 9/8, 17	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52, 54/53	NO
Industrial applicability (IA)	Claims	1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52, 54/53	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1, 2, 8, 9/1, 9/2 and 9/8 lack novelty under PCT Article 33(2) as being anticipated by US 3,870,801 A (TOMBS).

As per claim 1, Tombs discloses consumable product comprising an isolated and purified plant protein (a plant protein valuable in food products (consumable) such as arachin, a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21), wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM (mesophases (proteins) of the invention comprise undenatured plant protein in a dissolved state (soluble), dissolved in aqueous solution of 0.1 N (0.1 N = 0.1 M = 100 mM) NaCl (sodium chloride) and specifically 500 g rape protein is extracted in 5 L water (at least 1 mg/ml) adjusted to pH 7.1 and the temperature required to set the mesophase is generally set by heating at 90 degrees C to 100 degrees C; column 1, lines 64-65; column 3, lines 12-13; column 8, lines 56-57; column 19, lines 32-34).

As per claim 2, Tombs discloses the consumable product of claim 1, and Tombs further discloses wherein said product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5).

As per claim 8, Tombs discloses the consumable product of claim 2, and Tombs further discloses wherein said meat product is a pate, a sausage replica, or a meat substitute (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5).

As per claims 9/1, 9/2 and 9/8, Tombs discloses the consumable product of claims 1, 2 and 8, and Tombs further discloses wherein said isolated and purified plant protein is at least 10 kDa in size (a plant protein valuable in food products (consumable), such as arachin (21 kDa protein), a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21).

Claim 17 lacks novelty under PCT Article 33(2) as being anticipated by US 2013/0004617 A1 to Zhang, et al. (hereinafter 'Zhang').

As per claim 17, Zhang discloses a consumable product comprising a coacervate (abstract) comprising one or more isolated and purified proteins (an aqueous dispersion of complex coacervates in stable form suitable for use in foods (consumable products) is provided and can include whey protein, hydrolyzed protein, lauric arginate, polylysine, casein or any combination thereof; paragraphs [0004], [0006]).

Claims 3-7, 9/3-9/7, 52 and 54/52 lack an inventive step under PCT Article 33(3) as being obvious over Tombs in view of EP 1,361,264 B1 to Tsubaki, et al. (hereinafter 'Tsubaki').

As per claim 3, Tombs discloses the consumable product of claim 2, but Tombs does not disclose wherein said beverage is an alcoholic beverage or a protein drink. Tsubaki discloses wherein said beverage is an alcoholic beverage (foods also include beverages, such as alcoholic beverages; paragraph [0044]) or a protein drink which may prepared from grains (beer, paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of a plant protein in food that is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 4, Tombs and Tsubaki, in combination, disclose the consumable product of claim 3, but Tombs does not disclose wherein said alcoholic beverage is a cream liquor. Tsubaki discloses wherein said alcoholic beverage is a cream liquor (foods also include beverages, such as alcoholic beverages including lactic acid beverages (cream beverages); paragraph [0044]; gramineous plant fats and oils, paragraph [0039]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein in that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

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As per claim 5, Tombs and Tsubaki, in combination, disclose the consumable product of claim 4, but Tombs does not disclose said cream liquor further comprising a non-dairy lipid emulsion, wherein said cream liquor is free of animal products. Tsubaki discloses said cream liquor (foods also include beverages, such as alcoholic beverages including lactic acid beverages (cream beverages); paragraph [0044]; gramineous plant fats and oils, paragraph [0039]) further comprising a non-dairy lipid emulsion (fat (lipid) and oil composition containing beta-glucans extracted from a gramineous plant (non-dairy) can be used as a substitute in an emulsion; paragraph [0039]), wherein said cream liquor is free of animal products (fat (lipid) and oil composition containing glucans extracted from a gramineous plant can be used as a substitute (free of animal products) in an emulsion and can be alcoholic beverages including lactic acid beverages (cream beverages); paragraphs [0039], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 6, Tombs and Tsubaki, in combination, disclose the consumable product of claim 3, but Tombs does not disclose wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein. Tsubaki discloses wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein (fat (lipid) and oil composition containing glucans extracted from a gramineous plant can be used as a substitute (supplemented) in an emulsion and can be alcoholic beverages, including lactic acid beverages (cream beverages); paragraphs [0039], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with integrating protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of the plant in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), as a supplement, and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 7, Tombs discloses the consumable product of claim 2, but Tombs does not disclose wherein said condiment is a mayonnaise replica. Tsubaki discloses wherein said condiment is a mayonnaise replica (fat (lipid) and oil composition containing glucans extracted from a gramineous plant can be used as a substitute for a part of, or the whole of (replica) food which can include mayonnaise; paragraphs [0039], [0045]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of a plant protein as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product (Tsubaki; paragraphs [0001], [0039]), and a combination of these references would have resulted in a food product containing a replacement protein from a plant source.

As per claims 9/3-9/7, Tombs and Tsubaki, in combination, disclose the consumable product of claims 3-7, and Tombs further discloses wherein said isolated and purified plant protein is at least 10 kDa in size (a plant protein valuable in food products (consumable), such as arachin (21 kDa protein), a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21).

As per claim 52, Tombs discloses a method for making a consumable product comprising: a) preparing a solution comprising an isolated and purified plant protein (a plant protein valuable in food products (consumable) in its fluid form (solution), such as arachin, a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21), wherein said isolated and purified plant protein has (i) a solubility in said solution of at least 25 at a temperature between about 2 degrees C and 32 degrees C, wherein said solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in said solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein said solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM (mesophases (proteins) of the invention comprise undenatured plant protein in a dissolved state (soluble), dissolved in aqueous solution of 0.1 N (0.1 N = 0.1 M = 100 mM) NaCl (sodium chloride) and specifically 500 g rape protein is extracted in 5 L water (at least 1 mg/ml) adjusted to pH 7.1 and the temperature required to set the mesophase is generally set by heating at 90 degrees C to 100 degrees C; column 1, lines 64-65; column 3, lines 12-13; column 8, lines 56-57; column 19, lines 32-34). Tombs does not disclose adding said solution to a beverage. Tsubaki discloses adding said solution to a beverage (foods also include beverages, such as alcoholic beverages; paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 54/52, Tombs and Tsubaki, in combination, disclose the method of claim 52, but Tombs does not disclose wherein said beverage is clear. Tsubaki discloses wherein said beverage is clear (foods also include beverages, such as alcoholic beverages (many alcoholic beverages are clear); paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including the protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be utilized in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

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Claims 19-21, 22/20 and 22/21 lack an inventive step under PCT Article 33(3) as being obvious over Zhang in view of Tsubaki.

As per claim 19, Zhang discloses the consumable product of claim 17, but Zhang does not disclose said product further comprising a plant derived lipid or a microbial-derived lipid. Tsubaki discloses a product further comprising a plant derived lipid or a microbial-derived lipid (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells; paragraph [0029]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes the use of the coacervate to enhance food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food, which is uniformly dispersible throughout the food and can be used in part of, or as whole of a fat or lipid in the food product (Tsubaki; paragraphs [0001], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claim 20, Zhang and Tsubaki, in combination, disclose the consumable product of claim 19, but Zhang does not disclose wherein said plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil. Tsubaki discloses a plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells, and lecithin can be added; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate for enhancing food products, Tsubaki discloses the use of a plant protein as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claim 21, Zhang and Tsubaki, in combination, disclose the consumable product of claim 20, but Zhang does not disclose said product comprising up to about 1% lecithin by weight. Tsubaki discloses a product comprising up to about 1% lecithin by weight (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited, and include lipid of dry microbial cells and lecithin can be added in general amounts of 0.01 to 15%; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate to enhance food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product, and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claims 22/20 and 22/21, Zhang and Tsubaki, in combination, disclose the consumable product of claims 20 and 21, but Zhang does not disclose wherein said product comprises lecithin and said oil. Tsubaki discloses wherein said product comprises lecithin and said oil (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells and lecithin can be added in general amounts of 0.01 to 15%; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate for enhancing food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food, which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product, and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

Claim 18 lacks an inventive step under PCT Article 33(3) as being obvious over Zhang in view of Tombs.

As per claim 18, Zhang discloses the consumable product of claim 17, but Zhang does not disclose wherein said consumable product is a meat replica. Tombs discloses a consumable product that is a dried meat substitute formed wholly from vegetable protein using unset mesophase binder (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with method of extracting a plant protein, as previously disclosed by Tombs, as the previous disclosure by Zhang discloses an aqueous dispersion of complex coacervates in stable form suitable for use in foods (Zhang; paragraph [0004]), Tombs discloses the use of a plant protein in a food product, such as a meat replica (Tombs; abstract; column 6, lines 20-21), and a combination of these references would have resulted in a superior food product containing plant protein in a meat replica.

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WRITTEN OPINION OF THE
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Box No. V:

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Claims 53 and 54/53 lack an inventive step under PCT Article 33(3) as being obvious over Tombs and Tsubaki and further in view of Zhang.

As per claim 53, Tombs and Tsubaki, in combination, disclose the method of claim 52, but Tombs does not disclose wherein said solution comprises two or more isolated and purified plant proteins. Zhang discloses wherein said solution comprises two or more isolated and purified plant proteins (an aqueous dispersion of complex coacervates in stable form suitable for use in foods (consumable products) is provided and can include whey protein, hydrolyzed protein, lauric arginate, polylysine, casein or any combination thereof; paragraphs [0004], [0006]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the extracting a plant protein, as previously disclosed by Tombs, with the use of multiple proteins, as previously disclosed by Zhang, as the previous disclosure by Tombs includes the use of plant proteins in food products, Zhang discloses the use of multiple proteins in the form of a coacervate to add to food products (Zhang; paragraph [0004]), and a combination of these references would have resulted in a superior food product containing plant proteins.

As per claim 54/53, Tombs, Tsubaki and Zhang, in combination, disclose the method of claim 53, but Tombs does not disclose wherein said beverage is clear. Tsubaki discloses wherein said beverage is clear (foods also include beverages, such as alcoholic beverages (alcoholic beverages are clear); paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the the protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the integration of the protein in food, which is uniformly dispersible throughout the food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

Claims 1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52 and 54/53 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

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Supplemental Box

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Box No. IV: Lack of Unity of Invention:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-9, 17-21 and 52-54 are directed toward a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, and a method of making said consumable product.

Group II: Claims 17-21, 28-31, 37-40, 68-70 and 81-83 are directed toward a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins, said adipose tissue replica including one or more isolated plant proteins, one or more plant or algal derived oils, optionally a phospholipid, and a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica; and wherein the connective tissue replica comprises proteins assembled into fibrous structures by a solution spinning process.

Group III: Claims 32-36 and 61-65 are directed toward a consumable product comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, and a method of making said consumable product.

Group IV: Claims 47-49 are directed toward a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

Group V: Claim 51 is directed toward a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

Groups VI+: Claims 59, 60 and SEQ ID NO: 1 are directed toward a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. (SEQ ID NO: 1-27). + type Markush group.

The method for extending the shelf-life of a meat-free consumable product comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions can be searched to the extent that it encompasses a sequence at least 70% identical to SEQ ID NO: 1 (Vigna radiata amino acid sequence). Additional sequences will be searched upon the payment of additional fees. It is believed that Claims 59 and 60 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (Vigna radiata amino acid sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (Methyldacidophilum inferorum amino acid sequence).

Groups VII+: Claims 71-73 and SEQ ID NO: 1 are directed toward a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Group VIII: Claims 74-78 are directed toward a method of making a coacervate, said method comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and b) isolating said coacervate from said solution.

Group IX: Claims 91-93 are directed toward methods of minimizing undesirable odors in a composition comprising plant proteins.

Group X: Claim 94 is directed toward a chocolate flavored spread comprising: a) sugar; b) a chocolate flavoring; and c) a cream fraction from a plant based milk.

Group XI: Claims 95-99 are directed toward a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

Group XII: Claims 100-104 are directed toward tissue replicas comprising a freeze-aligned non-animal protein.

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Box No. IV: Lack of Unity of Invention:

The inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, not present in any other groups, the special technical features of Group II including a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, or a coacervate, which are not present in any other group, the special technical features of Group III including a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, which are not present in any other Group, the special technical features of Group IV including a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, not present in any other group, the special technical features of Group V including a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, which are not present in any other group, the special technical features of Groups VI+ including a method for extending the shelf-life of a meat-free consumable product, which are not present in any other group, the special technical features of Groups VII+ including a method for imparting a beef like flavor to a consumable product, which are not present in any other group, the special technical features of Group VIII including a method of making a coacervate comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, which are not present in any other group, the special technical features of Group IX including methods of minimizing undesirable odors in a composition comprising plant proteins, which are present in any other group, the special technical features of Group X including a chocolate flavored spread, which are not present in any other Group, the special technical features of Group XI including a method for altering the texture of a consumable, which not present in any other group, the special technical features of Group XII including a freeze-aligned non-animal protein.

Groups I-XII share the technical features including consumables. Groups I, II, V, VIII, IX, X and XI share the technical features including plants. Groups I, II, VIII, IX and XI share the technical features including plant proteins. Groups I-V, VI+, VII+, VIII, IX, XI and XII share the technical features including proteins. Groups I, III, V, VII+, XI and XII share the technical features including temperature. Groups I and VIII share the technical features including pH. Groups I, III and VIII share the technical features including sodium chloride. Groups I-III, VI+, VII+, VIII and XI share the technical features including a method of making a consumable product. Groups II, III and XII share the technical features including a meat replica. Groups II and VIII share the technical features including a coacervate. Groups II and V share the technical features including plant or algal derived oils or lipids. Groups II and V share the technical features comprising an emulsion. Groups III and V share the technical features including a gel. Groups IV, VI+ and VII+ share the technical features including a heme-containing protein. Groups IV and VI+ share the technical features including meat-free products. Groups VI+ and VII+ share the technical features including a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27.

Groups VI+ share the technical features including a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

Groups VII+ share the technical features including a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

However, these shared technical features are previously disclosed by WO 2001/015714 A1 (ZLOTKIN) in view of US 2010/0074998 A1 to Espeleta-Vega et al. (hereinafter 'Espeleta-Vega'), US 2011/0083681 A1 to Sengupta, et al. (hereinafter 'Sengupta'), the publication entitled 'Comparative Analysis Of Autoxidation Of Haemoglobin' by Jensen (hereinafter 'Jensen'), US 4,986,998 A to Yoo, et al. (hereinafter 'Yoo') and GenBank Accession AFK42304 by Krishnakumar, et al. (hereinafter 'Krishnakumar').

Zlotkin discloses consumables (compositions including micronutrients (consumables); abstract) including plants (chicory fructooligosaccharide (plants); page 11, lines 15-19); proteins (proteins; page 15, lines 20-28); temperature (temperature; page 17, lines 10-25); a method of making a consumable product (a process for producing a composition comprising micronutrients (a method of making a consumable product); page 8, lines 22-27); plant or algal derived oils or lipids (soybean oil (plant or algal derived oils or lipids); page 15, lines 1-4); meat-free products (cereals; purees of fruit, vegetables, milk, yogurt, cottage cheese (meat-free products); page 18, lines 1-11), and a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto (a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto; page 14, lines 21-28).

Zlotkin does not disclose plant proteins; pH, sodium chloride; a meat replica; a coacervate; an emulsion; a gel; a heme-containing protein; a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27; a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions; and a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

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Box No. IV: Lack of Unity of Invention:

Espeleta-Vega discloses plant proteins (vegetable protein (plant proteins); abstract), pH (a pH modifier; paragraph [0010]), sodium chloride (salt (sodium chloride); paragraph [0038]), a meat replica (substitute meat food product (a meat replica); abstract); an emulsion (an emulsion; paragraph [0011]); a gel (a gel; paragraph [0011]).

Sengupta discloses a process for preparing coacervate gel particles comprising plant materials including proteins (a process for preparing coacervate gel particles comprising plant materials including proteins; paragraph [0015]).

Jensen discloses a heme-containing protein (hemoglobin; abstract) wherein the oxidation of hemoglobin and myoglobin is significantly slower than free heme (oxidation of hemoglobin and myoglobin is significantly slower than free heme; page 2029, column 1, paragraph 2), and wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin (wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin; page 2030, column 1, paragraph 1).

Yoo discloses a method for imparting (flavoring (imparting); abstract) a beef (cow (beef); column 4, lines 26-32) like flavor to a consumable product (flavor to a consumable product; abstract, column 2, lines 49-50), comprising adding to said consumable composition a heme-containing protein (comprising adding to said consumable a powdered blood product (comprising adding to said consumable composition a heme-containing protein); abstract), wherein after cooking, a beef-like flavor is imparted to said consumable (providing a meat-like flavor (beef-like flavor is imparted to said consumable); column 3, lines 1-6).

Krishnakumar discloses a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1 (a globin (a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1); page 1).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin by combining the consumable disclosed by Zlotkin with a meat replica, such as that disclosed by Espeleta-Vega, for providing the micronutrients in the consumable in a form acceptable to a consumer, such as in a meat replica, for enhancing the nutrient content of a meat replica, particularly the iron content of said replica, providing the previous disclosure of Zlotkin. Also, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables with the coacervate preparation process of Sengupta, for enabling the preparation of particulates comprising the micronutrients disclosed by Zlotkin using a plant polysaccharide, such as pectin, as disclosed by Sengupta, for providing micronutrients in a consumable and easily digestible form to enable a consumer to absorb the nutrients. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin, for including the use of hemoglobin in preference to free iron or other forms of iron, in order to provide the iron to the consumer in a biologically useful form and oxidation state. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have therefore modified the disclosure of Zlotkin, for providing a method for extending the shelf-life of a meat-free consumable product, such as the consumables disclosed by Zlotkin, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions, such as adding hemoglobin, based on the disclosure of Jensen that tetrameric hemoglobin oxidizes more slowly than other forms of heme-containing proteins. Also, it would have been obvious to a person of ordinary skill in the art to have modified the disclosure of Zlotkin, for including a meat-like flavoring including an iron-containing protein based on the disclosure of Yoo, in order to provide a beef-like flavor to a consumable food, including a poultry or fish composition, to provide a beef-flavored product not containing beef, but with lower fat, such as a consumable comprising a plant protein, fish, or poultry protein. Finally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables comprising iron, for including a plant-based iron-comprising proteins, such as the globin disclosed by Krishnakumar, in order to provide iron from non-animal sources having high absorbability and utilization by a consumer.

Since none of the special technical features of the Groups I-XII inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zlotkin, Espeleta-Vega, Sengupta, Jensen, Yoo and Krishnakumar references, unity of invention is lacking.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 41778-701601	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US 12/46552	International filing date (<i>day/month/year</i>) 12 July 2012 (12.07.2012)	(Earliest) Priority Date (<i>day/month/year</i>) 12 July 2011 (12.07.2011)
Applicant LYRICAL FOODS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46552

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-33 and 89, directed to a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources; (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins; and (c) producing a cheese-replica from the gel.

Group II: claims 34, 38, 40-43 and 52, directed to a method comprising decomposing nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-33 and 89

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46552

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)
USPC - 426/573, 426/602, 426/632

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 426/573, 426/602, 426/632

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 426/573, 426/602, 426/632; 435/128

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Lyrical Foods, Casino, Voccola, Varadan, transglutaminase, cheese, replica, imitation, plant, nut, soy, milk, extract, lysyl oxidase,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,055,310 A (Nonaka et al.) 18 October 1991 (18.10.1991), especially col 1, ln 35-36; col 2, ln 9-59; col 4, ln 15-46;	1-33 and 89
Y	US 4,678,676 A (Ishizuka et al.) 07 July 1987 (07.07.1987), especially col 2, ln 50 to col 3, ln 2; col 3, ln 45-56;	1-33 and 89
Y	US 2008/0299254 A1 (Kim et al.) 04 December 2008 (04.12.2008), especially para [0013]	20-21 and 25-26
Y	US 2011/0064847 A1 (McCready et al.) 17 March 2011 (17.03.2011), especially para [0004], [0026]	8-16 and 33
Y	US 5,807,601 A (CARPENTER et al.) 15 September 1998 (15.09.1998) abstract; col 2, ln 1-5	8-16 and 33
Y	US 2003/0224476 A1 (CHOU) 4 December 2003 (04.12.2003) abstract; para [0003]; [0008]; [0042]; [0050]; [0176]	89
Y	US 2008/0292749 A1 (GOODWINS et al.) 27 November 2008 (27.11.2008) para [0021]; [0029]; [0034]	27

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

31 October 2012 (31.10.2012)

Date of mailing of the international search report

19 NOV 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46552

Continuation of Box III: Lack of Unity of Invention

Group III: claims 35-37, directed to a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

Group IV: claim 39, directed to a composition comprising a non-dairy milk with less than 30% polysaccharides

Group V: claims 44-51 and 53-59 directed to a composition comprising a non-dairy milk and a cross-linking enzyme.

Group VI: claims 60-66, and 82-85, directed to a composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.

Group VII: claims 67-72, directed to a cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

Group VIII: claims 73-79, directed to a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

Group IX: claim 80, directed to a cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and/or aroma and /or texture of the cheese replica.

Group X: claim 81, directed to a cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and/or aroma and/or texture of the cheese replica.

The inventions listed as Groups I - X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-X are indicated above in the Group descriptions.

There is no common technical element shared by all of the above groups. Groups I and VII-X are related to a cheese replica. Groups II-V are related to a non-dairy milk, which, in Groups II and III may be a nut milk. Groups I-III, V, and VI share the common technical element of a crosslinking enzyme, which, in Groups I-III and VI may be a transglutaminase. These common technical elements do not represent an improvement over the combined prior art of US 5,055,310 A to Nonaka et al. (hereinafter "Nonaka") and US 2005/0220937 A1 to Ito et al. (hereinafter "Ito"), as follows: Nonaka teaches a shelf-stable soybean curd product which is prepared by reacting a non-dairy milk (soy milk) with a solidifying agent and a transglutaminase (abstract); wherein the milk is produced by separating (filtering) plant solids from the liquid (col 9, ln 24-35). Although Nonaka does not specifically recite wherein the milk may also be a nut milk, it would have been obvious to a person of ordinary skill in the art to substitute a nut milk in place of the soy milk, as both were plant milk products in common use at the time of the invention. Further, Ito teaches producing a cheese-like food product (para [0058], [0071], [0072]) by using a transglutaminase (para [0059]) to enable crosslinking (shaping) of a soybean paste foodstuff (para [0059]), wherein the foodstuff comprises a non-dairy milk product (soy milk; para [0071]). It would have been obvious to a person skilled in the art to combine the teaching of Nonaka regarding the formation of a solid non-dairy milk product directly from the liquid with the teaching of Ito regarding the formation of a cheese replica made from plant material in order to make a cheese replica directly from the non-dairy milk without undue experimentation. A person of ordinary skill in the art would have desired to form a non-dairy cheese replica in a manner similar to dairy cheese making, while avoiding the use of allergenic dairy products to provide a cheese replica to users unable to consume dairy products.

Therefore, the inventions of Groups I-X lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITYTo: MICHAEL WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

19 NOV 2012

Applicant's or agent's file reference
41778-701601

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 12/46552

International filing date (day/month/year)

12 July 2012 (12.07.2012)

Priority date (day/month/year)

12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)

USPC - 426/573, 426/602, 426/632

Applicant LYRICAL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

31 October 2012 (31.10.2012)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43 *bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46552

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
- ☐ paid additional fees under protest and, where applicable, the protest fee
- ☐ paid additional fees under protest but the applicable protest fee was not paid
- ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

☐ complied with

☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-33 and 89, directed to a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources; (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins; and (c) producing a cheese-replica from the gel.

Group II: claims 34, 38, 40-43 and 52, directed to a method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.

Group III: claims 35-37, directed to a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

Group IV: claim 39, directed to a composition comprising a non-dairy milk with less than 30% polysaccharides

Group V: claims 44-51 and 53-59 directed to a composition comprising a non-dairy milk and a cross-linking enzyme.

Group VI: claims 60-66, and 82-85, directed to a composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.

Group VII: claims 67-72, directed to a cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

Group VIII: claims 73-79, directed to a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

Group IX: claim 80, directed to a cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and/or aroma and /or texture of the cheese replica.

Group X: claim 81, directed to a cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and/or aroma and/or texture of the cheese replica.

The inventions listed as Groups I - X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-X are indicated above in the Group descriptions.

- Please see first supplemental box for continuation -

4. Consequently, this opinion has been established in respect of the following parts of the international application:

☐ all parts

☒ the parts relating to claims Nos. 1-33 and 89

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46552

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-33 and 89	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-33 and 89	NO
Industrial applicability (IA)	Claims	1-33 and 89	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-7, 17-19, 22-24, and 28-32 lack an inventive step under PCT Article 33(3) as being obvious over US 5,055,310 A to Nonaka et al. (hereinafter 'Nonaka') in view of US 4,678,676 A to Ishizuka et al. (hereinafter 'Ishizuka').

Regarding claim 1, Nonaka teaches a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources (col 2, In 9-43: 'soy milk'); and (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins (col 2, In 44-59). Nonaka fails to expressly teach wherein the method further comprises: (c) producing a cheese-replica from the gel. However, in a related disclosure, Ishizuka teaches a method of forming a similar soy-milk-based gel by coagulating soybean solids (col 1, In 60 to col 2, In 14) and using a gelling agent to form moldable soybean-milk-based gels (col 2, In 50 to col 3, In 2); and the specific formation of 'cheese-like emulsified foods' from the soybean-milk-based gels (col 3, In 45-56). In light of the soybean-milk-based cheese-like emulsified foods taught in Ishizuka, one with ordinary skill in the art could confirm without undue experimentation the effectiveness of forming the soybean-milk-based gel taught in Nonaka into a cheese-replica product, as taught in Ishizuka.

Regarding claims 2 and 3, claim 1 is obvious as above. Nonaka further teaches wherein the emulsion contains no animal products (col 1, In 35: soy-milk was the source of proteins and fats in the product).

Regarding claims 4, 5 and 6, claim 1 is obvious as above. Nonaka also teaches wherein the inducing step comprises adding an enzyme (col 1, In 36); more specifically a transglutaminase from *Streptovorticillium mobaraense* (col 4, In 15).

Regarding claim 7, claim 1 is obvious as above. Nonaka additionally teaches wherein the emulsion is a non-dairy milk (col 1, In 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claims 17, 18 and 19, claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a sugar to the emulsion; more specifically a monosaccharide or a disaccharide. However, Nonaka does teach the use of various monosaccharides and disaccharides as carbon sources for the incubation of the transglutaminase-forming microorganisms (col 4, In 19-46). Ishizuka also teaches the inclusion of a 'sweetener' component within the cheese-like composition (col 3, In 60), wherein the use of monosaccharides and disaccharides as sweetener components in a food product is known and routine to those with ordinary skill in the art. A person of ordinary skill in the art would have desired to produce a flavor similar to dairy-based cheese, and would have known to use sweeteners, particularly sugars, based on the combined teachings of Nonaka and Ishizuka.

Regarding claim 22, claim 1 is obvious as above. Nonaka also discloses wherein the method further comprises cutting the gel (col 8, In 48-49).

Regarding claim 23, claim 22 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises draining and shaping the cut gel. However, the processing of draining and shaping a food product, especially gel-based food products, was known and routine to those with ordinary skill in the art as an effective way of optimizing the texture, palatability, and marketability of a gel-based food product, such as a cheese-like product; such that one with ordinary skill could have devised, without undue experimentation, a method of producing a gel-based food product comprising a step of draining and shaping the cut gel product, such as a product taught in Nonaka, such as to achieve a desired likeness to a cheese product.

Regarding claim 24, Ishizuka teaches the method further comprising adding a flavoring component (claim 6).

Regarding claim 28, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein at least one of the plant sources is a legume (col 1, In 35: teaching the use of soy-milk as the source of proteins and fats in the product).

Regarding claim 29, claim 1 is obvious as above. Furthermore, Nonaka teaches wherein the proteins are concentrated prior to preparing the emulsion (col 2, In 61-62).

----- continued in Second Supplemental Box -----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box IV: Lack of Unity of Invention

There is no common technical element shared by all of the above groups. Groups I and VII-X are related to a cheese replica. Groups II-V are related to a non-dairy milk, which, in Groups II and III may be a nut milk. Groups I-III, V, and VI share the common technical element of a crosslinking enzyme, which, in Groups I-III and VI may be a transglutaminase. These common technical elements do not represent an improvement over the combined prior art of US 5,055,310 A to Nonaka et al. (hereinafter "Nonaka") and US 2005/0220937 A1 to Ito et al. (hereinafter "Ito"), as follows: Nonaka teaches a shelf-stable soybean curd product which is prepared by reacting a non-dairy milk (soy milk) with a solidifying agent and a transglutaminase (abstract); wherein the milk is produced by separating (filtering) plant solids from the liquid (col 9, ln 24-35). Although Nonaka does not specifically recite wherein the milk may also be a nut milk, it would have been obvious to a person of ordinary skill in the art to substitute a nut milk in place of the soy milk, as both were plant milk products in common use at the time of the invention. Further, Ito teaches producing a cheese-like food product (para [0058], [0071], [0072]) by using a transglutaminase (para [0059]) to enable crosslinking (shaping) of a soybean paste foodstuff (para [0059]), wherein the foodstuff comprises a non-dairy milk product (soy milk; para [0071]). It would have been obvious to a person skilled in the art to combine the teaching of Nonaka regarding the formation of a solid non-dairy milk product directly from the liquid with the teaching of Ito regarding the formation of a cheese replica made from plant material in order to make a cheese replica directly from the non-dairy milk without undue experimentation. A person of ordinary skill in the art would have desired to form a non-dairy cheese replica in a manner similar to dairy cheese making, while avoiding the use of allergenic dairy products to provide a cheese replica to users unable to consume dairy products.

Therefore, the inventions of Groups I-X lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V.2. Citations and explanations:

Regarding claim 30, claim 29 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are each essentially homogeneous proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art could have produced without undue experimentation a food product using a mixture as taught by Nonaka using homogeneous proteins.

Regarding claim 31, claim 1 is obvious as above. Nonaka further discloses wherein the method further comprises concentrating, isolating, or purifying the proteins and fats from one or more plant sources (col 2, ln 9-43).

Regarding claim 32, claim 30 is obvious as above. Neither Nonaka nor Ishizuka explicitly recites wherein the concentrated proteins are all the same essentially homogeneous plant-derived proteins. However, the use of a homogeneous mixture of proteins in food processing was known and routine to those with ordinary skill in the art as an effective way of ensuring uniform cross-linking, texture, flavor, and density of protein components through the food product; such that one with ordinary skill in the art would have been able, without undue experimentation, to produce the food product mixture taught by Nonaka using a homogeneous mixture of proteins.

Claims 20-21 and 25-26 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0299254 A1 to Kim et al. (hereinafter 'Kim').

Regarding claim 20, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises inoculating with lactic acid bacteria. However, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Regarding claim 21, claim 20 is obvious as above. Kim teaches wherein the method further comprises incubating to allow growth of the bacterial cultures (para [0013]: 'aging the resulting composition for a period sufficient for fermentation by the microorganism').

Regarding claims 25 and 26, neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a lactic acid bacteria such as Lactobacillus species, however, in a related disclosure, Kim teaches a similar method of processing a soy-milk mixture into a gel product by inducing the emulsion to form a gel by enzymatic cross-linking of the proteins using a transglutaminase enzyme (para [0013]); wherein Kim further teaches wherein the method further comprises inoculating with lactic acid bacteria (para [0013]: 'Lactobacillus plantarum'). It would have been obvious to one with ordinary skill in the art to combine the lactic acid inoculation taught by Kim with the composition taught by Nonaka in order to improve the flavor and texture of the resulting cheese-like product.

Claim 27 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2008/0292749 A1 to Goodwins et al. (hereinafter "Goodwins").

Regarding claim 27; claim 1 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the method further comprises adding a halophile bacterium or achaea. However, in a related disclosure, Goodwins teaches the production of cheeses (para [0034]) using at least one bacterium producing bacteriocin (para [0029]), wherein the bacterium may be a halophilic bacterium (pediococcus halophiles; para [0021]). It would have been obvious to a person of ordinary skill in the art to apply a method of enhancing cheese production, as taught by Goodwin, to the cheese substitute taught by the combination of Nonaka and Ishizuka without undue experimentation, in order to improve the cheese substitute product and make it more cheese-like to a consumer.

Claim 89 lacks an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2003/0224476 A1 (Chou).

Regarding claim 89; claim 4 is obvious as above. Neither Nonaka nor Ishizuka expressly teaches wherein the enzyme is lysyl oxidase, in a related disclosure, Chou teaches transglutaminase-reactive compounds (abstract), including the use of cheesegelling of proteins (para [0003]); wherein the transglutaminase-reactive compound is produced by denaturing a compound and refolding the compound (para [0008]). The compound may also be cross-linked with a biological agent (para [0042]), wherein the biological agent may be a lysyl oxidase (para [0050]), and may be used in the gelling of cheese (para [0176]). It would have been obvious to a person of ordinary skill in the art to substitute lysyl oxidase for transglutaminase to gel a proteinaceous product, as taught by Chou, wherein the product was a substitute cheese product, as taught by the combination of Nonaka and Ishizuka, in order to provide a cheese substitute with a flavor that was not affected by the presence of the transglutaminase enzyme.

----- continued in next Supplemental Box -----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITYInternational application No.
PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Previous Supplemental Box

Claims 8-16 and 33 lack an inventive step under PCT Article 33(3) as being obvious over Nonaka in view of Ishizuka, further in view of US 2011/0064847 A1 to McCready et al. (hereinafter 'McCready'), and further in view of US 5,807,601 A to Carpenter et al. (hereinafter "Carpenter").

Regarding claim 8; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the utility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies.

Regarding claim 9; claim 8 is obvious as above. Further, McCready teaches wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]).

Regarding claim 10; claim 1 is obvious as above. Neither Nonaka nor Ishizuka explicitly teaches wherein at least one of the plant sources are nuts. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA".TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use a nut milk, as taught by McCready, for a protein source for a cheese alternative, as taught by Carpenter, using a process of formation of a non-dairy cheese product, as taught by the combination of Nonaka and Ishizuka. A person of ordinary skill in the art would have recognized the utility of nut milks as an alternative to soy-based milk as a source for the production of the cheese alternative, in order to provide a product useful for consumers with soy allergies. Neither Nonaka, Ishizuka, nor Carpenter teaches wherein the method further comprises the steps of:

(a) obtaining nuts or seeds, and
(b) surface sterilizing the nuts or seeds, wherein the nuts or seeds comprise the plant sources. However, McCready further teaches wherein preparation further includes the steps of: (a) obtaining nuts (para [0016]), and (b) surface sterilizing the nuts (para [0016]: 'blanching'). It would have been obvious to one with ordinary skill in the art to obtain and prepare the nuts for a nut milk, as taught by McCready, in order to minimize contamination of the nuts with known pathogens, such as salmonella, or fungi that produced known carcinogens, such as aflatoxin. A person of skill in the art would have desired to appropriately sterilize the raw materials used in the process as a first step.

Regarding claim 11; claim 10 is obvious as above. Furthermore, McCready teaches wherein the surface sterilization is blanching (para [0016]).

Regarding claim 12; claim 8 is obvious as above. Furthermore, McCready teaches wherein the method further comprises washing the nuts (para [0016]: 'blanching').

Regarding claims 13 and 14; claim 10 is obvious as above. Furthermore, McCready teaches wherein the preparation further comprising decomposing the nuts; more specifically wherein the decomposing is blending (para [0017]).

Regarding claim 15 and 16; claim 10 is obvious as above. Ishizuka further teaches centrifuging the soy materials (col 4, ln 38-44). Although Ishizuka does not explicitly recite wherein the centrifuging removes at least 85% of the suspended solids, it would have been obvious to a person of ordinary skill in the art to determine an appropriate centrifugation speed and duration to separate or remove a desired amount of solids that varied in density from the solvent from any solution without undue experimentation.

----- continued in next Supplemental Box -----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46552

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Previous Supplemental Box

Regarding claim 33; claim 30 is obvious as above, Neither Nonaka nor Ishizuka explicitly teaches wherein each concentrated protein is from a separate plant species. However, in a related disclosure, Carpenter teaches imitation cheese products (abstract), and wherein imitation cheese products were available that contained nut products (There are products which are sold as cheese substitute that do not contain casein. For example, "VEGAN RELLA". TM. is a product sold by Sharon's Finest, Santa Rosa, Calif., as a healthy cheese alternative. Its ingredients include brazil nuts, tapioca, rice, oats, canola oil and Irish moss: col 2, ln 1-5). Furthermore, in an additional related disclosure, McCready teaches a 'non-dairy, nut-based' milk (para [0004]) which can be used as a milk-replacement product 'by those who do not consume dairy products or soy products' for various reasons (para [0026]), wherein the nuts are selected from one or more of following group: almonds, cashews, brazil nuts, coconuts, macadamia nuts, peanuts, pistachios or walnuts (para [0011]). It would have been obvious to a person of ordinary skill in the art to use concentrated proteins from a variety of separate plant species, particularly different nut species in combination with soy, based on combining the disclosure of Carpenter regarding using a nut product to produce a cheese substitute, wherein the nut product could have been any of a number of nut milks, based on the disclosure of McCready, processed according to the method disclosed by the combination of Nonaka and Ishizuka. A person of skill in the art would have recognized the potential of the different nut proteins to alter and contribute to the flavor and texture of the product, and would have desired to produce different combinations thereof, either to identify a composition most resembling a known cheese product, or to create a series of imitation cheese products with varying flavors.

Claims 1-33 and 89 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used by industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 41751-701601	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/46560	International filing date (<i>day/month/year</i>) 12 July 2012 (12.07.2012)	(Earliest) Priority Date (<i>day/month/year</i>) 12 July 2011 (12.07.2011)
Applicant SAND HILL FOODS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

- a. the figure of the **drawings** to be published with the abstract is Figure No. _____
☐ as suggested by the applicant.
☐ as selected by this Authority, because the applicant failed to suggest a figure.
☐ as selected by this Authority, because this figure better characterizes the invention.
b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46560

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 21-26, 47-63, 70-71, and 88-104
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I claims 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.

(Continued on extra sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46560

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23J 1/00 (2012.01)

USPC - 426/656

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 426/656

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 426/656, 574, 573, 531, 615

(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase; Google; PubMed

Search terms: meat, substitute, isolated, purified, protein, gluten, raw, cook, recombinant, yeast

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0035003 A1 (MCMINDES et al.) 16 February 2006 (16.02.2006) para [0004], [0033], [0039], [0047], [0050], [0052]-[0054], [0058], [0065], [0110], [0118]	1-20
Y	US 2007/0269571 A1 (AKITA et al.) 22 November 2007 (22.11.2007) entire document.	1-20
Y	US 2010/0074998 A1 (ESPELETA VEGA et al.) 25 March 2010 (25.03.2010) entire document.	1-20
Y	US 2004/0161513 A1 (AKASHE et al.) 19 August 2004 (19.08.2004) entire document.	1-20
Y	US 2011/0008502 A1 (HOSOMI et al.) 13 January 2011 (13.01.2011) entire document.	1-20

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 December 2012 (05.12.2012)

Date of mailing of the international search report

14 DEC 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/46560

Continuation of Box III - observations where unity of invention is lacking:

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

The inventions listed as Groups I-XI do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflavored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat ("In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

+++++

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MICHAEL A. WILLIS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PCTWRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

14 DEC 2012

Applicant's or agent's file reference
41751-701601**FOR FURTHER ACTION**

See paragraph 2 below

International application No.

PCT/US 12/46560

International filing date (day/month/year)

12 July 2012 (12.07.2012)

Priority date (day/month/year)

12 July 2011 (12.07.2011)

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A23J 1/00 (2012.01)

USPC - 426/656

Applicant SAND HILL FOODS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

05 December 2012 (05.12.2012)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/46560

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/46560

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 21-26, 47-63, 70-71, and 88-104

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 21-26, 47-63, 70-71, 88-104 are so unclear that no meaningful opinion could be formed (*specify*):

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 21-26, 47-63, 70-71, and 88-104

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit
- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46560

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I claims 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

----- (Continued in Supplemental Box) -----

4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts
 - ☒ the parts relating to claims Nos. 1-20

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46560

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5-6, 11, 16-17, 19, 20	YES
	Claims	1-4, 7-10, 12-15, 18	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, 7-10, 12-15, and 18 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0035003 A1 to McMIndes et al. (hereinafter "McMIndes").

As to claims 1, 3-4 and 12, McMIndes teaches a meat substitute composition comprising a protein content (para [0033]), wherein one or more isolated and purified proteins accounts for 50% or more of said protein content by weight (para [0054] - 10-50% gluten), wherein said meat substitute accurately mimics the taste, texture, or color of a meat product derived from animal sources (para [0004] - "a need exists for structured meat products which closely simulate natural steaks and roasts in appearance, taste, texture and nutritional value, but which cost less. One object of the present invention is to provide such structured meat products."; para [0033]) wherein said one or more isolated and purified proteins is gluten (para [0054] - 10-50% gluten).

As to claim 2, McMIndes teaches that said composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking (para [0033]).

As to claim 7, McMIndes teaches that gluten does not account for 10% or more of said protein content by weight (para [0052]).

As to claim 8, McMIndes teaches that each of said isolated, purified proteins is separately isolated and purified (para [0039], [0047]).

As to claim 9, McMIndes teaches that said composition comprises 1-7 isolated and purified proteins (para [0039], [0047], [0054]).

As to claim 10, McMIndes teaches that said 1-7 isolated and purified proteins are each isolated from different plant species (para [0039], [0047], [0054] - soy, wheat).

As to claim 13, McMIndes teaches that said one or more isolated and purified proteins are not isolated from an animal (para [0039], [0047], [0054]).

As to claim 14, McMIndes teaches that said one or more isolated and purified proteins are isolated from a single plant source (para [0053]).

As to claim 15, McMIndes teaches that said one or more isolated and purified proteins are isolated from multiple plant sources (para [0039], [0047], [0054]).

As to claim 18, McMIndes teaches that the isolated, purified protein has been formed into fibers (para [0050], [0058], [0065] - formation of protein fibers)

Claims 5-6, 11, 16-17, 19, and 20 lack an inventive step under PCT Article 33(3) as being obvious over McMIndes.

As to claims 5-6, McMIndes teaches a meat substitute composition, wherein said one or more isolated and purified proteins accounts for 50% of said protein content by weight (see explanation above for claim 4). McMIndes does not teach that said one or more isolated and purified proteins accounts for 75% or 90% or more of said protein content by weight. However, methods for further optimizing the protein content of the disclosed composition were routine in the art, and one of ordinary skill in the art would have arrived at the claimed percentages via routine experimentation.

As to claim 11, McMIndes teaches a meat substitute composition (see explanation above for claim 10). McMIndes does not expressly teach that said protein content comprises no more than trace amounts of any other proteins derived from different plant species. However, it would have been obvious to one of ordinary skill in the art that, absent any express teachings of other added proteins, it could be assumed that said protein content comprises no more than trace amounts of any other proteins derived from different plant species.

------(Continued in Second Supplemental Box)-----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 12/46560

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box IV.3. Lack of Unity of Invention:

The inventions listed as Groups I-XI do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflavored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat ("In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

+++++

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/46560

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Box V.2. Citations and Explanations:

As to claim 16, McMindes teaches a meat substitute composition (see explanation above for claim 13). McMindes further teaches that a component of the composition may be isolated from a genetically modified organism (para [0110], [0118]). Although McMindes does not specifically teach that the one or more isolated, purified proteins are isolated from a genetically modified organism, McMindes does teach that said proteins may be soy or wheat (para [0039], [0047], [0054]), and it would have been obvious to one of ordinary skill in the art that genetically modified soy and wheat plants were well-known in the art and routinely used to provide the materials (e.g., protein) for food production. Accordingly, it would have been obvious to isolate and purify the disclosed proteins from a genetically modified plant.

As to claim 17, McMindes does not teach that said genetically modified organism is a genetically modified bacteria or yeast organism. However, genetically modified bacteria and yeast were well-known and routinely used to inexpensively produce proteins in large volumes for a wide variety of uses, and it thus would have been obvious to one of ordinary skill in the art that the isolated and purified proteins taught by McMindes could be substituted by or supplemented with isolated, purified proteins isolated from a genetically modified bacteria or yeast organism.

As to claims 19-20, McMindes teaches a meat substitute composition, wherein said isolated, purified protein has been formed into fibers (see explanation above for claim 18). McMindes does not teach that said fibers resemble skeletal muscle fibers, or that said fibers are asymmetric fibers. However, McMindes does teach that the disclosed composition simulates the texture of natural meat products comprising skeletal muscle (para [0004]). In order to achieve such simulation of natural meat products, it would have been obvious to one of ordinary skill in the art that the fibers comprising the isolated, purified protein would need to resemble natural skeletal muscle fibers, including asymmetric fibers.

Claims 1-20 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To: ELIZABETH N. KAYTOR
FISH & RICHARDSON P.C.
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year) **28 AUG 2015**

Applicant's or agent's file reference
387670026WO1

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US2015/023679

International filing date
(day/month/year) **31 March 2015**

Applicant **IMPOSSIBLE FOODS INC.**

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see PCT Applicant's Guide, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:**
☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide, National Chapters*.

Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45bis.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide, International Phase, paragraphs 8.006-8.032*.

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670026WO1	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2015/023679	International filing date (day/month/year) 31 March 2015	(Earliest) Priority Date (day/month/year) 31 March 2014
Applicant IMPOSSIBLE FOODS INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/023679

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-17, 37-74, 84, 95, 96, 105-109
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/023679

Box No. IV **Text of the abstract (Continuation of item 5 of the first sheet)**

This document relates to ground meat replicas, and more particularly to plant-based products that mimic ground meat, including the fibrousness, heterogeneity in texture, beefy flavor, and red-to-brown color transition during cooking of ground meat. For example, this document provides meat replicas that include proteins that are selected based upon the temperature at which they gel and/or denature to replicate the behavior and qualities of meat during cooking, i.e., the firming, syneresis (water release), chew texture, or mouthfeel.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/023679

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23J 3/22 (2015.01)

CPC - A23J 3/227 (2015.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A23J 3/14, 3/22 (2015.01)

CPC - A23J 3/14, 3/227 (2015.05) (keyword delimited)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 426/573, 574 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit, Google Patents, Public AppFT and PatFT, Google Scholar

Search terms used: "meat replica" isolated plant protein dough cucumis juice cucumber food flavor meat vegetarian

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0254199 A1 (ORCUTT et al) 16 October 2008 (16.10.2008) entire document	99, 100, 103, 104
Y		28-34, 77-83, 87-94, 101, 102, 113
Y	WO 2013/010042 A1 (SAND HILL FOODS INC) 17 January 2013 (17.01.2013) entire document	1-3, 18-27, 33-36, 85-94, 110-113
Y	US 2006/0204644 A1 (CAVALLINI et al) 14 September 2006 (14.09.2006) entire document	1-3, 18-36, 77-83, 110-113
Y	US 2002/0034570 A1 (KRAMMER et al) 21 March 2002 (21.03.2002) entire document	75, 76, 85, 86, 97, 98
Y	US 2003/0212281 A1 (SINHA et al) 13 November 2003 (13.11.2003) entire document	75, 76, 85, 86, 97, 98
Y	WO 2013/010037 A1 (LYRICAL FOODS INC) 17 January 2013 (17.01.2013) entire document	75, 76, 97, 98
Y	US 2010/0310738 A1 (LUDWIG) 09 December 2010 (09.12.2010) entire document	81, 82, 91, 92, 101, 102

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 May 2015

Date of mailing of the international search report

28 AUG 2015

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: ELIZABETH N. KAYTOR
FISH & RICHARDSON P.C.
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

PCTWRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)**28 AUG 2015**Applicant's or agent's file reference
387670026WO1**FOR FURTHER ACTION**

See paragraph 2 below

International application No.
PCT/US2015/023679International filing date (day/month/year)
31 March 2015Priority date (day/month/year)
31 March 2014International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23J 3/22 (2015.01)
CPC - A23J 3/227 (2015.05)Applicant
IMPOSSIBLE FOODS INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
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Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion
26 May 2015

Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2015/023679

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2015/023679

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 4-17, 37-74, 84, 95, 96, 105-109

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. See Below are so unclear that no meaningful opinion could be formed (*specify*):

Claims 4-17, 37-74, 84, 95, 96, and 105-109 are multiple dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 4-17, 37-74, 84, 95, 96, 105-109

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit
- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
 - ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
 - ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2015/023679

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims	1-3, 18-36, 75-83, 85-94, 97, 98, 101, 102, 110-113	YES
	Claims	99, 100, 103, 104	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 18-36, 75-83, 85-94, 97-104, 110-113	NO
Industrial applicability (IA)	Claims	1-3, 18-36, 75-83, 85-94, 97-104, 110-113	YES
	Claims	None	NO

2. Citations and explanations:

Claims 99, 100, 103 and 104 lack novelty under PCT Article 33(2) as being anticipated by Orcutt et al.

Regarding claim 99, Orcutt et al. teach a food replica product ("A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], and "methods for preparing acceptable meat-like food products, such as beef, pork, poultry, fish, and shellfish analogs, from a wide variety of plant proteins", Para. [0003]) comprising one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said one or more carotenoids are selected from the group consisting of beta-carotene, zeaxanthin, lutein, trans-beta-apo-8'-carotenal, lycopene, canthaxanthin, and combinations thereof (the composition may comprise "beet juice, beta-carotene (orange), beta-APO 8 carotenal (orange) ... canthaxanthin (pink-red) ... lutein (red-orange); lycopene (orange-red) ... and combinations thereof" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051]).

Regarding claim 100, Orcutt et al. teach a food replica product comprising a vegetable juice, a vegetable puree, a vegetable extract, a fruit juice, a fruit puree, or a fruit extract at a concentration from 0.0001% to 10% of said food product (the composition may comprise "beet juice ... red cabbage juice" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051], and "various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" "at levels of from about 0.01% to about 10%", Para. [0062]).

Regarding claim 103, Orcutt et al. teach the product of claim 100, wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was cooked or otherwise treated to denature proteins before being added to said food replica product (various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], then "The extruder heats the mixture as it passes through the extruder further denaturing the protein in the mixture. Passing through the extruder the denatured protein is restructured or reconfigured to produce a structured protein material with protein fibers substantially aligned.", Para. [0086]).

Regarding claim 104, Orcutt et al. teach the product of claim 103, wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was heated to a temperature of about 60°C to about 100°C before being added to said food replica product (various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], "The dry pre-mix or the conditioned pre-mix is then fed into an extruder to heat, shear, and ultimately plasticize the mixture.", Para. [0083]).

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 113 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 113 is indefinite for the following reason:

Regarding claim 113, the claim depends from claim 110 which fails to provide antecedent basis for "the denaturation temperature." For the purposes of the Written Opinion purposed, "the denaturation temperature" is best interpreted as "a denaturation temperature," to provide sufficient antecedent basis.

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In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Claims 1-3, 18 and 19 lack an inventive step under PCT Article 33(3) as being obvious over Sand Hill Foods Inc [hereinafter Sandhill] in view of Cavallini et al.

Regarding claim 1, Sandhill teaches a meat replica composition ("a plant-based meat replica" composition, Para. [00354]; Claims 163-166) comprising: (a) about 5% to about 88% by weight of a meat dough ("the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances", Para. [00354], where the protein may be any one of the extruded meat dough replicas "comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat", isolated plant proteins and edible fibers, such as cellulose, Paras. [0006];[00189]; [00244]-[00247];[00332]); (b) about 0% to about 40% by weight of a carbohydrate-based gel ("some or all of the components of the consumable are suspended in a gel ... The gel can be made thick using an agent based on polysaccharides or proteins.", Para. [00332]); (c) about 5% to about 35% by weight of a non-animal fat ("20-80% fat", Para. [00354], where "said one or more fats are derived from a plant source", Paras. [0034];[00322]); (e) about 0% to about 15% by weight of a binding agent ("crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable", Para. [00331]); and (f) about 0.01% to about 4% by weight of a heme-containing protein and/or an iron salt ("the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). Sandhill does not explicitly disclose (d) about 0.00001% to about 10% by weight of a flavoring agent. However, Cavallini et al. teach (d) about 0.00001% to about 10% by weight of a flavoring agent (Cavallini et al., "a process for making vegetable burger patties, using both the flavored emulsion base and the vegetable base meat analog. Generally, the process involves mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients. The vegetable burger mixture is subsequently shaped into patties, having any desired sizes.", Paras. [0032]-[0039]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of flavoring to the meat replica, as taught by Cavallini et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00362]-[00376]).

Regarding claim 2, Sandhill in view of Cavallini et al. teach the composition of claim 1, Sandhill teaches wherein said meat dough comprises a flavoring agent (the meat dough is a combination of "a muscle replica; a fat tissue replica; and a connective tissue replica", Paras. [0006];[00189];[00299];[00246]-[00247];[00332], and the fat replica contains plant oils that "can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking.", Paras. [00313]-[00317]).

Regarding claim 3, Sandhill in view of Cavallini et al. teach the composition of claim 1, Sandhill teaches wherein said non-animal fat comprises a flavoring agent ("The plant oils can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking.", Para. [00317]).

Regarding claim 18, Sandhill teaches a meat replica composition ("a plant-based meat replica" composition, Para. [00354]; Claims 163-166) comprising: (a) about 5% to about 80% by weight of a meat dough ("the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances", Para. [00354], where the protein may be any one of the extruded meat dough replicas "comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat"; isolated plant proteins and edible fibers, such as cellulose, Paras. [0006];[00189]; [00244]-[00247];[00332]); (b) about 5% to about 35% by weight of a non-animal fat ("20-80% fat", Para. [00354], where "said one or more fats are derived from a plant source", Paras. [0034];[00322]); (c) about 15% to about 40% by weight of an edible fibrous component (the connective tissue replica dough may be made from fibers that comprise "the one or more isolated, purified protein", Paras. [00299]; [00326]-[00327], "and said connective tissue replica accounts for 1-30% of said product by weight", Para. [00191]); and (g) about 0.1% to about 8% by weight of a heme-containing protein and/or an iron salt ("the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). Sandhill does not explicitly disclose (d) about 0.1% to about 18% by weight of a carbohydrate-based gel; (e) about 0% to about 10% by weight of a flavoring agent and (f) about 0.5% to about 15% by weight of a binding agent. However, Cavallini et al. teach (d) about 0.1% to about 18% by weight of a carbohydrate-based gel; (e) about 0% to about 10% by weight of a flavoring agent and (f) about 0.5% to about 15% by weight of a binding agent (Cavallini et al., "a process for making vegetable burger patties, using both the flavored emulsion base and the vegetable base meat analog. Generally, the process involves mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients. The vegetable burger mixture is subsequently shaped into patties, having any desired sizes.", Paras. [0032]-[0039]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of binder, flavor and gel as taught by Cavallini et al. The motivation for doing so would be to mimic the "look, texture, and taste" of meat, (Sandhill, Paras. [00284]-[00285]).

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Regarding claim 19, Sandhill in view of Cavallini et al. teach the composition of claim 18, Sandhill teaches comprising: (a) about 20% to about 30% by weight of a meat dough ("the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances", Para. [00354], where the protein may be any one of the extruded meat dough replicas "comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat" and isolated plant proteins and edible fibers, such as cellulose, Paras. [0006];[00189];[00244]-[00247];[00332]); (b) about 15% to about 25% by weight of a non-animal fat ("20-80% fat", Para. [00354], where "said one or more fats are derived from a plant source", Paras. [0034];[00322]); (c) about 15% to about 25% by weight of an edible fibrous component (the connective tissue replica dough may be made from fibers that comprise "the one or more isolated, purified protein", Paras. [00299];[00326]-[00327], "and said connective tissue replica accounts for 1-30% of said product by weight", Para. [00191]); and (g) about 2% to about 8% by weight of a heme-containing protein and/or an iron salt ("the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). Sandhill does not explicitly disclose (d) about 7% to about 18% by weight of a carbohydrate-based gel and (f) about 5% to about 15% by weight of a binding agent. However, Cavallini et al. teach (d) about 7% to about 18% by weight of a carbohydrate-based gel; (e) about 0% to about 10% by weight of a flavoring agent and (f) about 5% to about 15% by weight of a binding agent (Cavallini et al., "a process for making vegetable burger patties, using both the flavored emulsion base and the vegetable base meat analog. Generally, the process involves mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients. The vegetable burger mixture is subsequently shaped into patties, having any desired sizes.", Paras. [0032]-[0039]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of binder, flavor and gel as taught by Cavallini et al. The motivation for doing so would be to mimic the "look, texture, and taste" of meat, (Sandhill, Paras. [00284]-[00285]).

Claims 20-27, 35, 36 and 110-112 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Sandhill.

Regarding claim 20, Cavallini et al. teach a method of making a ground meat replica (a "ground meat patty", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021]), said method comprising: (a) heating a dough to a temperature ranging from 150°F to 250°F, said dough comprising an isolated plant protein (a vegetable dough comprising plant protein, flavorings and plant oil is heated "to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", Paras. [0024]-[0028];[0046]-[0047], where the plant protein is isolated, Para. [0031]), an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat; (b) combining said dough, after heating, with a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (c) combining the dough from step (b) with a carbohydrate-based gel, an optional edible fibrous component, an optional binding agent, and one or more optional flavoring agents to make said ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 21, Cavallini et al. in view of Sandhill teach the method of claim 20, Cavallini et al. teach said method further comprising breaking the dough from step (b) into pieces before combining with said carbohydrate-based gel, said optional edible fibrous component, said optional binding agent, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Regarding claim 22, Cavallini et al. teach a method of flavoring a meat dough ("This invention relates to a "two step" process wherein in the first step a flavored emulsion base and a vegetable base meat analog are prepared. These two components from the first step are combined in the second step with other components to produce a patty that maintains much of its original weight upon cooking.", Paras. [0022];[0046]-[0047]), said method comprising: (a) combining one or more flavor precursors and an optional non-animal fat ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base", Para. [0023]); (b) heating the mixture to form one or more flavor compounds ("and cooking the flavored emulsion base to produce a vegetable base meat analog", Para. [0023]); and (c) making a dough comprising an isolated plant protein, an optional edible fibrous component, and the mixture from step (b) ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Para. [0028], where the vegetable base comprises isolated plant protein, Paras. [0031];[0046]). Cavallini et al. do not explicitly disclose a first highly conjugated heterocyclic ring complexed to an iron ion and/or first iron salt. However, Sandhill teaches a first highly conjugated heterocyclic ring complexed to an iron ion and/or first iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 23, Cavallini et al. in view of Sandhill teach the method of claim 22, Cavallini et al. teach further comprising: (d) combining said dough, after heating, with a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (e) combining the dough of step (d) with a carbohydrate-based gel, an optional binding agent, and one or more optional flavoring agents to make a ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], where the vegetable base comprises isolated plant protein, Paras. [0031];[0046], and "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches a second highly conjugated heterocyclic ring complexed to a second iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 24, Cavallini et al. in view of Sandhill teach the method of claim 23, Cavallini et al. teach said method further comprising breaking the dough from step (d) into pieces before combining with said carbohydrate-based gel, said optional binding agent, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Regarding claim 25, Cavallini et al. teach a method of flavoring a meat dough ("This invention relates to a "two step" process wherein in the first step a flavored emulsion base and a vegetable base meat analog are prepared. These two components from the first step are combined in the second step with other components to produce a patty that maintains much of its original weight upon cooking.", Paras. [0022];[0046]-[0047]), said method comprising: (a) making a dough comprising an isolated plant protein, an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat (a dough comprising isolated protein, gluten, flavoring and vegetable oil, Paras. [0031];[0046]); (b) making a flavored non-animal fat by combining a non-animal fat with one or more flavor precursors and heating the mixture ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil, Paras. [0023]-[0025], and "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]); and (c) combining said dough, after heating, with said flavored non-animal fat ("mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients.", Para. [0039]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or a first iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or a first iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 26, Cavallini et al. in view of Sandhill teach the method of claim 25, Cavallini et al. teach further comprising combining the dough of step (c) with a carbohydrate-based gel, an optional binding agent, and one or more optional flavoring agents to make a ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches a second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 27, Cavallini et al. in view of Sandhill teach the method of claim 26, Cavallini et al. teach further comprising breaking the dough of step (c) before combining with said carbohydrate-based gel, said optional binding agent, said one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt. However, Sandhill teaches said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 35, Cavallini et al. in view of Sandhill teach the method of claim 22, Cavallini et al. teach wherein said one or more flavor precursors is a sugar, a sugar alcohol, a sugar acid, a sugar derivative, an oil, a free fatty acid, an amino acid or derivative thereof, a nucleoside, a nucleotide, a vitamin, an acid, a peptide, a phospholipid, a protein hydrolysate, a yeast extract, or a mixture thereof ("The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye.", Para. [0024]).

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Regarding claim 36, Cavallini et al. in view of Sandhill teach the method of claim 35, Cavallini et al. teach wherein said flavor precursor is selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, urea, pantothenic acid, ornithine, niacin, glycerol, citrulline, taurine, biotin, borage oil, fungal oil, blackcurrant oil, betaine, beta carotene, B-vitamins, N-Acetyl L-cysteine, iron glutamate and a peptone, or mixtures thereof ("Any vegetable oil such as soybean oil, canola oil, corn oil, or peanut oil is suitable. The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye. The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024], and "a sweetener such as dextrose or sucrose", Para. [0036]).

Regarding claim 110, Cavallini et al. teach a ground meat replica ("a vegetable base meat analog, which may be used in a variety of vegetarian food products", Abstract, such as a "ground meat patty", Para. [0004]) comprising: (a) a dough comprising an isolated plant protein, an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat (a dough comprising isolated protein, gluten, flavoring and vegetable oil, Paras. [0031];[0046]); (b) a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (c) a carbohydrate-based gel, a binding agent, an optional edible fibrous component, and one or more optional flavoring agents ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025], and "the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt. However, Sandhill teaches a highly conjugated heterocyclic ring complexed to an iron ion and/or an iron salt (Sandhill, "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 111, Cavallini et al. in view of Sandhill teach the replica of claim 110, Cavallini et al. teach wherein said binding agent is an isolated plant protein (wheat gluten and/or "soy protein concentrate") may be used as a binding agent, Paras. [0023]-[0024];[0040]; [0046]).

Regarding claim 112, Cavallini et al. in view of Sandhill teach the replica of claim 110, Cavallini et al. do not explicitly disclose wherein said isolated plant protein is a RuBisCO, an albumin, a gluten, a conglycinin, or mixtures thereof. However, Sandhill teaches wherein said isolated plant protein is a RuBisCO, an albumin, a gluten, a conglycinin, or mixtures thereof (Sandhill, "one or more isolated and purified proteins", such as "rubisco", "albumins", "gluten", and "conglycinins", Para. [0018]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to use the desired vegetable protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Claims 28-32, 77-80 and 83 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Orcutt et al.

Regarding claim 28, Cavallini et al. teach a method of making a ground meat replica (a "ground meat patty", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021]), said method comprising: (a) combining a salt with one or more flavor precursors and an optional non-animal fat; (b) heating the mixture to form one or more flavor compounds ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]); (c) making a dough comprising an isolated plant protein, an optional edible fibrous component, and the mixture from step (b); (d) combining said dough, after heating, with a non-animal fat, said non-animal fat optionally containing a flavoring agent and/or an isolated plant protein; and (e) combining the dough of step (d) with a carbohydrate-based gel, an optional binding agent, a salt, an optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents to make said ground meat replica ("mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients.", Para. [0039], where "the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047], and the vegetable base comprises isolated plant protein, Paras. [0031];[0046], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]). Cavallini et al. do not explicitly disclose an iron salt. However, Orcutt et al. teach an iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 29, Cavallini et al. in view of Orcutt et al. teach the method of claim 28, Cavallini et al. teach further comprising breaking the dough from step (d) into pieces before combining with said carbohydrate-based gel, said optional binding agent, said optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 30, Cavallini et al. teach a method of making a ground meat replica (a "ground meat patty", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021]), said method comprising: (a) making a dough comprising an isolated plant protein, an optional edible fibrous component, one or more optional flavoring agents, and an optional non-animal fat (a dough comprising isolated protein, gluten, flavoring and vegetable oil, Paras. [0031];[0046]); (b) making a flavored non-animal fat by combining a non-animal fat with a salt and one or more flavor precursors and heating the mixture ("the process for making the vegetable base meat analog includes the steps of: adding methyl cellulose into a water/ice mix and sequentially blending methyl cellulose and water/ice mix to form a cream; blending in a modified gluten, blending in a vegetable protein product having high solubility in water and capable of forming a gel with mild heat treatment forming a protein mixture; blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base" and "heating to a temperature of about 87.8.degree. C. (190.degree. F.) to about 90.6.degree. C. (195.degree. F.)", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]); (c) combining said dough, after heating, with said flavored non-animal fat ("mixing together 15-25 wt. % of the flavored emulsion base, 5-20 wt. % of the vegetable base meat analog pieces, 50-75 wt. % at least one food binding component, 0.1-1 wt. % of methyl cellulose, 7-12 wt. % of carrageenan gel mixture, and 0.01-0.25 wt. % of flavoring ingredients.", Para. [0039], where "The flavored emulsion base contains the vegetable protein product, oil, modified food starch, methyl cellulose, and flavoring ingredients.", Para. [0024]); and (d) combining the dough of step (c) with a carbohydrate-based gel, an optional binding agent, an optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents to make said ground meat replica ("the process of the invention includes the step of producing vegetable burger patties, by mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose an iron salt. However, Orcutt et al. teach an iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Regarding claim 31, Cavallini et al. in view of Orcutt et al. teach the method of claim 30, Cavallini et al. teach further comprising breaking the dough from step (c) before combining with said carbohydrate-based gel, said optional binding agent, said optional highly conjugated heterocyclic ring complexed to an iron ion, and one or more optional flavoring agents ("The cooked vegetable base meat analog may be sliced or chopped to small particles, preferably, about 3 to 12 mm in diameter to give vegetable base meat analog pieces. The flavored emulsion base or vegetable base meat analog pieces may be incorporated into a variety of vegetarian food products to improve the texture, mouthfeel and juiciness of the vegetarian food products.", such as "mixing the vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, carrageenan gel, and flavoring ingredients; and shaping the mixture into patties", Paras. [0028];[0047]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 32, Cavallini et al. in view of Orcutt et al. teach the method of claim 30, Cavallini et al. do not explicitly disclose wherein said iron salt is iron gluconate, iron chloride, iron oxalate, iron nitrate, iron citrate, iron ascorbate, ferrous sulfate, ferric pyrophosphate, or any other aqueous soluble salt. However, Orcutt et al. teach wherein said iron salt is any other aqueous soluble salt (Orcutt et al., "minerals include, without limitation, ... iron ... Suitable forms of any of the foregoing minerals include soluble mineral salts, slightly soluble mineral salts, insoluble mineral salts, chelated minerals, mineral complexes, non-reactive minerals such as carbonate minerals, reduced minerals, and combinations thereof.", Para. [0064]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 77, Cavallini et al. teach a method of increasing the meat flavor or masking off flavors from plant material in a food product (a "ground meat patty", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021], where the flavor may be a meat flavor and "The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye.", Para. [0024]; Claim 7). Cavallini et al. do not explicitly disclose said method comprising adding, to said food product, one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, zeaxanthin, lutein, trans-beta-apo-8', lycopene, canthaxanthin, and combinations thereof. However, Orcutt et al. teach said method comprising adding, to said food product, one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, lutein, beta-apo-8', lycopene, canthaxanthin, and combinations thereof (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice, beta-carotene (orange), beta-APO 8 carotenol (orange) ... canthaxanthin (pink-red) ... lutein (red-orange); lycopene (orange-red) ... and combinations thereof" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add carotene component, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture, color and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 78, Cavallini et al. in view of Orcutt et al. teach the method of claim 77, Cavallini et al. teach wherein said food product is a meat replica (a "ground meat patty", Para. [0004] from "a vegetable base meat analog", Abstract, and "a process for making vegetable burger patties", Paras. [0019]-[0021]).

Regarding claim 79, Cavallini et al. in view of Orcutt et al. teach the method of claim 78, Cavallini et al. teach wherein said meat replica is free of animal products ("a vegetable base meat analog", Abstract, "For making vegetarian burger patties", Para. [0037]).

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Regarding claim 80, Cavallini et al. teach a method of increasing the meat flavor of a meat replica (a "ground meat patty", Para. [0004], and "a process for making vegetable burger patties comprising the steps of: mixing vegetable base meat analog pieces with at least one food binding component, flavored emulsion base, methyl cellulose, a carrageenan gel mixture, and flavoring ingredients; and shaping the mixture into patties", Paras. [0019]-[0021], where the flavor may be a meat flavor and "The flavoring ingredients comprise at least one of a sweetener, spice, salt, beef flavor, garlic powder, or a coloring dye.", Para. [0024]; Claim 7). Caviling et al. do not explicitly disclose said method comprising adding, to said meat replica, a vegetable juice, a vegetable puree, a vegetable extract, a fruit juice, a fruit puree, or a fruit extract to said meat replica at a concentration from 0.0001% to 10% of said meat replica. However, Orcutt et al. teach said method comprising adding, to said meat replica, a vegetable juice, a vegetable extract to said meat replica at a concentration from 0.0001% to 10% of said meat replica (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice ... red cabbage juice" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051], and "various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" "at levels of from about 0.01% to about 10%", Para. [0062]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add a vegetable extract or juice component, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture, color and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 83, Cavallini et al. in view of Orcutt et al. teach the method of claim 80, Cavallini et al. do not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract is cooked or otherwise treated to denature proteins before adding to said meat replica. However, Orcutt et al. teach wherein said vegetable extract is cooked or otherwise treated to denature proteins before adding to said meat replica (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], then "The extruder heats the mixture as it passes through the extruder further denaturing the protein in the mixture. Passing through the extruder the denatured protein is restructured or reconfigured to produce a structured protein material with protein fibers substantially aligned.", Para. [0086]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture, color and taste of meat, (Cavallini et al., Abstract; Paras. [0024];[0045]).

Claims 87-90, 93 and 94 lack an inventive step under PCT Article 33(3) as being obvious over Sandhill in view of Orcutt et al.

Regarding claim 87, Sandhill teaches a food product or food replica product ("a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources", Paras. [0006];[00354]; Claims 163-166) comprising a heme-containing protein ("said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin", Para. [0018]). Sandhill does not explicitly disclose one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, zeaxanthin, lutein,trans-beta-apo-8', lycopene, canthaxanthin, and combinations thereof. However, Orcutt et al. teach one or more carotenoids at a concentration of between 0.00001% and 0.1% of said food product, wherein said carotenoids are selected from the group consisting of beta-carotene, lutein, beta-apo-8', lycopene, canthaxanthin, and combinations thereof (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice, beta-carotene (orange), beta-APO 8 carotenal (orange) ... canthaxanthin (pink-red) ... lutein (red-orange); lycopene (orange-red) ... and combinations thereof" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add a carotene component, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 88, Sandhill in view of Orcutt et al. teach the product of claim 87, Sandhill teaches wherein said food replica product is a meat replica (a plant-based meat replica" composition, Para. [00354]; Claims 163-166).

Regarding claim 89, Sandhill in view of Orcutt et al. teach the product of claim 88, Sandhill teaches wherein said meat replica is free of animal products ("the meat substitute compositions contains no animal protein", Para. [00353], and "said meat substitute product contains no animal products", Para. [0065]).

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Regarding claim 90, Sandhill teaches a food product or food replica product comprising (a) a heme-containing protein ("a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources", Paras. [0006];[00354]; Claims 163-166, where "said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin", Para. [0018]). Sandhill does not explicitly disclose (b) a vegetable juice, a vegetable puree, a vegetable extract, a fruit juice, a fruit puree, or a fruit extract at a concentration from 0.0001% to 10% of said food product. However, Orcutt et al. teach (b) a vegetable juice, a vegetable extract at a concentration from 0.0001% to 10% of said food product (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with "beet juice ... red cabbage juice" in concentrations ranging "from about 0.001% to about 5.0% by weight", Paras. [0050]-[0051], and "various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" "at levels of from about 0.01% to about 10%", Para. [0062]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add a vegetable extract or juice component, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 93, Sandhill in view of Orcutt et al. teach the product of claim 90, Sandhill does not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was cooked or otherwise treated to denature proteins before being added to said food replica product. However, Orcutt et al. teach wherein said vegetable extract was cooked or otherwise treated to denature proteins before being added to said food replica product (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], then "The extruder heats the mixture as it passes through the extruder further denaturing the protein in the mixture. Passing through the extruder the denatured protein is restructured or reconfigured to produce a structured protein material with protein fibers substantially aligned.", Para. [0086]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 94, Sandhill in view of Orcutt et al. teach the product of claim 93, Sandhill does not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, fruit juice, fruit puree, or fruit extract was heated to a temperature of about 60°C to about 100°C before being added to said food replica product. However, Orcutt et al. teach wherein said vegetable extract was heated to a temperature of about 60°C to about 100°C before being added to said food replica product (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], "The dry pre-mix or the conditioned pre-mix is then fed into an extruder to heat, shear, and ultimately plasticize the mixture.", Para. [0083]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

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Claims 101 and 102 lack an inventive step under PCT Article 33(3) as being obvious over Orcutt et al. in view of Ludwig.

Regarding claim 101, Orcutt et al. teach the product of claim 100, Orcutt et al. do not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, a fruit juice, a fruit puree, or a fruit extract is a Cucumis juice, puree, or extract. However, Ludwig teaches wherein said vegetable juice, vegetable puree, vegetable extract is a Cucumis juice, puree, or extract (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];[0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Orcutt et al. to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic ground "meat compositions" through use of the optimal plant extracts, (Orcutt et al., Paras. [0028];[0033];[0062]).

Regarding claim 102, Orcutt et al. in view of Ludwig teach the product of claim 101, Orcutt et al. do not explicitly disclose wherein said Cucumis juice, puree, or extract is from a cucumber or a melon. However, Ludwig teaches wherein said Cucumis juice, puree, or extract is from a cucumber (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];[0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Orcutt et al. to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic ground "meat compositions" through use of the optimal plant extracts, (Orcutt et al., Paras. [0028];[0033];[0062]).

Claims 33, 34 and 113 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Orcutt et al. and Sandhill.

Regarding claim 33, Cavallini et al. in view of Orcutt et al. teach the method of claim 28, Cavallini et al. teach wherein salt, said one or more flavor precursors, and said non-animal fat before heating said mixture ("blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base; and cooking the flavored emulsion base to produce a vegetable base meat analog", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion. However, Sandhill teaches said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]).

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Continuation of:

Regarding claim 34, Cavallini et al. in view of Orcutt et al. teach the method of claim 30, Cavallini et al. teach wherein combined with said non-animal fat, and said one or more flavor precursors before heating said mixture ("blending in an oil to form an emulsion base; blending in a modified food starch and flavoring ingredients; to form a flavored emulsion base; and cooking the flavored emulsion base to produce a vegetable base meat analog", where the oil is vegetable oil and the flavors may include salt, Paras. [0023]-[0025]). Cavallini et al. do not explicitly disclose said iron salt. However, Orcutt et al. teach said iron salt (Orcutt et al., "A variety of ingredients that contain protein may be utilized in a thermal plastic extrusion process to produce structured protein products suitable for use in the ground meat simulated meat compositions.", Para. [0028], such as iron salt minerals, Para. [0064]; Claims 1, 11 and 12). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing salt, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]). Cavallini et al. do not explicitly disclose a highly conjugated heterocyclic ring complexed to an iron ion. However, Sandhill teaches said second highly conjugated heterocyclic ring complexed to an iron ion and/or a second iron salt (Sandhill, "one or more isolated and purified proteins", Para. [0006], where "the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin.", Para. [00354]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add an iron containing protein, as taught by Sandhill. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 113 (as best interpreted), Cavallini et al. in view of Sandhill teach the replica of claim 110, Cavallini et al. do not explicitly disclose wherein a denaturation temperature of said binding agent is between about 40°C and about 80°C. However, Orcutt et al. teach wherein a denaturation temperature of said binding agent is between about 40°C and about 80°C (Orcutt et al., "ground meat simulated meat compositions", Para. [0028], with various plant extracts, such as those containing carotenoids, tocopherols, or flavonoids" are added to "the protein-containing materials that will be extruded", Para. [0062], and "Preferably the protein-containing mixture is heated to a temperature of from about 25.degree. C. to about 80.degree. C., more preferably from about 30.degree. C. to about 40.degree. C. in the preconditioner.", Para. [0079], "The dry pre-mix or the conditioned pre-mix is then fed into an extruder to heat, shear, and ultimately plasticize the mixture.", Para. [0083]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to denature the vegetable protein, as taught by Orcutt et al. The motivation for doing so would be to mimic the texture and taste of meat with one or more binding proteins, (Cavallini et al., Abstract; Paras. [0023]-[0024];[0040];[0045]-[0046]).

Claims 75, 76, 97 and 98 lack an inventive step under PCT Article 33(3) as being obvious over Lyrical Foods, Inc. [hereinafter Lyrical] in view of Krammer et al. and Sinha et al.

Regarding claim 75, Lyrical teaches a method of increasing the meat flavor or masking off flavors from plant material in a food product (a cheese replica, Abstract, where the replica is prepared from "proteins and fats from plants or other non-animal sources", and the replica incorporates flavor additives to mimic the flavor of cheese, Paras. [0012];[00125];[00127]). Lyrical does not explicitly disclose said method comprising adding, to said food product, one or more lactones, wherein said lactones are selected from the group consisting of tetrahydro-6-methyl-2H-pyran-2-one, delta-octalactone, 5-ethyl-dihydro-2(3H)-furanone, butyrolactone, dihydro-5-pentyl-2(3H)-furanone, dihydro-3-methylene-2,5-furandione, 1-pentoyllactone, tetrahydro-2H-pyran-2-one, 6-heptyltetrahydro-2H-pyran-2-one, gamma-octalactone, 5-hydroxymethyldihydrofuran-2-one, 5-ethyl-2(5H)-furanone, 5-acetyldihydro-2(3H)-furanone, trans-3-methyl-4-octanolide 2(5H)-furanone, 3-(1, 1-dimethylethyl)-2,5-urandione, 3,4-dihydroxy-5-methyl-dihydrofuran-2-one, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, delta-tetradecalactone, and dihydro-4-hydroxy-2(3H)-furanone. However, Krammer et al. teach said method comprising adding, to said food product, one or more lactones, wherein said lactones are selected from the group consisting of gamma-octalactone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, delta-tetradecalactone (Krammer et al., "Cheese flavoring containing both volatile and non-volatile components which comprise constituents which contribute to the taste sensation "cheese".", Abstract, where the Group 2 flavoring additives comprise "milk-like and creamy flavors are saturated and unsaturated .delta.- and .gamma.-lactone having 6 to 14 carbon atoms. Preferred compounds of this group are delta.-tetradecalactone gamma.-octalactone ... compounds having a caramel-like flavor. Preferred compounds are ... 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone.", Para. [0031], and are added to food products, Paras. [0068]-[0069]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired flavoring, as taught by Krammer et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]). Lyrical does not explicitly disclose adding, to said food product, one or more lactones at a concentration of 10-3 to 10-11 of said food product. However, Sinha et al. teach adding, to said food product, one or more lactones at a concentration of 10-3 to 10-11 of said food product (Sinha et al., flavoring food products with lactones, where lactones are added "in desired and acceptable amount varying from ppm to mg based on the type of products", Paras. [0010];[0021];[0061];[0069]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired amount of flavoring, as taught by Sinha et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]).

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Continuation of:

Regarding claim 76, Lyrical in view of Krammer et al. and Sinha et al. teach the method of claim 75, Lyrical does not explicitly disclose wherein said lactones are 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone. However, Krammer et al. teach wherein said lactones are 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone (Krammer et al., the Group 2 flavoring additives comprise "milk-like and creamy flavors are saturated and unsaturated .delta.- and .gamma.-lactone having 6 to 14 carbon atoms. Preferred compounds of this group are delta.-tetradecalactone gamma.-octalactone ... compounds having a caramel-like flavor. Preferred compounds are ... 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone.", Para. [0031]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired flavoring, as taught by Krammer et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]). Lyrical does not explicitly disclose butyrolactone. However, Sinha et al. teach butyrolactone (Sinha et al., flavoring food products with lactones, such as butyrolactone, Paras. [0010];[0021]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired flavoring, as taught by Sinha et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]).

Regarding claim 97, Lyrical teaches a food replica product (a cheese replica, Abstract, where the replica is prepared from "proteins and fats from plants or other non-animal sources", and the replica incorporates flavor additives to mimic the flavor of cheese, Paras. [0012]; [00125];[00127]). Lyrical does not explicitly disclose one or more lactones, wherein said one or more lactones are selected from the group consisting of tetrahydro-6-methyl-2H-pyran-2-one, deltaoctalactone, 5-ethyldihydro-2(3H)-furanone, butyrolactone, dihydro-5-pentyl-2(3H) furanone, dihydro-3-methylene-2,5-furandione, 1-pentoyllactone, tetrahydro-2H-pyran-2-one, 6-heptyltetrahydro-2H-pyran-2-one, gamma-octalactone, 5-hydroxymethyldihydrofuran-2-one, 5-ethyl-2(5H)-furanone, 5-acetyldihydro-2(3 H)-furanone, trans-3-methyl-4-octanolide 2(5H)-furanone, 3-(1, 1-dimethylethyl)-2,5-urandione, 3,4-dihydroxy-5-methyl-dihydrofuran-2-one, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, delta-tetradecalactone, and dihydro-4-hydroxy-2(3H)-furanone. However, Krammer et al. teach one or more lactones, wherein said one or more lactones are selected from the group consisting of gamma-octalactone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, delta-tetradecalactone (Krammer et al., "Cheese flavoring containing both volatile and non-volatile components which comprise constituents which contribute to the taste sensation "cheese".", Abstract, where the Group 2 flavoring additives comprise "milk-like and creamy flavors are saturated and unsaturated .delta.- and .gamma.-lactone having 6 to 14 carbon atoms. Preferred compounds of this group are delta.-tetradecalactone gamma.-octalactone ... compounds having a caramel-like flavor. Preferred compounds are ... 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone.", Para. [0031], and are added to food products, Paras. [0068];[0069]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired flavoring, as taught by Krammer et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]). Lyrical does not explicitly disclose one or more lactones at a concentration of 10-3 to 10-11 of said food product. However, Sinha et al. teach one or more lactones at a concentration of 10-3 to 10-11 of said food product (Sinha et al., flavoring food products with lactones, where lactones are added "in desired and acceptable amount varying from ppm to mg based on the type of products", Paras. [0010];[0021];[0061];[0069]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired amount of flavoring, as taught by Sinha et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]).

Regarding claim 98, Lyrical in view of Krammer et al. and Sinha et al. teach the product of claim 97, Lyrical does not explicitly disclose wherein said one or more lactones are 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone. However, Krammer et al. teach wherein said lactones are 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone (Krammer et al., the Group 2 flavoring additives comprise "milk-like and creamy flavors are saturated and unsaturated .delta.- and .gamma.-lactone having 6 to 14 carbon atoms. Preferred compounds of this group are delta.-tetradecalactone gamma.-octalactone ... compounds having a caramel-like flavor. Preferred compounds are ... 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone.", Para. [0031]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired flavoring, as taught by Krammer et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]). Lyrical does not explicitly disclose butyrolactone. However, Sinha et al. teach butyrolactone (Sinha et al., flavoring food products with lactones, such as butyrolactone, Paras. [0010];[0021]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lyrical to add the desired flavoring, as taught by Sinha et al. The motivation for doing so would be to mimic the taste of cheese, (Lyrical, Abstract; Paras. [0012];[00125];[00127]).

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Continuation of:

Claims 81 and 82 lack an inventive step under PCT Article 33(3) as being obvious over Cavallini et al. in view of Orcutt et al. and Ludwig.

Regarding claim 81, Cavallini et al. in view of Orcutt et al. teach the method of claim 80, Cavallini et al. do not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, a fruit juice, a fruit puree, or a fruit extract is a Cucumis juice, puree, or extract. However, Ludwig teaches wherein said vegetable juice, vegetable puree, vegetable extract is a Cucumis juice, puree, or extract. (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];[0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]).

Regarding claim 82, Cavallini et al. in view of Orcutt et al. and Ludwig teach the method of claim 81 Cavallini et al. do not explicitly disclose wherein said Cucumis juice, puree, or extract is from a cucumber or a melon. However, Ludwig teaches wherein said Cucumis juice, puree, or extract is from a cucumber (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];[0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Cavallini et al. to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic the texture and taste of meat with one or more "flavoring ingredients", (Cavallini et al., Abstract; Paras. [0024];[0045]).

Claims 85 and 86 lack an inventive step under PCT Article 33(3) as being obvious over Sandhill in view of Krammer et al. and Sinha et al.

Regarding claim 85, Sandhill teaches a food product or food replica product ("a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources", Paras. [0006];[00354]; Claims 163-166) comprising a heme-containing protein ("said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin", Para. [0018]). Sandhill does not explicitly disclose wherein said one or more lactones are selected from the group consisting of tetrahydro-6-methyl-2H-pyran-2-one, delta-octalactone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, butyrolactone, dihydro-5-pentyl-2(3 H)-furanone, dihydro-3-methylene-2,5-furandione, 1-pentoyllactone, tetrahydro-2H-pyran-2-one, 6-heptyltetrahydro-2H-pyran-2-one, gamma-octalactone, 5-hydroxymethyl dihydrofuran-2-one, 5-ethyl-2 (5H)-furanone, 5-acetyldihydro-2(3 H)furanone, trans-3-methyl-4-octanolide 2(5H)-furanone, 3-(1, 1-dimethylethyl)-2,5-urandione, 3,4-dihydroxy-5-methyl-dihydrofuran-2-one, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, delta-tetradecalactone, and dihydro-4-hydroxy-2 (3H)-furanone. However, Krammer et al. teach wherein said one or more lactones are selected from the group consisting of 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone (Krammer et al., the Group 2 flavoring additives comprise "milk-like and creamy flavors are saturated and unsaturated .delta.- and .gamma.-lactone having 6 to 14 carbon atoms. Preferred compounds of this group are delta.-tetradecalactone ... gamma.-octalactone ... compounds having a caramel-like flavor. Preferred compounds are ... 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone.", Para. [0031]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired flavoring, as taught by Krammer et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317]; [00362]-[00376]). Sandhill does not explicitly disclose one or more lactones at a concentration of 10-3 to 10-11 of said food product. However, Sinha et al. teach one or more lactones at a concentration of 10-3 to 10-11 of said food product (Sinha et al., flavoring food products with lactones, where lactones are added "in desired and acceptable amount varying from ppm to mg based on the type of products", Paras. [0010];[0021];[0061];[0069]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired amount of flavoring; as taught by Sinha et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 86, Sandhill in view of Krammer et al. and Sinha et al. teach the method of claim 85, Sandhill does not explicitly disclose wherein said one or more lactones are 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone. However, Krammer et al. teach wherein said one or more lactones are 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, gamma-octalactone, and delta-tetradecalactone (Krammer et al., the Group 2 flavoring additives comprise "milk-like and creamy flavors are saturated and unsaturated .delta.- and .gamma.-lactone having 6 to 14 carbon atoms. Preferred compounds of this group are delta.-tetradecalactone ... gamma.-octalactone ... compounds having a caramel-like flavor. Preferred compounds are ... 5-ethyl-4-hydroxy-2-methyl-3 (2H)-furanone.", Para. [0031]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired flavoring, as taught by Krammer et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]). Lyrical does not explicitly disclose butyrolactone. However, Sinha et al. teach butyrolactone (Sinha et al., flavoring food products with lactones, such as butyrolactone, Paras. [0010];[0021]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired flavoring, as taught by Sinha et al. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

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Continuation of:

Claims 91 and 92 lack an inventive step under PCT Article 33(3) as being obvious over Sandhill in view of Orcutt et al. and Ludwig.

Regarding claim 91, Sandhill in view of Orcutt et al. teach the product of claim 90, Sandhill does not explicitly disclose wherein said vegetable juice, vegetable puree, vegetable extract, a fruit juice, a fruit puree, or a fruit extract is a Cucumis juice, puree, or extract. However, Ludwig teaches wherein said vegetable juice, vegetable puree, vegetable extract is a Cucumis juice, puree, or extract (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];[0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Regarding claim 92, Sandhill in view of Orcutt et al. and Ludwig teach the product of claim 91, Sandhill does not explicitly disclose wherein said Cucumis juice, puree, or extract is from a cucumber or a melon. However, Ludwig teaches wherein said Cucumis juice, puree, or extract is from a cucumber (Ludwig, "A method of processing is disclosed that provides improved water retention and enhanced coloring and flavor, while preserving the meat and preventing bacterial contamination.", Abstract, where the process includes adding a material derived from a plant, i.e. an extract, juice or puree, such as "Cucumis sativus (cucumber)", and the concentration that contacts "meat product can be any suitable concentration", Paras. [0039];[0042];[0046]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Sandhill to add the desired vegetable extract, as taught by Ludwig. The motivation for doing so would be to mimic "the characteristic flavor and fragrance components" of meat, (Sandhill, Paras. [00317];[00362]-[00376]).

Claims 1-3, 18-36, 75-83, 85-94, 97-104, and 110-113 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

PCT Application No.: PCT/US2015/023679
Date of Search: 26 May 2015**Search History:****Limited Classification Search**

The Patent Analyst performed a limited classification search within the following US, IPC, CPC, ECLA, or F-Term classification areas:

IPC (8) Class/Subclass(es): A23J 3/14, 3/22 (2015.01)

CPC Class/Subclass(es): A23J 3/14, 3/227 (2015.05)

U.S. Class/Subclass(es): 426/573, 574

See Global Search Results.

Global Patent Literature Text Search

The Patent Analyst performed the following global text search, which was not limited by classification but may or may not have been limited by other criteria:

QUESTAL ORBIT:

Search Step	Result(s)	Query	Base
9	2	(PROTEIN AND MEAT AND REPLICA AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM	FAMPAT
8	739	(PROTEIN AND IRON AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM	FAMPAT
7	0	(PROTEIN AND IRON AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (426574)/PCL	FAMPAT
6	0	(PROTEIN AND IRON AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (426573)/PCL	FAMPAT
5	2	(PROTEIN AND IRON AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (A23J-003/22)/IPC	FAMPAT
4	2	(PROTEIN AND IRON AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (A23J-003/14)/IPC	FAMPAT
3	2	(PROTEIN AND IRON AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (A23J-003/227)/CPC	FAMPAT
2	0	(PROTEIN AND HEME AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (A23J-003/227)/CPC	FAMPAT
1	0	(PROTEIN AND HEME AND PLANT)/TI/AB/IW/OBJ/ADB/ICLM AND (A23J-003/14)/CPC	FAMPAT

PCT Application No.: PCT/US2015/023679
Date of Search: 26 May 2015**Google Patents: (<https://www.google.com/?tbn=pts>)**

No. of Hits	Text String
1	2014/0127358
1	2014/0193547
24	"meat dough" isolated plant protein
7	"meat replica" isolated plant protein
27	cucumis juice cucumber food flavor meat vegetarian

Computer Accessed Text Databases Searched

The Patent Analyst searched the following computer accessed text databases:

Public AppFT and PatFT:

No. of Hits	Text String	Other Limiting Criteria
3	IN/"varadan ranjani"	Patent Apps
2	IN/"solomatin sergey"	Patent Apps
0	IN/"holtz schietinger celeste"	Patent Apps
0	IN/"cohn elysia"	Patent Apps
0	IN/"klapholz brown ariel"	Patent Apps
0	IN/"shiu jennifer"	Patent Apps
69	IN/"kale aniket"	Patent Apps
0	IN/"karr jessica"	Patent Apps
0	IN/"fraser rachel"	Patent Apps
0	AN/"impossible foods"	Patent Apps
1	AN/"impossible foods"	Patents
1	IN/"varadan ranjani"	Patents
56	IN/"kale aniket"	Patents

Google Scholar (<http://scholar.google.com/>)

No. of Hits	Text String
1	"meat replica" isolated plant protein
>1,000 First 50 Searched	cucumis juice cucumber food flavor meat vegetarian

Date search was completed: 26 May 2015

YLK/BT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670010WO1	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/11361	International filing date (<i>day/month/year</i>) 13 January 2014 (13.01.2014)	(Earliest) Priority Date (<i>day/month/year</i>) 11 January 2013 (11.01.2013)
Applicant Maraxi, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☐

together with the international application in electronic form

☒

subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I: Claims 1-9, 17-22, 52-54

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

PCT/US14/11361

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 1/212 (2014.01)

USPC - 424/478, 479

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A23L 1/212, 1/10, 1/216; A23K 1/100 (2014.01)

USPC: 424/615, 481, 479, 478, 574, 531

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; Google Patents; Science Direct; ProQuest; consumable, plant, 'plant protein,' peptide, coacervate, 'food product,' 'meat substitute,' 'sodium chloride,' soluble, mayo, beverage

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 3,870,801 (TOMBS, MP) March 11, 1975; abstract; column 1, lines 64-65; column 3, lines 12-13; column 6, lines 20-21; column 8, lines 56-57; column 14, lines 3-5; column 19, lines 32-34	1, 2, 8, 9/1, 9/2, 9/8 ----- 3-7, 9/3-9/7, 18, 52, 53, 54/52, 54/53
Y	EP 1361264 B1 (TSUBAKI, K et al.) April 18, 2007; paragraphs [0001], [0029], [0038], [0039], [0044], [0045]	3-7, 9/3-9/7, 19-21, 22/20, 22/21, 52, 53, 54/52, 54/53
X --- Y	US 2013/0004617 A1 (ZHANG, N et al.) January 03, 2013; paragraphs [0004], [0006]	17 ----- 18-21, 22/20, 22/21, 53, 54/53

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 May 2014 (28.05.2014)

Date of mailing of the international search report

16 JUN 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

-***-Continued from Box No. III: Lack of Unity of Invention:-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-9, 17-22 and 52-54 are directed toward a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, and a method of making said consumable product.

Group II: Claims 28-31, 37-39, 68-70 and 81-83 are directed toward a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins, said adipose tissue replica including one or more isolated plant proteins, one or more plant or algal derived oils, optionally a phospholipid, and a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica; and wherein the connective tissue replica comprises proteins assembled into fibrous structures by a solution spinning process.

Group III: Claims 32-36 and 61-65 are directed toward a consumable product comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, and a method of making said consumable product.

Group IV: Claims 47-49 are directed toward a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

Group V: Claim 51 is directed toward a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

Groups VI+: Claims 59, 60 and SEQ ID NO: 1 are directed toward a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. (SEQ ID NO: 1-27). + type Markush group.

The method for extending the shelf-life of a meat-free consumable product comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions can be searched to the extent that it encompasses a sequence at least 70% identical to SEQ ID NO: 1 (Vigna radiata amino acid sequence). Additional sequences will be searched upon the payment of additional fees. It is believed that Claims 59 and 60 (in-part) encompass this first named invention and thus these claims can be searched, upon appropriate payment, to the extent that they encompass SEQ ID NO: 1 (Vigna radiata amino acid sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) may result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (Methylobacterium thermophilum amino acid sequence).

Groups VII+: Claims 71-73 and SEQ ID NO: 1 are directed toward a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Group VIII: Claims 74-78 are directed toward a method of making a coacervate, said method comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and b) isolating said coacervate from said solution.

Group IX: Claims 91-93 are directed toward methods of minimizing undesirable odors in a composition comprising plant proteins.

Group X: Claim 94 is directed toward a chocolate flavored spread comprising: a) sugar; b) a chocolate flavoring; and c) a cream fraction from a plant based milk.

Group XI: Claims 95-99 are directed toward a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

Group XII: Claims 100-104 are directed toward tissue replicas comprising a freeze-aligned non-animal protein.

-***-Continued Within the Next Supplemental Box-***-

***Continued from Previous Supplemental Page:

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, not present in any other groups; the special technical features of Group II including a meat replica comprising a muscle replica, a connective tissue replica, an adipose tissue replica, or a coacervate, which are not present in any other group; the special technical features of Group III including a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, which are not present in any other Group; the special technical features of Group IV including a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, not present in any other group; the special technical features of Group V including a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, which are not present in any other group; the special technical features of Groups VI+ including a method for extending the shelf-life of a meat-free consumable product, which are not present in any other group; the special technical features of Groups VII+ including a method for imparting a beef like flavor to a consumable product, which are not present in any other group; the special technical features of Group VIII including a method of making a coacervate comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, which are not present in any other group; the special technical features of Group IX including methods of minimizing undesirable odors in a composition comprising plant proteins, which are present in any other group; the special technical features of Group X including a chocolate flavored spread, which are not present in any other Group; the special technical features of Group XI including a method for altering the texture of a consumable, which not present in any other group; the special technical features of Group XII including a freeze-aligned non-animal protein.

Groups I-XII share the technical features including consumables. Groups I, II, V, VIII, IX, X and XI share the technical features including plants. Groups I, II, VIII, IX and XI share the technical features including plant proteins. Groups I-V, VI+, VII+, VIII, IX, XI and XII share the technical features including proteins. Groups I, III, V, VII+, XI and XII share the technical features including temperature. Groups I and VIII share the technical features including pH. Groups I, III and VIII share the technical features including sodium chloride. Groups I-III, VI+, VII+, VIII and XI share the technical features including a method of making a consumable product. Groups II, III and XII share the technical features including a meat replica. Groups II and VIII share the technical features including a coacervate. Groups II and V share the technical features including plant or algal derived oils or lipids. Groups II and V share the technical features comprising an emulsion. Groups III and V share the technical features including a gel. Groups IV, VI+ and VII+ share the technical features including a heme-containing protein. Groups IV and VI+ share the technical features including meat-free products. Groups VI+ and VII+ share the technical features including a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27.

Groups VI+ share the technical features including a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

Groups VII+ share the technical features including a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

However, these shared technical features are previously disclosed by WO 2001/015714 A1 (ZLOTKIN) in view of US 2010/0074998 A1 to Espeleta-Vega et al. (hereinafter 'Espeleta-Vega'), US 2011/0083681 A1 to Sengupta, et al. (hereinafter 'Sengupta'), the publication entitled 'Comparative Analysis Of Autoxidation Of Haemoglobin' by Jensen (hereinafter 'Jensen'), US 4,986,998 A to Yoo, et al. (hereinafter 'Yoo') and GenBank Accession AFK42304 by Krishnakumar, et al. (hereinafter 'Krishnakumar').

Zlotkin discloses consumables (compositions including micronutrients (consumables); abstract) including plants (chicory fructooligosaccharide (plants); page 11, lines 15-19); proteins (proteins; page 15, lines 20-28); temperature (temperature; page 17, lines 10-25); a method of making a consumable product (a process for producing a composition comprising micronutrients (a method of making a consumable product); page 8, lines 22-27); plant or algal derived oils or lipids (soybean oil (plant or algal derived oils or lipids); page 15, lines 1-4); meat-free products (cereals; purees of fruit, vegetables, milk, yogurt, cottage cheese (meat-free products); page 18, lines 1-11), and a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto (a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto; page 14, lines 21-28).

Zlotkin does not disclose plant proteins; pH, sodium chloride; a meat replica; a coacervate; an emulsion; a gel; a heme-containing protein; a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27; a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions; and a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Continued Within the Next Supplemental Box-

-***-Continued from Previous Supplemental Page:

Espeleta-Vega discloses plant proteins (vegetable protein (plant proteins); abstract), pH (a pH modifier; paragraph [0010]), sodium chloride (salt (sodium chloride); paragraph [0038]), a meat replica (substitute meat food product (a meat replica); abstract); an emulsion (an emulsion; paragraph [0011]); a gel (a gel; paragraph [0011]).

Sengupta discloses a process for preparing coacervate gel particles comprising plant materials including proteins (a process for preparing coacervate gel particles comprising plant materials including proteins; paragraph [0015]).

Jensen discloses a heme-containing protein (hemoglobin; abstract) wherein the oxidation of hemoglobin and myoglobin is significantly slower than free heme (oxidation of hemoglobin and myoglobin is significantly slower than free heme; page 2029, column 1, paragraph 2), and wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin (wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin; page 2030, column 1, paragraph 1).

Yoo discloses a method for imparting (flavoring (imparting); abstract) a beef (cow (beef); column 4, lines 26-32) like flavor to a consumable product (flavor to a consumable product; abstract, column 2, lines 49-50), comprising adding to said consumable composition a heme-containing protein (comprising adding to said consumable a powdered blood product (comprising adding to said consumable composition a heme-containing protein); abstract), wherein after cooking, a beef-like flavor is imparted to said consumable (providing a meat-like flavor (beef-like flavor is imparted to said consumable); column 3, lines 1-6).

Krishnakumar discloses a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1 (a globin (a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1); page 1).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin by combining the consumable disclosed by Zlotkin with a meat replica, such as that disclosed by Espeleta-Vega, for providing the micronutrients in the consumable in a form acceptable to a consumer, such as in a meat replica, for enhancing the nutrient content of a meat replica, particularly the iron content of said replica, providing the previous disclosure of Zlotkin. Also, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables with the coacervate preparation process of Sengupta, for enabling the preparation of particulates comprising the micronutrients disclosed by Zlotkin using a plant polysaccharide, such as pectin, as disclosed by Sengupta, for providing micronutrients in a consumable and easily digestible form to enable a consumer to absorb the nutrients. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin, for including the use of hemoglobin in preference to free iron or other forms of iron, in order to provide the iron to the consumer in a biologically useful form and oxidation state. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have therefore modified the disclosure of Zlotkin, for providing a method for extending the shelf-life of a meat-free consumable product, such as the consumables disclosed by Zlotkin, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions, such as adding hemoglobin, based on the disclosure of Jensen that tetrameric hemoglobin oxidizes more slowly than other forms of heme-containing proteins. Also, it would have been obvious to a person of ordinary skill in the art to have modified the disclosure of Zlotkin, for including a meat-like flavoring including an iron-containing protein based on the disclosure of Yoo, in order to provide a beef-like flavor to a consumable food, including a poultry or fish composition, to provide a beef-flavored product not containing beef, but with lower fat, such as a consumable comprising a plant protein, fish, or poultry protein. Finally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables comprising iron, for including a plant-based iron-comprising proteins, such as the globin disclosed by Krishnakumar, in order to provide iron from non-animal sources having high absorbability and utilization by a consumer.

Since none of the special technical features of the Groups I-XII inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zlotkin, Espeleta-Vega, Sengupta, Jensen, Yoo and Krishnakumar references, unity of invention is lacking.

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P. O. Box 1022
Minneapolis, MN 55440-1022
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

16 JUN 2014

Applicant's or agent's file reference
387670010WO1

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/US14/11361

International filing date (day/month/year)
13 January 2014 (13.01.2014)

Priority date (day/month/year)
11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/212 (2014.01)
USPC - 424/478, 479

Applicant
Maraxi, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

28 May 2014 (28.05.2014)

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11361

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☒ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☒ subsequently to this Authority for the purposes of search
4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11361

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. ***-Please See Below-*** are so unclear that no meaningful opinion could be formed (*specify*):

Claims 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80 and 84-90 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. ***-Please See Above-***

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit
- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13^{ter}.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11361

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:

-Please See Supplemental Page-

4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts
 - ☒ the parts relating to claims Nos. 1-9, 17-22, 52-54

**WRITTEN OPINION OF THE
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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	3-7, 9/3-9/7, 18-21, 22/20, 22/21, 52, 53, 54/52, 54/53	YES
	Claims	1, 2, 8, 9, 9/1, 9/2, 9/8, 17	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52, 54/53	NO
Industrial applicability (IA)	Claims	1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52, 54/53	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1, 2, 8, 9/1, 9/2 and 9/8 lack novelty under PCT Article 33(2) as being anticipated by US 3,870,801 A (TOMBS).

As per claim 1, Tombs discloses consumable product comprising an isolated and purified plant protein (a plant protein valuable in food products (consumable) such as arachin, a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21), wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM (mesophases (proteins) of the invention comprise undenatured plant protein in a dissolved state (soluble), dissolved in aqueous solution of 0.1 N (0.1 N = 0.1 M = 100 mM) NaCl (sodium chloride) and specifically 500 g rape protein is extracted in 5 L water (at least 1 mg/ml) adjusted to pH 7.1 and the temperature required to set the mesophase is generally set by heating at 90 degrees C to 100 degrees C; column 1, lines 64-65; column 3, lines 12-13; column 8, lines 56-57; column 19, lines 32-34).

As per claim 2, Tombs discloses the consumable product of claim 1, and Tombs further discloses wherein said product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5).

As per claim 8, Tombs discloses the consumable product of claim 2, and Tombs further discloses wherein said meat product is a pate, a sausage replica, or a meat substitute (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5).

As per claims 9/1, 9/2 and 9/8, Tombs discloses the consumable product of claims 1, 2 and 8, and Tombs further discloses wherein said isolated and purified plant protein is at least 10 kDa in size (a plant protein valuable in food products (consumable), such as arachin (21 kDa protein), a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21).

Claim 17 lacks novelty under PCT Article 33(2) as being anticipated by US 2013/0004617 A1 to Zhang, et al. (hereinafter 'Zhang').

As per claim 17, Zhang discloses a consumable product comprising a coacervate (abstract) comprising one or more isolated and purified proteins (an aqueous dispersion of complex coacervates in stable form suitable for use in foods (consumable products) is provided and can include whey protein, hydrolyzed protein, lauric arginate, polylysine, casein or any combination thereof; paragraphs [0004], [0006]).

Claims 3-7, 9/3-9/7, 52 and 54/52 lack an inventive step under PCT Article 33(3) as being obvious over Tombs in view of EP 1,361,264 B1 to Tsubaki, et al. (hereinafter 'Tsubaki').

As per claim 3, Tombs discloses the consumable product of claim 2, but Tombs does not disclose wherein said beverage is an alcoholic beverage or a protein drink. Tsubaki discloses wherein said beverage is an alcoholic beverage (foods also include beverages, such as alcoholic beverages; paragraph [0044]) or a protein drink which may prepared from grains (beer, paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of a plant protein in food that is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 4, Tombs and Tsubaki, in combination, disclose the consumable product of claim 3, but Tombs does not disclose wherein said alcoholic beverage is a cream liquor. Tsubaki discloses wherein said alcoholic beverage is a cream liquor (foods also include beverages, such as alcoholic beverages including lactic acid beverages (cream beverages); paragraph [0044]; gramineous plant fats and oils, paragraph [0039]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein in that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

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As per claim 5, Tombs and Tsubaki, in combination, disclose the consumable product of claim 4, but Tombs does not disclose said cream liquor further comprising a non-dairy lipid emulsion, wherein said cream liquor is free of animal products. Tsubaki discloses said cream liquor (foods also include beverages, such as alcoholic beverages including lactic acid beverages (cream beverages); paragraph [0044]; gramineous plant fats and oils, paragraph [0039]) further comprising a non-dairy lipid emulsion (fat (lipid) and oil composition containing beta-glucans extracted from a gramineous plant (non-dairy) can be used as a substitute in an emulsion; paragraph [0039]), wherein said cream liquor is free of animal products (fat (lipid) and oil composition containing -glucans extracted from a gramineous plant can be used as a substitute (free of animal products) in an emulsion and can be alcoholic beverages including lactic acid beverages (cream beverages); paragraphs [0039], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 6, Tombs and Tsubaki, in combination, disclose the consumable product of claim 3, but Tombs does not disclose wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein. Tsubaki discloses wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein (fat (lipid) and oil composition containing -glucans extracted from a gramineous plant can be used as a substitute (supplemented) in an emulsion and can be alcoholic beverages, including lactic acid beverages (cream beverages); paragraphs [0039], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with integrating protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of the plant in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), as a supplement, and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 7, Tombs discloses the consumable product of claim 2, but Tombs does not disclose wherein said condiment is a mayonnaise replica. Tsubaki discloses wherein said condiment is a mayonnaise replica (fat (lipid) and oil composition containing -glucans extracted from a gramineous plant can be used as a substitute for a part of, or the whole of (replica) food which can include mayonnaise; paragraphs [0039], [0045]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of a plant protein as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product (Tsubaki; paragraphs [0001], [0039]), and a combination of these references would have resulted in a food product containing a replacement protein from a plant source.

As per claims 9/3-9/7, Tombs and Tsubaki, in combination, disclose the consumable product of claims 3-7, and Tombs further discloses wherein said isolated and purified plant protein is at least 10 kDa in size (a plant protein valuable in food products (consumable), such as arachin (21 kDa protein), a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21).

As per claim 52, Tombs discloses a method for making a consumable product comprising: a) preparing a solution comprising an isolated and purified plant protein (a plant protein valuable in food products (consumable) in its fluid form (solution), such as arachin, a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21), wherein said isolated and purified plant protein has (i) a solubility in said solution of at least 25 at a temperature between about 2 degrees C and 32 degrees C, wherein said solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in said solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein said solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM (mesophases (proteins) of the invention comprise undenatured plant protein in a dissolved state (soluble), dissolved in aqueous solution of 0.1 N (0.1 N = 0.1 M = 100 mM) NaCl (sodium chloride) and specifically 500 g rape protein is extracted in 5 L water (at least 1 mg/ml) adjusted to pH 7.1 and the temperature required to set the mesophase is generally set by heating at 90 degrees C to 100 degrees C; column 1, lines 64-65; column 3, lines 12-13; column 8, lines 56-57; column 19, lines 32-34). Tombs does not disclose adding said solution to a beverage. Tsubaki discloses adding said solution to a beverage (foods also include beverages, such as alcoholic beverages; paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 54/52, Tombs and Tsubaki, in combination, disclose the method of claim 52, but Tombs does not disclose wherein said beverage is clear. Tsubaki discloses wherein said beverage is clear (foods also include beverages, such as alcoholic beverages (many alcoholic beverages are clear); paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including the protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be utilized in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

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Claims 19-21, 22/20 and 22/21 lack an inventive step under PCT Article 33(3) as being obvious over Zhang in view of Tsubaki.

As per claim 19, Zhang discloses the consumable product of claim 17, but Zhang does not disclose said product further comprising a plant derived lipid or a microbial-derived lipid. Tsubaki discloses a product further comprising a plant derived lipid or a microbial-derived lipid (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells; paragraph [0029]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes the use of the coacervate to enhance food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food, which is uniformly dispersible throughout the food and can be used in part of, or as whole of a fat or lipid in the food product (Tsubaki; paragraphs [0001], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claim 20, Zhang and Tsubaki, in combination, disclose the consumable product of claim 19, but Zhang does not disclose wherein said plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil. Tsubaki discloses a plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells, and lecithin can be added; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate for enhancing food products, Tsubaki discloses the use of a plant protein as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claim 21, Zhang and Tsubaki, in combination, disclose the consumable product of claim 20, but Zhang does not disclose said product comprising up to about 1% lecithin by weight. Tsubaki discloses a product comprising up to about 1% lecithin by weight (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited, and include lipid of dry microbial cells and lecithin can be added in general amounts of 0.01 to 15%; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate to enhance food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product, and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claims 22/20 and 22/21, Zhang and Tsubaki, in combination, disclose the consumable product of claims 20 and 21, but Zhang does not disclose wherein said product comprises lecithin and said oil. Tsubaki discloses wherein said product comprises lecithin and said oil (fat and oil compositions in which the β -glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells and lecithin can be added in general amounts of 0.01 to 15%; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate for enhancing food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food, which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product, and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

Claim 18 lacks an inventive step under PCT Article 33(3) as being obvious over Zhang in view of Tombs.

As per claim 18, Zhang discloses the consumable product of claim 17, but Zhang does not disclose wherein said consumable product is a meat replica. Tombs discloses a consumable product that is a dried meat substitute formed wholly from vegetable protein using unset mesophase binder (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with method of extracting a plant protein, as previously disclosed by Tombs, as the previous disclosure by Zhang discloses an aqueous dispersion of complex coacervates in stable form suitable for use in foods (Zhang; paragraph [0004]), Tombs discloses the use of a plant protein in a food product, such as a meat replica (Tombs; abstract; column 6, lines 20-21), and a combination of these references would have resulted in a superior food product containing plant protein in a meat replica.

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Box No. V:

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Claims 53 and 54/53 lack an inventive step under PCT Article 33(3) as being obvious over Tombs and Tsubaki and further in view of Zhang.

As per claim 53, Tombs and Tsubaki, in combination, disclose the method of claim 52, but Tombs does not disclose wherein said solution comprises two or more isolated and purified plant proteins. Zhang discloses wherein said solution comprises two or more isolated and purified plant proteins (an aqueous dispersion of complex coacervates in stable form suitable for use in foods (consumable products) is provided and can include whey protein, hydrolyzed protein, lauric arginate, polylysine, casein or any combination thereof; paragraphs [0004], [0006]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the extracting a plant protein, as previously disclosed by Tombs, with the use of multiple proteins, as previously disclosed by Zhang, as the previous disclosure by Tombs includes the use of plant proteins in food products, Zhang discloses the use of multiple proteins in the form of a coacervate to add to food products (Zhang; paragraph [0004]), and a combination of these references would have resulted in a superior food product containing plant proteins.

As per claim 54/53, Tombs, Tsubaki and Zhang, in combination, disclose the method of claim 53, but Tombs does not disclose wherein said beverage is clear. Tsubaki discloses wherein said beverage is clear (foods also include beverages, such as alcoholic beverages (alcoholic beverages are clear); paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the the protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the integration of the protein in food, which is uniformly dispersible throughout the food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

Claims 1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52 and 54/53 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

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Box No. IV: Lack of Unity of Invention:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-9, 17-21 and 52-54 are directed toward a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, and a method of making said consumable product.

Group II: Claims 17-21, 28-31, 37-40, 68-70 and 81-83 are directed toward a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins, said adipose tissue replica including one or more isolated plant proteins, one or more plant or algal derived oils, optionally a phospholipid, and a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica; and wherein the connective tissue replica comprises proteins assembled into fibrous structures by a solution spinning process.

Group III: Claims 32-36 and 61-65 are directed toward a consumable product comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, and a method of making said consumable product.

Group IV: Claims 47-49 are directed toward a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

Group V: Claim 51 is directed toward a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

Groups VI+: Claims 59, 60 and SEQ ID NO: 1 are directed toward a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. (SEQ ID NO: 1-27). + type Markush group.

The method for extending the shelf-life of a meat-free consumable product comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions can be searched to the extent that it encompasses a sequence at least 70% identical to SEQ ID NO: 1 (Vigna radiata amino acid sequence). Additional sequences will be searched upon the payment of additional fees. It is believed that Claims 59 and 60 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (Vigna radiata amino acid sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (Methyldacidophilum inferorum amino acid sequence).

Groups VII+: Claims 71-73 and SEQ ID NO: 1 are directed toward a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Group VIII: Claims 74-78 are directed toward a method of making a coacervate, said method comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and b) isolating said coacervate from said solution.

Group IX: Claims 91-93 are directed toward methods of minimizing undesirable odors in a composition comprising plant proteins.

Group X: Claim 94 is directed toward a chocolate flavored spread comprising: a) sugar; b) a chocolate flavoring; and c) a cream fraction from a plant based milk.

Group XI: Claims 95-99 are directed toward a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

Group XII: Claims 100-104 are directed toward tissue replicas comprising a freeze-aligned non-animal protein.

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Box No. IV: Lack of Unity of Invention:

The inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, not present in any other groups, the special technical features of Group II including a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, or a coacervate, which are not present in any other group, the special technical features of Group III including a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, which are not present in any other Group, the special technical features of Group IV including a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, not present in any other group, the special technical features of Group V including a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, which are not present in any other group, the special technical features of Groups VI+ including a method for extending the shelf-life of a meat-free consumable product, which are not present in any other group, the special technical features of Groups VII+ including a method for imparting a beef like flavor to a consumable product, which are not present in any other group, the special technical features of Group VIII including a method of making a coacervate comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, which are not present in any other group, the special technical features of Group IX including methods of minimizing undesirable odors in a composition comprising plant proteins, which are present in any other group, the special technical features of Group X including a chocolate flavored spread, which are not present in any other Group, the special technical features of Group XI including a method for altering the texture of a consumable, which not present in any other group, the special technical features of Group XII including a freeze-aligned non-animal protein.

Groups I-XII share the technical features including consumables. Groups I, II, V, VIII, IX, X and XI share the technical features including plants. Groups I, II, VIII, IX and XI share the technical features including plant proteins. Groups I-V, VI+, VII+, VIII, IX, XI and XII share the technical features including proteins. Groups I, III, V, VII+, XI and XII share the technical features including temperature. Groups I and VIII share the technical features including pH. Groups I, III and VIII share the technical features including sodium chloride. Groups I-III, VI+, VII+, VIII and XI share the technical features including a method of making a consumable product. Groups II, III and XII share the technical features including a meat replica. Groups II and VIII share the technical features including a coacervate. Groups II and V share the technical features including plant or algal derived oils or lipids. Groups II and V share the technical features comprising an emulsion. Groups III and V share the technical features including a gel. Groups IV, VI+ and VII+ share the technical features including a heme-containing protein. Groups IV and VI+ share the technical features including meat-free products. Groups VI+ and VII+ share the technical features including a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27.

Groups VI+ share the technical features including a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

Groups VII+ share the technical features including a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

However, these shared technical features are previously disclosed by WO 2001/015714 A1 (ZLOTKIN) in view of US 2010/0074998 A1 to Espeleta-Vega et al. (hereinafter 'Espeleta-Vega'), US 2011/0083681 A1 to Sengupta, et al. (hereinafter 'Sengupta'), the publication entitled 'Comparative Analysis Of Autoxidation Of Haemoglobin' by Jensen (hereinafter 'Jensen'), US 4,986,998 A to Yoo, et al. (hereinafter 'Yoo') and GenBank Accession AFK42304 by Krishnakumar, et al. (hereinafter 'Krishnakumar').

Zlotkin discloses consumables (compositions including micronutrients (consumables); abstract) including plants (chicory fructooligosaccharide (plants); page 11, lines 15-19); proteins (proteins; page 15, lines 20-28); temperature (temperature; page 17, lines 10-25); a method of making a consumable product (a process for producing a composition comprising micronutrients (a method of making a consumable product); page 8, lines 22-27); plant or algal derived oils or lipids (soybean oil (plant or algal derived oils or lipids); page 15, lines 1-4); meat-free products (cereals; purees of fruit, vegetables, milk, yogurt, cottage cheese (meat-free products); page 18, lines 1-11), and a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto (a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto; page 14, lines 21-28).

Zlotkin does not disclose plant proteins; pH, sodium chloride; a meat replica; a coacervate; an emulsion; a gel; a heme-containing protein; a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27; a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions; and a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

-Continued Within the Next Supplemental Box-

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

PCT/US14/11361

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box No. IV: Lack of Unity of Invention:

Espeleta-Vega discloses plant proteins (vegetable protein (plant proteins); abstract), pH (a pH modifier; paragraph [0010]), sodium chloride (salt (sodium chloride); paragraph [0038]), a meat replica (substitute meat food product (a meat replica); abstract); an emulsion (an emulsion; paragraph [0011]); a gel (a gel; paragraph [0011]).

Sengupta discloses a process for preparing coacervate gel particles comprising plant materials including proteins (a process for preparing coacervate gel particles comprising plant materials including proteins; paragraph [0015]).

Jensen discloses a heme-containing protein (hemoglobin; abstract) wherein the oxidation of hemoglobin and myoglobin is significantly slower than free heme (oxidation of hemoglobin and myoglobin is significantly slower than free heme; page 2029, column 1, paragraph 2), and wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin (wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin; page 2030, column 1, paragraph 1).

Yoo discloses a method for imparting (flavoring (imparting); abstract) a beef (cow (beef); column 4, lines 26-32) like flavor to a consumable product (flavor to a consumable product; abstract, column 2, lines 49-50), comprising adding to said consumable composition a heme-containing protein (comprising adding to said consumable a powdered blood product (comprising adding to said consumable composition a heme-containing protein); abstract), wherein after cooking, a beef-like flavor is imparted to said consumable (providing a meat-like flavor (beef-like flavor is imparted to said consumable); column 3, lines 1-6).

Krishnakumar discloses a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1 (a globin (a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1); page 1).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin by combining the consumable disclosed by Zlotkin with a meat replica, such as that disclosed by Espeleta-Vega, for providing the micronutrients in the consumable in a form acceptable to a consumer, such as in a meat replica, for enhancing the nutrient content of a meat replica, particularly the iron content of said replica, providing the previous disclosure of Zlotkin. Also, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables with the coacervate preparation process of Sengupta, for enabling the preparation of particulates comprising the micronutrients disclosed by Zlotkin using a plant polysaccharide, such as pectin, as disclosed by Sengupta, for providing micronutrients in a consumable and easily digestible form to enable a consumer to absorb the nutrients. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin, for including the use of hemoglobin in preference to free iron or other forms of iron, in order to provide the iron to the consumer in a biologically useful form and oxidation state. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have therefore modified the disclosure of Zlotkin, for providing a method for extending the shelf-life of a meat-free consumable product, such as the consumables disclosed by Zlotkin, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions, such as adding hemoglobin, based on the disclosure of Jensen that tetrameric hemoglobin oxidizes more slowly than other forms of heme-containing proteins. Also, it would have been obvious to a person of ordinary skill in the art to have modified the disclosure of Zlotkin, for including a meat-like flavoring including an iron-containing protein based on the disclosure of Yoo, in order to provide a beef-like flavor to a consumable food, including a poultry or fish composition, to provide a beef-flavored product not containing beef, but with lower fat, such as a consumable comprising a plant protein, fish, or poultry protein. Finally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables comprising iron, for including a plant-based iron-comprising proteins, such as the globin disclosed by Krishnakumar, in order to provide iron from non-animal sources having high absorbability and utilization by a consumer.

Since none of the special technical features of the Groups I-XII inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zlotkin, Espeleta-Vega, Sengupta, Jensen, Yoo and Krishnakumar references, unity of invention is lacking.

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670016WO1	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US14/11362	International filing date (<i>day/month/year</i>) 13 January 2014 (13.01.2014)	(Earliest) Priority Date (<i>day/month/year</i>) 11 January 2013 (11.01.2013)
Applicant MARAXI, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

NON-DAIRY CHEESE REPLICA COMPRISING A COACERVATE

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 11

☐ as suggested by the applicant.

☒ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 9-10, 15-48, 56-67, 72, 79, 89-90
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.

Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.

Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.

Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.

Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

---Continued Within the Next Supplemental Box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 8/1-4, 73-77, 78/73-77, and 91-93

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

PCT/US14/11362

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)

USPC - 426/615, 634; 530/378

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A23L 1/052, 1/36; A23C 19/055, 20/02; A23J 3/14 (2014.01)

USPC: 426/615, 634, 656; 530/378

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; Google; ProQuest; non-dairy, non-animal, coacervation, plant protein, chickpea protein, lentil protein, pea protein, lupine protein, pea vicilin, pea legumin, imitation cheese

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2004/0151778 A1 (RICHARD, J et al.) August 5, 2004; paragraphs [0011], [0019], [0022], [0025], [0028], [0039], [0040], [0048]	1-4, 91-93 ----- 8/1-4, 73-77, 78/73-77
Y	WO 2008/030089 A1 (BRANDSMA, JB et al.) March 13, 2008; page 5, lines 26-32; page 6, lines 1-3	8/1-4
Y	US 4435438 A (LEHNHARDT, WF et al.) March 6, 1984; column 4, lines 1-5; column 16, lines 20-30	73-77, 78/73-77
Y	US 2008/0226810 A1 (PASSE, D et al.) September 18, 2008; paragraphs [0005], [0007], [0014], [0015]	77, 78/77
A	US 5753295 A (GOLDMAN, MS) May 19, 1998; entire document	1-4, 8/1-4, 73-77, 78/73-77, 91-93
A	WO 2012/116703 A1 (ANDERSEN, KE et al.) September 7, 2012; entire document	1-4, 8/1-4, 73-77, 78/73-77, 91-93

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 May 2014 (14.05.2014)

Date of mailing of the international search report

13 JUN 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

-Continued from Box No. III: Observations where unity of invention is lacking-

The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosum*, and *Brevibacterium linens*; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non-animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

13 JUN 2014

Applicant's or agent's file reference
387670016WO1

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US14/11362

International filing date (day/month/year)

13 January 2014 (13.01.2014)

Priority date (day/month/year)

11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/052, 1/36; A23C 19/055 (2014.01)
USPC - 426/615, 634; 530/378

Applicant **MARAXI, INC.**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No: 571-273-3201

Date of completion of this opinion

14 May 2014 (14.05.2014)

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43 *bis*.1(a)).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US14/11362

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-99 are so unclear that no meaningful opinion could be formed (*specify*):

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 9-10, 15-48, 56-67, 72, 79, 89-90

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

- ☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

PCT/US14/11362

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

☐ complied with

☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 are directed toward a non-dairy cheese replica comprising a coacervate.

Group II: Claims 5-7, 8/5-7, 68-70, and 71/68-70 are directed toward a non-dairy cheese replica comprising a cold set gel.

Group III: Claims 11-13, 14/11-13, 49-53, 54/49-53, 55/54/49-53, 86-87, 88/86-87, 94-97, 98/96-97, 99-100, and 101/99-100 are directed toward a non-dairy cheese replica comprising a solidified mixture.

Group IV: Claims 80-83, 84/80-83, and 85/84/80-83 are directed toward a method of minimizing one or more undesirable odors.

Group V: Claim 102 is directed toward a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica.

---Continued Within Supplemental Box---

4. Consequently, this opinion has been established in respect of the following parts of the international application:

☐ all parts

☒ the parts relating to claims Nos. 1-4, 8/1-4, 73-77, 78/73-77, and 91-93

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

PCT/US14/11362

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	8/1-4, 73-77, 78/73-77	YES
	Claims	1-4, 91-93	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-4, 8/1-4, 73-77, 78/73-77, 91-93	NO
Industrial applicability (IA)	Claims	1-4, 8/1-4, 73-77, 78/73-77, 91-93	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-4 and 91-93 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0151778 A1 to Richard, et al. (hereafter "Richard").

As per claim 1, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated and purified proteins from a non-animal source (solubilization of at least one plant protein in an aqueous medium; the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used, are extracted from plants (extraction comprise process for isolation of pure proteins from the mixture); paragraphs [0022], [0025], [0028]; [0039], [0040]).

As per claim 2, Richard discloses the non-dairy cheese replica of claim 1, and Richard further discloses wherein said one or more isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 3, Richard discloses the non-dairy cheese replica of claim 2, and Richard further discloses wherein said one or more isolated and purified plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*); paragraph [0040]).

As per claim 4, Richard discloses the non-dairy cheese replica of claim 3, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claim 91, Richard discloses a non-dairy cheese replica (the preamble carries no patentable weight) comprising a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; vicilin, coacervation; paragraphs [0019], [0025]) comprising one or more isolated proteins from a non-animal source (the concentration of proteins in the coacervation solution is between 1.5 and 5 percent; microcapsules containing a material to be encapsulated, which comprises coacervating, in an aqueous medium and in the presence of the material to be encapsulated, at least one solubilized plant protein; tendency is to use natural products of plant origin as substitutes for animal proteins; plant proteins used in the context of the invention are extracted from plants; paragraphs [0022], [0025], [0039], [0040]).

As per claim 92, Richard discloses the non-dairy cheese replica of claim 91, and Richard further discloses wherein said one or more isolated proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants; paragraph [0040]).

As per claim 93, Richard discloses the non-dairy cheese replica of claim 92, and Richard further discloses wherein said one or more isolated plant proteins are selected from the group consisting of seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, and lentil proteins (the plant proteins used in the context of the invention are extracted from plants chosen from the group comprising lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*); paragraph [0040]).

-Continued Within the Next Supplemental Box-

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

****-Continued from Box V: Citations and Explanations-****

Claims 8/1-4 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of WO 2008/030089 A1 to Brandsma, et al. (hereafter 'Brandsma').

As per claims 8/1-4, Richard discloses the non-dairy cheese replica of any one of claims 1-4, but Richard does not disclose further comprising one or more microbes selected from bacteria, molds, and yeast. However, Brandsma discloses comprising one or more microbes selected from bacteria and yeast (adding at least two live food grade micro-organisms, said two micro-organisms are at least one a yeast strain selected from the genus Kluyveromyces and at least one bacterial strain selected from the genus Propionibacterium; to form a cheese; page 5, lines 26-32 to page 6, lines 1-3; claim 1). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide microbes such as bacteria or yeast, as disclosed by Brandsma, in order to have flavor formation in manufacture of fermented products, in order to provide non-dairy consumables.

Claims 73-76 and 78/73-76 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of US 4,435,438 A to Lehnhardt, et al. (hereafter 'Lehnhardt').

As per claim 73, Richard discloses a method of making a coacervate (a method for producing microcapsules containing a material to be encapsulated, which comprises coacervating; paragraph [0025]), said method comprising acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5 (method comprises, solubilization of at least one plant protein in an aqueous medium at a pH of between 2 and 7, and below the isoelectric pH of the protein; the pH of the medium is decreased, by adding IN hydrochloric acid; plant proteins used in the context of the invention are extracted from plants; paragraphs [0028],[0040], [0048]) and isolating said coacervate from said solution (encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; the wall formed is hardened by cooling the medium and crosslinked by the action of a crosslinking agent; paragraph [0011]). Richard does not disclose wherein said solution comprises 100 mM or less of a salt. However, Lehnhardt discloses wherein said solution comprises 100 mM or less of a salt (salt (sodium chloride); 2 parts by weight (2 pbw corresponds to 0.66 mM of sodium chloride); column 4, lines 1-5; column 16, lines 20-30). It would have been a matter of obvious experimentation to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein said solution comprises 100 mM or less of a salt, as disclosed by Lehnhardt, in order to provide plant protein coacervates having low salt content, in order to provide non-dairy protein consumables.

As per claim 74, Richard and Lehnhardt, in combination, disclose the method of claim 73, and Richard further discloses wherein said isolated and purified proteins are plant proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*) and cereals such as wheat, maize, barley, malt or oats; paragraph [0040]).

As per claim 75, Richard and Lehnhardt, in combination, disclose the method of claim 74, and Richard further discloses wherein said plant proteins are seed storage proteins, chickpea proteins, lentil proteins, pea proteins or Lupine proteins (plant proteins used in the context of the invention are extracted from plants chosen from the group comprising: lupin (genus *Lupinus*), soybean (genus *Glycine*), pea (genus *Pisum*), chickpea (*Cicer*), alfalfa (*Medicago*), broad bean (*Vicia*), lentil (*Lens*), bean (*Phaseolus*), rapeseed (*Brassica*), sunflower (*Helianthus*) and cereals such as wheat, maize, barley, malt or oats; plant proteins SWP100 and SWP50 and those marketed under the name Supro 670 and PISANE; paragraph [0040]).

As per claim 76, Richard and Lehnhardt, in combination, disclose the method of claim 75, and Richard further discloses wherein said pea proteins comprise pea vicilins (it is possible to produce nanoparticles and microparticles from vicilin (pea protein) by simple coacervation; paragraph [0019]).

As per claims 78/73-76, Richard and Lehnhardt, in combination, disclose the method of claims 73-76, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the pH of the medium is decreased, by adding in hydrochloric acid; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

****-Continued Within the Next Supplemental Box-****

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

-Continued from Previous Supplemental Box-

Claims 77 and 78/77 lack inventive step under PCT Article 33(3) as being obvious over Richard in view of Lehnhardt further in view of US 2008/0226810 A1 to Passe, et al (hereafter 'Passe').

As per claim 77, Richard and Lehnhardt, in combination, disclose the method of claim 76, but Richard does not disclose wherein said pea vicilins comprises convicilins. However, Passe discloses wherein said pea proteins comprise pea vicilins and/or pea legumins (pea proteins, like all proteins of leguminous plants, are constituted of three classes of proteins: globulins, albumins and insoluble proteins, the globulins (legumins, vicilins and convicilins) are the main storage proteins of the plant; legumins are hexameric proteins of 360 to 410 kDa composed of 6 double-stranded subunits of 60 kDa; vicilins are trimeric proteins whose mass ranges from 150 to 200 kDa; paragraphs [0005], [0007], [0014], [0015]). It would be obvious to a person of ordinary skill in the art at the time of the invention to modify Richard, to provide wherein pea proteins comprise pea vicilins and/or pea legumins, as disclosed by Passe, in order to provide other legumes, in order provide other suitable plant based proteins, in order to manufacture non-animal based imitation cheese.

As per claim 78/77, Richard, Lehnhardt, and Passe, in combination, disclose the method of claim 77, and Richard further discloses wherein said acidifying step is done in the presence of a plant based oil (the encapsulation of an oil by complex coacervation consists in emulsifying the oil in a solution of two polymers; the coacervation is induced by adjusting the pH of the medium; the polymeric complexes formed adsorb onto the droplets of oil and thus isolate them from the outside medium; paragraph [0011]).

Claims 1-4, 8/1-4, 73-77, 78/73-77, and 91-93 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

PCT/US14/11362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

-Continued from Box No. IV: Lack of unity of invention-

The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a non-dairy cheese replica comprising a coacervate of one or more isolated and purified proteins; a method of making a coacervate, said method comprising: acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of a salt; isolating said coacervate from said solution; and optionally subjecting said coacervate to high pressure processing, which are not present in Groups II-V; the special technical features of Group II include a non-dairy cheese replica comprising a cold set gel of one or more isolated and purified proteins from a non-animal source and a salt; a method for making a cold set gel comprising: denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4 degrees C and 25 degrees C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing, which are not present in Groups I and III-V; the special technical features of Group III include a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids; said cheese replica comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*; wherein at least 85 percent of the insoluble solids of the non-dairy milk have been removed, wherein said non-dairy milk is a nut milk, a bean milk, or a grain milk; wherein said non-dairy cheese replica has an increased creamy texture, an improved melting characteristic, or an increased stretching ability; and ricotta and blue cheese replicas comprising a solidified nut milk and microbes; and a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product, said method comprising a) adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a grain milk or a bean milk and culturing said microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is modulated, which are not present in Groups I-II and IV-V; the special technical features of Group IV include a method of minimizing one or more undesirable odors in a composition containing plant proteins, said method comprising contacting said composition with a ligand having binding affinity for lipoxygenases; contacting said composition with activated carbon then removing the activated carbon from said composition, and contacting said composition with a lipoxygenase inhibitor and/or an antioxidant, which are not present in Groups I-III and V; the special technical features of Group V include a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica, comprising: a) obtaining a starter culture comprising a heterogenous population of microbial strains; b) isolating one or more individual microbial strains from said heterogenous population; and c) determining a flavor contribution of each of said individual microbial strains to a non-dairy cheese replica, which are not present in Groups I-IV.

The common technical features of Groups I-V are a non-dairy cheese replica comprising one or more isolated and purified proteins from a non-animal source; and high pressure processing.

These common technical features are disclosed by US 6,419,975 B1 to Han, et al. Han discloses a non-dairy cheese replica (a cream cheese-like product (cheese replica) that does not require dairy caseins (non-dairy), where non-casein protein replaces casein protein, and does not require dairy caseins; abstract; column 2, lines 12-17) comprising one or more isolated and purified proteins from a non-animal source (food proteins, including soy protein isolate (isolated and purified protein from a non-animal source) may be used as protein sources to produce caseinless cream cheese-like products with similar texture; column 2, lines 12-17; claim 1); and high pressure processing (a process which combines high pressure homogenization and heat treatment to enhance or extend emulsifying capacity and which forms an irreversibly heat denatured protein matrix stabilized emulsion; column 2, lines 5-9).

Since the common technical features are previously disclosed by Han, these common features are not special and so Groups I-V lack unity.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: MONICA MCCORMICK GRAHAM
FISH & RICHARDSON P.C.
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year) 01 MAY 2015

Applicant's or agent's file reference
387670011WO1

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US 15/17147

International filing date
(day/month/year) 23 February 2015 (23.02.2015)

Applicant IMPOSSIBLE FOODS INC.

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

Shortly after the expiration of **18 months from the priority date**, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Within **19 months from the priority date**, the applicant may request that a supplementary international search be carried out by a different International Searching Authority that offers this service (Rule 45bis.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Lee W. Young

PCT Helpdesk: 571-272-4300
Telephone No. PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670011WO1	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 15/17147	International filing date (<i>day/month/year</i>) 23 February 2015 (23.02.2015)	(Earliest) Priority Date (<i>day/month/year</i>) 21 February 2014 (21.02.2014)
Applicant IMPOSSIBLE FOODS INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

- a. the figure of the **drawings** to be published with the abstract is Figure No. _____
☐ as suggested by the applicant.
☐ as selected by this Authority, because the applicant failed to suggest a figure.
☐ as selected by this Authority, because this figure better characterizes the invention.
- b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/17147

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8-40, 55-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/17147

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23C 19/00 (2015.01)

CPC - A23C 19/082; A23C 19/093; A23C20/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
cpc-A23C19/082; A23C19/093; A23C20/00Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC-426/582 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, PubWest, ProQuest Dialog, Google Scholar, Google

Search Terms: Soy, bean, milk, cheese, microbial, yeast, mold, strain, bacterial, micro, acid, amidated pectin, oil, sugar, starch, Lactococcus lactis lac tis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylos

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/013292 A1 (Lin et al.) 31 January 2013 (31.01.2013) pg2, ln7-8; pg4, ln1-2, 15-16; pg8, ln12, 17; pg11, ln26-29; pg12, ln12, 25, 32; pg14, ln22;	1-7, 47-54
Y	Herbstreith&Fox. Low Methylester, Amidated Pectins. Technical Application Information. 18 January 2007; pg1, col1, ln1-5	1-7, 41-54
Y	US 5,597,594 A (Matsuura et al.) 28 January 1997 (28.01.1997) abstract, col3, ln29, 35-40, 46-48; col4, ln3; col5, ln53-54	3-7, 41-46
Y	US 2010/0196575 A1 (Sanchez et al.) 5 August 2010 (05.08.2010) para [0065], [0098]	41-46
Y,E	WO 2014/110540 A1 (Holz-Schietinget et al.) 17 July 2014 (17.07.2014) abstract, pg4, ln1-30	1-7

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 April 2015 (17.04.2015)

Date of mailing of the international search report

01 MAY 2015

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

To: MONICA MCCORMICK GRAHAM
FISH & RICHARDSON P.C.
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

Date of mailing
(day/month/year) 01 MAY 2015

Applicant's or agent's file reference
387670011WO1

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/US 15/17147

International filing date (day/month/year)
23 February 2015 (23.02.2015)

Priority date (day/month/year)
21 February 2014 (21.02.2014)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23C19/00 (2015.01)
CPC - A23C19/082; A23C19/093; A23C20/00

Applicant IMPOSSIBLE FOODS INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

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Facsimile No. 571-273-8300

Date of completion of this opinion
16 April 2015 (16.04.2015)

Authorized officer:
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PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 15/17147

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

- ☐ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

- ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 15/17147

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 8-40, 55-68

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (*specify*):

Claims 8-40, 55-68 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 8-40, 55-68

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims	1-7, 41-54	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-7, 41-54	NO
Industrial applicability (IA)	Claims	1-7, 41-54	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-2, 47-54 lack inventive step under PCT Article 33(3) as being obvious over WO 2013/013292 A1 to Lin et al. (hereinafter 'Lin') in view of the article entitled "Low Methyl ester, Amidated Pectins" to Herbstreith&Fox (hereinafter 'Herbstreith').

As per claim 1, Lin teaches a soy-based cheese (Lin-pg2, ln7-8) comprising: a) soy milk (Lin-pg2, ln7-8) b) a microbial strain (Lin-pg4, ln15-16) d) an oil (Lin-pg11, ln28) e) optionally one or more of a salt, a starch, a sugar, a flavoring (Lin-pg11, ln27), an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, coconut milk, coconut cream, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose. Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach a soy-based cheese comprising a pectin as a stabilizer (Lin-pg12, ln32). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to use a low methyl ester, amidated pectin to replace pectin in the cheese-making because it offers a very high product safety.

As per claim 2, Lin teaches the soy-based cheese of claim 1, wherein said microbial strain is a bacterial strain (Lin-pg4, ln15-16) a yeast strain, or a mold strain.

As per claim 47, Lin teaches a soy-based cheese comprising: a) soy milk (Lin pg2, ln7-8); b) an acid (Lin-pg4, ln1-2); d) an oil (Lin-pg14, ln22); and e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, one or more proteins, lecithin, a flour, a fiber, or cellulose. Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach a cheese comprising a pectin as a stabilizer (Lin-pg12, ln32) and Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to incorporate a low methyl ester, amidated pectin as taught by Herbstreith into the cheese as taught by Lin because it offers a very high product safety.

As per claim 48, Lin teaches the soy-based cheese of claim 47, wherein said acid is selected from the group consisting of citric acid (Lin-pg4, ln2), lemon juice, HCl, and pyruvic acid.

As per claim 49, Lin teaches a soy-based cheese comprising: a) soy milk (Lin-pg2, ln7-8); b) a microbial strain (Lin-pg4, ln15-16); d) one or more proteins (Lin-pg8, ln12-two proteins); e) an oil (Lin-pg11, ln28); f) a salt (Lin-pg11, ln28); g) a sugar (Lin-pg11, ln29); h) a gum (Lin-pg12, ln25); i) a coloring agent (Lin-pg11, ln26); and j) a flavoring (Lin-pg11, ln27). Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach the cheese comprising a pectin as a stabilizer (Lin-pg12, ln32). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to use a low methyl ester, amidated pectin to replace pectin in the cheese-making because it offers a very high product safety.

As per claim 50, Lin teaches a soy-based cheese comprising: a) soy milk (Lin pg2, ln7-8); b) one or more proteins (Lin-pg8, ln12, two proteins) d) an oil (Lin-pg11, ln28); and e) optionally one or more of a salt, a starch, a sugar, a flavoring, an antioxidant, a coloring agent, a gum, an amino acid, quinoa, pyruvic acid or a salt form thereof, a preservative, lactic acid, acetic acid, yeast extract, a dried yeast, lecithin, a flour, a fiber, or cellulose. Although Lin doesn't teach the cheese comprising c) a low methyl ester, amidated pectin, it does teach the cheese comprising a pectin as a stabilizer (Lin-pg12, ln32). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, ln1-5). It would have been obvious to one skilled in the art to use a low methyl ester, amidated pectin to replace pectin in the cheese making because of it offers a very high product safety.

-----continued in Supplemental Box-----

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient:

Continuation of:

Box No. V 2. Citations and explanations:

As per claim 51, Lin teaches the soy-based cheese of claim 49 or claim 50, wherein said one or more proteins comprise soy protein isolate or the soluble proteins from soy flour or soy protein (Lin-pg8, ln29) isolate.

As per claim 52, Lin teaches the soy-based cheese of claim 49 or claim 50, wherein said one or more proteins is an isolated protein (Lin-pg8, ln22).

As per claim 53, Lin teaches the soy-based cheese of claim 52, wherein said isolated protein is selected from the group consisting of a lentil protein, a soy protein (Lin-pg8, ln29), or a pea protein.

As per claim 54, Lin teaches the soy-based cheese of claim 53, wherein said protein is pea vicilin, pea legumin, soy B-conglycinin, soy glycinin (Lin-pg8, ln22), or a dehydrin protein.

Claims 3-7 lack inventive step under PCT Article 33(3) as being obvious over Lin in view of Herbstreith in further view of US 5,597,594 A to Matsuura et al. (hereinafter 'Matsuura').

As per claim 3, although neither Lin nor Herbstreith teaches the soy-based cheese of claim 2, wherein said bacterial strain is selected from the group consisting of Lactococcus lactis lactis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylosus (SX), and Streptococcus thermophilus (ST). Matsuura teaches the soy-based cheese of claim 2, wherein said bacterial strain is selected from the group consisting of Lactococcus lactis lactis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylosus (SX), and Streptococcus thermophilus (ST) (Matsuura-col3, ln37-38). It would have been obvious to one skilled in the art to incorporate Streptococcus thermophilus (ST) as taught by Matsuura into the cheese as taught by Lin and Herbstreith since Matsuura teaches Streptococcus thermophilus (ST) is good for making cheese.

As per claim 4, Matsuura further teaches the soy-based cheese of claim 3, said soy-based cheese comprising two bacterial strains (Matsuura-col3, ln36, mixed bacteria).

As per claim 5, Matsuura teaches the soy-based cheese of claim 4, wherein said bacterial strains are ST (Matsuura-col3, ln37-38), but no mentioning SX. It would have been obvious to one skilled in the art to use ST and SX together since both are common strains for fermentation and may achieve synergistic effect when using together.

As per claim 6, Lin teaches the soy-based cheese of any one of claims 1-5, said soy-based cheese comprising said starch (Lin-pg12, ln12).

As per claim 7, Lin teaches further teaches the soy-based cheese of claim 6, wherein said starch is potato starch (Sanchez-para [0085]), corn starch, tapioca, rice starch (Lin-pg12, ln17), pea starch, arrowroot starch, or a modified food starch.

-----continued in Supplemental Box-----

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INTERNATIONAL SEARCHING AUTHORITY

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
previous box

Claims 41-46 lack inventive step under PCT Article 33(3) as being obvious over Matsuura in view of US 2010/0196575 A1 to Sanchez et al. (hereinafter 'Sanchez') further in view of Herbstreith

As per claim 41, Matsuura teaches a method of making a soy-based cheese (Matsuura-abstract) teaches, said method comprising: a) incubating (Matsuura-col3, In46-47) mixture of (i) soy milk (Matsuura-col3, In26), (ii) an optional sugar (Matsuura-col3, In29) and/or optional one or more proteins, and (iii) a microbial strain (Matsuura-col3, In35) or an acid for a period of time sufficient (Matsuura-col3, In46-48) to reach a pH of 5.4 to 6.4 (Matsuura-col5, In53); Although Matsuura doesn't explicitly teach b) blending said mixture, c) adding to said blended mixture of b), a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional preservative, and an optional coloring agent and blending; d) heating said mixture of c) to a temperature of 60C to 100C; and e) adding to the heated mixture of d), one or more optional sugars and one or more flavorings to produce said soy-based cheese, Sanchez teaches b) blending said mixture (Sanchez-para [0098]), c) adding to said blended mixture of b), an oil (Sanchez-para [0098]-coconut oil), an optional salt, an optional starch, an optional preservative, and an optional coloring agent and blending (Sanchez-para [0098]-combined); d) heating said mixture of c) to a temperature of 60C to 100C (Sanchez-para [0098]-90C); and e) adding to the heated mixture of d), one or more optional sugars and one or more flavorings to produce said soy-based cheese. Although Sanchez doesn't explicitly teach adding b), a low methylester, amidated pectin, it does teach a pectin can be added as a hydrocolloid (Sanchez-para [0065]). Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, In1-5). It would have been obvious to one skilled in the art to add a low methyl ester, amidated pectin to the mixture because it offers a very high product safety.

As per claim 42, Matsuura teaches the method of claim 41, further comprising forming said soy-based cheese in a mold (Matsuura-col4, In3).

As per claim 43, Matsuura teaches the method of claim 41 or claim 42, wherein said microbial strain is a bacterial strain (Matsuura-col3, In35-40).

As per claim 44, Matsuura teaches a method of making a soy-based cheese (Matsuura-abstract), said method comprising: a) incubating (Matsuura-col3, In46-47) a mixture of (i) soy milk (Matsuura-col3, In26), (ii) an optional sugar (Matsuura-col3, In29) and/or optional one or more proteins, and (iii) a microbial strain (Matsuura-col3, In35) or an acid for a period of time sufficient to reach a pH of 5.4 to 5.6 (Matsuura-col3, In46-48 and col5, In53-54), c) separating the curd and whey (Matsuura-col3, In53-67) in said heated mixture of b) and optionally combining the curd:whey to obtain a ratio of 35 :65 to 100:0 curd:whey; Although Matsuura doesn't teach b) heating said mixture to a temperature between 70C and 90C d) adding to said curd:whey of c), a low methylester, amidated pectin, an oil, an optional salt, an optional starch, an optional sugar, an optional coloring agent, and optional preservative; e) cooking said mixture of d) to a temperature between 80C and 100C with blending; f) adding to the cooked mixture of d), one or more flavorings; and g) blending said cooked mixture off) to obtain said soy-based cheese, Sanchez teaches b) heating said mixture to a temperature between 70C and 90C (Sanchez-para [0098]-75C) d) adding to said curd:whey of c) a hydrocolloid (Sanchez-para [0065] and [0098]), an oil (Sanchez-para [0098]), an optional salt, an optional starch, an optional sugar, an optional coloring agent, and optional preservative; e) cooking said mixture of d) to a temperature between 80C and 100C with blending (Sanchez-para [0098]-90C); f) adding to the cooked mixture of d), one or more flavorings (Sanchez-para [0098]-cheese flavorant); and g) blending said cooked mixture off) to obtain said soy-based cheese (Sanchez-para [0098]). Although Sanchez doesn't teaches the hydrocolloid is a low methylester, amidated pectin, it doesn't teach a pectin can be used as a hydrocolloid (Sanchez-para [0065]) and Herbstreith teaches a low methyl ester, amidated pectin can offer a very high product safety (Herbstreith -pg1, col1, In1-5). It would have been obvious to one skilled in the art to add a low methyl ester, amidated pectin to the mixture because it offers a very high product safety.

As per claim 45, Matsuura teaches the method of claim 44, further comprising forming said soy-based cheese in a mold (Matsuura-col4, In3).

As per claim 46, Matsuura teaches the method of claim 44 or claim 45, wherein said microbial strain is a bacterial strain (Matsuura-col3, In35-40).

Claims 1-7, and 41-54 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

SEARCH HISTORY

Application Number	PCT/US 15/17147
Search Conducted By	QW
Search Approved By	SV

US/IPC Classifications Searched	IPC (8)- A23C19/00 (2015.01) CPC - A23C19/082; A23C19/093; A23C20/00
Date Conducted	April 14-17, 2015

Documentation Searched	CPC -A23C19/082; A23C19/093; A23C20/00
Search Terms Used	Soy, bean, milk, cheese, microbial, yeast, mold, strain, bacterial, micro, acid, amidated pectin, oil, sugar, starch, Lactococcus lactis lac tis (LLL), Lactococcus lactis cremoris (LLC), Lactococcus lactis biovar diacetylactis (LLBD), Staphylococcus xylosus (SX), Streptococcus thermophilus (ST)
Date Conducted	April 14-17, 2015

Electronic Database Searched	Patbase
Files Searched	Patents: US, WIPO/PCT, EP, JP, CN, IN, KR, GB, FR, DE, CA, BR, IE, HU, GE, FI, IL, HK, MY, PH, AT, BA, BE, BG, CH, CS, CZ, DK, EE, ES, GR, HR, HU, IE, EA, GC, ID, IN, KZ, MN, CR, CU, DO, AR, CL, AU, AP, DZ, EG, KE),
Date Conducted	April 14-17, 2015
Search Logic:	

- 1) ipc=A23C19/00 (6067)
- 2) uc=426/582 (1094)
- 3) cpc=A23C19/082 (511)
- 4) cpc= A23C19/093 (227)
- 5) cpc=A23C20/00 (208)
- 6) 1 or 2 or 3 or 4 or 5 (6668)
- 7) PA=("IMPOSSIBLE" OR "IMPOSSIBLE_FOODS_INC" OR "IMPOSSIBLE_INC" OR "IMPOSSIBLE_OBJECTS_LLC" OR "IMPOSSIBLE_S_R_O") (16)
- 8) 6 and (soy or soybean or saya*) (835)
- 9) 8 and cheese (794)
- 10) 9 and (bacterial or yeast or mold) (428)

- 11) 10 and ((Lactococcus lactis lactis) or LLL or (Lactococcus lactis cremoris) or LLC or (Lactococcus lactis biovar diacetylactis) or LLBD or (Staphylococcus xylosus) or SX or (Streptococcus thermophilus) or ST) (153)
- 12) 11 and (Amidated pectin) (0)
- 13) 11 and (oil or fat) (144)
- 14) 13 not 7 (143)
- 15) 6 and ((soy or soybean or saya*) W10 cheese) (194)
- 16) 15 and (Amidated pectin) (0)
- 17) 15 and (microbial or yeast or mold or strain or bacterial or micro*) (146)
- 18) 17 and (oil or fat) (138)
- 19) 18 and pectin (24)
- 20) PN=WO2013/010037 (1)
- 21) ctf 20 or ctb 20 (10)
- 22) ((soy or soybean or saya*) W10 cheese) (1584)
- 23) 22 and (microbial or yeast or mold or strain or bacterial or micro*) (1335)
- 24) 23 and (Amidated pectin) (2)
- 25) 22 and acid (1343)
- 26) 25 and (Amidated pectin) (2)
- 27) 26 not 24 (0)
- 28) 23 and pectin (157)
- 29) 28 not 19 (133)
- 30) 29 not 11 (132)
- 31) 25 and pectin (164)
- 32) 31 not 28 (9)
- 33) PN=WO13152406 (1)
- 34) (Staphylococcus xylosus) and (Streptococcus thermophilus) (164)
- 35) 34 and 22 (2)
- 36) (soy or soybean or saya*) and 34 (85)
- 37) 36 and cheese (35)
- 38) PN=WO14110540 (1)
- 39) ctf 38 or ctb 38 (6)
- 40) PN=WO2013/010037 (1)
- 41) ctf 40 or ctb 40 (10)
- 42) PN=US5597594 (1)
- 43) ctf 42 or ctb 42 (14)

Electronic Database Searched	PubWest
Files Searched	(US Pat, PgPub, EPO, JPO: class, keyword)
Date Conducted	April 15, 2015

DATE: Wednesday, April 15, 2015 [Purge Queries](#) [Printable Copy](#) [Create Case](#)

Order	<u>Set</u> <u>Name</u>	<u>Query</u>	<u>Hit</u> <u>Count</u>	<u>Set</u> <u>Name</u> Result Set	<u>Set</u> <u>Name</u> Grid	<u>Set Name</u> Classification
By	Side by Side					
DB=PGPB,USPT,USOC,EPAB,JPAB,DWPL,TDBD,FPRS; PLUR=YES; OP=OR						
Date	<u>L28</u>	L27 not L14	35	<u>L28</u>	<u>L28</u>	<u>L28</u>
Date	<u>L27</u>	L26 not L17	38	<u>L27</u>	<u>L27</u>	<u>L27</u>

Date	<u>L26</u>	L24 and pectin	38	<u>L26</u>	<u>L26</u>	<u>L26</u>
Date	<u>L25</u>	L24 and (Amidated ADJ1 pectin)	1	<u>L25</u>	<u>L25</u>	<u>L25</u>
Date	<u>L24</u>	L18 and acid	951	<u>L24</u>	<u>L24</u>	<u>L24</u>
Date	<u>L23</u>	L22 not L14	7	<u>L23</u>	<u>L23</u>	<u>L23</u>
Date	<u>L22</u>	L21 not 17	8	<u>L22</u>	<u>L22</u>	<u>L22</u>
Date	<u>L21</u>	L19 and pectin	30	<u>L21</u>	<u>L21</u>	<u>L21</u>
Date	<u>L20</u>	L19 and (Amidated ADJ1 pectin)	1	<u>L20</u>	<u>L20</u>	<u>L20</u>
Date	<u>L19</u>	L18 and (yeast or mold or strain or bacterial or micro\$)	880	<u>L19</u>	<u>L19</u>	<u>L19</u>
Date	<u>L18</u>	(soy or soybean or saya\$ or bean) near cheese	1212	<u>L18</u>	<u>L18</u>	<u>L18</u>
Date	<u>L17</u>	L16 not L14	28	<u>L17</u>	<u>L17</u>	<u>L17</u>
Date	<u>L16</u>	L15 and pectin	215	<u>L16</u>	<u>L16</u>	<u>L16</u>
Date	<u>L15</u>	L10 and acid	813	<u>L15</u>	<u>L15</u>	<u>L15</u>
Date	<u>L14</u>	L13 not L7	192	<u>L14</u>	<u>L14</u>	<u>L14</u>
Date	<u>L13</u>	L12 and pectin	192	<u>L13</u>	<u>L13</u>	<u>L13</u>
Date	<u>L12</u>	L10 and (yeast or mold or strain or bacterial or micro\$)	661	<u>L12</u>	<u>L12</u>	<u>L12</u>
Date	<u>L11</u>	L10 and (Amidated ADJ1 pectin)	0	<u>L11</u>	<u>L11</u>	<u>L11</u>
Date	<u>L10</u>	L9 and cheese	950	<u>L10</u>	<u>L10</u>	<u>L10</u>
Date	<u>L9</u>	L6 and (soy or soybean or saya\$ or bean)	1056	<u>L9</u>	<u>L9</u>	<u>L9</u>
Date	<u>L8</u>	L7 and L6	0	<u>L8</u>	<u>L8</u>	<u>L8</u>
Date	<u>L7</u>	IMPOSSIBLE\$.as.	14	<u>L7</u>	<u>L7</u>	<u>L7</u>
Date	<u>L6</u>	L5 or L4 or L3 or L2 or L1	7311	<u>L6</u>	<u>L6</u>	<u>L6</u>
Date	<u>L5</u>	A23C20/00.cpc.	1188	<u>L5</u>	<u>L5</u>	<u>L5</u>
Date	<u>L4</u>	A23C19/093.cpc.	1146	<u>L4</u>	<u>L4</u>	<u>L4</u>
Date	<u>L3</u>	A23C19/082.cpc.	2349	<u>L3</u>	<u>L3</u>	<u>L3</u>
Date	<u>L2</u>	426/582.ccls.	2167	<u>L2</u>	<u>L2</u>	<u>L2</u>
Date	<u>L1</u>	A23C19/00.ipc.	1976	<u>L1</u>	<u>L1</u>	<u>L1</u>

END OF SEARCH HISTORY

Electronic Database Searched	ProQuest Dialog
Files Searched	ABI/INFORM® Professional Market Research, ABI/INFORM® Professional Standard, AGRICOLA, AGRIS, Allied & Complementary Medicine™, Analytical Abstracts, British Library Inside Conferences, British Nursing Index, Business & Industry, Business Monitor International, Chemical Business Newsbase, Chemical Engineering & Biotechnology Abstracts, Current Contents® Search, Dialog Global Reporter, Economist Intelligence Unit, Ei EnCompassLIT, Emerging Markets Direct, Energy Science and Technology, ESPICOM Pharmaceutical & Medical Device News, FDAnews, FLUIDEX (Fluid Engineering Abstracts), Gale Group Computer Database™, Gale Group

	Health Periodicals Database, Gale Group New Product Announcements / Plus®, Gale Group Newsletter Database™, Gale Group PharmaBiomed Business Journals, Gale Group PROMT®, Gale Group Trade & Industry Database™, GEOBASE, HSELINE: Health and Safety, ICONDA - International Construction Database, Inspec®, Jane's Defense & Aerospace News, Lancet Titles, Material Safety Datasheets -OHS™, MEDLINE®, New England Journal of Medicine, Paperbase, PAPERCHEM, PASCAL, PIRABASE, ProQuest Biological & Health Science Professional, ProQuest Environmental Science Professional, ProQuest Newsstand Professional, ProQuest Research, ProQuest Technology Research Professional, Registry of Toxic Effects of Chemical Substances (RTECS®), Toxfile®, Transport Research International Documentation, TULSA™ (Petroleum Abstracts), UBM Computer Full Text, Weldasearch®, World News
Date Conducted	April 16, 2015
Search Logic:	

Set#: S1
Searched for: (soy or soybean or saya?)
Results: 1589013*
Set#: S2
Searched for: S1 and cheese
Results: 104284*
Set#: S3
Searched for: (yeast or mold or strain or bacterial or micro?)
Results: 12399525*
Set#: S4
Searched for: S2 and S3
Results: 13391*
Set#: S5
Searched for: S4 and pectin
Results: 304°
Set#: S6
Searched for: S5 and oil
Results: 252°
Set#: S7
Searched for: S6 and starch
Results: 160°
Set#: S8
Searched for: S6 and sugar
Results: 202°

Electronic Database Searched	Google web
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Files Searched	Google Web
Date Conducted	April 16, 2015
Search Logic:	

- 1). soy milk cheese bacterial
About 304,000 results
- 2). soy milk cheese bacterial pectin
About 551,000 results
- 3). soy milk cheese bacterial low methyl ester, amidated pectin
About 1,160 results
- 4). soy milk cheese acid oil low methyl ester, amidated pectin
About 1,310 results

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To: Monica McCormick Graham
Fish & Richardson P.C.
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INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

(PCT Article 17(3)(a) and Rules 40.1 and 40.2(e))

Date of mailing (day/month/year)	10 APR 2014
Applicant's or agent's file reference 387670010WO1	PAYMENT DUE within ONE MONTH from the above date of mailing
International application No. PCT/US14/11361	International filing date (day/month/year) 13 January 2014 (13.01.2014)
Applicant MARAXI, INC.	

1. This International Searching Authority

- (i) considers that there are 12+ (number of) inventions claimed in the international application covered by the claims indicated below/on an extra sheet:

-***-Please See Below-***-

- (ii) therefore considers that the international application does not comply with the requirement of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below/on an extra sheet:

-***-Please See Below-***-

- (iii) ☐ has carried out a partial international search (see Annex) ☒ will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

Group I: Claims 1-9, 17-22, 52-54

- (iv) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid.

2. Consequently, the applicant is hereby invited to pay, within the time limit indicated above, additional fees in the amount indicated below:

\$2,080.00 x 12+ = \$24,960.00+ (See Item 2 of annex)
Fee per additional invention number of additional inventions total amount of additional fees/currency

3. The applicant is informed that, according to Rule 40.2(c), the payment of any additional fees may be made under protest, that is, a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fees is excessive, where applicable, subject to the payment of a protest fee.

Where the applicant pays additional fees under protest, the applicant is hereby invited, within the time limit indicated above, to pay a protest fee (Rule 40.2(e)) in the amount of (amount/currency)

Where the applicant has not, within the time limit indicated above, paid the required protest fee, the protest will be considered not to have been made and the International Searching Authority will so declare.

4. ☒ Claim(s) Nos. -***-Please See Supplemental Page-***- have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300
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Form PCT/ISA/206 (April 2005)

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Item 2 (continued). For International Applications filed on or after 01 January 2014, Applicant is reminded that the search fee per additional invention indicated in item 2 is the undiscounted fee per additional invention. An Applicant may pay the search fee per additional invention fee reduced by 50% (small entity assertion) or 75% (micro entity certification), as appropriate. See 37 CFR 1.27 and 1.29.

****-Continued from 4.: Claim(s) Nos. 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90 have been found to be unsearchable under Article 17(2)(b) because defects under Article 17(2)(a) and thereof have not been included with any invention.

-Continued from 1. (i):

Group I: Claims 1-9, 17-22, 52-54; Group II: Claims 28-31, 37-39, 68-70, 81-83; Group III: Claims 32-36, 61-65; Group IV: Claims 47-49; Group V: Claim 51; Groups VI+: Claims 59, 60, SEQ ID NO: 1 (Vigna radiata amino acid sequence); Groups VII+: Claims 71-73, SEQ ID NO: 1 (vigna radiata amino acid sequence); Group VIII: Claims 74-78; Group IX: Claims 91-93; Group X: Claim 94; Group XI: Claims 95-99; Group XII: Claims 100-104

-Continued from 1. (ii):

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-9, 17-22 and 52-54 are directed toward a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, and a method of making said consumable product.

Group II: Claims 28-31, 37-39, 68-70 and 81-83 are directed toward a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins, said adipose tissue replica including one or more isolated plant proteins, one or more plant or algal derived oils, optionally a phospholipid, and a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica; and wherein the connective tissue replica comprises proteins assembled into fibrous structures by a solution spinning process.

Group III: Claims 32-36 and 61-65 are directed toward a consumable product comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, and a method of making said consumable product.

Group IV: Claims 47-49 are directed toward a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

Group V: Claim 51 is directed toward a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

Groups VI+: Claims 59, 60 and SEQ ID NO: 1 are directed toward a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. (SEQ ID NO: 1-27). + type Markush group.

The method for extending the shelf-life of a meat-free consumable product comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions can be searched to the extent that it encompasses a sequence at least 70% identical to SEQ ID NO: 1 (Vigna radiata amino acid sequence). Additional sequences will be searched upon the payment of additional fees. It is believed that Claims 59 and 60 (in-part) encompass this first named invention and thus these claims can be searched, upon appropriate payment, to the extent that they encompass SEQ ID NO: 1 (Vigna radiata amino acid sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group (s) may result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (Methylococcus thermophilus amino acid sequence).

Groups VII+: Claims 71-73 and SEQ ID NO: 1 are directed toward a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Group VIII: Claims 74-78 are directed toward a method of making a coacervate, said method comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and b) isolating said coacervate from said solution.

Group IX: Claims 91-93 are directed toward methods of minimizing undesirable odors in a composition comprising plant proteins.

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Group X: Claim 94 is directed toward a chocolate flavored spread comprising: a) sugar; b) a chocolate flavoring; and c) a cream fraction from a plant based milk.

Group XI: Claims 95-99 are directed toward a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

Group XII: Claims 100-104 are directed toward tissue replicas comprising a freeze-aligned non-animal protein.

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, not present in any other groups; the special technical features of Group II including a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, or a coacervate, which are not present in any other group; the special technical features of Group III including a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, which are not present in any other Group; the special technical features of Group IV including a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, not present in any other group; the special technical features of Group V including a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, which are not present in any other group; the special technical features of Groups VI+ including a method for extending the shelf-life of a meat-free consumable product, which are not present in any other group; the special technical features of Groups VII+ including a method for imparting a beef like flavor to a consumable product, which are not present in any other group; the special technical features of Group VIII including a method of making a coacervate comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, which are not present in any other group; the special technical features of Group IX including methods of minimizing undesirable odors in a composition comprising plant proteins, which are present in any other group; the special technical features of Group X including a chocolate flavored spread, which are not present in any other Group; the special technical features of Group XI including a method for altering the texture of a consumable, which not present in any other group; the special technical features of Group XII including a freeze-aligned non-animal protein.

Groups I-XII share the technical features including consumables. Groups I, II, V, VIII, IX, X and XI share the technical features including plants. Groups I, II, VIII, IX and XI share the technical features including plant proteins. Groups I-V, VI+, VII+, VIII, IX, XI and XII share the technical features including proteins. Groups I, III, V, VII+, XI and XII share the technical features including temperature. Groups I and VIII share the technical features including pH. Groups I, III and VIII share the technical features including sodium chloride. Groups I-III, VI+, VII+, VIII and XI share the technical features including a method of making a consumable product. Groups II, III and XII share the technical features including a meat replica. Groups II and VIII share the technical features including a coacervate. Groups II and V share the technical features including plant or algal derived oils or lipids. Groups II and V share the technical features comprising an emulsion. Groups III and V share the technical features including a gel. Groups IV, VI+ and VII+ share the technical features including a heme-containing protein. Groups IV and VI+ share the technical features including meat-free products. Groups VI+ and VII+ share the technical features including a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27.

Groups VI+ share the technical features including a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

Groups VII+ share the technical features including a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

However, these shared technical features are previously disclosed by WO 2001/015714 A1 (ZLOTKIN) in view of US 2010/0074998 A1 to Espeleta-Vega et al. (hereinafter 'Espeleta-Vega'), US 2011/0083681 A1 to Sengupta, et al. (hereinafter 'Sengupta'), the publication entitled 'Comparative Analysis Of Autoxidation Of Haemoglobin' by Jensen (hereinafter 'Jensen'), US 4,986,998 A to Yoo, et al. (hereinafter 'Yoo') and GenBank Accession AFK42304 by Krishnakumar, et al. (hereinafter 'Krishnakumar').

Zlotkin discloses consumables (compositions including micronutrients (consumables); abstract) including plants (chicory fructooligosaccharide (plants); page 11, lines 15-19); proteins (proteins; page 15, lines 20-28); temperature (temperature; page 17, lines 10-25); a method of making a consumable product (a process for producing a composition comprising micronutrients (a method of making a consumable product); page 8, lines 22-27); plant or algal derived oils or lipids (soybean oil (plant or algal derived oils or lipids); page 15, lines 1-4); meat-free products (cereals; purees of fruit, vegetables, milk, yogurt, cottage cheese (meat-free products); page 18, lines 1-11), and a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto (a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto; page 14, lines 21-28).

Zlotkin does not disclose plant proteins; pH, sodium chloride; a meat replica; a coacervate; an emulsion; a gel; a heme-containing protein; a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27; a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions; and a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

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Espeleta-Vega discloses plant proteins (vegetable protein (plant proteins); abstract), pH (a pH modifier; paragraph [0010]), sodium chloride (salt (sodium chloride); paragraph [0038]), a meat replica (substitute meat food product (a meat replica); abstract); an emulsion (an emulsion; paragraph [0011]); a gel (a gel; paragraph [0011]).

Sengupta discloses a process for preparing coacervate gel particles comprising plant materials including proteins (a process for preparing coacervate gel particles comprising plant materials including proteins; paragraph [0015]).

Jensen discloses a heme-containing protein (hemoglobin; abstract) wherein the oxidation of hemoglobin and myoglobin is significantly slower than free heme (oxidation of hemoglobin and myoglobin is significantly slower than free heme; page 2029, column 1, paragraph 2), and wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin (wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin; page 2030, column 1, paragraph 1).

Yoo discloses a method for imparting (flavoring (imparting); abstract) a beef (cow (beef); column 4, lines 26-32) like flavor to a consumable product (flavor to a consumable product; abstract, column 2, lines 49-50), comprising adding to said consumable composition a heme-containing protein (comprising adding to said consumable a powdered blood product (comprising adding to said consumable composition a heme-containing protein); abstract), wherein after cooking, a beef-like flavor is imparted to said consumable (providing a meat-like flavor (beef-like flavor is imparted to said consumable); column 3, lines 1-6).

Krishnakumar discloses a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1 (a globin (a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1); page 1).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin by combining the consumable disclosed by Zlotkin with a meat replica, such as that disclosed by Espeleta-Vega, for providing the micronutrients in the consumable in a form acceptable to a consumer, such as in a meat replica, for enhancing the nutrient content of a meat replica, particularly the iron content of said replica, providing the previous disclosure of Zlotkin. Also, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables with the coacervate preparation process of Sengupta, for enabling the preparation of particulates comprising the micronutrients disclosed by Zlotkin using a plant polysaccharide, such as pectin, as disclosed by Sengupta, for providing micronutrients in a consumable and easily digestible form to enable a consumer to absorb the nutrients. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin, for including the use of hemoglobin in preference to free iron or other forms of iron, in order to provide the iron to the consumer in a biologically useful form and oxidation state. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have therefore modified the disclosure of Zlotkin, for providing a method for extending the shelf-life of a meat-free consumable product, such as the consumables disclosed by Zlotkin, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions, such as adding hemoglobin, based on the disclosure of Jensen that tetrameric hemoglobin oxidizes more slowly than other forms of heme-containing proteins. Also, it would have been obvious to a person of ordinary skill in the art to have modified the disclosure of Zlotkin, for including a meat-like flavoring including an iron-containing protein based on the disclosure of Yoo, in order to provide a beef-like flavor to a consumable food, including a poultry or fish composition, to provide a beef-flavored product not containing beef, but with lower fat, such as a consumable comprising a plant protein, fish, or poultry protein. Finally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables comprising iron, for including a plant-based iron-comprising proteins, such as the globin disclosed by Krishnakumar, in order to provide iron from non-animal sources having high absorbability and utilization by a consumer.

Since none of the special technical features of the Groups I-XII inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zlotkin, Espeleta-Vega, Sengupta, Jensen, Yoo and Krishnakumar references, unity of invention is lacking.

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(81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))

(54) **Title:** SOY/MILK CHEESE-TYPE AND YOGHURT-TYPE PRODUCTS AND METHOD OF MAKING

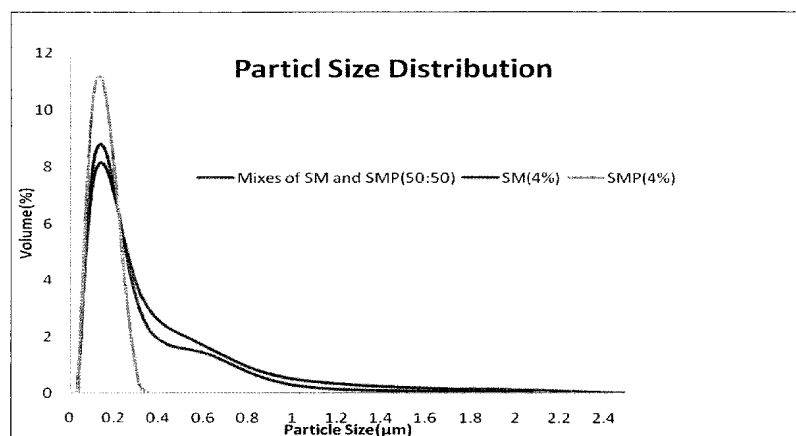


Figure 1

(57) **Abstract:** Novel soy/milk gels are provided useful for making cheese-type and yoghurt type products. Method for preparing such products are also disclosed herein. In particular, the invention relates to a soy/milk cheese-type product which is a blend of soy milk and milk and to a method for the preparation thereof. In further aspects, is a soy/milk yoghurt-type product and a method for the preparation thereof.

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Soy/Milk Cheese-type and Yoghurt-type Products and Method of Making

Field of the Invention

The present invention relates to soy/milk gels useful for making soy/dairy products. In particular, the invention relates to a soy/milk cheese-type product which is a blend of soy milk and milk and also relates to a soy/milk yoghurt type product which is a blend of soy milk and milk, both such products made from a soy/milk gel. The invention also relates to methods for the preparation thereof.

Background of the Invention

Soy products are becoming increasingly accepted by consumers due to the health benefits associated with the consumption of soy protein. Soy ingredients are widely used in a number of food products as they have good processing functionality. The protein obtained from soybeans is easily digestible, and thus it is valuable as a substitute for animal protein. Soy products containing high proteins have been associated with cholesterol lowering abilities. Furthermore, soy contains isoflavones which have been shown to be beneficial in the prevention of osteoporosis and inhibition of spreading of various cancers. Soybeans incorporated into food products could substantially lower the cost, while delivering the benefit of increasing the nutritional value of the final food products.

In the past decades, many attempts have been made in the production of soy cheese using soy milk. Examples of apparatus and processes for the processing of soy and or the production of soy milk may be found in U.S. 5,183,681, U.S. 5,270,450, U.S. 4,906,482, U.S. 4,971,825, U.S. 4,971,810, U.S. 5,077,062 and U.S. 5,137,736 (the disclosures of which are incorporated herein by reference in their entirety). Examples of soy cheese processes are found in U.S. 6,495,187, U.S. 6,455,081, U.S. 6,413,569, U.S. 6,399,135, U.S. 6,383,531 and U.S. 6,254,900 (the disclosures of which are incorporated herein by reference in their entirety). However, in most cases the soy cheeses are not commercialized to any extent because it is difficult to obtain a soy cheese with body and texture comparable to that of natural cheese. There are large differences in the structure and processing functionality between soy proteins and milk proteins, and therefore it is not possible

to obtain similar textures with matrices composed with soy protein. In addition other functions of the cheese, for example, crumbliness, color or meltability are also not matched with tofu like products.

It is therefore desirable to develop a soy milk cheese product that overcomes at least some of the deficiencies of the prior art soy cheeses.

Summary of the Invention

Novel soy/milk gels are provided that are used to make soy/milk cheese type products. The gels are also suitable for use to make soy/milk yoghurt type products.

The present invention in an aspect provides a novel soy milk and milk cheese-type product (herein referred to as "soy/milk cheese-type product"). The soy milk and milk cheese-type product is a combination of soy milk and milk that has been simultaneously gelled (co-gelled). The soy milk and milk cheese-type product is a soy/milk curd that can be used for a variety of soft ripened cheese products, having good texture and body characteristics. The soy/milk cheese-type product has a favorable texture that is comparable to commercial regular cheese products, and has a synergy of health benefits associated with the presence of milk proteins, soy proteins and isoflavones.

The invention in further aspects provides a novel soy milk and milk yoghurt type product (herein referred to as "soy/milk yoghurt-type product"). The soy milk and milk yoghurt-type product is a combination of soy milk and milk that has been simultaneously gelled (co-gelled) and then syneresis (water released from the gel) is minimized such that the soy/milk gel retains a high water holding capacity to have a consistency of a yoghurt.

The invention also encompasses a novel method for making the soy/milk cheese-type product and the soy/milk yoghurt-type product of the present invention whereby acidification of soy protein and renneting of casein occurs simultaneously. These two gel forming processes as combined and occurring essentially simultaneously lead to the formation of a soy/milk gels used and then treated to make either a cheese-type product (soy/milk curd) with desirable properties or a yoghurt-type product with desirable properties.

According to an aspect of the present invention is a soy/milk cheese-type product.

According to another aspect of the present invention is a soy/milk cheese-type product having desired hardness, springiness, cohesiveness and chewiness similar to a milk-only soft cheese.

According to an aspect of the present invention is a soy/milk curd.

According to an aspect of the present invention is a soy/milk yoghurt-type product. Said soy/milk yoghurt-type product being similar in characteristics to dairy type yoghurts.

According to another aspect of the present invention is a soy/milk yoghurt-type product having desirable hardness and water holding capacity similar to a milk only yoghurt.

According to an aspect of the present invention is a soy/milk gelled slurry (gel).

In aspects of the invention, the novel soy/milk gelled slurry (gel) can be treated to provide a cheese-type or a yoghurt-type product.

According to yet another aspect of the present invention is a soy/milk cheese-type product comprising mixed gels of rennet coagulated casein micelles and acid coagulated soy protein.

According to still another aspect of the present invention is a soy/milk cheese-type product comprising gel structures of strands of interacting soy protein and milk protein.

According to yet another aspect of the present invention is a soy/milk yoghurt-type product comprising mixed gels of rennet coagulated casein micelles and acid coagulated soy protein.

According to still another aspect of the present invention is a soy/milk yoghurt-type product comprising gel structures of strands of interacting soy protein and milk protein.

According to another aspect of the present invention is a method for making a soy/milk cheese-type product, the method comprising:

- (a) mixing heated soy milk with cold renneted milk;
- (b) cooling and acidifying to cause simultaneous gelation of (a) ;
- (c) coagulating cooled (b) and setting;
- (d) incubating (c) overnight; and
- (e) dry salting (d) to form said cheese-type product.

Alternatively, direct acidification of the hot soya milk may be effected. In this case, citric acid, lactic acid or glucono-delta-lactone (GdL) is added to the warm soy milk/milk mixture first.

In aspects, the method further comprises allowing the dry salted cheese-type product to dry refrigerated and then immersing in a brine solution.

According to another aspect of the invention is a method for making a soy/milk cheese-type product, the method comprising:

- (a) mixing heated soy milk with cold renneted milk;
- (b) acidifying and cooling (a) to effect simultaneous gelation of the soy milk and the milk;
- (c) coagulating cooled (b) and setting;
- (d) allowing (c) to incubate overnight; and
- (e) dry salting (d) to form said cheese-type product.

In aspects of the method, the acidifying comprises adding lactic acid, citric acid, or GdL to (a). In further aspects, the acidifying comprises adding lactic acid-producing bacteria to the cold renneted milk prior to mixing with the heated soy milk.

According to another aspect of the present invention is a soy milk and milk cheese-type product made by a method comprising:

- (a) mixing heated soy milk with cold renneted milk that comprises an acidifying agent;
- (b) cooling to effect gelation of (a);
- (c) coagulating cooled (b) and setting;
- (d) allowing (c) to incubate overnight; and
- (e) dry salting (d) to form said cheese-type product.

According to another aspect of the invention is a soy/milk cheese-type product prepared by a method comprising:

- (a) mixing hot soy milk with cold renneted milk;
- (b) acidifying and adding a firming agent to said mixture from (a);
- (c) mixing the product resulting from (b) for a time sufficient to cool (b) and effect simultaneous gelation of soy milk and milk;
- (d) transfer the product resulting from (c) into molds at a temperature of less than about 35°C; and
- (e) optionally salting (d).

In aspects, the acidifying agent is a lactic bacterial culture. As the cold renneted milk heats when mixed with the hot soy milk, culturing of the milk takes place as the bacteria then grow and multiply and the soy milk gels simultaneously with the renneted milk.

According to another aspect of the present invention is a method for making a soy milk and milk cheese-type product, the method comprising;

- adding lactic culture to renneted milk and storing refrigerated overnight without coagulation;
- adding hot soy milk at about 80-85°C and mixing during which time the temperature of the mixture cools to about 38-40°C;
- slowly adding a gelation agent and stirring to allow the mixture to coagulate and form a curd;
- incubating the curd overnight and salting; and
- optionally immersing the salted curd in a brine solution.

In aspects of the method, when a soy/milk gel is formed, the soy/milk gel can be treated such that the extent of syneresis is minimized so that there is a high water holding capacity. In this manner, the soy/milk gel with a high water holding capacity has a texture more in keeping with a milk yoghurt product.

According to a further aspect of the invention is a soy/dairy yoghurt- type product comprising mixed gels of rennet coagulated casein micelles and acid coagulated soy protein.

According to another aspect of the invention is a method for making a soy/milk gel, the method comprising:

- (a) mixing heated soy milk with cold renneted milk;
- (b) acidifying and cooling (a) to effect simultaneous gelation of the soy milk and the milk; and
- (c) optionally fermenting (b).

In further aspects, the method further comprises the step of decreasing syneresis to increase water holding capacity of said gel. The invention encompasses a soy/milk yoghurt product made such method.

According to yet a further aspect of the invention is a soy/milk cheese-type product or a soy/milk yoghurt-type product, said products comprising mixed gels of rennet coagulated casein micelles and acid coagulated soy protein.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from said detailed description.

Brief Description of the Drawings

The present invention will become more fully understood from the detailed description given herein and from the accompanying drawings, which are given by way of illustration only and do not limit the intended scope of the invention.

Figure 1 shows the particle size distribution of soy milk (SM), skim milk (SMP) and a 50:50 mixture of SM and SMP determined by light scattering;

Figure 2 shows acidification as a function of time for gels made with T1, T2, T5, T6, and the mixes of SM/SMP (50/50) with rennet or GDL without CaCl_2 . Treatment codes are defined in Table 1;

Figure 3 shows averaged storage modulus (G') as a function of pH for gels made with T1, T3, T5, T6, and the mixes of SM/SMP (50/50) with rennet or GDL without CaCl_2 . Treatment codes are defined in Table 1;

Figure 4 shows the average measurement of storage modulus (G') as a function of time for gels made with T1, T2, T5, T6 and the mixes of SM/SMP (50/50) with rennet and CaCl_2 . Treatment codes are defined in Table 1;

Figure 5 shows confocal scanning micrographs of soy, milk and mixed soy/milk gels. T1 SMP, 2% protein, rennet; T2 SM, 2% protein, GDL; T3 SMP, 4% protein, GDL; T4 SM, 4% protein, GDL; T5, 50:50 SM:SMP, 4% protein, GDL, rennet; T6 50:50 SM:SMP, 4% protein, GDL. Bars represent 6 μM ;

Figure 6 shows syneresis of soy, milk and mixed soy/milk gels. Treatment codes are defined in Table 1;

Figure 7 shows the textural properties of the soy/milk cheese-type product of the invention;

Figure 8 shows SEM imaging of the soy/milk cheese-type product of the invention; and

Figures 9A-9B show various process steps in the making of the soy/milk cheese-type product.. Figure 9A shows mixture of co-gelled soy and milk; Figure 9B

shows the curd appearing separated from the whey after 30 minutes; Figure 9C shows curd poured into molds; Figure 9D shows curd incubated overnight at 30°C under gravity; Figure 9E shows soy/milk cheese-type product sample; Figure 9F shows the soy/milk cheese-type product salted; Figure 9G shows the salted product brined; and Figure 9H shows the soy/milk cheese-type product after two weeks.

Detailed Description of the Invention

Broadly stated, the invention is a novel soy/milk gel that can be further treated to provide novel and desirable soy/milk cheese-type products and novel and desirable soy/milk yoghurt-type products.

The present invention in an embodiment provides a novel and desirable soy/milk cheese-type product that is similar to conventional milk based soft ripened natural cheeses with respect to taste and physical characteristics and yet provides additional health benefits such as reduced cholesterol and containing beneficial isoflavones. The soy/milk cheese-like product is a curd that can be used as a basis for a variety of soft ripen cheese products. It is believed to be useful for the manufacture of cheeses that are designated as "Soft" according to the CODEX General Standard for Cheese (A6) Firmness Designators. These include, for example but are not limited to, feta, cream cheese, fresh fermented cheeses, and surface ripened varieties such as brie and camembert.

The invention also encompasses a novel method for making a soy/milk cheese-type product having similar physical characteristics to natural milk cheeses, but with novel taste profiles and a different protein composition. The novel method is based on creation of an interacting gel by virtue of having two gel forming processes (acidification of soy protein and renneting of casein) occurring simultaneously. This results in a novel soy/milk cheese-type product having rheological properties and desired gel microstructure and therefore being in the same product category as conventional type cheeses.

Generally, the method comprises forming a gel from mixtures of heated soy milk (SM) and cold reconstituted skimmed milk (SMP). This is accomplished by adding heated soy milk to cold renneted milk and acidifying. In one aspect of the method, hot soy milk is added to cold renneted milk to which an acidifying agent is added (renneted/acidified milk). Gelling via acidification will not take place until mixing with the hot soy milk. The cold renneted/acidified milk does not gel until the

hot soy milk is added thereto. In another aspect of the method, the acidification is done by adding an acidifying agent to the mixture of the hot milk and cold renneted milk. Still in another aspect, the acidification can occur by directly acidifying the hot soy milk via acid fermentation, by addition of calcium and/or magnesium, or by addition of citric, lactic acid or GdL.

Acidification with the use of lactic culture to the cold renneted milk as the acidifying agent occurs as the temperature of the mixture warms as the lactic culture will grow in warmer temperatures only, reaches to about 40°C or less. Mixing the hot soy milk with cold renneted/acidified (i.e. cultured) milk brings the mixture to a temperature that affects the gelation process of both the soy milk and the renneted acidified milk, causing both to occur simultaneously and to interact to form a mixed gelled network of the two proteins, and a gelled slurry. Culture then occurs with the warmer temperature of the gel. To aid in the gelation of the soy protein or the milk protein a firming agent can be utilized while stirring is maintained. This gelled slurry is then allowed to set to form a curd which is poured without breaking into a cheese mold and incubated overnight at suitable temperatures, depending on the starter culture used. The curd may then be pressed and/or dry salted by rubbing all sides of the curd surface with dry salt, to allow for some syneresis to occur while refrigerated. The curd is turned a few times. After a couple of days the salted curd may be immersed in a brine comprising salt and stored at suitable cool temperatures of about 4°C.

It is believed that gelation of both principal soy globulins, β -conglycinin and glycinin is induced by the addition of the cool renneted/cultured milk (but not yet coagulated) to the hot soy milk. During cooling, soy proteins rearrange mainly through non-covalent interactions, causing stiffening of the gel network. Gelation of heated soy milk can be effected by quiescent acidification via acid fermentation or addition of glucono-delta-lactone which slowly converts to gluconic acid when dissolved in an aqueous medium, or by addition of ions such as magnesium or calcium. The gelation of soy proteins occurs because of charge neutralization, and bridging by cations present in the serum phase. During acidification from neutral pH, coagulation begins at about pH 6.2. The principal milk proteins, caseins, can be coagulated by acidification to pH less than about 5.0 or at higher pH values (lower acidity) via enzymatic coagulation, namely, renneting. Soy-dairy mixed gels with differing functionalities can be created by combining coagulation (i.e. gelation) of soy

proteins in the pH range of about 5.2-6.4 and rennet coagulation of caseins. These gelation processes are made to occur simultaneously in the mixed gels and without being bound to theory, three gel structures are possible: (1) a gel structure formed predominately of soy proteins with coagulated caseins as inert (not interacting with the soy protein) particles within the soy gel matrix; (2) a gel structure formed predominately of renneted caseins with particles of acid coagulated soy proteins as inert particles within the milk gel matrix; or (3) a gel structure formed with interacting soy and dairy proteins.

In further embodiments of the invention, the soy/milk gel made from mixtures of heated soy milk (SM) and cold reconstituted skimmed milk (SMP) can be processed in a manner to provide a yoghurt-type product. In this aspect (see example one for a non-limiting example) the gel is not subjected to syneresis or minimally subjected to syneresis such that a high water holding capacity is provided. In this manner, novel soy dairy products can be made that are comparable to milk yoghurt products in texture, and yet have the benefits of soy.

The soy milk can be purchased or made from dry soybeans. The soy milk is typically filtered to eliminate fiber material. A soy protein extract as a base for the process may also be made from mixing soy flour (dehulled soy flour or defatted soy flour), soy powder, soybean flakes, soy concentrate, and/or soy protein isolate with water. In general, any source of soy protein can be used in the present invention as would be understood by one of skill in the art. The soy milk may have a concentration of up to about 10% protein and in aspects about 3-6% protein. If soybeans are used as starting material, then the soy milk should be heated to a high enough temperature and for a sufficient time to denature any anti-nutritional compounds (such as trypsin inhibitors). If soy protein suspensions are to be prepared, then heating is desired to solubilise the proteins from the powder. Suitable temperatures are about 60°C to about 95°C for up to about 10 minutes. One of skill in the art would recognize suitable temperatures and times. The heated soy milk is then prepared and ready to add to the milk.

The milk used in the present invention may comprise from about 0% to about 10% fat and in aspects from about 0.5% to about 5% fat. It may be desirable to use milk with a low fat content and optionally add fat from other sources. In the present invention skim milk is used in one aspect to reduce the fat content however, it is encompassed that higher fat milk including homogenized milk can be used as is

desired and understood by one of skill in the art. It is expected that the addition of fat to the mixture may improve the appearance, texture and mouth feel of the cheese curd. Any type of milk that could be used in cheese making can be used as understood by one of skill in the art. The milk used may be from different sources such as for example pasteurized cow's milk, buffalo milk or goat's milk (including mixtures thereof) that is kept refrigerated at about 4°C.

The milk is prepared for adding to the hot soy milk. To cold milk diluted rennet (125µl concentrated Chy-mx Extra rennet Milwaukee, WI U.S.A.) is added and then the mixture stored overnight for the rennet to go to completion. The amount of rennet for use is understood by one of skill in the art. In aspects, the concentration of rennet for use can be about 0.01-0.002IMC/g. In aspects the amount of rennet for use may be about 0.020IMC/g, sufficient for the renneting reaction to reach completion. Indirect acidification (i.e. culturing) of the cold renneted milk is performed microbially by the addition of a starter culture of one or more lactic acid-producing bacteria to the milk, and then allowing the bacteria to grow and multiply (culture) when the mixture is warmed by adding to the hot soy milk. Milk with lactic acid-producing bacteria added thereto is referred to as cultured milk herein. In one aspect of the present method a freeze-dried concentrated lactic cheese culture (Choozit MA 16 LYO, Danisco France) is used at a dosage of about 0.625DCU (0.005%) bacteria per 10kg/milk. One of skill in the art would recognize that a variety of bacteria can be added that impart a desired flavour and other desired character to the final product. For example, lactic acid cultures and suitable adjuncts such as probiotics and flavour enhancing cultures can be used. Probiotic cultures may be selected from the group consisting of *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Bifidobacterium* and combinations thereof. Once renneted and cultured (i.e. acidified), the milk is kept cold until ready for mixing with the hot soy milk. At this point the renneted and cultured cold milk has not coagulated. Alternatively, direct acidification may be used instead of lactic acid bacteria. In this case, citric acid, lactic acid or glucono-delta-lactone (GdL), for example, is added to the warm soy milk/milk mixture.

To effect simultaneous gelation, the cold renneted milk is mixed with the hot soy milk causing the temperature of the soy milk to quickly decrease and the temperature of the milk to quickly increase to less than about 40°C. At this temperature, the lactic acid bacteria are able to grow and thus act to acidify the

mixture. Alternatively, if lactic acid bacteria were not used, direct acidification may be done by adding citric acid, lactic acid, or GdL directly to the warm mixture.

To increase gelation of the soy protein, firming agents may optionally be added to firm the curd. Preferably, calcium chloride is added to help gel and form a curd and can be added in amounts of from about 0% to about 0.5%, in aspects from about 0% to about 0.2%. In aspects, $MgCl_2$ (0.25g/ml) is slowly added while stirring the gelling (coagulating is synonymous) slurry. After stirring, the coagulated mixture is left undisturbed to set for a sufficient time to form a curd and incubated at a certain temperature for sufficient time to develop the required acidity. The quiescent time for curd formation can be up to 60 minutes or so as understood by one of skill in the art. The gelled curd is poured into a mold and incubated at about 30°C or higher, overnight after which dry salting of the curd takes place. The temperature will be the optimal growth temperature for the starter culture employed. The dry salted curd can be partially dried for days and refrigerated. To maintain a soft ripened cheese-type product of soy and milk, the drying curd is not subject to pressure. To form a firmer cheese-type soy/milk product pressure can be applied to remove more moisture from the product as it dries. The salted cheese-type product is then optionally brined and stored.

The invention provides the above and below described methods wherein said soy protein is present in the final soy/milk cheese-type product in amounts of from about 10% to 60% by weight, and preferably from about 15% to about 50%. By "percent" it is meant weight percent based on the calculated amount of casein solids in the milk. Milk is present in amounts of from about 10% to about 90%.

Ingredients may be included in the slurry such as, but are not limited to, non-fat dry milk, a milk protein, an acidity regulator, an acid, an anticaking agent, an antifoaming agent, a coloring agent, an emulsifier, an enzyme preparation, a flavoring agent, a firming agent, a food protein, a gelling agent, a preservative, sequestrants, a stabilizer, a starch, a thickener, an oil, a fat, a cheese powder, a salt, a nutritional supplement, an acid, an enzyme, a nutraceutical, a carbohydrate, a vitamin, a mineral, soluble fiber sources. Examples may further include procream, whey cream, a dairy solid, and foodstuffs of vegetable, fruit and/or animal source. The foodstuffs may include fruit, vegetables, nuts, meat, and spices, among other foodstuffs.

Depending on the character desired in the end soy milk/milk cheese-type product, a sweetener or sweeteners may be added to the gelled curd (i.e. the slurry). Examples of suitable sweeteners include artificial and natural sweeteners such as saccharin, sucrose, fructose, glucose, corn syrup, maltose, honey, glycerin, fructose, aspartame, sucralose, high fructose corn syrup, crystallized fructose, acesulfame potassium, and mixtures thereof. The amount of sweetener used in the acidified compositions will vary depending on the desired taste and the perceived sweetness of the specific sweetener selected.

If desired, bulking agents may be added to the curd to enhance the textural properties. Suitable bulking agents include, but are not limited to, maltodextrin, corn syrup solids, dextrose, lactose, whey solids, and mixtures thereof.

Food starches can also be used in the manufacture of the soy milk/milk cheese-type product of the present invention to aid in water management. In general, starch can be incorporated into the final product in the range of about 0.5-4.0 wt. %.

Suitable starches include, for example, modified and unmodified food starches, corn starch (dent or waxy), rice starch, tapioca, wheat starch, flour, potato starch, native food starches having cross-linked polysaccharide backbones, and mixtures thereof.

A number of different types of generally recognized as safe (GRAS) ingredients can be incorporated into the slurry and optionally added at other stages of the overall manufacturing process as described herein. If added at a stage other than the slurry, most ingredients can generally be added as a powder, coating or as a dressing. The ingredients that are incorporated are selected, for example, to tailor the performance, nutritional, and taste characteristics of the final soft or firm/semi-hard cheese product. Different types of gums or stabilizers can also be incorporated into the cheese. The cellulose can be either natural or modified. One cellulose or combinations of different celluloses can be utilized. Types of celluloses that can be utilized include, but are not limited to, microcrystalline cellulose, powdered cellulose, methylcellulose, propylene glycol alginate, and sodium alginate. Examples of suitable gums that can be incorporated include, but are not limited to, xanthan gum, guar gum, konjac flour and locust bean gum. Examples of suitable stabilizers include chondrus extract (carrageenan), pectin, gelatin, and agar, or the soluble fraction of the soybean polysaccharides. The total amount of gums and stabilizers included in the final cheese product is typically up to about 0.01, about 0.50, or about 3.0% by

weight. More specifically, the amount of gums and/or stabilizers can range from about 0.01 to 3.0%, from about 0.25 to 2.5%, from about 0.5 to 2.0%, or about 0.75-1.5% by weight of the final cheese-type product. Gums and stabilizers concentrations in the slurry are typically in the range of about 0.02-6.0, or 0.50-5.0 wt. %.

Any colorants known in the art, including all certified colorants and natural colorants may be used in the soy milk/milk cheese-type product to impart a cheese color to the compositions. If the end product desired is to be a yellow/orange imitation cheese composition, the preferred colorants are Certified Yellow #5, Certified Yellow #6, annatto, carotenels, or oleoresin paprika. Additionally, it may be desirable to include titanium dioxide in the composition, to increase overall opacity. If desired, preservatives may be included in the acidified food composition to prevent discoloration or decay, and to further ensure avoidance of microbial or fungal spoilage, or other degradation of the composition's components. Such preservatives include, for example, sodium benzoate, potassium sorbate, sorbic acid and EDTA.

In addition to cheese flavorings discussed above, additional flavorings or flavor-enhancing additives may be included in the soy milk/milk cheese-type product, as long as such additions do not substantially alter the character of the product. Such flavorings may include, for example, spices, such as black pepper, white pepper, salt, paprika, garlic powder, onion powder, oregano, thyme, chives, basil, curry, Worcestershire sauce, soy sauce, mustard flower, yeast extracts, cumin and mixtures thereof. Additionally, particulate components such as fruit or vegetable matter, meat, tofu, or nuts may be added. Flavoring agents are typically added in an amount such that the concentration in the final cheese product is within the range of about 0.01 to 5 wt. %. If incorporated into the slurry, the concentration of the flavoring agent in the slurry is generally in the range of about 0.02 to 5 wt. %.

Salts of various types, but typically sodium chloride, can be added to tailor the flavor of the final cheese. The salt can be incorporated into the final cheese product by including it in the heated slurry or by adding it in granular form or as an unheated solution apart from the slurry. Regardless of how introduced, the salt concentration in the final cheese product is usually added at a level of about 0.1-5 wt. %. When added as an ingredient of the slurry, this means that the salt concentration in the slurry is generally about 0.0 to 25.0 wt. %, for example about 0.5-22%, or about 1-18% by wt.

Neutraceuticals may be included to deliver nutrients not normally present in cheese. Examples of neutraceuticals include, but are not limited to lycopene, antioxidants, probiotics, prebiotics, phosphatidylserine, vegetable sterols, immunoglobulins. These products in particular may be added as part of the slurry or to the final cheese-type product.

We note that the term “gel” and “slurry” are used interchangeably in this description.

Although preferred amounts of the various components of the soy milk/milk cheese-type product have been detailed herein, it will be apparent to one of skill in the art that the amounts of the components can be varied depending on the taste, texture, viscosity, color, and/or other organoleptic properties desired in the final soy/milk cheese-type product of the invention.

Similarly, the soy/milk yoghurt type product of the invention may be varied with respect to the amounts of the components depending on the desired taste, texture, viscosity, color, and/or other organoleptic properties desired in the final soy/milk yoghurt-type product of the invention. The soy/milk type product of the invention can also incorporate any or all of the other components as described herein for soy/milk cheese-type products such as one or more of nonfat dry milk, a milk protein, an acidity regulator, an acid, an anticaking agent, an antifoaming agent, a coloring agent, an emulsifier, an enzyme preparation, a flavoring agent, a firming agent, a food protein, a gelling agent, a preservative, sequestrants, a stabilizer, a starch, a thickener, an oil, a fat, flavour powders, a salt, a nutritional supplement, an acid, an enzyme, a neutraceutical, a carbohydrate, a vitamin, a mineral, soluble fiber, fruit, vegetables, nuts, meat, spices, and other foodstuffs.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Examples

Example One: Production and Characterization of Soy/Milk Gels

Table 1 summarizes the various treatments used in the study. The protein content of the soymilk (SM) and milk (from reconstituted skim milk powder, SMP) mixes was kept constant at 4%, and controls containing only 2% of skim milk proteins or soymilk proteins were also used. The mixes were aggregated by a combination of rennet and acidification (using GDL). CaCl_2 was also added to all the samples.

Table 1

Treatment	4%SM (ml)	4%SMP (ml)	Distilled Water (mL)	GDL (g)	Rennet (μ l)
T1	0	12.5	12.5	0	200
T2	12.5	0	12.5	0.25	0
T3	0	25	0	0	200
T4	25	0	0	0.25	0
T5	12.5	12.5	0	0.25	200
T6	12.5	12.5	0	0.25	0
T7	17.5	7.5	0	0.25	200
T8	22.5	2.5	0	0.25	200

Note:

4%SM is the soymilk(SM) containing 4% soy protein

4%SMP is the skim milk powder (SMP) solution containing 4% milk protein

GDL is Glucono delta-lactone at a concentration of 1.0% (w/v)

Rennet concentration was 0.001% (v/v).

All treatments included CaCl_2 at concentration in the final mix of 2mM.

Soymilk Preparation

Soymilk was prepared according to the procedure of Mullin et al. (2001) with slight modifications. Soybeans (Harovinton variety, grown at the Agriculture and Agri-Food Canada Harrow research station, Harrow ON) were weighed (100 g) and soaked in excess water overnight at room temperature, drained and rinsed with cold water and drained again, then reweighed to determine the amount of water absorbed by the beans. The water uptake was calculated by dividing the weight of the soaked beans by the initial weight of the dry beans. The amount of additional water needed to obtain a ratio of 18:1 water to protein was then calculated by subtracting the amount of absorbed water.

Approximately half of additional water needed was then added to the beans at 20°C and blended (commercial blender, WARING, New Hartford, CT) at high speed for 3 min. The remaining water was heated to 60°C and added to the slurry for better

protein extraction, and the whole mixture was blended at high speed for another 30 s. A two-step filtration was then carried out to remove the coarse material (okara, which is mainly composed of fiber material). The slurry was filtered through a juice extractor (Juiceman, professional series 211, Korea) and the okara was collected and passed through the juice extractor again. The soymilk obtained from the juice extractor was filtered through cheese cloth to remove fines (Mullin et al., 2001). A portion of raw soymilk was then divided in test tubes (10 ml each tube) and heated in boiling water (90-100°C) for 7 min, following a previously published procedure (Ono et al., 1991) and cooled to room temperature in an ice-water bath. The heated soymilk was then centrifuged at 10,000g for 30 min at 20 °C in a refrigerated ultracentrifuge (Optima LE-80K Beckman Coulter, CA, USA) to eliminate any additional large particles. The protein content of collected supernatants was measured using Dumas method (LECO, FP-528, Mississauga, ON, Canada) using EDTA as standard for calibration of the instrument. The protein concentration was calculated using 6.25 as a conversion factor.

Low heat skim milk powder, purchased from Dairy Farmers of America (Kansas City, MO), was used for the study. Skim milk powder was suspended (12% solid total solids w/v) in high-purity water containing 0.02% sodium azide (to avoid microbial growth), stirred for 2 h, and incubated overnight at 4°C to ensure complete hydration.

Particle Size Analysis: Mastersizer 2000 (Malvern Instruments Ltd, UK) was used to determine the protein particle sizes of 2% soymilk, 2% skim milk powder solution and the mixes of 2% soymilk and 2% skim milk powder solution. The values of refractive index used were 1.39 for skim milk particles, 1.46 for soymilk and 1.42 for the mixes of soymilk and skim milk powder solution.

Figure 1 illustrates the particle size distribution of various samples of soymilk (SM), reconstituted skim milk (SMP) and a 50:50 (v/v) mix. All samples showed a monomodal size distribution with most particles in the range between 0.05 µm and 0.3 µm. Soymilk showed a shoulder with particles of larger diameter and a similar particle size distribution was also noted for the 50:50 mixture.

Rheological Measurements: A controlled stress rheometer and Rheology Advanced Data Analysis software version 5.0.38, both from TA instruments Ltd., New Castle, Canada, were used to collect and analyze the experimental data. A conical concentric cylinder geometry (20 ml sample size, 5920 µm fixed gap, 15 mm

radius, 14 mm rotor outer radius and 42 mm cylinder immersed height) was used to measure time sweeps and frequency sweeps at a constant temperature of 30°C, controlled by a temperature-controlled water bath. A 20 mL sample was loaded into the rheometer.

A water trap was used to minimize evaporation during the measurement. Time sweep measurements were carried out with a constant maximum strain of 0.01% and a frequency of 0.1 Hz. After four hours, a frequency sweep test was performed applying a constant stress of 1 Pa in the frequency range from 10- 0.005 Hz.

Dynamic rheological measurements provide information on the viscoelastic nature of food materials. Dynamic rheological tests can be used to study the characteristics of the gel as well as of gelation (solution-gel) and melting (gel-solution transition). From dynamic rheological tests the elastic modulus (G'), the viscous modulus (G''), and $\tan \delta = (G'/G'')$ where δ is the phase angle) were obtained (Rao, 1992). Three types of dynamic tests can be conducted to obtain useful properties of gels and gel formation: a) Frequency sweep tests in which G' and G'' are determined as a function of frequency at fixed temperatures. (b) Temperature sweep tests in which G' and G'' are determined as a function of temperature at fixed ω . (3) Time sweep tests in which G' and G'' are determined as a function of time at fixed ω and temperature (Rao and Steffe, 1992). Figure 3 illustrates the time sweep test carried out on the mixture of soy protein and milk protein,

When soy protein and milk protein were combined together, after acidification the mixes showed a faster onset of gelation than control samples containing only casein micelles (Figure 3). Moreover, when rennet was added into the mixes, it greatly affected the G' value. Compare T5 to T6. This suggests that GdL was a prerequisite for onset of the gelation of the mixes (Figure 2), and that renneting is needed to increase gel firmness, and to make sure caseins contribute to the gel network.

Figure 4 depicts the development of the elastic modulus (G') at 30°C versus time for different mixtures treated by GdL and rennet as well as the corresponding control samples. The G' used in the discussion refers to the final G' achieved after 11000s of incubation, and gelation time was reported as the time at which $G' > 1$ Pa. The G' versus time curves of T5 is different from T6 and the others. That means gelation was affected by the presence of rennet. But the mixes of SM/SMP (50/50) did not gel during rennet in the absence of GdL, possibly because the presence of

soy protein disrupted the gelation of milk protein. Compared to T5, T6 and T2, T1 had less G' , which means that soy protein can form stronger gels than milk protein. Statistical analysis demonstrated that the final G' value of the SM/SMP mixtures with addition of rennet was significantly different from that of the mixtures without rennet. In particular, note that the soy-milk mixtures gelled with GdL+Rennet (T5) had a greater final G' (firmer gel) at 4000 s than the same mixture gelled with only GdL (Treatment 6). This suggests that renneted caseins are contributing to the structure of mixed gels more than non-renneted caseins. This was confirmed by confocal microscopy.

Confocal scanning laser microscopy: Confocal scanning laser microscopy (CSLM) measurements of soymilk, skim milk, and soymilk/skim milk mixture gels were performed on a Leica TCS SP Confocal Scanning Light Microscope (Leica, Heidelberg, Germany), in single photon mode, configured with an inverted microscope (model Leica DM IRBE), and using an Ar/Kr laser. Slices of approximately 0.2–0.5 cm thick were cut from the cylindrical shaped gels and the protein was labeled with two drops of a 0.1% aqueous solution of fluorescein isothiocyanate (FITC). The labeled slices were incubated at ambient temperature for 30 min to allow the dye to diffuse uniformly throughout the sample volume before direct microscopic observation. The light wavelength used to visualize the dye labeled sample was 488 nm. The emission maximum of the dye was 518 nm. The following Leica objective lenses were used: 20×/0.7NA/dry/HC PL APO, 63×/UV/1.25NA/water immersion/PL APO. In addition to 2D images, 3D image stacks were recorded and presented in 2D projections of the sample volume.

In a confocal laser scanning microscope (CLSM) a beam of light is focused on a small portion of the specimen, and a confocal point detector is used to recollect the signal from the sample. In-focus plane images are obtained and the out-of-focus regions appear as black background (Brakenhoff et al., 1988). It is possible to distinguish the spatial distribution of the components present in a sample by using fluorescent labeling dyes, such as FITC or rhodamine B for protein, Bodipy or Nile red for lipids, and conjugates of lectins for polysaccharides. Confocal microscopy has several advantages: it can work in two modes (fluorescence and reflectance), it has the ability to scan samples at different depths; it is possible to obtain 3-D images without damage to the sample, high resolution images can be obtained, and it does not require major sample preparation (Vodovotz, 1996). Confocal microscopy is

useful tool in the study of physical aggregation and phase separation, and to identify, albeit at times qualitatively effects of processing conditions and ingredient variation on the microstructure of gel with much less sample manipulation, at least compared to other conventional microscopy measurements (Vodovotz, 1996).

Confocal images in Figure 5 show that SM coagulated with GdL (T1) forms larger strands than rennet coagulated SMP (T2). Figure 5 also shows that increased protein concentration creates larger pores in soy gels formed with GdL and smaller pores in renneted skim milk proteins. Figure 5 clearly shows that soy-dairy mixtures gelled with rennet and GdL have a much finer network structure (smaller pores) than mixed gels formed with only GdL. The network formed by the mixes with rennet during acidification (see T5 in Figure 5) contained smaller pores, larger aggregates and denser clusters of protein than that of mixed gels without rennet during acidification (see T6 in Figure 5).

SEM images (not shown here) confirm that the protein strands forming the gel structure in mixed gels formed with both GdL and rennet are thicker and appear more dense than in mixed gels formed with only rennet, suggesting that renneted caseins and soy proteins are interacting to form mixed strands of protein, which further interact to form the gel structure. In other words, two distinct types of gels can be formed with soy-dairy protein blends with a final pH in the range of 5.2 – 5.4 (note this is the pH of many rennet coagulated cheese varieties). First, using only GdL a gel based mainly on acid coagulated soy proteins is formed. In this case, the caseins apparently contribute little to gel structure. Second, using both GdL and rennet a gel is formed where rennet destabilized caseins interact with acid destabilized soy proteins and actively participate in gel formation.

Syneresis tests on the formed gels: Mixes were incubated in water baths at 30°C for 1h, and then the gel formed was placed on a plastic net and drained onto a balance to measure the amount of whey released over time. The whole system was placed into an environmental chamber at 0~4°C in order to control evaporation. The water weight excluded from the gel measured by the balance was recorded.

Factors such as pH, ionic strength, protein concentration, and temperature history and time influence the microstructure of protein gels (Kinsella 1982; Offer and Trinick 1983; Schnepf 1989; Hermansson 1986 and 1994; Damodaran 1996). During gelation a 3-dimensional network stabilizes water physically and chemically within the gel structure. So, protein–protein interactions are necessary to form the gel and

stabilize water. However, stronger protein-protein interactions can also lead to thicker protein strands and larger pores in which the water is less firmly held and more easily pressed out. A more open structure with larger water pores has lower water holding capacity than a dense network structure (Stanley and Yada 1992). A gel will develop a dense network structure if protein-protein interaction is uniform throughout the gel network (Hermansson 1986, 1988; Niwa 1986). The extent of syneresis (water released from the gel) due to temperature changes or physical disruption of the gel can be predicted from the gel structure such as the size of water pores and the integrity of the gel network. Syneresis is desirable or undesirable depending on the further processing and the desired type of final product. For example, in a soy-dairy yoghurt type product, high water holding capacity is desirable. This would decrease the amount of stabilizers needed. However, if desired to make cheese like products, syneresis is desirable, as some whey needs to be released before the curd is manipulated.

Figure 7 shows syneresis of soy, milk and mixed soy/milk gels. Treatment codes are defined in Table 1. Mixes including coagulants were incubated at 30°C for 60 min. The resulting gels were placed on a plastic net at 4°C and the whey released was weighed at 10 min intervals for 3 hours. Note results are not normalized for protein concentration, so treatments T1 and T2 which contain only 2% protein are valid comparators for each other, but not for the other treatments which contain 4% protein. Note also that there is missing data for T8; however, the available data confirms the trend that illustrated by T5, T6, T7 and T8 that syneresis of mixed soy-dairy gels improves with increasing amounts of renneted casein.

These observations suggest that the mixed gels with both GdL and rennet may hold more water than mixed gels with GdL because they have smaller pore sizes and apparently stronger strands forming the gel matrix. Preliminary results suggest the opposite is true. Note that although soy gels are much firmer than rennet SMP gels, rennet gels had much greater syneresis. This is seen when comparing T2 versus T1 and T4 versus T3 in Figure 4. In mixed gels, renneted caseins improved the rate and extent of syneresis relative to mixed gels formed with GDL only. Higher amounts of soy protein relative to skim milk proteins in the mixes reduced the amount of syneresis (compare T5, T6, T7 and T8). Note that the syneresis versus time curve for the 50:50 soy-dairy mixture coagulated with GDL and rennet (T5, total

protein 4%) is almost identical to the syneresis curve for renneted SMP (T3, protein 4%).

Example Two: Preparation and Characterization of Soy/Milk Cheese-type Product (Curd)

Food grade soybeans were obtained from a local grocery store (30 kg bags). Pasteurized skim milk was obtained from a local supplier (Crown's Dairy, Agropur division) and stored in fridge ($\pm 4^{\circ}\text{C}$). Rennet (125 μl) concentrated Chy-max Extra rennet (Chr. Hansen. Inc., Milwaukee, WI, U.S.A) was diluted in 10 ml ultrapure water, and then added into 5 kg skim milk at 4°C . The milk containing rennet was stored in fridge overnight. The concentration of rennet was 0.020IMCU/g. A freeze-dried concentrated lactic cheese culture (Choozit MA 16 LYO, Danisco, France) was used. The dosage was 0.625DCU (0.005%) bacteria per 10kg milk.

Soy/milk Cheese-type product making and Storage of the Curd:

2 kg dry soybeans were soaked in 5 kg of deionized water overnight at room temperature, drained and rinsed once with water. The weight of soaked soybeans was 4.75 kg. Additional water (11.25 kg) was added to the soaked soybeans, and the mixture was processed with a Stephan Microcut MC-15 (Hameln, Germany) using 0.05 mm cutting ring for 4 times. The soybean slurry was filtered using a 5-speed juice extractor (Breville Ikon, Australia) at speed 3. The okara (filtered fiber material) was collected and passed through the juice extractor again. Soymilk is filtered through cloth fabric to remove fines, and heated to 95°C with stirring in jacketed pot. The temperature was held for 5 min.

The milk was stored overnight with rennet added, so that the rennet would act on the proteins, without coagulation. The culture was then added into cold renneted milk and mixed for 2 min. At this point, the milk and soymilk were mixed together. The hot soymilk at about 85°C was added into cold milk at about 6°C , and the mixture temperature reached very quickly 38°C . To aid in the gelation of soy protein, MgCl_2 (0.25g/ml) was added as slowly as possible while manually stirring, and the coagulated mixture was left undisturbed to set for 30 min. The curd obtained was poured into cheese molds and incubated at 30°C overnight. The next day, dry salt (3% of the total wet weight) was added to the curd, by rubbing it on all sides of the curd surface. The cheese was placed in plastic tubs with the lids partially open to allow some drying off of the cheese, and stored in fridge for two days. There was no

pressure applied to the molds and the curd was turned a few times. After 2 days, the salted cheese was immersed into a brine composed of 8% salt, 0.5% CaCl_2 , and 0.9% vinegar, pH=4.6, and stored at 4°C.

Characterization of the soy/milk cheese curd, chemical analysis, texture and microstructure: The pH of cheese was determined using a portable digital pH meter (pHTestr.20, Eutech Instruments) by inserting the probe into a block of cheese and penetrating to a depth of 2 cm. The protein content in soymilk, milk and soy cheese was measured by Dumas method using Nitrogen Analyzer (LECO, FP-528, Mississauga, On, Canada). About 0.2 g ground cheese samples was collected from the centre of cheese. Protein values of soymilk were calculated by multiplying the nitrogen content by 6.25, and for milk by 6.38 (for the cheese, 6.25). Total solids were estimated using the vacuum oven drying method. About 3 g of ground curd or cheese was placed into aluminum tins, and dried with vacuum oven at 50°C for 18 h. After cooling for 30 min in a desiccator at room temperature, the weight was recorded.

As skim milk was used for the preparation, fat content was not measured. The textural properties of the cheese-product were measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp., UK, Model TA.XT2, version 05.16) of Stable micro System equipped with a cylindrical probe (25mm in diameter) with a 5 kg load cell. A texture profile analysis was carried out, using a pre-test Speed of 2.0mm/s, a test speed of 2.0mm/s, a post-test speed: 2.0mm/s and a distance of 6mm. The time was 3 s and the data acquisition rate: 200pps.

The Texture Analyzer was calibrated before testing. The test protocol was summarized as follows: texture analysis is carried out at ambient temperature on cylindrical cheese blocks with 125 mm in diameter and 30 mm in height, immediately after removal from refrigerator at 4°C. Samples are compressed axially in two consecutive cycles without yield, with 20% deformation (6mm) from the initial sample's height. Each sample was analyzed three times. Mean and standard deviations were reported.

Microstructural analysis was carried out by preparing 3mm^3 cheese samples, taken from with the cheese block, and mounted vertically on each of the copper holders designed for the Emitech 1250X cryo- preparation unit (Ashford, Kent, UK). Tissue-Tek®, a cryo- mounting gel, was used to ensure that the samples were affixed to the holder. The copper holders were plunged into liquid nitrogen slush (-

207 °C) which was prepared by pulling a vacuum on the liquid nitrogen. Liquid nitrogen slush provides a faster freezing rate to minimize ice crystals which result in less distortion of the sample. The copper holders are withdrawn from the freezing chamber through argon to prevent frost forming on the surface of the samples. Samples are transferred frozen and under vacuum into the preparation chamber of the cryo unit where the frozen cheese is fractured. Samples were fractured by pre-cooled razor blade, thus provided a better surface for sublimation. Samples were sublimated for 1 hour at -80 °C. After sublimation completed, samples were coated with 30nm of gold. The thin coat of the high atomic number element provides conductivity to prevent the sample from absorbing the electron beam and also results in more secondary electrons being created, which are the primary signal for SEM imaging. Samples were transferred, frozen and under vacuum, into the SEM (Hitachi S-570) cryo-stage for observation at 10KV accelerating voltage. The images were captured digitally using Quartz PCI imaging software (Quartz Imaging Corp. Vancouver, BC).

Soy/milk cheese-type product composition:

Table 2. Compositional and physicochemical properties of soy/milk cheese.

Composition/property	Soy cheese
Protein (%)	16.0
Total solid (%)	30.2
Fat (%)	Less than 1%
pH	4.4

Texture Analysis:

Hardness is the peak force of the first curve.

Springiness is a ratio of the two peak force.

Cohesiveness is a ratio of the total areas under the two curves.

Chewiness is the product of **Hardness* Cohesiveness* Springiness**.

	Force	Co-efficiency of variation (%)
Hardness	2.88kg	3.7
Springiness	90.8%	1.7
Cohesiveness	83.3%	0.4

Chewiness	2.18kg	2.6
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Example Three: Generation of mixed soy-dairy gels using soy milk and making of yoghurt.

Dairy yogurt consumption has been steadily increasing over the years as consumers are increasingly searching for new healthy snack options with high protein and low fat contents. Thus it is now possible to generate novel yogurt products. Such yogurts deliver the health benefits of both dairy products (high calcium content, high protein quality and soy products (isoflavones) with high protein quality. In addition to the individual benefits of dairy and soy, the addition of skim milk powder to soy yoghurts enhances isoflavone glycoside transformation to a biologically active form during yogurt storage. Dual gelation as herein described allows for selective gelation of the proteins for improved understanding of the participation or interference of soy and milk proteins during mixed gelation.

Soymilk preparation. Soymilk (5% soy protein) was prepared according to the procedure of Malaki Nik, *et al.*, {{88 Malaki Nik, A. 2010}} with slight modifications. In brief, Harovinton soybeans were soaked overnight in milli Q water for hydration. The hydrated soybeans were blended (Osterizer BLSTMG-WOO-033, Oster®) with a measured amount of water at room temperature (calculated to obtain the desired protein content) before being passed through a kitchen juicer (Professional Series 211, The Juiceman®) to further liquefy the sample. The soymilk was then passed through a cheesecloth to remove the okara (mainly composed of insoluble fibre material) and heated at 95°C for 7 minutes before cooling it in ice and storing in a refrigerator at 4°C until use. The soymilk was then used as is, without further centrifugation. Soy serum was prepared by first removing large particles by centrifugation (Optima™ LE-80K, Beckman Coulter) of the soymilk for 30 min at 20°C and at 7980 g. Next, the soymilk was transferred to Macrosep Centrifugal Devices (10 kDa molecular weight cut-off) from Pall Corporation and centrifuged for two hours at 5000 g and 10°C. Following centrifugation, soy serum was poured out from the filtrate receivers and kept in the refrigerator (4°C) until further use, within 5 days.

Skim milk preparation. Fresh milk was collected from the Elora dairy research station of the University of Guelph. Sodium azide was added at a concentration of 0.02% (w/v) to prevent bacterial growth. Milk was centrifuged at 6000 g for 20 min at 4 °C

using a Beckman J2-21 centrifuge and JA-10 rotor (Beckman Coulter, Mississauga, ON, Canada). Milk was then filtered four times through Whatman glass fiber filters (Fisher Scientific, Whitby, ON, Canada) before being subjected to ultrafiltration (PLGC 10k regenerated cellulose cartridge, Millipore Corp., Bedford, MA). During ultrafiltration, both the milk permeate (devoid of protein) and the retentate (concentrated milk containing all the proteins) were collected. Ultrafiltration was continued until a protein concentration of 4% was reached, as determined by the volume of permeate removed. The skim milk was stored in the refrigerator at 4°C until use.

Protein determination. Protein contents of skim milk and soymilk were analyzed using the DC protein assay kit (BIO RAD).

Soy milk characterization. Mineral content of soymilk serum was determined using atomic absorption spectroscopy and the serum was found to contain the following: 80 µg calcium, 190 µg magnesium, 200 µg phosphorus, 1400 µg potassium, <95 µg sodium and 83 µg sulfur.

Gel preparation. Samples were generated by mixing equal volumes of unheated concentrated skim milk (SM, 4% protein) and soymilk (SOY, 5% protein) resulting in a total protein concentration of 4.5%. Calcium chloride (Fisher Scientific, Whitby, ON, Canada) was added to all samples at a concentration of 1mM as reported by Li *et al* {{89 Li,Jie 2006}}. Glucono-delta-lactone (GDL) (Sigma-Aldrich Co., St. Louis, MO, USA) was added at 0.6% at 30°C to slowly reduce the pH from ~6.6 to ~5.5 over the course of the 3 hour experiments. Rennet (Chymax Ultra Rennet (790 IMCU/mL), Chr. Hansen, Milwaukee, WI, USA) was added in concentrations of 0.1074 IMCU/mL (high rennet) as well as 0.0537 IMCU/mL (low rennet) at 30°C. It was anticipated that the interpretation of binary gelation of a mixed system would be very challenging considering the lack of information available and the possible synergistic/competitive effects. Thus, a large number of controls were deemed essential to provide a better understanding of the gelation behaviour of the mixed system. Controls included the mixed system with each of the gelling conditions alone (high rennet alone, low rennet alone or GDL alone). Additionally, every combination of the gelling agents (Table 1) was applied to 4% skim milk, 2% skim milk (diluted 1:1 with soy serum), 5% soymilk and 2.5% soymilk (diluted 1:1 with milk permeate). Gelation experiments were repeated in triplicate if they gelled and in duplicate if they did not gel.

Gelation studies

Each sample for rheology and DWS was prepared in volumes of 35mL and distributed as follows: 1.5mL for light scattering experiment, 20mL for rheology and the remaining amount was used for pH measurement. Rheology, light scattering and pH measurement were carried out simultaneously.

Diffusing Wave Spectroscopy. Diffusing wave spectroscopy (DWS) was employed to measure the temporal fluctuations of light that have been scattered by a system, without the deleterious effect of dilution and in a steady (undisturbed) state. It is based on the measurement of temporal fluctuations of light due to the (Brownian) motion of its scatterers. By careful interpretation of the signal, it can yield information on the dynamic properties of the colloidal system via the diffusion coefficient, which can then be used to calculate the hydrodynamic particle radius with the Stokes-Einstein relation. A more detailed description of DWS theory is found elsewhere {{103 Weitz,D. 1993}}. The light source was a solid diode pumped Nd:YAG laser emitting light with a wavelength of 532 nm and a power of 350mW. For a detailed set-up of the experiment please refer to Alexander *et al.* {{100 Alexander,Marcela 2004}}. All samples except the 2% skim milk were placed into a 5 mm, 1.5 mL glass cuvette and measured at 30°C controlled by an external water bath. The 2% skim milk samples were analyzed in a 10 mm (3 mL) glass cuvette to ensure sufficient multiple scattering. Each treatment was measured in triplicate (i.e., three separate milk or soymilk batches) and analysis was carried out until gelation. In all cases, the light scattering measurements were collected for 2 minutes with intervals of 1 second. Data was analyzed using DWS-Fit software (Mediavention Engineering Inc., Guelph, ON, Canada) and Sigma Plot 10.0 (SPSS Inc., Chicago, IL, USA). The gel point was extrapolated from the plot of increase in radius as a function of pH/time.

Rheology. Experiments were carried out using a controlled stress rheometer at a constant strain of 0.01, a frequency of 0.1 Hz and an initial stress of 6 mPa. The temperature was controlled with an external water bath and kept at 30 °C. Rheological measurements were carried out in triplicate and each experiment was continued for 3 hours. Rheology was not performed on 2% skim milk samples due to the difficulty in obtaining large volumes of soy serum. The gel point was taken as the pH/time at $\tan \delta=1$.

pH Measurement. Samples were kept at 30°C in a circulating waterbath during pH measurements. pH measurements were recorded every 11 s into an excel

spreadsheet automatically by AR15 pH recorder software (Mediavention Engineering Inc.) during the course of the 3 hour experiments.

Confocal microscopy of final gels. 20 μ L of Rhodamine B (0.2% w/v in milliQ water) was added to 5 mL of sample for staining. Two drops of sample were placed into grooves of a concave microscope slide and a cover slip was placed overtop and sealed. Slides were incubated at 30°C for three hours before analysis. Images were taken using an inverted confocal laser scanning microscope (Leica TCS SP2, model Leica DM IRE2, Leica Microsystems CMS GmbH, Mannheim, Germany) with an Ar/Kr visible light laser, 63x (oil) objective. Experiments were repeated in triplicate.

Gelation Behaviour

Mixtures of soymilk (5% protein) and concentrated dairy milk (4% protein) were prepared in a 1:1 ratio and analyzed by DWS and rheology to investigate the sol-gel transition times and subsequent formation of the gels. While DWS looks at changes and rearrangements happening at the microstructural level, rheology detects the macro-characteristics of a sample. By combining both techniques, along with microscopy, a more complete picture of the gelling behaviour of the samples could be obtained.

All samples were supplemented with 0.1 mM CaCl_2 to compensate for the lower calcium content of soymilk as it is known that casein micelles dissociate at low levels of calcium. As mentioned earlier, soy proteins have a higher isoelectric point than milk proteins. Thus, if only acidification is used, soy proteins will aggregate far earlier than milk proteins. For this reason, rennet was added to induce aggregation of casein proteins closer to the gel point of soy proteins. Gelation was induced by addition of 0.6% GDL and two concentrations of rennet. The relatively low concentration of GDL resulted in a gradual decrease in the pH of the sample allowing for more careful observation of the early stages of gelation. Two concentrations of rennet were used (high and low) to examine the effect of modulating the timing of casein aggregation. To distinguish between the activity of rennet and acid, control experiments were conducted wherein GDL and rennet (at both concentrations) were added to the system in isolation.

In addition to observing the activity of each gelation mechanism in isolation, it was also important to observe the behaviour of each protein source in isolation. Thus additional control experiments were conducted to observe gelation of soymilk and skim milk alone to determine the contribution of each protein source to the gelation

behaviour in the mixed system. The actual concentration of soymilk and milk proteins in the mixed system was 2.5 and 2%, respectively. However these individual low protein concentration systems do not accurately represent the actual conditions of the mixed system where the total protein content is higher and proteins are in closer proximity to each other. Thus, all of the controls were measured at high protein concentrations (4% protein skim milk and 5% protein soymilk) and low protein concentrations (2% protein skim milk and 2.5% protein soymilk), reflecting the actual amount of soy and dairy proteins in the mixed system. Control experiments for addition of rennet only (no acidification) to either skim milk or soymilk in isolation were done and the results show an initial casein micelle size of approximately 120 nm radius. As the time after rennet addition progressed, the size of the micelles decreased slightly as the k-casein was cleaved by the action of chymosin. Eventually, the casein micelles were destabilized and coagulation took place. This is perceived as an increase in the radius measured by DWS, changing from about 120 nm to over 3 microns in the span of 10 minutes. Similarly, when the gelation behaviour was followed by rheology the elastic modulus, G' , remained low and constant during the initial minutes until the gel point, at which the G' increased rapidly. There was a noticeable difference in coagulation point for the skim milk renneted with the low rennet concentration (~29 min) and high rennet concentration (~18 min) as more enzyme resulted in a higher rate of cleavage taking place. Gelation points were measured by DWS and rheology for all the systems were investigated in this study. There was a slight difference in coagulation time between DWS and rheology. This has been observed before and it is due to the different length scales probed by the two techniques {{98 Holland,Ben 2011}}. While DWS looks at the movement of individual colloids, rheology can only measure the combined and cooperative effect of the colloids in the system. The final G' , 180 minutes after rennet addition was approximately 185 Pa for both the high rennet and low rennet samples.

Overall, the results suggest that if rennet were added to a mixed soymilk-dairy milk sample, only the dairy component would be affected by the action of rennet. Control experiments for addition of only GDL (no renneting) to either skim milk or soymilk in isolation were completed. There was an initial period of invariance in size, until the pH was progressively lowered to near the isoelectric point of soy proteins. At this point, there was sufficient neutralization of surface charges on soy proteins to

allow them to approach each other and begin to aggregate, as expressed by the sudden increase in radius. The gelation pH for the low and high protein samples was around 6.0 and 5.9, respectively. Although the gel points are statistically different, in reality a pH difference of 0.1 would not be considered important. Although the 2.5% and 5% soymilk controls gelled at a similar pH, rheological measurements revealed that the final G' of the 5% soymilk (~130 Pa) was around five times higher than that of the 2.5% soymilk (~25 Pa). This result should be expected as the higher concentration of protein allows for a higher number of bonds to be created between particles, thereby increasing the stiffness of the gel.

When only rennet was added to the mixed system, this sample did not result in any increase in radius during the three hours of observation (figure not shown). It is known from control experiments that soy proteins were not affected by rennet addition. Thus only milk proteins could have been acted upon in this instance.

However, the mixed system contained 2% skim milk proteins which, as mentioned before revealed that there was likely insufficient number of milk proteins available to build the gel network. Furthermore, the scattering signal arising from the milk protein component was also mostly overshadowed by the soymilk scattering in DWS.

GDL was observed alone on the mixed soymilk-dairy milk system. From the control samples it is known that skim milk proteins did not aggregate at these levels of GDL and only soy proteins were affected. DWS from this acidified mixed system show the initial radius of this sample was similar to that of soymilk particles alone, around 500 nm. As the pH dropped to pH 6.1, the size of the proteins remained constant. At pH 6.16, there was a clear increase in radius. This gelation point is slightly earlier than that shown by DWS on 2% soymilk alone. However, this discrepancy could be due to the method by which gelling point is calculated in DWS. The extrapolation of a straight line following the radius increase down to the x-axis was difficult to accomplish accurately at the shoulder. Nevertheless, the difference in gelation pH was minimal. The radius continued to increase down to pH 5.6 where there was a change of slope, and a drastic increase in radius. It is worthwhile to point out, that the kinetics of this acidified system, as shown by DWS, differed from that of the soymilk alone, as the shoulder seen in this mixed milk was not present when only soymilk proteins were aggregating. Interestingly, the rheological measurements showed gelation at pH 6.1, which was statistically equal to the gel point of soymilk alone in the control experiments and in agreement with that shown by the shoulder in

DWS. It could be speculated that protein aggregation in this system, as shown by DWS, was manifested as the initial increase in radius at the beginning of the shoulder, but aggregation did not ensue rapidly because of interference from unaggregated milk proteins. Although soy proteins may have begun to aggregate around pH 6.1, rapid and full coagulation may not have taken place until the net charge of the soy proteins had been sufficiently neutralized to overcome the disturbances of the milk proteins, in this case around pH 5.6.

The reason why the rapid increase in radius only occurs at pH 5.6 may be explained by the reduction of net charge in soy particles due to the combined effect of a reduction in pH and calcium release from casein micelles. This would demonstrate a synergistic effect in gelation of the mixed system wherein calcium release from casein micelles accelerates gelation of soy proteins.

The desired system of interest is the mixed soy milk-dairy milk system with added GDL and rennet. DWS plots of the soymilk-skim milk mixture show a dual gelling system. Once more, the initial radius was measured to be around 500 nm which remained constant for the initial pHs. Regardless of whether the high or low rennet concentration was used in combination with GDL, the mixed soy-milk systems showed a drastic increase in radius and gelled around pH 6.0. As explained earlier, the agreement in gel point between samples with the low and high rennet concentrations can be attributed to the excess rennet in solution. From the control experiments, and under the conditions used in this experiment, acidified soy proteins gelled at a pH 6.0 and 2% milk proteins at a pH of 6.1, however, these were not significantly different. Thus, it is clear that at the gelation point of the mixed system (which is also not significantly different from the control systems just mentioned) both milk and soy proteins participate in a mixed gel network when the system is acidified and renneted simultaneously. Thus, the presence of rennet was effective in making milk proteins available to aggregation and therefore enabled them to participate in network formation rather than to interfere with network formation.

Rheology data also shows the sol-gel transition around a pH of 6.1, in agreement with the DWS data. This gel point is slightly later than the systems with skim milk alone but slightly earlier than that of soymilk. It could be interpreted as a compromise between the two gelling forces. Although the pH of gelation of the mixed system was the same with high and low rennet, the elastic modulus (G') indicates that the gel structures differed. The G' with high rennet and acid was around 86 Pa

whereas with the lower concentration of rennet it was not possible to obtain a consistent (final) G' . This suggests that, although the system contained excess rennet to fully cleave the k-casein in the micelles, a desired concentration of rennet may be required to generate a self-supporting gel structure in the mixed system, possibly due to competing elements or structure formation. Furthermore, if the elastic modulus of the mixed system was compared to the controls, it can be observed that even when both proteins participated in the gel network (4.5% total protein), the gel stiffness (~86 Pa) was still far lower than that of 4% skim milk or 5% soymilk alone (~190 Pa and 130 Pa, respectively). This might be further indication that there may be some antagonistic effects which impede gel stiffening in the mixed system.

Microstructure

Confocal images show that the soymilk gel, produced with GDL, appears as densely packed aggregates of soy proteins. The milk gel, produced with GDL and high rennet, exhibited a very different structure: the milk gel had a network-like appearance with large pores and interconnecting strands. The mixed soymilk-skim milk gels had appearances which were in between, exhibiting a network of strands of aggregated protein. These images indicate that the mixed systems may have microstructures different from those of pure soy gels and pure milk gels. When confocal images of the mixed system with the different gelling agents were compared, no obvious differences were observed between the samples using imaging technology. In general, the structures were more open than those of soymilk alone but more closely packed than those of skim milk alone.

Claims:

1. A soy/milk cheese-type product prepared by a method comprising:
 - (a) mixing hot soy milk with cold renneted milk;
 - (b) acidifying and adding a firming agent to said mixture from (a);
 - (c) mixing the product resulting from (b) for a time sufficient to cool (b) and effect simultaneous gelation of soy milk and milk;
 - (d) transfer the product resulting from (c) into molds at a temperature of less than about 35°C; and
 - (e) optionally salting (d).
2. The product of claim 1, wherein said method further comprises subjecting the molded product from (d) to external pressure.
3. The product of claim 1 or 2, wherein said product is further brined.
4. The product of claim 1, 2 or 3, wherein said milk is present in amounts of from about 10% to about 90%.
5. The product of any one of claims 1 to 4, wherein said milk comprises 0% to 10% fat.
6. The product of any one of claims 1 to 5, wherein said firming agent is calcium chloride.
7. The product of claim 6, wherein said firming agent is present in amounts from about 0% to about 0.5%.
8. The product of any one of claims 1 to 7, wherein said milk is renneted using rennet or a synthetic rennet equivalent.
9. The product of any one of claims 1 to 8, wherein acidification is effected by the addition of a lactic bacterial culture.

10. The product of claim 9, wherein said lactic bacterial culture is added to the cold renneted milk prior to step (b).
11. The product of any one of claims 1 to 8, wherein acidification is effected by the addition of GdL, magnesium and/or calcium ions to (a).
12. A method for making a soy/milk cheese-type product, the method comprising:
 - (a) mixing heated soy milk with cold renneted milk;
 - (b) acidifying and cooling (a) to effect simultaneous gelation of the soy milk and the milk;
 - (c) coagulating cooled (b) and setting;
 - (d) allowing (c) to incubate overnight; and
 - (e) dry salting (d) to form said cheese-type product.
13. The method of claim 12, wherein the acidifying comprises adding lactic acid, citric acid, or GdL to (a).
14. The method of claim 12, wherein the acidifying comprises adding lactic acid-producing bacteria to the cold renneted milk prior to mixing with the heated soy milk.
15. A method for making a soy/milk gel, the method comprising:
 - (a) mixing heated soy milk with cold renneted milk;
 - (b) acidifying and cooling (a) to effect simultaneous gelation of the soy milk and the milk; and
 - (c) optionally fermenting (b).
16. The method of claim 15, further comprising the step of decreasing syneresis to increase water holding capacity of said gel.
17. A soy/milk yoghurt product made the method of claim 15 or 16.
18. A method for making a soy/milk curd, the method comprising:
 - (a) mixing heated soy milk with cold renneted milk;

(b) acidifying and cooling (a) to effect simultaneous gelation of the soy milk and the milk;

(c) coagulating cooled (b), and setting; and

(d) allowing (c) to incubate overnight.

19. A soy/milk cheese-type product or a soy/milk yoghurt-type product, said products comprising mixed gels of rennet coagulated casein micelles and acid coagulated soy protein.

20. The products of claim 19, wherein said coagulated soy protein is present as strands of interacting soy protein with said casein.

21. The products of claim 19 or 20, wherein the product is a soy/milk cheese-type product having hardness, springiness, cohesiveness and chewiness similar to milk only soft cheese.

22. The products of claim 19 or 20, wherein said product is a soy/milk yoghurt-type product with a high water holding capacity.

23. The products of any one of claims 19 to 22, wherein said soy protein is present in an amount of about up to 60% of said product.

24. The products of any one of claims 19 to 23, wherein said milk protein is present in an amount of about up to 90% of said product.

25. The products of any one of claims 19 to 24, wherein said product further comprises one or more of nonfat dry milk, a milk protein, an acidity regulator, an acid, an anticaking agent, an antifoaming agent, a coloring agent, an emulsifier, an enzyme preparation, a flavoring agent, a firming agent, a food protein, a gelling agent, a preservative, sequestrants, a stabilizer, a starch, a thickener, an oil, a fat, a cheese powder, a salt, a nutritional supplement, an acid, an enzyme, a neutraceutical, a carbohydrate, a vitamin, a mineral, soluble fiber, fruit, vegetables, nuts, meat, spices, and other foodstuffs.

26. A soy/dairy yoghurt- type product comprising mixed gels of rennet coagulated casein micelles and acid coagulated soy protein.
27. The soy/dairy yoghurt- type product of claim 26, wherein said coagulated soy protein is present as strands of interacting soy protein with said casein.
28. The soy/dairy yoghurt- type product of claim 26 or 27 having less degree of syneresis leading to high water holding capacity similar to yoghurt.
29. The soy/dairy yoghurt- type product of any one of claims 26 to 28, wherein said soy protein is present in an amount of about up to 60% of said product.
30. The soy/dairy yoghurt- type product of any one of claims 26 to 29, wherein said milk protein is present in an amount of about up to 90% of said product.
31. The soy/dairy yoghurt- type product of any one of claims 26 to 30, wherein said product further comprises one or more of nonfat dry milk, a milk protein, an acidity regulator, an acid, an anticaking agent, an antifoaming agent, a coloring agent, an emulsifier, an enzyme preparation, a flavoring agent, a firming agent, a food protein, a gelling agent, a preservative, sequestrants, a stabilizer, a starch, a thickener, an oil, a fat, a cheese powder, a salt, a nutritional supplement, an acid, an enzyme, a nutraceutical, a carbohydrate, a vitamin, a mineral, soluble fiber, fruit, vegetables, nuts, meat, spices, and other foodstuffs.

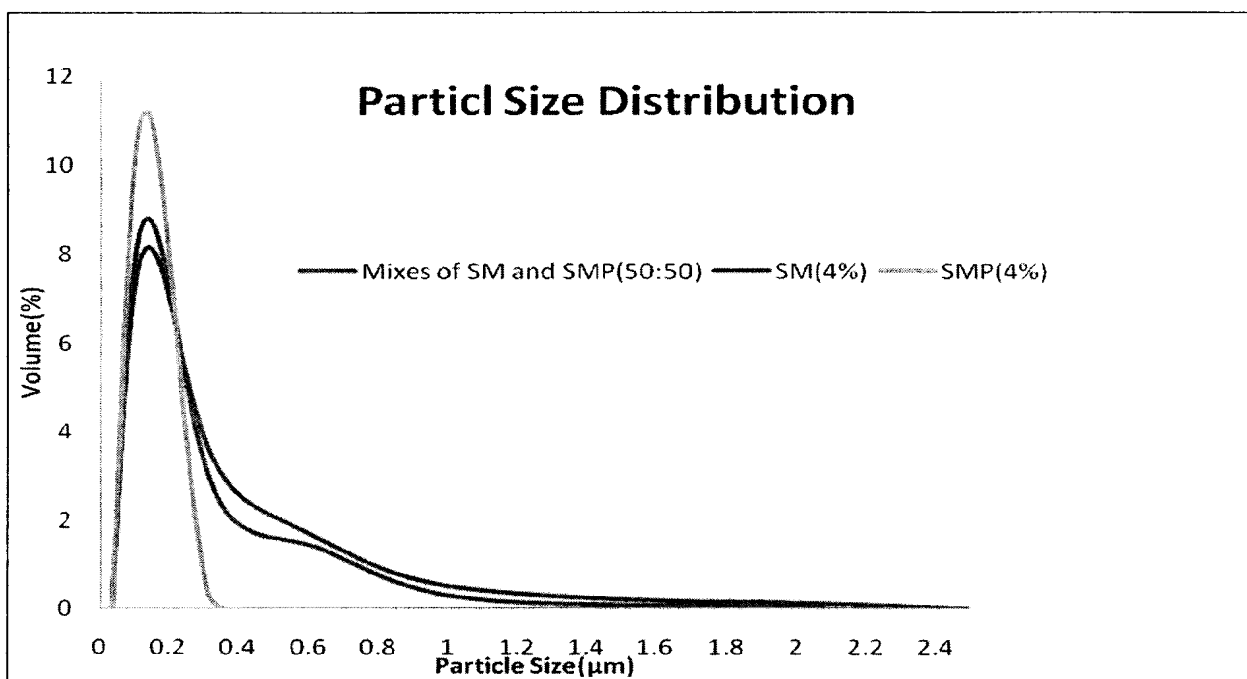


Figure 1

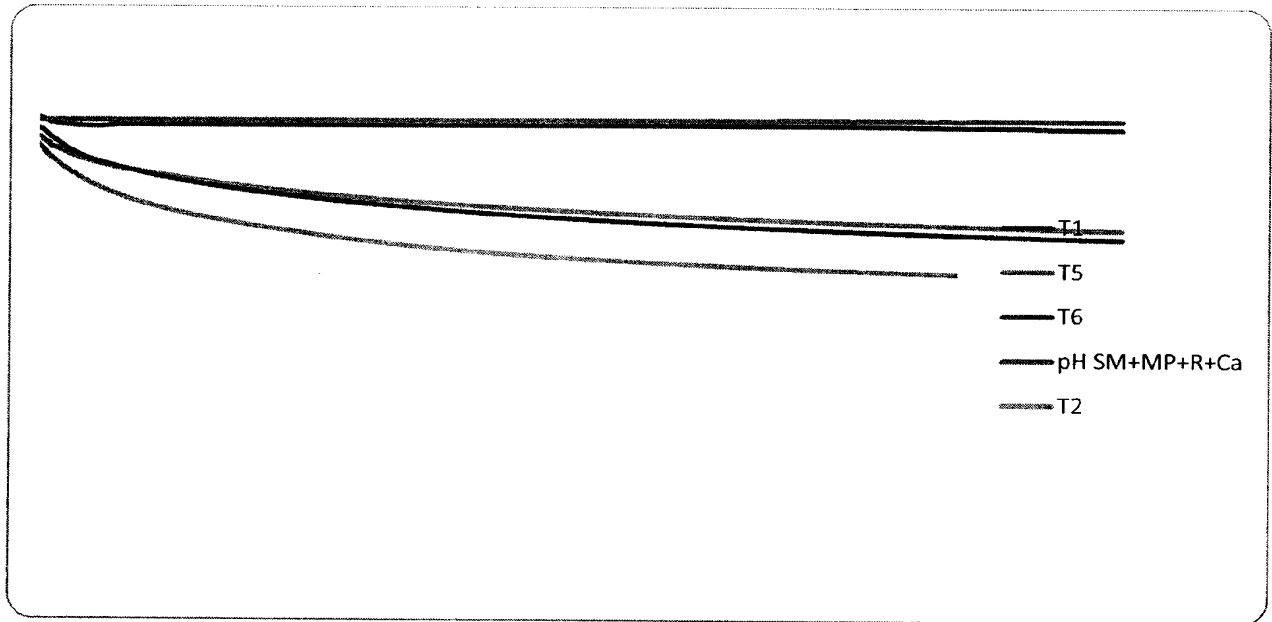


Figure 2

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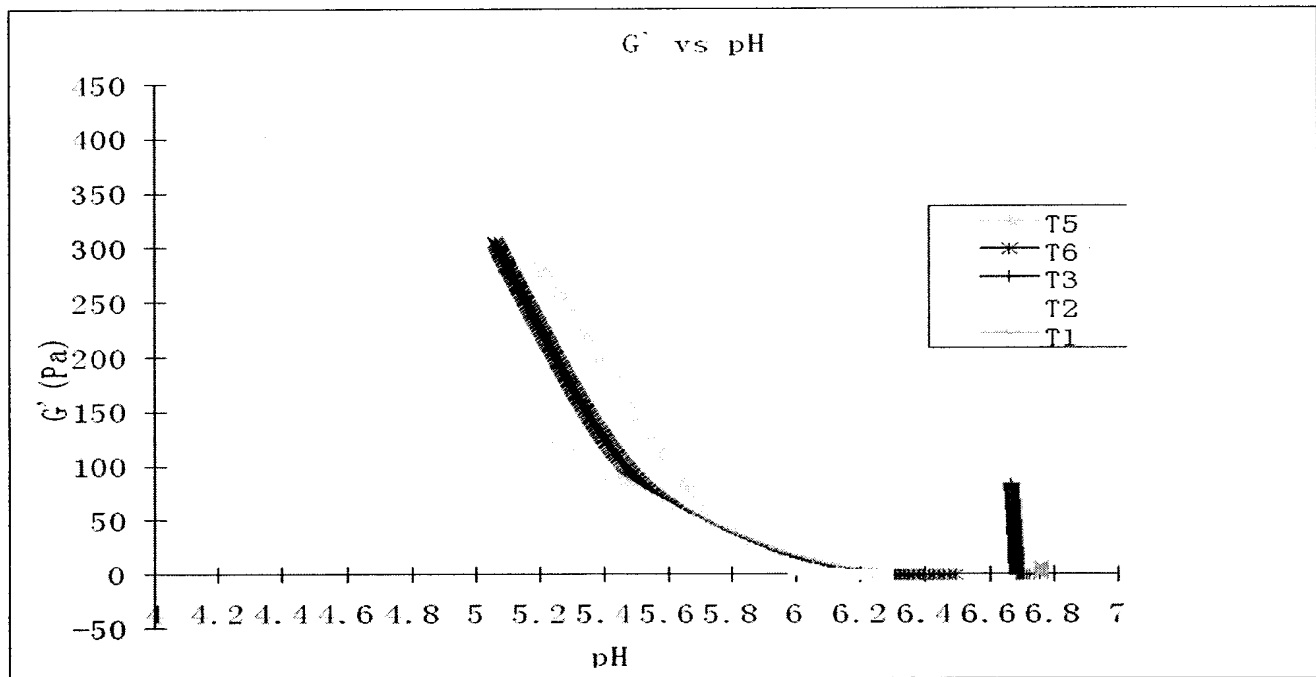


Figure 3

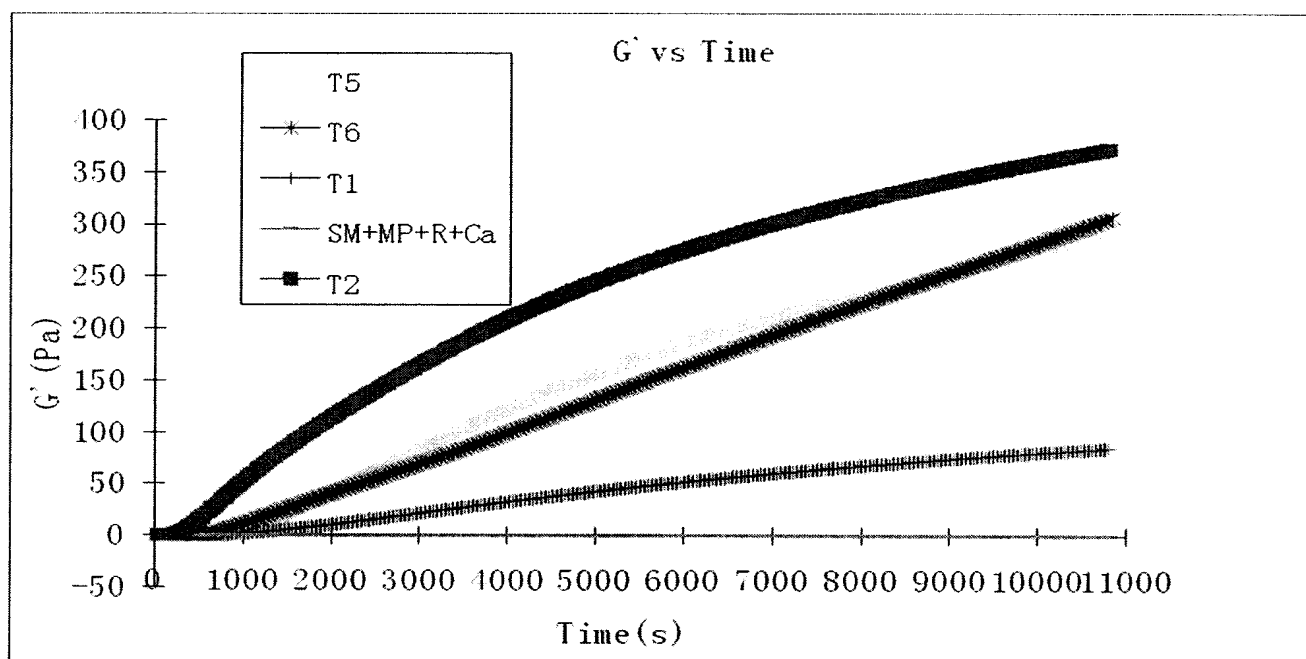


Figure 4

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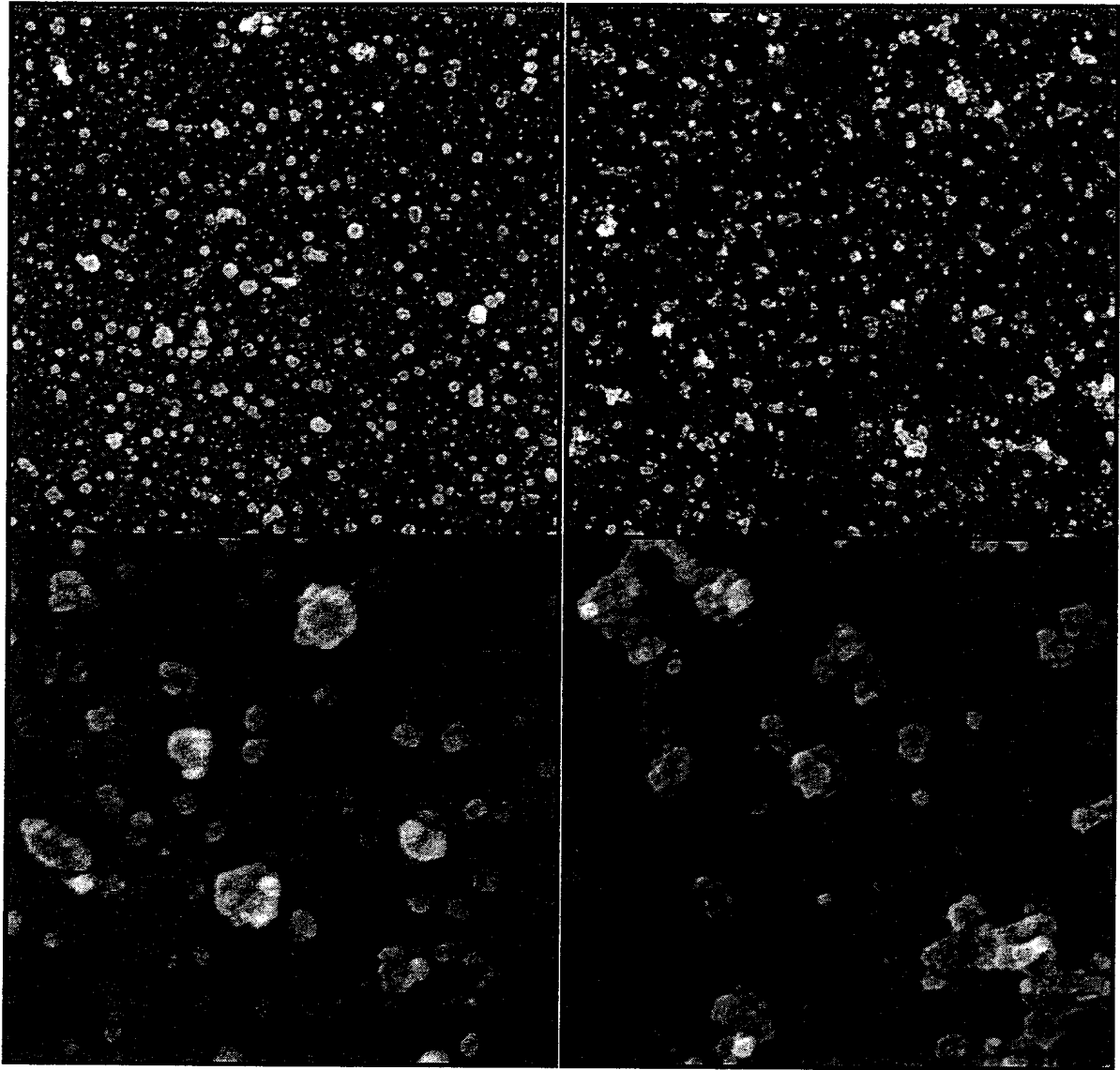


Figure 5

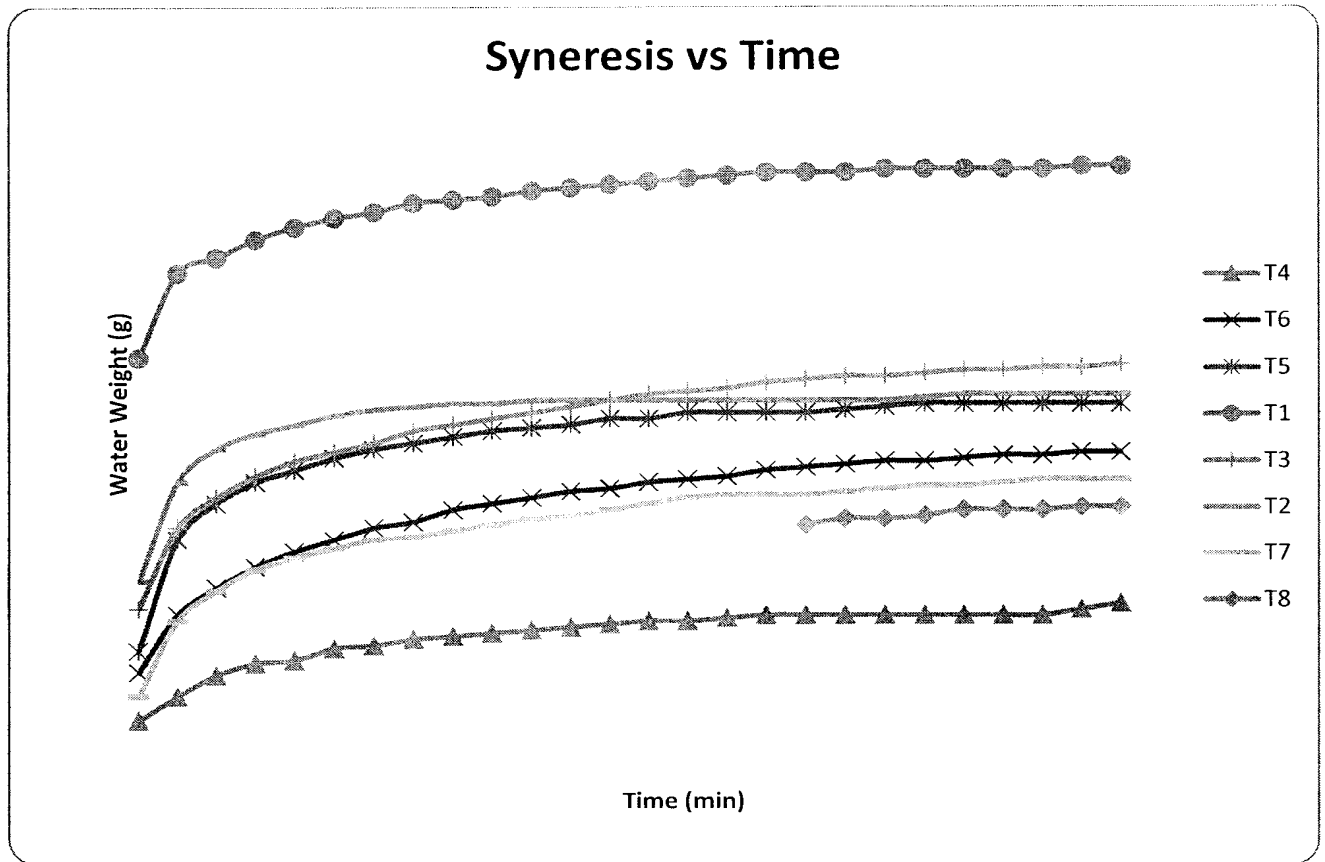


Figure 6

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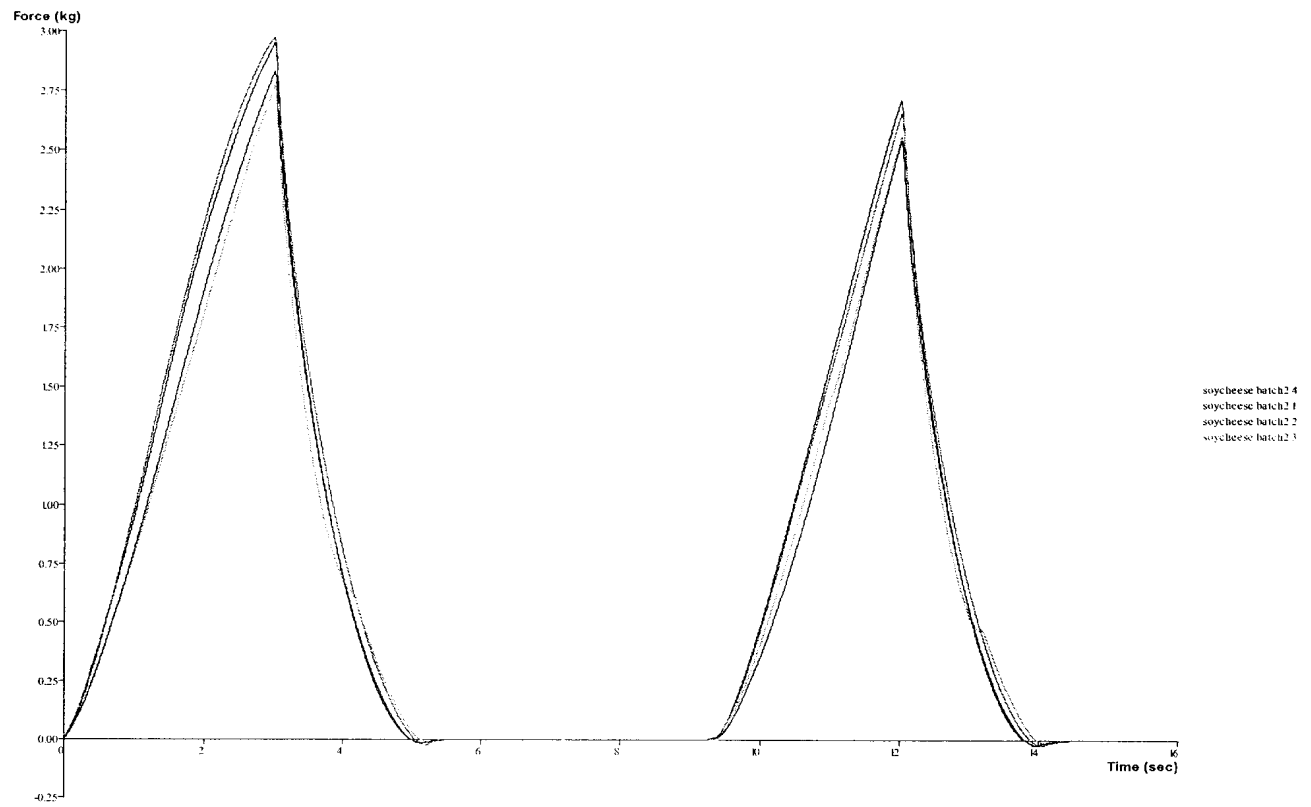


Figure 7

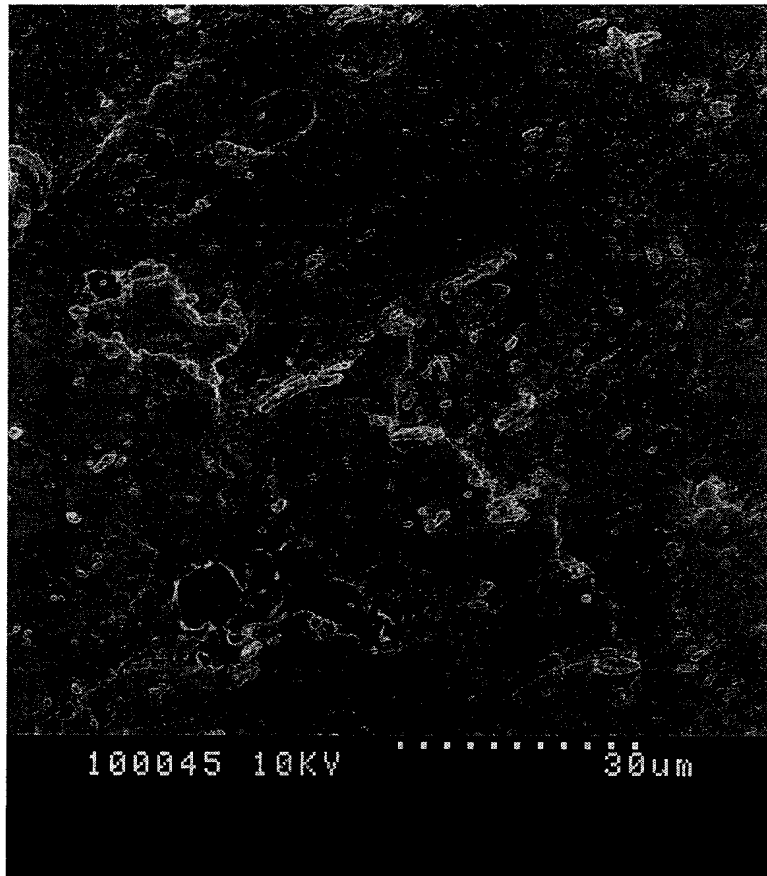


Figure 8

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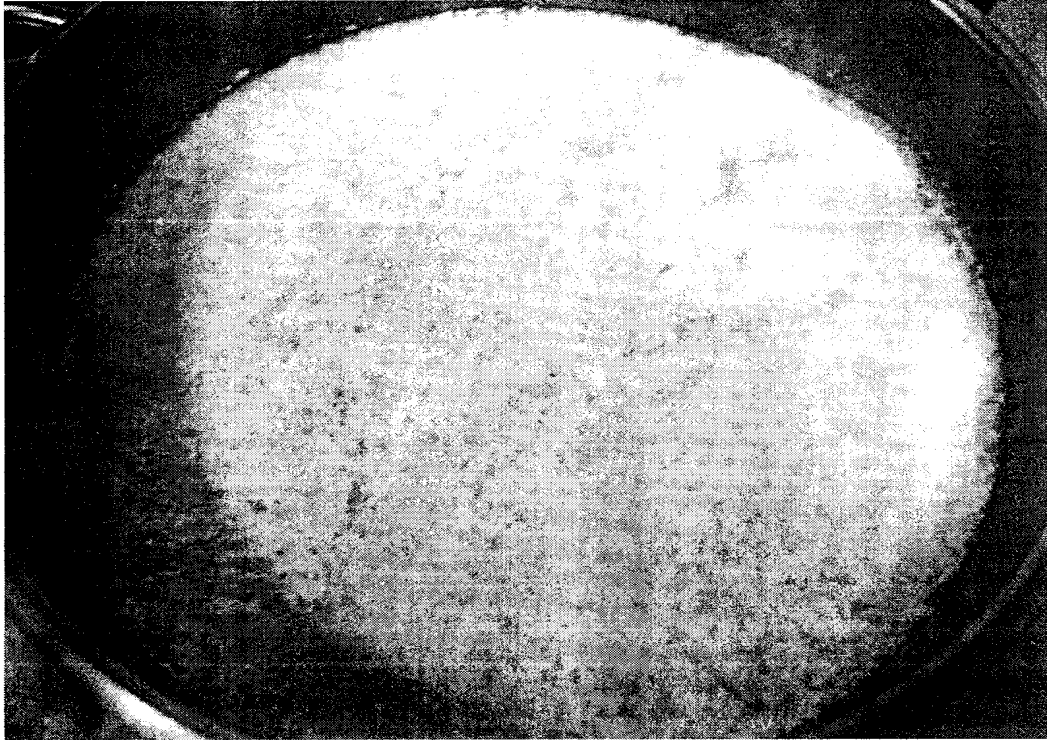


Figure 9A

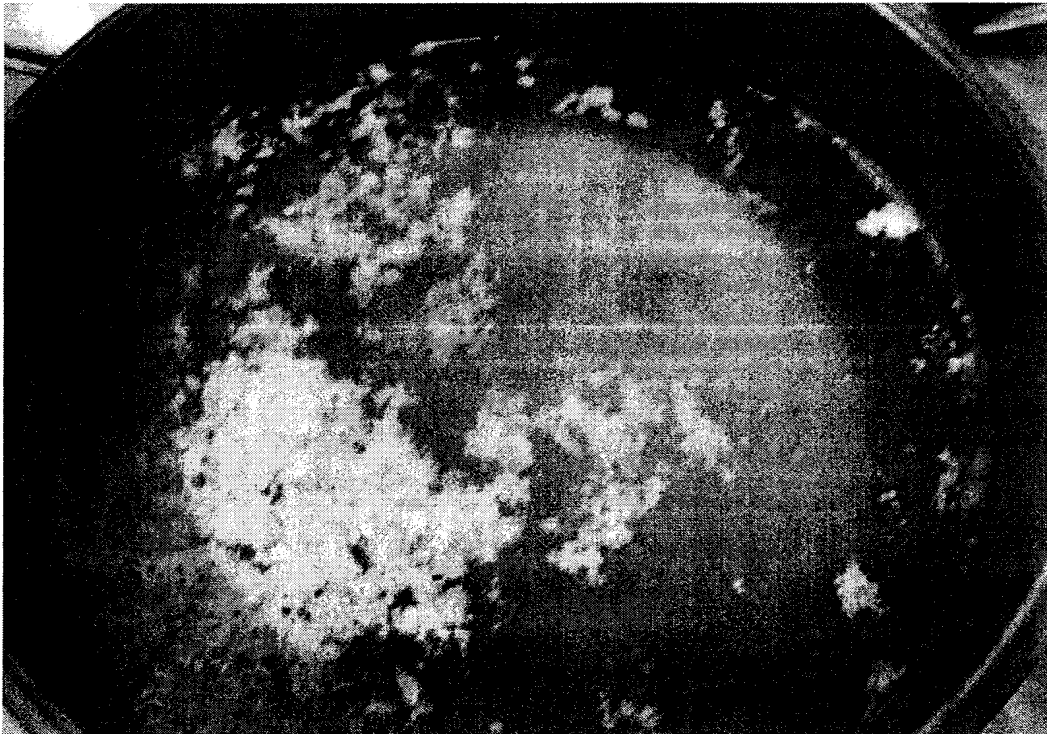


Figure 9B

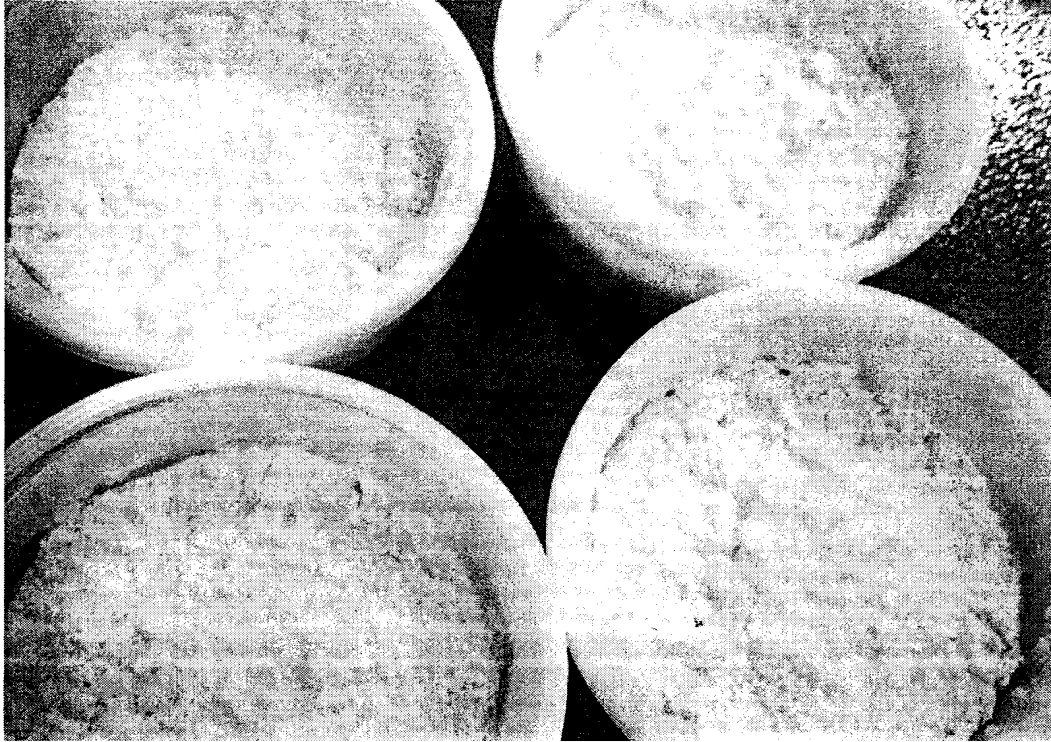


Figure 9C



Figure 9D

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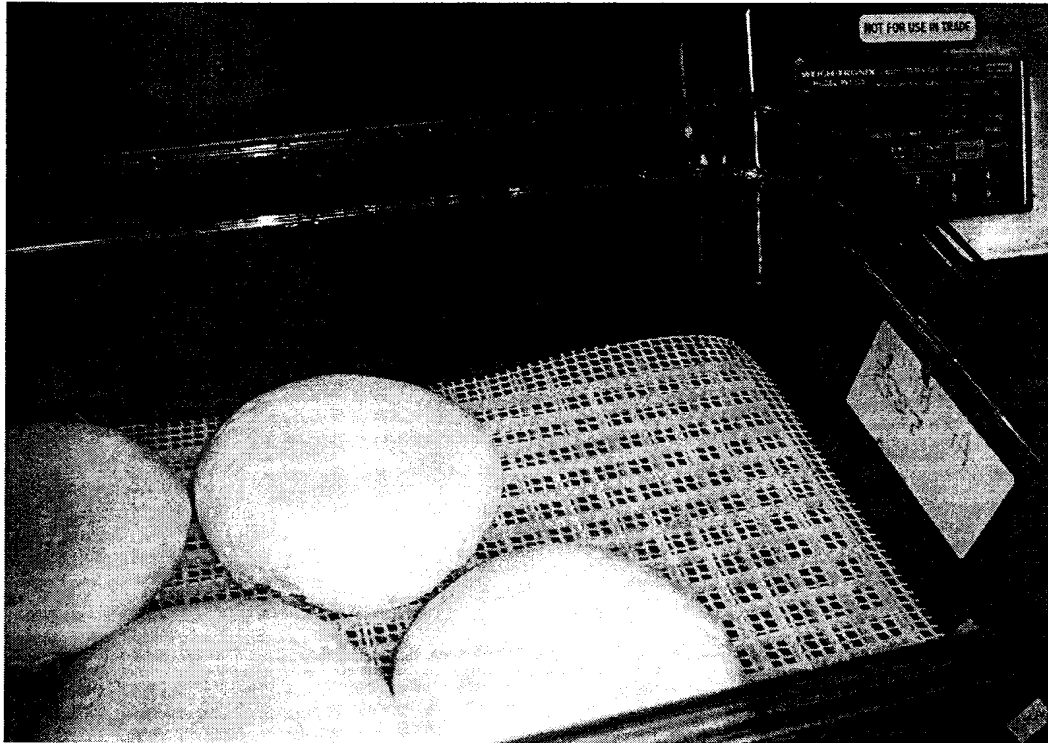


Figure 9E

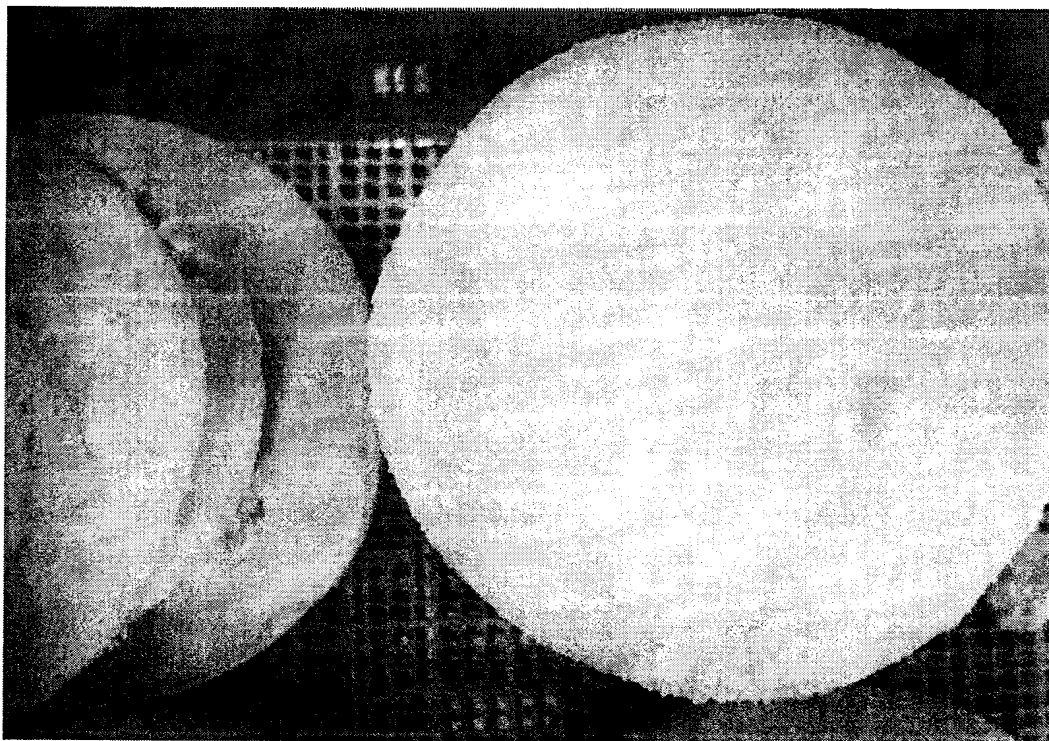


Figure 9F

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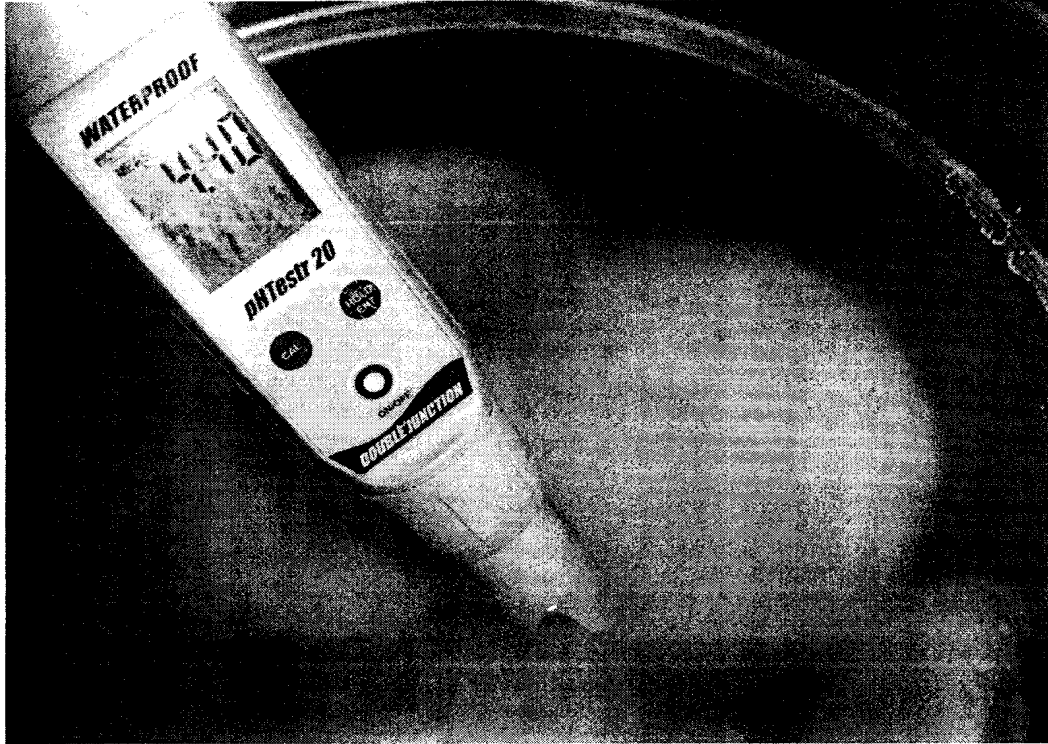


Figure 9G

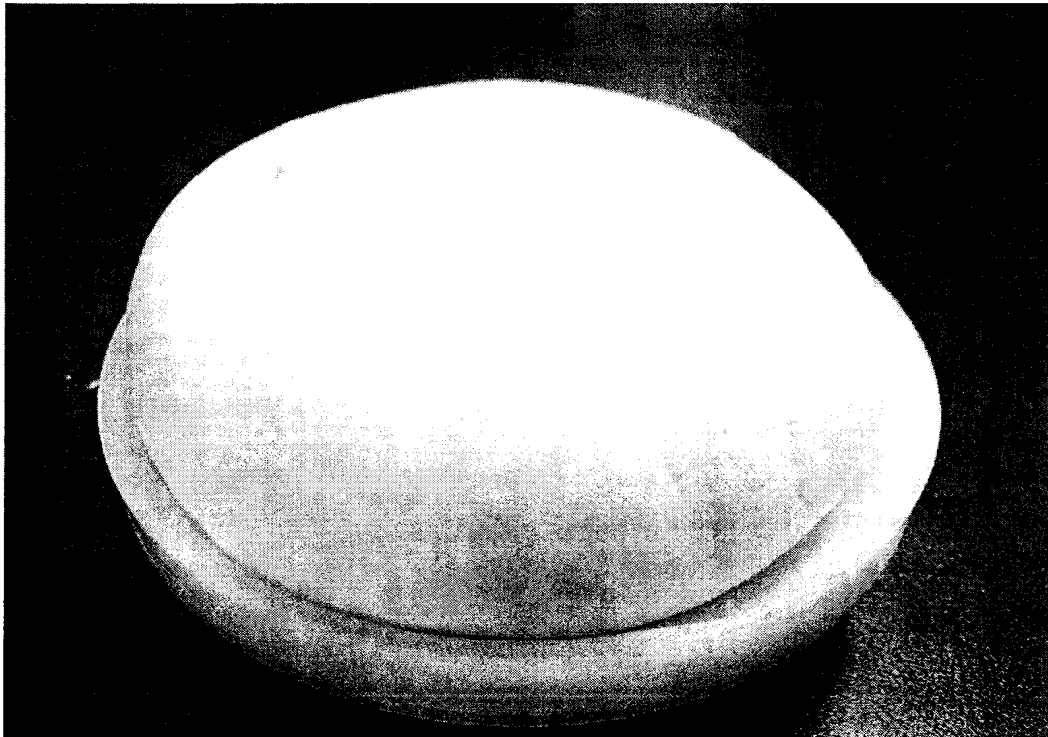


Figure 9H

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2012/000445

A. CLASSIFICATION OF SUBJECT MATTER IPC: A23C 19/055 (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC: A23C														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched IPC: A23L														
Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used) Epoque (Full Text, Epodoc), Canadian Patent Database Keywords: soy, milk, cheese, yogurt, rennet, enzyme, gel, slurry, matrix, network, coagulation, analog, heat, cool, acid, casein, glycinin														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X	US 6 495 187 B1 (BORDERS ET AL.) 17 December 2002 (17-12-2002) Abstract Column 1 line 38 - Column 4 line 33 Examples, Claims	19-31												
X	US 2001/0046531A1 (GOTTEMOLLER ET AL.) 29 November 2001 (29-11-2002) Entire Document	19-31												
X	US 6 455 081 B1 (HAN ET AL.) 24 September 2002 (24-09-2002) Abstract Column 4 line 8 - Column 5 line 67	19-31												
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents :</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
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Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476		Authorized officer Michael Kapur (819) 953-4859												

INTERNATIONAL SEARCH REPORT
Information on patent family membersInternational application No.
PCT/CA2012/000445

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61/751,818 11 January 2013 (11.01.2013) US
- (71) Applicant: **MARAXI, INC.** [US/US]; 525 Chesapeake Drive, Redwood City, California 94063 (US).
- (72) Inventors; and
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- (74) Agents: **MCCORMICK GRAHAM, Monica** et al.; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440 (US).
- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).
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[Continued on next page]

(54) Title: NON-DAIRY CHEESE REPLICA COMPRISING A COACERVATE

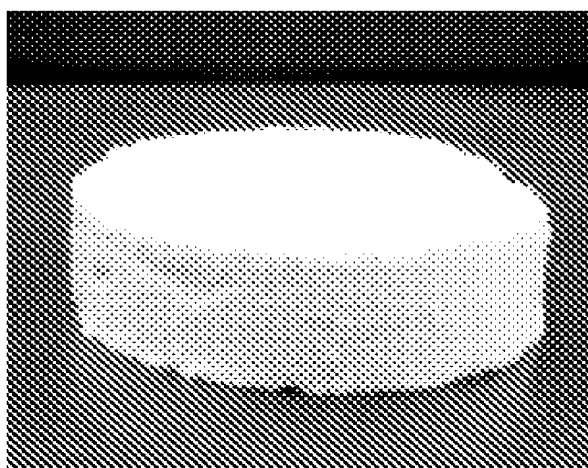


FIG. 11

(57) Abstract: Provided herein are methods and compositions for the production of cheese replicas. Generally the cheese replicas are produced by inducing the enzymatic curdling of non-dairy milks.

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- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

NON-DAIRY CHEESE REPLICA COMPRISING A COACERVATE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/751,818, filed January 11, 2013, and is related to co-pending Application Serial No PCT/US12/46552, filed July 12, 2012, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Cheese making has relied on dairy milks as the major ingredient for more than 4000 years. Dairy cheese is usually made from curds formed from dairy milk. Dairy milks can readily be made to form curds suitable for making cheese by contacting the dairy milk with rennet (an aspartic protease which cleaves kappa-casein) at mildly acidic pH. Some cheeses, e.g., cream cheese, cottage cheese and paneer, are made without rennet. In the absence of rennet, dairy cheese may be induced to curdle with acid (e.g., lemon juice, vinegar, etc.) or a combination of heat and acid. Acid coagulation can also occur naturally from starter culture fermentation. The strength of the curds depends on the type of coagulation. Most commercially produced cheeses use some type of rennet (animal, vegetable or microbial-derived) in their production. Commodity cheeses or “processed cheeses” such as bulk cheddar, food-service mozzarella pizza and “cheese products,” or “cheese foods” such as American cheese, American singles, Velveeta, and Cheese Whiz are typically produced from dairy-derived ingredients and other additives using industrial processes which sometimes little resemble traditional cheese making.

The global dairy sector contributes an estimated 4 percent to the total global anthropogenic green house gas emissions. Producing 1 kg of cheddar cheese requires an average of 10,000 L of fresh water. Additionally, many individuals cannot digest and metabolize lactose. In these individuals enteric bacteria ferment the lactose, resulting in various abdominal symptoms, which can include abdominal pain, bloating, flatulence, diarrhea, nausea, and acid reflux. Additionally, the presence of lactose and its fermentation products raises the osmotic pressure of the colon contents. Approximately 3.4% of children in the U.S.A. are reported to have allergies to dairy milks. Many individuals choose to avoid milk for ethical or religious reasons.

Non-dairy milks, including plant-derived milks avoid many of the environmental, food sensitivity, ethical and religious problems associated with dairy milk and they can be made free of lactose, making the generation of dairy substitutes using the plant derived milks attractive. However, rennet is not an effective agent for inducing non-dairy

5 proteins or emulsions, including plant-derived milks (e.g., almond milk, chestnut milk, pecan milk, hazelnut milk, cashew milk, pine nut milk, or walnut milk), to curdle. Consequently, traditional cheese making techniques have not been successfully used to produce non-dairy cheese replicas.

Flavor and aroma in dairy cheese results in part from the degradation of lactose, proteins and fats, carried out by ripening agents, which include bacteria and enzymes in

10 the milk, bacterial cultures added during the cheese-making process, rennet, other proteases, lipases, added molds and/or yeasts and bacteria and fungi that opportunistically colonize the cheese during ripening and aging. In addition, the bacterial cultures and fungi used in traditional dairy cheesemaking use microorganisms adapted to growing and

15 producing flavors in dairy milks. Hence, traditional cheese culturing techniques have not been successfully used to produce non-dairy cheese replicas.

Cheese replicas made principally of non-dairy ingredients are commercially available. Many of these cheese replicas include some dairy ingredients, for example, casein. Some commercially available cheese replicas contain no animal products. These

20 include fermented cheese replicas made from nut milks from which insoluble carbohydrates have not been effectively removed, and made without using a protein crosslinking agent and several products in which a starch is a principal ingredient or containing agar, carrageenan or tofu to provide the desired texture. The few fermented products contain *Lactobacillus acidophilus*, a microbe often used in dairy yogurts. Most

25 tasters consider none of the currently available cheese replicas to adequately replicate the taste, aroma and mouthfeel of dairy cheeses.

Complex carbohydrates in currently available cheese replicas made from nut milks have unfavorable effects on the texture, resulting in a product with a grainy mouthfeel and lacking the creaminess of dairy cheeses.

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Starches that comprise the major gelling agent in many currently available cheese replicas lead to a relatively high carbohydrate content, which may be undesirable to consumers, for example those wishing to limit carbohydrate intake.

Because of these deficiencies, there is currently no cheese replica that is
5 acceptable to most consumers as an alternative to traditional dairy cheeses.

Thus, it is clear that there is a great need in the art for an improved method and system for producing non-dairy cheese replicas while avoiding the shortcomings and drawbacks of the cheese replicas that have previously been available to consumers.

SUMMARY OF THE INVENTION

10 The invention relates to methods and compositions for producing non-dairy milk and cheese products, including without limitation, plant-derived milk and cheese products, as an alternative to dairy products for human consumption.

In one aspect, this document features a non-dairy cheese replica that includes a coacervate comprising one or more isolated and purified proteins from a non-animal source. The one or more isolated and purified proteins can be plant proteins (e.g., seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, or lentil proteins. The pea proteins can include pea vicilins and/or pea legumins. The non-dairy cheese replica can include one or more microbes selected from bacteria, molds, and yeast.

This document also features a non-dairy cheese replica that includes a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt. The non-dairy cheese replica can include one or more heat-labile ingredients such as one or more fats, microbes, volatile compounds, or enzymes. The non-dairy cheese replica can include one or more microbes selected from bacteria, molds, and yeast.

The one or more microbes in the non-dairy cheese replicas can be selected from the group consisting of a *Penicillium* species, a *Debaryomyces* species, a *Geotrichum* species, a *Corynebacterium* species, a *Streptococcus* species, a *Verticillium* species, a *Kluyveromyces* species, a *Saccharomyces* species, a *Candida* species, a *Rhodospiridium* species, a *Cornybacteria* species, a *Micrococcus* species, a *Lactobacillus* species, a *Lactococcus* species, a *Staphylococcus* species, a *Halomonas* species, a *Brevibacterium* species, a *Psychrobacter* species, a *Leuconostocaceae* species, a *Pediococcus* species, a *Propionibacterium* species, and a lactic acid bacterium.

In some embodiments, the one or more microbes are selected from the group consisting of *Lactococcus lactis lactis* (LLL), *Leuconostoc mesenteroides cremoris* (LM), *Lactococcus lactis cremoris* (LLC), *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus* (SX), *Lactococcus lactis biovar diacetylactis* (LLBD), *Penicillium roqueforti*, *Penicillium candidum*, *Penicillium camemberti*, *Penicillium nalgiovensis*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Streptococcus thermophiles* (TA61), *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Rhodospiridium infirmominiatum* and *Brevibacterium linens*.

This document also features a non-dairy cheese replica comprising a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids, said cheese replica comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*,
5 *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*.

In another aspect, this document features a non-dairy cheese replica that includes a solidified non-dairy milk, nut milk, and one or microbes selected from the group
10 consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*, wherein at least 85% of the insoluble solids of the non-dairy milk have been removed, wherein the non-dairy milk is a nut milk, a bean milk, or a grain
15 milk. Such a non-dairy replica further include one of more microbes selected from the group consisting of *Penicillium roqueforti*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Penicillium candidum*, *Corynebacteria*, *Streptococcus thermophiles*, *Penicillium camemberti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Rhodospiridium infirmominiatum*,
20 *Cornybacteria*, a *Micrococcus species*, a *Lactobacillus species*, a *Lactococcus species*, *Lactococcus lactis lactis* (LLL), *Leuconostoc mesenteroides cremoris* (LM), *Lactococcus lactis cremoris* (LLC), a *Staphylococcus species*, a *Halomonas species*, a *Brevibacterium sps*, a *Psychrobacter sps*, a *Leuconostocaceae sps*, a *Pediococcus species*, *Leuconostoc mesenteroides*, *Lactococcus lactis biovar. diacetylactis* (LLBD) or a *Propionibacterium*
25 species.

In another aspect, this document features a non-dairy cheese replica that includes an isolated and solidified non-dairy cream fraction and one or microbes selected from the group consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*,
30 *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*. Such a non-dairy replica further include one of more

microbes selected from the group consisting of *Penicillium roqueforti*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Penicillium candidum*, *Corynebacteria*, *Streptococcus thermophiles*, *Penicillium camemberti*, *Penicillium nalgiovensis*, *Verticillium lecanii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Rhodospiridium infirmominiatum*, *Cornybacteria*, a *Micrococcus species*, a *Lactobacillus species*, a *Lactococcus species*, *Lactococcus lactis lactis* (LLL), *Leuconostoc mesenteroides cremoris* (LM), *Lactococcus lactis cremoris* (LLC), a *Staphylococcus species*, a *Halomonas species*, a *Brevibacterium sps*, a *Psychrobacter sps*, a *Leuconostocaceae sps*, a *Pediococcus species*, *Leuconostoc mesenteroides*, *Lactococcus lactis biovar. diacetylactis* (LLBD) or a *Propionibacterium species*.

In any of the non-dairy replicas described herein, the replica can include two of LLL, LLC, and LLBD (e.g., LLC and LLL, LLL and LLBD, or LLL, LLC, and LLBD) or can include SX and TA61. The non-dairy cheese replica further can include *Penicillium roqueforti* and *Debaryomyces hansenii*.

The one or more non-animal based proteins can be plant proteins (e.g., seed storage protein or an oil body protein). The seed storage protein can be an albumin, glycinin, conglycinin, globulin, legumin, vicilin, conalbumin, gliadin, glutelin, glutenin, hordein, prolamin, phaseolin, proteinoplast, secalin, triticeae gluten, or zein. The oil body protein can be an oleosin, a caloleosin, or a steroleosin. The plant protein can be selected from the group consisting of ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase), collagens, kafirin, avenin, dehydrins, hydrophilins, and natively unfolded proteins.

In any of the non-dairy replicas described herein, the replica further can include one or more sugars (e.g., sucrose, glucose, fructose, and/or maltose), one or more purified enzymes (a lipase, a protease, and/or an amylase), a melting salt (e.g., sodium citrate, trisodium pyrophosphate, sodium hexametaphosphate, disodium phosphate, or any

combination thereof), a divalent cation (e.g., Fe^{2+} , Mg^{2+} , Cu^{2+} , or Ca^{2+}), an isolated amino acid (e.g., methionine, leucine, isoleucine, valine, proline, or alanine) or other additive selected from the group consisting of a food product, a yeast extract, miso, molasses, a nucleobase, an organic acid, a vitamin, a fruit extract, coconut milk, and a malt extract, one or more plant-derived lipids, one or more oils derived from an algae, fungus, or bacterium, or one or more free fatty acids. The plant-derived lipids can include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, palm fruit oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil (e.g., canola oil, cocoa butter, and/or coconut oil).

In any of the non-dairy cheese replicas described herein, the replica further can include a cross-linking enzyme (e.g., a transglutaminase or a lysyl oxidase).

In any of the non-dairy cheese replicas described herein, the cheese replica can have one or more of: a) increased creamy, milky, buttery, fruity, cheesy, free fatty acids, sulfury, fatty, sour, floral, or mushroom flavor or aroma notes; 2) reduced nutty, planty, beany, soy, green, vegetable, dirty, or sour flavor or aroma notes; 3) an increased creamy texture; 4) an improved melting characteristic; and 5) an increased stretching ability, relative to a corresponding cheese replica lacking the one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, a cheese replica can have an increase in one or more of acetoin, diacetyl, 2,3-hexandione, or 5-hydroxy-4-octanone, or a decrease in one or more of benzaldehyde, 1-hexanol, 1-hexanal, furan, benzaldehyde or 2-methyl-2-propanol, pyrazine, or heptanal relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in methional and/or dimethyl trisulfide relative to a corresponding cheese replica lacking said one or more microbes, sugar, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in one or more of butanoic acid, propanoic acid, hexanoic acid, octanoic acid, or decanoic acid relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in one or more of 2-heptanone, 2-undecanone, 2-nonanone, 2-butanone, 2-methyl propanoic acid, 2-methyl butanoic acid, or 3-methyl butanoic acid relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in one or more of ethyl butanoate or methyl hexanoate relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have (i) an increase in ethyl octanoate and/or 2-ethyl-1-hexanol or (ii) an increase in 2-methyl butanal and/or 3-methyl butanal relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in acetic acid relative to a corresponding cheese replica lacking the one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids or other additive, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in one or more of gamma-octalactone, delta-octalactone, gamma-nonolactone, butyrolactone, or methyl isobutyl ketone relative to a corresponding cheese replica lacking said one or more microbes, sugars, divalent cations, isolated enzymes, isolated amino acids, yeast extract, plant-derived lipids, or combinations thereof.

For example, the cheese replica can have an increase in one or more of nonanol or 1-octen-3-ol relative to a corresponding cheese replica lacking said one or more

microbes, sugars, divalent cations, isolated enzymes, isolated amino acids, yeast extract, plant-derived lipids, or combinations thereof.

This document also features a method of making a non-dairy cheese replica. The method includes solidifying a mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated fats, the mixture comprising one or more microbes selected from the group consisting of *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*. The solidifying can include cross-linking the proteins using a transglutaminase or a lysyl oxidase, subjecting the mixture to a heat / cool cycle, forming a cold set gel, forming a coacervate comprising one or more isolated and purified proteins from a non-animal source. The method further can include adding one or more of the following: the replica further can include one or more sugars (e.g., sucrose, glucose, fructose, and/or maltose), one or more purified enzymes (a lipase, a protease, and/or an amylase), a melting salt (e.g., sodium citrate, trisodium pyrophosphate, sodium hexametaphosphate, disodium phosphate, or any combination thereof), a divalent cation (e.g., Fe²⁺, Mg²⁺, Cu²⁺, or Ca²⁺), an isolated amino acid (e.g., methionine, leucine, isoleucine, valine, proline, or alanine) or other additive selected from the group consisting of a food product, a yeast extract, miso, molasses, a nucleobase, an organic acid, a vitamin, a fruit extract, coconut milk, and a malt extract, one or more plant-derived lipids, one or more oils derived from an algae, fungus, or bacterium, or one or more free fatty acids. The method further can include aerating the mixture. The method can include incubating said mixture with one microbe for a period of time and then adding a second microbe to said mixture.

This document also features a method for making a cold set gel. The method includes denaturing a solution comprising at least one isolated and purified plant proteins under conditions wherein said isolated and purified protein does not precipitate out of said solution; optionally adding any heat-labile components to said solution of denatured protein; gelling said solution of denatured protein between 4°C and 25°C by increasing the ionic strength; and optionally subjecting said cold set gel to high pressure processing. The one or more isolated and purified proteins can be plant proteins (e.g., seed storage

proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, or lentil proteins. The pea proteins can include pea vicilins and/or pea legumins. The heat labile components can include one or more microbes. Gelling can be induced using 5 to 100 mM sodium or calcium chloride.

5 This document also features a method of making a coacervate. The method includes acidifying a solution of one or more isolated and purified proteins from a non-animal source to a pH between 3.5 and 5.5 (e.g., pH 4-5, wherein the solution comprises 100 mM or less of a salt; and isolating the coacervate from said solution; and optionally
10 subjecting said coacervate to high pressure processing. The isolated and purified proteins can be plant proteins (e.g., seed storage proteins, chickpea proteins, lentil proteins, pea proteins or Lupine proteins). The pea proteins can include pea vicilins and/or pea legumins. The pea vicilins can include convicilins. The acidifying step can be done in the presence of a plant based oil.

This document also features a method of minimizing one or more undesirable
15 odors in a composition containing plant proteins. The method includes contacting the composition with a ligand having binding affinity for lipoxygenases. The ligand can be bound to a solid substrate. The composition can be a food composition (e.g. a cheese replica)

In another aspect, this document features a method of minimizing one or more
20 undesirable odors in a composition containing plant proteins. The method includes contacting the composition with activated carbon then removing the activated carbon from the composition. The composition can be a food composition (e.g. a cheese replica)

This document also features a method of minimizing one or more undesirable odors in a composition containing plant proteins. The method includes contacting the
25 composition with a lipoxygenase inhibitor and/or an antioxidant. The composition can be a food composition (e.g. a cheese replica).

This document also features a method for modulating a flavor profile and/or an aroma profile of a cultured non-dairy product. The method includes adding one or more microbes to a non-dairy milk source selected from the group consisting of a nut milk, a
30 grain milk or a bean milk and culturing the microbe-containing non-dairy milk and modulating 1) the aeration rate and/or timing of aeration during the culturing; 2) the

timing of adding the microbes to the mixture; 3) the order of adding the one or more microbes; 4) the cell density of the contacted microbes prior to or after addition to the mixture; or 5) the microbial growth phase of the microbes prior to or after addition to the mixture, whereby the flavor profile and/or aroma profile of the non-dairy milk is

5 modulated. The method further can include adding one or more sugars, divalent cations, isolated enzymes, isolated amino acids or other additives, plant-derived lipids, algal oils, or oil derived from bacteria, oil derived from fungi, or free fatty acids to the mixture during said culturing step. The method further can include solidifying the microbe containing mixture. The product can be a cheese replica, yogurt or sour cream, crème
10 fraiche, or kefir.

This document also features a non-dairy cheese replica comprising a coacervate comprising one or more isolated proteins from a non-animal source. The one or more isolated and purified proteins can be plant proteins (e.g., seed storage proteins, pea proteins, Lupine proteins, proteins from a legume, chickpea proteins, or lentil proteins.

15 The pea proteins can include pea vicilins and/or pea legumins.

In another aspect, this document features a non-dairy cheese replica comprising (i) a solidified mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated plant based lipids or (ii) a solidified non-dairy milk, nut milk, and one or microbes; wherein the non-dairy cheese replica has a) an increased
20 creamy texture; b) an improved melting characteristic; or an increased stretching ability.

This document also features a method of making a non-dairy cheese replica. The method includes solidifying a mixture of one or more isolated and purified proteins from a non-animal source and one or more isolated fats using high pressure processing. The mixture can include one or more microbes comprising one or more microbes selected
25 from the group consisting of a *Penicillium* species, a *Debaryomyces* species, a *Geotrichum* species, a *Corynebacterium* species, a *Streptococcus* species, a *Verticillium* species, a *Kluyveromyces* species, a *Saccharomyces* species, a *Candida* species, a *Rhodospiridium* species, a *Cornybacteria* species, a *Micrococcus* species, a *Lactobacillus* species, a *Lactococcus* species, a *Staphylococcus* species, a *Halomonas* species, a
30 *Brevibacterium* species, a *Psychrobacter* species, a *Leuconostocaceae* species, a *Pediococcus* species, a *Propionibacterium* species, and a lactic acid bacterium.

This document also features a ricotta cheese replica comprising a solidified nut milk, *Lactococcus lactis lactis*, and *Lactococcus lactis cremoris*. The ricotta cheese of further can include a transglutaminase. The nut milk can be made with almond milk. In some embodiments, it is made with a mixture of almond milk and macadamia nut milk.

5 The ricotta replica has a white and creamy appearance with a buttery appearance. It can be smooth and sweet with toasted almond overtones. The ricotta cheese replica can be whipped or firm. The whipped ricotta has a smooth texture, and a higher moisture content than the firm ricotta. The whipped ricotta replica can be used as substitute for mascarpone. The firm ricotta can be used as a cottage cheese substitute.

10 In yet another aspect, this document features a blue cheese replica comprising a solidified nut milk, *Lactococcus lactis cremoris*, *Lactococcus lactis diacetylactis*, *Lactococcus lactis lactis*; *Penicillium roqueforti*, and *Debaryomyces hansenii*. The blue cheese of further can include a transglutaminase. The nut milk can include a mixture of almond milk and macadamia nut milk.

15 In yet another aspect, this document features a method for creating a library of isolated microbial strains for use in flavoring a non-dairy cheese replica. comprising: obtaining a starter culture comprising a heterogenous population of microbial strains; isolating one or more individual microbial strains from said heterogenous population; and determining a flavor contribution of each of said individual microbial strains to a non-
20 dairy cheese replica.

In practicing the invention, in some embodiments the cheese replica is comprised of less than 5% complex carbohydrates.

In practicing the invention, in some embodiments the cheese replica is comprised of less than 5% polysaccharides

25 In practicing the invention, in some embodiments the cheese exhibits smooth melting.

In some embodiments the invention provides a hard cheese replica and method of making the same. In some embodiments, a non-dairy milk is inoculated with thermophilic cultures before formation of the gel. The hard cheese replica is optionally
30 aged.

In another aspect, the invention provides a blue cheese replica and method of making the same. In some embodiments, the blue cheese replica is prepared from a nut milk. In some embodiments, the nut milk is prepared from almonds and macadamia nuts. In some embodiments, the nut milk is a 50:50 composition of almond and macadamia milk. In some embodiments, the nut milk has 28% cream. In some embodiments, the nut milk is pasteurized. In some embodiments, the nut milk is heated, e.g., to $83 \pm 3^\circ \text{F}$. In some embodiments, microbial cultures are then added to the nut milk. In some embodiments, the microbial cultures comprise MA11 and penicillium roquefortii. In particular embodiments, the microbial cultures are allowed to hydrate on top of milk for about 5 minutes before they are stirred into the milk). In some embodiments, proteases or lipases are added. In some embodiments, the proteases or lipases are dissolved in water prior to adding to the milk.

In another aspect the invention provides a washed rind cheese replica and methods of making the same. In some embodiments, the washed rind cheese replica is prepared from a nut milk. In some embodiments, the nut milk is prepared from almonds and macadamia nuts. In some embodiments, the nut milk is a 50:50 composition of almond and macadamia milk. In some embodiments, the nut milk has 28% cream.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. The word “comprising” in the claims may be replaced by “consisting essentially of” or with “consisting of,” according to standard practice in patent law.

BRIEF DESCRIPTION OF THE DRAWINGS

The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

FIG. 1 is a bar graph of the average texture score of each of the soft ripened cheese replicas with the addition of proteases and lipases, as determined by the taste testers.

FIG. 2 is a bar graph of the average firmness of each of the soft ripened cheeses with the addition of proteases, as determined by the texture analyzes.

FIG. 3A and FIG. 3B are bar graphs of the average preference scores and flavor scores, respectively, for the cheese replicas made by varying proteases added and time of protease addition, as determined by taste testers.

FIG. 4 is a bar graph of the average butteriness scores for the cheese replicas made by varying proteases added and time of protease addition, as determined by taste testers. Error bars are the standard deviation of the taste tester scores.

FIG. 5 is a bar graph of the average acidity scores for the cheese replicas made by varying proteases added and time of protease addition, as determined by taste testers.

FIG. 6 is a line graph depicting the effect of individual LF2 and LF5 bacterial strains on the pH of filtered nut media.

FIG. 7 is a bar graph depicting buttery and sour flavor scores for individual LM and LLBD samples.

FIG. 8 is a bar graph depicting the combined buttery and sour flavor scores for LM and LLBD samples.

FIG. 9 is a bar graph depicting nutty and sweet flavor scores for individual LM and LLBD samples.

FIG. 10 is a bar graph depicting the combined nutty and sweet flavor scores for LM and LLBD samples.

FIG. 11 depicts a cheese replica prepared using crosslinked isolated proteins.

FIG. 12 is a line graph depicting the concentration of 2,3-butanedione detected in each sample with 10 mM, 50 mM, or 200 mM glucose and increasing concentrations of citrate.

FIG. 13 is a line graph depicting the concentration of acetoin detected in each sample with 10 mM, 50 mM, or 200 mM glucose and increasing concentrations of citrate.

FIG. 14 is a line graph depicting the concentration of 2,3-hexanedione detected in each sample with 10 mM, 50 mM, or 200 mM glucose and increasing concentrations of citrate.

FIG. 15 is a bar graph depicting the signal intensity of free fatty acids as detected by GCMS in samples cultured with SX.

FIG. 16 is a bar graph depicting the signal intensity of 2-methyl and 3-methyl butanoic acids as detected by GCMS in samples cultured with SX.

FIG. 17 is a bar graph depicting the signal intensity of the cheese acids (butanoic acid, propanoic acid, 3-methyl butanoic acid, and 2-methyl propanoic acid) as detected by GCMS in *Brevibacterium* cultured yeast extract media with additional substrates (citrate, oxalic acid, or pyruvate).

FIG. 18 is a bar graph depicting the signal intensity of “buttery” compounds (acetoin and 2,3-butanedione) as detected by GCMS in soymilk samples cultured with MD88.

FIG. 19 is a bar graph depicting the signal intensity of “buttery” compounds (acetoin and 2,3-butanedione) as detected by GCMS in soymilk samples cultured with TA61.

FIG. 20 is a bar graph depicting the signal intensity of 3-methyl-butanoic acid, as detected by GCMS, produced by SX in soymilk with various branched chain amino acids supplemented.

FIG. 21 is a bar graph depicting the signal intensity of 2-methyl-butanoic acid, as detected by GCMS, produced by SX in soymilk with various branched chain amino acids supplemented.

FIG. 22 is a bar graph depicting the signal intensity of 2-methyl-propanoic acid, as detected by GCMS, produced by SX in soymilk with various branched chain amino acids supplemented.

FIG. 23 is a bar graph depicting the signal intensity of 3-methyl-butanoic acid as detected by GCMS in soymilk samples cultured with different concentrations of leucine.

FIG. 24 is a bar graph depicting the signal intensity of dimethyl trisulfide as detected by GCMS in *Brevibacterium* cultured samples.

5 FIG. 25 is bar graph depicting the signal intensity of acetoin as detected by GCMS in cultured PV with coconut milk.

FIG. 26 is bar graph depicting the signal intensity of 2,3-butanedione as detected by GCMS in cultured PV with coconut milk.

10 FIG. 27 is bar graph depicting the signal intensity of free fatty acids as detected by GCMS in cultured PV with coconut milk.

DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses methods and compositions for producing desired flavors and textures in cheese made from non-dairy sources. One of skill in the art will recognize that any combination of the embodiments described herein are within the scope
15 of the invention. Broadly, the invention provides methods for making non-dairy cheeses by solidifying a non-dairy cheese source using a variety of techniques, including crosslinking, using a heat / cool cycle, forming a cold set gel, forming a coacervate, or using high pressure processing. The solidifying process can allow the separation of the crosslinked proteins and associated fats into solid curds that can be separated from the
20 “whey,” e.g., the liquid remaining after curdling. The solidified proteins can hold a fat emulsion, and have the essential physical characteristics needed for pressing, culturing and ripening a cheese replica derived from non-dairy milk.

As described herein, the texture and/or flavor of the cheese replica, as well as melting characteristic or stretchability of the cheese replica, can be modified by adding
25 one or more specific enzymes (e.g., lipases and/or protease), sugars, proteins, amino acids, divalent cations, melting salts, yeast extract, food product, miso, molasses, nucleobases, organic acids, vitamins, fruit extracts, coconut milk, malt extracts, plant-based lipids, free fatty acids, and one or more microbes. In addition, culture parameters can be adjusted to alter the flavor, texture, melting characteristics, and stretchability of a
30 cheese replica. For example, the aeration rate and/or timing of aeration during the culturing; the timing of adding the microbes to the mixture; the order in which two or

more microbes are added, e.g., together or sequentially; the relative amounts of two or more microbes; the absolute number of microbes inoculated; or the microbial growth phase of the microbes prior to or after addition to the mixture,

In some embodiments, one or more specific enzymes (e.g., lipases and/or protease), sugars, proteins, amino acids, divalent cations, melting salts, yeast extract, food product, miso, molasses, nucleobases, organic acids, vitamins, fruit extracts, coconut milk, malt extracts, plant-based lipids, free fatty acids, can be used to affect emulsion stability, protein solubility, suspension stability, or ability to support growth of microbial cultures used in making cheese replicas, yogurt replicas, or other food replicas of cultured dairy products.

In various embodiments, the current invention includes cheese replicas principally, entirely or partially composed of ingredients derived from non-animal sources. In additional embodiments the present invention includes methods for making cheese replicas from non-animal sources. In various embodiments these results are achieved by replicating the curdling process of cheese making in non-dairy milks using a transglutaminase.

Definitions.

The term “isolated protein” as used herein refers to a preparation in which a protein or population of proteins is substantially isolated from a source, wherein non-proteinaceous components have been substantially reduced in the preparation. Non-proteinaceous components may be reduced by a factor of 3 or more, a factor of 5 or more, or a factor of 10 or more relative to the source material from which the protein or proteins have been isolated. The population of proteins may be heterogenous, or the population of proteins may be heterogenous. A non-limiting example of an isolated protein preparation comprising a heterogenous population of proteins is soy protein isolate.

The term “isolated and purified protein” refers to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or

1000 or more relative to the source material from which the specified protein was said to be purified. For clarity, the isolated and purified protein is described as isolated and purified relative to its starting material (e.g., plants or other non-animal sources). In some embodiments, the term “isolated and purified” can indicate that the preparation of the protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. The fact that composition can include materials in addition to the isolated and purified protein does not change the isolated and purified nature of the protein as this definition typically applies to the protein before addition to the composition.

The term “homogeneous” can mean a single protein component comprises more than 90% by mass of the total protein constituents of a preparation.

The term “resemble” can mean one composition having characteristics recognizably similar to another composition by an ordinary human observer.

The term “indistinguishable” can mean that an ordinary human observer would not be able to differentiate two compositions based on one or more characteristics. It is possible that two compositions are indistinguishable based on one characteristic but not based on another, for example two compositions can have indistinguishable taste while having colors that are different. Indistinguishable can also mean that the product provides an equivalent function as or performs an equivalent role as the product for which it is substituting.

The term cheese “substitute” or “replica” can be any non-dairy product that can be used in any role commonly served by traditional dairy cheese. A cheese “substitute” or “replica” can be a product that shares visual, olfactory, textural or taste characteristics of cheese such that an ordinary human observer of the product is induced to think of traditional dairy cheese.

The term “controlled”, “controlling”, and “defined” are used interchangeably herein to refer to the manipulation of a method or components of a composition to achieve a desired characteristic or keep said desired characteristic within certain bounds defined by a user. By way of example only, a controlled fat profile refers to a fat profile wherein the fat content or content of specific fat classes (e.g., saturated or unsaturated) are kept within user-defined limits. By way of other example only, a controlled amount refers to an amount kept within certain bounds defined by a user. For instance, adding a

controlled amount of bacteria may refer to adding bacterial cultures comprising a known population and/or known amount of bacterial strains. By contrast, Rejuvelac is an exemplary composition that comprises an uncontrolled amount of, e.g., bacteria.

Rejuvelac is prepared by incubating a liquid containing a bacterial food source in an environment that is conducive to the growth of bacteria, but the amount of bacteria or the types of bacteria that grow in said environment is not kept to within user-defined bounds, e.g., is not controlled.

Non-dairy milks

In one aspect, the invention provides a non-dairy cheese source that can be used as a starting material for preparing a non-dairy cheese. The term “non-dairy cheese source” refers to an emulsion comprising proteins and fats, wherein said proteins and fats are prepared from a non-dairy source. In some embodiments, the non-dairy cheese source can be a non-dairy milk obtained from nuts or seeds. In other embodiments, one or more isolated proteins from a non-animal source as used as a non-dairy cheese source.

In some embodiments, the plant source comprises one or more nuts or seeds. In some embodiments, the plant source is a flour compounded from one or more nuts or seeds (e.g., almonds and macadamia nuts). The term “nut” generally refers to any hard-walled, edible kernel. A nut can be a composite of a hard-shelled fruit and a seed, where the hard-shelled fruit does not open to release the seed. Exemplary nuts include, but are not limited to almonds, butternut, hickory nuts, wingnuts (*Pterocarya*), beech nuts, oak nuts, filberts, hornbeam nuts, soy nuts, cashews, brazil nuts, chestnuts, coconuts, hazelnuts, macadamia nuts, mongongo (*Schinziophyton rautanenii*), peanuts, pecans, pine nuts, pistachios, or walnuts. Any large, oily kernel found within a shell and used in food may be regarded as a nut. Plant seeds can include any embryonic plant enclosed in a seed coat. Exemplary plant seeds include, e.g., legumes such as, e.g., alfalfa, clover, peas, beans, lentils, lupins, mesquite, carob, soybeans, peanuts, cereals such as, e.g., corn, rice, wheat, barley, sorghum, millet, oats, triticale, rye, buckwheat, fonio, teff, amaranth, spelt, quinoa, angiosperms (e.g., flowering plants such as, for example, sunflowers) and gymnosperms. “Gymnosperm” generally refer to plant species that produce seeds that are generally not enclosed in a nut or fruit. Exemplary gymnosperms include conifers such

as, e.g., pine trees, spruce trees, and fir trees. In some embodiments, the non-dairy milk is not soy milk.

Non-dairy products or compositions include products or compositions where the constituent proteins, fats and/or small molecules can be isolated from, or secreted by, plants, bacteria, viruses, archaea, fungi, or algae. The non-dairy proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast cells, plant cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce proteins synthetically. In some cases, *in vitro* transcription/translation reactions are used to produce the proteins. Non-dairy products are generally not derived from cows, goats, buffalo, sheep, horses, camels, or other mammals. In some embodiments non-dairy products do not contain dairy proteins. In some embodiments non-dairy products do not contain dairy fats.

The non-dairy milk can be made by a method comprising preparing the nuts or plant seeds with processing steps such as sterilizing, blanching, shocking, decompounding, centrifugation, or washing. The nuts or seeds can be decompounded for example, by grinding or blending or milling the nuts in a solution comprising water. Alternative methods for decompounding the nuts or dried seeds can include crushing, tumbling, crumbling, atomizing, shaving, pulverizing, grinding, milling, water eroding (for example with a water jet), or finely chopping the nuts or plant seeds. In some embodiments, the decompounding step takes place in a blender, a continuous flow grinder, or a continuous flow mill. The decompounding can be followed by a sorting, filtering, screening, air-classification, or separation step. In some embodiments the decompounded nuts or seeds can be stored prior to the formation of a non-dairy milk. The aqueous solution can be added before, during, or after the decompounding.

The nuts or seeds used in some embodiments of the invention to make non-dairy milks may have contaminants on the surface which would make a non-dairy milk unsafe or unpalatable. Accordingly the nuts or seeds can be washed or blanched prior to use.

The nuts or seeds can also be sterilized to remove, reduce, or kill any contaminants on the surface of the nuts or seeds. A sterilization step can be an irradiation step, a heat step

(e.g. steam sterilization, flaming, or dry heat), or a chemical sterilization (e.g., exposure to ozone). In some embodiments the sterilization step kills more than 95% or more than 99% of microbes on the nuts or seeds.

In some embodiments, the non-dairy milk is centrifuged to remove insoluble solids. The non-dairy milk can have less than 1%, 5%, 10%, 20%, 30%, 40% or 50% of insoluble solids found in the non-dairy milk before centrifugation. The non-dairy milk can have 99%, 95%, 90%, 80%, 70%, 60% or 50% of insoluble solids removed by centrifugation. Centrifugation of the non-dairy milk is described herein.

In some embodiments the non-dairy milk is pasteurized or sterilized. The pasteurization can be high-temperature, short-time (HTST), “extended shelf life” (ESL) treatment, or ultra-high temperature (UHT or ultra-heat-treated). In some embodiments the pasteurization procedure includes pasteurizing the non-dairy milk at 164°F -167°F for 10 to 20 seconds (e.g., 10, 12, 14, 16, 18, or 20) seconds. In some embodiments, the microbial load in the non-dairy milk is reduced by exposure to UV light or high pressure pasteurization. A controlled chilling system can be used to rapidly bring non-dairy milk temperature down rapidly and store in a refrigerator at 36°F.

In some embodiments, the non-dairy milk is a non-dairy cream fraction.

In some embodiments, the non-dairy milk is an emulsion comprising one or more isolated and purified proteins and one or more isolated fats. In some embodiments the isolated and purified proteins are contained in a protein solution. The solution can comprise EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), NaSO₄ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), sucrose (0-50%), Urea (0-2M) or any combination thereof. The solution can have a pH of 3 to 11. In some embodiments, the one or more isolated and purified proteins accounts for 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein content of said protein solution. In some embodiments, the one or more isolated and purified proteins accounts for 0.1-5%, 1-10%, 5-20%, 10-40%, 30-60%, 40-80%, 50-90%, 60-95%, or 70-100% of the protein content of said protein solution. In some embodiments, the total protein content of the protein solution is about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75%, 1%, 1.5%, 2%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, or more

than 20% weight/volume. In some embodiments, the total protein content of the protein solution is 0.1-5%, 1-10%, 5-20%, or more than 20% weight/volume. In some embodiments, the protein content of the protein solution contains 1-3 isolated and purified proteins, 2-5 isolated and purified proteins, 4-10 isolated and purified proteins, 5-20 isolated and purified proteins, or more than 20 isolated and purified proteins.

In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. In some embodiments, the non-dairy milk is isolated from a non-dairy source (e.g., a plant). Non-limiting examples of plant sources include grain crops such as, e.g., maize, oats, rice, wheat, barley, rye, triticale (a wheat rye hybrid), teff (*Eragrostis tef*); oilseed crops including cottonseed, sunflower seed, safflower seed, *Crambe*, *Camelina*, mustard, rapeseed (*Brassica napus*); leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, or cabbage; or green matter not ordinarily consumed by humans, including biomass crops such as switchgrass (*Panicum virgatum*), *Miscanthus*, *Arundo donax*, energy cane, *Sorghum*, or other grasses, alfalfa, corn stover, kelp or other seaweeds, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, or turnips; plants from the legume family, such as, e.g., clover, *Stylosanthes*, *Sesbania*, vetch (*Vicia*), *Arachis*, *Indigofera*, *Leucaena*, *Cyamopsis*, peas such as cowpeas, english peas, yellow peas, or green peas, or beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts (*Arachis hypogaea*); coconut; or *Acacia*. One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used in the present invention. In some embodiments, the plant source is not soybeans.

Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the cheese products. Accordingly, in some embodiments, the one or more isolated proteins comprise an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70% of the total protein content of the source plant.

In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, or about 20-50% of the total weight of the dry matter of the source plant.

In particular embodiments, the one or more isolated proteins comprise an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo). RuBisCo is a particularly useful protein for cheese replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase). In particular embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

In some embodiments, the one or more isolated proteins comprise an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins,

globulins, vicilins, convicilins, legumins, conalbumin, gliadin, glutelin, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, or oil body proteins such as oleosins, caloleosins, or steroleosins.

In some embodiments, the one or more isolated proteins comprise proteins that
5 interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the cheese product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are
10 found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from moong bean, and 8S globulins from kidney bean.

In some embodiments, the one or more isolated and purified proteins is selected
15 from the group consisting of ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation
20 factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase), albumins, glycinins, conglycinins, globulins, legumins, vicilins, conalbumin, gliadin, glutelin, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, collagens, zein, kafirin, avenin, dehydrins, hydrophilins, late embryogenesis
25 abundant proteins, natively unfolded proteins, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin, pea globulins, pea albumins, or any other protease described herein.

In some embodiments, the isolated and purified proteins are concentrated using
30 any methods known in the art. The proteins may be concentrated 2-fold, five-fold, 10-fold, or up to 100 fold. The proteins may be concentrated to a final concentration of

0.001-1%, 0.05-2%, 0.1-5%, 1-10%, 2-15%, 4-20%, or more than 20%. Exemplary methods include, e.g., ultrafiltration (or tangential flow filtration), lyophilisation, spray drying, or thin film evaporation.

The fats used in preparing the emulsion can be from a variety of sources. In some
5 embodiments, the sources are non-animal sources (e.g., oils obtained from plants, algae, fungi such as yeast or filamentous fungi, seaweed, bacteria, Archae), including genetically engineered bacteria, algae, archaea or fungi. The oils can be hydrogenated (e.g., a hydrogenated vegetable oil) or non-hydrogenated. Non-limiting examples of
10 plant oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil; or margarine.

In some embodiments, the fat can be triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phospholipids such as phosphatidic
15 acids, lysophosphatidic acids, phosphatidyl cholines, phosphatidyl inositols, phosphatidyl ethanolamines, or phosphatidyl serines; sphingolipids such as sphingomyelins or ceramides; sterols such as stigmasterol, sitosterol, campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol, or episterol; free fatty acids such as palmitoleic acid, palmitic
20 acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid (C18:2), eicosanoic acid (C22:0), arachidonic acid (C20:4), eicosapentanoic acid (C20:5), docosapentaenoic acid (C22:5), docosahexanoic acid (C22:6), erucic acid (C22:1), conjugated linoleic acid, linolenic acid (C18:3), oleic acid (C18:1), elaidic acid (trans isomer of oleic acid), trans-vaccenic
25 acid (C18:1 *trans* 11), or conjugated oleic acid; or esters of such fatty acids, including monoacylglyceride esters, diacylglyceride esters, and triacylglyceride esters of such fatty acids.

The fat can comprise phospholipids, sterols or lipids. The phospholipids can comprise a plurality of amphipathic molecules comprising fatty acids (e.g., see above),
30 glycerol and polar groups. In some embodiments, the polar groups are, for example, choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol or inositol

phosphates. In some embodiments, the lipids are, for example, sphingolipids, ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

In some embodiments, the fat are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflower seeds, safflower seeds, sesame seeds, rape seeds, almonds, macadamia nuts, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, mango, butternut squash, cashews, brazilnuts, chestnuts, hazelnuts, peanuts, pecans, walnuts, and pistachios. Methods for preparing a cream fraction are described herein.

The addition of controlled amounts of one or more fats can result in different cheese properties, including but not limited to firmness, water retention, oil leakage, melt-ability, stretching, color, and creaminess. The fats can be in the form of unsaturated oil, saturated oil, washed cream fraction, and/or unwashed cream fraction. The unsaturated oils can include, e.g., olive oil, palm oil, soybean oil, canola oil (rapeseed oil), pumpkin seed oil, corn oil, sunflower oil, safflower oil, avocado oil, other nut oils, peanut oil, grape seed oil, sesame oil, argan oil, and rice bran oil. The saturated oils can include, e.g., coconut, palm, cocoa, cottonseed, mango oil, etc. The cream fraction can be made from, by way of example only, sunflower seeds, safflower, sesame seeds, rape seeds, almonds, macadamia, and pistachios. Preparation and isolation of cream fractions are described herein.

In some embodiments, an emulsion is prepared by isolating and purifying one or more proteins, preparing a solution comprising the one or more isolated and purified proteins, admixing said solution with one or more fats, thereby creating said emulsion. The ratio of protein solution to fats can be about 1:10, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, or 10:1. The ratio of protein solution to fats can be about 10:1-1:2, 1:4-2:1, 1:1-4:1, or 2:1-10:1. The emulsion can be used as a non-dairy milk for the preparation of a non-dairy cheese. By way of example only, 0%- 50% fat can be added to a protein solution by weight/weight or weight/volume.

Method of isolating and mixing cream and skim fractions

In some embodiments, the non-dairy milk can be further separated into a cream fraction and a skim fraction. In some embodiments, defined amounts of cream fraction can be mixed with defined amounts of skim fraction to produce a non-dairy milk with a controlled fat profile. In one aspect, the invention provides a method for creating a non-dairy cheese replica with a controlled fat profile. In some embodiments, the method comprises isolating a cream and skim fraction from a non-dairy milk, and mixing a defined amount of said cream and optionally said skim fraction to produce a mixture with a controlled fat profile. In some embodiments, said isolating comprises separating a non-dairy milk into a cream and skim fraction. In some embodiments the cream fraction is enriched in fats relative to the skim fraction. The cream fraction may contain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more of the fat content of said non-dairy milk prior to separation. The cream fraction may comprise a fat content that is at greater than the fat content of a skim fraction. The cream fraction may comprise a fat content that is increased by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or greater than 100% as compared to the skim fraction. The cream fraction may comprise a fat content that is 0.2-fold greater, 0.5-fold greater, 0.75-fold greater, 1-fold greater, 1.2-fold greater, 1.3-fold greater, 1.4-fold greater, 1.5-fold greater, 2-fold greater, 3-fold greater, 4-fold greater, 5-fold greater, 7.5-fold greater, 10-fold greater, 15-fold greater, 20-fold greater, or more than 20-fold greater than the fat content of the skim fraction.

The cream and skim fractions can, for example, be separated by gravity or by centrifugation. Centrifugation generally refers to a process of separating components in a composition using centrifugal force. The rate of centrifugation is specified by the angular velocity measured in revolutions per minute (RPM), or acceleration expressed as g. The term “g” generally refers to the acceleration produced by gravity at the Earth's surface. In some embodiments, the cream and skim fractions are separated by centrifugation at 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, or 10,000 RPM. In some embodiments, the cream and skim fraction are separated by centrifugation at about 500-2000, 1000-5000, 2000-7000, 4000-10,000, or greater than 10,000 RPM. In some embodiments, the cream and skim fractions are separated by centrifugation for about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50,

55, 60, or more than 60 minutes. In some embodiments, the cream and skim fractions are separated by centrifugation for 1-10, 5-30, 10-45, 30-60, or more than 60 minutes. In one embodiment, the cream and skim fractions are centrifuged in a JS-5.0 rotor at 5000 RPM for 30 mins. In some embodiments, the cream fraction and skim fraction are separated by centrifugal separation in a Flotwegg ac1500 or GEA ME55.

In some embodiments the cream fraction and the skim fraction are incompletely separated. The skim fraction and the cream fraction can be separated from the insoluble solids in a separation process. In some embodiments the skim fraction and the cream fraction are stored separately.

In some embodiments, a cream fraction can be used as a non-dairy cheese source.

In some embodiments, a protein solution can be admixed with a cream fraction to produce a non-dairy cheese source. Exemplary protein solutions are described herein.

In some embodiments, the method further comprises admixing the protein solution with a cream fraction isolated from a plant source. As used herein, the term “cream fraction” refers to an emulsion comprising fats, proteins and water that is enriched in fats as compared to an original emulsion (i.e., non-dairy milk). Exemplary cream fractions and methods of preparing the same are described herein. In some embodiments, a cream fraction is purified from a plant source, e.g., seeds, nuts, or legumes such as sunflowers, safflower, sesame seeds, rape seeds, almonds, macadamia, and pistachios. In some embodiments, the cream fraction is purified by blending seeds or nuts in water or a solution to create a slurry. Some embodiments includes blending seeds, nuts or legumes from 1 minute up to 30 minutes which could include a blending method by increasing the speed gradually to maximum speed over 4 minute, and blending at maximum speed for 1 minute. The solution can comprise EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), NaSO₄ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), sucrose (0-50%), urea (0-2M) or any combination thereof. The solution can have a pH of 3 to 11. See, Example 11. The slurry can be centrifuged by any method known in the art or as described herein.

Centrifugation can result in a separation of liquid layers and an insoluble solid pellet. The top layer can be used as the cream fraction. The lower layer can be used as whey, and the pellet is removed. The cream fraction can then be used as is immediately

after centrifugation, or can be further washed with the solutions described above or heated in solution. Washing and heating removes the unwanted color and flavor molecules, or unwanted grainy particles to improve the mouth feel. In particular, washing with a high pH buffer (e.g., above pH 9) can remove bitter tasting compounds and improve mouth feel, washing with urea can remove storage proteins, washing below pH 9, followed by washing with a pH above pH 9 can remove unwanted color molecules, and/or washing with salts can decrease taste compounds. Heating increases the removal of grainy particles, color and flavor compounds. Heating can be from 0-24 hours, at 25°C to 80°C. Washing and heating can remove unwanted colors and flavor notes, and can remove unwanted grainy particles. In some embodiments, washing and heating improves mouthfeel. In some embodiments, the resulting creamy fraction comprises seed storage proteins. In some embodiments, the seed storage proteins are substantially removed from the resulting creamy fraction.

In some embodiments, a defined amount of a cream fraction and a defined amount of a skim fraction are mixed to produce a mixture. The cream and/or skim fractions can be pasteurized or not pasteurized. In some embodiments, the defined amounts are defined by a user to result in a mixture with a controlled fat profile. In some embodiments, the cream and skim fractions are mixed at a defined ratio to result in a mixture with a controlled fat profile. In some embodiments the ratio of cream layer to skim layer in the non-dairy milk is about 100:1, 90:1, 80:1, 70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, or 1:60. In some embodiments the methods described herein comprise measuring the amount of skim layer and cream layer being added to a non-dairy milk.

The mixture may then be used as a non-dairy cheese source to prepare a cheese replica. It is understood that any of the non-dairy cheese sources as described herein may be used individually, or in any combination thereof, when practicing the methods described herein.

Flavoring components/methods

In another aspect, the invention provides methods for flavoring cultured non-dairy products, including sour cream, crème fraîche, yogurt, or cheese replicas. In some embodiments, the method comprises comparing a flavor note profile of a test non-dairy product with one or more flavor additives and/or one or more individual microbial strains described herein to a flavor note profile of a control non-dairy product without the additives and/or individual microbial strain. The texture and flavor profile of the non-dairy product (e.g. cheese replica) can be ascertained by any method known in the art or described herein. Exemplary methods of ascertaining flavor and texture can be by a taste test, e.g., a blind taste test, or using gas chromatography–mass spectrometry (GCMS).

GCMS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a dairy cheese and a cheese replica. For example volatile chemicals can be detected from the head space around a dairy cheese or a cheese replica. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around cheese is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the cheese replicas. The GCMS could be used to refine the cheese replica. In some embodiments the cheese replica has a GCMS profile similar to that of dairy cheese. In some embodiments the cheese replica has a GCMS profile identical to that of dairy cheese.

The flavor profile can be characterized by the presence and/or intensity of one or more flavor notes. Exemplary flavor notes include, but are not limited to butteriness, fruitiness, nuttiness, dairy, milky, chessy, fatty, fruity, pinnapple, waxy, buttery, tonka, dark fruit, citrus, sour, banana-like, sweet, bitter, musty, floral, goaty, sweaty, woody, earthy, mushroom, malty, spicy, pear, green, balsamic, pungent, oily, rose, fatty, butterscotch, orange, pine, carnation, melon, pineapple, vanilla, garlic, herbaceous, woody, cinnamon, rue, yogurt, peach, vanilla, hawthorn, and herbaceous. The flavor notes may be associated with the release of one or more volatile compounds. The flavor profile can be characterized by the absence or reduction in the intensity of one or more

flavor notes. Exemplary flavor notes include: planty, beany, soy, green, vegetable, nutty, dirty, and sour.

Exemplary volatile compounds include, e.g., gamma-nonanoic lactone, gamma-undecalactone, gamma-decalactone, delta-tetradecalactone, S-methyl thiopropionate, 5 delta-tridecalactone, delta-tetradecalactone, δ -tetradecalactone, butyl butyryllactate, 2,3-hexandione, methyl hexanoate, butyrolactone, propanoic acid, 2-methyl propanoic acid, methyl isobutyl ketone, gamma octalactone, delta octalactone, gamma nonalactone, 5-hydroxy-4-octanone, 2-ethyl-1-hexanol, octane, ethanol, 2,3-butanedione, 2 heptanone, 1-butan-2-ol, acetoin, butanoic acid, nonanal, acetic acid, 1,3 butanediol, methyl-3-buten-1-ol, 10 methanol, hexanol, dimethyl-benzene, ethyl-benzene, indole, limonene, toluene, acetophenone, pentan-2,3-dione, 2-pentanone, 2-heptanone, 2-nonanone, acetone, butanone, 2-methylpropionic acid, butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, pentanoic acid, 4-methylpentanoic acid, hexanoic acid, octanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, 15 octadecanoic acid, oleic acid, linoleic acid, linolenic acid, propanol, butanol, pentanol, hexanol, heptanol, octanol, propan-2-ol, butan-2-ol, pentan-2-ol, hexan-2-ol, heptan-2-ol, nonan-2-ol, undecan-2-ol, octen-3-ol, octa-1,5-dien-3-ol, 3-methyl-2-cyclohexenol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol, 3-methylpentanol, phenylmethanol, 2-phenylethanol, 2-phenyl-ethan-2-ol, propan-2-one, butan-2-one, 20 pentan-2-one, hexan-2-one, heptan-2-one, octan-2-one, nonan-2-one, decan-2-one, undecan-2-one, dodecan-2-one, tridecan-2-one, pentadeca-2-one, pentan-3-one, octan-3-one, 3-methylpentan-2-one, 4-methylpentan-2-one, methylhexan-2-one, hydroxypropan-2-one, hept-5-en-2-one, 4-methylpent-3-en-2-one, octen-3-one, octa-1,5-dien-3-one, nonen-2-one, undecen-2-one, methylfuryl ketone, phenylpropan-2-one, propiophenone, 25 methyl butanoate, methyl hexanoate, methyl octanoate, methyl decanoate, methyl tetradecanoate, methyl hexadecanoate, methyl cinnamate, ethyl formate, ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl-3-methyl butanoate, propyl acetate, propyl butanoate, butyl formate, butyl acetate, amyl acetate, isoamyl formate, isoamyl acetate, 30 isoamyl propanoate, isoamyl butanoate, diethyl phthalate, dimethyl phthalate, 2-phenylethyl acetate, 2-phenylethyl propanoate, 2-phenylethyl butanoate, 3-

methylthiopropanol, methanethiol, hydrogen sulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide, methylethyl disulfide, diethyl disulfide, 2,4-dithiapentane, methional, 3-methylthio-2,4-dithiapentane, 2,4,5-trithiahexane, 1,1-bis-methylmercaptodisulfide, methanethiol acetate, methyl thiopropanoate, methyl
5 thiobenzoate, thiophen-2-aldehyde, methylindole, p-ethylphenol, p-cresol, acetaldehyde, butanal, 2-methylbutanal, 3-methylbutanal, 2-methylpropanal, hexanal, heptanal, nonanal, 2-methylbuten-2-al, benzaldehyde, 3-methylheptyl acetate, 1-butanol, 1-butanol, 3-methyl, 1-heptanol, formic acid, 1-hexanol-2,ethyl, 1-octanol, 2-butanone, 2-hepten-1-ol, 2-hexanone, heptanal, 2-octen-1-ol, 1-octen-3-ol, 2-pentanone, 2,3-butanedione, 3-
10 buten-1-ol, 5-Hepten-2-one, octane, ethanol, 2,3-butanedione, 2 heptanone, 1-butanol, butanoic acid, nonanal, acetic acid, 1,3 butanediol, methyl-3-buten phenylethyl alcohol, toluene, 1-pentanol, 3-octene-1-ol, 2 octene-1-ol, 2-undecanone, 1-octanol, Benzaldehyde, 1-heptanol, 2-heptanone, 4-methyl-2-nonanone, 2-methyl-2-nonanol, 1-hexanol, 2-methyl 2-propanol, Ethanol, 3 methyl 1-butanol, 1-hexanol, 2-methyl 2-
15 nonanol, 2-nonanone, 2-heptanone, 4-methyl, 1-heptanol, 1-octanol, 2 octene-1-ol, 3-octene-1-ol, 1-octanol, 1-heptanol, 2-heptanone, 4-methyl-2-nonanone, 2-dodecanol, 2-dodecanone, 3-decene 1-ol acetate, benzyl alcohol, phenylethyl alcohol, 2-methoxy 4-vinylphenol, 3-decene 1-ol acetate, 2-dodecanone, 2-dodecanol, or 2-methoxy 4-vinylphenol.

20 In some embodiments, the improved flavors are due to the decreased levels of volatile flavor compounds, such as, e.g., benzaldehyde, 2-methyl-2-propanol, acetophenone, octane, ethanol, 2-pentanone, pentanal, 2 heptanone, 1-butanol, 1-hexanol, 3-methyl-1-butanol, 2-methyl-2-nonanol, 2-nonanone, 1-octanol, 2-undecanone, 2-octene-1-ol (Z), 1-octene-3-ol, acetophenone, 4-methyl-2 heptanone, nonanal, acetic acid,
25 3-methyl furan, 2-methyl furan, 1-hexanal, furan, 2-methyl-2-propanol, pyrazine, 1-heptanal, 2-ethyl furan, 2-pentyl furans, or 1,3 butanediol.

In some embodiments, the method further comprises preparing a cultured non-dairy product such as a cheese replica, yogurt, sour cream, or crème fraiche with a controlled flavor profile, by the controlled addition of defined combinations of flavor
30 additives, described herein, to the non-dairy e source at any time point of the replica making process. Exemplary additives and specific combinations are described herein.

Flavor Generators

Control of flavor by addition of bacteria/microbes

Flavor compounds can be generated by microbes in the non-animal derived material used for producing many different non-dairy products described herein, including cheese replicas. The methods of flavoring generally include contacting a non-dairy milk or protein solution with one or more microbes, and preparing a cultured non-dairy product from the non-dairy milk. Microbes such as bacteria, yeast, or mold can be used to create a product with a desired flavor profile or be used as a component of the flavor in a product, as bacteria can create desirable flavors (e.g., buttery, creamy, dairy, or cheesy) in a neutral, planty, or beany product.

Exemplary non-dairy milks are described herein. Any of the non-dairy cheese milks or combinations thereof may be contacted with one or more microbes (e.g., a controlled amount of bacteria) to control the flavor of a resulting cultured non-dairy product such as a cheese replica. The microbes can be selected from bacteria, yeast, or molds. The bacteria can comprise mesophilic and/or thermophilic bacteria. The bacteria can comprise bacteria from a commercial starter. Exemplary commercial starters are described herein.

Flavor production in the replicas can be controlled by the use of one or more microbes e.g., one or more bacteria, yeast, or molds, including but not limited to Flavor production in the replicas can be controlled by the use of one or more microbes e.g., one or more bacteria, yeast, or molds, including but not limited to *Lactococcus* species such as *Lactococcus lactis lactis* (LLL, used alone or as a component of commercial mix MA11), *Lactococcus lactis cremoris* (LLC, used alone or as a component of commercial mix MA11), or *Lactococcus lactis biovar diacetylactis* (LLBD, often used as commercial culture MD88), a *Lactobacillus* species such as *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, or *Lactobacillus rhamnosus*, a *Leuconostocaceae* species such as *Leuconostoc mesenteroides cremoris* (LM), a *Streptococcus* species such as *Streptococcus thermophiles* (ST, often used as commercial culture TA61) a *Pediococcus* species such as *Pediococcus pentosaceus*, a *Clostridium* species such as *Clostridium butyricum*, a

Staphylococcus species such as *Staphylococcus xylosus* (SX), a *Brevibacterium* species such as *Brevibacterium linens*, a *Propionibacteria* species, a *Penicillium* species such as *Penicillium candidum*, *Penicillium camemberti*, or *Penicillium roqueforti*, a *Debaryomyces* species such as *Debaryomyces hansenii*, a *Geotrichum* species such as *Geotrichum candidum*, a *Corynebacteria* species, a *Verticillium* species such as *Verticillium lecanii*, a *Kluyveromyces* species such as *Kluyveromyces lactis*, a *Saccharomyces* species such as *Saccharomyces cerevisiae*, a *Candida* species such as *Candida jefer* or *Candida utilis*, a *Rhodospiridium* species such as *Rhodospiridium infirmominiatum*, a *Micrococcus* species., a *Halomonas* species., a *Psychrobacter* species. In some embodiments, lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, or *Streptococcus* are used. In some embodiments, the bacteria do not comprise *Lactobacillus acidophilus* strains. In some embodiments, a yeast such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and/or *Debaromyces hansenii* can be used. In some embodiments, a mold can be *Penicillium candidum*, *Penicillium camemberti*, *Penicillium roqueforti*, *Geotrichum candidum*, or a combination thereof.

In some embodiments, one or more of the follow microbes are used: *Pediococcus pentosaceus*, *Clostridium butyricum*, *Lactobacillus delbrueckii lactis*, *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Staphylococcus xylosus*, and *Brevibacterium linens*.

The one or more microbes can be cultured alone (e.g., bacteria, yeast, or mold alone), or in combination with two ore more microbes (e.g., two different bacteria, two different yeast, two different molds, a bacteria and a yeast, a bacteria and a mold, or a yeast and a mold). When two or more microbes are used, the microbes can be co-cultured or sequentially cultured, i.e., one microbe can be cultured for a length of time before adding another microbe. Particular good combinations for flavor generation in replicas are pre-culturing with SX, followed by either TA61 or MD88, or MD88 co-cultured with MA11.

The growth conditions of microbes also can control flavor generation in replicas. The temperature of microbes growth ranging from 4°C to 45°C can control the amount and type of flavor compounds produced in replicas. The amount of aeration by shaking

(e.g., 0 to 300 rpm) changes the flavor productions of many different bacteria in non-dairy media. Greater aeration during culturing by either SX, TA61, or MD88 generates more desired cheese and buttery compounds. Aeration also decreases the undesired flavor compounds. Desired cheese compounds such as 2-heptanone increase when SX, MD88, or TA61 are cultured with aeration. MD88's production of hexanoic methyl ester in cheese replicas is also modulated by aeration. An increase in aeration of SX during culturing in soymilk greatly increases 3-methyl and 2-methyl butanoic acid production and decreases the amounts of undesirable aroma compounds such as 2-ethyl furan or 2-pentyl furan in cheese replicas.

The amount of time the one or more microbes is cultured also can modulate the amount and types of flavor compounds. Culturing can range from 1 hour to multiple days. In some embodiments, one or more microbes and the non-dairy milk are incubated together for a length of time ranging from 1 min-60 minutes, 0.5-5 hours, 3-10 hours, 6-15 hours, 10-20 hours, or more than 20 hours. Most buttery compounds are created within the first 10 hours, while additional cheese compounds typically require 24-48 hours or more hours. Butyrolactone, a creamy, milky note compound is created in non-dairy media by MD88 and MA11 only after 20 hours of culturing in soymilk.

The one or more microbes also can be added at different inoculums, e.g., 10^2 - 10^9 cfu/mL or even greater. The phase of growth (i.e., stationary phase versus exponential phase) and the cell density of the bacterial culture affect the flavor compound profile of the medium. Higher inocula of a starter culture can protect the replica from unwanted microbial contamination (e.g., bacterial contamination). Therefore, an inoculum of 10^6 - 10^9 cfu/mL is usually used.

Flavor production by the one or more microbes also can be modulated by directing the metabolic pathways, e.g., by modulating their nitrogen source, carbon source, additional available nutrients, and growth conditions. Non-limiting examples of additives that can be used are shown in Table A. The additives can be added to the media at the same time as the bacteria or any time during the creation of the replica. When sequential culturing occurs additional additives can be added at the same time that the additional strains are inoculated.

TABLE A**Additives used to control flavor production by microbes in non-dairy replicas**

FeCl ₂	Asp	C8:0, Caprylic acid
MgCl ₂	Cys	C10:0, Capric acid
CaCl ₂	Glutamine	C12:0, lauric acid
MnSO ₄	Glutamate	C14:0, myristic acid
CoCl ₂	Gly	C16:0, palmitic acid
CuSO ₄	His	C18:0, stearic acid
ZnSO ₄	Ile	C16:1, palmitoleic acid
Adenine	Lue	Coconut Oil
Guanine	Lys	Castor Oil
Inosine	Met	Palm Oil
Uracil	Phe	Palm Fruit Oil
Xanthine	Pro	Jobba Oil
Pyridoxamine	Ser	Sunflower Oil
Pyridoxine	The	Mango Butter
D-sorbitol	Trp	Ala
Citric Acid	Tyr	Arg
Lactic Acid	Val	Asn
α -ketoglutarate	Riboflavin	FAD
Pyruvic Acid	Thiamine	NAD
Ororic acid	Lipoic Acid	Biotin
oxalic acid	Nicotinic acid	Pantothenate
Ascorbic Acid	CoA hydrate	B12
Succinic acid	propanoic acid	Folic Acid
p-aminobenzoic acid	C4:0, butyric acid	
DL-Malate	C6:0, caproic acid	

The amount and type of sugar is a large driver of the type of flavors produced, including buttery compounds. In some embodiments, sugars are naturally present in the non-dairy milk. By way of example only, sucrose is present in a variety of nuts such as, e.g., almonds, which can be used to provide a non-dairy milk used for preparing the cheese replica. In some embodiments, a controlled amount of one or more sugars is added to the non-dairy milk or non-dairy cheese source.

In some embodiments, the sugar is a monosaccharide, including but not limited to glucose (dextrose), fructose (levulose), galactose, mannose, arabinose, xylose (D- or L-

xylose), and ribose, a disaccharide including but not limited to sucrose, lactose, melibiose, trehalose, cellobiose and maltose, a sugar alcohol such as arabitol, mannitol, dulcitol, or sorbitol, sugar acids such as galacturonate, glucuronate, or gluconate, oligosaccharides and polysaccharides such as glucans, starches such as corn starch, potato starch, pectins such as apple pectin or orange pectin, raffinose, stachyose and dextrins, a plant cell wall degradation product, β -galactosides, β -glucosides such as salicin, and/or sugar derivatives such as N-acetylglucosamine. In particular embodiments, the sugars are chosen from the group consisting of sucrose, maltose, glucose and fructose.

The relative growth of each isolated strain can be controlled by the addition of said one or more sugars. The addition of said one or more sugars can result in nondairy cheese replicas with significantly improved texture and flavor. A user, e.g., an individual or plurality of individuals practicing the invention, can select specific isolated strains and add controlled amounts of the selected strains to create a non-dairy cheese replica with a desired flavor and texture profile. A user can additionally select specific sugars and add controlled amounts of the selected sugars along with controlled amounts of specific isolated strains to create a non-dairy cheese replica with a desired flavor and texture profile. Table B provides non-limiting examples of bacteria culturing conditions for producing dairy flavor notes created in non-dairy cheese or other non-dairy products.

TABLE B

Genus	species	sub species	growth temperature	Aeration	sugar preference (based on generation time)
<i>Lactococcus</i>	<i>lactis</i>	<i>cremoris</i>	30C (25-40C)	yes	glucose=fructose >maltose >sucrose*
<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	30C (25-40C)	yes	glucose=fructose=sucrose >maltose*
<i>Leuconostoc</i>	<i>mesenteroides</i>	<i>cremoris</i>	30C (25-35C)*	yes	glucose=fructose=maltose >sucrose
<i>Lactococcus</i>	<i>lactis</i>	<i>biovar diacetylactis</i>	30C (25-40C)	yes	glucose >fructose=sucrose=maltose
<i>Streptococcus</i>	<i>thermophilus</i>		37C	no	glucose >maltose
<i>Lactobacillus</i>	<i>delbrueckii</i>	<i>lactis</i>	37C	no	glucose >maltose
<i>Lactobacillus</i>	<i>delbrueckii</i>	<i>bulgaricus</i>	37C	no	glucose >maltose
<i>Lactobacillus</i>	<i>helveticus</i>		37C	no	glucose >maltose
<i>Lactobacillus</i>	<i>plantarum</i>		37C	no	glucose=maltose
<i>Lactobacillus</i>	<i>casei</i>		37C	no	glucose >maltose
<i>Lactobacillus</i>	<i>rhamnosus</i>		37C	no	glucose >maltose
<i>Staphylococcus</i>	<i>xylosus</i>		30C	yes	glucose >maltose
<i>Pediococcus</i>	<i>pentosaceus</i>		30C		maltose >glucose
<i>Clostridium</i>	<i>butyricum</i>		37C	no	glucose >maltose

* Large variation in different strains of this subspecies

The effect of different sugars and microbes on flavor production also depends on the type and composition of the starting material (e.g., the starting material can include any non-animal derived material, including but not limited to soymilk, pea protein, moong protein, soy protein, coconut milk, yeast extract, protein hydrolysate, derived media, and synthetic media), and the amino acid composition of the proteins in the starting materials, the types of sugars and carbohydrates included, and the types of fats, triglycerides, and/or free fatty acids present. The amino acid composition of the protein can be broken down by enzymes, such as those created by the microbes and those added as part of the recipe, and the resulting amino acids or peptides can serve as precursors to particular flavor molecules. Synthetic media refers to using ammonium for the nitrogen source, and a defined sugar for the carbon source, with any other additives. The starting material can comprise isolated purified proteins, or crude plant extracts.

Acetoin and diacetyl (“buttery” compounds) are created by MD88 (LLBD) in the greatest abundance in a yeast extract medium with maltose added. On the other hand MD88 creates more of these buttery compounds in soymilk with glucose added. Acetoin and diacetyl are created in even higher amounts by MD88 and TA61 (ST) with the addition of citrate or pyruvate. Acetoin/diacetyl and 2,3-hexandione concentrations all increase in response to increased citrate concentration in cheese replicas made with strain MD88.

The addition of amino acids (see Table A) can directly control the production of particular flavor compounds in non-dairy replicas. The creation of these flavor compounds contributes to the overall flavor profile of the replica. Methionine can be added to cheese replica or media to produce methional by SX or dimethyl trisulfide by *Brevibacterium*. Methional and dimethyl trisulfide are two sulfur compounds that are found in many dairy cheeses, and contribute to the aged character of cheddar. Leucine added to soymilk, yeast extract media, or pea proteins significantly increase the 3-methyl butanoic acid production by SX bacteria, adding cheesy notes. Adding multiple compounds can further control flavor production by bacteria, e.g. alpha-ketoglutarate with leucine increases the 3-methyl butanoic acid production by SX bacteria. 3-methyl butanoic acid has a cheesy aroma and taste, it is similar to butanoic acid, which is a key flavor in American and cheddar cheeses. Organic acids can also control flavor production

by bacteria in non-dairy replicas. Oxalate added to yeast extract media and cultured by *Brevibacterium* creates cheese compounds butanoic acid, 3-methyl butanoic acid, and 2,6-nonadienal, and the flavors generated were described as “aged cheese”. The creation of buttery compounds can be controlled by the addition of citrate, xanthine, and pyruvate, when added to cultures of TA61. Adding citric acid, pyruvate, riboflavin, and copper can affect the cheese and buttery flavor production by MD88. Other important aroma compounds like butanoic acid derivatives and 2-heptanone are produced when isoleucine, proline, alanine, malate, inosine, Fe^{2+} , Mg^{2+} , serine, and thiamine are added into replicas. Additionally, ZnSO_4 , citric acid, lactic acid, pyruvic acid, succinic acid, malic acid, aspartic acid, lysine, tyrosine, valine, and jojoba oil allow for the creation of desired cheese flavor compounds by TA61 in replicas. The affect of each additive depends on the composition of the other stating materials.

Other additives can be added before bacteria culturing or after culturing. These include but are not limited to fruit extracts (0.0001% -0.2% wt/vol), fruit puree (peach, pineapple strawberry, mango, papaya, plum, etc) (0.001% -2% wt/vol), vegetable puree (potato, yam, onion, garlic, or broccoli) (0.001% -2% wt/vol), molasses (0.001% -2% wt/vol), yeast extracts (0.001% -2% wt/vol), protein hydrolysates (0.01% -10% wt/vol), red or white miso (0.01% -2% wt/vol), coconut milk (0.5%-60%), malt extracts (0.01% -2% wt/vol), or coconut cream (0.5%-60%), and combinations thereof. Malt extracts and molasses can add cheese flavor molecules like 3-methyl butanal, 2-methyl butanal, 2-heptanone, butanoic acid, or butyrolactone. Replicas with the addition of peach extract or puree were described by trained flavor scientists as having more cream flavor. Replicas with the addition of papaya were described as more cheesy by trained flavor scientist and had an increase in 3-methyl butanoic acid by GCMS. Red miso added to MD88 cultures, and white miso added to TA61 cultures resulted in improved flavor complexity and a perceived decrease in astringency by a trained flavor scientist.

Yeast extract can control bacteria growth and flavor generation, and contribute different starting flavors. There are many vitamins in yeast extracts that improve the growth of TA61 in replicas. All yeast extracts are not the same. TA61 growth was greater with Flavor House Flavor Spark, and BioSpringer Yeast Extract 2020, compared to other yeast extracts. BioSpringer Yeast Extract 2020 supports good growth and decent

flavor development for MD88 and TA61. Yeast extract can be added between 0.01% -2% wt/vol of media to improve growth and flavor production by the bacteria. Yeast extract can be added at 0.02%-0.1% improve growth and flavor production by the bacteria. Yeast extracts themselves can also provide certain flavors to the product including, brothy, whey, nutty, savory, roasted, malty, caramel, cooked-milk, light-sulfurous, and slight cheese-like. Yeast extracts also supports the production of flavor compounds by TA61, MA88, and SX that lead to a more cheesy and complexly flavored replicas.

In some embodiments, the one or more microbes, the non-dairy cheese source, and the one or more optional components that can be used to alter flavor (e.g., sugars, fats, carbohydrates, vitamins, organic acids, nucleotides, or food products) are incubated together for a sufficient period of time to achieve a desired pH. The pH can range from pH 3-5, 4-6, or 4.3-5.7. The desired pH can be pH 6 or lower, pH 5 or lower, or pH 4 or lower. Culturing the material by bacteria in some cases decreases the pH to 6.5, 6, 5.5, 5, 4.5, 4, or 3.5, while in other cases, flavors are generated with no change in pH. Culturing with *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and/or *Streptococcus* typically results in a decrease in pH with most starting material, while culturing with *Staphylococcus*, *Brevibacterium*, and/or *Clostridium* typically has little or no effect on the pH.

In some embodiments, the method further comprises solidifying said non-dairy cheese source. Methods of solidifying are described herein.

In some embodiments, the method comprises isolating a plurality of microbial strains from a heterogeneous population, e.g., a commercial starter or a probiotic (e.g., Rejuvelac). In some embodiments, the isolated microbial strains are each characterized according to defined criteria. The defined criteria may include, for example, growth rates in non-dairy cheese sources comprising different sugars added therein. The defined criteria may include the contribution of each isolated strain to a flavor palette by characterizing flavor notes from a cheese replica with the isolated strain added therein as compared to a control cheese replica without the isolated strain added therein. The isolated microbial strains may be characterized by genetic sequencing, and/or determining sequences unique to each of the isolated strains.