ascorbate, sodium bisulfite, sodium erythorbate, sodium metabisulfite, sodium sulfite, superoxide dismutase, sodium thioglycolate, sorbityl furfural, thiodiglycol, thiodiglycolamide, thiodiglycolic acid, thioglycolic acid, thiolactic acid, thiosalicylic acid, tocophereth-5, tocophereth-10, tocophereth-12, tocophereth-18, tocophereth-50, tocopherol, tocophersolan, tocopheryl acetate, tocopheryl linoleate, tocopheryl nicotinate, tocopheryl succinate, and tris(nonylphenyl)phosphite.

*e. Thickening Agents*

[0049] Thickening agents, including thickener or gelling agents, include substances which that can increase the viscosity of a composition. Thickeners include those that can increase the viscosity of a composition without substantially modifying the efficacy of the ingredients within the composition. Thickeners can also increase the stability of the compositions of the present invention. Non-limiting examples of additional thickeners that are known to those of ordinary skill in the art can be used in the context of the present invention (*e.g.*, U.S. Patent Nos. 5,087,445; 4,509,949; 2,798,053; International Cosmetic Ingredient Dictionary and Handbook, 10th Ed., 2004). Examples include carboxylic acid polymers, crosslinked polyacrylate polymers, polyacrylamide polymers, polysaccharides, and gums. Examples of carboxylic acid polymers include crosslinked compounds containing one or more monomers derived from acrylic acid, substituted acrylic acids, and salts and esters of these acrylic acids and the substituted acrylic acids, wherein the crosslinking agent contains two or more carbon-carbon double bonds and is derived from a polyhydric alcohol. Examples of commercially available carboxylic acid polymers include carbomers, which are homopolymers of acrylic acid crosslinked with allyl ethers of sucrose or pentaerytritol (*e.g.*, Carbopol™ 900 series from B. F. Goodrich).

*f. Silicone Containing Compounds*

[0050] In non-limiting aspects, silicone containing compounds include any member of a family of polymeric products whose molecular backbone is made up of alternating silicon and oxygen atoms with side groups attached to the silicon atoms. By varying the -Si-O- chain lengths, side groups, and crosslinking, silicones can be synthesized into a wide variety of materials. They can vary in consistency from liquid to gel to solids.

[0051] The silicone containing compounds that can be used in the context of the present invention include those described in this specification or those known to a person of ordinary skill in the art. Non-limiting examples include silicone oils (*e.g.*, volatile and non-

volatile oils), gels, and solids. The silicon containing compound can be a silicone oil such as a polyorganosiloxane. Non-limiting examples of polyorganosiloxanes include dimethicone, cyclomethicone, polysilicone-11, phenyl trimethicone, trimethylsilylamodimethicone, stearoxytrimethylsilane, or mixtures of these and other organosiloxane materials in any given ratio in order to achieve the desired consistency and application characteristics depending upon the intended application (*e.g.*, to a particular area such as the skin, hair, or eyes). A "volatile silicone oil" includes a silicone oil have a low heat of vaporization, *i.e.* normally less than about 50 cal per gram of silicone oil. Non-limiting examples of volatile silicone oils include: cyclomethicones such as Dow Corning 344 Fluid, Dow Corning 345 Fluid, Dow Corning 244 Fluid, and Dow Corning 245 Fluid, Volatile Silicon 7207 (Union Carbide Corp., Danbury, Conn.); low viscosity dimethicones, *i.e.* dimethicones having a viscosity of about 50 cst or less (*e.g.*, dimethicones such as Dow Corning 200-0.5 cst Fluid). The Dow Corning Fluids are available from Dow Corning Corporation, Midland, Michigan. Cyclomethicone and dimethicone are described in International Cosmetic Ingredient Dictionary, 10th Ed., 2004 as cyclic dimethyl polysiloxane compounds and a mixture of fully methylated linear siloxane polymers end-blocked with trimethylsiloxy units, respectively. Other non-limiting volatile silicone oils that can be used in the context of the present invention include those available from General Electric Co., Silicone Products Div., Waterford, N.Y. and SWS Silicones Div. of Stauffer Chemical Co., Adrian, Michigan.

**2. Pharmaceutical Actives**

[0052] Pharmaceutical active agents are also contemplated as being useful with the compositions of the present invention. Non-limiting examples of pharmaceutical active agents include anti-acne agents, agents used to treat rosacea, analgesics, anesthetics, anorectals, antihistamines, anti-inflammatory agents including non-steroidal anti-inflammatory drugs, antibiotics, antifungals, antivirals, antimicrobials, anti-cancer actives, scabicides, pediculicides, antineoplastics, antiperspirants, antipruritics, antipsoriatic agents, antiseborrheic agents, biologically active proteins and peptides, burn treatment agents, cauterizing agents, depigmenting agents, depilatories, diaper rash treatment agents, enzymes, hair growth stimulants, hair growth retardants including DFMO and its salts and analogs, hemostatics, kerotolytics, canker sore treatment agents, cold sore treatment agents, dental and periodontal treatment agents, photosensitizing actives, skin protectant/barrier agents, steroids including hormones and corticosteroids, sunburn treatment agents, sunscreens, transdermal

actives, nasal actives, vaginal actives, wart treatment agents, wound treatment agents, wound healing agents, *etc.*

**D Vehicles**

[0053] Compositions of the present invention can be incorporated into all types of vehicles. Non-limiting examples of suitable vehicles include emulsions (*e.g.*, water-in-oil, water-in-oil-in-water, oil-in-water, oil-in-water-in-oil, oil-in-water-in-silicone, water-in-silicone, silicone-in-water emulsions), creams, lotions, solutions (both aqueous and hydro-alcoholic), anhydrous bases (such as lipsticks and powders), gels, and ointments or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (*see, e.g.*, Remington's, 1990 and International Cosmetic Ingredient Dictionary and Handbook, 10th Ed., 2004)). Variations and other appropriate vehicles will be apparent to the skilled artisan and are appropriate for use in the present invention. In certain aspects, it is important that the concentrations and combinations of the compounds, ingredients, and agents be selected in such a way that the combinations are chemically compatible and do not form complexes which precipitate from the finished product.

**E Cosmetic Products and Articles of Manufacture**

[0054] The composition of the present invention can also be used in many cosmetic products including, but not limited to, sunscreen products, sunless skin tanning products, hair products, finger nail products, moisturizing creams, skin benefit creams and lotions, softeners, day lotions, gels, ointments, foundations, night creams, lipsticks, cleansers, toners, masks, or other known cosmetic products or applications. Additionally, the cosmetic products can be formulated as leave-on or rinse-off products. In certain aspects, the compositions of the present invention are stand-alone products.

**F Kits**

[0055] Kits are also contemplated as being used in certain aspects of the present invention. For instance, emulsion compositions of the present invention can be included in a kit. A kit can include a container. Containers can include a bottle, a metal tube, a laminate tube, a plastic tube, a dispenser, a pressurized container, a barrier container, a package, a compartment, a lipstick container, a compact container, cosmetic pans that can hold cosmetic compositions, or other types of containers such as injection or blow-molded plastic containers

into which the dispersions or compositions or desired bottles, dispensers, or packages are retained. The kit and/or container can include indicia on its surface. The indicia, for example, can be a word, a phrase, an abbreviation, a picture, or a symbol.

[0056] The containers can dispense a pre-determined amount of the emulsion composition. In other embodiments, the container can be squeezed (*e.g.*, metal, laminate, or plastic tube) to dispense a desired amount of the emulsion composition. The emulsion composition can be dispensed as a spray, an aerosol, a liquid, a fluid, or a semi-solid. The containers can have spray, pump, or squeeze mechanisms. A kit can also include instructions for employing the kit components as well the use of any other emulsion compositions included in the container. Instructions can include an explanation of how to apply, use, and maintain the emulsion compositions.

## EXAMPLES

[0057] The following examples are included to demonstrate certain non-limiting aspects of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow representative techniques discovered by the inventor to function well in the practice of the invention. However, those of skill in the art should appreciate that changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

### EXAMPLE 1
### Methods of Making Glyceryl/Acid or Glycol Acid Compounds

[0058] An esterification process can be used for producing glyceryl/acid and glycol/acid compounds. A glyceryl or glycol molecule and an acid molecule are added to a multi-necked round bottom flask equipped with a heating mantle, a condenser, a Dean-Stark trap and a thermometer. A solvent is chosen that will allow a high enough reflux temperature to accommodate the reaction. The reaction is run until the calculated amount of water is collected in the trap signifying that the limiting reagent is exhausted. The by-product can be compounds other than water in some processes. For instance, if a methyl salicylate is used in place of salicylic acid, methanol will be the by-product rather than water, and the methanol may escape through the condenser or remain in the reaction flask. The methanol can be removed when the ester is extracted.

## EXAMPLE 2
## UV Absorption Data for Glyceryl Mono-Salicylate

[0059] UV absorption data for glyceryl mono-salicylate is summarized in Table 1 below:

**Table 1 (UV absorption data*)**

| Glyceryl Mono-Salicylate | Data |
|---|---|
| Avg. Mol. Wt. | 242.95 |
| Molar Absorptivity (λ max 238nm) | 12065.7 |
| Molar Absorptivity (λ max 307nm) | 6126.4 |

*Data obtained by using a UV/Vis spectrometer using standard USP 197U testing procedures.

## EXAMPLE 3
## Compositions

[0060] Non-limiting examples of compositions of the present invention are described in Tables 1-3.

**Table 2* (Exfoliation composition)**

| Ingredient | % Concentration (by weight) |
|---|---|
| SD Alcohol 40-B | 56.00 |
| Glyceryl mono-salicylate** | 7.50 |
| Water | 29.00 |
| Mineral oil | 3.00 |
| Sepigel 305 (thickener) | 4.00 |

*The composition was prepared as follows: Ingredients were cold-mixed and added to a beaker in the listed order prior to the addition of the next ingredient. The pH of the composition was adjusted to approximately 4.3 by using 1N HCl solution.
** Glyceryl mono-salicylate can be substituted with other glyceryl/acid and glycol/acid compounds.

**Table 3* (Moisturizer**)**

| Ingredient | % Concentration (by weight) |
|---|---|
| **Phase A** | |
| Water | 66.44 |
| Xanthum gum | 0.10 |
| Methyl paraben | 0.15 |
| Propyl paraben | 0.10 |
| Citric acid | 0.01 |
| **Phase B** | |
| Cetyl alcohol | 4.00 |
| Glyceryl stearate + PEG 100 | 4.00 |
| Octyl palmitate | 4.00 |
| Dimethicone | 1.00 |
| Tocopheryl acetate | 0.20 |
| **Phase C** | |
| SD Alcohol 40-B | 15.00 |
| Glyceryl mono-salicylate*** | 5.00 |

*The composition was prepared as follows: Phases A and B were heated to approximately 70°C-75°C in separate beakers while mixing. Phase B was added to Phase A when both phases were at approximately 70°C-75°C. The Phase A+B mixture was cooled to <40°C while mixing. Phase C was subsequently added to the Phase A+B mixture, and the mixture was cooled to room temperature (approximately 20°C-25°C) while mixing.
**Formulation showed statistically significant moisturization for 6 hours after application to skin when compared to the baseline control ($p<0.05$) (data not shown).
***Glyceryl mono-salicylate can be substituted with other glyceryl/acid and glycol/acid compounds.

**Table 4 (Generic formulation)***

| Ingredient | % Concentration (by weight) |
|---|---|
| **Phase A** | |
| Water | 84.44 |
| Xanthum gum | 0.1 |
| M-paraben | 0.15 |
| P-paraben | 0.1 |
| Citric acid | 0.01 |
| **Phase B** | |
| Cetyl alcohol | 4.0 |
| Glyceryl stearate + PEG 100 | 4.0 |
| Octyl palmitate | 4.0 |
| Dimethicone | 1.0 |
| Tocopheryl acetate | 0.2 |
| **Phase C** | |
| Glyceryl mono-salicylate** | 2.0 |

*The composition can be prepared as follows: Sprinkle Xanthum gum in water and mix for 10 min. Subsequently, add all ingredients in phase A and heat to 70-75°C. Add all items in phase B to a separate beaker and heat to 70-75°C. Mix phases A and B at 70-75°C. Continue mixing and allow composition to cool to 30°C. Subsequently, add phase C ingredient while mixing.
**Glyceryl mono-salicylate can be substituted with other glyceryl/acid and glycol/acid compounds.

**Table 5 (Generic formulation)***

| Ingredient | % Concentration (by weight) |
|---|---|
| **Phase A** | |
| Water | 78.6 |
| M-paraben | 0.2 |
| P-paraben | 0.1 |
| Na2 EDTA | 0.1 |
| Shea butter | 4.5 |
| Petrolatum | 4.5 |
| Glycerin | 4.0 |
| Propylene Glycol | 2.0 |
| Finsolve TN | 2.0 |
| **Phase B** | |
| Sepigel 305 | 2.0 |
| **Phase C** | |
| Glyceryl mono-salicylate** | 2.0 |

*Add ingredients in phase A to beaker and heat to 70-75°C while mixing. Subsequently, add the phase B ingredient with phase A and cool to 30°C with mixing. Subsequently, add phase C ingredient while mixing.
**Glyceryl mono-salicylate can be substituted with other glyceryl/acid and glycol/acid compounds.

**Table 6 (Generic formulation)***

| Ingredient | % Concentration (by weight) |
|---|---|
| **Phase A** | |
| Water | 84.44 |
| Xanthum gum | 0.1 |
| M-paraben | 0.15 |
| P-paraben | 0.1 |
| Citric acid | 0.01 |
| **Phase B** | |
| Cetyl alcohol | 4.0 |
| Glyceryl stearate + PEG 100 | 4.0 |
| Octyl palmitate | 4.0 |
| Dimethicone | 1.0 |
| Tocopheryl acetate | 0.2 |
| **Phase C** | |
| Glyceryl mono-salicylate** | 1.0 |
| Glycol mono-salicylate** | 1.0 |

*The composition can be prepared as follows: Sprinkle Xanthum gum in water and mix for 10 min. Subsequently, add all ingredients in phase A and heat to 70-75°C. Add all items in phase B to separate beaker and heat to 70-75°C. Mix phases A and B at 70-75°C. Continue mixing and allow composition to cool to 30°C. Subsequently, add phase C ingredient while mixing.
**Glyceryl mono-salicylate and/or glycol mono-salicylate can be substituted with other glyceryl/acid and glycol/acid compounds.

**Table 7 (Generic formulation)***

| Ingredient | % Concentration (by weight) |
|---|---|
| **Phase A** | |
| Water | 78.6 |
| M-paraben | 0.2 |
| P-paraben | 0.1 |
| Na2 EDTA | 0.1 |
| Shea butter | 4.5 |
| Petrolatum | 4.5 |
| Glycerin | 4.0 |
| Propylene Glycol | 2.0 |
| Finsolve TN | 2.0 |
| **Phase B** | |
| Sepigel 305 | 2.0 |
| **Phase C** | |
| Glyceryl mono-salicylate** | 1.0 |
| Glycol mono-salicylate** | 1.0 |

*Add ingredients in phase A to beaker and heat to 70-75°C while mixing. Subsequently, add the phase B ingredient with phase A and cool to 30°C with mixing. Subsequently, add phase C ingredient while mixing.
**Glyceryl mono-salicylate and/or glycol mono-salicylate can be substituted with other glyceryl/acid and glycol/acid compounds.

## EXAMPLE 4
## Determining Efficacy of Glyceryl/Acid or Glycol/Acid Compounds and Compositions

[0061] The efficacy of the glyceryl/acid or glycol/acid compounds and compositions containing the same can be determined by methods known to those of ordinary skill in the art. The following are non-limiting procedures that can be used in the context of the present invention. It should be recognized that other testing procedures can be used, including, for example, objective and subjective procedures.

[0062] Skin moisture/hydration can be measured by using impedance measurements with the Nova Dermal Phase Meter. The impedance meter measures changes in skin moisture content. The outer layer of the skin has distinct electrical properties. When skin is dry it conducts electricity very poorly. As it becomes more hydrated increasing conductivity results. Consequently, changes in skin impedance (related to conductivity) can be used to assess changes in skin hydration. The unit can be calibrated according to instrument instructions for each testing day. A notation of temperature and relative humidity can also be made. Subjects can be evaluated as follows: prior to measurement they can equilibrate in a room with defined humidity (*e.g.*, 30-50%) and temperature (*e.g.*, 68-72C). Three separate impedance readings can be taken on each side of the face, recorded, and averaged. The T5 setting can be used on the impedance meter which averages the impedance values of every

five seconds application to the face. Changes can be reported with statistical variance and significance.

[0063] Skin clarity and the reduction in freckles and age spots can be evaluated using a Minolta Chromometer. Changes in skin color can be assessed to determine irritation potential due to product treatment using the a* values of the Minolta Chroma Meter. The a* value measures changes in skin color in the red region. This is used to determine whether a composition is inducing irritation. The measurements can be made on each side of the face and averaged, as left and right facial values. Skin clarity can also be measured using the Minolta Meter. The measurement is a combination of the a*, b, and L values of the Minolta Meter and is related to skin brightness, and correlates well with skin smoothness and hydration. Skin reading is taken as above. In one non-limiting aspect, skin clarity can be described as L/C where C is chroma and is defined as $(a^2+b^2)^{1/2}$.

[0064] Skin dryness, surface fine lines, skin smoothness, and skin tone can be evaluated with clinical grading techniques. For example, clinical grading of skin dryness can be determined by a five point standard Kligman Scale: (0) skin is soft and moist; (1) skin appears normal with no visible dryness; (2) skin feels slightly dry to the touch with no visible flaking; (3) skin feels dry, tough, and has a whitish appearance with some scaling; and (4) skin feels very dry, rough, and has a whitish appearance with scaling. Evaluations can be made independently by two clinicians and averaged.

[0065] Clinical grading of skin tone can be performed *via* a ten point analog numerical scale: (10) even skin of uniform, pinkish brown color. No dark, erythremic, or scaly patches upon examination with a hand held magnifying lens. Microtexture of the skin very uniform upon touch; (7) even skin tone observed without magnification. No scaly areas, but slight discolorations either due to pigmentation or erythema. No discolorations more than 1 cm in diameter; (4) both skin discoloration and uneven texture easily noticeable. Slight scaliness. Skin rough to the touch in some areas; and (1) uneven skin coloration and texture. Numerous areas of scaliness and discoloration, either hypopigmented, erythremic or dark spots. Large areas of uneven color more than 1 cm in diameter. Evaluations were made independently by two clinicians and averaged.

[0066] Clinical grading of skin smoothness can be analyzed *via* a ten point analog numerical scale: (10) smooth, skin is moist and glistening, no resistance upon dragging

finger across surface ; (7) somewhat smooth, slight resistance; (4) rough, visibly altered, friction upon rubbing; and (1) rough, flaky, uneven surface. Evaluations were made independently by two clinicians and averaged.

[0067] Skin smoothness and wrinkle reduction can also be assessed visually by using the methods disclosed in Packman and Gams (1978). For example, at each subject visit, the depth, shallowness and the total number of superficial facial lines (SFLs) of each subject can be carefully scored and recorded. A numerical score was obtained by multiplying a number factor times a depth/width/length factor. Scores are obtained for the eye area and mouth area (left and right sides) and added together as the total wrinkle score.

[0068] Skin firmness can be measured using a Hargens ballistometer, a device that evaluates the elasticity and firmness of the skin by dropping a small body onto the skin and recording its first two rebound peaks. The ballistometry is a small lightweight probe with a relatively blunt tip (4 square mm-contact area) was used. The probe penetrates slightly into the skin and results in measurements that are dependent upon the properties of the outer layers of the skin, including the stratum corneum and outer epidermis and some of the dermal layers.

[0069] Skin softness/suppleness can be evaluated using the Gas Bearing Electrodynamometer, an instrument that measures the stress/strain properties of the skin. The viscoelastic properties of skin correlate with skin moisturization. Measurements can be obtained on the predetermined site on the cheek area by attaching the probe to the skin surface with double-stick tape. A force of approximately 3.5 gm can be applied parallel to the skin surface and the skin displacement is accurately measured. Skin suppleness can then be calculated and is expressed as DSR (Dynamic Spring Rate in gm/mm).

[0070] The appearance of lines and wrinkles on the skin can be evaluated using replicas, which is the impression of the skin's surface. Silicone rubber like material can be used. The replica can be analyzed by image analysis. Changes in the visibility of lines and wrinkles can be objectively quantified *via* the taking of silicon replicas form the subjects' face and analyzing the replicas image using a computer image analysis system. Replicas can be taken from the eye area and the neck area, and photographed with a digital camera using a low angle incidence lighting. The digital images can be analyzed with an image processing program and the are of the replicas covered by wrinkles or fine lines was determined.

[0071] The surface contour of the skin can be measured by using the profilometer/Stylus method. This includes either shining a light or dragging a stylus across the replica surface. The vertical displacement of the stylus can be fed into a computer *via* a distance transducer, and after scanning a fixed length of replica a cross-sectional analysis of skin profile can be generated as a two-dimensional curve. This scan can be repeated any number of times along a fix axis to generate a simulated 3-D picture of the skin. Ten random sections of the replicas using the stylus technique can be obtained and combined to generate average values. The values of interest include Ra which is the arithmetic mean of all roughness (height) values computed by integrating the profile height relative to the mean profile height. Rt which is the maximum vertical distance between the highest peak and lowest trough, and Rz which is the mean peak amplitude minus the mean peak height. Values are given as a calibrated value in mm. Equipment should be standardized prior to each use by scanning metal standards of know values. Ra Value can be computed by the following equation: $R_a$ = Standardize roughness; $l_m$ = the traverse (scan) length; and y = the absolute value of the location of the profile relative to the mean profile height (x-axis).

[0072] In other non-limiting aspects, the efficacy of the compositions of the present invention can be evaluated by using a skin analog, such as, for example, MELANODERM™. Melanocytes, one of the cells in the skin analog, stain positively when exposed to L-dihydroxyphenyl alanine (L-DOPA), a precursor of melanin. The skin analog, MELANODERM™, can be treated with a variety of bases containing the compositions and whitening agents of the present invention or with the base alone as a control. Alternatively, an untreated sample of the skin analog can be used as a control.

[0073] UV absorption of glyceryl/acid or glycol/acid compounds and compositions containing the same can be determined by UV absorption assays that are generally known to those of ordinary skill in the art.

* * * * * * * * * * * * * *

[0074] All of the compounds, compositions, and/or methods disclosed and claimed can be made and executed without undue experimentation in light of the present disclosure. While the compounds, compositions, and methods of this invention have been described in terms of certain embodiments, it will be apparent to those of skill in the art that variations may be applied to the compounds, compositions, and/or methods and in the steps or in the

sequence of steps of the method without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention.

## REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

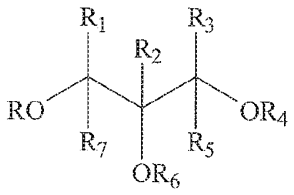
U.S. Patent 2,798,053
U.S. Patent 4,509,949
U.S. Patent 5,087,445

CTFA International Cosmetic Ingredient Dictionary and Handbook, 10th Ed., (2004).
Organic Chemistry, 5th Ed.
Packman and Gams, *J. Soc. Cos. Chem.*, 29:70-90, 1978.
Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1289-1329, 1990.

## CLAIMS

1. A topical skin care composition comprising a glyceryl salicylate compound or a glycol salicylate compound.

2. The composition of claim 1, wherein the composition comprises a glyceryl salicylate compound having the following structure:



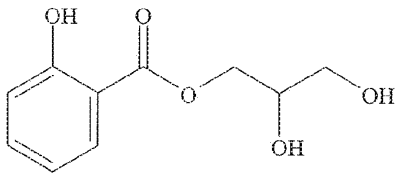

where

R, $R_4$, and $R_6$ are each independently an H, a salicylic acid molecule, or an alkyl group, with the proviso that at least one of R, $R_4$, and $R_6$ is a salicylic acid molecule; and

$R_1$, $R_2$, $R_3$, $R_5$, and $R_7$ are each independently an H or an alkyl group.

3. The composition of claim 2, wherein R is a salicylic acid and $R_4$ and $R_6$ are each an H.

4. The composition of claim 2, wherein the compound has the following structure:

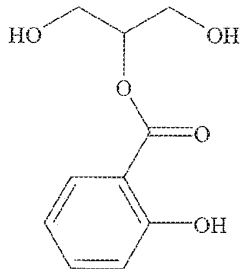



5. The composition of claim 4, wherein the composition further comprises a glyceryl di-salicylate compound or a glyceryl tri-salicylate compound.

6. The composition of claim 5, wherein the composition further comprises a glyceryl di-salicylate compound and a glyceryl tri-salicylate compound.

7. The composition of claim 2, wherein $R_6$ is a salicylic acid and R and $R_4$ are each an H.

8. The composition of claim 7, wherein the compound has the following structure:

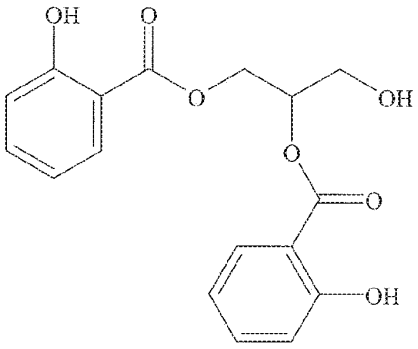



9. The composition of claim 2, wherein R and $R_6$ are each a salicylic acid and $R_4$ is H.

10. The composition of claim 9, wherein the composition further comprises a glyceryl di-salicylate compound or a glyceryl tri-salicylate compound.

11. The composition of claim 10, wherein the composition further comprises a glyceryl di-salicylate compound and a glyceryl tri-salicylate compound.

12. The composition of claim 9, wherein the compound has the following structure:

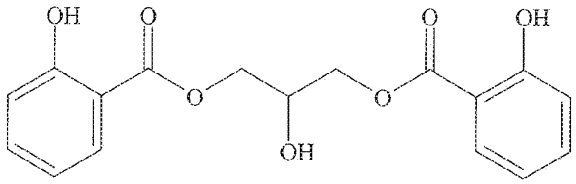



13. The composition of claim 12, wherein the composition further comprises a glyceryl mono-salicylate compound or a glyceryl tri-salicylate compound.

14. The composition of claim 13, wherein the composition further comprises a glyceryl mono-salicylate compound and a glyceryl tri-salicylate compound.

15. The composition of claim 2, wherein R and $R_4$ are each a salicylic acid and $R_6$ is H.

16. The composition of claim 15, wherein the compound has the following structure:

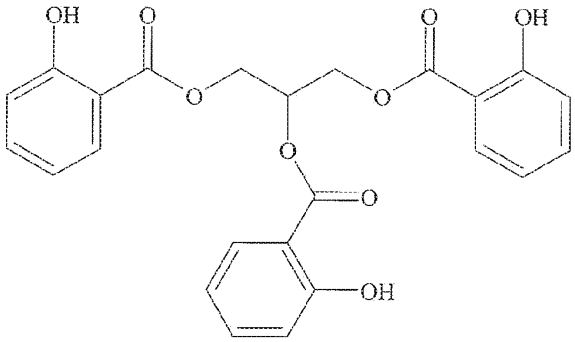


17. The composition of claim 16, wherein the composition further comprises a glyceryl mono-salicylate compound or a glyceryl tri-salicylate compound.

18. The composition of claim 17, wherein the composition further comprises a glyceryl mono-salicylate compound and a glyceryl tri-salicylate compound.

19. The composition of claim 2, wherein R, $R_4$, and $R_6$ are each salicylic acid.

20. The composition of claim 19, wherein the compound has the following structure:

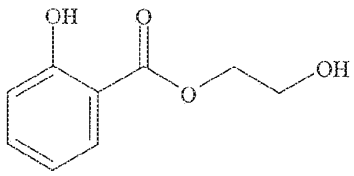


21. The composition of claim 20, wherein the composition further comprises a glyceryl mono-salicylate compound or a glyceryl di-salicylate compound.

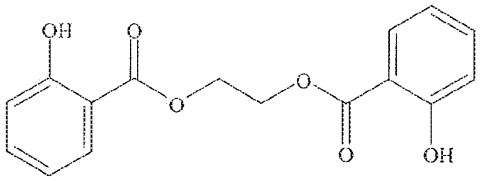
22. The composition of claim 21, wherein the composition further comprises a glyceryl mono-salicylate compound and a glyceryl di-salicylate compound.

23. The composition of claim 1, wherein the composition comprises a glycol salicylate compound selected from the group consisting of an ethylene glycol salicylate and a propylene glycol salicylate.

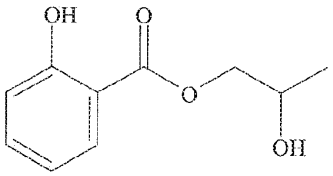
24. The composition of claim 23, wherein the composition comprises an ethylene glycol salicylate compound selected from the group consisting of:



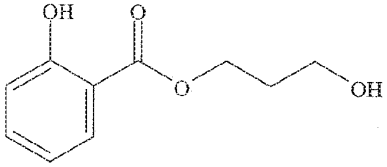
and



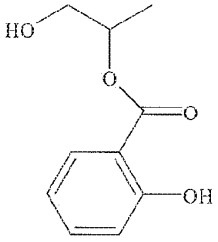
25. The composition of claim 23, wherein the composition comprises a propylene glycol salicylate compound selected from the group consisting of:



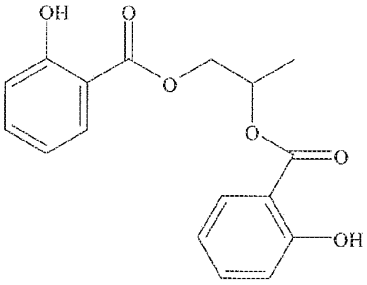
,



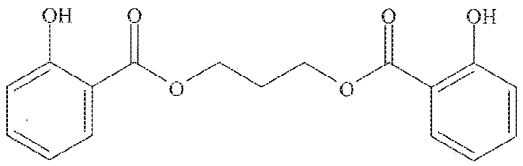
,



,



and



26. The composition of claim 1, wherein the composition comprises a glyceryl salicylate compound and a glycol salicylate compound.

27. The composition of claim 1, wherein the composition is an emulsion, a cream, a lotion, or an ointment.

28. The composition of claim 1, wherein the composition is an anhydrous composition.

29. The composition of claim 1, wherein the composition comprises from about 0.001% to about 20%, by weight, of the compound.

30. The composition of claim 1, wherein the composition absorbs UV light.

31. A method of treating a skin condition comprising topically applying a composition of claim 1 to skin, wherein the topical application of the composition to skin treats the skin condition.

32. The method of claim 31, wherein the skin condition is fine lines or wrinkles.

33. The method of claim 31, wherein the skin condition is dry skin.

34. A method of reducing the amount of moisture evaporated from skin comprising topically applying a composition of claim 1 to skin, wherein topical application of the composition creates a film on the skin that reduces the amount of moisture evaporated from the skin.

35. A method of protecting skin from UV light comprising topically applying the composition of claim 1 to skin, wherein the composition absorbs UV light.

36. A method of protecting a composition from chemical or physical deterioration induced by UV light comprising adding to the composition an effective amount of a

glyceryl salicylate molecule or a glycol salicylate molecule, wherein the glyceryl salicylate or glycol salicylate molecule absorbs UV light.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/069160

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/37 A61Q17/04

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)
A61K A61Q

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 practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2 081 117 A (R. HALL)<br>18 May 1937 (1937-05-18)<br>the whole document | 1-36 |
| X | EP 1 421 940 A (SEKISUI CHEMICAL CO)<br>26 May 2004 (2004-05-26)<br><br>paragraphs [0001], [0005], [0012], [0013], [0040]; claims 1-3; tables 1-4 | 1-4,8, 23-25, 27-36 |
| X | WO 02/26207 A (PROVINCIA ITALIANA DELLA CONGREGAZIONE DEI FIGLI DELL'IMMACOLATA CONC.) 4 April 2002 (2002-04-04)<br>page 1, lines 1-6; claims; examples 2-10<br>page 3, lines 28-31<br>page 4, lines 9-16 | 1,23-25, 27-36 |

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* 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 document 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 | Date of mailing of the international search report |
|---|---|
| 11 October 2007 | 23/10/2007 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Boeker, Ruth |

3

Form PCT/ISA/210 (second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/069160

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GB 2 283 173 A (SANSHO SEIYAKU KK [JP])<br>3 May 1995 (1995-05-03)<br>page 4, last paragraph; claim 1; examples<br>5,14,23 | 1,23,24,<br>27-36 |
| X | US 2 279 468 A (GUSTAVE LAHOUSSE JOSEPH<br>EDOUAR ET AL) 14 April 1942 (1942-04-14)<br>claims 1,3; example 1 | 1,23,<br>27-36 |
| X | US 4 009 254 A (A. RENOLD)<br>22 February 1977 (1977-02-22)<br>claims 1,4; example 7<br>column 5, lines 54,55<br>column 4, lines 37-45 | 1,2,23,<br>26-36 |

3

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/069160

**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. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an 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 reportcovers 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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 31 and 35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Continuation of Box II.1

Claims Nos.: -

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/069160

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 2081117 A | 18-05-1937 | NONE | |
| EP 1421940 A | 26-05-2004 | WO 03013548 A1 | 20-02-2003 |
| | | US 2004213749 A1 | 28-10-2004 |
| | | US 2007128287 A1 | 07-06-2007 |
| WO 0226207 A | 04-04-2002 | AU 9588501 A | 08-04-2002 |
| | | CA 2423713 A1 | 04-04-2002 |
| | | EP 1322285 A2 | 02-07-2003 |
| | | IT RM20000520 A1 | 28-03-2002 |
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| GB 2283173 A | 03-05-1995 | NONE | |
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| US 4009254 A | 22-02-1977 | NONE | |

Form PCT/ISA/210 (patent family annex) (April 2005)

Espacenet

## Bibliographic data: KR20090009990 (A) — 2009-01-23

# A PROTEIN COMPOSITION AND ITS USE IN RESTRUCTURED MEAT AND FOOD PRODUCTS

**Inventor(s):** MCMINDES MATTHEW K [US]; GODINEZ EDUARDO [US]; MUELLER IZUMI [US]; ORCUTT MAC [US]; ALTEMUELLER PATRICA A [US] + (MCMINDES MATTHEW K, ; GODINEZ EDUARDO, ; MUELLER IZUMI, ; ORCUTT MAC, ; ALTEMUELLER PATRICA A)

**Applicant(s):** SOLAE LLC [US] + (SOLAE LLC)

**Classification:** - **international:*A23J3/14; A23J3/26; A23L13/40; A23L13/60***
- **cooperative:** **A23J3/04**; **A23J3/14**; **A23J3/18**; **A23J3/227**; **A23J3/26**; **A23L13/426**; **A23L13/52**; **A23L13/67**; **A23L19/09**; **A23P30/20**

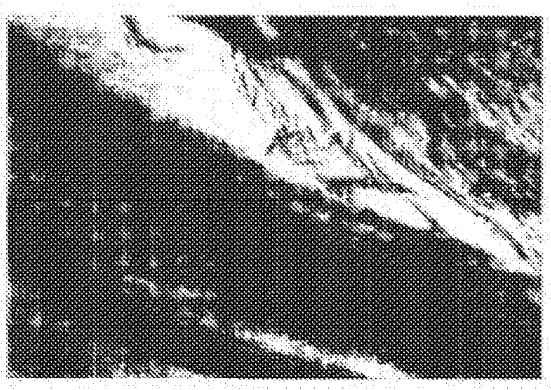
**Application number:** KR20087030897 20070521 Global Dossier

**Priority number(s):** US20060437164 20060519 ; US20070749540 20070516

**Also published as:** BRPI0711212 (A2) CA2652384 (A1) CA2652384 (C) DK2020875 (T3) EP2020875 (A2) more

## Abstract of KR20090009990 (A)

The invention provides protein compositions containing structured protein products having protein fibers that are substantially aligned.



(19) 대한민국특허청(KR)
(12) 공개특허공보(A)

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(43) 공개일자 2009년01월23일

(51) Int. Cl.[9]
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(21) 출원번호 10-2008-7030897
(22) 출원일자 2008년12월19일
심사청구일자 2008년12월19일
번역문제출일자 2008년12월19일
(86) 국제출원번호 PCT/US2007/069157
국제출원일자 2007년05월21일
(87) 국제공개번호 WO 2007/137125
국제공개일자 2007년11월29일
(30) 우선권주장
11/437,164 2006년05월19일 미국(US)
11/748,540 2007년05월16일 미국(US)

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*(뒷면에 계속)*
(74) 대리인
**김영, 양영준**

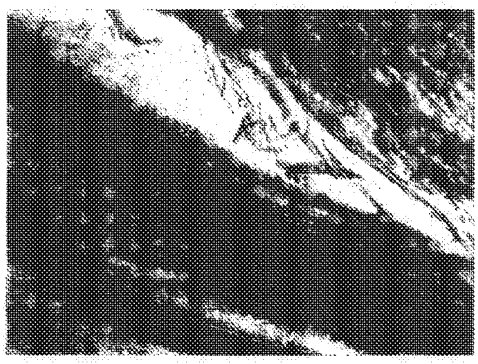
전체 청구항 수 : 총 55 항

## (54) 단백질 조성물 및 그의 재구성 고기 및 식품 제품에서의 용도

*(57) 요 약*

본 발명은 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품을 함유하는 단백질 조성물을 제공한다.

*대 표 도 - 도1*



(72) 발명자

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*특허청구의 범위*

**청구항 1**

높은 온도 및 압력의 조건 하에서 다이 조립체를 통해 식물 단백질 물질을 압출시켜 실질적으로 정렬된 단백질 섬유를 갖는 구조화 식물 단백질 제품을 형성하는 것을 포함하는, 재구성 고기 조성물의 제조 방법.

**청구항 2**

제1항에 있어서, 다이 조립체가

a. 공동으로 내부 챔버를 한정하는 뒷부분 및 앞부분을 갖는 다이 슬리브;

b. 내부 챔버 내에 배치된 다이 삽입물 (여기에서 다이 삽입물은 전면부 및 후면부를 갖는 삽입물 본체를 포함하고, 상기 전면부가 바닥부 및 다수의 유동 전환장치를 한정하며, 바닥부와 어느 2개의 인접한 다수의 유동 전환장치 사이에서 점점 가늘어지는 유동 경로가 한정된다); 및

c. 상기 다이 슬리브에 결합된 다이 원뿔

을 포함하고, 상기 다이 원뿔과 상기 유동 경로가 공동으로 충분히 가늘어지는 유동 채널을 한정하는 방법.

**청구항 3**

제2항에 있어서, 점점 가늘어지는 유동 경로가 3개의 측면을 따라 가늘어지는 방법.

**청구항 4**

제3항에 있어서, 충분히 가늘어지는 유동 채널이 4개의 측면을 따라 가늘어지는 방법.

**청구항 5**

제4항에 있어서, 충분히 가늘어지는 유동 채널이 한쪽 끝 입구 및 반대쪽 끝 출구와 통해 있는 방법.

**청구항 6**

제5항에 있어서, 충분히 가늘어진 유동 채널이 상기 입구로부터 상기 출구로 안쪽으로 가늘어지는 방법.

**청구항 7**

제6항에 있어서, 구조화 식물 단백질 제품을 동물 고기와 조합하는 것을 더 포함하는 방법.

**청구항 8**

제6항에 있어서, 구조화 식물 단백질 제품이 2000 그램 이상의 평균 전단 강도 및 17% 이상의 평균 절단 특징을 갖는 방법.

**청구항 9**

제8항에 있어서, 구조화 식물 단백질 제품이 도 1의 현미경 사진에 나타낸 방식으로 실질적으로 정렬된 단백질 섬유를 포함하는 방법.

**청구항 10**

제1항에 있어서, 식물 단백질 물질이 콩류, 옥수수, 완두콩, 카놀라, 해바라기, 수수, 쌀, 아마란스, 감자, 타피오카, 칡, 칸나, 루핀, 평지, 밀, 귀리, 호밀, 보리 및 이들의 혼합물로 구성된 군에서 선택되는 방법.

**청구항 11**

제1항에 있어서, 하나 이상의 동물 단백질 물질을 압출 전에 식물 단백질 물질과 조합하여, 실질적으로 정렬된 단백질 섬유를 포함하는 구조화 식물 단백질 제품을 제조하는 것을 더 포함하는 방법.

**청구항 12**

제11항에 있어서, 동물 단백질 물질이 카제인, 카제이네이트, 유장 단백질, 오브알부민, 오보글로불린, 오보뮤신, 오보뮤코이드, 오보트랜스페린, 오보비텔라, 오보비텔린, 알부딘 글로불린 및 비텔린으로 구성된 군에서 선택되는 방법.

**청구항 13**

제1항에 있어서, 식물 단백질 물질이 콩 단백질 및 밀 단백질을 포함하는 방법.

**청구항 14**

제13항에 있어서, 유장 단백질을 더 포함하는 방법.

**청구항 15**

제13항에 있어서, 식물 단백질 물질이 건조 물질 기준으로 약 40% 내지 약 75% 단백질을 갖는 방법.

**청구항 16**

제15항에 있어서, 식물 단백질 물질이 단백질, 전분, 글루텐 및 섬유를 포함하는 방법.

**청구항 17**

제16항에 있어서, 식물 단백질 물질이

a. 건조 물질 기준으로 약 45% 내지 약 65% 콩 단백질;

b. 건조 물질 기준으로 약 20% 내지 약 30% 밀 글루텐;

c. 건조 물질 기준으로 약 10% 내지 약 15% 밀 전분; 및

d. 건조 물질 기준으로 약 1% 내지 약 5% 섬유

를 포함하는 방법.

**청구항 18**

제16항에 있어서, 식물 단백질 물질이 인산이칼슘 및 L-시스테인을 더 포함하는 방법.

**청구항 19**

제1항에 있어서, 압출 온도가 약 90 ℃ 내지 약 150 ℃이고, 압력이 약 500 psig 내지 약 1500 psig인 방법.

**청구항 20**

제7항에 있어서, 동물 고기가 통근육 조각, 분쇄된 고기, 기계적으로 뼈 제거된 고기 및 이들의 조합으로 구성된 군에서 선택되는 방법.

**청구항 21**

제7항에 있어서, 동물 고기가 돼지, 소, 양, 가금류, 야생 사냥동물 및 어류로 구성된 군에서 선택된 동물로부터 유래되는 방법.

**청구항 22**

제1항에 있어서, 고기 조성물이 물을 더 포함하는 방법.

**청구항 23**

제6항에 있어서, 구조화 식물 단백질 제품을 분쇄된 야채와 조합하는 것을 더 포함하는 방법.

**청구항 24**

제6항에 있어서, 구조화 식물 단백질 제품을 분쇄된 과일과 조합하는 것을 더 포함하는 방법.

**청구항 25**

실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품을 포함하는 재구성 고기 조성물.

**청구항 26**

제25항에 있어서, 동물 고기를 더 포함하는 재구성 고기 조성물.

**청구항 27**

제26항에 있어서, 조성물이 약 40 내지 약 60 중량%의 구조화 단백질 제품 및 약 40 내지 약 60 중량%의 고기를 포함하는 재구성 고기 조성물.

**청구항 28**

제27항에 있어서, 조성물의 약 10 내지 약 20 중량% 범위의 양으로 지방 원료를 더 포함하는 재구성 고기 조성물.

**청구항 29**

제28항에 있어서, 구조화 단백질 제품이 도 1의 현미경 사진에 나타낸 방식으로 실질적으로 정렬된 단백질 섬유를 포함하는 재구성 고기 조성물.

**청구항 30**

제28항에 있어서, 구조화 단백질 제품이 콩 단백질, 전분, 글루텐 및 섬유를 포함하는 재구성 고기 조성물.

**청구항 31**

제28항에 있어서, 구조화 단백질 제품이

a. 건조 물질 기준으로 약 45% 내지 약 65% 콩 단백질;

b. 건조 물질 기준으로 약 20% 내지 약 30% 밀 글루텐;

c. 건조 물질 기준으로 약 10% 내지 약 15% 밀 전분; 및

d. 건조 물질 기준으로 약 1% 내지 약 5% 섬유

를 포함하는 재구성 고기 조성물.

**청구항 32**

제30항에 있어서, 고기가 쇠고기, 돼지고기, 양고기, 칠면조고기 및 닭고기로부터 선택되는 재구성 고기 조성물.

**청구항 33**

제29항에 있어서, 착색 조성물을 더 포함하는 재구성 고기 조성물.

**청구항 34**

제33항에 있어서, 착색 조성물이 비트, 안나토, 카라멜 색소 및 아미노산 원료를 포함하는 재구성 고기 조성물.

**청구항 35**

제34항에 있어서, 단리된 콩 단백질을 더 포함하는 재구성 고기 조성물.

**청구항 36**

제35항에 있어서, 항산화제, 물, 향신료 및 향미제를 더 포함하는 재구성 고기 조성물.

**청구항 37**

제25항에 있어서, 분쇄된 야채를 더 포함하는 재구성 고기 조성물.

**청구항 38**

제37항에 있어서, 조성물이 약 40 내지 약 60 중량%의 구조화 단백질 제품 및 약 40 내지 약 60 중량%의 분쇄된 야채를 포함하는 재구성 고기 조성물.

**청구항 39**

제38항에 있어서, 구조화 단백질 제품이 도 1의 현미경 사진에 나타낸 방식으로 실질적으로 정렬된 단백질 섬유를 포함하는 재구성 고기 조성물.

**청구항 40**

제38항에 있어서, 구조화 단백질 제품이 콩 단백질, 전분, 글루텐 및 섬유를 포함하는 재구성 고기 조성물.

**청구항 41**

제38항에 있어서, 구조화 단백질 제품이

a. 건조 물질 기준으로 약 45% 내지 약 65% 콩 단백질;

b. 건조 물질 기준으로 약 20% 내지 약 30% 밀 글루텐;

c. 건조 물질 기준으로 약 10% 내지 약 15% 밀 전분; 및

d. 건조 물질 기준으로 약 1% 내지 약 5% 섬유

를 포함하는 재구성 고기 조성물.

**청구항 42**

제40항에 있어서, 착색 조성물이 비트, 안나토, 카라멜 색소 및 아미노산 원료를 포함하는 재구성 고기 조성물.

**청구항 43**

제42항에 있어서, 단리된 콩 단백질을 더 포함하는 재구성 고기 조성물.

**청구항 44**

제43항에 있어서, 항산화제, 물, 향신료 및 향미제를 더 포함하는 재구성 고기 조성물.

**청구항 45**

제25항에 있어서, 분쇄된 과일을 더 포함하는 재구성 고기 조성물.

**청구항 46**

제45항에 있어서, 조성물이 약 40 내지 약 60 중량%의 구조화 단백질 제품 및 약 40 내지 약 60 중량%의 분쇄된 과일을 포함하는 재구성 고기 조성물.

**청구항 47**

제46항에 있어서, 구조화 단백질 제품이 도 1의 현미경 사진에 나타낸 방식으로 실질적으로 정렬된 단백질 섬유를 포함하는 재구성 고기 조성물.

**청구항 48**

제46항에 있어서, 구조화 단백질 제품이 콩 단백질, 전분, 글루텐 및 섬유를 포함하는 재구성 고기 조성물.

**청구항 49**

제46항에 있어서, 구조화 단백질 제품이

a. 건조 물질 기준으로 약 45% 내지 약 65% 콩 단백질;

b. 건조 물질 기준으로 약 20% 내지 약 30% 밀 글루텐;

c. 건조 물질 기준으로 약 10% 내지 약 15% 밀 전분; 및

d. 건조 물질 기준으로 약 1% 내지 약 5% 섬유

를 포함하는 재구성 고기 조성물.

**청구항 50**

제48항에 있어서, 착색 조성물이 비트, 안나토, 카라멜 색소 및 아미노산 원료를 포함하는 재구성 고기 조성물.

**청구항 51**

제50항에 있어서, 단리된 콩 단백질을 더 포함하는 재구성 고기 조성물.

**청구항 52**

제51항에 있어서, 항산화제, 물, 향신료 및 향미제를 더 포함하는 재구성 고기 조성물.

**청구항 53**

제25항에 있어서, 구조화 단백질 제품이 다이 조립체를 통해 압출되어 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품이 얻어지는 재구성 고기 조성물.

**청구항 54**

제37항에 있어서, 구조화 단백질 제품이 다이 조립체를 통해 압출되어 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품이 얻어지는 재구성 고기 조성물.

**청구항 55**

제45항에 있어서, 구조화 단백질 제품이 다이 조립체를 통해 압출되어 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품이 얻어지는 재구성 고기 조성물.

## *명 세 서*

<1> **관련 출원과의 상호참조**

<2> 본 출원은 미국 특허출원 일련번호 11/437,164 (2006년 5월 19일 출원)의 부분 연속 특허출원이다.

## *기 술 분 야*

<3> 본 출원은 단백질 조성물 및 야채 제품, 과일 제품 및 재구성 고기 제품에서 단백질 조성물의 용도를 제공한다. 또한, 본 발명은 함수 및 절단 단백질 조성물의 제조 방법을 제공한다.

## *배 경 기 술*

<4> 본 발명의 중요한 측면은 비구조화 단백질 제품을 구조화 단백질 제품으로 발전시키는데 있다. 특히, 하나의 구현양태에서, 본 발명은 눈에 보이는 알갱이나 조직을 갖지 않은 비구조화 단백질 제품을 취하고 이것을 조리된 근육 고기의 점조도를 지닌 한정된 형태의 구조화 단백질 제품으로 전환시키기 위한 제품 및 방법을 제공한다.

<5> 용어 "구조"는 식품 제품의 각종 물리적 성질을 설명한다. 허용가능한 구조의 제품은 보통 제품의 품질과 유의어이다. 구조는 "물리적 성질의 조합으로부터 비롯되고, 근감각 및 입안 느낌을 포함한 촉각, 시각 및 청각의 감각에 의해 인지되는 물질의 속성"으로서 정의된다. 국제 표준화 기구에 의해 정의된 바와 같이, 구조는 "기계적, 촉각 및 적절하다면 시각 및 청각 수용체에 의하여 인지가능한 식품 제품의 모든 유동학적 및 구조적 (기하 및 표면) 속성"이다. 포괄적인 "구조"에 속하는 제품 특징을 설명하기 위하여 하기 용어들이 사용되었다.

표 I

<6>

| 식품 구조를 나타내는 형용사의 요약 목록 | | | |
|---|---|---|---|
| 점착성의(Adhesive) | 다육질의(Fleshy) | 죽 같은(Mushy) | 부드러운(Soft) |
| 탄력있는(Bouncy) | 솜털 같은(Fluffy) | 유성의(Oily) | 진득진득한(Soggy) |
| 부서지기 쉬운(Brittle) | 거품이 많은(Foamy) | 풀같은(Pasty) | 발포성의(Sparkly) |
| 거품이 많은(Bubbly) | 깨지기 쉬운(Fragile) | 소성의(Plastic) | 찢어지기 쉬운 (Splintery) |
| 잘씹히지 않는(Chewy) | 감칠맛이 있는 (Full-bodied) | 다공성의(Porous) | 해면질의(Spongy) |
| 점착성의(Clingy) | 들러붙는(Gooey) | 가루모양의(Powdery) | 탄력이 있는(Springy) |
| 도포되는(Coating) | 낱알이 많은(Grainy) | 부푼(Puffy) | 끈적거리는(Sticky) |
| 응집성의(Cohesive) | 껄끄러운(Gritty) | 과육질의(Pulpy) | 힘줄이 많은(Stringy) |
| 크림 같은(Creamy) | 고무질의(Gummy) | 기름진(Rich) | 시럽 같은(Syrupy) |
| 바삭바삭한(Crisp) | 단단한(Hard) | 거친(Rough) | 연한(Tender) |
| 푸석푸석한(Crumbly) | 무거운(Heavy) | 탄성이 있는(Rubbery) | 걸쭉한(Thick) |
| 두꺼운 껍질이 있는(Crusty) | 불균질한(Heterogeneous) | 액상의(Runny) | 묽은(Thin) |
| 조밀한(Dense) | 즙이 많은(Juicy) | 모래 같은(Sandy) | 따가운(Tingly) |
| 말랑말랑한(Doughy) | 지방이 적은 (Lean) | 까칠거리는(Scratchy) | 질긴(Tough) |
| 건조한(Dry) | 연한(Light) | 부족한(Short) | 고른(Uniform) |
| 탄력있는(Elastic) | 나긋나긋한(Limp) | 부드러운(Silky) | 점성의(Viscous) |
| 지방성의(Fatty) | 우둘두둘한(Lumpy) | 미끄러운(Slippery) | 물 같은(Watery) |
| 굳은(Firm) | 축축한(Moist) | 쓰개지는(Slivery) | 밀납성의(Waxy) |
| 박편으로된(Flaky) | 입안을 덮는(Mouth coating) | 매끄러운(Smooth) | 물결치는(Wiggly) |
| | | | |

<7> 구조는 가공 및 모방 제품, 형성된 고기 및 어류 제품을 포함하여 새로운 식품 물질에 관계되기 때문에 관심이 쏠리고 있고, 원래의 또는 다른 천연 식품 물질의 성질을 복제하기 위한 공정에 의해 매우 전지한 노력이 행해지고 있다. 비-전통적 원료, 합성 향료, 충진재, 신장제 및 연장제의 사용은 최종 제품의 특정한 조직 특징을 변경시키는 경향이 있다. 종종, 조직 성질의 모방은 맛, 냄새 및 색의 복제에 있어서 훨씬 더 어렵다. 천연 구조 성질을 모방하기 위하여 압출 구조화를 포함한 다수의 조작 공정이 개발되었다. 공정은 일반적으로 초기 시장 허용성을 촉진하기 위하여 기술적 및 경제적으로 실행가능한 정도로 원래의 물질의 성질을 복제하는데 세심한 것으로 밝혀졌다. 구조는 외관에 관련된 속성을 갖긴 하지만, 이것은 또한 입과 접촉될 때의 촉감 및 입안 느낌 또는 식품의 상호작용에 관련된 속성을 갖는다. 종종, 씹는 것과 관련된 이러한 감각적 인지는 호감 또는 비호감의 인상과 관련될 수 있다.

<8> 따라서, 구조에 관련된 용어는 응력 또는 변형 하에서 재료의 거동에 관련된 용어를 포함하고, 예를 들어 굳은(firm), 단단한(hard), 부드러운(soft), 질긴(tough), 연한(tender), 잘씹히지 않는(chewy), 탄성이 있는(rubbery), 탄력있는(elastic), 소성의(plastic), 끈적거리는(sticky), 점착성의(adhesive), 끈적끈적한(tacky), 바삭바삭한(crispy), 아삭아삭한(crunchy), 등을 포함한다. 두 번째로, 구조에 관련된 용어는 재료의 구조에 관련될 수도 있다: 매끄러운(smooth), 미세한(fine), 가루모양의(powdery), 잘부서지는(chalky), 우둘두둘한(lumpy), 굵은가루의(mealy), 거친(coarse), 껄끄러운(gritty) 등. 세 번째로, 구조에 관련된 용어는 구조 요소의 형태 및 배열, 예컨대 박편으로된(flaky), 섬유질의(fibrous), 힘줄이 많은(stringy), 과육질의(pulpy), 다공성의(cellular), 결정성(crystalline), 유리질(glassy), 해면질(spongy) 등에 관련될 수도 있다. 마지막으로, 구조에 관련된 용어는 입안 느낌(mouth feel), 덩어리(body), 건조한(dry), 축축한(moist), 젖은(wet), 물같은(watery), 밀랍같은(waxy), 질척한(slimy), 죽같은(mushy) 등을 포함하여 입안 느낌의 특징에 관련될 수도 있다.

<9> 여기에서 사용된 바와 같이, "비구조화" 및 "구조화"는 하기 표 II에 나타낸 바와 같이 식품 제품의 특징을 설명한다.

표 II

<10>

| | 비구조화 특징 | 구조화 특징 |
|---|---|---|
| 응력 또는 변형 하에서 재료의 거동 | 끈적거리는(sticky ) | 굳은(firm ) |
| | 들러붙는(gooey ) | 잘씹히지 않는(chewy ) |
| | 소성의(plastic) | |
| | | |
| 재료의 구조 | 매끄러운(smooth) | 거친(coarse ) |
| | | |
| | | |
| 구조 요소의 형태 및 배열 | 아교질의(gelatinous ) | 섬유질의(fibrous ) |
| | 과육질의(pulpy ) | 두꺼운 껍질이 있는(crusty ) |
| | 풀 같은(pasty) | |
| | | |
| 입안 느낌 | 크림 같은(creamy ) | 축축한(moist ) |
| | 점성있는 즉같은(mushy with body) | 건조한(dry) |
| | | |

<11> **발명의 요약**

<12> 본 발명의 한가지 측면은 재구성 고기 조성물의 제조 방법을 제공한다. 방법은 일반적으로 식물 단백질 물질을 높은 온도 및 압력 조건 하에 다이 조립체를 통해 압출하여 실질적으로 정렬된 단백질 섬유를 갖는 구조화 식물 단백질 제품을 형성하는 것을 포함한다.

<13> 본 발명의 다른 측면은 재구성 고기 조성물을 제공한다. 재구성 고기 조성물은 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품을 포함한다.

*발명의 상세한 설명*

<20> 본 발명은 함수 및 절단 단백질 조성물 및 각각의 조성물의 제조 방법을 제공한다. 전형적으로, 단백질 조성물은 실질적으로 정렬된 단백질 섬유를 갖는 동물 고기 및 구조화 단백질 제품을 포함할 것이다. 대안적으로, 단백질 조성물은 실질적으로 정렬된 단백질 섬유를 갖는 분쇄된 야채 또는 분쇄된 과일 및 구조화 단백질 제품을 포함할 것이다.

<21> **(I) 구조화 단백질 제품**

<22> 본 발명의 단백질 조성물은, 하기 I(e)에 더욱 상세히 기재된 바와 같이, 각각 실질적으로 정렬된 단백질 섬유를 포함하는 구조화 단백질 제품을 포함한다. 일례의 구현양태에서, 구조화 단백질 제품은 하기 I(d)에 상세히 기재된 압출 공정으로 처리되어진 단백질 물질의 압출물이다. 구조화 단백질 제품이 동물 고기와 유사한 방식으로 실질적으로 정렬된 단백질 섬유를 갖기 때문에, 본 발명의 단백질 조성물은 일반적으로 100% 동물 고기로 이루어진 조성물의 조직 및 식용 품질 특징을 갖는다.

<23> **(a) 단백질-함유 물질**

<24> 단백질을 함유한 각종 성분들을 열 가소성 압출 공정에 사용하여 단백질 조성물에서 사용하기 위해 적절한 구조화 단백질 제품을 제조할 수 있다. 식물로부터 유래된 단백질을 포함하는 성분들이 전형적으로 사용되긴 하지만, 본 발명의 범위에서 벗어나지 않으면서 다른 원료, 예컨대 동물 원료로부터 유래된 단백질을 사용할 수도 있는 것으로 생각된다. 예를 들어, 카제인, 카제이네이트, 유장 단백질 및 이들의 혼합물로 구성된 군에서 선택되는 유제품 단백질이 사용될 수도 있다. 일례의 구현양태에서, 유제품 단백질은 유장 단백질이다. 추가의 예로서, 오브알부민, 오브글로불린, 오브뮤신, 오브뮤코이드, 오브트랜스페린, 오브비텔라, 오브비텔린, 알부민 글로불린 및 비텔린으로 구성된 군에서 선택되는 난 단백질이 사용될 수도 있다. 또한, 고기 단백질 또는 콜라겐, 혈액, 기관 고기, 기계적으로 분리된 고기, 부분 탈지된 조직 및 혈액 혈청 단백질로 구성된 단백질 성분이 구조화 단백질 제품의 하나 이상의 성분으로서 포함될 수도 있다.

<25> 단백질에 추가로 다른 성분 유형이 사용될 수도 있는 것으로 생각된다. 이러한 성분의 비-제한적인 예는 당,

전분, 올리고당, 콩 섬유 및 기타 식이 섬유를 포함한다.

<26> 단백질-함유 물질이 무-글루텐일 수도 있는 것으로 생각된다. 전형적으로 압출 공정에서 글루텐이 필라멘트 형성에 사용되기 때문에, 무-글루텐 출발 물질이 사용된다면, 필라멘트 형성을 촉진하기 위하여 식용 가교제가 사용될 수도 있다. 적절한 가교제의 비-제한적인 예는 곤약 글루코만난(KGM) 가루, 베타 1,3-글루칸 (다케다-기린 푸드에 의해 제조된 퓨어글루칸(Pureglucan)™ 또는 커들란(Curdlan)), 트랜스글루타미나제, 칼슘 염 및 마그네슘 염을 포함한다. 당업자라면 존재하는 경우 무-글루텐 구현양태에서 필요한 가교 물질의 양을 쉽게 결정할 수 있다.

<27> 그의 원료 또는 성분 분류와는 무관하게, 압출 공정에서 사용된 성분들이 실질적으로 정렬된 단백질 섬유를 갖는 압출물을 형성할 수 있다. 이러한 성분의 적절한 예는 이하에서 충분히 상술된다.

<28> (i) 식물 단백질 물질

<29> 일례의 구현양태에서, 단백질-함유 물질을 형성하기 위하여 식물로부터 유래된 하나 이상의 성분을 사용할 것이다. 일반적으로 말하자면, 성분은 단백질을 포함할 것이다. 사용된 성분(들)에 존재하는 단백질의 양은 그들의 용도에 의존하여 변할 수 있고 변할 것이다. 예를 들어, 사용된 성분(들)에 존재하는 단백질의 양은 약 40 내지 약 100 중량%의 범위일 수도 있다. 다른 구현양태에서, 사용된 성분(들)에 존재하는 단백질의 양은 약 50 내지 약 100 중량%의 범위일 수도 있다. 추가의 구현양태에서, 사용된 성분(들)에 존재하는 단백질의 양은 약 60 내지 약 100 중량%의 범위일 수도 있다. 추가의 구현양태에서, 사용된 성분(들)에 존재하는 단백질의 양은 약 70 중량% 내지 약 100 중량%의 범위일 수도 있다. 다른 구현양태에서, 사용된 성분(들)에 존재하는 단백질의 양은 약 80 중량% 내지 약 100 중량%의 범위일 수도 있다. 추가의 구현양태에서, 사용된 성분(들)에 존재하는 단백질의 양은 약 90 내지 약 100 중량%의 범위일 수도 있다.

<30> 압출에서 사용된 성분(들)은 각종 적절한 식물로부터 유래될 수도 있다. 비-제한적인 예로서, 적절한 식물은 콩류, 옥수수, 완두콩, 카놀라, 해바라기, 수수, 쌀, 아마란스, 감자, 타피오카, 칡, 칸나, 루핀, 평지, 밀, 귀리, 호밀, 보리 및 이들의 혼합물을 포함한다.

<31> 하나의 구현양태에서, 성분들은 밀 및 대두로부터 단리된다. 다른 일례의 구현양태에서, 성분들은 대두로부터 단리된다. 적절한 밀 유래 단백질-함유 성분들은 밀 글루텐, 밀가루 및 이들의 혼합물을 포함한다. 본 발명에서 사용될 수도 있는 통상적으로 입수가능한 밀 글루텐의 예는 젬 오브 더 스타 글루텐(Gem of the Star Gluten), 바이탈 밀 글루텐(Vital Wheat Gluten) (유기) (이들의 각각은 마닐드라 밀링(Manildra Milling)으로부터 입수가능함)을 포함한다. 적절한 대두 유래 단백질-함유 성분 ("콩 단백질 물질")은 대두 단백질 단리물, 콩 단백질 농축물, 콩가루 및 이들의 혼합물 (각각은 이하에서 상세히 설명된다)을 포함한다. 적절한 옥수수 유래 단백질-함유 성분들은 옥수수 글루텐 가루, 예를 들어 제인을 포함한다. 상기 각각의 구현양태에서, 대두 물질을 전분, 곡분, 글루텐, 식이 섬유 및 이들의 혼합물로 구성된 군에서 선택되는 하나 이상의 성분과 조합할 수도 있다.

<32> 각종 원료로부터 단리된 단백질-함유 물질의 적절한 예를 표 III에 상술하고, 다양한 조합을 나타낸다.

*표 III*

<33>

| 단백질 조합 | |
|---|---|
| 제1 단백질 원료 | 제2 성분 |
| 대두 | 밀 |
| 대두 | 유채풀 |
| 대두 | 난 |
| 대두 | 옥수수 |
| 대두 | 쌀 |
| 대두 | 보리 |
| 대두 | 수수 |
| 대두 | 귀리 |
| 대두 | 기장 |
| 대두 | 호밀 |
| 대두 | 라이밀(triticale) |

| | |
|---|---|
| 대두 | 메밀 |
| 대두 | 완두콩 |
| 대두 | 땅콩 |
| 대두 | 편두 |
| 대두 | 루핀 |
| 대두 | 칸나(이집트콩(garbonzo)) |
| 대두 | 평지씨 (카놀라) |
| 대두 | 카사바 |
| 대두 | 해바라기 |
| 대두 | 유창 |
| 대두 | 타피오카 |
| 대두 | 참 |
| 대두 | 아마란스 |
| 대두 | 밀 및 유제품 |
| 대두 | 밀 및 난 |
| 대두 | 밀 및 옥수수 |
| 대두 | 밀 및 쌀 |
| 대두 | 밀 및 보리 |
| 대두 | 밀 및 수수 |
| 대두 | 밀 및 귀리 |
| 대두 | 밀 및 기장 |
| 대두 | 밀 및 호밀 |
| 대두 | 밀 및 라이밀 |
| 대두 | 밀 및 메밀 |
| 대두 | 밀 및 완두콩 |
| 대두 | 밀 및 땅콩 |
| 대두 | 밀 및 편두 |
| 대두 | 밀 및 루핀 |
| 대두 | 밀 및 칸나 (이집트콩) |
| 대두 | 밀 및 평지씨 (카놀라) |
| 대두 | 밀 및 카사바 |
| 대두 | 밀 및 해바라기 |
| 대두 | 밀 및 감자 |
| 대두 | 밀 및 타피오카 |
| 대두 | 밀 및 참 |
| 대두 | 밀 및 아마란스 |
| 대두 | 옥수수 및 밀 |
| 대두 | 옥수수 및 유제품 |
| 대두 | 옥수수 및 난 |
| 대두 | 옥수수 및 쌀 |
| 대두 | 옥수수 및 보리 |
| 대두 | 옥수수 및 수수 |
| 대두 | 옥수수 및 귀리 |
| 대두 | 옥수수 및 기장 |
| 대두 | 옥수수 및 호밀 |
| 대두 | 옥수수 및 라이밀 |
| 대두 | 옥수수 및 메밀 |
| 대두 | 옥수수 및 완두콩 |
| 대두 | 옥수수 및 땅콩 |
| 대두 | 옥수수 및 편두 |
| 대두 | 옥수수 및 루핀 |
| 대두 | 옥수수 및 칸나 (이집트콩) |

| | |
|---|---|
| 대두 | 옥수수 및 평지씨 (카놀라) |
| 대두 | 옥수수 및 카사바 |
| 대두 | 옥수수 및 해바라기 |
| 대두 | 옥수수 및 감자 |
| 대두 | 옥수수 및 타피오카 |
| 대두 | 옥수수 및 쌀 |
| 대두 | 옥수수 및 아마란스 |

<34>

<35> 표 III에 묘사된 각각의 구현양태에서, 단백질-함유 물질의 조합은 전분, 곡분, 글루텐, 식이 섬유 및 이들의 혼합물로 구성된 군에서 선택되는 하나 이상의 성분과 조합할 수도 있다. 하나의 구현양태에서, 단백질-함유 물질은 단백질, 전분, 글루텐 및 섬유를 포함한다. 일례의 구현양태에서, 단백질-함유 물질은 건조 물질 기준으로 약 45% 내지 약 65% 콩 단백질; 건조 물질 기준으로 약 20% 내지 약 30% 및 글루텐; 건조 물질 기준으로 약 10% 내지 약 15% 및 전분; 및 건조 물질 기준으로 약 1% 내지 약 5% 섬유를 포함한다. 각각의 상기 구현양태에서, 단백질-함유 물질은 인산이칼슘, L-시스테인 또는 인산이칼슘과 L-시스테인의 조합물을 포함할 수도 있다.

<36> (ii) 콩 단백질 물질

<37> 상기 상술된 바와 같이 일례의 구현양태에서, 콩 단백질 단리물, 콩 단백질 농축물, 콩가루 및 이들의 혼합물을 압출 공정에서 사용할 수도 있다. 콩 단백질 물질은 당 기술분야에 공지된 방법에 따라 전 대두로부터 유래될 수도 있다. 전 대두는 표준 대두 (즉, 유전자 조작되지 않은 대두), 상품 대두, 유전자 조작 대두 및 이들의 조합일 수도 있다.

<38> 일반적으로 말하자면, 콩 단리물이 사용될 때, 고 가수분해 콩 단백질 단리물이 아닌 단리물이 바람직하게 선택된다. 그러나, 특정한 구현양태에서, 조합된 콩 단백질 단리물의 고 가수분해 콩 단백질 단리물 함량이 일반적으로 조합된 콩 단백질 단리물의 약 40 중량% 미만인 전제 조건하에서, 고 가수분해 콩 단백질 단리물이 다른 콩 단백질 단리물과 조합하여 사용될 수도 있다. 추가로, 사용된 콩 단백질 단리물은 바람직하게는, 단리물에 있는 단백질이 압출 시에 실질적으로 정렬된 섬유를 형성할 수 있도록 하기 위해 충분한 에멀젼 강도 및 젤 강도를 갖는다. 본 발명에서 유용한 콩 단백질 단리물의 예는 예를 들어 솔레 LLC (미국 미조리주 세인트루이스)로부터 통상적으로 입수가능하고, 수프로® 500E, 수프로® EX33, 수프로® 620, 수프로® 630 및 수프로® 545를 포함한다. 일례의 구현양태에서, 수프로® 620의 형태가 실시예 3에 상술된 바와 같이 사용된다.

<39> 대안적으로, 콩 단백질 물질의 원료로서 콩 단백질 단리물의 일부를 대체하기 위하여 콩 단백질 농축물을 콩 단백질 단리물과 함께 배합할 수도 있다. 전형적으로, 콩 단백질 농축물이 콩 단백질 단리물의 일부를 대체한다면, 콩 단백질 농축물은 최대 콩 단백질 단리물의 약 40 중량%이하를 대체하고, 더욱 바람직하게는 콩 단백질 단리물의 약 30 중량%이하를 대체한다. 본 발명에서 유용한 적절한 콩 단백질 농축물의 예는 프로민(Promine) DSPC, 프로콘(Procon), 알파 12 및 알파 5800 (이것은 솔레 LLC (미국 미조리주 세인트루이스)로부터 통상적으로 입수가능하다)를 포함한다.

<40> 콩 떡잎 섬유는 섬유 원료로서 임의로 사용될 수도 있다. 전형적으로, 콩 단백질 및 콩 떡잎 섬유의 혼합물이 공압출될 때, 적절한 콩 떡잎 섬유가 일반적으로 물을 효과적으로 결합한다. 이 명세서에서, "물을 효과적으로 결합한다"는 일반적으로 콩 떡잎 섬유가 콩 떡잎 섬유 그램 당 적어도 5.0 내지 약 8.0 그램의 물의 물 보유 능력을 갖고, 바람직하게는 콩 떡잎 섬유가 콩 떡잎 섬유 그램 당 적어도 약 6.0 내지 약 8.0 그램의 물의 물 보유 능력을 갖는 것을 의미한다. 콩 떡잎 섬유는 일반적으로 무 수분 기준으로 약 1 중량% 내지 약 20 중량%, 바람직하게는 약 1.5 중량% 내지 약 20 중량%, 가장 바람직하게는 약 2 중량% 내지 약 5 중량% 범위의 양으로 콩 단백질 물질에 존재할 수도 있다. 적절한 콩 떡잎 섬유가 통상적으로 입수가능하다. 예를 들어, 퍼브림(FIBRIM)® 1260 및 퍼브림® 2000이 솔레 LLC (미국 미조리주 세인트 루이스)로부터 통상적으로 입수가능한 콩 떡잎 섬유 물질이다.

<41> **(b) 추가의 성분**

<42> 본 발명의 범위에서 벗어나지 않으면서 상기 상술된 단백질-함유 물질에 다양한 추가의 성분을 첨가할 수도 있

다. 예를 들어, 항산화제, 항균제 및 이들의 조합이 포함될 수도 있다. 항산화제 첨가제는 BHA, BHT, TBHQ, 비타민 A, C 및 E 및 이들의 유도체를 포함한다. 추가로, 저장 수명을 증가시키거나 단백질 조성물을 영양적으로 증진시키기 위하여 항산화 성질을 갖는 카로티노이드, 토코페롤 또는 플라보노이드를 함유하는 것과 같은 다양한 식물 추출물이 포함될 수도 있다. 항산화제 및 항균제는 단백질-함유 물질의 약 0.01 중량% 내지 약 10 중량%, 바람직하게는 약 0.05 중량% 내지 약 5 중량%, 더욱 바람직하게는 약 0.1 중량% 내지 약 2 중량%의 수준으로 조합되어 존재할 수도 있다.

<43> **(c) 수분 함량**

<44> 당업자에 의해 이해될 수 있듯이, 단백질-함유 물질 및 임의의 추가의 성분의 수분 함량이 변할 수 있고 변할 것이다. 물의 목적은 단백질 조성물의 성분을 수화시키는 것이다. 일반적으로 말하자면, 수분 함량은 약 1 중량% 내지 약 80 중량%의 범위일 수도 있다. 저 수분 압출 응용에서, 단백질-함유 물질의 수분 함량은 약 1 중량% 내지 약 35 중량%의 범위일 수 있다. 대안적으로, 고 수분 압출 응용에서, 단백질-함유 물질의 수분 함량은 약 35 내지 약 80 중량%의 범위일 수도 있다. 일례의 구현양태에서, 압출물을 형성하기 위해 사용된 압출 응용은 저 수분이다. 실질적으로 정렬된 섬유를 갖는 단백질을 갖는 압출물을 재조하기 위해 저 수분 압출 공정의 일례를 I(d) 및 실시예 3에 상술한다.

<45> **(d) 단백질-함유 물질의 압출**

<46> 구조화 단백질 제품의 제조를 위해 적절한 압출 공정은, 단백질 물질 및 기타 성분을 혼합 용기(즉, 성분 배합기)에 도입하여 성분을 조합하고, 건식 배합된 단백질 물질 예비혼합물을 형성하는 것을 포함한다. 건식 배합된 단백질 물질 예비혼합물을 호퍼로 옮길 수 있고, 그로부터 건식 배합된 성분들을 수분과 함께 예비-조절장치에 도입하여 상태조절된 단백질 물질 혼합물을 형성한다. 이어서, 상태조절된 물질을 압출기에 공급하고, 여기에서 압출기의 축에 의해 발생된 기계적 압력 하에 혼합물을 가열하여 용융된 압출 덩어리를 형성한다. 대안적으로, 건식 배합된 단백질 물질 예비-혼합물을 압출기에 직접 공급하고, 여기에서 수분 및 열을 도입하여 용융된 압출 덩어리를 형성한다. 용융된 압출 덩어리는 압출 다이 조립체를 통해 압출기를 나와서 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품을 포함하는 압출물을 형성한다.

<47> <u>(i) 압출 공정 조건</u>

<48> 본 발명의 실행에서 유용한 적절한 압출 장치 중에는 예를 들어 미국 특허 4,600,311호에 기재된 것과 같은 이중 통 이축 압출기가 있다. 적절한 통상적으로 입수가능한 압출 장치의 추가의 예는 클렉스트랄(CLEXTRAL) 모델 BC-72 압출기 (클렉스트랄 인코포레이티드 (미국 플로리다주 탬파) 제조); 웬저(WENGER) 모델 TX-57 압출기, 웬저 모델 TX-168 압출기 및 웬저 모델 TX-52 압출기 (모두 웬저 매뉴팩처링 인코포레이티드 (미국 캔사스주 사베타) 제조)를 포함한다. 본 발명에서 사용하기 위해 적절한 다른 통상적인 압출기는 예를 들어 미국 특허 4,763,569호, 4,118,164호 및 3,117,006호 (이것은 여기에서 그 전체내용이 참고문헌으로 포함된다)에 기재되어 있다.

<49> 이축 압출기의 축은 동일하거나 반대 방향으로 통 안에서 회전할 수 있다. 동일한 방향으로의 축의 회전은 단일 유동이라 일컬어지는 반면, 반대 방향으로의 축의 회전은 이중 유동이라 일컬어진다. 압출기의 축 또는 축들의 속도는 구체적인 장치에 의존하여 변할 수 있다. 그러나, 축 속도는 전형적으로 약 250 내지 약 400 회전/분 (rpm)이다. 일반적으로, 축 속도가 증가함에 따라 압출물의 밀도는 감소한다. 압출 장치는 식물 단백질 물질을 압출하기 위해 압출 장치 제조업자에 의해 추천된 바와 같이 샤프트 및 나사 톱니바퀴로부터 조립된 축 뿐만 아니라 혼합 로브 및 고리형 시어락(shearlock) 부품을 보유한다.

<50> 압출 장치는 일반적으로 다수의 온도 조절 대역을 포함하고, 이것을 통하여 압출 다이 조립체를 거쳐 압출 장치를 빠져 나오기까지 단백질 혼합물이 기계적 압력 하에 운반된다. 각각의 연속 가열 대역에서의 온도는 이전의 가열 대역의 온도보다 약 10 ℃ 내지 약 70 ℃ 만큼 초과한다. 하나의 구현양태에서, 상태조절된 예비-혼합물이 압출 장치 내에서 4개의 가열 대역을 통해 전달되고, 용융된 압출 덩어리가 약 100 ℃ 내지 약 150 ℃의 온도에서 압출 다이 조립체에 들어가도록 약 100 ℃ 내지 약 150℃의 온도까지 단백질을 가열하였다.

<51> 통 압력은 예를 들어 압출기 축 속도, 통으로 혼합물의 공급 속도, 통으로 물의 공급 속도 및 통 안에서 용융된 덩어리의 점도를 포함하여 다수의 요인에 의존된다.

<52> 식물 단백질 물질 혼합물을 수화시키고 단백질의 조직화를 촉진하기 위하여 압출기 통 안에 물을 주입할 수도 있다. 용융된 압출 덩어리를 형성함에 있어서 보조재로서 물이 가소제로서 작용할 수도 있다. 가열 대역과 통해 있는 하나 이상의 주입 분출구를 통하여 물이 압출기 통에 도입될 수도 있다. 전형적으로, 통 안의 혼합물

은 약 1 중량% 내지 약 30 중량%의 물을 함유한다. 하나의 구현양태에서, 통 안의 혼합물은 약 5 중량% 내지 약 20 중량% 물을 함유한다. 원하는 특징을 갖는 압출물의 생성을 촉진하기 위하여 가열 대역으로의 물의 도입 속도를 조절한다. 통으로 물의 도입 속도가 저하됨에 따라 압출물의 밀도가 감소되는 것으로 관찰되었다.

<53> (ii) 임의의 예비상태조절

<54> 예비-상태조절장치에서, 단백질-함유 물질 및 임의의 추가의 성분들 (단백질-함유 혼합물)을 예열하고, 수분과 접촉시키고, 수분이 개별 입자를 침투하고 연화하도록 하는 온도 및 압력 조건 하에서 유지시킨다. 예비상태조절 단계는 입상 섬유 둘질 혼합물의 벌크 밀도를 증가시키고 그의 유동 특징을 개선시킨다. 예비상태조절장치는 단백질의 균질한 혼합을 촉진하고 예비상태조절장치를 통해 단백질 혼합물의 전달을 촉진하기 위하여 하나 이상의 패들을 함유한다. 패들의 배열 및 회전 속도는 예비상태조절장치의 용량, 압출기 산출량 및/또는 예비상태조절장치 또는 압출기 통에서 혼합물의 바람직한 체류 시간에 의존하여 넓은 범위로 변한다. 일반적으로, 패들의 속도는 분 당 약 500 내지 약 1300 회전수 (rpm)이다.

<55> 전형적으로, 예비-혼합물을 수분 (즉, 증기 및/또는 물)과 접촉시킴으로써 단백질-함유 혼합물을 압출 장치로 도입되기 전에 예비-상태조절한다. 바람직하게는, 단백질-함유 혼합물을 예비상태조절장치에서 약 20 ℃ 내지 약 60 ℃, 더욱 바람직하게는 약 30 ℃ 내지 약 45 ℃의 온도로 가열한다.

<56> 전형적으로, 예비상태조절장치의 속도 및 크기에 의존하여 단백질-함유 예비-혼합물을 약 0.5분 내지 약 10.0분의 기간 동안 상태조절한다. 일례의 구현양태에서, 단백질-함유 예비-혼합물을 약 3.0분 내지 약 5.0분의 기간 동안 상태조절한다. 예비-혼합물을 증기 및/또는 물과 접촉시키고 일반적으로 일정한 증기 유동으로 예비-상태조절장치에서 가열하여 원하는 온도를 달성한다. 물 및/또는 증기는 예비-혼합물을 상태조절(즉, 수화)하고, 그의 밀도를 증가시키고, 단백질을 조직화하는 압출기 통에 도입하기 전에 방해없이 건조된 혼합물의 유동성을 촉진한다. 저 수분 예비-혼합물을 원한다면, 상태조절된 예비-혼합물은 약 1% 내지 약 35% (중량 기준) 물을 함유할 수도 있다. 고 수분 예비-혼합물을 원한다면, 상태조절된 예비-혼합물은 약 35% 내지 약 80% (중량 기준) 물을 함유할 수도 있다.

<57> 상태조절된 예비-혼합물은 전형적으로 약 0.25 g/cm$^3$ 내지 약 0.60 g/cm$^3$의 벌크 밀도를 갖는다. 일반적으로, 예비-상태조절된 단백질 혼합물의 벌크 밀도가 이 범위 내에서 증가하기 때문에, 단백질 혼합물은 가공이 용이하다. 현재, 이것은 압출기 축 간의 모든 공간 또는 대부분의 공간을 차지하는 혼합물에 기인하는 것으로 생각되고, 이에 의해 통을 통해 압출 덩어리를 운반하는 것을 수월하게 한다.

<58> (iii) 압출 공정

<59> 건조 예비-혼합물 또는 상태조절된 예비-혼합물을 압출기에 공급하여 혼합물을 가열하고 전단하고 궁극적으로 가소화한다. 통상적으로 입수가능한 압출기로부터 압출기를 선택할 수도 있고, 단축 압출기 또는 바람직하게는 축 요소로 혼합물을 기계적 전단시키는 이축 압출기일 수도 있다.

<60> 예비-혼합물이 압출 장치에 일반적으로 도입되는 속도는 특정한 장치에 의존하여 변할 것이다. 일반적으로, 예비-혼합물은 약 25 킬로그램/분 이하의 속도로 도입된다. 일반적으로, 압출기로 예비-혼합물의 공급 속도가 증가함에 따라 압출물의 밀도가 감소되는 것으로 관찰되었다.

<61> 예비-혼합물을 압출기에 의해 전단 및 압력 처리하여 혼합물을 가소화한다. 압출기의 축 요소는 혼합물을 전단시킬 뿐만 아니라 압출기를 통해 그리고 다이 조립체를 통해 혼합물을 전방으로 강제로 보냄으로써 압출기에서 압력을 발생시킨다. 축 모터 속도는 축(들)에 의해 혼합물에 적용되는 전단 및 압력의 양을 결정한다. 바람직하게는, 축 모터 속도를 약 200 rpm 내지 약 500 rpm, 더욱 바람직하게는 약 300 rpm 내지 약 400 rpm의 속도로 설정하고, 이것은 약 20 킬로그램/시간 이상, 더욱 바람직하게는 약 40 킬로그램/시간 이상의 속도로 압출기를 통해 혼합물을 이동시킨다. 바람직하게는, 조리 압출기는 약 500 내지 약 1500 psig의 압출기 통 배출 압력을 발생시키고, 더욱 바람직하게는 약 600 내지 약 1000 psig의 압출기 통 배출 압력이 발생된다.

<62> 압출기는 혼합물이 압출기를 통해 통과할 때 혼합물을 가열하여 혼합물 내의 단백질을 변성시킨다. 압출기는 혼합물을 약 100 ℃ 내지 약 180 ℃의 온도로 가열 및/또는 냉각하기 위한 수단을 포함한다. 바람직하게는, 압출기에서 혼합물을 가열 또는 냉각하는 수단은 압출기 통 외피를 포함하고, 압출기를 통과하는 혼합물의 온도를 조절하기 위하여 그 안에 증기 또는 물과 같은 가열 또는 냉각 매질을 도입할 수도 있다. 압출기는 압출기 내에서 혼합물 내에 증기를 직접 주입하기 위한 증기 주입구를 포함할 수도 있다. 압출기는 바람직하게는 독립적인 온도로 조절될 수 있는 다수의 가열 대역을 포함하고, 이곳에서 혼합물이 압출기를 통해 진행될 때 혼합물의

온도를 증가시키기 위하여 가열 대역의 온도를 바람직하게 설정한다. 하나의 구현양태에서, 압출기는 4개의 온도 대역 배열로 설정될 수도 있고, 이곳에서 첫 번째 대역 (압출기 유입구 근처)을 약 80 ℃ 내지 약 100 ℃의 온도로 설정하고, 두 번째 대역을 약 100 ℃ 내지 135 ℃의 온도로 설정하고, 세 번째 대역을 135 ℃ 내지 약 150 ℃로 설정하고, 네 번째 대역 (압출기 출구 근처)을 150 ℃ 내지 180 ℃의 온도로 설정한다. 원한다면, 압출기를 다른 온도 대역 배열로 설정할 수도 있다. 다른 구현양태에서, 압출기는 5개 온도 대역 배열로 설정될 수도 있고, 이곳에서 첫 번째 대역을 약 25 ℃의 온도로 설정하고, 두 번째 대역을 약 50 ℃의 온도로 설정하고, 세 번째 대역을 약 95 ℃의 온도로 설정하고, 네 번째 대역을 약 130 ℃의 온도로 설정하고, 다섯 번째 대역을 약 150 ℃의 온도로 설정한다. 또 다른 구현양태에서, 압출기는 6개 온도 대역 배열로 설정될 수도 있고, 이곳에서 첫 번째 대역을 약 90 ℃의 온도로 설정하고, 두 번째 대역을 약 100 ℃의 온도로 설정하고, 세 번째 대역을 약 105 ℃의 온도로 설정하고, 네 번째 대역을 약 100 ℃의 온도로 설정하고, 다섯 번째 대역을 약 120 ℃의 온도로 설정하고 여섯 번째 대역을 약 130 ℃의 온도로 설정한다.

<63> 혼합물은 압출기에서 용융된 가소화 덩어리를 형성한다. 가소화 혼합물이 압출기 출구로부터 다이 조립체 내로 유동되도록 하고, 다이 조립체를 통해 유동될 때 가소화 혼합물 내에서 단백질 섬유의 실질적인 정렬을 일으키는 배열로 다이 조립체를 압출기에 부착한다. 다이 조립체는 전면판 다이 또는 주변 다이를 포함할 수도 있다. 16 메쉬 망을 통해 5% 이하, 그리고 1/2-인치 망 위에서 65% 이하로 조각이 크기에 따라 분류되도록 절단기 속도를 설정한다.

<64> 하나의 구현양태는 도 3 내지 5에서 (10)으로 일반적으로 도시 및 표시된 주변 다이 조립체를 포함한다.

<65> 도 3 및 4에 나타낸 것과 같이, 주변 다이 조립체(10)는 원통형 2-부분 슬리브 다이 본체(17)를 갖는 다이 슬리브(12)를 포함할 수도 있다. 슬리브 다이 본체(17)는 앞 부분(20)에 결합된 뒷 부분(18)을 포함하고, 대향 개구부(72, 74)와 통해 있는 내부 챔버(31)를 공동으로 한정한다. 다이 슬리브(12)는, 압출 공정 동안에 주변 다이 조립체(10)를 통한 가소화 혼합물의 흐름을 촉진하기 위해 필요한 구조 요소를 제공하기 위하여, 다이 삽입물(14) 및 다이 원뿔(16)을 수용하는데 적합할 수도 있다.

<66> 추가로, 주변 다이 조립체(10)의 조립 동안에 앞 부분(20)이 다이 슬리브(12)의 뒷 부분(18)에 고정될 때, 다이 슬리브(12)의 앞 부분(20)을 다이 삽입물(14)과 조화되기에 적합한 다이 원뿔(16)에 고정시킬 수도 있다. 더 나타낸 바와 같이, 다이 슬리브(12)의 뒷 부분(18)은 슬리브 본체(17)를 따라 다수의 원형 출구(24)를 한정하며, 이것은 압출 공정 동안에 주변 다이 조립체(10)로부터 압출물의 배출을 위한 도관을 제공하는데 적합하다. 대안적으로, 다수의 출구(24)는 상이한 형태, 예컨대 정사각형, 직사각형, 부채모양 또는 불규칙 형태를 가질 수도 있다. 더 나타낸 바와 같이, 다이 슬리브(12)의 뒷 부분(18)은 개구부(72)를 둘러싸고 있고 압출기에 다이 슬리브(12)를 맞물릴 때 다이 슬리브(12)를 적절히 정렬하기 위해 사용되는 한 쌍의 대향 슬롯(82A, 82B)을 한정하는 원형 플랜지(37)를 포함할 수도 있다.

<67> 도 5에 나타낸 바와 같이, 주변 다이 조립체(10)가 완전히 조립될 때, 다이 슬리브(12)의 앞 부분(20)에 고정된 다이 슬리브(12)의 뒷 부분(18) 안에 다이 삽입물(14)이 배치되고, 따라서 다이 원뿔(16)의 원뿔 측면(56)이 챔버(31) 쪽으로 배향되고 뒷 부분(18)과 앞 부분(20) 사이에서 통에 넣어진다. 이러한 배향에서, 원뿔 측면(56)이 다이 삽입물(14)의 전면부(27)와 작동적으로 연합된다. 그로써, 각각의 인접한 유동 전환장치(38)의 대향 측벽(30), 다이 삽입물(14)의 바닥부(64) 및 다이 원뿔(16)의 원뿔 측면(16)이, 공동으로 개개의 출구(24)와 통해 있는 개개의 유동 채널(40)을 한정한다. 상기 기재된 바와 같이 다이 슬리브(12), 다이 삽입물(14) 및 다이 원뿔(16) 사이에서 한정된 유동 채널(40)은 유동 채널(40)의 모두 4개의 측면에서 점점 가늘어질 수도 있다. 따라서, 유동 채널(40)은 각각의 유동 채널(40)의 입구(84)로부터 출구(24)까지 모두 4개의 측면 위에서 안쪽으로 점차적으로 가늘어진다.

<68> 도 5A를 참조하면, 유동 채널(40)을 통해 유동 경로 "A"를 나타내는 확대도를 나타낸다. 구체적으로, 유동 채널(40)은 다이 삽입물(14)에 의해 한정된 개구부(70)를 거쳐 출구(24)와 통해 있다.

<69> 압출 공정 동안에, 주변 다이 조립체(10)가 압출기와 작동적으로 맞물리고, 다이 삽입물(14)의 후면부(29)에 의해 한정되는 웰(52)과 접촉하고 유동 경로 "A"에 의해 표시되는 바와 같이 좁은 통로(34) 안으로 유동되어 내부 공간 개구부(36)에 들어가는 가소화 혼합물을 생성한다. 가소화 혼합물은 다이 삽입물(14)에 의해 한정되는 내부 공간(44)에 들어가고 점점 가늘어지는 각각의 유동 채널(42)의 입구(84)에 들어갈 수도 있다. 이어서, 가소화 혼합물이 각각의 유동 채널(42)을 통해 유동되고, 주변 다이 조립체(10)에 의해 제조된 압출물에서 단백질 섬유의 실질적인 정렬을 일으키는 방식으로 개개의 출구(24)로부터 배출된다.

<70> 원하는 치수를 갖는 섬유성 물질 압출물을 제공하기 위해 혼합물을 압출하기 전에 출구(들) (24)의 폭 및 높이 치수를 선택하고 설정한다. 압출물이 고기의 입방체 덩어리 내지 스테이크 필레를 닮도록 출구(들) (24)의 폭을 설정할 수도 있고, 여기에서 출구(들) (24)의 폭을 넓히면 압출물의 입방체 덩어리-유사 성질이 줄고 압출물의 필레-유사 성질을 증가시킨다. 일례의 구현양태에서, 약 10 밀리미터 내지 약 40 밀리미터의 폭으로 출구(들)(24)의 폭을 설정할 수도 있다.

<71> 압출물의 원하는 두께를 제공하기 위하여 출구(들)(24)의 높이 치수를 설정할 수도 있다. 매우 얇은 압출물 또는 두꺼운 압출물을 제공하기 위하여 출구(들)(24)의 높이를 설정할 수도 있다. 예를 들어, 출구(들)(24)의 높이를 약 1 밀리미터 내지 약 30 밀리미터로 설정할 수도 있다. 일례의 구현양태에서, 출구(들)(24)의 높이를 약 8 밀리미터 내지 약 16 밀리미터로 설정할 수도 있다.

<72> 또한, 출구(들)(24)이 둥글 수도 있다고 생각된다. 압출물의 원하는 두께를 제공하기 위하여 출구(들)(24)의 직경을 설정할 수도 있다. 매우 얇은 압출물 또는 두꺼운 압출물을 제공하기 위하여 출구(들)(24)의 직경을 설정할 수도 있다. 예를 들어, 출구(들)(24)의 직경을 약 1 밀리미터 내지 약 30 밀리미터로 설정할 수도 있다. 일례의 구현양태에서, 출구(들)(24)의 직경을 약 8 밀리미터 내지 약 16 밀리미터로 설정할 수도 있다.

<73> 본 발명에서 사용하기 위해 적절한 다른 주변 다이 조립체는 미국 특허출원 60/882,662호 (여기에서 그 전체내용이 참고문헌으로 포함됨)에 기재되어 있다.

<74> 다이 조립체에서 나온 후에 압출물을 자른다. 압출물을 자르기 위해 적절한 장치는 웬저 매뉴팩처링 인코포레이티드 (미국 캔사스주 사베타) 및 클렉스트랄 인코포레이티드 (미국 플로리다주 탬파)에 의해 제조된 가요성 나이프를 포함한다. 전형적으로, 절단 장치의 속도는 약 1000 rpm 내지 약 2500 rpm이다. 일례의 구현양태에서, 절단 장치의 속도는 약 1600 rpm이다.

<75> 건조기가 사용된다면, 건조기는 일반적으로 공기 온도가 변할 수도 있는 다수의 건조 대역을 포함한다. 일반적으로, 하나 이상의 대역 내에서 공기의 온도는 약 100 ℃ 내지 약 185 ℃이다. 전형적으로, 원하는 수분 함량을 갖는 압출물을 제공하기에 충분한 시간 동안에 압출물이 건조기에 존재한다. 일반적으로, 압출물을 약 5분 이상 동안 건조시키고, 더 일반적으로 약 10분 이상 동안 건조시킨다. 대안적으로, 더 장시간 동안 저온, 예컨대 약 70 ℃에서 압출물을 건조시킬 수도 있다. 적절한 건조기는 월버린 프록터 앤드 슈와츠 (Wolverine Proctor & Schwartz) (미국 매사추세츠주 메리맥), 내셔널 드라잉 머쉬너리 컴퍼니 (National Drying Machinery Co.) (미국 펜실바니아주 필라델피아), 웬저 (미국 캔사스주 사베타), 클렉스트랄 (미국 플로리다주 탬파) 및 뷔흘러(Buehler) (미국 일리노이주 레이크 블러프)에 의해 제조된 것을 포함한다.

<76> 압출물의 원하는 용도에 의존하여 바람직한 수분 함량이 넓은 범위로 변할 수 있다. 일반적으로 말하자면, 압출된 물질은 약 6 중량% 내지 약 13 중량%의 수분 함량을 갖고, 건조된다면 물이 흡수되고 섬유가 분리될 때까지 물에서 수화되는 것이 필요하다. 단백질 물질이 건조되지 않거나 충분히 건조되지 않는다면, 그의 수분 함량은 더욱 높고, 일반적으로 약 16 중량% 내지 약 30 중량%이다.

<77> 압출물의 평균 입자 크기를 감소시키기 위하여, 건조된 압출물을 더욱 분쇄할 수도 있다. 적절한 분쇄장치는 해머 밀, 예컨대 마크로 해머 밀스(Makro Hammer Mills) (영국 호소까와 마이크론 리미티드 제)를 포함한다.

<78> **(e) 구조화 단백질 제품의 특징결정**

<79> I(d)에서 제조된 압출물은 전형적으로 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품을 포함한다. 본 발명의 내용에서, "실질적으로 정렬된"은 구조화 단백질 제품을 형성하는 단백질 섬유의 상당히 높은 퍼센트가 일반적으로 수평면에서 볼 때 약 45도 미만의 각으로 서로 간에 연속되는 단백질 섬유의 배열을 가리킨다. 전형적으로, 구조화 단백질 제품을 포함하는 단백질 섬유의 적어도 55%의 평균이 실질적으로 정렬된다. 다른 구현양태에서, 구조화 단백질 제품을 구성하는 단백질 섬유의 적어도 60%의 평균이 실질적으로 정렬된다. 추가의 구현양태에서, 구조화 단백질 제품을 구성하는 단백질 섬유의 적어도 70%의 평균이 실질적으로 정렬된다. 추가의 구현양태에서, 구조화 단백질 제품을 구성하는 단백질 섬유의 적어도 80%의 평균이 실질적으로 정렬된다. 또 다른 구현양태에서, 구조화 단백질 제품을 구성하는 단백질 섬유의 적어도 90%의 평균이 실질적으로 정렬된다. 단백질 섬유 정렬 정도를 결정하는 방법은 당 기술분야에 공지되어 있고 현미경 사진을 기초로 하여 육안으로 결정하는 것을 포함한다.

<80> 일례로서, 도 1 및 도 2는 상당히 크로스해치된 단백질 섬유를 갖는 단백질 제품에 비하여 실질적으로 정렬된 단백질 섬유를 갖는 구조화 단백질 제품 간의 차이를 보여주는 현미경 사진을 나타낸다. 도 1은 실질적으로 정

렬된 단백질 섬유를 갖는 I(a)-I(d)를 따라 제조된 구조화 단백질 제품을 나타낸다. 반대로, 도 2는 상당히 크로스해치되고 실질적으로 정렬되지 않은 단백질 섬유를 함유하는 단백질 제품을 나타낸다. 도 1에 나타낸 것과 같이 단백질 섬유가 실질적으로 정렬되기 때문에, 본 발명에서 사용된 구조화 단백질 제품은 일반적으로 조리된 근육 고기의 조직 및 점조도를 갖는다. 반대로, 랜덤하게 배향되거나 크로스해치된 단백질 섬유를 갖는 압출물은 일반적으로 부드럽거나 해면질의 조직을 갖는다.

<81> 실질적으로 정렬된 단백질 섬유를 갖는 것에 추가로, 구조화 단백질 제품은 전형적으로 전 고기 근육과 실질적으로 유사한 전단 강도를 갖는다. 본 발명의 내용에서, 용어 "전단 강도"는 구조화 단백질 제품에 전-근육 유사 조직 및 외관을 부여하기 위해 충분한 섬유 망상의 형성을 정량화하는 한가지 수단을 제공한다. 전단 강도는 주어진 샘플을 통해 전단시키기 위해 필요한 최대 힘(그램)이다. 전단 강도의 측정 방법은 실시예 1에 기재된다.

<82> 일반적으로 말하자면, 본 발명의 구조화 단백질 제품은 1400 그램 이상의 평균 전단 강도를 가질 것이다. 추가의 구현양태에서, 구조화 단백질 제품은 약 1500 내지 약 1800 그램의 평균 전단 강도를 가질 것이다. 또 다른 구현양태에서, 구조화 단백질 제품은 약 1800 내지 약 2000 그램의 평균 전단 강도를 가질 것이다. 추가의 구현양태에서, 구조화 단백질 제품은 약 2000 내지 약 2600 그램의 평균 전단 강도를 가질 것이다. 추가의 구현양태에서, 구조화 단백질 제품은 2200 그램 이상의 평균 전단 강도를 가질 것이다. 추가의 구현양태에서, 구조화 단백질 제품은 2300 그램 이상의 평균 전단 강도를 가질 것이다. 또 다른 구현양태에서, 구조화 단백질 제품은 2400 그램 이상의 평균 전단 강도를 가질 것이다. 또 다른 구현양태에서, 구조화 단백질 제품은 2500 그램 이상의 평균 전단 강도를 가질 것이다. 추가의 구현양태에서, 구조화 단백질 제품은 2600 그램 이상의 평균 전단 강도를 가질 것이다.

<83> 구조화 단백질 제품에서 형성된 단백질 섬유의 크기를 정량화하는 수단은 절단(shred) 특징결정 시험에 의해 수행될 수도 있다. 절단 특징결정은 일반적으로 구조화 단백질 제품에서 형성된 큰 조각의 퍼센트를 결정하는 시험이다. 간접적인 방식에서, 절단 특징의 퍼센트는 구조화 단백질 제품에서 단백질 섬유 정렬 정도를 정량하는 추가의 수단을 제공한다. 일반적으로 말하자면, 큰 조각의 퍼센트가 증가함에 따라 구조화 단백질 제품 내에서 정렬된 단백질 섬유의 정도가 전형적으로 증가한다. 역으로, 큰 조각의 퍼센트가 감소함에 따라 구조화 단백질 제품 내에서 정렬된 단백질 섬유의 정도는 전형적으로 저하된다.

<84> 절단 특징을 결정하기 위한 방법을 실시예 2에서 상세히 설명한다. 본 발명의 구조화 단백질 제품은 전형적으로 10중량% 이상의 큰 조각의 평균 절단 특징을 갖는다. 추가의 구현양태에서, 구조화 단백질 제품은 약 10 내지 약 15 중량%의 큰 조각의 평균 절단 특징을 갖는다. 다른 구현양태에서, 구조화 단백질 제품은 약 15 중량% 내지 약 20 중량%의 큰 조각의 평균 절단 특징을 갖는다. 또 다른 구현양태에서, 구조화 단백질 제품은 약 20 내지 약 25 중량%의 큰 조각의 평균 절단 특징을 갖는다. 추가의 구현양태에서, 평균 절단 특징은 20 중량% 이상, 21 중량% 이상, 22 중량% 이상, 23 중량% 이상, 24 중량% 이상, 25 중량% 이상, 또는 26 중량% 이상의 큰 조각이다.

<85> 본 발명의 적절한 구조화 단백질 제품은 일반적으로 실질적으로 정렬된 단백질 섬유를 갖고, 1400 그램 이상의 평균 전단 강도를 갖고, 10 중량% 이상의 큰 조각의 평균 절단 특징을 갖는다. 더욱 전형적으로, 구조화 단백질 제품은 55% 이상 정렬된 단백질 섬유를 갖고, 1800 그램 이상의 평균 전단 강도를 갖고, 15 중량% 이상의 큰 조각의 평균 절단 특징을 갖는다. 일례의 구현양태에서, 구조화 단백질 제품은 55% 이상 정렬된 단백질 섬유를 갖고 2000 그램 이상의 평균 전단 강도를 갖고 17 중량% 이상의 큰 조각의 평균 절단 특징을 갖는다. 다른 일례의 구현양태에서, 구조화 단백질 제품은 55% 이상 정렬된 단백질 섬유를 갖고 2200 그램 이상의 평균 전단 강도를 갖고 20 중량% 이상의 큰 조각의 평균 절단 특징을 갖는다.

<86> **(II) 재구성 고기 조성물**

<87> 본 발명에서 구조화 단백질 제품을 재구성 고기 조성물에서 성분으로서 사용한다. 재구성 고기 조성물은 동물 고기 및 구조화 단백질 제품의 혼합물을 포함할 수도 있거나, 또는 고기 및 1차 구조화 단백질 제품을 포함하지 않을 수도 있다. 재구성 고기 조성물의 제조 방법은 일반적으로 이것을 동물 고기와 임의로 혼합하고, 구조화 단백질 제품을 착색 및 수화하고, 그의 입자 크기를 감소시키고 고기를 포함한 식품 제품으로 조성물을 더욱 가공하는 것을 포함한다.

<88> **(a) 동물 고기와의 임의 배합**

<89> 구조화 단백질 제품을 하기 상술된 착색 조성물과 접촉시키기 전 또는 후에 동물 고기 조성물을 제조하기 위하

여 구조화 단백질 제품을 동물 고기와 임의로 배합할 수도 있다. 일반적으로, 구조화 단백질 제품을 유사한 입자 크기를 갖는 동물 고기와 배합할 것이다.

<90> 동물 조직으로부터 뼈를 분리하는 고압 기계를 사용하여, 먼저 동물 조직에 붙어있는 뼈를 부수고 이어서 뼈가 아닌 동물 조직을 체 또는 유사한 선별 장치를 통해 강제로 통과시킴으로써 기계적으로 뼈 제거되거나 분리된 날고기를 만드는 것이 당 기술분야에 공지되어 있다. 본 발명에서 동물 조직은 근육 조직, 기관 조직, 연결 조직 및 피부를 포함한다. 공정은 반죽과 유사한 점조도를 갖는 연질 동물 조직의 비구조화 페이스트-유사 배합물을 형성하고, 이것은 보통 기계적으로 뼈 제거된 고기 또는 MDM이라 일컬어진다. 이러한 페이스트-유사 배합물은 약 0.25 내지 약 15 밀리미터의 입자 크기, 바람직하게는 약 5 밀리미터 이하, 가장 바람직하게는 약 3 밀리미터 이하의 입자 크기를 갖는다.

<91> 가공 이전에 미생물 오염을 피하기 위하여 날 고기로 알려진 동물 조직이 적어도 실질적으로 냉동된 형태로 제공되는 것이 바람직하긴 하지만, 일단 고기가 분쇄되면 개개의 긴 조각 또는 조각으로의 절단성을 제공하기 위해 그것을 반드시 냉동할 필요는 없다. 고기 음식물과 달리, 날 고기는 약 1:3.6 내지 1:3.7의 단백질 대 수분 비율을 갖는 천연 고 수분 함량을 갖는다.

<92> 본 발명에서 사용된 날 고기는 인간 소비를 위해 적절한 어떠한 식용 고기일 수도 있다. 고기는 비-정제, 비-건조, 날 고기, 날 고기 제품, 날 고기 부산물 및 이들의 혼합물일 수도 있다. 고기 또는 고기 제품을 분쇄하고, 미생물 오염을 피하기 위하여, 일반적으로 완전히 냉동되거나 적어도 실질적으로 냉동된 상태로 매일 공급한다. 일반적으로, 분쇄된 고기의 온도는 약 40 ℃ (104 °F) 미만, 바람직하게는 약 10 ℃ (50 °F) 미만, 더욱 바람직하게는 약 -4 ℃ (25 °F) 내지 약 6 ℃ (43 °F), 가장 바람직하게는 약 -2 ℃ (28 °F) 내지 약 2 ℃ (36 °F)이다. 냉장 또는 냉각된 고기를 사용할 수도 있지만, 공장 설비에서 다량의 냉동되지 않은 고기를 장기간 보관하는 것이 일반적으로 비실용적이다. 냉동된 제품은 냉장되거나 냉각된 제품에 비하여 더욱 긴 보관기간을 제공한다.

<93> 냉동된 분쇄 고기 대신에, 새로 준비된 분쇄 고기가 약 40 ℃ (104 °F) 이하의 온도 조건을 충족하는 이상, 분쇄된 고기를 재구성 고기 제품의 제조를 위해 새로 준비할 수도 있다.

<94> 냉동 또는 비냉동 날 고기의 수분 함량은 날 고기의 중량을 기준으로 하여 일반적으로 약 50 중량% 이상, 가장 종종 약 60 중량% 내지 약 75 중량%이다. 본 발명의 구현양태에서, 냉동 또는 비냉동 날 고기의 지방 함량은 2 중량% 이상이다. 일반적으로, 냉동되거나 냉동되지 않은 날고기의 지방 함량은 약 3 중량% 내지 약 95 중량%이다. 다른 구현양태에서, 냉동되거나 냉동되지 않은 날 고기의 지방 함량은 약 20 중량% 내지 약 95 중량%이다. 본 발명의 다른 구현양태에서, 고기 제품을 조합하여 약 15 중량% 내지 약 30 중량%의 지방 함량을 갖는 고기 조성물을 생산할 수 있다. 다른 구현양태에서, 고기 조성물은 약 10 중량% 미만의 지방 함량을 가질 수도 있고 탈지된 고기 제품이 사용될 수도 있다.

<95> 냉동 또는 냉각된 고기는 약 -18 ℃ (4 °F) 내지 약 0 ℃ (32 °F)의 온도에서 저장될 수도 있다. 이것은 일반적으로 20 킬로그램 블록으로 공급된다. 사용 시에, 블록을 약 10 ℃ (50 °F) 이하에서 해동하고, 다시 말해서 완화된 환경에서 녹인다. 따라서, 여전히 냉동된 동안에 블록의 나머지 내부를 계속 해동시키고 외부를 약 10 ℃ (50 °F) 미만으로 유지하면서, 블록의 외층, 예를 들어 약 1/4"의 깊이까지의 외층을 약 0 ℃ (32 °F)의 온도에서 여전히 녹이거나 해동할 수도 있다.

<96> 다양한 동물 고기가 재구성 고기 조성물에서 사용하기 위해 적절하다. 예를 들어, 고기는 양, 소, 염소, 돼지, 들소 및 말로 구성된 군에서 선택되는 농장 동물로부터 얻어질 수도 있다. 동물 고기는 가금류, 예컨대 닭, 오리, 거위 또는 칠면조로부터 얻어질 수도 있다. 대안적으로, 동물 고기는 사냥 동물로부터 얻어질 수도 있다. 이러한 사냥 동물의 비-제한적인 예는 물소, 사슴, 엘크, 큰사슴, 순록, 카리부, 영양, 토끼, 다람쥐, 비버, 사향뒤쥐, 주머니쥐, 미국너구리, 아르마딜로, 호저, 악어 및 뱀을 포함한다. 추가의 구현양태에서, 동물 고기는 어류 또는 갑각류로부터 얻어질 수도 있다. 적절한 어류 또는 어류 제품의 비-제한적 예는 염수 및 담수 어류, 예컨대 메기, 촙치, 연어, 베스, 고등어, 대구류(pollack), 대구류(hake), 틸라피아, 대구(cod), 그루퍼(grouper), 송어, 보우핀(bowfin), 동갈치(gar), 패들피쉬(paddlefish), 철갑상어, 잉어과의 식용어(bream), 잉어과 물고기(carp), 송어(trout), 수리미(surimi), 눈알이 큰 물고기(walleye), 패모의 일종(snakehead) 및 상어를 포함한다. 일례의 구현양태에서, 동물 고기는 쇠고기, 돼지고기 또는 칠면조고기이다.

<97> 고기는, 겹쳐 있는 지방 및 보통 고기 살을 동반하는 피부, 힘줄, 신경 및 혈관의 일부를 동반하거나 동반하지 않으면서, 골격이거나 예를 들어 혀, 횡격막, 심장 또는 식도에서 발견되는 횡문근을 포함한다. 고기 부산물의

예는 기관 및 조직, 예컨대 폐, 비장, 신장, 뇌, 간, 혈액, 뼈, 부분 탈지된 저온 지방 조직, 위, 그들의 내용물을 갖지 않은 소장 등이다.

<98> 용어 "고기 부산물"은 이에 한정되지 않지만 포유류, 가금류 등을 포함하고 [Definitions of Feed Ingredients (the Association of American Feed Control Officials, Incorporated 발행)]에서 용어 "고기 부산물"에 포함되는 성분을 포함하는, 도살된 동물 사체의 비-정제 부분을 가리키는 것으로 해석된다.

<99> 전형적으로, 동물 고기 조성물에서 동물 고기의 양에 관련된 구조화 단백질 제품의 양은, 조성물의 원하는 용도에 의존하여 변할 수 있고 변할 것이다. 일례로서, 비교적 작은 정도의 동물 향미를 갖는 채식주의자용 조성물을 원할 때, 재구성 고기 조성물에서 동물 고기의 농도는 약 45 중량%, 40 중량%, 35 중량%, 30 중량%, 25 중량%, 20 중량%, 15 중량%, 10 중량%, 5 중량%, 2 중량% 또는 0 중량%일 수도 있다. 대안적으로, 비교적 높은 정도의 동물 고기 향미를 갖는 재구성 고기 조성물을 원할 때, 재구성 고기 조성물에서 동물 고기의 농도는 약 50 중량%, 55 중량%, 60 중량%, 65 중량%, 70 중량%, 75 중량%, 80 중량%, 85 중량%, 90 중량% 또는 95 중량%일 수도 있다. 그 결과, 재구성 고기 조성물에서 함수 구조화 식물 단백질 제품의 농도는 약 5 중량%, 10 중량%, 15 중량%, 20 중량%, 25 중량%, 30 중량%, 35 중량%, 40 중량%, 45 중량%, 50 중량%, 55 중량%, 60 중량%, 65 중량%, 70 중량%, 75 중량%, 80 중량%, 85 중량%, 90 중량%, 95 중량% 또는 99 중량%일 수도 있다. 일례의 구현양태에서, 재구성 고기 조성물은 일반적으로 약 40 중량% 내지 60 중량%의 함수 구조화 단백질 제품 및 약 40 중량% 내지 약 60 중량%의 동물 고기를 가질 것이다.

<100> 또한, 다양한 고기 품질이 본 발명에서 사용될 수도 있는 것으로 생각된다. 예를 들어, 분쇄되거나 덩어리 또는 스테이크 형태의 전 고기 근육이 사용될 수도 있다. 고기는 넓은 범위로 변하는 지방 함량을 가질 수도 있다.

<101> **(b) 분쇄된 야채 또는 분쇄된 과일과의 임의 배합**

<102> 구조화 단백질 제품을 하기 상술된 착색 조성물과 접촉시키기 전 또는 후에 구조화 단백질 제품을 임의로 분쇄된 야채 또는 분쇄된 과일과 배합하여 재구성 고기 조성물을 제조할 수 있다. 일반적으로, 구조화 단백질 제품을 유사한 입자 크기를 갖는 분쇄된 야채 또는 분쇄된 과일과 배합할 것이다.

<103> 각종 야채 또는 과일이 재구성 고기 조성물에서 사용하기 위해 적절하다. 전형적으로, 재구성 고기 조성물에서 분쇄된 야채 또는 분쇄된 과일의 양에 관련된 구조화 단백질 제품의 양은 조성물의 원하는 용도에 의존하여 변할 수 있고 변할 것이다. 일례로, 재구성 고기 조성물에서 분쇄된 야채 또는 분쇄된 과일의 농도는 약 95 중량%, 90 중량%, 85 중량%, 80 중량%, 75 중량%, 70 중량%, 65 중량%, 60 중량%, 55 중량%, 50 중량%, 45 중량%, 40 중량%, 35 중량%, 30 중량%, 25 중량%, 20 중량%, 15 중량%, 10 중량%, 5 중량%, 2 중량% 또는 0 중량%일 수도 있다. 그 결과, 재구성 고기 조성물에서 함수 구조화 식물 단백질 제품의 농도는 약 5 중량%, 10 중량%, 15 중량%, 20 중량%, 25 중량%, 30 중량%, 35 중량%, 40 중량%, 45 중량%, 50 중량%, 55 중량%, 60 중량%, 65 중량%, 70 중량%, 75 중량%, 80 중량%, 85 중량%, 90 중량%, 95 중량% 또는 99 중량%일 수도 있다. 일례의 구현양태에서, 재구성 고기 조성물은 일반적으로 약 40 중량% 내지 약 60 중량%의 함수 구조화 단백질 제품 및 약 40 중량% 내지 약 60 중량%의 분쇄된 야채 또는 분쇄된 과일을 가질 것이다.

<104> **(c) 구조화 단백질 제품의 수화 및 착색**

<105> 구조화 단백질 제품을 날고기 및/또는 조리된 고기와 비슷하게 되도록 하기 위하여 일반적으로 착색 조성물로 착색한다. 본 발명의 착색 조성물은 열 불안정성 안료, 열 안정성 안료, 및 갈색화 제를 포함할 수도 있다. 착색 조성물에 존재하는 안료의 유형 및 양의 선택은, 재구성 고기 조성물의 원하는 색에 의존하여 변할 수 있고 변할 것이다. 재구성 고기 조성물이 "예비-조리된 제품"을 모방할 때, 구조화 식물 제품을 전형적으로 갈색화 제 및/또는 열 안정성 안료와 접촉시킨다. 대안적으로, 재구성 고기 조성물이 날고기를 모방할 때, 구조화 단백질 제품을 일반적으로 열 불안정성 적색 안료 및 갈색화 제 및/또는 열 안정성 안료와 접촉시키고, 그 결과 재구성 고기 조성물이 조리될 때, 그의 외관이 날고기 색으로부터 완전히 조리된 색으로 바뀐다. 적절한 열 불안정성 적색 안료, 열 안정성 안료 및 갈색화 제를 하기에 기재한다.

<106> 압출기에 공급되기 이전에 착색제(들)를 단백질-함유 물질 및 기타 성분과 혼합할 수도 있다. 대안적으로, 압출기에 공급한 후에, 착색제(들)를 단백질-함유 물질 및 기타 성분과 조합할 수도 있다. 압출 공정 동안에 사용되는 열 또는 열 및 압력의 존재에서, 착색제 및 단백질-함유 물질의 일부 조합은 예상 밖의 색을 가져오기도 한다. 일례로서, 압출 공정 동안에 카민 (또는 락(lac))을 단백질-함유 물질과 접촉시킬 때, 색은 적색으로부터 보라색/자색으로 바뀐다.

<107> 착색제(들)는 천연 착색제, 천연 착색제들의 조합, 인공 착색제, 인공 착색제들의 조합, 또는 천연 및 인공 착색제의 조합일 수도 있다. 식품에서 사용하기 위해 승인된 천연 착색제의 적절한 예는 안나토(적색을 띤 오렌지색), 안토시아닌 (적색 내지 청색, pH에 의존), 비트 즙, 베타-카로틴(오렌지색), 베타-APO 8 카로테날 (오렌지색), 블랙 커런트, 탄 설탕(burnt sugar), 칸타잔틴(분홍-적색), 카라멜, 카민/카민산 (밝은 적색), 양홍 추출물 (적색), 큐커민 (황색-오렌지색), 랙 (진홍색), 루테인(적색-오렌지색), 리코펜(오렌지색-적색), 혼합된 카로티노이드 (오렌지색), 모나스커스 (적색-자색, 발효된 적색 쌀로부터), 파프리카, 적양배추 즙, 리보플라빈 (황색), 사프란, 이산화티탄 (백색) 및 심황 (황색-오렌지색)을 포함한다. 미국에서 식품 용도를 위해 승인된 인공 착색제의 적절한 예는 FD & C 적색 3호 (에리트로신), FD & C 적색 40호 (알루어 레드), FD & C 황색 5호 (타르트라진), FD & C 황색 6호 (선셋 엘로우 FCF), FD & C 청색 1호 (브릴리언트 블루), FD & C 청색 2호 (인디고틴)을 포함한다. 다른 나라에서 사용될 수 있는 인공 착색제는 CI 식품 적색 3호 (카르모이신), CI 식품 적색 7호 (폰슈 4R), CI 식품 적색 9호 (아마란스), CI 식품 황색 13호 (퀴놀린 엘로우) 및 CI 식품 청색 5호 (패이턴트 블루 V)를 포함한다. 식품 착색제는 염료일 수 있고, 이것은 물에 가용성인 분말, 과립 또는 액체이다. 대안적으로, 천연 및 인공 식품 착색제는 염료 및 불용성 물질의 조합인 진홍색일 수도 있다. 진홍색은 가용성이 아니고, 유 분산성이며 분산에 의해 엷게 된다.

<108> 적절한 착색제(들)를 다양한 형태로 단백질-함유 물질과 조합할 수도 있다. 비-제한적인 예는 고체, 반-고체, 분말, 액체 및 젤라틴을 포함한다. 사용되는 착색제(들)의 유형 및 농도는 사용된 단백질-함유 물질 및 착색된 구조화 단백질 제품의 원하는 색에 의존하여 변할 수도 있다. 전형적으로, 착색제(들)의 농도는 약 0.001 내지 약 5.0 중량%의 범위일 수도 있다. 하나의 구현양태에서, 착색제(들)의 농도는 약 0.01 내지 약 4.0 중량%의 범위일 수도 있다. 다른 구현양태에서, 착색제(들)의 농도는 약 0.05 내지 약 3.0 중량%의 범위일 수도 있다. 다른 구현양태에서, 착색제(들)의 농도는 약 0.1 내지 약 3.0 중량%의 범위일 수도 있다. 추가의 구현양태에서, 착색제(들)의 농도는 약 0.5 내지 약 2.0 중량%의 범위일 수도 있다. 다른 구현양태에서, 착색제(들)의 농도는 약 0.75 내지 약 1.0 중량%의 범위일 수도 있다.

<109> 조리되지 않은 날고기의 적색을 제공하기 위하여, 착색 조성물에서 열 불안정성 안료를 사용할 수도 있다. 열 불안정성 안료는 전형적으로 조리되지 않은 상태에 있는 갈색화 고기 (즉, 날고기)의 붉은 자연색을 닮은 적색을 갖는 식품 착색용 염료 또는 분말이다. 일반적으로 말하자면, 열 불안정성 안료는 구조화 단백질 제품을 조리하는데 효과적인 온도에 노출시킬 때 분해되는 구조를 갖는 식품 착색용 염료 또는 분말이다. 이러한 방식으로, 안료가 열적으로 분해되고, 따라서 조리될 때 구조화 단백질 제품에 실질적인 색을 제공하는데 비효과적이다. 열 불안정성 안료는 전형적으로 약 100 ℃ 이상의 온도, 더욱 바람직하게는 약 75 ℃ 이상의 온도, 가장 바람직하게는 약 50 ℃ 이상의 온도에서 분해된다. 하나의 구현양태에서, 열 불안정성 안료는 베타닌, 불량한 열 안정성을 갖는 적색 식품 착색 염료 또는 분말이다. 베타닌은 적색 비트로부터 유래되고, 전형적으로 적색 비트 즙 또는 비트 분말로부터 제조된다. 열 불안정성 안료는 착색 조성물의 건조 중량 기준으로 약 0.005 내지 약 30 중량%로 착색 조성물에 존재할 수도 있다. 열 불안정성 안료가 베타닌일 때, 베타닌은 바람직하게는 건조 중량 기준으로 착색 조성물의 약 0.005% 내지 약 0.5%를 형성하고, 더욱 바람직하게는 건조 중량 기준으로 착색 조성물의 약 0.01% 내지 약 0.05%를 형성한다. 대안적으로, 베타닌을 함유하는 비트 분말 또는 비트 추출물 재료는 건조 중량 기준으로 조성물의 약 5% 내지 약 30%로 착색 조성물에 존재할 수 있고, 더욱 바람직하게는 착색 조성물의 약 10% 내지 약 25%로 존재할 수 있다. 일례로서, 착색 조성물은 0.0087% 안나토, 21.68% 비트 분말, 52.81% 덱스트로스 및 25.43% NFE (모든 퍼센트는 중량 기준이다)로 구성될 수 있다.

<110> 하나 이상의 열 안정성 식품 착색 염료로 이루어진 열 안정성 안료가 착색 조성물에서 사용될 수도 있다. 적절한 열 안정성 안료는 조리되지 않은 상태 및 조리된 상태 양쪽 모두에서 갈색화된 고기를 닮은 색을 갖는 구조화 단백질 제품을 제공하는데 효과적인 것을 포함한다. 적절한 열 안정성 안료는 카라멜 식품 착색 재료, 및 황색, 갈색 및/또는 오렌지색 식품-착색 재를 포함한다. 각종 카라멜 식품 착색제가 본 발명에서 유용하고, 카라멜 색 번호 602 (미국 켄터키주 루이스빌 윌리암슨 컴퍼니로부터 입수가능함) 및 5440 카라멜 분말 D.S. (미국 미조리주 세인트루이스 센시엔트 컬러스 인코포레이티드로부터 입수가능함)를 포함하여 분말화 형태 또는 액체 형태로 통상적으로 입수가능하다.

<111> 몇 가지 유형의 상업적으로 입수가능한 황색/오렌지색 식품 색소가 열 안정성 안료에서 사용될 수도 있다. 적절한 황색/오렌지색 식품 색소는 안나토, 투메릭 및 인공 황색 염료, 예컨대 FD & C 황색 5호를 포함한다. 착색 조성물에 존재하는 열 안정성 안료의 양은 착색 조성물의 건조 중량 기준으로 약 0% 내지 약 7%, 더욱 바람직하게는 착색 조성물의 건조 중량 기준으로 약 0.1% 내지 약 3%이다. 황색/오렌지색 식품 착색 물질, 바람직하게는 안나토가 건조 중량 기준으로 착색 조성물의 약 0% 내지 약 2%를 구성할 수도 있고, 바람직하게는 건조

중량 기준으로 착색 조성물의 약 0.1% 내지 약 1%로 존재한다. 카라멜 식품 착색 물질은 전형적으로 착색 조성물의 건조 중량 기준으로 약 0% 내지 약 5%, 바람직하게는 건조 중량 기준으로 약 0.5% 내지 약 3%를 구성한다.

<112> 착색 조성물은 아민 원료 및 환원 당을 포함한 갈색화 제를 포함할 수도 있다. 상기 상술된 바와 같이, 갈색화 제는 일반적으로, 착색 조성물이 혼합되어 있는 단백질 함유 물질을 단백질 물질이 조리될 때의 조리 갈색화 고기와 유사한 갈색으로 만든다. 대안적인 구현양태에서, 착색 조성물의 갈색화 제는 아민 원료를 포함할 수도 있다. 아민 화합물은 환원 당과 반응하여 갈색화를 유도한다. 적절한 아민 원료는 폴리펩티드 물질, 가수분해된 단백질 물질 또는 아미노산 물질을 포함한다. 특정한 이론에 의해 한정되지 않으면서, 폴리펩티드 물질, 가수분해된 단백질 및/또는 아미노산 물질이 갈색화 제에서 아민 원료로서 바람직하게 포함되어 바람직한 갈색화를 증진시킨다. 일례의 구현양태에서, 단리된 콩 단백질이 갈색화 제에서 아민 기의 원료이다. 착색 조성물에 포함될 때, 아민 원료는 일반적으로 건조 중량 기준으로 착색 조성물의 약 20% 내지 55%로 착색 조성물에 존재한다. 일례의 갈색화 제는 환원 당이다. 단백질 함유 물질이 조리될 때 바람직한 갈색화를 제공하기 위하여, 적절한 환원 당이 전형적으로 아민 기를 함유하는 화합물의 존재하에서 마일라드 갈색화 반응을 겪을 수 있다. 적절한 환원 당의 대표적인 예는 자일로스, 아라비노스, 갈락토스, 프럭토스, 글리세르알데히드, 만노스, 덱스트로스, 락토스 및 말토스를 포함한다. 일례의 구현양태에서, 환원 당은 덱스트로스이다. 환원 당은 착색 조성물의 건조 중량 기준으로 약 25% 내지 약 95%로 착색 조성물에 존재할 수도 있고, 바람직하게는 착색 조성물의 건조 중량 기준으로 약 30% 내지 약 60%로 존재할 수도 있다.

<113> 일례의 구현양태에서, 착색 조성물은 비트 안료, 안나토, 카라멜 색소, 환원 당 및 아미노산 원료를 포함한다. 이러한 구현양태의 하나의 대안에서, 아미노산 원료는 아미노산 및 2차 아미노산으로 이루어진 펩티드를 포함한다. 다른 대안적인 구현양태에서, 아미노산 원료는 단리된 콩 단백질이다.

<114> 착색 조성물은 착색제를 위한 최적의 범위로 pH를 유지하기 위해 산도 조절제를 더 포함할 수도 있다. 산도 조절제는 산성화제(acidulent)일 수도 있다. 식품에 첨가될 수도 있는 산성화제의 예는 시트르산, 아세트산 (식초), 타르타르산, 말산, 푸마르산, 락트산, 글루콘산, 인산, 소르브산, 염산, 프로피온산 및 벤조산을 포함한다. 착색 조성물에서 산성화제의 최종 농도는 착색 조성물의 약 0.001 중량% 내지 약 5 중량%의 범위일 수도 있다. 산도 조절제는 pH-상승제, 예컨대 이인산이나트륨, 탄산나트륨, 중탄산나트륨, 수산화나트륨 및 수산화칼륨일 수도 있다.

<115> 본 발명의 착색 조성물은 당업자에게 공지된 방법 및 절차를 사용하여 성분들을 조합함으로써 제조될 수도 있다. 성분은 전형적으로 액체 형태 또는 분말 형태로 이용될 수 있고, 종종 양쪽 형태로 이용된다. 성분들을 직접 혼합하여 착색 조성물을 형성할 수도 있지만, 바람직하게는 착색 조성물의 성분들을 약 10 중량% 내지 약 25 중량%의 총 농도로 수용액에서 조합하고, 여기에서 착색 수용액을 구조화 단백질 제품과 혼합하고 착색하기 위한 양의 물에 편리하게 첨가할 수 있다.

<116> **(d) 임의 성분들의 첨가**

<117> 재구성 고기 조성물은 각종 향미제, 향신료, 항산화제 또는 바람직한 향미 또는 조직을 부여하거나 최종 식품 제품을 영양학적으로 증진시키기 위한 기타 성분을 임의로 포함할 수도 있다. 당업자에게 이해되듯이, 재구성 고기 조성물에 첨가된 성분들의 선택은 제조되는 식품 제품에 의존될 수 있고 의존될 것이다.

<118> 재구성 고기 조성물은 향미를 부여하기 위하여 약 1 중량% 내지 약 30 중량%의 지방 원료를 포함할 수도 있다. 전형적으로, 지방 원료는 동물 지방이다. 적절한 동물 지방은 소 지방, 돼지 지방, 가금류 지방 및 어린 양 지방을 포함한다. 일례의 구현양태에서, 재구성 고기 조성물은 약 10 내지 약 20 중량%의 지방 원료를 포함할 것이다. 추가의 구현양태에서, 식물 유래 지방 원료가 사용될 수 있고, 그것의 비-제한적인 예는 카놀라유, 면실유, 포도씨유, 올리브유, 땅콩유, 팜유, 대두유, 해바라기유, 식물성유 및 이들의 조합을 포함한다. 식물 유래 지방 원료의 중량 퍼센트는 약 10% 내지 20%일 수 있다.

<119> 재구성 고기 조성물은 항산화제를 더 포함할 수도 있다. 항산화제는 동물 고기에서 다중불포화 지방산 (예를 들어 오메가-3 지방산)의 산화를 막을 수 있고, 또한 항산화제는 재구성 고기 조성물에서 산화적 변색을 막을 수도 있다. 항산화제는 천연 또는 합성일 수도 있다. 적절한 항산화제는 이에 한정되지 않지만 아스코르브산 및 그의 염, 아스코르빌 팔미테이트, 아스코르빌 스테아레이트, 아녹소머, N-아세틸시스테인, 벤질 이소티오시아네이트, m-아미노벤조산, o-아미노벤조산, p-아미노벤조산(PABA), 부틸화 히드록시아니솔 (BHA), 부틸화 히드록시톨루엔(BHT), 카페인산, 칸탁산틴, 알파-카로틴, 베타-카로틴, 베타-카라오텐, 베타-아포-카로텐산, 카르노솔, 카르바크롤, 카테킨, 세틸 갈레이트, 클로로겐산, 시트르산 및 그의 염, 클로브 추출물, 커피콩 추출물, p-

코우마린산, 3,4-디히드록시벤조산, N,N'-디페닐-p-페닐렌디아민 (DPPD), 디라우릴 티오디프로피오네이트, 디스테아릴 티오디프로피오네이트, 2,6-디-tert-부틸페놀, 도데실 갈레이트, 에테르산, 엘라긴산, 에리토르빈산, 에리토르빈산나트륨, 에스쿨레틴, 에스쿨린, 6-에톡시-1,2-디히드로-2,2,4-트리메틸퀴놀린, 에틸 갈레이트, 에틸 말톨, 에틸렌디아민테트라아세트산(EDTA), 유칼립투스 추출물, 유게놀, 페룰산, 플라보노이드 (예, 카테킨, 에피카테킨, 에피카테킨 갈레이트, 에피갈로카테킨(EGC), 에피갈로카테킨 갈레이트(EGCG), 폴리페놀 에피갈로카테킨-3-갈레이트), 플라본 (예, 에피게닌, 크리신, 루테올린) 플라보놀 (예, 다티스세틴, 미리세틴, 다엘페토), 플라바논, 플락세틴, 푸마르산, 갈산, 겐타안 추출물, 글루콘산, 글리신, 구아이아컴 고무, 헤스페레틴, 알파-히드록시벤질 포스핀산, 히드록시신남산, 히드록시글루타르산, 히드로퀴논, N-히드록시숙신산, 히드록시트리로졸, 히드록시우레아, 쌀겨 추출물, 락트산 및 그의 염, 레시틴, 레시틴 시트레이트; R-알파-리포산, 루테인, 리코펜, 말산, 말톨, 5-메톡시 트립타민, 메틸 갈레이트, 모노글리세리드 시트레이트; 모노이소프로필 시트레이트; 모린; 베타-나프토플라본, 노르디히드로구아이아레트산 (NDGA), 옥틸 갈레이트, 옥살산, 팔미틸 시트레이트, 페노티아진, 포스파티딜콜린, 인산, 인산염, 피틴산, 피틸루비크로멜, 판댄토 추출물, 프로필 갈레이트, 폴리포스페이트, 퀘르세틴, 트랜스-레스베라트롤, 로즈마리 추출물, 로스마린산, 세이지 추출물, 세사몰, 실리마린, 시나프산, 숙신산, 스테아릴 시트레이트, 시린지산(syringic acid), 타르타르산, 티몰, 토코페롤 (즉, 알파-, 베타-, 감마- 및 델타-토코페롤), 토코트리에놀 (즉, 알파-, 베타-, 감마- 및 델타-토코트리에놀), 티로졸, 바닐린산, 2,6-디-tert-부틸-4-히드록시메틸페놀 (즉, 이오녹스 100), 2,4-(트리스-3',5'-비-tert-부틸-4'-히드록시벤질)-메시틸렌 (즉, 이오녹스 330), 2,4,5-트리히드록시부티로페논, 유비퀴논, 3급 부틸 히드로퀴논(TBHQ), 티오디프로피온산, 트리히드록시 부티로페논, 트립타민, 티라민, 요산, 비타민 K 및 유도체, 비타민 Q10, 및 배아 유, 제아크산틴 또는 이들의 조합을 포함한다.

<120> 재구성 고기 조성물에서 항산화제의 농도는 약 0.0001 중량% 내지 약 20 중량%의 범위일 수도 있다. 다른 구현양태에서, 재구성 고기 조성물에서 항산화제의 농도는 약 0.001 중량% 내지 약 5 중량%의 범위일 수도 있다. 또 다른 구현양태에서, 재구성 고기 조성물에서 항산화제의 농도는 약 0.01 중량% 내지 약 1 중량%의 범위일 수도 있다.

<121> 추가의 구현양태에서, 재구성 고기 조성물은 하나 이상의 향미제를 더 포함할 수도 있다. 향미제는 천연일 수도 있거나 또는 인공일 수도 있다. 향미제는 지방이 적은 고기 또는 지방 조직에서 발견되는 성분, 예컨대 혈청 단백질, 근육 단백질, 가수분해된 동물 단백질, 수지, 지방산 등을 모방하거나 대체할 수도 있다. 향미제는 동물 고기 향미, 그릴 고기 향미, 덜 구워진 쇠고기 향미 등을 제공할 수도 있다. 향미제는 동물 고기 유, 향신료 추출물의 올레오레진 또는 아루아레진, 향신유, 천연 훈연 용액, 천연 훈연 추출물, 효모 추출물 또는 표고버섯 추출물일 수도 있다. 추가의 향미제는 양파 향미, 마늘 향미 또는 허브 향미를 포함할 수도 있다. 재구성 고기 조성물은 향미 증진제를 더 포함할 수도 있다. 사용될 수도 있는 향미 증진제의 예는 염 (염화나트륨), 글루탐산 염 (예, 모노소듐 글루타메이트), 글리신 염, 구아닐산 염, 이노신산 염, 5'-리보뉴클레오티드 염, 가수분해된 동물 단백질 및 가수분해된 식물 단백질을 포함한다.

<122> 재구성 고기 조성물은 각종 향미제를 임의로 포함할 수도 있다. 적절한 향미제는 동물 고기 향미, 동물 고기 유, 향신료 추출물, 향신유, 천연 훈연 용액, 천연 훈연 추출물, 효모 추출물, 셰리, 민트, 갈색당, 꿀을 포함한다. 향미제 및 향신료는 올레오레진 및 아루아레진의 형태로 이용될 수도 있다. 다른 향미제는 양파 향미, 마늘 향미 또는 허브 향미를 포함한다. 대안적인 구현양태에서, 향미제는 견과맛, 단맛 또는 과일맛일 수도 있다. 적절한 과일 향미의 비-제한적인 예는 사과, 살구, 아보카도, 바나나, 블랙베리, 블랙체리, 블루베리, 보이센베리, 캔탈로프, 체리, 코코넛, 크랜베리, 무화과, 포도, 자몽, 녹색 사과, 감로 멜론, 키위, 레몬, 라임, 망고, 혼합 베리, 오렌지, 복숭아, 감, 파인애플, 라스베리, 딸기 및 수박을 포함한다. 재구성 고기 조성물은 향미 증진제를 더 포함할 수도 있다. 적절한 향미 증진제의 비-제한적인 예는 염화나트륨 염, 글루탐산 염, 글리신 염, 구아닐산 염, 이노신산 염 및 5-리보뉴클레오타드 염, 효모 추출물, 표고버섯 추출물, 건조 가다랑이 추출물 및 켈프 추출물을 포함한다. 재구성 고기 조성물은 발효 또는 향미제, 향신료, 기름, 물, 향미 증진제, 항산화제, 산성화제, 보존제 및 감미제의 배합에 의해 만들어질 수 있는 다양한 소스 및 마리네이드를 사용할 수도 있다.

<123> 추가의 구현양태에서, 재구성 고기 조성물은 증점제 또는 겔화제, 예컨대 알긴산 및 그의 염, 한천, 카라기난 및 그의 염, 가공된 홍조류(Eucheuma), 고무 (아라비아 고무, 캐럽 콩(carob bean), 로커스트 콩, 구아, 트라가칸트 및 크산탄), 펙틴, 소듐 카르복시메틸셀룰로스, 메틸셀룰로스 및 개질 전분을 더 포함할 수도 있다.

<124> 추가의 구현양태에서, 재구성 고기 조성물은 비타민, 미네랄, 항산화제, 오메가-3 지방산 또는 허브와 같은 영양소를 더 포함할 수도 있다. 적절한 비타민은 비타민 A, C 및 E (또한, 항산화제임) 및 비타민 B 및 D를 포함

한다. 첨가될 수도 있는 미네랄의 예는 알루미늄, 암모늄, 칼슘, 마그네슘, 철 및 칼륨의 염을 포함한다. 적절한 오메가-3 지방산은 도코사헥사에논산(DHA), EPA (에이코사펜타논산) 및 ALA (알파-리놀렌산)을 포함한다. 첨가될 수도 있는 허브는 월계수 잎, 바질, 셀러리 잎, 파슬리류, 골파, 고수 잎, 고수풀, 커민, 시라, 생강, 육두구껍질, 마요라나, 후추, 심황(tumeric), 파슬리, 오레가노, 사철쑥류 및 타임을 포함한다.

<125> **식품 제품**

<126> 재구성 고기 조성물을 다양한 형태를 갖는 각종 식품 제품으로 가공할 수도 있다. 단백질 조성물이 젤화 단백질, 동물 지방, 염화나트륨, 인산염 (트리폴리인산나트륨, 피로인산 나트륨산, 헥사 메타포스페이트 등), 착색제, 경화제, 항산화제, 항균제, 향미제, 또는 이들의 혼합물로 구성된 군에서 선택되는 하나 이상의 성분을 더 포함할 때, 구조화 단백질 제품, 동물 고기 및 물 만을 이용하는 제품 및 공정과 유사한 절차로 제품 및 공정을 완결한다. 단백질 조성물을 먼저 물로 수화시키고 절단하여 섬유를 노출시키고 분리한다. 수화 및 절단이 완결될 때 착색제를 첨가한다. 동물 고기 및 물을 첨가하고, 균질한 덩어리가 수득될 때까지 내용물을 혼합한다. 이어서, 동물 지방, 향미제, 염화나트륨, 인산염 및 젤화 단백질을 첨가한다. 추가의 구현양태에서, 염 및 인산염과 함께 질산나트륨을 첨가할 수도 있다.

<127> 얻어진 균질한 재구성 고기 제품을 손으로 또는 기계로 긴 조각, 스테이크, 커틀릿, 패티 또는 일반적으로 캐쥬얼 입방체 형태로 형성할 수 있다. 재구성 고기 제품을 고기 스틱으로 형성할 수도 있다. 재구성 고기 제품을 투과성 또는 비투과성 포장에 채워넣어 소시지를 형성할 수도 있다.

<128> 일단 형성된 재구성 고기 제품을 조리하거나, 이후의 사간에 마무리하기 위해 부분 조리하거나 또는 비조리 상태, 부분 조리 상태 또는 조리된 상태로 냉동시킨다. 조리는 살짝튀기거나 또는 강하게 튀기는 튀김, 굽기, 훈연 및 충격 조리 및 찜을 포함한다. 충분히 조리된 재구성 고기 제품을 얇게 저미거나 절단하거나 분쇄할 수도 있다.

<129> 또한, 재구성 고기 제품을 발효시킬 수도 있다. 고기 제품의 pH를 약 4.0 내지 약 5.2로 조절함으로써 고기 제품을 발효시킨다. 락트산 배양물의 첨가에 의해 발효를 달성한다. 시트르산, 락트산, 글루코노 델타 락톤 및 이들의 혼합물을 사용하는 직접적인 산성화에 의하여 산성화를 수행할 수도 있다.

<130> 재구성 고기 제품 (건조 전, 부분 건조, 건조, 조리 또는 비조리)를 그 자체로 포장할 수도 있다. 재구성 고기 제품의 추가의 가공 (건조 전, 부분 건조, 건조, 조리 또는 비조리)을 예를 들어 냉동 터널에서 쇼크-냉동시키고, 이어서 적절한 유형의 용기, 예를 들어 플라스틱 파우치 등에서 자동 부분 포장할 수도 있다. 제품이 소비전에 일반적으로 강하게 튀기거나 구워지는 경우에, 제품이 패스트푸드 판매점 또는 식품 서비스 응용을 위해 계획된다면 상기 유형의 추가의 가공 및 포장이 적절하다.

<131> 대안적으로, 강한 튀김 또는 굽기 동안에 균일한 갈색화를 얻기 위하여, 재구성 고기 제품 (건조 전, 부분 건조, 건조, 조리 또는 비조리)의 형성 후에, 탄수화물 용액 또는 관련 물질로 제품의 표면을 분무할 수도 있다. 이어서, 제품을 쇼크 냉동시키고 부분 포장 (즉, 파우치)으로 판매할 수 있다. 재구성 고기 제품은 소비자에 의하여 강한 튀김 대신에 대류 오븐에서 굽거나 가공될 수 있다. 또한, 재구성 고기 제품을 조리 전 또는 후에 빵부스러기를 묻힐 수도 있거나 다른 유형의 코팅물로 코팅할 수 있다. 추가로, 존재할 수도 있는 어떠한 미생물이라도 사멸시키기 위해 재구성 고기 제품을 레토르트 조리할 수 있다.

<132> 조리 또는 비조리된 재구성 고기 제품을 통상적인 밀봉 절차를 사용하여 통상적인 방식으로 통조림에 포장하고 밀봉할 수도 있다. 보통, 이 단계에서 통조림을 65 ℃ 내지 77 ℃의 온도로 유지하고, 레토르트 또는 조리 단계 동안에 통조림 제조 및 살균 사이의 기간 동안에 미생물 오염의 위험을 막기 위해 가능한 한 빨리 레토르트 또는 조리 단계로 운반한다. 레토르트 조리를 위해 설계된 비투과성 포장에 채워 넣어진 재구성 고기를 레토르트 조리기에서 조리하여 저장 안정성 소시지를 만들 수 있다.

<133> 일단 형성된 재구성 고기 제품이 원상태 근육의 조직을 갖도록 보장하기 위하여, 약 75 중량% 이상의 단백질 조성물이 약 4 cm 이상의 길이의 식물성 단백질 섬유, 약 3 cm 이상의 길이의 식물성 단백질 가닥 및 약 2 cm 이상의 길이의 식물성 단백질 덩어리를 포함하는 큰 조각을 15 중량% 이상 함유하고, 약 75 중량% 이상의 단백질 조성물이 약 1400 그램 이상의 전단 강도를 갖는 것이 필요하다.

<134> 야채 제품은, 단백질 조성물, 바람직하게는 함수 및 절단 콩 단백질 조성물을 분쇄된 야채와 조합하고, 여기에서 약 75 중량% 이상의 단백질 조성물이 약 4 cm 이상의 길이의 단백질 섬유, 약 3 cm 이상의 길이의 단백질 가닥 및 약 2 cm 이상의 길이의 단백질 덩어리를 포함하는 단편을 약 15 중량% 이상 포함하고, 약 75 중량% 이상

의 단백질 조성물이 약 1400 그램 이상의 전단 강도를 가지며;

<135> 바람직한 함수 및 절단 콩 단백질 조성물 및 분쇄된 야채를 혼합하여, 실질적으로 정렬된 단백질 섬유를 갖는 균질한 섬유성 구조화 야채 제품을 제조하는

<136> 방법에 의해 제조될 수도 있다.

<137> 상기 방법에 의해 제조된 야채 제품의 예는 채식주의자용 패티, 채식주의자용 핫도그, 채식주의자용 소시지 및 채식주의자용 크럼블을 포함한 채식주의자용 식품 제품이다. 채식주의자용 식품 제품의 다른 예는 함수 및 절단 단백질 조성물이 첨가된 치즈이다.

<138> 과일 제품은, 단백질 조성물, 바람직하게는 함수 및 절단 콩 단백질 조성물을 분쇄된 과일과 조합하고, 여기에서 약 75 중량% 이상의 단백질 조성물이 약 4 ㎝ 이상의 길이의 단백질 섬유, 약 3 ㎝ 이상의 길이의 단백질 가닥 및 약 2 ㎝ 이상의 길이의 단백질 덩어리를 포함하는 단편을 약 15 중량% 이상 포함하고, 약 75 중량% 이상의 단백질 조성물이 약 1400 그램 이상의 전단 강도를 가지며;

<139> 바람직한 함수 및 절단 콩 단백질 조성물 및 분쇄된 과일을 혼합하여, 실질적으로 정렬된 단백질 섬유를 갖는 균질한 섬유성 구조화 과일 제품을 제조하는

<140> 방법에 의해 제조될 수도 있다.

<141> 상기 방법에 의해 제조된 과일 제품의 예는 과일 롤업, 시리얼, 및 과일 크럼블을 포함하는 스낵 식품 제품이다.

<142> **정의**

<143> 여기에서 사용된 용어 "동물 고기"는 동물로부터 유래된 살, 전 고기 근육 또는 그의 일부를 가리킨다.

<144> 여기에서 사용된 용어 "분쇄된 과일"은 단일 과일의 퓌레 또는 하나 이상의 과일의 퓌레의 혼합물을 가리킨다.

<145> 여기에서 사용된 용어 "분쇄된 고기"는 동물 사체로부터 회수된 고기 페이스트를 가리킨다. 뼈에 붙어있거나 뼈에서 멀리 떨어져 있는 고기를, 뼈로부터 고기를 분리하고 크기를 감소시키는 뼈제거 장치를 통해 강제로 보낸다.

<146> 여기에서 사용된 용어 "분쇄된 야채"는 단일 야채의 퓌레 또는 하나 이상의 야채의 퓌레의 혼합물을 가리킨다.

<147> 여기에서 사용된 용어 "압출물"은 압출 제품을 가리킨다. 본 명세서에서, 일부 구현양태에서 실질적으로 정렬된 단백질 섬유를 포함하는 식물 단백질 제품이 압출물일 수도 있다.

<148> 여기에서 사용된 용어 "섬유"는 실시예 2에 상술된 절단 특징결정 시험을 수행한 후에 대략 4 ㎝ 길이 내지 0.2 ㎝ 폭의 크기를 갖는 식물 단백질 제품을 가리킨다. 본 명세서에서, 용어 "섬유"는 대두 떡잎 섬유와 같은 섬유의 영양소 부류를 포함하지 않고, 식물 단백질 제품을 포함하는 실질적으로 정렬된 단백질 섬유의 구조적 형성을 가리키지 않는다.

<149> 여기에서 사용된 용어 "글루텐"은 높은 함량의 단백질뿐만 아니라 특유의 구조적 및 응집 성질을 갖는 곡물 가루, 예컨대 밀에서의 단백질 분획을 가리킨다.

<150> 여기에서 사용된 용어 "무-글루텐 전분"은 변형된 타피오카 전분과 같은 다양한 전분 제품을 가리킨다. 무 글루텐 또는 실질적으로 무 글루텐 전분은 밀, 옥수수, 및 타피오카 기재 전분으로부터 만들어진다. 이들은 밀, 귀리, 호밀 또는 보리로부터의 글루텐을 함유하지 않기 때문에 "무-글루텐"이다.

<151> 여기에서 사용된 용어 "수화 시험"은 공지된 양의 단백질 조성물을 수화시키기 위해 필요한 시간 (분)의 양을 측정한다.

<152> 여기에서 사용된 용어 "큰 조각"은 착색되거나 비착색된 구조화 식물 단백질 제품의 절단 퍼센트가 특징화되는 방식이다. 절단 특징의 결정을 실시예 2에 상술한다.

<153> 여기에서 사용된 용어 "기계적으로 뼈 제거된 고기(MDM)"은 통상적으로 이용가능한 장치를 사용하여 소, 돼지 및 닭 뼈로부터 회수된 고기 페이스트를 가리킨다. MDM은 원상태 근육에서 발견되는 천연 섬유 조직이 없는 분쇄된 제품이다.

<154> 여기에서 사용된 용어 "수분 함량"이란 물질 중의 수분의 양을 가리킨다. 물질의 수분 함량은 A.O.C.S. (아메

리칸 오일 케미스츠 소사이어티) 방법 Ba 2a-38 (1997) (여기에서 그 전체내용이 참고문헌으로 포함된다)에 의해 결정될 수 있다.

<155> 여기에서 사용된 용어 "단백질 함량", 예를 들어 콩 단백질 함량은 A.O.C.S. (아메리칸 오일 케미스츠 소사이어티) 공인 방법 Bc 4-91 (1997), Aa 5-91 (1997), 또는 Ba 4d-90 (1997) (각각 그 전체내용이 참고문헌으로 포함된다)에서 확인된 물질의 상대적 단백질 함량을 가리키며 이것은 암모니아로서 물질 샘플의 총 질소 함량을 결정하고, 단백질 함량이 샘플의 총 질소 함량 곱하기 6.25로서 결정된다.

<156> 여기에서 사용된 용어 "단백질 섬유"는 본 발명의 식물 단백질 제품의 구조를 함께 한정하는 다양한 길이의 개별 연속 필라멘트 또는 별개의 신장된 조각을 가리킨다. 추가로, 본 발명의 착색 및 비착색 구조화 식물 단백질 제품이 실질적으로 정렬된 단백질 섬유를 갖기 때문에, 단백질 섬유의 배열은 전 고기 근육의 조직을 착색 및 비착색 구조화 식물 단백질 제품에 부여한다.

<157> 여기에서 사용된 용어 "전단 강도"는, 형성된 제품에 고기-유사 조직 및 외관을 부여하기에 충분히 높은 강도를 지닌 섬유성 망상 구조를 형성하는 조직화 단백질의 능력을 측정한다. 전단 강도는 그램으로 측정된다.

<158> 여기에서 사용된 용어 "모방된"은 동물 고기를 함유하지 않는 동물 고기 조성물을 가리킨다.

<159> 여기에서 사용된 용어 "콩 떡잎 섬유"는 약 70% 이상의 식이 섬유를 함유하는 콩 떡잎의 다당류 부분을 가리킨다. 콩 떡잎 섬유는 전형적으로 일부 소량의 콩 단백질을 함유하지만 또한 100% 섬유일 수도 있다. 여기에서 사용된 콩 떡잎 섬유는 콩 껍질 섬유를 가리키지 않거나 포함하지 않는다. 일반적으로, 콩 떡잎 섬유는 대두의 껍질 및 배아를 제거하고, 떡잎을 박편화하거나 분쇄하고, 박편화되거나 분쇄된 떡잎으로부터 기름을 제거하고, 콩 물질 및 떡잎의 탄수화물로부터 콩 떡잎 섬유를 분리함으로써 대두로부터 형성된다.

<160> 여기에서 사용된 용어 "콩 단백질 농축물"은 무-수분 기준으로 약 65% 내지 약 90% 미만의 단백질 함량을 갖는 콩 물질이다. 콩 단백질 농축물은 또한 콩 떡잎 섬유를 함유하고, 전형적으로 무-수분 기준으로 약 3.5% 내지 약 20% 이하의 콩 떡잎 섬유를 함유한다. 대두의 껍질 및 배아를 제거하고, 떡잎을 박편화하거나 분쇄하고, 박편화되거나 분쇄된 떡잎으로부터 오일을 제거하고, 떡잎의 가용성 탄수화물로부터 콩 단백질 및 콩 떡잎 섬유를 분리함으로써 대두로부터 콩 단백질 농축물이 형성된다.

<161> 여기에서 사용된 용어 "콩 가루"는 바람직하게는 약 1% 미만의 오일을 함유하고, No.100 메쉬 (미국 표준) 망을 통해 입자가 통과할 수 있는 크기를 갖는 입자로 형성된 탈지 대두 물질의 분쇄된 형태를 가리킨다. 콩 케이크, 얇은 조각, 박편, 거친 가루 또는 이들의 혼합물을 통상적인 콩 분쇄 공정을 사용하여 콩 가루로 분쇄한다. 콩 가루는 무 수분 기준에서 약 49% 내지 약 65%의 콩 단백질 함량을 갖는다. 바람직하게는, 가루는 매우 미세하게 분쇄되고, 가장 바람직하게는 가루의 약 1% 미만이 300 메쉬 (미국 표준) 망 위에 유지되도록 분쇄된다.

<162> 여기에서 사용된 용어 "콩 단백질 단리물"은 무-수분 기준으로 약 90% 이상의 콩 단백질의 단백질 함량을 갖는 콩 물질이다. 떡잎으로부터 대두의 껍질 및 배아를 제거하고, 떡잎을 박편화 하거나 분쇄하고, 박편화되거나 분쇄된 떡잎으로부터 오일을 제거하고, 떡잎 섬유로부터 떡잎의 콩 단백질 및 탄수화물을 분리하고, 이어서 탄수화물로부터 콩 단백질을 분리함으로써 대두로부터 콩 단백질 단리물이 형성된다.

<163> 여기에서 사용된 용어 "가닥"은, 실시예 2에 상술된 절단 특징결정 시험을 수행한 후에, 대략 2.5 내지 약 4 ㎝ 길이 및 대략 0.2 ㎝ 초과의 폭의 크기를 갖는 식물 단백질 제품을 가리킨다.

<164> 여기에서 사용된 용어 "전분"은 임의 천연 원료로부터 유래된 전분을 가리킨다. 전분을 위한 원료는 전형적으로 곡류, 괴경, 근경, 콩류 및 과일이다.

<165> 여기에서 사용된 용어 "무-수분 기준 중량"이란 모든 수분을 완전히 제거하도록 건조된 후에, 예를 들어 물질의 수분 함량이 0%일 때 물질의 중량을 가리킨다. 구체적으로, 물질의 무-수분 기준 중량은, 물질이 일정한 중량에 이를 때까지 물질을 45℃ 오븐에 놓아둔 후에 물질의 무게를 잼으로써 수득될 수 있다.

<166> 여기에서 사용된 용어 "밀가루"는 밀의 제분으로부터 수득된 가루를 가리킨다. 일반적으로 말하면, 밀가루의 입자 크기는 약 14 내지 약 120 ㎛이다.

<167> 하기 실시예는 본 발명의 바람직한 구현양태를 증명하기 위해 포함된다. 당업자라면, 하기 실시예에 개시된 기술들이 본 발명의 실행에서 양호하게 작용하기 위해 본 발명자들에 의해 발견된 기술을 나타낸다는 것을 이해해야 한다. 그러나, 본 개시내용의 관점에서, 당업자라면 개시된 특정한 구현양태에 많은 변화가 만들어질 수 있

고 본 발명의 의도 및 범주에서 벗어나지 않으면서 비슷하거나 유사한 결과를 얻을 수 있으며, 따라서 첨부된 도면에 개시되거나 나타낸 모든 사항이 예증을 위한 것으로 해석되고 제한적인 의미가 아님을 이해해야 한다.

*실 시 예*

<168> 실시예 1 내지 105는 본 발명의 다양한 구현양태를 예증한다.

<169> **실시예 1 전단 강도의 결정**

<170> 샘플의 전단 강도는 그램으로 측정되고 하기 절차에 의해 결정될 수 있다. 구조화 단백질 제품의 샘플의 무게를 재고 열 밀봉성 파우치에 그것을 놓고 샘플 중량의 3배의 실온 수돗물로 샘플을 수화시켰다. 약 0.01 바아의 압력까지 파우치의 공기를 빼고 파우치를 밀봉하였다. 샘플을 약 12 내지 약 24시간 동안 수화시켰다. 함수 샘플을 꺼내고, 이것을 조직 분석기로부터의 나이프가 샘플의 직경을 통해 자르도록 배향된 조직 분석기 기본판 위에 놓았다. 또한, 나이프가 조직화된 조각의 장 축에 수직으로 자르도록 샘플을 조직 분석기 나이프 아래에 배향시켜야 한다. 압출물을 절단하기 위해 사용된 적절한 나이프는 모델 TA-45 앞쪽 날개 (택스처 테크놀로지스(USA) 제조)이다. 이 시험을 수행하기 위해 사용된 조직 분석기는 25, 50 또는 100 kg 하중 셀이 설치된 스테이블 마이크로 시스템스 리미티드 (영국)에 의해 재조된 모델 TA, TXT2이다. 이 시험의 내용에서, 전단 강도는 샘플의 구멍을 뚫기 위해 필요한 최대 힘 (그램)이다.

<171> **실시예 2 절단 특징의 결정**

<172> 절단 특징을 결정하는 절차는 다음과 같이 수행될 수도 있다. 전체 조각을 사용하여 구조화 단백질 제품의 약 150 그램을 무게를 쟀다. 샘플을 열-밀봉성 플라스틱 주머니에 놓고 25 ℃의 물 약 450 그램을 첨가하였다. 주머니를 약 150 mmHg에서 진공 밀봉하고 내용물을 약 60 분 동안 수화시켰다. 함수 샘플을 단일 날개 패들이 설치된 키친 에이드 혼합기 모델 KM14G0의 용기에 넣고, 130 rpm에서 2 분 동안 내용물을 혼합하였다. 패들 및 용기의 측면을 긁어내고 긁어낸 부스러기를 용기의 바닥으로 다시 보냈다. 혼합 및 긁어냄을 2회 반복하였다. 용기에서 약 200 g의 혼합물을 제거하였다. 모든 섬유 또는 2.5cm보다 더욱 긴 가닥이 절단된 혼합물로부터 이탈되도록 혼합물을 분리하였다. 절단된 혼합물로부터 분류된 섬유 집단의 무게를 재고 이 중량을 출발 중량 (예를 들어 ~200 g)으로 나누고 이 값에 100을 곱하였다. 이것은 샘플 내의 큰 조각의 퍼센트를 결정하였다. 얻어진 값이 15% 미만이거나 20% 초과라면 시험이 완결하였다. 값이 15% 내지 20%이라면, 용기로부터 다른 ~200 g의 무게를 재고, 절단된 혼합물로부터 2.5 cm보다 긴 섬유 또는 가닥을 분리하고 다시 재산을 수행하였다.

<173> **실시예 3 식물 단백질 제품의 제조**

<174> 본 발명의 착색된 구조화 식물 단백질 제품을 제조하기 위해 하기 압출 방법을 사용할 수도 있다. 건식 배합 혼합 용기에 다음 성분을 첨가하였다: 1000 킬로그램 수프로 820 (콩 단리물), 440 kg 밀 글루텐, 236 kg 밀 전분, 34 kg 콩 떡잎 섬유, 8 kg 인산이칼슘, 및 2 kg L-시스테인. 내용물을 혼합하여 건식 배합된 콩 단백질 혼합물을 형성하였다. 건조 배합물을 호퍼로 옮기고, 호퍼로부터의 건조 배합물을 480 kg의 물과 함께 예비-상태조절장치에 도입하여 상태조절된 콩 단백질 예비-혼합물을 형성하였다. 이어서, 상태조절된 콩 단백질 예비-혼합물을 이축 압출 장치에 25 kg/분 이하의 속도로 공급하였다. 압출 장치는 5개 온도 조절 대역을 포함하고, 단백질 혼합물을 첫 번째 대역에서 약 25 ℃, 두 번째 대역에서 약 50 ℃, 세 번째 대역에서 약 95 ℃, 네 번째 대역에서 약 130 ℃ 및 다섯 번째 대역에서 약 150 ℃로 조절하였다. 압출 덩어리를 첫 번째 대역에서 적어도 약 400 psig 내지 다섯 번째 대역에서 약 1500 psig까지의 압력으로 처리하였다. 물 60 kg을 가열 대역과 통해 있는 하나 이상의 주입 제트를 통하여 압출기 통에 주입하였다.

<175> 가소화된 혼합물이 압출기 출구로부터 다이 조립체로 유동되도록 하는 배열로 다이 조립체를 압출기에 부착하고, 가소화된 혼합물이 다이 조립체를 통해 유동될 때 가소화된 혼합물 내에서 단백질 섬유의 실질적인 정렬을 생성하였다.

<176> 실질적으로 정렬된 단백질 섬유를 함유하는 압출물이 다이 조립체를 나올 때, 이것을 나이프로 자르고 잘린 덩어리를 약 10 중량%의 수분 함량까지 건조시켰다. 일단 건조되면, 잘린 덩어리의 일부를 작은 조각 및 큰 조각으로 크기를 분류하였다. 각각의 크기에 대해 총 25개 조각을 수득한다. 이어서, 이러한 조각을 측정하고 표 IV에 표로 나타낸다.

표 IV

| 샘플 번호 | 작은 조각 | | | | 큰 조각 | |
|---|---|---|---|---|---|---|
| | L (mm) | W (mm) | | | L (mm) | W (mm) |
| 1 | 11 | 10 | | | 16 | 11 |
| 2 | 10 | 6 | | | 16 | 13 |
| 3 | 8 | 8 | | | 22 | 11 |
| 4 | 11 | 8 | | | 19 | 11 |
| 5 | 14 | 9 | | | 17 | 11 |
| 6 | 10 | 8 | | | 20 | 13 |
| 7 | 14 | 4 | | | 15 | 10 |
| 8 | 8 | 6 | | | 21 | 12 |
| 9 | 10 | 8 | | | 19 | 12 |
| 10 | 11 | 8 | | | 12 | 12 |
| 11 | 13 | 8 | | | 15 | 10 |
| 12 | 19 | 9 | | | 17 | 9 |
| 13 | 12 | 9 | | | 11 | 10 |
| 14 | 14 | 6.5 | | | 14 | 10 |
| 15 | 10 | 7 | | | 14 | 10 |
| 16 | 10 | 7 | | | 17 | 12 |
| 17 | 10 | 6 | | | 15 | 13 |
| 18 | 12 | 8 | | | 14 | 14 |
| 19 | 10 | 7 | | | 19 | 12 |
| 20 | 9 | 6 | | | 18 | 10 |
| 21 | 10 | 8 | | | 14 | 12 |
| 22 | 9 | 5 | | | 19 | 12 |
| 23 | 10 | 7 | | | 12 | 11 |
| 24 | 11 | 9 | | | 16 | 10 |
| 25 | 9 | 8 | | | 16 | 9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 평균 | **11.0** | **7.4** | | 평균 | **16.3** | **11.2** |
| 표준 편차 | **2.3** | **1.4** | | 표준 편차 | **2.8** | **1.3** |
| 최대 | **19.0** | **10.0** | | 최대 | **22.0** | **14.0** |
| 최소 | **8.0** | **4.0** | | 최소 | **11.0** | **9.0** |

<177>

<178> **실시예 4-94 식물 단백질 제품의 제조**

<179> 실시예 4-94는 실시예 3의 반복이다. 하기 표 V는 실시예 3-94의 분석을 서술한다.

표 V

| 실시예 번호 # | % 큰 조각 | 전단 강도(g) | 수화 | 밀도 (g/cc) |
|---|---|---|---|---|
| 3 | 30.2 | 2150 | 80 | 0.27 |
| 4 | 24.2 | 2366 | 80 | 0.24 |
| 5 | 29.4 | 2341 | 60 | 0.30 |
| 6 | 26.0 | 2142 | 70 | 0.29 |
| 7 | 27.1 | 2291 | 70 | 0.28 |
| 8 | 32.7 | 2442 | 70 | 0.23 |
| 9 | 27.4 | 2568 | 70 | 0.27 |
| 10 | 26.1 | 2511 | 90 | 0.26 |

<180>

| 실시예 번호 # | % 큰 조각 | 관단 강도(g) | 수화 | 밀도 (g/cc) |
|---|---|---|---|---|
| 11 | 21.1 | 2260 | 80 | 0.28 |
| 12 | 22.3 | 2421 | 80 | 0.24 |
| 13 | 21.9 | 2490 | 75 | 0.28 |
| 14 | 22.4 | 2438 | 104 | 0.28 |
| 15 | 7.8 | 59 | 81 | 0.30 |
| 16 | 7.3 | 675 | 83 | 0.28 |
| 17 | 9.3 | 553 | 100 | 0.24 |
| 18 | 7.3 | 226 | 90 | 0.23 |
| 19 | 3.5 | 412 | 72 | 0.24 |
| 20 | 0.0 | 055 | 100 | 0.23 |
| 21 | 2.6 | 511 | 75 | 0.25 |
| 22 | 2.7 | 168 | 100 | 0.25 |
| 23 | 2.0 | 207 | 102 | 0.25 |
| 24 | 7.7 | 247 | 62 | 0.29 |
| 25 | 1.2 | 51 | 73 | 0.28 |
| 26 | 0.2 | 164 | 63 | 0.27 |
| 27 | 6.6 | 966 | 68 | 0.28 |
| 28 | 4.9 | 164 | 50 | 0.31 |
| 29 | 5.0 | 812 | 58 | 0.28 |
| 30 | 9.6 | 108 | 60 | 0.31 |
| 31 | 5.8 | 864 | 70 | 0.27 |
| 32 | 6.5 | 473 | 58 | 0.25 |
| 33 | 0.7 | 879 | 65 | 0.28 |
| 34 | 5.4 | 688 | 70 | 0.29 |
| 35 | 0.3 | 038 | 74 | 0.26 |
| 36 | 9.3 | 074 | 73 | 0.28 |
| 37 | 1.5 | 937 | 70 | 0.39 |
| 38 | 2.5 | 462 | 77 | 0.40 |

<181>

| 실시예 번호 # | % 큰 조각 | 전단 강도(g) | 수화 | 밀도 (g/cc) |
|---|---|---|---|---|
| 39 | 0.1 | 051 | 66 | 0.28 |
| 40 | 7.9 | 384 | 54 | 0.31 |
| 41 | 8.1 | 064 | 58 | 0.28 |
| 42 | 9.2 | 158 | 60 | 0.27 |
| 43 | 0.0 | 834 | 58 | 0.28 |
| 44 | 6.8 | 202 | 58 | 0.28 |
| 45 | 2.8 | 363 | 57 | 0.26 |
| 46 | 3.9 | 361 | 57 | 0.28 |
| 47 | 6.9 | 293 | 103 | 0.25 |
| 48 | 6.3 | 205 | 73 | 0.28 |
| 49 | 9.0 | 286 | 53 | 0.29 |
| 50 | 2.6 | 206 | 63 | 0.25 |
| 51 | 0.5 | 125 | 63 | 0.31 |
| 52 | 5.5 | 290 | 55 | 0.29 |
| 53 | 8.2 | 274 | 55 | 0.26 |
| 54 | 1.5 | 205 | 42 | 0.33 |
| 55 | 1.3 | 185 | 55 | 0.31 |
| 56 | 1.8 | 969 | 40 | 0.30 |
| 57 | 9.1 | 028 | 55 | 0.31 |
| 58 | 7.2 | 598 | 63 | 0.37 |
| 59 | 8.3 | 869 | 60 | 0.31 |
| 60 | 9.7 | 044 | 50 | 0.29 |
| 61 | 7.6 | 216 | 52 | 0.28 |
| 62 | 5.0 | 001 | 53 | 0.28 |
| 63 | 8.1 | 096 | 45 | 0.27 |
| 64 | 9.0 | 796 | 53 | 0.27 |
| 65 | 0.0 | 924 | 51 | 0.27 |
| 66 | 3.7 | 295 | 51 | 0.28 |

<182>

| 실시예 번호 # | % 큰 조각 | 절단 강도(g) | 수화 | 밀도 (g/cc) |
|---|---|---|---|---|
| 67 | 7.4 | 259 | 50 | 0.29 |
| 68 | 9.2 | 204 | 43 | 0.28 |
| 69 | 5.3 | 059 | 38 | 0.31 |
| 70 | 6.1 | 284 | 70 | 0.32 |
| 71 | 3.6 | 085 | 70 | 0.30 |
| 72 | 5.6 | 279 | 44 | 0.28 |
| 73 | 3.7 | 170 | 44 | 0.32 |
| 74 | 1.2 | 128 | 49 | 0.29 |
| 75 | 2.4 | 068 | 50 | 0.29 |
| 76 | 0.1 | 939 | 40 | 0.30 |
| 77 | 8.7 | 592 | 50 | 0.30 |
| 78 | 9.6 | 812 | 68 | 0.28 |
| 79 | 5.2 | 848 | 64 | 0.28 |
| 80 | 3.6 | 973 | 70 | 0.30 |
| 81 | 3.7 | 078 | 66 | 0.36 |
| 82 | 5.6 | 940 | 44 | 0.31 |
| 83 | 8.5 | 339 | 33 | 0.29 |
| 84 | 0.2 | 366 | 50 | 0.24 |
| 85 | 8.1 | 425 | 40 | 0.29 |
| 86 | 9.6 | 122 | 59 | 0.27 |
| 87 | 7.5 | 193 | 56 | 0.16 |
| 88 | 1.1 | 186 | 56 | 0.28 |
| 89 | 2.4 | 061 | 56 | 0.27 |
| 90 | 1.3 | 143 | 50 | 0.27 |
| 91 | 4.4 | 108 | 54 | 0.26 |
| 92 | 9.9 | 101 | 53 | 0.30 |
| 93 | 2.3 | 551 | 55 | 0.25 |
| 94 | 4.3 | 164 | 57 | 0.28 |

<183>

| 실시예 번호 # | % 큰 조각 | 절단 강도(g) | 수화 | 밀도 (g/cc) |
|---|---|---|---|---|
| | | | | |
| 첫번째 4분위수 | 2.6 | 045 | 53 | 0.27 |
| 중앙값 | 6.5 | 164 | 60 | 0.28 |
| 세번째 4분위수 | 0.2 | 291 | 70 | 0.30 |
| 평균 | 6.6 | 156 | 63 | 0.28 |

<184>

<185> **실시예 95 단백질 조성물의 제조**

<186> 약 10 ℃ (50 °F)의 수돗물 3625 그램을 혼합 용기에 첨가하고, 콩 단백질 조성물이 수화되고 섬유가 분리될 때까지, 교반하면서 콩 단백질 단리물, 콩 떡잎 섬유, 밀 글루텐 및 전분을 포함하는 건조된 저 수분 (약 7% 내지 약 12%) 콩 단백질 조성물 (FXP M0339로 확인됨, 미국 미조리주 세인트루이스 솔래 LLC로부터 입수가능함) 1160 그램을 첨가하였다. 약 50% 이상의 수분 함량을 갖는 기계적으로 뼈제거된 닭고기의 분쇄된 고기 5216 그램을 혼합기에 첨가하였다. 기계적으로 뼈제거된 닭고기는 약 2 ℃ (36 °F) 내지 약 4 ℃ (39 °F)의 온도이다. 균질한 재구성 고기 제품이 수득될 때까지 내용물을 혼합하였다. 재구성 고기 제품을 홀리매틱(Hollymatic) 형성 기계로 옮기고 여기에서 재구성 고기 제품을 스테이크 또는 커틀릿으로 형성한 다음 냉동시켰다.

<187> 콩 단백질 단리물, 콩 떡잎 섬유, 밀 글루텐 및 전분을 포함하는 비-건조 저 수분 (약 28 내지 약 35%) 콩 단백질 조성물 1500 그램을 3175 그램의 물로 수화시키는 것 이외에는, 실시예 3의 절차를 반복하였다. 재구성 고기 제품을 채워넣는 기계로 옮기고 여기에서 재구성 고기 제품을 비투과성 포장에 채워넣은 다음 냉동시켰다.

채워넣는 기계는 HITEC 푸드 이큅프먼트 인코포레이티드 (미국 일리노이주 엘크 그로브 빌리지에 위치), 타운센드 엔지니어링 컴퍼니 (미국 아이오와 댁스 모인스에 위치), 로버트 레이저 앤드 컴퍼니 인코포레이티드 (미국 매사추세츠주 캔톤에 위치) 및 핸트만 인코포레이티드 (미국 일리노이주 버팔로 그로브에 위치)를 포함하여 다양한 상업적 제조업자로부터 입수가능하다.

<188> **실시예 97**

<189> 약 12 ℃ (54 °F)의 수돗물 2127 그램을 첫 번째 혼합 용기에 첨가하고, 콩 단백질 조성물이 수화되고 섬유가 분리될 때까지, 교반하면서 건조된 저 수분 (약 7% 내지 약 12%) 콩 단백질 조성물 1000 그램을 첨가하였다. 이어서, 카라멜 색소 43 g을 함수 콩 단백질 조성물에 첨가하였다. 약 2 ℃ (36 °F)에서, 약 50%의 수분 함량을 갖는 기계적으로 뼈제거된 닭고기의 분쇄된 고기 4500 그램을 첨가하였다. 이어서, 100 그램의 염화나트륨 및 30 그램의 트리폴리인산나트륨을 첨가하여 결합을 위해 분쇄된 고기에서 골격근 단백질을 추출/가용화하였다. 혼합이 계속될 때, 500 그램의 쇠고기 지방 및 100 그램의 쇠고기 향미제를 첨가하고 혼합을 계속하였다. 두 번째 혼합 용기에서 수프로® 620의 600그램의 젤화 단백질을 1000 그램 물에서 수화시키고 첫 번째 혼합 용기에 첨가하였다. 균질한 재구성 고기 제품이 수득될 때까지 내용물을 혼합하였다. 재구성 고기 제품을 홀리매틱 (미국 일리노이주 파크 포리스트 홀리매틱 코포레이션) 형성 기계로 옮기고 이곳에서 재구성 고기 제품을 패티로 형성하고 이어서 냉동시켰다.

<190> **실시예 98**

<191> 약 10 ℃ (50 °F)의 수돗물 3000 그램을 혼합 용기에 첨가하고, 콩 단백질 조성물이 수화되고 절단에 의해 섬유가 분리될 때까지, 교반하면서 수프로® 620으로부터 제조된 콩 단백질 압출물 1500 그램을 첨가하였다. 이어서, 약 50% 이상의 수분 함량을 갖는 기계적으로 뼈제거된 닭고기의 분쇄된 고기 3000 그램을 혼합기에 첨가하였다. 기계적으로 뼈제거된 닭고기는 약 2 ℃ (36 °F) 내지 약 4 ℃ (39 °F)의 온도이다. 이어서, 균질한 재구성 고기 제품이 수득될 때까지 내용물을 혼합하였다. 재구성 고기 제품을 홀리매틱 형성 기계로 옮기고 이곳에서 재구성 고기 제품을 스테이크 또는 커틀릿으로 형성하고 이어서 냉동시켰다.

<192> **실시예 99**

<193> 함수 및 절단 콩 단백질 조성물이 콩 단백질 단리물, 쌀가루 및 무 글루텐 전분을 포함하는 것 이외에는 실시예 98의 절차를 반복하였다.

<194> **실시예 100**

<195> 함수 및 절단 콩 단백질 조성물이 콩 단백질 단리물 및 쌀가루를 포함하는 것 이외에는 실시예 98의 절차를 반복하였다.

<196> **실시예 101**

<197> 함수 및 절단 콩 단백질 조성물이 콩 단백질 단리물 및 무 글루텐 전분을 포함하는 것 이외에는 실시예 98의 절차를 반복하였다.

<198> **실시예 102**

<199> 함수 및 절단 콩 단백질 조성물이 콩 단백질 단리물, 밀가루 및 전분을 포함하는 것 이외에는 실시예 98의 절차를 반복하였다.

<200> **실시예 103**

<201> 함수 및 절단 콩 단백질 조성물이 콩 단백질 단리물 및 콩 떡잎 섬유를 포함하는 것 이외에는 실시예 98의 절차를 반복하였다.

<202> **실시예 104**

<203> 함수 및 절단 콩 단백질 조성물이 콩 단백질 단리물, 콩 떡잎 섬유 및 밀 글루텐을 포함하는 것 이외에는 실시예 98의 절차를 반복하였다.

<204> **실시예 105**

<205> 약 10 ℃ (50 °F)의 수돗물 3383 그램을 혼합 용기에 첨가하고, 콩 단백질 추출물이 수화되고 절단에 의해 섬유

가 분리될 때까지, 교반하면서 건조된 저 수분 (약 7% 내지 약 12%) 콩 단백질 압출물 (수프로맥스(SUPROMAX)(R) 5050으로 확인됨) 1208 그램을 첨가하였다. 이어서, 약 50% 이상의 수분 함량을 갖는 기계적으로 뼈제거된 닭고기의 분쇄된 고기 3340 그램 및 약 10%의 지방 함량을 갖는 1/2 인치로 분쇄된 쇠고기 3383 그램을 혼합기에 첨가하였다. 기계적으로 뼈제거된 닭고기 및 분쇄된 쇠고기는 약 2 ℃ (36 °F) 내지 약 4 ℃ (39 °F)의 온도이다. 또한, 염, 에리트로베이트, 아질산나트륨, 덱스트로스, 부숴진 블랙페퍼, 넛맥, 육두구, 과립 마늘, 고수풀, 레드페퍼 및 재함수 LHP 즙탈 배양액의 다양한 착색제 및 향미제를 첨가하였다. 균질한 재구성 고기 제품이 수득될 때까지 내용물을 혼합하였다. 이어서, 재구성 고기 제품을 고기 스틱으로 형성하였다.

<206> 바람직한 구현양태에 관해 본 발명을 설명하였으나, 명세서를 읽을 때 그의 다양한 변형이 당업자에게 명백할 것으로 이해된다. 따라서, 여기에 개시된 발명은 본 발명의 첨부된 청구범위의 범위 내에 속하는 변형을 포함하는 것으로 해석된다는 것을 이해해야 한다.

## 도면의 간단한 설명

<14> 도 1은 실질적으로 정렬된 단백질 섬유를 갖는 본 발명의 구조화 식물 단백질 제품을 나타내는 현미경사진의 사진 화상을 나타낸다.

<15> 도 2는 본 발명의 방법에 의해 제조되지 않는 식물 단백질 제품을 나타내는 현미경사진의 사진 화상을 나타낸다. 여기에 기재된 바와 같이, 식물 단백질 제품을 구성하는 식물 섬유가 크로스해치되어 있다.

<16> 도 3은 단백질 함유 물질의 압출 공정에서 사용될 수도 있는 주변 다이 조립체의 투시도를 나타낸다.

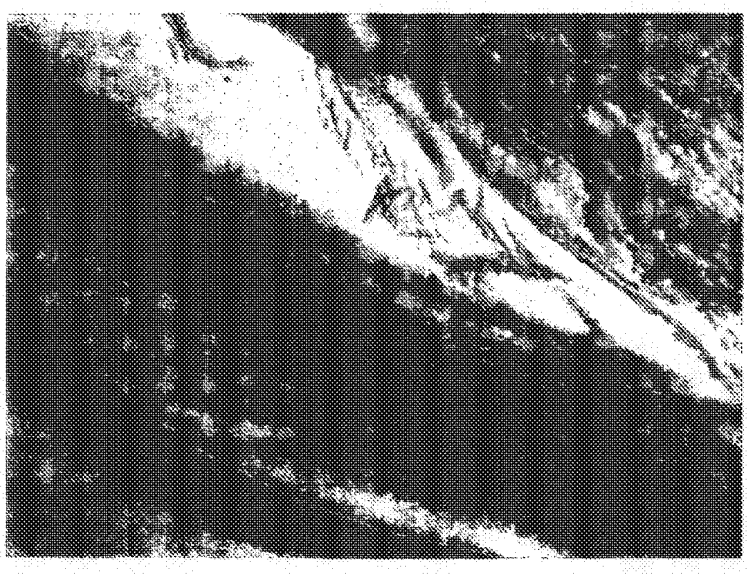
<17> 도 4는 다이 삽입물, 다이 슬리브 및 다이 원뿔을 나타내는 도 3의 주변 다이 조립체의 분해조립도를 나타낸다.

<18> 도 5는 다이 슬리브, 다이 삽입물 및 다이 원뿔 배열 사이에 한정된 유동 채널을 보여주는 도 3의 선 9-9를 따라 자른 단면도를 나타낸다.

<19> 도 5A는 유동 채널과 다이 슬리브의 출구 사이의 계면을 보여주는 도 5의 확대된 단면도이다.

## 도면

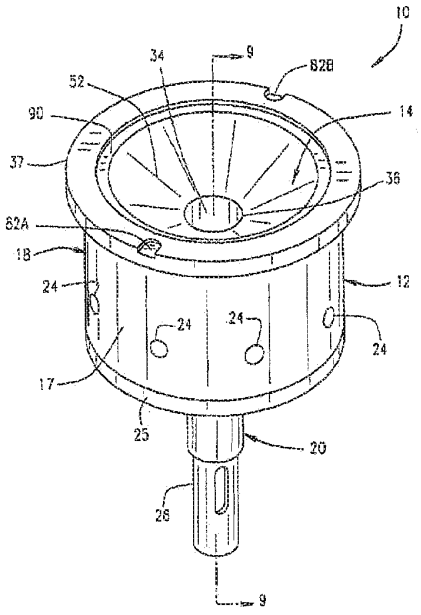
### 도면1



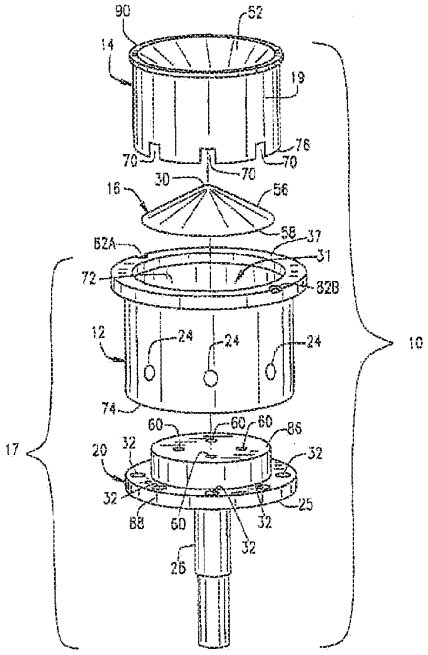
도면2



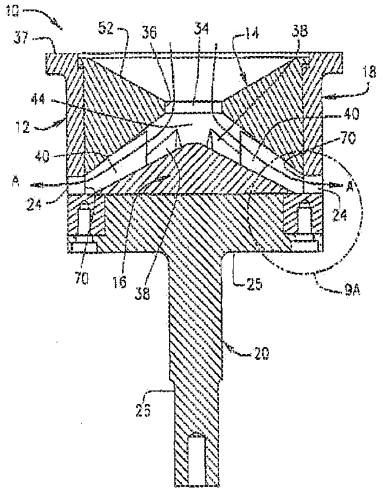
도면3



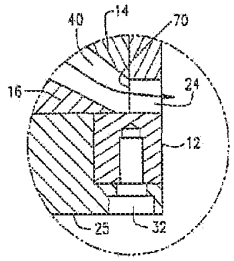
도면4


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52A
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도면5


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9A
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도면4



# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 34907436 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Kirsten Neuman |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 18-JAN-2019 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 22:15:51 |
| **Application Type:** | Utility under 35 USC 111(a) |

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| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Non Patent Literature | Macleod_1981_Part3.pdf | 25646451<br>0fe4b767c053c83cb11c4632451ba5a8ae46232f | no | 26 |

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| **Information:** | | | | | |
| 2 | Non Patent Literature | Macleod_1981_Part4.pdf | 25986560<br>64a9095a01d02c39aa8a5bbad7dcc76f5c4199ae | no | 24 |
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| 3 | Non Patent Literature | Macleod_1981_Part5.pdf | 5850514<br>d90c2b898315324188l0d5fc6ed0f9be9da08b8f | no | 5 |
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| 4 | Non Patent Literature | Nielsen_2001.pdf | 9471959<br>aea5d86f304672e66bb01664ffe6e8552774 2801 | no | 5 |
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| 5 | Non Patent Literature | Ofori_Hsieh_2012.pdf | 631125<br>9146259a608b4ca4a4af817d10d22b6acc0 3eaa5 | no | 30 |
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| **Information:** | | | | | |
| 6 | Non Patent Literature | Oshodi_1997.pdf | 1055674<br>c94dafce70162ac28bf17b2f1cc1a0016277 7be3 | no | 5 |
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| 7 | Non Patent Literature | Usami_2011.pdf | 12764212<br>4b1a92942234107ad45e70acdc76de87b42 d8681 | no | 9 |
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| 8 | Non Patent Literature | Wansink_2003.pdf | 5927159<br>9c8db570992a391230d75ca26a8b1b79cff 13b7e | no | 3 |
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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**
**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**
**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

This statement is being filed before the receipt of a first action on the merits. Therefore, no fees are believed due. If this is incorrect, please apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: May 8, 2019

/Kimberly M. Maize, Reg. No. 76,370/
Kimberly M. Maize
Reg. No. 76,370

Customer Number 26191
Fish & Richardson P.C.
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61907615.doc

Sheet 1 of 2

| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Class | Subclass | Filing Date If Appropriate |
|---|---|---|---|---|---|---|---|
| | 1. | 9700067 | 7/11/2017 | Fraser | | | |
| | 2. | 10039306 | 8/7/2018 | Vrljic et al. | | | |
| | 3. | 10172380 | 1/8/2019 | Varadan et al. | | | |
| | 4. | 10172381 | 1/8/2019 | Vrljic et al. | | | |
| | 5. | 20080026128 | 1/31/2008 | Yamaguchi | | | |
| | 6. | 20140220217 | 8/7/2014 | Brown et al. | | | |
| | 7. | 20150289541 | 10/15/2015 | Brown et al. | | | |
| | 8. | 20160305361 | 10/29/2015 | Holz-Schietinger et al. | | | |
| | 9. | 20170172169 | 6/22/2017 | Grzanich | | | |
| | 10. | 20170188612 | 9/29/2016 | Varadan et al. | | | |
| | 11. | 20180168209 | 6/21/2018 | Fraser et al. | | | |
| | 12. | 20190116855 | 4/25/2019 | Vrljic 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 | Translation No |
|---|---|---|---|---|---|---|---|---|
| | 13. | JP 2010-523125 | 7/15/2010 | JAPAN | | | English Abstract | |
| | 14. | JP 2013-192528 | 9/3/2013 | JAPAN | | | English Abstract | |
| | 15. | WO 2010/110493 | 10/4/2012 | WIPO | | | English Abstract | |

**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|
| | 16. | EPO Opposition, "Proprietor's observations and amendments under Rule 79(1) EPC," February 27, 2019, 50 pages |
| | 17. | Fountoulakis and Lahm, "Hydrolysis and amino acid composition analysis of proteins", J. Chromatogr A, 826: 109-134, 1998 |
| | 18. | Konermann et al., "Acid-induced denaturation of myoglobin studied by time-resolved electrospray ionization mass spectrometry", Biochemistry, 1997, vol. 36, pages 6448-6454 |
| | 19. | MacMunn, "An address on some of the applications of the spectroscope to medicine", The British Medical Journal, January 3, 1891, pages 3-9 |

| Examiner Signature | Date Considered |
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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

| Substitute Disclosure Form (37 CFR §1.98(b)) | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. | Application No. |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | 38767-0015008 | 15/913,090 |
| | | Applicant: Impossible Foods Inc. | |
| | | Filing Date: March 6, 2018 | Group Art Unit: 1791 |

| Other Documents (include Author, Title, Date, and Place of Publication) | | |
|---|---|---|
| Examiner Initial | Desig. ID | Document |
| | 20. | Oellingrath, "Heat Degradataion of Heme In Methemoglobin and Metmyoglobin Model Systems Measured by Reversed-Phase Ion-Pair High Performance Liquid Chromatography," J Food Science, 1998, 53(1): 40-42 |
| | 21. | Peterson, "The Impact of the Enzymatic Hydrolysis Process on Recovery and Use of Proteins", In: Birch G.G., Blakebrough N., Parker K.J. (eds) Enzymes and Food Processing. Springer, Dordrecht (1981) |
| | 22. | United States Environmental Protection Agency Document EPA-745-B-99-0145, 1999 |
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| 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

Espacenet

# Bibliographic data: JP2010523125 (A) — 2010-07-15

## Colored Structured Protein Products

**Inventor(s):**

**Applicant(s):**

**Classification:** **- international:** ***A23J3/22***
**- cooperative:** **A23J3/26**; **A23L11/07**; **A23L5/43**; **A23P30/20**; **D01D4/02**; **D01D5/08**; **D01F1/06**; **D01F4/00**

**Application number:** JP20100502328 20080404 Global Dossier

**Priority number (s):** US20070910261P 20070405 ; US20080059961 20080331 ; WO2008US59450 20080404

**Also published as:** AT544888 (T) BRPI0809069 (A2) CN101680123 (A) CN101680123 (B) EP2137343 (A1) more

**Abstract not available for JP2010523125 (A)**
**Abstract of corresponding document: US2008254199 (A1)**

The present invention provides a process for producing a colored structured protein product with protein fibers that are substantially aligned and the resultant product. Specifically, the plant protein is combined with a colorant and extruded, forming a colored structured protein product with protein fibers that are substantially aligned and the resultant product.

Espacenet

## Bibliographic data: JP2013192528 (A) — 2013-09-30

# METHOD FOR MASKING RETORT ODOR

**Inventor(s):** WATANABE TOSHIYUKI ± (WATANABE TOSHIYUKI, ; 渡辺 俊之)

**Applicant(s):** EZAKI GLICO CO ± (EZAKI GLICO CO LTD, ; 江崎グリコ株式会社)

**Classification:** **- international:** ***A23L13/00; A23L35/00***
**- cooperative:**

**Application number:** JP20120064725 20120322 Global Dossier

**Priority number(s):** JP20120064725 20120322

**Also published as:** JP5877743 (B2)

**Abstract of JP2013192528 (A)**

PROBLEM TO BE SOLVED: To mask retort odor of a sauce for a retort bowl.SOLUTION: A production method for masking the retort odor of a sauce for a retort bowl including meat selected from beef, pork and chicken, is a masking method of a retort odor of a retort bowl in which a sauce for the retort bowl includes at least one fruit juice selected from the group consisting of apple juice, peach juice, mandarin orange juice and muscat juice.

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## Bibliographic data: JPWO2010110493 (A1) — 2012-10-04

# FLAVORING MATERIAL

**Inventor(s):** 高倉 友紀子, ; 林 和寛, ; 伊賀 まゆこ, ; 増沢 拓也

**Applicant(s):** 味の素株式会社

**Classification:** **- international:** ***A23L1/221; A23L1/226; A23L1/231; A23L1/31; A23L13/00; A23L23/00; A23L27/10; A23L27/20; A23L27/26***
**- cooperative:** **A23L27/2022**; **A23L27/2024**; **A23L27/2056**

**Application number:** JP20110506171 20100325 Global Dossier

**Priority number (s):** JP20090078329 20090327 ; WO2010JP55995 20100325 ; JP20110506171 20100325

**Also published as:** BRPI1016028 (A2) CN102365033 (A) CN102365033 (B) EP2412249 (A1) EP2412249 (A4) more

**Abstract not available for JPWO2010110493 (A1)**
**Abstract of corresponding document: EP2412249 (A1)**

[Summary] [Problem] Provision of a method of imparting aroma or flavor to food. [Solving Means]An aroma or flavor-imparting composition containing methional, dienals and thiazoles in proportions satisfying 0 ¤A ¤100, 0 ¤B ¤100, 0 ¤C ¤60 and A+B+C=100 wherein A shows parts by weight of niethional, B shows parts by weight of dienals and C shows parts by weight of thiazoles.

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 35948959 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Kirsten Neuman |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 08-MAY-2019 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 09:59:43 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Information Disclosure Statement (IDS) Form (SB08) | Transmittal.pdf | 70905<br>efe220b9feaeef0c9b3d29488f0d0f36fc13f428 | no | 1 |

**Warnings:**

| | | | | | |
|---|---|---|---|---|---|
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 2 | Information Disclosure Statement (IDS) Form (SB08) | PTO_1449.pdf | 135215<br>0138b75a21f4ce964a81dda3a8fe57fe76a87f40 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 3 | Foreign Reference | JP2010523125.pdf | 65167<br>5dc9301fe2633120b9df8d60818e0fd64f6b1f7d | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Foreign Reference | JP2013192528.pdf | 83676<br>3aae66827f89a5df464e63ab388b9a80bd7722b5 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Foreign Reference | WO2010110493.pdf | 88097<br>769eb4037558e2d57a3b7e80b75329c0fee9b8ff | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Non Patent Literature | EPO_Opposition_ProprietorsObservations_2019.pdf | 13734674<br>db2fc4d53741aaecca9d2b608f1d310ba51fb287 | no | 60 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 7 | Non Patent Literature | Fountoulakis_Lahm_Hydrolysis_1998.pdf | 15170087<br>96fbd4f396796ec1524a02ce4cfbb0985fcb513c | no | 26 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 8 | Non Patent Literature | Konermann_1997.pdf | 221943<br>79022bce00f1fc3c89a6afee6f9e67eb9e51e5dc | no | 7 |
| **Warnings:** | | | | | |

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|---|---|---|---|---|---|
| **Information:** | | | | | |
| 9 | Non Patent Literature | MacMunn_1989.pdf | 14671671<br>82f625767af3d92cff5df1ebc67ea09413e2cc6b | no | 7 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 10 | Non Patent Literature | Oelligrath_1998.pdf | 695732<br>f3cf28baef3d2e3b5de83e06ed3ec6ca554469bc | no | 3 |
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| **Information:** | | | | | |
| 11 | Non Patent Literature | Peterson_1981.pdf | 1373582<br>5b20d2370a3d5e349729df4df742b2183f5db21f | no | 27 |
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| **Information:** | | | | | |
| 12 | Non Patent Literature | UnitedStatesEnvironmentalProtectionAgency_1999.pdf | 1725437<br>6afd15e67149337b98268163332cf97011ae901d | no | 31 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 13 | Non Patent Literature | Wisuthiphaet_KongruangProduction_2015.pdf | 5531504<br>803be7fd815c34446abfcfff74d50c9d8fb56557 | no | 5 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 53567690 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
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Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 CFR § 1.98(a)(2).

This statement is being filed before the receipt of a first Office Action on the merits. Therefore, no fees are believed due. If this is incorrect, please apply any charges applicable under 37 CFR § 1.17(p), or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

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

Respectfully submitted,

Date: August 23, 2019

/M. Angela Parsons/
M. Angela Parsons
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
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Facsimile: (877) 769-7945

62054107.doc

Sheet 1 of 4

| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) (37 CFR §1.98(b)) | | Applicant Impossible Foods Inc. | |
| | | Filing Date March 6, 2018 | Group Art Unit 1791 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Class | Subclass | Filing Date If Appropriate |
|---|---|---|---|---|---|---|---|
| | 1. | 3857970 | 12/31/1974 | Tsumura | | | |
| | 2. | 20190133162 | 5/9/2019 | Varadan et al. | | | |
| | 3. | 20190133163 | 5/9/2019 | Varadan et al. | | | |
| | 4. | 20190200658 | 7/4/2019 | Vrljic 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 | Translation No |
|---|---|---|---|---|---|---|---|---|
| | 5. | GB 1364747 | 8/29/1974 | GB | | | | |
| | 6. | JP 2001/037434 | 2/13/2001 | JP | | | | |
| | 7. | JP 2001/346533 | 12/18/2001 | JP | | | | |
| | 8. | JP 2002/101835 | 4/9/2002 | JP | | | | |
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**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|
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| | 15. | "www.jackkruse.com", [online], "Brain Gut 6: Epi-Paleo Rx", dated July 2012, Internet URL: https://jackkruse.com/brain-gut-6-epi-paleo-rx/?print=pdf, 12 pages |
| | 16. | "www.maraseaweed.com", [ online], "SMOKED DULSE", Applewood Smoked Dulse - Seaweed Flakes \| Mara Seaweed, NO DATE, Retrieved from the Internet July 15, 2019, Internet URL: https://maraseaweed.com/collections/products/products/smoked-dulse, 3 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

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|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

| Other Documents (include Author, Title, Date, and Place of Publication) | | |
|---|---|---|
| Examiner Initial | Desig. ID | Document |
| | 17. | "www.myliquidsupplements.com", " Red seaweed is a rich source of nutrients, essential fatty acids, fiber, protein, complex cards, and minerals and now red sea vegetables are easy to find", Health Benefits of Red Seaweed, dated 2013, Retrieved from Internet July 29, 2019, Internet URL: https//:www.myliquidsupplements.com/red-seaweed/, 10 pages |
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| | 19. | "www.shop.healthyhealing.com", [online], "What Are Sea Vegetables? (/healthyhealing-safety/what-are-sea-vegetables)", dated October 25, 2012, Retrieved from the Internet July 15, 2019, Internet URL: (https://shop.healthyhealing.com/Seaweed-Products_c_140.html), 5 pages |
| | 20. | "www.universal-tao.com", [online], "Sprouts", NO DATE, Retrieved from Internet on July 29, 2019, Internet URL: https://www.universal-tao.com/article/sprouts/html, 9 pages |
| | 21. | "www.wildfoodplants.com", [online], "Teriyaki Weed", dated September 9, 2007, Retrieved from Internet July 15, 2019, Internet URL: https://wildfoodplants.com/2007/09/teriyaki-weed/, 2 pages |
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| | 24. | Ames, "Volatile Sulfur Compounds in Yeast Extracts", Chapter 12, Sulfur compounds in foods. Washington DC: ACS, pp. 147-159, 1994 |
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| | 26. | Baek et al., "Aroma Extract Dilution Analysis of a Beef like Process Flavor from Extruded Enzyme-Hydrolyzed Soybean Protein", J. Agric. Food Chem., 49, 790-793, 2001 |
| | 27. | Bodnar, "Novel approaches to improving qualities of maize endosperm", Graduate Theses and Dissertations. Paper 10271. Ames, Iowa, USA: Iowa State University Digital Repository @ Iowa State University, 2011, 147 pages |
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| | 29. | Buttery, "FLAVOR CHEMISTRY AND ODOR THRESHOLDS", In: Teranishi, R, Wick, EL, Hornstein, I, editors. Flavor chemistry: thirty years of progress, pp. 353-65, 1999 |
| | 30. | Chohan et al., "Catalytic Effect of Cobalt, Copper, Iron, Magnesium, Manganese, Nickel and Zinc Metal ions on the Conversion of Glucose into 5-Bydroxymethylfurfuraldehyde.", Jour. Chem. Soc. Pak. Vol. 19, No. 3, pages 221-223, 1997 |
| | 31. | Collman et al., "Regioselective and Enantioselective Epoxidation Catalyzed by Metalloporphyrins", SCIENCE, VOL. 261, pages 1404-1411, 1993 |
| | 32. | Derwent, "Cheese-like, soybean-fermented foodstuff prodn. - by inoculating soybean milk with lactic acid bacteria and protein decomposing yeast", 1979 |
| | 33. | Duff et al., "Hemoglobin expression in germinating barley.", Seed Science Research, 431-436, 1998 |
| | 34. | Erickson et al., "Lipid Oxidation of Muscle Foods", Chapter 12, Food Lipids Chemistry, Nutrition, and Biotechnology Second Edition,48 pages, 2002 |

| 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.

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| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

| Other Documents (include Author, Title, Date, and Place of Publication) | | |
|---|---|---|
| Examiner Initial | Desig. ID | Document |
| | 35. | Evers et al., "Furans Substituted at the Three Position with Sulfur", American Chemical Society: Washington, pp 184-119, 1976 |
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| | 37. | Extended European Search Report in Application No. 18203176.5, dated May 31, 2019 |
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| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
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| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

| Other Documents (include Author, Title, Date, and Place of Publication) | | |
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| Examiner Initial | Desig. ID | Document |
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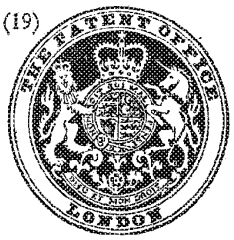
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# PATENT SPECIFICATION (11) 1364747

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## (54) FLAVORING COMPOSITIONS AND PROCESSES UTILIZING SIX MEMBERED HETEROCYCLIC-POLY-S-COMPOUNDS

(71) We, INTERNATIONAL FLAVORS & FRAGRANCES INC., a corporation organized and existing under the laws of the State of New York, United States of America whose address is 521 West 57th Street, New York, New York 10019, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention has to do with the use of certain trithianes and dithiazines to alter the flavor and aroma of consumable materials.

The term "consumable material" means (1) foodstuffs and beverages, (2) perfumed materials and (3) tobacco.

Examples of foodstuffs to which the present invention may be applied are meats, gravies, soups, convenience foods, nut butters (such as peanut butter) and other spreads, seafoods (including fish, crustaceans and mollusks) confectionery, breakfast foods, baked goods, vegetables, cereals, snack foods and pet foods (such as dog and cat foods).

Examples of beverages to which the invention may be applied are alcoholic and non-alcoholic drinks based on melt, and soft drinks.

Examples of perfumed materials to which the invention may be applied are perfumes, colognes, soaps and after-shave lotions.

The term "tobacco" will be understood herein to mean natural products, for example burley, Turkish tobacco, Maryland tobacco and flue-cured tobacco, and tobacco-based products such as reconstituted or homogenized leaf, as well as tobacco substitutes intended to replace natural tobacco, such as lettuce and cabbage leaves. Examples of tobaccos and tobacco products are those designed or used for smoking such as in cigarette, cigar, and pipe tobacco, as well as such products as snuff and chewing tobacco.

The term "alter" (in its application to foodstuffs and tobacco) in its various forms will be understood herein to mean the supplying or imparting of a flavour character or note to an otherwise bland, relatively tasteless substance, or augmenting an existing flavour characteristic where the natural flavor is deficient in some regard, or supplementing the existing flavor impression to modify the organoleptic character.

There is a continuing search for compositions which can vary, fortify, modify, enhance or otherwise improve (i.e. "alter") the flavor and aroma of a foodstuff. To be fully satisfactory, such compositions should be stable, non-toxic, and blendable with other ingredients to provide their own unique flavor and aroma nuance without detracting from the co-ingredients. Preferably, such compositions should be naturally occurring or present in natural foodstuffs (although unrecognized as flavor components thereof) so that their ingestible safety can be readily recognized. Additionally, these materials should be capable of being synthesized in a simple and economic manner.

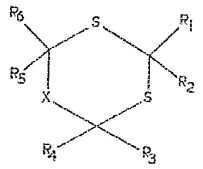
Various heterocyclic poly - S - compounds have been shown in the literature to have flavor implications. For example, in United States Letters Patent 3,503,758 to Wada et al, pentathiepane, tetrathiane and tetrathiepane are said to have a flavor and aroma; and Chang in *Chemistry and Industry*, 1639, November 23, 1968, reports 3,5 - dimethyl - 1,2,4 - trithiolane as present in the volatiles of boiled beef. Other investigators have commented that poly alkyl trithianes are odorless or have an intensely putrid odor. See Barch, United States Letters Patent 2,594,379 and Breslav and Skolnik, "Heterocyclic Com-

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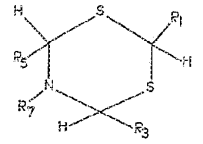
pounds", p. 726, *Interscience* (1966). Pippen in *Journal of Food Science, 34*, p. 443—446 (1969) speculated that $H_2S$ reacted with a carbonyl compound in chicken fat and postulated that 2,4,6 - trimethyl - S - trithiane could be one of the products formed.

It has now been found that the flavor of a foodstuff can be altered by adding thereto at least one heterocyclic poly - S - compound having the formula



wherein X is S or —$NR_7$—; and (1) when X is S, then $R_1$, $R_3$, and $R_5$ are the same and are alkyl of from 1 to 5 carbon atoms and $R_2$, $R_4$, and $R_6$ are the same and are hydrogen or alkyl from 1 to 5 carbon atoms; or (2) when X is —$NR_7$—, then $R_1$, $R_3$, and $R_5$ are the same and are alkyl of from 1 to 5 carbon atoms, $R_2$, $R_4$, and $R_6$ are hydrogen, and $R_7$ is hydrogen or alkyl of from 1 to 5 carbon atoms. The invention also contemplates flavoring compositions containing such heterocyclic poly - S - compounds.

A particularly preferred class of such compounds includes the dithiazines of the formula

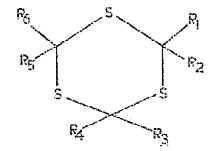


wherein $R_1$, $R_5$ and $R_5$ are the same alkyl radical of 1 to 5 carbon atoms and $R_7$ is hydrogen or alkyl radical of 1 to 5 carbon atoms. Suitable dithiazines include:

2,4,6 - trimethyldihydro - 1,3,5 - dithiazine
2,4,5,6 - tetramethyldihydro - 1,3,5 - dithiazine
2,4,6 - tri - isopropyldihydro - 1,3,5 - dithiazine
2,4,6 - tri - n - butyldihydro - 1,3,5 - dithiazine
2,4,6 - tri - isobutyldihydro - 1,3,5 - dithiazine
2,4,6 - tri - n - pentyldihydro - 1,3,5 - dithiazine

and mixtures thereof. The dithiazines have a pleasant sweet cereal, nutty taste and are especially suitable for beef, meat, nut and burnt flavors.

Another class of preferred heterocyclic poly - S - compounds include the trithianes having the formula



wherein $R_1$, $R_3$, and $R_5$ are the same and are alkyl of from 1 to 5 carbon atoms and $R_2$, $R_4$, and $R_6$ are the same and are hydrogen or alkyl from 1 to 5 carbon atoms. Suitable trithianes are:

2,4,6 - trimethyl - s - trithiane
2,2,4,4,6,6 - hexamethyl - s - trithiane
2,4,6 - triethyl - s - trithiane
2,4,6 - triethyl - 2,4,6 - trimethyl - s - trithiane
2,4,6 - tri - t - butyl - s - trithiane
2,4,6 - tri - n - butyl - s - trithiane
2,4,6 - tri - isopropyl - s - trithiane
2,4,6 - tri - n - propyl - s - trithiane
2,2,4,4,6,6 - hexaethyl - s - trithiane
2,2,4,4,6,6 - hexaisopropyl - s - trithiane
2,4,6 - triethyl - 2,4,6 - tri - n - pentyl - s - trithiane

The trithianes have a sweet nutty aroma and taste and are suitable for fruit (gooseberry, black currant, grape, raspberry) nut and meat flavors.

The structural formulae given herein contemplate and include cis- and trans- and other conformational isomers.

The dithiazines are prepared by using conventional processes, such as the methods shown in Beilstein, *27*, pp. 460—462 and 2d *Suppl.*, p. 524. The trithianes are similarly known compounds and can be prepared in a known manner such as that described by Campaigne, *Chem. Reviews, 39*, No. 1, pp. 1—77, August 1946.

Such heterocyclic - poly - S - compounds are useful in flavoring compositions and to alter the flavor of foodstuffs. They are especially adapted for adding sweet, meaty and nut nuances to consumable materials and in rounding out and improving the character and quality of meat flavors.

When the heterocyclic poly - S - compounds according to this invention are used in a flavouring composition, they can be combined with conventional flavouring materials or adjuvants. Such co-ingredients or flavouring adjuvants are well known in the art for such use and have been extensively described in the literature. Apart from the requirement that any such adjuvant material be ingestibly acceptable, and thus non-toxic or otherwise non-deleterious, conventional materials can be

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used and broadly include other flavor materials, vehicles, stabilizers, thickeners, surface active agents, conditioners and flavor intensifiers.

Such conventional flavoring materials include saturated and unsaturated fatty and amino acids, alcohols, including primary and secondary alcohols; esters, carbonyl compounds including ketones and aldehydes; lactones, other cyclic organic materials including benzene derivatives, alicyclics, heterocyclics such as furans, pyridines and pyrazines; sulfur-containing materials including thiols, sulfides and disulfides; proteins; lipids; carbohydrates; so-called flavor potentiators such as mono-sodium glutamate, guanylates, and inosinates; natural flavoring materials such as cocoa, vanilla and caramel; essential oils and extracts such as anise oil and clove oil; artificial flavoring materials such as vanillin. Particularly useful flavoring agents and adjuncts are cyclopentane thiol, protein hydrolysate, such as hydrolyzed vegetable protein, cysteine, salts of cysteine such as cysteine hydrochloride, thiamine, salts of thiamine, 2,5 - dimethyl - 3 - hydroxy - 4 - oxo - 4,5 - dihydrofuran, and products resulting from heating a mixture of at least two differing materials of the foregoing and high pressure reaction products of $H_2S$ and 2,5 - dimethyl - 3 - hydroxy - 4 - oxo - 4,5 - dihydrofuran.

Stabilizers include preservatives such as sodium chloride; antioxidants such as calcium and sodium ascorbate, ascorbic acid, butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate; sequestrants such as citric acid, EDTA and phosphates.

Thickeners include carriers, binders, protective colloids, suspending agents and emulsifiers, such as agar-agar, carrageenan, cellulose and cellulose derivatives such as carboxymethyl cellulose and methyl cellulose, natural and synthetic gums such as gum arabic and gum tragacanth, and other proteinaceous materials, lipids, carbohydrates, starches and pectins.

Surface active agents include emulsifying agents such as mono- and/or diglycerides of fatty acids such as caproic acid, caprylic acid, palmitic acid, myristic acid and oleic acid; lecithin; defoaming and flavor-dispersing agents such as sorbitan monostearate, potassium stearate and hydrogenated tallow alcohol.

Conditioners include compounds such as bleaching and maturing agents such as benzoyl peroxide, calcium peroxide and hydrogen peroxide; starch modifiers such as peracetic acid, sodium chlorite, sodium hypochlorite, propylene oxide and succinic anhydride; buffers and neutralizing agents such as sodium acetate, sodium diacid phosphate, ammonium bicarbonate, ammonium phosphate, citric acid, lactic acid and vinegar; colorants such as carminic acid, cochineal, turmeric, curcumin and approved food and drug dyes; firming agents such as aluminum sodium sulfate, calcium chloride and calcium gluconate; texturizers; anti-caking agents such as aluminum calcium sulfate and tribasic calcium phosphate; enzymes, yeast foods such as calcium lactate and calcium sulfate; nutrient supplements such as iron salts (e.g. ferric phosphate, ferric pyrophosphate and ferrous gluconate), riboflavin, vitamins; zinc sources such as zinc chloride and zinc sulfate.

The heterocyclic - poly - S - compounds, or the compositions incorporating them, as mentioned above, can be combined with one or more vehicles or carriers for adding them to the particular product. Vehicles can be edible or otherwise suitable materials such as ethyl alcohol, propylene glycol and water. Carriers include materials such as gum arabic, carrageenan and other gums. The heterocyclic - poly - S - compounds can be incorporated with the carriers by conventional means such as spray-drying and drum drying. Such carriers can also include materials for coacervating the heterocyclic - poly - S - compounds (and other flavoring ingredients, as present) to provide encapsulated products. When the carrier is an emulsion, the flavoring composition can also contain emulsifiers such as mono- and diglycerides of fatty acids. With these carriers or vehicles, the desired physical form of the composition can be prepared.

It will be understood by those skilled in the art that the heterocyclic - poly - S - compounds can be added to the materials to be flavored at any convenient point in the production of the finished product. Thus, when they are used to alter or otherwise vary the flavor of a foodstuff, they can be added in the original mixture (e.g. dough, emulsion or batter), prior to any cooking or heating operation. Alternatively, they can be added at a later stage of processing if volatilization losses would be excessive during the earlier processing.

When the materials are used to treat tobacco products, for example, the additive can be applied in a suitable manner by spraying, dipping or otherwise. The heterocyclic - poly - S - compounds can be applied during the "casing" or final spray treatment of the tobacco, or they can be applied at some earlier stage of curing. The quantity of heterocyclic - poly - S - compounds or mixtures thereof utilized should be sufficient to impart the desired flavor characteristic to the product, but on the other hand, the use of an excessive amount of the heterocyclic - poly - S - compounds is not only wasteful and uneconomical but in some instances too large a quantity may unbalance the flavor or other organoleptic property of the product to be consumed. The quantity used will vary depending upon the ultimate foodstuff, tobacco product, or other consumable product; the

amount and type of flavor initially present in the product; the further process or treatment steps to which the product will be subjected; regional and other preference factors; the type of storage, if any, to which the product will be subjected; and the pre-consumption treatment, such as baking, frying, and so on, given to the product by the ultimate consumer.

It is accordingly preferred that the foodstuff, beverage or tobacco after addition of the compounds used according to the invention should contain from 0.02 parts per million (ppm) to 100 ppm of the heterocyclic - poly - S - compounds. More particularly, in foodstuffs and beverages it is desirable to use from 0.02 to 20 ppm and in certain preferred embodiments of the invention, from 0.1 to 15 ppm of the heterocyclic - poly - S - compounds are included in the finished foodstuff or beverage. On the other hand, tobaccos can contain as little as 0.02 ppm and as much as 100 ppm, depending upon whether a cigarette tobacco, a pipe tobacco, a cigar tobacco, a chewing tobacco, or snuff is being prepared.

All parts, proportions, percentages, and ratios herein are by weight unless otherwise indicated.

The amount of heterocyclic - poly - S - compounds to be utilized in flavoring or flavor-enhancing compositions can be varied over a wide range depending upon a particular quality to be added to the foodstuff, beverage or tobacco. Thus, amounts of one or more heterocyclic - poly - S - compounds according to the present invention from 0.1 up to 80 or 90% can be incorporated in such compositions. It is generally found to be desirable to include from 0.5 to 25% of the heterocyclic - poly - S - compounds in such compositions.

The heterocyclic poly - S - compounds of this invention can also be added to perfume compositions in their pure form or they can be added to mixtures of materials in fragrance-imparting compositions to provide a desired fragrance character to a finished perfume material. The perfume and fragrance compositions obtained according to this invention are suitable in a wide variety of perfumed articles and can also be used to improve, enhance, modify, alter or reinforce fragrance materials. It will thus be appreciated that the heterocyclic poly - S - compounds and mixtures thereof of this invention are useful as olfactory agents and fragrances.

The term "perfume composition" is used herein to mean a mixture of compounds, including for example, natural oils, synthetic oils, alcohols, aldehydes, ketones, esters, lactones, and frequently hydrocarbons which are admixed so that the combined odors of the individual components produce a pleasant or desired fragrance. Such perfume compositions usually contain: (a) the main note or the "bouquet" or foundation-stone of the composition; (b) modifiers which round-off and accompany the main note; (c) fixatives which include odorous substances which lead a particular note to the perfume throughout all stages of evaporation, and substances which retard evaporation; and (d) top-notes which are usually low-boiling, fresh-smelling materials. Such perfume compositions or the novel materials of this invention can be used in conjunction with a perfume base, generally comprising one or more carriers, vehicles, solvents, dispersants, emulsifiers, surface-active agents and/or aerosol propellants. In perfume compositions, the individual components contribute their particular olfactory characteristics, but the overall effect of the perfume composition will usually at least be the sum of the effect of each ingredient. Thus, the heterocyclic poly - S - compounds of this invention can be used to alter the aroma characteristics of a perfume composition, for example, by highlighting or moderating the olfactory reaction contributed by another ingredient of the composition.

The amount of one or more compounds of this invention which will be effective in perfume compositions depends on many factors, including the other ingredients, their amounts and the effects which are desired. It has been found that perfume compositions containing as little as 1.0% by weight of the compounds of this invention, or even less can be used to intensify or augment and enhance various types of fragrance compounds, the odors of which may be desired to be imparted to colognes, perfumes, bath oils and other cosmetic products. The amount employed will depend on considerations of cost, nature of the end product, the effect desired in the finished product, and the particular fragrance sought. Higher concentrations (e.g. 2% by weight) of the heterocyclic - poly - S - compounds of this invention will intensify the green leafy note and green nutty notes of the compositions.

The heterocyclic poly - S - compounds disclosed herein can be used in a composition as an olfactory component of a fragrance which in turn can be used in perfumes, colognes, bath preparations (such as bath oils and bath salts) and the like. When the heterocyclic poly - S - compounds of this invention are used in finished perfumed articles, such as the foregoing, they can be used in amounts of 0.04% by weight or lower.

The following examples are given to illustrate embodiments of the invention as it is presently preferred to practise it. It will be understood that these examples are illustrative, and the invention is not to be considered as restricted thereto except as indicated in the appended claims.

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EXAMPLE I

The following materials are homogeneously mixed at 25°C:

| Ingredients | Parts |
|---|---|
| Vegetable shortening | 622.7 |
| Salt | 321.7 |
| Glutamic acid | 5.1 |
| L-Cysteine hydrochloride | 10.3 |
| Glycine | 5.1 |
| β-Alanine | 1.3 |
| Taurine | 20.0 |
| Mixture of di-sodium inosinate and di-sodium guanylate | 3.3 |

The mixture is heated at 300°F for thirty seconds. After cooling to 100°F, 0.12 part of diacetyl and 0.10 part of hexanal are added. After aging the resulting mixture for three hours, 0.1 part of 2,4,6 - trimethyldihydro - 1,3,5 - dithiazine is added.

The resulting mixture is aged for ten hours to provide a material having an excellent chicken aroma.

EXAMPLE II

| Ingredients | Parts |
|---|---|
| Vegetable shortening | 622.7 |
| Salt | 321.7 |
| Glutamic acid | 5.1 |
| L-Cysteine hydrochloride | 10.3 |
| β-Alanine | 1.3 |
| Taurine | 20.0 |
| Mixture of di-sodium inosinate and di-sodium guanylate | 3.3 |
| 2,4,6-trimethyl-s-trithiane | 0.1 |

The mixture is heated to 300°F for thirty seconds. After cooling to 100°F, 0.12 part of diacetyl and 0.10 part of hexanal are added.

The resulting mixture has an excellent chicken aroma.

EXAMPLE III

The following ingredents are homogeneously mixed at 25°C:

| Ingredients | Parts |
|---|---|
| Vegetable shortening | 622.7 |
| Salt | 321.7 |
| Glutamic acid | 5.1 |
| L-Cysteine hydrochloride | 10.3 |
| Glycine | 5.1 |
| β-Alanine | 1.3 |
| Taurine | 20.0 |
| Mixture of di-sodium inosinate and di-sodium guanylate | 3.3 |

The mixture is heated to 300°F for thirty seconds. After cooling to 100°F, 0.12 part of diacetyl, 0.10 part of hexanal, and 0.1 part of 2,4,5,6 - tetramethyl - dihydro - 1,3,5 - dithiazine are added.

The resulting mixture has an excellent chicken aroma.

EXAMPLE IV

The following ingredients are homogeneously mixed at 25°C:

| Ingredients | Parts |
|---|---|
| Vegetable shortening | 622.7 |
| Salt | 321.7 |
| Glutamic acid | 5.1 |
| L-Cysteine hydrochloride | 10.3 |
| Glycine | 5.1 |
| β-Alanine | 1.3 |
| Taurine | 20.0 |
| Mixture of di-sodium inosinate and di-sodium guanylate | 2.0 |

The mixture is heated to 300°F for sixty seconds. After cooling to 100°F, 0.12 part of diacetyl and 0.10 part of hexanal are added. After aging the mixture for a period of three hours at a temperature of 65°F, 0.1 part of 2,4,6 - triethyl - 2,4,6 - tri - n - pentyl - s - trithiane are added. The resulting mixture is then aged for ten hours to yield a compocition having an excellent chicken aroma and flavor.

EXAMPLE V

Cysteine hydrochloride in the amount of 8.8 g is refluxed at 215°F under atmospheric pressure for four hours with a mixture of 309 g of hydrolyzed vegetable protein and 674 g of water. Subsequent to the reflux, the mixture is cooled and 0.05 g of 2,4,6 - trimethyldihydro - 1,3,5 - dithiazine is added and intimately admixed with the composition. The mixture has an excellent beef flavor.

EXAMPLE VI

Cyclopentane thiol and 2,2,4,4,6,6 - hexamethyl - s - trithiane (1:1 mole ratio) in the amount of 0.05 g. are refluxed at 215°F under atmospheric pressure for four hours with the following premix composition:

| Ingredients | Amount |
|---|---|
| Hydrolyzed vegetable protein (Maggi 4BE) | 309 g |
| Water | 674 g |
| Thiamine-HCl | 8.8 g |
| Cysteine-HCl | 8.8 g |

(The word "Maggi" is a Registered Trade Mark).

Subsequent to the reflux period, the resulting mixture is aged for 72 hours at 60—75°C. The mixture has an excellent beef aroma.

EXAMPLE VII

2,4,6 - Trimethyl - s - trithiane in the amount of 0.05 g is refluxed at 215°F under atmospheric pressure for four hours with the following pre-mix composition:

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| Ingredients | Amount |
|---|---|
| Hydrolyzed vegetable protein (Maggi 4BE) | 309 g |
| Cysteine-HCl | 8.8 g |
| Thiamine-HCl | 8.8 g |
| Water | 674 g |

Subsequent to the reflux, the resulting mixture is aged for 72 hours at 60—75°C. The mixture has an excellent beef aroma.

## EXAMPLE VIII

The composition prepared in Example I is dissolved in propylene glycol to provide a 0.1% solution. This solution in the amount of 0.966 g is added to 7.3 g of a soup base consisting of:

| Ingredients | Parts |
|---|---|
| Fine ground sodium chloride | 35.62 |
| Hydrolyzed vegetable protein | 27.40 |
| Monosodium glutamate | 17.81 |
| Sucrose | 10.96 |
| Beef fat | 5.48 |
| Sethness caramel color (powder B & C) | 2.73 |

The resulting mixture is added to 12 ounces of boiling water to obtain a soup having an excellent chicken flavor.

The composition of Example VI (0.005 g) when added to the above soup base also provides a soup having good meat flavor.

## EXAMPLE IX

The composition prepared in Example V is dissolved in propylene glycol to provide a 0.1% solution. This solution in the amount of 0.966 g is added to 7.3 g of a soup base consisting of:

| Ingredients | Parts |
|---|---|
| Fine ground sodium chloride | 35.62 |
| Hydrolyzed vegetable protein (Maggi 4BE) | 27.40 |
| Monosodium glutamate | 17.81 |
| Sucrose | 10.96 |
| Beef fat | 5.48 |
| Caramel color, bakers and confectioner's powder grade | 2.73 |

The resulting mixture is added to 12 ounces of boiling water to obtain a soup having an excellent chicken flavor.

The composition of Example II (0.005 g) when added to the above soup base also provides a soup having good meat flavor.

## EXAMPLE X

One-half gram of the soup base mixture of Example VIII is emulsified in a solution containing 100 g gum arabic and 300 g water. The emulsion is spray-dried with a Bowen Lab Model Drier utilizing 250 cfm of air with an inlet temperature of 500°F, an outlet temperature of 200°F, and a wheel speed of 50,000 rpm.

Twelve grams of the spray-dried material is mixed with 29.2 g of the soup base set forth in Example VIII. The resulting mixture is then added to 12 ounces of boiling water, and an excellent meat flavored soup is obtained.

## EXAMPLE XI

Example I is repeated except that the dithiazine is replaced by .003 part of a mixture comprising the following ingredients:

| Ingredients | Parts |
|---|---|
| 2,4,6-trimethyl dihydro-1,3,5-dithiazine | 10 |
| 2,4,6-trimethyl-s-trithiane | 26 |
| Corn Oil | 50 |
| Gum arabic | 20 |

The resulting mixture has an excellent meat flavor.

## EXAMPLE XII

Preparation of Perfume Composition Containing 2,4,6 - Trimethyl - S - Trithiane

The following composition is prepared:

| Ingredients | Parts by Weight |
|---|---|
| 2,4,6-trimethyl-s-trithiane | 4 |
| Benzyl butyrate | 4 |
| Bois de Rose | 10 |
| Citronellyl formate | 30 |
| Citronellyl acetate | 20 |
| Geraneol coeur | 200 |
| Citronellol coeur | 300 |
| Menthone | 5 |
| Menthol natural | 5 |
| Rose oxide | 10 |
| Geranyl acetate | 30 |
| Dimethyl Benzylcarbinyl-acetate | 2 |
| | 620 |

The addition of the 2,4,6 - trimethyl - s - trithiane in the quantity given imparts a green leafy note similar to that of natural geranium Bourbon to the composition without detracting from the quality of the odor.

## EXAMPLE XIII

The following formulations are produced:

Formulation A

1.9 gm Natural black currant juice, concentrate
0.1 gm Natural black currant esters
10.0 ml Sugar Syrup 32°Be
q.s. 100 ml Spring water

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Formulation B
1.9 gm Natural black currant juice, concentrate
0.1 gm Buchu leaf oil 0.1% (ethanol 95%)
10.0 ml Sugar Syrup 32°Be
q.s. 100 ml Spring water

Formulation C
1.9 gm Natural black currant juice, concentrate
0.1 gm Niribine* 10% (ethanol 95%)
10.0 ml Sugar Syrup 32°Be
q.s. 100 ml Spring water

*Niribine is produced by distilling an alcoholic macerate of black currant buds.

Formulation D
1.9 gm Natural black currant juice, concentrate
0.1 gm 2,4,6 - trimethyl - s - trithiane 0.1% (ethanol 95%)
10.0 ml Sugar Syrup 32°Be
q.s. 100 ml Spring water

Formulation E
1.9 gm Natural black currant juice, concentrate
0.1 gm 2,4,6 - trimethyl - s - trithiane 0.01% (ethanol 95%)
q.s. 100 ml Spring water

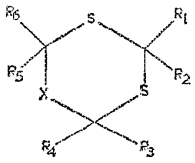
Formulation F
1.9 gm Natural black currant juice, concentrate
10.0 ml Sugar Syrup 32°Be
q.s. 100 ml Spring water

Each of the above-mentioned formulations is compared with one another by a panel composed of 10 tasters. Formulation F is generally considered by the panel to be flat and not very characteristic for fresh black currant. Formulations B, C, D and E are considered as having substantially fresh and more pleasant notes than formulation F. In summary, formulations D and E were preferred as the best black currant juice formulations. In conclusion, for use in black currant flavor, the material 2,4,6 - trimethyl - s - trithiane can be used at rates of one-tenth of that of Buchu leaf oil in black currant juice.

It is further to be concluded that 2,4,6 - trimethyl - s - trithiane can successfully replace Buchu leaf oil, Niribine and/or natural black currant esters wherever the ingredient is used in reinforced black currant juices, substituted black currant juices and imitation black currant flavors.

WHAT WE CLAIM IS:—

1. A method for altering the flavour or aroma of a consumable material which comprises adding to the consumable material a compound of the general formula:—



in which X is S or —$NR_7$; and

(1) when X is S, then $R_1$, $R_3$ and $R_5$ are the same and are alkyl groups of from 1 to 5 carbon atoms and $R_2$, $R_4$ and $R_6$ are the same and are hydrogen atoms or alkyl groups of from 1 to 5 carbon atoms; or

(2) when X is $NR_7$, then $R_1$, $R_3$ and $R_5$ are the same and are alkyl groups of from 1 to 5 carbon atoms, $R_2$, $R_4$ and $R_6$ are hydrogen atoms, and $R_7$ is a hydrogen atom or an alkyl group of from 1 to 5 carbon atoms.

2. A method according to claim 1 in which X is —NH and each of $R_1$, $R_3$ and $R_5$ is an alkyl group having from 1 to 5 carbon atoms.

3. A method according to claim 2 in which $R_1$, $R_3$ and $R_5$ are methyl groups.

4. A method according to claim 1 in which X is S, and each of $R_2$, $R_4$ and $R_6$ is an alkyl group having from 1 to 5 carbon atoms.

5. A method according to claim 4 in which X is S; $R_1$, $R_3$ and $R_5$ are methyl groups and $R_2$, $R_4$ and $R_6$ are hydrogen atoms.

6. A method according to claim 4 in which $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ and $R_6$ are all methyl groups.

7. A method according to claim 1 in which X is S and $R_2$, $R_4$ and $R_6$ are hydrogen atoms.

8. A method according to claim 1 in which the compound is one of those hereinbefore specifically mentioned.

9. A method according to any preceding claim in which the final concentration of the said compound in the material is 0.02 to 100 ppm.

10. A method according to claim 9 in which the final concentration of the said compound of the material is 0.1 to 15 ppm.

11. A method according to any preceding claim in which the material is a foodstuff.

12. A method according to any preceding claim in which the foodstuff is one of those hereinbefore specifically mentioned.

13. A method according to claim 11 or 12 in which the foodstuff is a reinforced, substituted or imitation black currant juice and the compound is 2,4,6 - trimethyl - S - trithiane.

14. A method according to claim 11 or 12 in which the foodstuff comprises hydrolysed vegetable protein.

15. A method according to any of claims 1 to 10 in which the material is tobacco.

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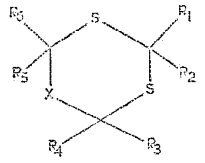
16. A method according to any of claims 1 to 10 in which the material is a perfume base.

17. The method of any of claims 1 to 10 in which the material is a toilet preparation.

18. A method according to claim 1 for altering the flavour or aroma of a consumable material substantially as hereinbefore described in the Examples.

19. Consumable materials having a flavour or aroma altered by the method of any preceding claim.

20. A flavouring composition containing an organoleptically modifying amount of a compound of the general formula:—



in which X is S or —$NR_7$; and

(1) when X is S, then $R_1$, $R_3$ and $R_5$ are the same and are alkyl groups of from 1 to 5 carbon atoms and $R_2$, $R_4$ and $R_6$ are the same and are hydrogen atoms or lower alkyl groups of from 1 to 5 carbon atoms; or

(2) when X is —$NR_7$, then $R_1$, $R_3$ and $R_5$ are the same and are alkyl groups of from 1 to 5 carbon atoms, $R_2$, $R_4$ and $R_6$ are hydrogen atoms, and $R_7$ is a hydrogen atom or an alkyl group of from 1 to 5 carbon atoms; and an auxiliary flavouring adjuvant.

21. A composition according to claim 20 in which X is —NH— and each of $R_1$, $R_3$ and $R_5$ is an alkyl group having from 1 to 5 carbon atoms.

22. A flavouring composition according to claim 21 in which $R_1$, $R_3$ and $R_5$ are methyl groups.

23. A flavouring composition according to claim 20 in which X is S and each of $R_1$, $R_3$ and $R_5$ is an alkyl group having from 1 to 5 carbon atoms.

24. A flavouring composition according to claim 23 in which $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ and $R_6$ are all methyl groups.

25. A flavouring composition according to claim 23 in which $R_1$, $R_3$ and $R_5$ are methyl groups and $R_2$, $R_4$ and $R_6$ are hydrogen atoms.

26. A flavouring composition according to claim 20 in which X is S and $R_2$, $R_4$ and $R_6$ are hydrogen atoms.

27. A flavouring composition according to claim 20 in which the compound is one of those hereinbefore specifically mentioned.

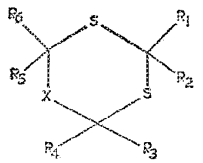
28. A composition according to any of claims 20 to 27 in which the adjuvant is a further flavouring material, a vehicle, a stabiliser, a thickener, a surfactant, a conditioner or a flavour intensifier.

29. A composition according to claim 28 in which the adjuvant is one of those hereinbefore specifically mentioned.

30. Flavouring compositions according to claim 20 substantially as hereinbefore described in the Examples.

31. A method according to any of claims 1 to 19 in which the compound is added in the form of a flavouring composition according to any of claims 20 to 30.

32. A perfume composition containing an organoleptically modifying amount of a compound of the general formula:—



in which X is S or —$NR_7$—; and

(1) when X is S, then $R_1$, $R_3$ and $R_5$ are the same and are alkyl groups of from 1 to 5 carbon atoms and $R_2$, $R_4$ and $R_6$ are the same and are hydrogen atoms or alkyl groups of from 1 to 5 carbon atoms; or

(2) when X is —$NR_7$—; then $R_1$, $R_3$ and $R_5$ are the same and are alkyl groups of from 1 to 5 carbon atoms, $R_2$, $R_4$ and $R_6$ are hydrogen atoms, and $R_7$ is a hydrogen atom or an alkyl group of from 1 to 5 carbon atoms; and an auxiliary perfume adjuvant.

33. A composition according to claim 32 in which the compound is one of those hereinbefore specifically mentioned.

34. A composition according to claim 32 or 33 in which the adjuvant is one of those hereinbefore specifically mentioned.

35. Perfume compositions according to claim 34 substantially as hereinbefore described in the Examples.

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# Bibliographic data: JP2001037434 (A) — 2001-02-13

## FROZEN COOKED FOOD AND ITS PRODUCTION

**Inventor(s):** ISHII SHOJI ± (ISHII SHOJI)

**Applicant(s):** FUJI SHOKUHIN KK ± (FUJI SHOKUHIN:KK)

**Classification:** **- international:** ***A23L23/00; A23L3/36; A23L35/00; A23L7/10; A23L7/109;*** (IPC1-7): A23L1/10; A23L1/16; A23L1/39; A23L1/48; A23L3/36

**- cooperative:**

**Application number:** JP19990211469 19990727

**Priority number(s):** JP19990211469 19990727

### Abstract of JP2001037434 (A)

PROBLEM TO BE SOLVED: To obtain a frozen cooked food producible in high efficiency and having the appearance and palatability of newly cooked food by bringing a cooked main food material into contact with a gelatinous seasoning liquid added with gelatin and filling the mixture in a package. SOLUTION: (A) A main food material such as cooked noodle, pasta or rice is filled in a package in a state contacting with (B) a gelatinous seasoning liquid obtained by adding gelatin to a seasoning liquid such as sauce or soup. When the objective food is e.g. chow mein, the steamed noodle for chow mein is prepared first, the noodle is mixed with a seasoning sauce, ingredients, oil, etc., and the mixture is heat-treated to sufficiently fry the noodle and cooled to normal temperature. Separately, a sauce same as or similar to the above seasoning sauce is incorporated with gelatin and cooled to solidify the sauce in gelatinous state. A prescribed amount of the cooked chow mein 4 is brought into contact with a prescribed amount of the gelatinous sauce 5, filled in a package 1 and frozen to obtain the objective frozen food.

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# Bibliographic data: JP2001346533 (A) — 2001-12-18

## PASTA AND METHOD FOR PRODUCING THE SAME

**Inventor(s):** NISHIWAKI KEISUKE; ISHIBASHI HIDEAKI ± (NISHIWAKI KEISUKE, ; ISHIBASHI HIDEAKI)

**Applicant(s):** NIPPON FLOUR MILLS ± (NIPPON FLOUR MILLS CO LTD)

**Classification:** **- international:** ***A23L7/109;*** (IPC1-7): A23L1/16
**- cooperative:**

**Application number:** JP20000166646 20000602

**Priority number(s):** JP20000166646 20000602

**Abstract of JP2001346533 (A)**

PROBLEM TO BE SOLVED: To obtain pasta with good palate feeling. SOLUTION: The method for producing pasta is characterized by comprising the following process: a chip-like dough is produced by extruding a dough 25-45 wt.% in water content after kneading under a reduced pressure, i.e., >=55 kPa less than the atmospheric pressure, and the chip-like dough thus produced is kneaded again and extruded.

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## Bibliographic data: JP2002101835 (A) — 2002-04-09

# METHOD FOR PRODUCING NOODLES AND NOODLE QUALITY IMPROVER

**Inventor(s):** KAWAI TAKANORI; HANEKI TAKASHI; KONDO KEIICHI; YOKOTA YUURI; SAKIDA SHUICHIRO; MORIMOTO ERIKO; KAWAI AKIFUSA; YAMAZAKI NAGAHIRO ± (KAWAI TAKANORI, ; HANEKI TAKASHI, ; KONDO KEIICHI, ; YOKOTA YUURI, ; SAKIDA SHUICHIRO, ; MORIMOTO ERIKO, ; KAWAI AKIFUSA, ; YAMAZAKI NAGAHIRO)

**Applicant(s):** TAIYO KAGAKU KK ± (TAIYO KAGAKU CO LTD)

**Classification:** **- international:** ***A23L7/109;*** (IPC1-7): A23L1/16
**- cooperative:**

**Application number:** JP20010226061 20010726

**Priority number(s):** JP20010226061 20010726 ; JP20000224717 20000726

**Abstract of JP2002101835 (A)**

PROBLEM TO BE SOLVED: To provide a method for producing noodles good in taste of the noodles, having a moderate hardness, elasticity (viscoelasticity), chewiness and smoothness (smooth feeling) with texture good in throat passage and scarcely losing firmness with hot water and by boiling without deteriorating the taste of the noodles and to obtain a quality improver for the noodles and the noodles capable of maintaining the texture even during the production or after storage thereof not only in the noodles eaten just after cooking but also in cooked noodles, raw type instant noodles (hereinafter referred to LL noodles), frozen noodles or instant noodles. SOLUTION: A vegetable protein or a milk protein having <=20 [mu]m average particle diameter is added.

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## Bibliographic data: JP2009284893 (A) — 2009-12-10

# SEASONING LIQUID IMPARTING BAKING FLAVOR, STEAMED NOODLE COATED WITH THE SAME, AND METHOD FOR PRODUCING THE SAME

**Inventor(s):** SHIIBASHI YUSUKE; KAWADA RYOICHI; HIRAKAWA TOSHIAKI; NASU YASUNOBU; OTA KEIJI + (SHIIBASHI YUSUKE, ; KAWADA RYOICHI, ; HIRAKAWA TOSHIAKI, ; NASU YASUNOBU, ; OTA KEIJI)

**Applicant(s):** SHIMADAYA CORP + (SHIMADAYA CORP)

**Classification:** **- international:** ***A23L27/00; A23L7/109***
**- cooperative:**

**Application number:** JP20080167927 20080530 Global Dossier

**Priority number(s):** JP20080167927 20080530

**Also published as:** JP4993216 (B2)

## Abstract of JP2009284893 (A)

PROBLEM TO BE SOLVED: To provide steamed noodles coated with a seasoning liquid, making a nice-smelling baking flavor by heating in a microwave oven compatible with a smooth texture of just boiled raw noodles. ;SOLUTION: The top surface of a lump of steamed noodles is coated with a whipped seasoning liquid obtained by dissolving a reducing sugar activating an aminocarbonylation reaction by being heated, and gelatin as a gelling agent in a seasoning liquid containing a protein hydrolyzate and heating, and then whipping while cooling. Since flowing down into gaps of the noodles is hardly caused even after heating and melting, and heat is readily generated by heating in a microwave oven to obtain the nice-smelling baking flavor in a short time, the steamed noodles are not excessively heated. ;COPYRIGHT: (C)2010,JPO&INPIT

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# Bibliographic data: JPH0970272 (A) — 1997-03-18

## INSTANT NOODLE

**Inventor(s):** INAOKA TOKIJIROU; FUKITA TOMOHIRO; SHIIBA DAISUKE; KAMEO YOJI; GENJI MIWA; KOMIKADO MASANORI ± (INAOKA TOKIJIROU, ; FUKITA TOMOHIRO, ; SHIIBA DAISUKE, ; KAMEO YOJI, ; GENJI MIWA, ; KOMIKADO MASANORI)

**Applicant(s):** KAO CORP ± (KAO CORP)

**Classification:** **- international:*A23L7/109; A23L7/113;*** (IPC1-7): A23L1/16; A23L1/162

**- cooperative:**

**Application number:** JP19950226323 19950904

**Priority number(s):** JP19950226323 19950904

### Abstract of JPH0970272 (A)

PROBLEM TO BE SOLVED: To obtain instant noodle having excellent instant cookability and resembling genuine noodle in taste and palatability by adding a diglycerol disaturated fatty acid ester to raw material flour, kneading the mixture, forming to noodle form and gelatinizing the noodle. SOLUTION: Raw material flour produced by mixing wheat flour with potato starch in the form of powder is incorporated with a diglycerol fatty acid ester composition containing >=80wt.% of a diglycerol saturated fatty acid ester having a structure obtained by esterifying either one of a terminal hydroxyl group or adjacent hydroxyl group of diglycerol and either one of the other terminal hydroxyl group or adjacent hydroxyl group with a saturated fatty acid, the mixture is kneaded after the addition of salt and water, the prepared noodle dough is formed by a roll mill, the obtained noodle strip is slit to noodle strings with a disk blade and the strings are gelatinized and dried to obtain the objective instant noodles having excellent instant cookability and resembling genuine noodles in the taste and palatability.

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— 国際調査報告（条約第 21 条(3)）

WO 2012/157544 A1

(54) Title: FROZEN PASTA

(54) 発明の名称 : 冷凍パスタ

(57) Abstract: Provided is a frozen pasta in which the original flavor of the pasta is retained. A frozen pasta characterized by having a ground product of a pasta and/or a durum wheat flour attached thereon. A process for producing a frozen pasta, characterized by comprising cooling a cooked pasta, attaching a ground product of a pasta and/or a durum wheat flour on the surface of the pasta, and then freezing the resultant product.

(57) 要約:　パスタ本来の風味が保持されている冷凍パスタの提供。パスタ粉砕物及び／又はデュラム小麦粉が付着していることを特徴とする冷凍パスタ。茹で処理したパスタを冷却した後、その表面にパスタ粉砕物及び／又はデュラム小麦粉を付着させて、冷凍することを特徴とする冷凍パスタの製造方法。

# 明 細 書

## 発明の名称 ： 冷凍パスタ

## 技術分野

[0001] 本発明は、パスタ本来の風味が保持されている冷凍パスタ及びその製造方法に関する。

## 背景技術

[0002] 従来、冷凍パスタとしては、茹で上げた乾燥パスタを冷却し、その後冷凍することにより製造されたものが一般に提供されている。斯かる従来の冷凍パスタの製造過程においては、茹で上げたパスタを、通常水で冷却しているが、この場合、冷却の際に、パスタからパスタの風味が抜けてしまうことがあり、特に十分な冷却が求められる冷凍パスタにおいては、それが顕著であった。そのため、従来の冷凍パスタは、これを解凍して喫食しても、茹で上げたパスタと比較して、パスタ本来の風味に劣るという問題があった。

[0003] パスタの風味を増強する方法としては、例えば、粉砕デンプン生成物、デンプン、乳化剤、ゲル化剤及び風味付け組成物と軟水を含む混合物を押し出し加工してパスタに成形し、これをカルシウム水溶液に接触させ、ついで乾燥させることを特徴とする、風味付けパスタの製造法などが提案されている（特許文献１）。

[0004] しかしながら、上記特許文献１の方法で製造されたパスタも、風味付け組成物が麺生地として混合されているため、これを用いて冷凍パスタを製造すると、やはり水冷中にパスタの風味が抜けてしまうことが避けられなかった。そのため、製造した冷凍パスタを解凍して喫食すると、茹で上げたパスタと比較して、パスタ本来の風味に劣り、満足のいくものが得られないのが実状であった。

## 先行技術文献

## 特許文献

[0005] 特許文献1：特開平４－２６２７５４号公報

## 発明の概要

[0006] 本発明は、パスタ粉砕物及び／又はデュラム小麦粉が付着していることを特徴とする冷凍パスタを提供する。

また本発明は、茹で処理したパスタを冷却する工程と、該パスタの表面にパスタ粉砕物及び／又はデュラム小麦粉を付着させる工程と、該パスタを冷凍する工程とを含む、冷凍パスタの製造方法を提供する。

## 発明を実施するための形態

[0007] 本発明は、解凍して喫食するときに、茹で立てのパスタのようなパスタ本来の風味を保持している冷凍パスタを提供する。

[0008] 本発明者らは、種々研究を重ねた結果、茹で処理したパスタを冷却した後、その表面に、パスタ粉砕物及び／又はデュラム小麦粉を付着させて、冷凍すれば、極めて良い結果が得られることを見出し、本発明を完成した。

[0009] 本発明によれば、解凍して喫食するときに、茹で立てのパスタのようなパスタ本来の風味を保持している冷凍パスタを提供することができる。

[0010] 本発明の冷凍パスタ類の製造方法においては、まず、パスタを茹で処理する。ここに用いられるパスタとしては、デュラム小麦粉を原料とするパスタであれば、特に制限されず、スパゲティ等のロングパスタ；マカロニ、ペンネ等のショートパスタ；ラザニア；ラビオリ等のあらゆる種類のパスタが挙げられる。当該パスタは、乾燥パスタでも、半生パスタでも、生パスタでもよく、また、必要に応じて、肉や野菜等の具材、又は調味料等が包み込まれているか、あるいは練り込まれているものであってもよい。

[0011] パスタの茹で処理は、茹でた後のパスタが、茹でる前のパスタ１００質量部に対して、乾燥パスタの場合は２２０～２４５質量部（いわゆる歩留まり２２０～２４５％）程度になるように、半生パスタ又は生パスタの場合は２００～２３０質量部（いわゆる歩留まり２００～２３０％）程度になるように茹でると、喫食時の食感が向上するため好ましい。さらに、パスタを茹でる際には、食塩を添加して茹でたほうが、パスタの組織が引き締まり、パスタの食感がさらに向上するため好ましい。

[0012] 次に、茹で処理したパスタを冷却する。冷却は、水冷、風冷等の任意の方法により行うことができるが、冷却されたパスタ類の喫食時の食感がより向上するため、水冷が好ましい。水冷の際には、１５～２５℃程度の常温の水で冷却しても問題ないが、０℃～１０℃程度の冷水で冷却したほうが、パスタの組織が引き締まることにより喫食時の食感が向上するため、好ましい。パスタを冷却する時間は、パスタの組織を引き締めて喫食時の食感を向上させる観点から、３０～２４０秒程度が好ましい。

[0013] 次いで、冷却されたパスタの表面に、パスタ粉砕物及び／又はデュラム小麦粉を付着させる。パスタ粉砕物は、パスタを粉砕することにより得ることができる。当該パスタ粉砕物としては、デュラム小麦粉を原料とするパスタの粉砕物であれば、特に制限されず、スパゲティ等のロングパスタ；マカロニ、ペンネ等のショートパスタ；ラザニアなどのあらゆる種類のパスタの粉砕物が挙げられる。当該パスタ粉砕物は、乾燥パスタの粉砕物でも、生パスタ若しくは半生パスタの粉砕物でもよいが、パスタ本来の風味を保持する上で、乾燥パスタを粉砕したものが好ましい。粉砕物の粒径は、１ｍｍ以下であることが、パスタ表面への付着性の点から見て好ましい。

また、デュラム小麦粉としては、特に制限はなく、公知のデュラム小麦粉を用いることができる。

[0014] 上記パスタ粉砕物とデュラム小麦粉は、そのいずれか一方だけを含んでいてもよく、あるいは両方を含んでもよいが、パスタ本来の風味を保持する上で、少なくともパスタ粉砕物を含んでいるのが好ましい。また好ましくは、上記パスタ粉砕物とデュラム小麦粉は、α化されている。

[0015] 上記パスタ粉砕物及び／又はデュラム小麦粉をパスタ表面に付着させる手段は、特に限定されないが、水と上記パスタ粉砕物及び／又はデュラム小麦粉とを混合して調製したコーティング液を用いるのが、特に簡便で効果的であるため好ましい。

好ましくは、当該コーティング液は、水とパスタ粉砕物及び／又はデュラム小麦粉とを混合し、加熱することにより調製される。この加熱により、当

該コーティング液に含まれるパスタ粉砕物及び／又はデュラム小麦粉をα化させてもよい。

上記コーティング液中のパスタ粉砕物及び／又はデュラム小麦粉の含有量は、コーティング液の全質量に対して、１～１５質量％が好ましく、５～１０質量％がより好ましい。含有量が１質量％未満であると、多くのコーティング液を付着させなければならず、結果としてパスタが液を吸って柔らかくなり、風味及び食感が低下する。他方、含有量が１５質量％を超えると、コーティング液の粘性が高くなるため操作性が低下したり、コーティング液が量が少ないためパスタ全体に付着させにくくなることがある。

また、当該コーティング液中には、更に食塩、糖類等を配合してもよい。

[0016] 斯かるコーティング液をパスタ表面に付着させる手段としては、パスタ表面全体にコーティング液を付着させることができる手段であれば特に限定されず、噴霧、塗布、浸漬等の任意の手段が挙げられる。このうち、経済性及び簡便性の点からは、噴霧が好ましい。

[0017] パスタ粉砕物及び／又はデュラム小麦粉をパスタ表面に付着させる量としては、冷凍前の茹でパスタ１００質量部に対して、０．１～２．０質量部、好ましくは０．２５～２．０質量部、より好ましくは０．５～２．０質量部を付着させればよい。付着させる量が０．１質量部未満であると、解凍して喫食した際の風味に劣り、他方、２．０質量部を超えると、パスタの食感が悪くなることがある。

上記コーティング液を用いる場合、パスタに付着させるべきパスタ粉砕物及び／又はデュラム小麦粉の量と、コーティング液中のそれらの含有量とを考慮して、使用すべきコーティング液の量を決定する。茹でパスタ１００質量部に対して１～２０質量部、好ましくは１～１５質量部程度の液量となるように、コーティング液の濃度を調整することが望ましい。

[0018] 次いで、パスタ粉砕物及び／又はデュラム小麦粉を付着させたパスタを冷凍することにより、本発明の冷凍パスタを製造することができる。パスタを冷凍する温度は、約－１０℃以下であればよい。あるいはさらに、このよう

にして得られた冷凍パスタに水を噴霧し、その後再冷凍してもよい。当該水の噴霧及び再冷凍により、電子レンジで再加熱調理をする際にパスタの過加熱を防止することができるとともに、付着させたパスタ粉砕物及び／又はデュラム小麦粉をパスタ表面に閉じ込めて脱落を防ぐことができるので、パスタ本来の風味をより向上させることができる。噴霧する水の量は、茹でパスタ１００質量部に対して１～２０質量部、好ましくは１～１５質量部程度であればよい。

[0019] 本発明の製造方法により得られた冷凍パスタは、解凍し、必要に応じて味付けして、喫食されるものである。解凍の方法は、湯せん、電子レンジ加熱等、特に限定されないが、電子レンジ加熱が簡便であり好ましい。斯くして解凍された冷凍パスタを喫食すれば、茹で立てのパスタのようなパスタ本来の風味を味わうことができる。

## 実施例

[0020] 以下、実施例を挙げて、本発明をさらに詳細に説明するが、本発明はこれらの実施例にのみ限定されるものではない。

[0021] 製造例　コーティング液

デュラム小麦粉（日清製粉（株）製「デュエリオ」）、又は乾燥パスタ（日清フーズ（株）製「ブルー」：直径１．７ｍｍ、長さ２５０ｍｍ）を粒径が１ｍｍ以下になるように粉砕して得られたパスタ粉砕物を、水と混合した。得られた混合物を１００℃になるまで加熱して、デュラム小麦粉又はパスタ粉砕物をα化させた。加熱した混合物に水を添加して混合物中におけるデュラム小麦粉又はパスタ粉砕物の含有量が以下の表１に記載の量になるように調整し、コーティング液を得た。

[0022] [表1]

| 含有量（質量%） | コーティング液 | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| デュラム小麦粉 | 0 | 5 | 10 | 0 | 0 |
| パスタ粉砕物 | 0 | 0 | 0 | 5 | 10 |

[0023] 試験例１

（１）乾燥パスタ（日清フーズ（株）製「ブルー」：直径１．７ｍｍ、長さ２５０ｍｍ）２００ｇを２Ｌの沸騰水に入れ、９分間茹でた後、５℃に冷却して、歩留まり２４０％の茹でパスタを調製した。次いで、該茹でパスタ２００ｇを容器に充填し、その上から上記コーティング液１～５のいずれかを１０ｇ噴霧して、該パスタの表面全体に該コーティング液を満遍なく付着させた後、該パスタを－３０℃の冷凍庫に入れて完全に凍結させ、冷凍パスタを製造した（実験例１～５）。

[0024] （２）比較のため、上記と同じ乾燥パスタ（日清フーズ（株）製「ブルー」）２００ｇを２Ｌの沸騰水に入れて９分間茹で、茹で直後のパスタを製造した（参考例１）。

[0025] （３）実験例１～５の冷凍パスタを１晩冷凍庫で保存した後、電子レンジ（５００Ｗ）で７０℃まで加熱解凍し、調理後の風味を以下の評価基準で評価した。同じ評価基準で参考例１の茹で直後のパスタの風味も評価した。評価結果を表２に示す。なお、評価の数値は、１０名のパネラーによる平均値で示した。

[0026] 評価基準

風味

５：茹でた直後のパスタの風味が感じられる

４：茹でた直後のパスタとほぼ同等の風味が感じられる

３：茹でた直後のパスタの風味がやや感じられる

２：茹でた直後のパスタの風味がほのかに感じられる

１：茹でた直後のパスタの風味があまり感じられない

[0027]

[表2]

| 実験例 | コーティング液 | デュラム小麦粉又はパスタ粉砕物（茹でパスタ100質量部当りの質量部） | | 評価項目 風味 |
|---|---|---|---|---|
| 1 | 1 | − | 0 | 1.3 |
| 2 | 2 | デュラム小麦粉 | 0.25 | 3.3 |
| 3 | 3 | デュラム小麦粉 | 0.50 | 3.8 |
| 4 | 4 | パスタ粉砕物 | 0.25 | 4.3 |
| 5 | 5 | パスタ粉砕物 | 0.50 | 4.6 |
| 参考例1（茹で直後） | − | − | − | 5.0 |

[0028] 試験例２

表３の組成で製造例と同様の手順により調製したコーティング液を用いて、試験例１（１）と同様の手順で冷凍パスタ（実験例６～１１）を製造した。製造した冷凍パスタを試験例１（３）と同様の手順で評価した。評価結果を表４に示す。尚、表４中には、試験例１における実験例１、４及び５、ならびに参考例１の評価結果を併せて記した。

[0029] [表3]

| コーティング液（パスタ粉砕物10質量%含有） | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|
| 噴霧量(g/茹でパスタ100g) | 1 | 2.5 | 7.5 | 10 | 15 | 20 |

[0030] [表4]

| 実験例 | コーティング液 | デュラム小麦粉又はパスタ粉砕物（茹でパスタ100質量部当りの質量部） | | 評価項目 風味 |
|---|---|---|---|---|
| 1 | 1 | − | 0 | 1.3 |
| 6 | 6 | パスタ粉砕物 | 0.10 | 4.0 |
| 7 | 7 | パスタ粉砕物 | 0.25 | 4.2 |
| 4 | 4 | パスタ粉砕物 | 0.25 | 4.3 |
| 5 | 5 | パスタ粉砕物 | 0.50 | 4.6 |
| 8 | 8 | パスタ粉砕物 | 0.75 | 4.6 |
| 9 | 9 | パスタ粉砕物 | 1.00 | 4.7 |
| 10 | 10 | パスタ粉砕物 | 1.50 | 4.8 |
| 11 | 11 | パスタ粉砕物 | 2.00 | 4.8 |
| 参考例1（茹で直後） | − | − | − | 5.0 |

[0031] 試験例３

試験例１（１）と同様の手順でコーティング液４及び５をそれぞれ付着させた冷凍パスタを製造した。その後、該冷凍パスタに水５ｇを噴霧し、－３０℃の冷凍庫にて再冷凍して、再冷凍パスタを製造した（実験例１２～１３）。

これらの再冷凍パスタを、試験例１（３）と同様の手順で評価した。結果を表５に示す。尚、表５中には、試験例１における実験例４、５及び参考例１の評価結果を併せて記した。

[0032] [表5]

| 実験例 | コーティング液 | デュラム小麦粉又はパスタ粉砕物（茹でパスタ100質量部当りの質量部） | | 評価項目 |
|---|---|---|---|---|
| | | | | 風味 |
| 4 | 4 | パスタ粉砕物 | 0.25 | 4.3 |
| 5 | 5 | パスタ粉砕物 | 0.50 | 4.6 |
| 12(再冷凍) | 4 | パスタ粉砕物 | 0.25 | 4.4 |
| 13(再冷凍) | 5 | パスタ粉砕物 | 0.50 | 4.7 |
| 参考例1(茹で直後) | – | – | – | 5.0 |

# 請求の範囲

[請求項1] パスタ粉砕物及び／又はデュラム小麦粉が付着していることを特徴とする冷凍パスタ。

[請求項2] 前記パスタ粉砕物及び／又はデュラム小麦粉の付着量が、冷凍前の前記パスタ１００質量部に対して０．１～２．０質量部である、請求項１に記載の冷凍パスタ。

[請求項3] 前記パスタ粉砕物及び／又はデュラム小麦粉を含むコーティング液が付着しており、該コーティング液は、水とパスタ粉砕物及び／又はデュラム小麦粉とを混合し、加熱して調製される、請求項１又は２に記載の冷凍パスタ。

[請求項4] 前記コーティング液の付着量が、冷凍前の前記パスタ１００質量部に対して１～２０質量部である、請求項３に記載の冷凍パスタ。

[請求項5] 茹で処理したパスタを冷却する工程と、該パスタの表面にパスタ粉砕物及び／又はデュラム小麦粉を付着させる工程と、該パスタを冷凍する工程とを含む、冷凍パスタの製造方法。

[請求項6] 前記パスタ粉砕物及び／又はデュラム小麦粉の付着量が、冷凍前の前記パスタ１００質量部に対して０．１～２．０質量部である、請求項５に記載の方法。

[請求項7] 前記付着させる工程が、前記パスタの表面に前記パスタ粉砕物及び／又はデュラム小麦粉を含むコーティング液を付着させる工程であり、該コーティング液は、水とパスタ粉砕物及び／又はデュラム小麦粉とを混合し、加熱して調製される、請求項５又は６に記載の方法。

[請求項8] 前記コーティング液の付着量が、冷凍前の前記パスタ１００質量部に対して１～２０質量部である、請求項７に記載の方法。

[請求項9] さらに、冷凍されたパスタに水を噴霧し、再冷凍することを特徴とする、請求項５～８のいずれか１項に記載の方法。

# INTERNATIONAL SEARCH REPORT

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Minimum documentation searched (classification system followed by classification symbols)
A23L1/16-1/162

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

| | | | |
|---|---|---|---|
| Jitsuyo Shinan Koho | 1922-1996 | Jitsuyo Shinan Toroku Koho | 1996-2012 |
| Kokai Jitsuyo Shinan Koho | 1971-2012 | Toroku Jitsuyo Shinan Koho | 1994-2012 |

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA/BIOSIS/MEDLINE/WPIDS(STN), JSTPlus/JMEDPlus/JST7580(JDreamII), G-Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* 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
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| Date of the actual completion of the international search<br>02 August, 2012 (02.08.12) | Date of mailing of the international search report<br>14 August, 2012 (14.08.12) |
| Name and mailing address of the ISA/<br>Japanese Patent Office<br><br>Facsimile No. | Authorized officer<br><br>Telephone No. |

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**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/JP2012/062097

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国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２０１２／０６２０９７

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl.　A23L1/16(2006.01)i

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　A23L1/16-1/162

最小限資料以外の資料で調査を行った分野に含まれるもの

| | |
|---|---|
| 日本国実用新案公報 | １９２２－１９９６年 |
| 日本国公開実用新案公報 | １９７１－２０１２年 |
| 日本国実用新案登録公報 | １９９６－２０１２年 |
| 日本国登録実用新案公報 | １９９４－２０１２年 |

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

CA/BIOSIS/MEDLINE/WPIDS(STN), JSTPlus/JMEDPlus/JST7580(JDreamII), G-Search

Ｃ．　関連すると認められる文献

| 引用文献のカテゴリー＊ | 引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示 | 関連する請求項の番号 |
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☑　Ｃ欄の続きにも文献が列挙されている。　　☐　パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
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「Ｏ」口頭による開示、使用、展示等に言及する文献
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| 国際調査を完了した日 | 国際調査報告の発送日 |
|---|---|
| ０２．０８．２０１２ | １４．０８．２０１２ |

| 国際調査機関の名称及びあて先 | 特許庁審査官（権限のある職員） | 4B | 3964 |
|---|---|---|---|
| 日本国特許庁（ＩＳＡ／ＪＰ）<br>郵便番号１００－８９１５<br>東京都千代田区霞が関三丁目４番３号 | 戸来　幸男 | | |
| | 電話番号　０３－３５８１－１１０１　内線　３４４８ | | |

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（２００９年７月）

国際調査報告　　国際出願番号　ＰＣＴ／ＪＰ２０１２／０６２０９７

Ｃ（続き）．　関連すると認められる文献

| 引用文献のカテゴリー＊ | 引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示 | 関連する請求項の番号 |
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# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 36960754 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | M. Angela Parsons/Kirsten Neuman |
| **Filer Authorized By:** | M. Angela Parsons |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 23-AUG-2019 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 12:02:02 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Information Disclosure Statement (IDS) Form (SB08) | Transmittal.pdf | 71379<br>cc6d3a656ceb099ce960eafd6b1ac0182b7b1890 | no | 1 |

**Warnings:**

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| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 2 | Information Disclosure Statement (IDS) Form (SB08) | PTO_1449.pdf | 161567<br>657043ce568653ea1af9b8ffefa66b217ffdfb27 | no | 4 |
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| 3 | Foreign Reference | GB1364747.pdf | 14005514<br>bc27893d382e598f5cc3caebca6f023a4a60ad4a | no | 8 |
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| 5 | Foreign Reference | JP2001346533.pdf | 68789<br>d9ad3ca14b217f1bbf4288d86dd63c554316559c | no | 1 |
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| 6 | Foreign Reference | JP2002101835.pdf | 72001<br>dec47c7b728bf1049b3a1c59100ab79738f41ee2 | no | 1 |
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| **Warnings:** | | | | | |
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| **Information:** | | | | | |
| 9 | Foreign Reference | WO2012157544.pdf | 1004187<br>a4ed9ecb38beb1ed9687da3c5acb5a98fbaf3f29 | no | 14 |
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| 54 | Non Patent Literature | MacLeod_TheScientific_1986.pdf | 19290758<br>90447893fa1b39bc0fcb0bc758e6219dc2648b27 | no | 36 |
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| 55 | Non Patent Literature | Meunier_Metal-oxoSpecies_2002.pdf | 379502<br>bc787c5a9c27e54da25cadf9b8d02ad121453bfd | no | 8 |
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| 56 | Non Patent Literature | Mintel_2012.pdf | 7749601<br>16b8ea1bc395758c3cc41a5253a296b0362df51d | no | 5 |
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| 57 | Non Patent Literature | Mottram_FlavorCompoundsFormed_1993.pdf | 24955254<br>21081927ca77c1bc281e31a35439e2e6bd70aacf | no | 23 |
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| 58 | Non Patent Literature | Mouritsen_SeaweedsForUmami_2012.pdf | 1197753<br>c0a6047f9cf3ccf62c140654ca0680a589b99ea0 | no | 12 |
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| 59 | Non Patent Literature | Pittet_ComparativeStudy_1974.pdf | 6623345<br>2eea2141cb61f409772ab310259c48403c41d066 | no | 6 |
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| 60 | Non Patent Literature | Shaw_Bioavailability_2000X.pdf | 2358892<br>21ea58039228de98480a745f7a1cf0c37e88602a | no | 4 |
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| | | **Total Files Size (in bytes):** | 341161736 | | |

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**New Applications Under 35 U.S.C. 111**
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**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**
**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

# Electronic Acknowledgement Receipt

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| **EFS ID:** | 36960816 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | M. Angela Parsons/Kirsten Neuman |
| **Filer Authorized By:** | M. Angela Parsons |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 23-AUG-2019 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 12:05:00 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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| Submitted with Payment | no |

## File Listing:

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| 1 | Non Patent Literature | Strelec_AminopeptidasesOfGerminated_2009.pdf | 162344<br>dae355aa678f81873cfebe0972ba4fec385e89b0 | no | 8 |

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| 2 | Non Patent Literature | VanDenOuweland_ComponentsContributing_1975.pdf | 4290345<br>352cf9429e896a841ad3b110d16d0226a2a68598 | no | 5 |
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| 3 | Non Patent Literature | Vernin_MechanismsOfFormation_1982.pdf | 14955103<br>554a11fb2c1b1e5f186d82b9cbf49617ac048278 | no | 57 |
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| 4 | Non Patent Literature | Wajcman_Lhemoglobine_2002.pdf | 1796026<br>086a4f9c020abffc361abc85e4bb9bc4a8ea6b69 | no | 16 |
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| 5 | Non Patent Literature | Weenen_ProcessFlavorings_1998.pdf | 20318474<br>d8be91848ed3ca47655dbaa7cc5792dc01c06e94 | no | 28 |
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| 6 | Non Patent Literature | Weinberger_2007.pdf | 5153838<br>fa5c7c0fc15afc90f06df46372a5d212c98e3408 | no | 8 |
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| 7 | Non Patent Literature | Werkhoff_IsolationAndCharacterization_1990.pdf | 13934296<br>11aea54f88d2cb68ead58db4d52a72d7049b4958 | no | 15 |
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| 8 | Non Patent Literature | Whitfield_EffectOfPhospholipid_1988.pdf | 14570915<br>fb2f311f1a91e7d248874cdeb685c0931680d8b4 | no | 12 |
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| 9 | Non Patent Literature | Wu_CharacterizationOfTheAroma_2002.pdf | 760584<br>08c0c952620465fdb035340ce5443aac10235cad | no | 8 |
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| 10 | Non Patent Literature | Yaylayan_MicrowaveAndThermally_1993.pdf | 9187341<br>8e987a83149c493b666d3bc7ea43e74c0bf4a4c8 | no | 8 |
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| 11 | Non Patent Literature | Zampronio_CatalyticOxidation_2005.pdf | 84043<br>55d6e173730c95feba12fd5258858aec467766a6 | no | 4 |
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| 12 | Non Patent Literature | Zhang_ComparisonOfTheVolatile_1991.pdf | 4806022<br>22096755aeea8ce4663a08cadd1057790075ff69 | no | 4 |
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**New Applications Under 35 U.S.C. 111**

**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**

**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Sheet 1 of 1

| Substitute Disclosure Form (37 CFR §1.98(b)) | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. | Application No. |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | 38767-0015008 | 15/913,090 |
| | | Applicant: Impossible Foods Inc. | |
| | | Filing Date: March 6, 2018 | Group Art Unit: 1791 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Class | Subclass | Filing Date If Appropriate |
|---|---|---|---|---|---|---|---|

**Foreign Patent Documents or Published Foreign Patent Applications**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Class | Subclass | Translation Yes | Translation No |
|---|---|---|---|---|---|---|---|---|

**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|
| | 1. | Analysis of soy hydrolysate, Avomen Analytical Services Laboratory Test Results, July 25, 2019 |
| | 2. | Biede, et al., "Swiss cheese flavor: I. chemical analysis," Journal of Dairy Science, 1979, 62:227-237 |
| | 3. | Declaration of Dr. Jorge Ruiz-Carrascal, dated 7/29/2019, 72 pages |
| | 4. | EP Opposition Letter from Opponent in EP Appln. No. 14737766.7 dated 8/6/2019, 55 pages |
| | 5. | Estevez, et al., "Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS," Journal of Agricultural and Food Chemistry, 2003, 51:3429-3435 |
| | 6. | Friedman and Brandon, "Nutritional and health benefits of soy proteins," J. Agric. Food Chem., 2001, 49(3):1069-1086 |
| | 7. | Ibrahim, et al., "Mechanism of the CO-sensing heme protein CooA: new insights from the truncated heme domain and UVRR spectroscopy," Journal of Inorganic Biochemistry," 2007, 101:1776-1785 |
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| | 9. | Mahajan, et al., "Aroma compounds in sweet whey powder," Journal of Dairy Science, 2004, 87:4057-4063 |
| | 10. | Meisinger, et al., Flavor relationships among muscles from the beef chuck and round," Journal of Animal Science, 2006, 84:2826-2833 |
| | 11. | Singh, et al., "Functional and edible uses of soy protein products," Comp. Rev. Food Sci. Food Safety, 2008, 7:14-28 |
| | 12. | Vaghefi, et al., "Influence of the extent of hemoglobin hydrolysis on the digestive absorption of heme iron. An in vitro study," Journal of Agricultural and Food Chemistry, 2002, 50:4969-4973 |
| | 13. | Yeast Metabolom Database, "Heme (YMDB0041)," (http://www.ymdb.ca/compounds/YMDB00041) |
| | 14. | Young and Pellett, "Plant proteins in relation to human protein and amino acid nutrition," Am. J. Clin. Nutr., 1994, 59(Suppl):1203S-1212S |

| 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

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 CFR § 1.98(a)(2).

This statement is being filed before the receipt of a first Office Action on the merits. Therefore, no fees are believed due. If this is incorrect, please apply any charges applicable under 37 CFR § 1.17(p), or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

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

Respectfully submitted,

Date: September 26, 2019

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

62109055.doc

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 37282672 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | M. Angela Parsons/Kristyn Waldhauser |
| **Filer Authorized By:** | M. Angela Parsons |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 26-SEP-2019 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 11:45:04 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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| 2 | Non Patent Literature | AnalysisofSoyHydrolysate.pdf | 1617720<br>ceba5ff84a3daebfa78ad18b726e5d862db3ddc9 | no | 9 |
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| 8 | Non Patent Literature | Ibrahim_2007.pdf | 8893186<br>cb15590ca53b7f3b20ba5c32f503443ee5189a9c | no | 20 |
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| 10 | Non Patent Literature | Mahajan_2004.pdf | 3543408<br>c6d3fe365e56764b144c9e6c6335bea8b566d95a | no | 7 |
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| 12 | Non Patent Literature | Singh_2008.pdf | 6205037<br>730fc27f0a90e45aff7a77f486ecc35c53d80a4f | no | 15 |
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**New Applications Under 35 U.S.C. 111**

**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**

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**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Sheet 1 of 2

| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Class | Subclass | Filing Date If Appropriate |
|---|---|---|---|---|---|---|---|

**Foreign Patent Documents or Published Foreign Patent Applications**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Class | Subclass | Translation Yes | Translation No |
|---|---|---|---|---|---|---|---|---|

**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|
| | 1. | Charkin, et al., "Fragmentation of heme and hemin+ with sequential loss of carboxymethyl groups: A DFT and mass-spectrometry study," Chemical Physics Letters, 2005, 415:362–369 |
| | 2. | FDA Statement and Response, GRAS Notice 540, dated May 29, 2015, 13 pages |
| | 3. | FDA Statement, "Structural comparison of plant hemoglobins and animal myoglobins," annex to communication in opposition to EP Patent No. 2943072, 2019, 5 pages |
| | 4. | Fyrestam and Ostman, "Determination of heme in microorganisms using HPLC-MS/MS and cobalt(III) protoporphyirin IX inhibition of heme acquisition in Escherichia coli," Anal. Bioanal. Chem., 2017, 409:6999-7010 |
| | 5. | GenBank Accession No. AFB70892.1, Sariam, "Non-symbiotic hemoglobin [Vigna radiata]," dated 2/26/2012, 1 page |
| | 6. | Gledhill, "The detection of iron protoporphyrin (heme b) in phytoplankton and marine particulate material by electrospray ionisation mass spectrometry - comparison with diode array detection," Anal. Chim. Acta., 2014, 841:33-43 |
| | 7. | Impossible Foods Response to Opponent's Reply in opposition to EP Patent No. 2943072, dated 11/21/2019, 28 pages |
| | 8. | Jenschke, et al., "Unsaturated fatty acids and sodium affect the liver-like off-flavor in cooked beef," J. Anim. Sci., 2007, 85:3072-3078 |
| | 9. | Jianjun Jiang, et al., "Food technology (Volume 1)," Higher Education Press, 2002, 1:31-32, English translation |
| | 10. | Kohler, et al., "Physiological Characterization of a Heme-Deficient Mutant of Staphylococcus aureus by a Proteomic Approach," Journal of Bacteriology 2003, 185:6928-6937 |
| | 11. | Lane and Nursten, "The Variety of Odors Produced in Maillard Model Systems and How They Are Influenced by Reaction Conditions," in: The Maillard Reaction in Foods and Nutrition, Waller and Feather, eds., ACS Symposium Series, 1983, No. 215, pp. 141-158 |
| | 12. | Opponent's Reply Letter in opposition to EP Patent. No. 2943072, dated 11/21/2019, 15 pages |
| | 13. | Pashynska, et al., "Characterization of Noncovalent Complexes of Antimalarial Agents of the Artemisinin-Type and FE(III)-Heme by Electrospray Mass Spectrometry and Collisional Activation Tandem Mass Spectrometry," J. Am. Soc. Mass Spectrom., 2004, 15:1181-1190 |
| | 14. | Song, et al., "Agricultural and sideline products processing value-added technology II: Processing of soybean products," Henan Science and Technology Press, 2009, 1:64-69, English translation |
| | 15. | Wang and Wang, "Principles and techniques of food nutrition and health care," China Light Industry Press, 1998, 1:446-449, English translation |

| 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<br>U.S. Department of Commerce<br>Patent and Trademark Office<br><br>**Information Disclosure Statement by Applicant**<br>(Use several sheets if necessary)<br><br>(37 CFR §1.98(b)) | Attorney Docket No.<br>38767-0015008 | Application No.<br>15/913,090 |
|---|---|---|
| | Applicant<br>Impossible Foods Inc. | |
| | Filing Date<br>March 6, 2018 | Group Art Unit<br>1791 |

| Examiner Signature | Date Considered |
|---|---|
| | |

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Substitute Disclosure Form

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

This statement is being filed within three months of the filing date of the application or before the receipt of a first action on the merits; therefore, no fee is believed due.

Please apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: November 26, 2019

/Kimberly M. Maize, Reg. No. 76,370/
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62179616.doc

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 37863487 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Emma Johnson |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 26-NOV-2019 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 13:22:37 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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| **Information:** | | | | | |
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**New Applications Under 35 U.S.C. 111**
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**National Stage of an International Application under 35 U.S.C. 371**
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**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Sheet 1 of 1

| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Filing Date If Appropriate | Class | Subclass |
|---|---|---|---|---|---|---|---|

**Foreign Patent Documents or Published Foreign Patent Applications**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Translation | Class | Subclass |
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| Examiner Signature | Date Considered |
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| | |

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

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

The applicant wishes to make the Examiner aware of the following related applications. The prosecution histories for these applications and patents are available on PAIR, and are not provided with this statement.

| Application No. | Filing Date | First Named Inventor | Attorney Docket No. |
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| 15/300,339 | 9/29/2016 | Ranjani Varadan | 38767-0026US1 |

First Named Inventor : Rachel Fraser
Application No. : 15/913,090
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Page : 2 of 2

Attorney Docket: 38767-0015008

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| 16/238,698 | 1/3/2019 | Ranjani Varadan | 38767-0026002 |
| 16/238,749 | 1/3/2019 | Ranjani Varadan | 38767-0026003 |

This statement is being filed before the receipt of a first action on the merits. Therefore, no fee is believed due.

Please apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: March 19, 2020

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JPH08289761A AGENT FOR IMPARTING THICK TASTE

Applicants: AJINOMOTO KK

Inventors: OMURA HARUKI,SHIBUYA KOUJI

Classifications:

IPC ***A23L17/00; A23L27/20; A23L27/22; A23L27/23;*** (IPC1-7): A23L1/226; A23L1/228; A23L1/229; A23L1/325;

Priorities: JP9706595A 1995-04-21

Application: JP9706595A·1995-04-21

Publication: JPH08289761A· 1996-11-05

Published as: JPH08289761A;**JP3467901B2**

AGENT FOR IMPARTING THICK TASTE

Abstract

PURPOSE: To obtain an inexpensive agent for imparting thick taste, containing a sulfone-containing compound as the exclusive component or the compound in combination with betaine and/or a phosphoric acid salt as active components and imparting thick delicious taste to a food or drink by using in combination with a tasty substance. CONSTITUTION: This thick taste imparting agent contains a sulfone-containing compound (preferably taurine) as the exclusive component or in combination with betaine (e.g. glycine betaine) and/or a phosphoric acid salt (secondary sodium orthophosphate) as active components. The amounts of the active components to be compounded to a tasty substance are e.g. 0.01-20 pts.wt. each of taurine, disodium phosphate and glycine betaine based on 1 pt.wt. of sodium glutamate used as the tasty substance.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報**（Ａ）　(11)特許出願公開番号

**特開平8－289761**

(43)公開日　平成８年(1996)11月５日

| (51)Int.Cl.[6] | | 識別記号 | 庁内整理番号 | ＦＩ | | | 技術表示箇所 |
|---|---|---|---|---|---|---|---|
| Ａ２３Ｌ | 1/226 | | | Ａ２３Ｌ | 1/226 | Ｚ | |
| | 1/228 | | | | 1/228 | | |
| | 1/229 | | | | 1/229 | Ａ | |
| | 1/325 | １０１ | | | 1/325 | １０１Ｄ | |

審査請求　未請求　請求項の数９　ＯＬ　（全　８　頁）

(21)出願番号　特願平7－97065

(22)出願日　平成７年(1995)４月21日

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(74)代理人　弁理士　川口　義雄　（外２名）

(54)【発明の名称】　こく味付与剤

(57)【要約】
【構成】　旨味物質に加えてスルホン基含有化合物およびリン酸塩を利用して、またはこれらに加えてベタインを利用して飲食品の調味を行なう。
【効果】　飲食品に容易にこく味を付与することができる。

【特許請求の範囲】
【請求項１】　スルホン基含有化合物を単独にもしくはスルホン基含有化合物およびベタインを併用して、またはスルホン基含有化合物およびリン酸塩を併用してもしくはスルホン基含有化合物、リン酸塩およびベタインを併用して有効成分とすることを特徴とするこく味付与剤。
【請求項２】　旨味物質とスルホン基含有化合物、リン酸塩および／またはベタインとを有効成分とすることを特徴とするこく味調味料。
【請求項３】　スルホン基含有化合物がタウリンであることを特徴とする請求項１または２記載のこく味付与剤またはこく味調味料。
【請求項４】　請求項１～３のいずれかに記載のこく味付与剤またはこく味調味料でこく味を付与することを特徴とする飲食品の調味方法。
【請求項５】　旨味物質、スルホン基含有化合物およびリン酸塩を有効成分としてこく味を付与されたことを特徴とする飲食品。
【請求項６】　旨味物質、スルホン基含有化合物、リン酸塩およびベタインを有効成分としてこく味を付与されたことを特徴とする飲食品。
【請求項７】　タウリンとリン酸塩、魚骨粉および／またはベタインとを有効成分として含有することを特徴とする練製品用こく味付与剤。
【請求項８】　旨味物質に加えて請求項７記載の練製品用こく味付与剤を使用して調味をすることを特徴とする練製品の調味方法。
【請求項９】　旨味物質に加えてスルホン基含有化合物とリン酸塩および／または魚骨粉とベタインとを有効成分としてこく味を付与されたことを特徴とする練製品。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は、旨味物質、スルホン基（－$SO_3H$）含有化合物およびリン酸塩の３者の併用作用により、またはこれら３者にベタインを加えた４者の併用作用により発現するこくのある旨味を活用するこく味付与剤およびこく味調味料、ならびにこのようなこく味付与剤またはこく味調味料を使用する飲食品の調味方法、およびこのようにして調味された飲食品に関する。
【０００２】
【従来の技術】Ｌ－グルタミン酸モノソーダ（コンブの味）を代表とする特有の味は、旨味（Flavor-enhancer）と称される。甘酸苦塩味を四原味と称するが、旨味は電気生理学的にもこれとは異なる味であることが証明されている。旨味を呈する物質としては、このほかに、アスパラギン酸、オキシグルタミン酸、イボテン酸、トリコロミン酸、イノシン酸ソーダ（かつお節の味）、グアニル酸ソーダ（椎茸の味）等の核酸関連物質、コハク酸ソーダ（二枚貝の味）も、それぞれ個性をもった旨味をもっている。また、グルタミン酸と核酸系旨味物質との間にはきわめて強い相乗効果を呈することが知られている。このことを利用した調味料が実際に市販されている。欧米では旨味に相当する言葉がなく、Ｕｍａｍｉで通用するようになってきている。以上、日本食品工業学会編「食品工業総合辞典」（（株）光琳発行（昭和５４年））。
【０００３】そして、グルタミン酸ナトリウム、イノシン酸ナトリウムおよびグアニル酸ナトリウムで代表される旨味は、国際的にもＵｍａｍｉと命名されるに至り、今日まで数多くの研究がなされてきており、また、これらの旨味物質を配合した多種多様の調味料が市販されている。
【０００４】しかしながら、このような調味料は、なるほど旨味の付与性は充分であるが、ビーフエキス、ポークエキス、チキンエキス、魚介エキス、酵母エキスなどの天然エキスあるいは蛋白加水分解物（ＨＡＰ、ＨＶＰなど）等の調味料が有するこく味の付与性はなお充分でなく、この点が問題点とされ、これを改善する手段の開発提供が強く望まれている。
【０００５】因みに、スルホン基含有化合物の１種であるタウリンは、ウシの胆汁およびイカ、タコ、貝類などの軟体動物の肉エキス中に多量に存在し、その薬理作用については、胆汁の分泌を促し、脂肪の吸収をよくし、また制汗作用のあることが知られている（共立出版（株）「化学大辞典」（昭和３６年）参照）。しかしながら、従来、タウリンの飲食品分野における実用用途にはみるべきものがなく、僅かに上記薬理作用を利用した健康飲料の用途（特開平４－２６２７６７）等が知られているに過ぎない。特にその呈味性については、水産物エキスの呈味試験の結果は、タウリンとアルギニンは量的に多いが、味とは無関係であると言われ（「調理科学」 Vol.5, No.1, p.p. 2～7 (1982)）、従ってこれを調味料として実際に使用した例のないことはもちん、その調味料としての用途が示唆されることもなかった。
【０００６】
【発明が解決しようとする課題】前項記載の従来技術の背景下に、本発明は、こく味を付与または強化する調味料の開発を目的とする。
【０００７】
【課題を解決するための手段】本発明者は、前項記載の目的を達成すべく鋭意研究の結果、従来味とは無関係であると考えられていたタウリンが極めて驚くべきことにリン酸塩との併用作用により旨味を前提とした、すなわち旨味のこく味付与剤となり得ること、また、タウリンと同様にスルホン基を有する他の化合物にもタウリンと同様の作用があること、さらにはスルホン基含有化合物およびリン酸塩による旨味に対するこく味付与効果がベタインにより顕著に改善されることを見出し、このよう

な知見に基いて本発明を完成した。

【０００８】以下、本発明を逐次詳細に説明する。

【０００９】本発明の具体的態様の１つは、スルホン基含有化合物を単独にもしくはスルホン基含有化合物およびベタインを併用して、またはスルホン基含有化合物およびリン酸塩を併用してもしくはスルホン基含有化合物、リン酸塩およびベタインを併用して有効成分とすることを特徴とするこく味付与剤である。

【００１０】このようなこく味付与剤の成分であるスルホン基含有化合物は、可食性でなければならないことはもちろんであり、タウリンの他に、脱炭酸を受けてタウリンを生成するシステイン酸、またナフタリン－１，５－ジスルホン酸およびチアミンのナフタリン－１，５－ジスルホン酸塩などのその塩類を例示することができる。また、これらの化合物は必ずしも純品である必要はなく、例えば、スルホン基含有化合物を含む天然物からの抽出物の形態であってもよい。

【００１１】他の成分であるリン酸塩も、同じく可食性でなければならないことはもちろんであり、いわゆる正リン酸の第一、第二または第三ナトリウム塩、カリウム塩、カルシウム塩などを例示することができる。また、カルシウム塩は、例えば、魚骨粉の形態であってもよい。本発明者の確認したところによれば、ポリリン酸塩は、スルホン基含有化合物と併用してもこく味を呈さない。

【００１２】ベタインとしてはグリシンベタインなどのベタインを挙げることはできる。ベタインは必ずしも純品である必要はなく、ビートモラセス、ケインモラセスなどの廃糖蜜からのベタインの高濃度画分やその濃縮物の形態であってもよい。

【００１３】本発明者の確認したところによれば、リン酸塩は、スルホン基化合物と併用しただけではこく味を呈さない。旨味物質にリン酸塩およびスルホン基含有化合物を同時に使用して初めてこくのある旨味となるのである。そして、ベタインにより、このこくのある旨味が強化される。

【００１４】このようなこく味付与剤の製造方法自体には、有効成分としてスルホン基含有化合物を単独に使用するかまたはスルホン基含有化合物およびリン酸塩を併用して配合するか、あるいはこれらにそれぞれベタインを併用して配合することを除いては、特別の制限はなく、適宜公知の調味料の製造方法を採用することができる。従って、本発明のこく味付与剤は、例えば、適当な賦形剤などの添加剤を使用して、液体および粉状、顆粒状などの固体の商品形態（流通形態）に製造することができる。

【００１５】先に説明したように、本発明は、スルホン基含有化合物およびリン酸塩の２者が、またはこれらの２者に加えてベタインの３者が同時に作用して旨味をこくのある旨味に変えること、を見出したことに基ずくものである。すなわち、３要素または４要素の併用作用による。従って、このような本発明のこく味付与剤の使用方法は、これらを考えると当業者に自から明らかであるが、若干例示する。

【００１６】例えば、スルホン基含有化合物、リン酸塩および旨味物質の３要素によりこくのある旨味を得ようとするとき、スルホン基含有化合物を単独に配合したこく味付与剤の場合は、旨味物質を既に原材料として使用されている飲食品（旨味調味料そのものを含む）に別途調達したリン酸塩と併用してこく味を呈せしめることができ、また旨味物質およびリン酸塩が既に原材料として使用されている飲食品の場合は、このようなこく味付与剤を使用するだけで飲食品にこく味を付与することができる。そして、スルホン基含有化合物とリン酸塩とを併用したこく味付与剤の場合は、旨味物質を含有するがスルホン基含有物質もリン酸塩も含有しない飲食品にこく味を呈せしめるのに有効に使用することができる。本発明者の新たなる知見に係わる、こくのある旨味を発現すべき３要素中、リン酸塩は既に含有するが他の２要素は含有しない原材料の場合は、スルホン基含有化合物を単独に有効成分とするこく味付与剤と別途調達した旨味物質とを併用することで、または旨味物質とスルホン基含有化合物とを有効成分とする本発明のこく味調味料（後述）を使用することで、こくのある旨味を発現せしめることができる。

【００１７】また、前記４要素、すなわちスルホン基含有化合物、リン酸塩、ベタインおよび旨味物質の４者により顕著にこく味の強化された旨味を得ようとするときも、上に説明したところに準じて使用することで、すなわち、飲食品中においてこれら４者が共存するように使用することで目的を達成することができる。

【００１８】スルホン基含有化合物およびリン酸塩を併用したこく味付与剤またはこれらにベタインの追加されたこく味付与剤における有効成分の配合割合は、こく味を付与すべき対象の飲食品が定まれば、当業者であれば簡単な事前の呈味試験により極めて容易に定めることができる。

【００１９】このようなこく味付与剤を使用することにより、飲食品にこく味が付与され、または強化される。

【００２０】本発明の具体的態様のその２は、旨味物質とスルホン基含有化合物、リン酸塩および／またはベタインとを有効成分とすることを特徴とするこく味調味料である。

【００２１】先に説明したように、スルホン基含有化合物およびリン酸塩は、それぞれ単独に旨味物質とともに使用してもこれにこく味を付与せず、これらを併用して旨味物質とともに使用することにより、すなわち３要素の併用作用により初めてこく味をし、しかもこのこく味はベタインを使用することで顕著に強化されるのである。従って、本発明の、このような具体的態様による、

スルホン基含有化合物、リン酸塩および／またはベタインを、旨味物質とともに有効成分とするこく味調味料は、例えば、既に旨味物質およびリン酸塩は含有するがスルホン基含有化合物は含まない、同様に既に旨味物質およびスルホン基含有化合物は含有するがリン酸塩は含有しない、または旨味物質は含有するがリン酸塩もスルホン基含有化合物も含有しない原材料の飲食品の調味に使用すると、このような飲食品にこく味が付与されまたは強化されるので、こく味調味料と称するのがふさわしい。ベタインの関与する場合も同様である。

【００２２】このようなこく味調味料の製造法および商品形態にも特別の制限はなく、先に説明したこく味付与剤の製造法および商品形態に準ずることができる。有効成分の配合割合も、要するに、こく味調味料としての目的を達成し得るものであればよく、例えば、グルタミン酸ナトリウム１重量部に対し、タウリン０．０１～２０重量部およびリン酸二ナトリウム０．０１～２０重量部の割合とすることができる。ベタインを併用する場合は、グルタミン酸ナトリウム１重量部に対し、グリシンベタイン０．０１～２０重量部の割合とすることができる。

【００２３】また、使用法にも特別の困難はなく、先に説明した３要素または４要素の併用によるこくのある旨味の発現ということを考えて、例えば、既にリン酸塩を含有する原材料からの、またはリン酸塩を含有しない原材料からの場合は、それぞれ、適宜本発明のリン酸塩を配合していないこく味調味料、またはこれと別途調達したリン酸塩とを併用して飲食品を製造する、適宜の工程で使用することにより、こく味が付与されまたは強化された飲食品を容易に製造することができる。

【００２４】本発明の具体的態様のその３は、上に説明したこく味付与剤またはこく味調味料を使用してこくのある旨味を付与することを特徴とする飲食品の調味方法である。

【００２５】このような調味方法は、こく味を付与しまたは強化するために、調味料として上に説明した本発明のこく味付与剤またはこく味調味料を使用することを除いては、特別の制限はなく、従来公知の調味方法を適宜採用することで行なうことができる。飲食品にこく味を付与しまたはこれを強化するには、例えば、飲食品製造の原材料の一部として本発明のこく味付与剤またはこく味調味料を使用することもできるし、既に製品となっている飲食品にこく味を付与しまたはこれを強化するために本発明のこく味付与剤またはこく味調味料を使用することもできることはもちろんである。

【００２６】本発明の具体的態様のその４は、旨味物質、スルホン基含有化合物およびリン酸塩の３者をまたはこれにベタインを加えた４者を有効成分としてこく味を付与されたことを特徴とする飲食品である。尚、これらの飲食品には、調味料、畜肉エキス、家禽エキス、その他の各種エキス類、各種加工食品類が含まれる。

【００２７】このような飲食品には、例えば、旨味物質に加えてスルホン基含有化合物およびリン酸塩の両者を使用して、またはこれらに加えてベタインを使用してこく味を付与した飲食品が含まれることは言うまでもないが、更に、例えば、原材料などに既に旨味物質に加えてスルホン基含有化合物およびリン酸塩のいずれか一方が含有されている場合に、残りの一方を添加使用してこく味を付与した飲食品も含まれることはもちろんである。要するに、３成分（３要素）または４成分（４要素）の少なくも１つを外来物質として使用してこくのある旨味を発現せしめた飲食品は、全て本発明の、このような飲食品の範囲に包含される。

【００２８】なお、上に説明した本発明のこく味付与剤もしくはこく味調味料または調味方法に関しては、本発明の目的とするこく味の発現の妨げとならない限りは、旨味物質、例えばグルタミン酸ナトリウムをはじめとするアミノ酸系および／またはイノシン酸ナトリウムやグアニル酸ナトリウムをはじめとする核酸系旨味物質の他に、有機酸、無機酸、これらの塩類、糖類等の、従来使用されている添加物を適宜併用できることはもちろんである。

【００２９】本発明の具体的態様の最後は、タウリンとリン酸塩および／または魚骨粉を、およびこれらに加えてベタインを有効成分として含有することを特徴とする練製品用こく味付与剤である。ここに、練製品には、かまぼこ、竹輪などの水産練製品、ハム、ソーセージなどの魚畜肉練製品等が含まれる。

【００３０】魚骨粉にはリン酸がそのカルシウム塩の形態で存在している。本発明の練製品用こく味付与剤においては、リン酸塩に加えてまたはこれに代えて魚骨粉を併用配合することができる。この場合、練製品のナトリウム量を減少でき、かつカルシウムを強化できる。

【００３１】このような練製品用こく味付与剤における有効成分の含有量比は、これを使用してこく味を付与すべき対象の練製品が定まれば、先に説明した場合と同じく、当業者であれば容易に定めることができる。また、先に説明した本発明者の知見に係わる３要素または４要素の併用作用によるこくのあるまたはこくの強化された旨味の発現ということを考えると、このようなこく味付与剤の商品形態、使用方法なども、先に説明した場合と同様にできる。

【００３２】また、旨味物質に加えて、スルホン基含有化合物とリン酸塩および／または魚骨粉とベタインとを有効成分としてこく味を付与されたことを特徴とする練製品の範囲も、先に説明した本発明の具体的態様のその４の場合に準じて考えることができる。

【００３３】

【実施例】以下、本発明を実施例により更に説明する。なお、実施例において、％は重量％を意味する。

【００３４】実施例１（３要素の併用作用の検査）
スルホン基含有化合物、リン酸塩および旨味物質として、それぞれ、タウリン、燐酸二ナトリウムおよびグルタミン酸ナトリウムを選び、これら３要素の併用によるこくのある旨味の発現を検査した。

【００３５】（ａ）すなわち、下記第１表に示す評価系（各種の水溶液）を試飲温度５０～６０℃での官能検査に付した。なお、官能検査は、熟練した５名よりなるパネルにより行ない、その結果も同表に併記する。評価系（２）以降において、本発明の３要素によるこく味の発現が見られる。

【００３６】

【表１】

第　１　表

| | 評　価　系 | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>タウリン　0.2 %<br>燐酸二ナトリウム　0.2 %<br>食　塩　0.5 %<br>蒸留水　残 余 | 塩味＋燐酸塩の味、少し旨味様の味有り |
| (2) | (1)＋0.5%（上乗せ）グルタミン酸ナトリウム | 塩味＋旨味、こく味発現 |
| (3) | (2) ＋0.4%（同上）50%乳酸ナトリウム | (2) の呈味からさらにエキス様の呈味に変化 |
| (4) | (3)＋0.2%（同上）結晶ブドウ糖 | (3) の呈味に少し甘味が加わる |
| (5) | (4) ＋0.1%（同上）グリシン | (4) の呈味にさらにエキス様の呈味が増す |
| (6) | (5) ＋0.1%（同上）アラニン | (5) の呈味にさらにエキス感が強まる |

【００３７】さらに比較のために、（ｂ）タウリン及び燐酸塩をともに抜いた場合、（ｃ）燐酸塩を抜いた場合、（ｄ）タウリンを抜いた場合、そして（ｅ）グルタミン酸ナトリウムを抜いた場合のそれぞれについて同様の検査を行なった。

【００３８】評価系と官能検査の結果（呈味の特徴）をそれぞれ下記第２～５表に示す。これらの結果から、３要素の１つでも欠けるとこく味の発現が見られないことが判る。

【００３９】

【表２】

第　２　表

| | 評　価　系 | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>食　塩　0．5%<br>蒸留水　残 余 | 塩味のみ |
| (2) | (1)＋0.5%（上乗せ）グルタミン酸ナトリウム | 塩味 ＋ 旨味（こくのある旨味発現せず） |

【００４０】　　【表３】

第　3　表

| | 評　価　系 | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>タウリン　0．2%<br>食　塩　0．5%<br>蒸留水　残余 | ソフトな塩味 |
| (2) | (1)+0．5%（上乗せ）グルタミン酸ナトリウム | ソフトな旨味と塩味（こくのある旨味発現せず） |

【0041】

【表4】

第　4　表

| | 評　価　系 | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>燐酸二ナトリウム　0．2%<br>食　塩　0．5%<br>蒸留水　残余 | 塩味中心の弱いこく味 |
| (2) | (1)+0．5%（上乗せ）グルタミン酸ナトリウム | グルタミン酸ナトリウムのソフトな旨味と塩味（こくのある旨味発現せず） |

【0042】

【表5】

第　5　表

| | 評　価　系 | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>タウリン　0．2%<br>燐酸二ナトリウム　0．2%<br>食　塩　0．5%<br>蒸留水　残余 | 柔らかい塩味 |
| (2) | (1)+0．4%（上乗せ）50%乳酸ナトリウム | (1) の呈味とほぼ同じ呈味 |
| (3) | (2)+0．2%（上乗せ）結晶ブドウ糖 | (2) の呈味よりいくらか濃厚感あり |
| (4) | (3)+0．1%（上乗せ）グリシン | 甘味系のこく味少しでてくるも、旨味系のこく味発現せず |
| (5) | (4) +0．1%（上乗せ）アラニン | 甘味系のこく味が(4) の呈味より強まるが、旨味系のこく味なし |

【0043】実施例2（ヴィヨン系における評価）
ビーフヴィヨン（これにはもちろん旨味物質は含まれている。）に対するこく味の付与の検査を実施例1におけると同じ試飲温度で検査した。

【0044】評価系と評価結果を下記第6および7表に示す。これらの表から、本発明によれば、ビーフヴィヨンは、配合系および天然系をとわず、こく味の強化されることが判る。そして、配合系の場合、ベタインの併用により天然感の強化されることが判る。

【0045】

【表6】

第　6　表

| 評　価　系 | | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>濃度１％の配合系ヴイヨン | 水っぽいビーフヴイヨンの味 |
| (2) | (1)＋0.2%（上乗せ）タウリン<br>＋0.2%（上乗せ）燐酸二ナトリウム | 濃厚感、こく味のかなり強まったビーフヴイヨンの味に変化 |
| (3) | (2)＋1%　（上乗せ）ベタイン | 甘味を伴い、厚みが強まり、まろやかで天然感のあるエキス感が強まる |

【００４６】

【表７】

第　7　表

| 評　価　系 | | 呈味の特徴 |
|---|---|---|
| (1) | ベース：<br>濃度１％の天然系ヴイヨン | 水っぽいビーフヴイヨンの味 |
| (2) | (1)＋0.2%（上乗せ）タウリン<br>＋0.2%（上乗せ）燐酸二ナトリウム | 濃厚感、こく味、厚味の著しく強化されたビーフヴイヨンの味に変化 |

【００４７】実施例３（蒲鉾系における評価）
（ａ）常法により、下記第８表に示す原材料の配合により蒲鉾（対照）を試作した（試作品Ａ）。

【００４８】

【表８】

第　8　表

| 原　材　料 | 配合量（g） |
|---|---|
| 助宗すり身ＳＡ級 | １０００ |
| グルタミン酸ナトリウム | １０ |
| 食　塩 | ３０ |
| 砂　糖 | ３０ |
| でんぷん | ５０ |
| みりん | ２０ |
| 水 | ５００ |

【００４９】試作品Ａの呈味の特徴は、試食温度２０℃においてグルタミン酸ナトリウムの旨味のみ感じる、というものであった。

【００５０】（ｂ）原材料の配合を第８表に示す原材料にタウリンおよび燐酸二ナトリウムを各３ｇ加えたものとした以外は（ａ）におけると全く同様にして蒲鉾を試作した（試作品Ｂ）。

【００５１】試作品Ｂの呈味の特徴は、旨味の強さは、対照（試作品Ａ）より弱まり自然なこく味系の味に変化するが、味全体は弱めに感じる、というものであった。

【００５２】（ｃ）原材料の配合を（ｂ）における原材料にＨＡＰ由来オリゴペプチド（平均分子量４００）３ｇを加えたものとした以外は、（ｂ）におけると全く同様にして蒲鉾を試作した（試作品Ｃ）。

【００５３】試作品Ｃの呈味の特徴は、噛んでいるうちに味がでて来る、グルタミン酸ナトリウムのシャープな旨味はない、直接食べるには丁度良い手ごろなこく味系の味である、というものであった。

【００５４】（ｄ）原材料の配合を（ｂ）における原材料をタウリンおよび燐酸二ナトリウムについて、これらを各２倍とした、すなわち、両者を各６ｇとしたものとした以外は、（ｂ）におけると全く同様にして蒲鉾を試作した（試作品Ｄ）。

【００５５】試作品Ｄの呈味の特徴は、試作品Ｂに較べて先味は強くないが、こくのある旨味を呈し、すっきりした味で美味しい蒲鉾である、というものであった。

【００５６】（ｅ）原材料の配合を（ｃ）における原材料をタウリン、燐酸二ナトリウムおよびオリゴペプチドについて、これらを各２倍とした、すなわち、これら３者を各６ｇとしたものとした以外は、（ｃ）おけると全く同様にして蒲鉾を試作した（試作品Ｅ）。

【００５７】試作品Ｅの呈味の特徴は、オリゴペプチドの使用量を増やしたことによるものと思われるが、試作品Ｃに較べて味が強すぎて、くどくなり過ぎであるが、こくのある旨味の付与されていることは変わりはない、というものであった。

【００５８】

【発明の効果】本発明により、こく味付与剤が安価に容易に提供することが可能となり、また飲食品にこくのある旨味を付与することが容易に行なうことができるようになった。

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 38916353 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Emma Johnson |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
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| **Application Type:** | Utility under 35 USC 111(a) |

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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Information Disclosure Statement (IDS) Form (SB08) | IDS.pdf | 711172<br>e06d60c3fcdf60268412d3d23b2c04ecb12e6dbf | no | 3 |

**Warnings:**

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| **Information:** | | | | | |
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| 2 | Foreign Reference | JPH08289761.pdf | 3683614<br>b65ff947571c4a686c6ed66332fdf3921e71f28b | no | 9 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Non Patent Literature | Berrios_2010.pdf | 1253457<br>219eb62325b9f28ea79e72b1ea1e75779353ef27 | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Non Patent Literature | Declaration_Ruiz-Carrascal_2020.pdf | 1081930<br>a3a45a8b94df24079a5f9e3b8c7f3a8fb1c4b1ed | no | 12 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Non Patent Literature | EPOpposition_Jul2019.pdf | 886594<br>0ca03421ec8337bc89658bf6f33716686dc5217b | no | 8 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Non Patent Literature | FDC_Peas_2019.pdf | 447751<br>ff6912035605e561f6d36d2ed609d1a45339a261 | no | 8 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 7 | Non Patent Literature | Jahreis_2016.pdf | 1679291<br>371b6c437500458e17857bfb6bc5b191b43b3bf0 | no | 7 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 8 | Non Patent Literature | Liu_2011.pdf | 3092927<br>69d32c9c9cb4b018f70a5b94d7757dc742188f71 | no | 10 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |

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| 9 | Non Patent Literature | Macnicol_1983.pdf | 1272411<br>fd732b082bac569a66b89af903fe19fee7c624ac | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 10 | Non Patent Literature | Oldach_2019.pdf | 2344528<br>9348d3d5f3485a9ffc543567d979bb65a99f302a | no | 12 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 11 | Non Patent Literature | OpponentsReply_Jan2020.pdf | 1963806<br>92a5250c51ae5275524b0b03874cde7e7951cfcb | no | 18 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 12 | Non Patent Literature | ProprietorsResponse_Nov2019.pdf | 3230574<br>643132547efc5279620009dcf6ca51a8ae9c0554 | no | 28 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 13 | Non Patent Literature | Wood_2008.pdf | 3224649<br>6a8b90238575b68b30248627657a7348a5c3c84e | no | 16 |
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**New Applications Under 35 U.S.C. 111**

**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**

**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**



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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 15/913,090 | 03/06/2018 | Rachel Fraser | 38767-0015008 | 9878 |

26191 7590 05/12/2020
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

| EXAMINER | |
|---|---|
| GWARTNEY, ELIZABETH A | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1796 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 05/12/2020 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com

| *Office Action Summary* | **Application No.** 15/913,090 | **Applicant(s)** Fraser et al. | |
|---|---|---|---|
| | **Examiner** ELIZABETH A GWARTNEY | **Art Unit** 1796 | **AIA (FITF) Status** No |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims***

5) ☑ Claim(s) 31-53 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 31-53 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 06 March 2018 is/are: a)☐ accepted or b)☑ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

## DETAILED ACTION

### *Notice of Pre-AIA or AIA Status*

1. The present application is being examined under the pre-AIA first to invent provisions.

### *Drawings*

2. The drawings are objected to because the Y-axis of Figure 2 is not labeled. Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

### *Claim Rejections - 35 USC § 112*

3. The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. The following is a quotation of 35 U.S.C. 112(b):

> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. **Claims 31-53 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.**

New **claims 31-53** are directed to a "meat-like food product." The present specification uses the term "meat-like" to describe the flavors produced upon cooking the inventive food product ([0010]). While the present specification is broadly directed to creating meat-like flavor in animal or non-animal based food product, including food products in the form of hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets or cubes ([0010]-[0011]), there is no description of a "meat-like food product.

6. **Claims 31-50 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.**

Regarding **claims 31-50**, the term "meat-like food product" renders the claim indefinite. It is not clear what properties define a "meat-like food product." Is a meat-like food product any consumable food product that exhibits a meat flavor or product exhibiting the texture, appearance, nutritional profile and flavor of a pork tenderloin?

***Claim Rejections - 35 USC § 103***

7. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

9. This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the

various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

10. **Claims 31-53 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Hsieh et al. (US 2012/0093994) in view of Proulx et al. ("Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model" - J. Agric. Food Chem." 2006, p. 1518-1522) as evidenced by Dinh et al. ("Effects of USDA quality grade and cooking on water-soluble precursors of beef flavor", Meat Science, Volume 146, December 2018, pp. 122-130).**

Regarding **claims 31, 34-36, 38-41, 46 and 49-53,** Hsieh et al. disclose a structured plant protein product having protein fibers that are substantially aligned and has meat-like moisture, texture, mouthfeel, flavor and color (i.e. meat-like product) comprising: (a) dry ingredients that comprise: (i) a protein component that comprises a plant-derived protein material, wherein the protein component is at an amount that is no more than about 90% by weight of the dry ingredients; (ii) a carbohydrate component at an amount that is in the range of about 2 to about 50% by weight of the dry ingredients; and (iii) a lipid component at an amount that is in the range of about 0.1 to about 5% by weight of the dry ingredients; and (b) wet ingredients that comprise water (Abstract, [0013]). Hsieh et al. disclose wherein the protein component may also include animal meat, including beef ([0079]).

As evidenced by Dinh et al., beef is known to comprise; (a) the free amino acids, cysteine, cysteine, and methionine (p. 125/Table 1); and (b) the reducing sugars, glucose, glucose-6-phosphate, fructose and ribose (p. 126/Table 2).

Given Hsieh et al. disclose a structured plant protein product comprising animal meat, e.g. beef, it necessarily follows, cooking the structured plant protein product, wherein cooking includes heating to a temperature of 150ºC for about 3 to about 5 minutes, results in the production of the claimed meat associated aroma compounds.

While Hsieh et al. does not disclose the precise amount of free amino acid or reducing sugar in the added animal meat (e.g. beef) and thus the final structured plant protein product, given beef is known to comprise free amino acids including cysteine, cysteine and methionine and reducing sugars including glucose, glucose-6-phosphate, fructose and ribose, since beef would necessarily exhibit beef flavor when cooked, one of ordinary skill in the art would have adjusted to the amount of added animal meat, and therefore the amount of free amino acid and reducing sugar, to obtain the desired beef or meat-like flavor.

Hsieh et al. is silent with respect to a plant, yeast, fungal, or bacterial heme-containing protein.

Proulx et al. teach that iron deficiency is a major nutritional problem and fortification of foods with iron has been a successful strategy for improving iron content of foods (p. 1518/Introduction). Proulx et al. teach that iron bioavailability is higher from heme iron sources because of lack of inhibition from chelating compounds (p. 1518/Introduction). Proulx et al. teach that heme iron in the human diet is almost exclusively from animal sources and its intake has been shown to have a positive correlation with iron status (p. 1520/Results and Discussion). Proulx et al. teach that the incorporation of animal sourced heme iron is often unfeasible because

of economic costs and because cultural and religious barrier forbid the consumption of meat (p. 1520-1521/Results and Discussion).

Proulx et al. teach that heme iron in the human diet is generally present in animal sources as part of hemoglobin and myoglobin but is also found in many invertebrates, bacteria, fungi and in the plant kingdom (p. 1518/Introduction). Proulx et al. teach ***leghemoglobin*** is a heme protein identified in soybean root nodules (p.1518/Introduction). Proulx et al. disclose a purified leghemoglobin protein (i.e. 73% by weight in the extract – p. 1520/Results and Discussion). Proulx et al. teach that the bioavailability of hemoglobin from soy root nodules is similar to that of heme iron from animal sources (p. 1521/Results and Discussion). Proulx et al. teach using plant hemoglobin, i.e. leghemoglobin, as a heme iron source in diets that are consumed by humans (p. 1518/Introduction).

Given Proulx et al. teach that iron deficiency is a major nutritional problem and that fortification of foods with iron has been a successful strategy from improving iron content, since Proulx et al. teach that bioavailability is higher from heme iron sources including, leghemoglobin from soy root nodules, it would have been obvious to one of ordinary skill in the art to have fortified the structured plant protein product of Hsieh et al., with leghemoglobin from soy root nodules, to obtain a product with improved nutritional status, i.e. bioavailable source of iron. One of ordinary skill in the art would have added an amount of the leghemoglobin depending on the level of iron fortification desired.

Regarding **claims 32 and 33**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Hsieh et al. disclose that the protein component is texturized in an extruder ([0006], [0009]), since Hsieh et al. disclose the protein component includes plant

protein, it follows that the structured plant protein product would comprise textured plant (i.e. vegetable) protein.

Regarding **claim 37**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Proulx et al. teach leghemoglobin from soybean, intrinsically the plant heme-containing protein would comprise an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NO: 1-26.

Regarding **claims 42-45**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Hsieh et al. also disclose wherein the structured plant protein product may comprise flavoring agents such as an animal meat flavor, yeast extract, hydrolyzed proteins, glutamic acid salts, guanylic acid salts (e.g. guanosine monophosphate – GMP) and inosinic acid salts (e.g. inosine monophosphate- IMP) ([0090]) and lipids such as corn oil, palm oil, and cottonseed oil ([0052]).

Hsieh et al. disclose wherein the structured plant protein product may comprise coloring agents, including for example, beta carotene ([0086]).

Hsieh et al. disclose wherein the structured plant protein product may comprise antioxidants, including for example, propyl gallate, alpha tocopherol and caffeic acid ([0089]).

Hsieh et al. disclose where in the structured plant protein product may comprise a pH-lowering agent, including for example, citric acid and tartaric acid ([0058]).

Regarding **claims 47 and 48**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Hsieh et al. disclose a structured plant protein product comprising animal meat, including beef, it necessarily follows that the product would display the raw and cooked color properties as presently claimed.

***Double Patenting***

11. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the

form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

12. **Claims 31-53 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31-50 of U.S. Patent No. 10,327,464. Although the claims at issue are not identical, they are not patentably distinct from each other.**

U.S. Patent No. **10,327,464** is directed to a ground beef-like food product comprising heme-containing protein, a compound selected from glucose, ribose, fructose, lactose, xylose, arabinoise, glucose-6-phosphate, maltose and galactose, and mixtures of two or more thereof; and at least 10 mM of a compound selected from cysteine, cysteine, selenocysteine, thiamine, methionine and mixtures of two or more thereof, one or plant proteins, wherein the ground beef like food product contains no animal product; and wherein upon cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma (claim 31). While the present claims are directed to a meat-like food product, given a meat-like food product is considered to encompass ground beef-like food products, the claimed subject matter of the present claim set is considered obvious from claims 31-50 of U.S. Patent No. 10,327,464.

13. **Claims 31-53 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31-49 of U.S. Patent No. 9,826,772. Although the claims at issue are not identical, they are not patentably distinct from each other.**

U.S. Patent No. **9,826,772** is directed to a ground beef-like food product comprising heme-containing protein, a compound selected from glucose, ribose, fructose, lactose, xylose, arabinoise, glucose-6-phosphate, maltose and galactose, and mixtures of two or more thereof; and at least 10 mM of a compound selected from cysteine, cysteine, selenocysteine, thiamine, methionine and mixtures of two or more thereof, one or plant proteins, wherein the ground beef like food product contains no animal product; and wherein upon cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma (claim 31). While the present claims are directed to a meat-like food product, given a meat-like food product is considered to encompass ground beef-like food products, the claimed subject matter of the present claim set is considered obvious from claims 31-49 of U.S. Patent No. 9,826,772.

14. **Claims 31-53 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 9,808,029. Although the claims at issue are not identical, they are not patentably distinct from each other.**

U.S. Patent No. **9,808,029** is directed to a ground beef-like food product comprising heme-containing protein, a compound selected from glucose, ribose, fructose, lactose, xylose, arabinoise, glucose-6-phosphate, maltose and galactose, and mixtures of two or more thereof; and at least 10 mM of a compound selected from cysteine, cysteine, selenocysteine, thiamine, methionine and mixtures of two or more thereof, one or plant proteins, wherein the ground beef like food product contains no animal product; and wherein upon cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma (claim 1). While the present claims are directed

to a meat-like food product, given a meat-like food product is considered to encompass ground beef-like food products, the claimed subject matter of the present claim set is considered obvious from claims 1-20 of U.S. Patent No. 9,808,029.

15. **Claims 31-53 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-8 and 11-23 of U.S. Patent No. 9,700,067. Although the claims at issue are not identical, they are not patentably distinct from each other.**

U.S. Patent No. **9,700,067** is directed to a ground beef-like food product comprising heme-containing protein, a compound selected from glucose, ribose, fructose, lactose, xylose, arabinoise, glucose-6-phosphate, maltose and galactose, and mixtures of two or more thereof; and at least 10 mM of a compound selected from cysteine, cysteine, selenocysteine, thiamine, methionine and mixtures of two or more thereof, one or plant proteins, wherein the ground beef like food product contains no animal product; and wherein upon cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma (claim 1). While the present claims are directed to a meat-like food product, given a meat-like food product is considered to encompass ground beef-like food products, the claimed subject matter of the present claim set is considered obvious from claims 1-8 and 11-23 of U.S. Patent No. 9,700,067.

16. **Claim 31-53 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31-50 of copending Application No. 15/913,018. Although the claims at issue are not identical, they are not patentably distinct from each other.** Claim 31 of copending Application No. 15/913,018 is directed to a meat-like

food product comprising, one or more plant proteins; and a source of a heme-containing protein, a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof; a compound selected from cysteine, cysteine, thiamine, methionine, and mixtures of two or more thereof in a concentration of at least 1.5 mM; and wherein cooking the meat-like food product results in the product of at least two volatile compounds which have a meat-associated aroma. This reference makes obvious the claimed meat-like composition of the present application (claim 31) which requires all of the same components.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

17. **Claims 31, 33, 34 and 46-53 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5-8, 13 and 14 of copending Application No. 16/238,802. Although the claims at issue are not identical, they are not patentably distinct from each other.**

Claim 2 of copending Application No. 16/238,802 is directed to a meat replica matrix comprising one or more plant proteins; 0.01% -5% of a non-animal heme-containing protein, a sugar selected from selected from glucose, ribose, fructose, xylose, arabinose, maltodextrin and combinations thereof; at least one compound selected from cysteine, cysteine, thiamine, methionine; and wherein cooking the meat replica matrix, at least two volatile compounds are generated that are associated with a meat-like aroma. While the present claims are directed to a meat-like food product, given a meat-like food product is considered to encompass a meat

replica matrix, the claimed subject matter of the present claim set is considered obvious from claims 2, 3, 5-8, 13 and 14 of U.S. Application No. 16.238,802.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

***Conclusion***

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELIZABETH A GWARTNEY whose telephone number is (571)270-3874. The examiner can normally be reached on M-F: 8 a.m. - 4 p.m..

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Donald (Larry) Tarazano can be reached on 571-272-1515. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to

the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ELIZABETH A. GWARTNEY
Primary Examiner
Art Unit 1791

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1796

| *Notice of References Cited* | Application/Control No. 15/913,090 | Applicant(s)/Patent Under Reexamination Fraser et al. | |
|---|---|---|---|
| | Examiner ELIZABETH A GWARTNEY | Art Unit 1796 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | CPC Classification | US Classification |
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| | A | | | | | |
| | B | | | | | |
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| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | Examiner | Art Unit |
| | ELIZABETH A GWARTNEY | 1796 |

| CPC - Searched* | | |
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| **Symbol** | **Date** | **Examiner** |
| A23J3/227 | 05/06/2020 | EG |

| CPC Combination Sets - Searched* | | |
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| US Classification - Searched* | | | |
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| **Class** | **Subclass** | **Date** | **Examiner** |
| | | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| **Search Notes** | **Date** | **Examiner** |
| Inventor search completed | 05/05/2020 | EG |
| Review of prosecution history for US App 14797006 and 15913018 | 05/05/2020 | EG |
| A23J3/227 (text limited by heme) | 05/06/2020 | EG |

| Interference Search | | | |
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| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
| | | | |

| Index of Claims | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | **Examiner** | **Art Unit** |
| | ELIZABETH A GWARTNEY | 1796 |

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**CLAIMS**

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

| CLAIM | | DATE | | | | | | | | | |
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| Final | Original | 05/07/2020 | | | | | | | | | |
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| Index of Claims | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | **Examiner** | **Art Unit** |
| | ELIZABETH A GWARTNEY | 1796 |

| CLAIM | | DATE | | | | | | | | |
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| Final | Original | 05/07/2020 | | | | | | | | |
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| | 53 | ✓ | | | | | | | | |

Receipt date: 09/26/2019

Sheet 1 of 1

| Substitute Disclosure Form / U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. | Application No. |
|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) (37 CFR §1.98(b)) | 38767-0015008 | 15/913,090 |
| | Applicant: Impossible Foods Inc. | |
| | Filing Date: March 6, 2018 | Group Art Unit: 1791 |

| U.S. Patent Documents | | | | | | | |
|---|---|---|---|---|---|---|---|
| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Class | Subclass | Filing Date If Appropriate |

| Foreign Patent Documents or Published Foreign Patent Applications | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Class | Subclass | Translation Yes | Translation No |

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| Examiner Signature | Date Considered |
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| /ELIZABETH GWARTNEY/ | 05/07/2020 |

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.

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /E.G./

Receipt date: 09/26/2019

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 CFR § 1.98(a)(2).

This statement is being filed before the receipt of a first Office Action on the merits. Therefore, no fees are believed due. If this is incorrect, please apply any charges applicable under 37 CFR § 1.17(p), or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

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

Respectfully submitted,

Date: September 26, 2019

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
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# BIB DATA SHEET

**CONFIRMATION NO. 9878**

| SERIAL NUMBER | FILING or 371(c) DATE | CLASS | GROUP ART UNIT | ATTORNEY DOCKET NO. |
|---|---|---|---|---|
| 15/913,090 | 03/06/2018<br>RULE | 426 | 1796 | 38767-0015008 |

**APPLICANTS**

Impossible Foods Inc., Redwood City, CA;

**INVENTORS**

Rachel Fraser, San Francisco, CA;
Patrick O'Reilly Brown, Stanford, CA;
Jessica Karr, San Francisco, CA;
Celeste Holz-Schietinger, East Palo Alto, CA;
Elysia Cohn, Sunnyvale, CA;

**** CONTINUING DATA ***************************

This application is a CON of 15/398,479 01/04/2017 PAT 9943096
which is a CON of 14/797,006 07/10/2015 PAT 9700067
which is a CON of PCT/US2014/011347 01/13/2014
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and claims benefit of 61/908,634 11/25/2013
and claims benefit of 61/751,816 01/11/2013

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

04/06/2018

| Foreign Priority claimed ☐ Yes ☑ No<br>35 USC 119(a-d) conditions met ☐ Yes ☑ No<br>Verified and Acknowledged /ELIZABETH A GWARTNEY/ Examiner's Signature | ☐ Met after Allowance<br>Initials | STATE OR COUNTRY | SHEETS DRAWINGS | TOTAL CLAIMS | INDEPENDENT CLAIMS |
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| | | CA | 5 | 23 | 1 |

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PO BOX 1022
MINNEAPOLIS, MN 55440-1022
UNITED STATES

**TITLE**

METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

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| 2020 | FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following: | ☐ All Fees<br>☐ 1.16 Fees (Filing)<br>☐ 1.17 Fees (Processing Ext. of time)<br>☐ 1.18 Fees (Issue)<br>☐ Other _____<br>☐ Credit |

BIB (Rev. 05/07).

Receipt date: 08/23/2019



| Substitute Disclosure Form (37 CFR §1.98(b)) | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. | Application No. |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | 38767-0015008 | 15/913,090 |
| | | Applicant: Impossible Foods Inc. | |
| | | Filing Date: March 6, 2018 | Group Art Unit: 1791 |

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| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Class | Subclass | Filing Date If Appropriate |
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| Examiner Signature | Date Considered |
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| /ELIZABETH GWARTNEY/ | 05/06/2020 |

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Receipt date: 08/23/2019



| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

| Other Documents (include Author, Title, Date, and Place of Publication) | | |
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| Examiner Initial | Desig. ID | Document |
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| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
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Sheet 1 of 1

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| | Applicant: Impossible Foods Inc. | |
| | Filing Date: March 6, 2018 | Group Art Unit: 1791 |

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| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. 38767-0015008 | Application No. 15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | Applicant Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date March 6, 2018 | Group Art Unit 1791 |

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|---|---|---|
| | Applicant<br>Impossible Foods Inc. | |
| | Filing Date<br>March 6, 2018 | Group Art Unit<br>1791 |

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Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| Title | : | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES | | | |

**MAIL STOP AMENDMENT**
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Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

This statement is being filed within three months of the filing date of the application or before the receipt of a first action on the merits; therefore, no fee is believed due.

Please apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: November 26, 2019

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Sheet 1 of 1

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| **Information Disclosure Statement by Applicant**<br>(Use several sheets if necessary) | | Applicant<br>Impossible Foods Inc. | |
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| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | 38767-0015008 | 15/913,090 |
| | | Applicant: Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date: March 6, 2018 | Group Art Unit: 1791 |

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Receipt date: 01/18/2019



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|---|---|---|---|
| **Information Disclosure Statement by Applicant**<br>(Use several sheets if necessary) | | Applicant<br>Impossible Foods Inc. | |
| | | Filing Date<br>March 6, 2018 | Group Art Unit<br>1791 |

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Receipt date: 06/06/2018



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| **Information Disclosure Statement by Applicant**<br>(Use several sheets if necessary) | | Applicant<br>Impossible Foods Inc. | |
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| | Applicant<br>Impossible Foods Inc. | |
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| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Class | Subclass | Translation | |
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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
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| S1 | 1 | "15913090" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2020/05/05 12:26 |
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**EAST Search History (Interference)**

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Sheet 1 of 1

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| Substitute Disclosure Form<br>(37 CFR §1.98(b)) | U.S. Department of Commerce<br>Patent and Trademark Office | Attorney Docket No.<br>38767-0015008 | Application No.<br>15/913,090 |
|---|---|---|---|
| **Information Disclosure Statement by Applicant**<br>(Use several sheets if necessary) | | Applicant<br>Impossible Foods Inc. | |
| | | Filing Date<br>March 6, 2018 | Group Art Unit<br>1791 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Filing Date If Appropriate | Class | Subclass |
|---|---|---|---|---|---|---|---|

**Foreign Patent Documents or Published Foreign Patent Applications**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Translation | Class | Subclass |
|---|---|---|---|---|---|---|---|
| | 1. | JP H08-289761 | 11/5/1996 | JP | Abstract | | |

**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|
| | 2. | "Peas, green raw," FoodData Central, US Department of Agriculture, retrieved from URL <https:/fdc.nal.usda.gov/fdc-app.html#/food-details/170419/nutrients>, April 1, 2019, 8 pages |
| | 3. | Berrios, et al., "Carbohydrate composition of raw and extruded pulse flours," Food Research International, 2010, 43:531-536 |
| | 4. | EP Opposition, in Application No. 14737766.7, dated July 4, 2019, 8 pages |
| | 5. | Jahreis, et al., "Legume flours: nutritionally important sources of protein and dietary fiber," Ernahrungs Umschau, 2016, 63(2):36-42 |
| | 6. | Liu, "Comparison of lipid content and fatty acid composition and their distribution within seeds of 5 small grain species," J. Food Sco., 2011, 76(2):C334-C342 |
| | 7. | Macnicol, "Developmental changes in the free amino acid pool and total protein amino acids of pea cotyledons (Pisum sativum L.)," Plant Physiol., 1983, 72:492-497 |
| | 8. | Oldach, "Biochemistry of a Burger: Impossible Foods does science, makes food and tries to change the world," ASBMB Today, retrieved from URL <https://www.asbmb.org/asbmb-today/industry/100119/biochemistry-of-a-burger>, October 1, 2019, 12 pages |
| | 9. | Opponent's Reply Letter in opposition to EP Patent. No. 2943072, dated 1/20/2020, 18 pages |
| | 10. | Rebuttal Declaration of Dr. Jorge Ruiz-Carrascal and Supporting Exhibits, dated January 20, 2020, 12 pages |
| | 11. | Warendorf, et al., "The flavour of bouillon: 2. Sensory analysis of non-volatiles and imitation of a bouillon," Z. Lebensm. Unters. Forsch., 1992, 195(3):215-223, English Abstract |
| | 12. | Wood, et al., "Fatty deposition, fatty acid composition and meat quality: a review," Meat Science, 2008, 78:343-358 |

| Examiner Signature | Date Considered |
|---|---|
| /ELIZABETH GWARTNEY/ | 05/07/2020 |

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

Receipt date: 03/19/2020

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1791 |
| Application No. | : | 15/913,090 | Examiner | : | Unknown |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

The applicant wishes to make the Examiner aware of the following related applications. The prosecution histories for these applications and patents are available on PAIR, and are not provided with this statement.

| Application No. | Filing Date | First Named Inventor | Attorney Docket No. |
|---|---|---|---|
| 14/796,970 | 7/10/2015 | Marija Vrljic | 38767-0010001 |
| 15/786,776 | 10/18/2017 | Marija Vrljic | 38767-0010002 |
| 16/238,802 | 1/3/2019 | Marija Vrljic | 38767-0010003 |
| 16/238,769 | 1/3/2019 | Marija Vrljic | 38767-0010004 |
| 14/152,499 | 1/10/2014 | Patrick O'Reilly Brown M.D., Ph.D. | 38767-0012001 |
| 14/752,540 | 6/26/2015 | Patrick O'Reilly Brown M.D., Ph.D. | 38767-0012002 |
| 15/985,694 | 5/21/2018 | Patrick O'Reilly Brown M.D., Ph.D. | 38767-0012003 |
| 14/797,006 | 7/10/2015 | Rachel Fraser | 38767-0015001 |
| 15/398,479 | 1/4/2017 | Rachel Fraser | 38767-0015002 |
| 15/624,505 | 6/15/2017 | Rachel Fraser | 38767-0015003 |
| 15/624,513 | 6/15/2017 | Rachel Fraser | 38767-0015004 |
| 15/839,994 | 12/13/2017 | Rachel Fraser | 38767-0015005 |
| 15/913,018 | 3/6/2018 | Rachel Fraser | 38767-0015006 |
| 15/912,763 | 3/6/2018 | Rachel Fraser | 38767-0015007 |
| 13/941,211 | 7/12/2013 | Patrick O'Reilly Brown M.D., Ph.D. | 38767-0019001 |
| 15/985,697 | 5/21/2018 | Patrick O'Reilly Brown M.D., Ph.D. | 38767-0019002 |
| 15/300,339 | 9/29/2016 | Ranjani Varadan | 38767-0026US1 |

Receipt date: 03/19/2020

First Named Inventor : Rachel Fraser
Application No. : 15/913,090
Filed : March 6, 2018
Page : 2 of 2

Attorney Docket: 38767-0015008

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|---|---|---|---|
| 16/238,698 | 1/3/2019 | Ranjani Varadan | 38767-0026002 |
| 16/238,749 | 1/3/2019 | Ranjani Varadan | 38767-0026003 |

This statement is being filed before the receipt of a first action on the merits. Therefore, no fee is believed due.

Please apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: March 19, 2020

/Kimberly M. Maize, Reg. No. 76,370/
Kimberly M. Maize
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62274507.doc

DocCode – SCORE

# SCORE Placeholder Sheet for IFW Content

Application Number: 15913090 Document Date: 11/12/2020

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Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1796 |
| Application No. | : | 15/913,090 | Examiner | : | Elizabeth A Gwartney |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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

REPLY TO ACTION OF MAY 12, 2020

Please consider the following reply.

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 15913090 |
| **Filing Date:** | 06-Mar-2018 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Filer:** | Kimberly M. Maize/Emily Palmer |
| **Attorney Docket Number:** | 38767-0015008 |

Filed as Large Entity

**Filing Fees for Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| Extension - 3 months with $0 paid | 1253 | 1 | 1480 | 1480 |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **1480** |

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 41109412 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Emily Palmer |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 12-NOV-2020 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 16:31:00 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | DA |
| Payment was successfully received in RAM | $1480 |
| RAM confirmation Number | E2020ABG31147954 |
| Deposit Account | |
| Authorized User | |
| The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Drawings-only black and white line drawings | Replacement-Fig.pdf | 157333<br>a31995748abd1846b577d2d5a236a199fc64357b | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | | Response.pdf | 171304<br>af7c0d0155a6e9f2c7e4434768fef488b7362e23 | yes | 19 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
|---|---|---|
| Applicant Arguments/Remarks Made in an Amendment | 11 | 19 |
| Drawings-only black and white line drawings | 10 | 10 |
| Claims | 2 | 9 |
| Amendment/Req. Reconsideration-After Non-Final Reject | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 31233<br>e78e8a6b42c27e05c8c48acb4cc2cce45cf92174 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 359870 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**

**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

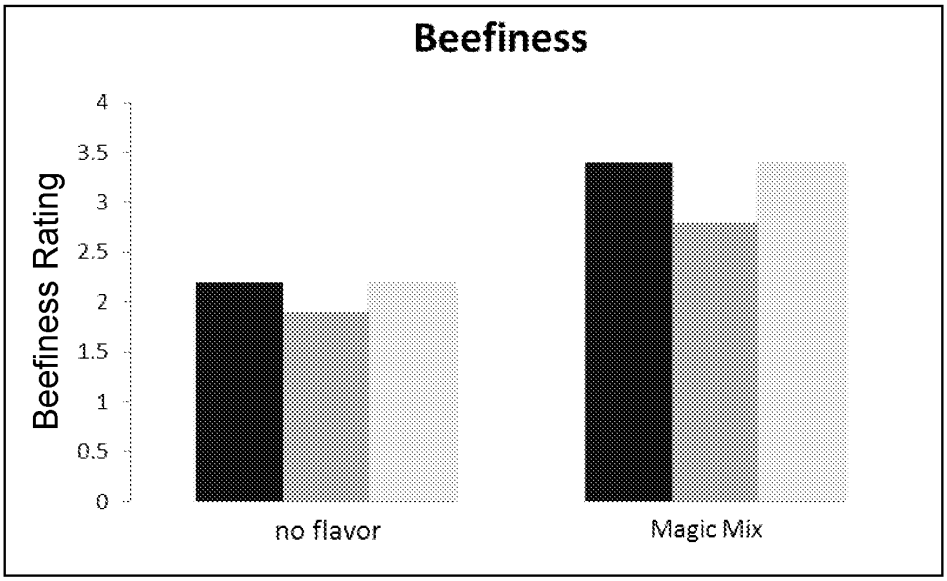
**National Stage of an International Application under 35 U.S.C. 371**

**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

FIG. 2


Beefiness
Beefiness Rating
4
3.5
3
2.5
2
1.5
1
0.5
0
no flavor
Magic Mix

Amendments to Claims:

Listing of Claims.

1-30. (Cancelled)

31. (Currently Amended) A meat-like-food product produced by a method comprising:

providing one or more plant proteins; and

combining, with the one or more plant proteins:

a ~~source of~~ composition comprising a heme-containing protein, wherein the heme-containing protein is free of animal heme-containing proteins and wherein the ~~source of the heme-containing protein~~ composition comprises at least 60% of the heme-containing protein on a dry weight basis;

a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof;

a compound selected from cysteine, cystine, thiamine, methionine, and mixtures of two or more thereof in to a concentration of at least 1.5 mM; [[and]]

wherein cooking the meat-like-food-product results in the production of at least two volatile compounds which have a meat-associated aroma; and

wherein the meat-like food product is in a form selected from the group consisting of a hotdog, a burger, ground meat, a sausage, a steak a filet, a roast, a meatball, a meatloaf, or bacon.

32. (Previously Presented) The meat-like-food product of claim 31, wherein the one or more plant proteins comprises a textured vegetable protein.

33. (Previously Presented) The meat-like-food product of claim 31, wherein the texture vegetable protein is textured soy protein.

34. (Previously Presented) The meat-like-food product of claim 31, wherein the source of the heme-containing protein comprises at least 70% of the heme-containing protein on a dry weight basis.

35. (Previously Presented) The meat-like-food product of claim 31, wherein the source of the heme-containing protein comprises at least 80% of the heme-containing protein on a dry weight basis.

36. (Previously Presented) The meat-like-food product of claim 31, wherein the heme-containing protein is a globin.

37. (Previously Presented) The meat-like-food product of claim 31, wherein the heme-containing protein comprises an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs. 1-26.

38. (Previously Presented) The meat-like-food product of claim 31, wherein the glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, or mixtures of two or more thereof is added to a concentration of about 5.6 to about 20 mM.

39. (Previously Presented) The meat-like-food product of claim 31, wherein the cysteine, cystine, thiamine, methionine, and mixtures of two or more thereof is added to a concentration of at least 10 mM.

40. (Previously Presented) The meat-like-food product of claim 31, wherein the cysteine is added to a concentration of about 0.8 mM to about 10 mM.

41. (Previously Presented) The meat-like-food product of claim 31, wherein the thiamine is added to a concentration of about 0.1 mM to about 2 mM.

42. (Previously Presented) The meat-like-food product of claim 31, further comprising adding one or more of inosine, inosine monophosphate (IMP), guanosine, guanosine monophosphate (GMP), and adenosine monophosphate (AMP).

43. (Previously Presented) The meat-like-food product of claim 31, further comprising adding one or more of beta-carotene, alpha-tocopherol, caffeic acid, propyl gallate, and epigallocatechin gallate.

44. (Previously Presented) The meat-like-food product of claim 31, further comprising adding one or more of a vegetable oil, an 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, canola oil, flaxseed oil, coconut oil, and mango oil.

45. (Previously Presented) The meat-like-food product of claim 31, further comprising adding one or more of acetic acid, lactic acid, glycolic acid, citric acid, succinic acid, tartaric acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, and erucic acid.

46. (Previously Presented) The meat-like-food product of claim 31, wherein cooking comprises heating the meat-like food product at 150°C for about 3 to about 5 minutes.

47. (Previously Presented) The meat-like-food product of claim 31, wherein the meat-like food product has a pink to red color before cooking to indicate a raw or uncooked state.

48. (Previously Presented) The meat-like-food product of claim 31, wherein at least a portion of the meat-like food product, upon cooking, transitions in color from a pink to red color in a raw or uncooked state to a lighter pink to brown color in a partially cooked to fully cooked state.

49. (Currently Amended) The meat-like-food product of claim 31, wherein cooking the meat-like-food product results in the production of at least five volatile compounds ~~which have a meat-associated aroma~~selected from the group consisting of (1-ethyl-1-propenyl)-benzene, (E)-2-decenal, (E)-2-heptenal, (E)-2-hexenal, (E)-2-nonenal, (E)-2-octen-1-ol, (E)-2-octenal, (E)-2-

tridecen-1-ol, (E)-3-penten-2-one, (E)-4-octene, (E)-5-decene, (E,E)-3,5-octadien-2-one, (Z)-2-decenal, (Z)-2-heptenal, (Z)-3-decen-1-ol, acetate, [(methylsulfonyl)methyl]-benzene, 1-(ethylthio)-2-(methylthio)-buta-1,3-diene, 1-(methylthio)-propane, 1,1'-(1,2-cyclobutanediyl)bis-cis-benzene, 1,10-undecadiene, 1,3,5-cycloheptatriene, 1,3-bis(1,1-dimethylethyl)-benzene, 1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane, 1,3-dimethyl-benzene, 1-decen-3-one, 1-heptanol, 1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene, 1-hexanol, 1-hydroxy-2-propanone, 1-nonanol, 1-octanol, 1-octen-3-ol, 1-octen-3-one, 1-octene, 1-pentanol, 1-penten-3-one, 1-undecanol, 2,2,4,6,6-pentamethyl-heptane, 2,3,4-trimethyl-pentane, 2,3-butanedione, 2,3-dihydro-5-methyl-furan, 2,3-dimethyl-pyrazine, 2,4-decadienal, 2,4-dimethyl-1-heptene, 2,5-dimethyl-pyrazine, 2-acetatyl-1-propene, 2-acetyl-propen-2-ol, 2-acetylthiazole, 2-butanone, 2-butenal, 2-butyl-1-decene, 2-decanone, 2-decenal, 2-ethyl-5-methyl-pyrazine, 2-ethylacrolein, 2-ethyl-furan, 2-heptanone, 2-heptenal, 2-hexyl furan, 2-methyl-1(H)-pyrrole, 2-methyl-1-heptene, 2-methyl-1-penten-1-one, 2-methyl-3-(methylthio) furan, 2-methyl-3-furanthiol, 2-methyl-5-(methylthio)-furan, 2-methyl-butanal, 2-methyl-cyclopentanone, 2-methyl-furan, 2-methyl-heptane, 2-methyl-pentanal, 2-methyl-propanal, 2-methyl-thiazole, 2-n-octylfuran, 2-nonanone, 2-nonen-4-one, 2-octanone, 2-octenal, 2-pentanone, 2-pentyl-furan, 2-pentyl-thiophene, 2-phenylpropenal, 2-propenal, 2-propenoic acid, methyl ester, 2-thiophenecarboxaldehyde, 2-tridecen-1-ol, 2-undecanone, 2-undecenal, 3-(methylthio)-propanenitrile, 3-(phenylmethyl)-2,5-piperazinedione, 3,3'-dithiobis-2-methyl-furan, 3,5-bis(1,1-dimethylethyl)-phenol, 3,5-di-tert-butylbenzoic acid, 3-decen-2-one, 3-ethylcyclopentanone, 3-ethyl-pentane, 3-methyl-2-butenal, 3-methyl-2-thiophenecarboxaldehyde, 3-methyl-3-buten-2-one, 3-methyl-butanal, 3-methyl-furan, 3-methylheptyl acetate, 3-methyl-pentanal, 3-pentyl-furan, 3-phenyl-furan, 3-thiophenecarboxaldehyde, 3-thiophenemethanol, 4,7-dimethyl-undecane, 4-chloro-2,6-bis(1,1-dimethylethyl)-phenol, 4-ethoxy-benzoic acid, ethyl ester, 4-ethyl-2-methyl-pyrrole, 4-ethyl-benzaldehyde, 4-methyl-2-heptanone, 4-methyl-5-thiazoleethanol, 4-methylthiazole, 4-nitrophenyl 2-thiophenecarboxylic acid ester, 4-penten-2-one, 4-quinolinecarboxaldehyde, 5-(2-chloroethyl)-4-methylthiazole, 5-ethenyl-4-methyl-thiazole, 5-ethyldihydro-2(3H)-furanone, 5-formyl-4-methylthiazole, 5-methyl-1-undecene, 6-methyl-2-heptanone, 7-methyl-(E)-5-undecene, 9-octadecenal, acetaldehyde, acetic acid, acetone, acetonitrile, acetophenone, acetyl valeryl, aminophenylacetylene, benzaldehyde, benzene, benzoic acid, benzyl alcohol, bis(2-

methyl-4,5-dihydro-3-furyl) disulfide, butanal, butanone, butyrolactone, carbon disulfide, decanal, dihydro-3-(2H)-thiophenone, dihydro-5-pentyl-2(3H)-furanone, dimethyl selenone, dimethyl sulfide, dimethyl trisulfide, dodecanal, ethanol, ethyl acetate, formic acid, heptyl ester, furan, furfural, heptanal, hexanoic acid, isopropyl alcohol, isothiazole, isothiocyanato-methane, m-aminophenylacetylene, methacrolein, methanethiol, methional, methyl (methylthio)methyl disulfide, methyl isopropyl disulphide, methyl thiolacetate, methyl vinyl ketone, methyl-pyrazine, methyl-thiirane, nonanal, octanal, octane, oxalic acid, diallyl ester, oxalic acid, isobutyl pentyl ester, p-Cresol, p-dithiane-2,5-diol, pentanal, pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester, phenol, phenylacetaldehyde, propanal, propyl-cyclopropane, p-xylene, pyrazine, pyrrole, styrene, thiazole, thiocyanic acid, methyl ester, thiophene, toluene, trans-2-(2-pentenyl)furan, and tridecane.

50. (Currently Amended) The meat-like food product of claim 31, wherein cooking the meat-like food product results in the production of at least ten volatile compounds ~~which have a meat-associated aroma~~selected from the group consisting of (1-ethyl-1-propenyl)-benzene, (E)-2-decenal, (E)-2-heptenal, (E)-2-hexenal, (E)-2-nonenal, (E)-2-octen-1-ol, (E)-2-octenal, (E)-2-tridecen-1-ol, (E)-3-penten-2-one, (E)-4-octene, (E)-5-decene, (E,E)-3,5-octadien-2-one, (Z)-2-decenal, (Z)-2-heptenal, (Z)-3-decen-1-ol, acetate, [(methylsulfonyl)methyl]-benzene, 1-(ethylthio)-2-(methylthio)-buta-1,3-diene, 1-(methylthio)-propane, 1,1'-(1,2-cyclobutanediyl)bis-cis-benzene, 1,10-undecadiene, 1,3,5-cycloheptatriene, 1,3-bis(1,1-dimethylethyl)-benzene, 1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane, 1,3-dimethyl-benzene, 1-decen-3-one, 1-heptanol, 1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene, 1-hexanol, 1-hydroxy-2-propanone, 1-nonanol, 1-octanol, 1-octen-3-ol, 1-octen-3-one, 1-octene, 1-pentanol, 1-penten-3-one, 1-undecanol, 2,2,4,6,6-pentamethyl-heptane, 2,3,4-trimethyl-pentane, 2,3-butanedione, 2,3-dihydro-5-methyl-furan, 2,3-dimethyl-pyrazine, 2,4-decadienal, 2,4-dimethyl-1-heptene, 2,5-dimethyl-pyrazine, 2-acetatyl-1-propene, 2-acetyl-propen-2-ol, 2-acetylthiazole, 2-butanone, 2-butenal, 2-butyl-1-decene, 2-decanone, 2-decenal, 2-ethyl-5-methyl-pyrazine, 2-ethylacrolein, 2-ethyl-furan, 2-heptanone, 2-heptenal, 2-hexyl furan, 2-methyl-1(H)-pyrrole, 2-methyl-1-heptene, 2-methyl-1-penten-1-one, 2-methyl-3-(methylthio) furan, 2-methyl-3-furanthiol, 2-methyl-5-(methylthio)-furan, 2-methyl-butanal, 2-methyl-cyclopentanone, 2-methyl-furan, 2-methyl-

heptane, 2-methyl-pentanal, 2-methyl-propanal, 2-methyl-thiazole, 2-n-octylfuran, 2-nonanone, 2-nonen-4-one, 2-octanone, 2-octenal, 2-pentanone, 2-pentyl-furan, 2-pentyl-thiophene, 2-phenylpropenal, 2-propenal, 2-propenoic acid, methyl ester, 2-thiophenecarboxaldehyde, 2-tridecen-1-ol, 2-undecanone, 2-undecenal, 3-(methylthio)-propanenitrile, 3-(phenylmethyl)-2,5-piperazinedione, 3,3'-dithiobis-2-methyl-furan, 3,5-bis(1,1-dimethylethyl)-phenol, 3,5-di-tert-butylbenzoic acid, 3-decen-2-one, 3-ethylcyclopentanone, 3-ethyl-pentane, 3-methyl-2-butenal, 3-methyl-2-thiophenecarboxaldehyde, 3-methyl-3-buten-2-one, 3-methyl-butanal, 3-methyl-furan, 3-methylheptyl acetate, 3-methyl-pentanal, 3-pentyl-furan, 3-phenyl-furan, 3-thiophenecarboxaldehyde, 3-thiophenemethanol, 4,7-dimethyl-undecane, 4-chloro-2,6-bis(1,1-dimethylethyl)-phenol, 4-ethoxy-benzoic acid, ethyl ester, 4-ethyl-2-methyl-pyrrole, 4-ethyl-benzaldehyde, 4-methyl-2-heptanone, 4-methyl-5-thiazoleethanol, 4-methylthiazole, 4-nitrophenyl 2-thiophenecarboxylic acid ester, 4-penten-2-one, 4-quinolinecarboxaldehyde, 5-(2-chloroethyl)-4-methylthiazole, 5-ethenyl-4-methyl-thiazole, 5-ethyldihydro-2(3H)-furanone, 5-formyl-4-methylthiazole, 5-methyl-1-undecene, 6-methyl-2-heptanone, 7-methyl-(E)-5-undecene, 9-octadecenal, acetaldehyde, acetic acid, acetone, acetonitrile, acetophenone, acetyl valeryl, aminophenylacetylene, benzaldehyde, benzene, benzoic acid, benzyl alcohol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, butanal, butanone, butyrolactone, carbon disulfide, decanal, dihydro-3-(2H)-thiophenone, dihydro-5-pentyl-2(3H)-furanone, dimethyl selenone, dimethyl sulfide, dimethyl trisulfide, dodecanal, ethanol, ethyl acetate, formic acid, heptyl ester, furan, furfural, heptanal, hexanoic acid, isopropyl alcohol, isothiazole, isothiocyanato-methane, m-aminophenylacetylene, methacrolein, methanethiol, methional, methyl (methylthio)methyl disulfide, methyl isopropyl disulphide, methyl thiolacetate, methyl vinyl ketone, methyl-pyrazine, methyl-thiirane, nonanal, octanal, octane, oxalic acid, diallyl ester, oxalic acid, isobutyl pentyl ester, p-Cresol, p-dithiane-2,5-diol, pentanal, pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester, phenol, phenylacetaldehyde, propanal, propyl-cyclopropane, p-xylene, pyrazine, pyrrole, styrene, thiazole, thiocyanic acid, methyl ester, thiophene, toluene, trans-2-(2-pentenyl)furan, and tridecane.

51. (Currently Amended) The meat-like-food product of claim 31, wherein cooking the meat-like-food product results in the production of at least twenty volatile compounds ~~which~~

~~have a meat-associated aroma~~selected from the group consisting of (1-ethyl-1-propenyl)-benzene, (E)-2-decenal, (E)-2-heptenal, (E)-2-hexenal, (E)-2-nonenal, (E)-2-octen-1-ol, (E)-2-octenal, (E)-2-tridecen-1-ol, (E)-3-penten-2-one, (E)-4-octene, (E)-5-decene, (E,E)-3,5-octadien-2-one, (Z)-2-decenal, (Z)-2-heptenal, (Z)-3-decen-1-ol, acetate, [(methylsulfonyl)methyl]-benzene, 1-(ethylthio)-2-(methylthio)-buta-1,3-diene, 1-(methylthio)-propane, 1,1'-(1,2-cyclobutanediyl)bis-cis-benzene, 1,10-undecadiene, 1,3,5-cycloheptatriene, 1,3-bis(1,1-dimethylethyl)-benzene, 1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane, 1,3-dimethyl-benzene, 1-decen-3-one, 1-heptanol, 1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene, 1-hexanol, 1-hydroxy-2-propanone, 1-nonanol, 1-octanol, 1-octen-3-ol, 1-octen-3-one, 1-octene, 1-pentanol, 1-penten-3-one, 1-undecanol, 2,2,4,6,6-pentamethyl-heptane, 2,3,4-trimethyl-pentane, 2,3-butanedione, 2,3-dihydro-5-methyl-furan, 2,3-dimethyl-pyrazine, 2,4-decadienal, 2,4-dimethyl-1-heptene, 2,5-dimethyl-pyrazine, 2-acetatyl-1-propene, 2-acetyl-propen-2-ol, 2-acetylthiazole, 2-butanone, 2-butenal, 2-butyl-1-decene, 2-decanone, 2-decenal, 2-ethyl-5-methyl-pyrazine, 2-ethylacrolein, 2-ethyl-furan, 2-heptanone, 2-heptenal, 2-hexyl furan, 2-methyl-1(H)-pyrrole, 2-methyl-1-heptene, 2-methyl-1-penten-1-one, 2-methyl-3-(methylthio) furan, 2-methyl-3-furanthiol, 2-methyl-5-(methylthio)-furan, 2-methyl-butanal, 2-methyl-cyclopentanone, 2-methyl-furan, 2-methyl-heptane, 2-methyl-pentanal, 2-methyl-propanal, 2-methyl-thiazole, 2-n-octylfuran, 2-nonanone, 2-nonen-4-one, 2-octanone, 2-octenal, 2-pentanone, 2-pentyl-furan, 2-pentyl-thiophene, 2-phenylpropenal, 2-propenal, 2-propenoic acid, methyl ester, 2-thiophenecarboxaldehyde, 2-tridecen-1-ol, 2-undecanone, 2-undecenal, 3-(methylthio)-propanenitrile, 3-(phenylmethyl)-2,5-piperazinedione, 3,3'-dithiobis-2-methyl-furan, 3,5-bis(1,1-dimethylethyl)-phenol, 3,5-di-tert-butylbenzoic acid, 3-decen-2-one, 3-ethylcyclopentanone, 3-ethyl-pentane, 3-methyl-2-butenal, 3-methyl-2-thiophenecarboxaldehyde, 3-methyl-3-buten-2-one, 3-methyl-butanal, 3-methyl-furan, 3-methylheptyl acetate, 3-methyl-pentanal, 3-pentyl-furan, 3-phenyl-furan, 3-thiophenecarboxaldehyde, 3-thiophenemethanol, 4,7-dimethyl-undecane, 4-chloro-2,6-bis(1,1-dimethylethyl)-phenol, 4-ethoxy-benzoic acid, ethyl ester, 4-ethyl-2-methyl-pyrrole, 4-ethyl-benzaldehyde, 4-methyl-2-heptanone, 4-methyl-5-thiazoleethanol, 4-methylthiazole, 4-nitrophenyl 2-thiophenecarboxylic acid ester, 4-penten-2-one, 4-quinolinecarboxaldehyde, 5-(2-chloroethyl)-4-methylthiazole, 5-ethenyl-4-methyl-thiazole, 5-ethyldihydro-2(3H)-furanone, 5-

formyl-4-methylthiazole, 5-methyl-1-undecene, 6-methyl-2-heptanone, 7-methyl-(E)-5-undecene, 9-octadecenal, acetaldehyde, acetic acid, acetone, acetonitrile, acetophenone, acetyl valeryl, aminophenylacetylene, benzaldehyde, benzene, benzoic acid, benzyl alcohol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, butanal, butanone, butyrolactone, carbon disulfide, decanal, dihydro-3-(2H)-thiophenone, dihydro-5-pentyl-2(3H)-furanone, dimethyl selenone, dimethyl sulfide, dimethyl trisulfide, dodecanal, ethanol, ethyl acetate, formic acid, heptyl ester, furan, furfural, heptanal, hexanoic acid, isopropyl alcohol, isothiazole, isothiocyanato-methane, m-aminophenylacetylene, methacrolein, methanethiol, methional, methyl (methylthio)methyl disulfide, methyl isopropyl disulphide, methyl thiolacetate, methyl vinyl ketone, methyl-pyrazine, methyl-thiirane, nonanal, octanal, octane, oxalic acid, diallyl ester, oxalic acid, isobutyl pentyl ester, p-Cresol, p-dithiane-2,5-diol, pentanal, pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester, phenol, phenylacetaldehyde, propanal, propyl-cyclopropane, p-xylene, pyrazine, pyrrole, styrene, thiazole, thiocyanic acid, methyl ester, thiophene, toluene, trans-2-(2-pentenyl)furan, and tridecane.

52. (Previously Presented) The meat-like-food product of claim 31, wherein the at least two volatile compounds are selected from 2-methyl-furan, bis(2-methyl-3-furyl)disulfide, 2-pentyl- furan, 3,3'-dithiobis-2-methyl-furan, 2,5-dimethyl-pyrazine, 2-methyl-3-furanthiol, dihydro-3- (2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl sulfide, decanal, 5-ethyldihydro-2(3H)-furanone, dihydro-5-pentyl- 2(3H)-furanone, 2-octanone, 3,5-octadien-2-one, p-Cresol, and hexanoic acid.

53. (Previously Presented) The meat-like-food product of claim 31, wherein the at least two volatile compounds are 2-methyl-furan and bis(2-methyl-3-furyl)disulfide.

Amendments to the Drawings:

The attached replacement sheet of drawings includes changes to Fig. 2 and replaces the original sheet including Fig. 2.

In Figure 2, the y-axis is now labeled.

Attachments following last page of this Amendment: Replacement Sheet (1 pages)

## REMARKS

Applicants respectfully request entry of the amendments and remarks submitted herein. Claims 31 and 49-51 have been amended. Support for new claim amendments can be found in US 2018/0199606 at least at paragraph [0062], paragraph [0068], Table 3; Table 8; Table 9; Table 12; Table 14; Table 16; Table 17; and Table 18. No new matter has been introduced by these amendments.

Claims 31-53 are currently pending. Reconsideration of the pending claims and allowance of the same are respectfully requested.

### Drawings

The drawings are objected to because the Examiner indicated that Figure 2 does not contain a label for the Y-axis. Filed herewith is a Replacement Sheet for FIG. 2 addressing the present rejection. Reconsideration and withdrawal of the objection is requested.

### Double Patenting

Claims 31-53 are rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 31-50 of U.S. Patent No. 10,327,464. According to the Examiner, although the claims at issue are not identical, they are not patentably distinct from each other.

The Examiner recites the following: "[c]laims 31-53 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims **31-50**….". Applicant believes that this was a typographical error and assumes that the Examiner intended to reject claims 31-53 over claims **1-34** of U.S. Patent No. 10,327, 464.

Without conceding to the merits of the rejection, Applicant will file a terminal disclaimer over U.S. Patent No. 10,327,464, thus rendering the rejection moot. Applicant therefore requests that the rejection of claims 31-53 be withdrawn.

Claims 31-53 are rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 31-49 of U.S. Patent No. 9,826,772. According to the Examiner, although the claims at issue are not identical, they are not patentably distinct from each other.

The Examiner recites the following: "[c]laims 31-53 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims **31-50**....". Applicant believes that this was a typographical error and assumes that the Examiner intended to reject claims 31-53 over claims **1-19** of U.S. Patent No. 9,826,772.

Without conceding to the merits of the rejection, Applicant will file a terminal disclaimer over U.S. Patent No. 9,826,772, thus rendering the rejection moot. Applicant therefore requests that the rejection of claims 31-53 be withdrawn.

Claims 31-53 are rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 1-20 of U.S. Patent No. 9,808,029. According to the Examiner, although the claims at issue are not identical, they are not patentably distinct from each other.

Without conceding to the merits of the rejection, Applicant will file a terminal disclaimer over U.S. Patent No. 9,808,029, thus rendering the rejection moot. Applicant therefore requests that the rejection of claims 31-53 be withdrawn.

Claims 31-53 are rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 1-8 and 11-23 of U.S. Patent No. 9,700,067. According to the Examiner, although the claims at issue are not identical, they are not patentably distinct from each other.

Without conceding to the merits of the rejection, Applicant will file a terminal disclaimer over U.S. Patent No. 9,700,067, thus rendering the rejection moot. Applicant therefore requests that the rejection of claims 31-53 be withdrawn.

Claim 31-53 are provisionally rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 31-50 of copending Application No. 15/913,018. According to the Examiner, although the claims at issue are not identical, they are not patentably distinct from each other.

Without conceding to the merits of the rejection, Applicant will file a terminal disclaimer over copending Application No. 15/913,018, thus rendering the rejection moot. Applicant therefore requests that the rejection of claims 31-53 be withdrawn.

Claims 31, 33, 34 and 46-53 are provisionally rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 2, 3, 5-8, 13 and 14 of copending Application No. 16/238,802. According to the Examiner, although the claims at issue are not identical, they are not patentably distinct from each other.

Without conceding to the merits of the rejection, Applicant will file a terminal disclaimer over copending Application No. 16/238,802, thus rendering the rejection moot. Applicant therefore requests that the rejection of claims 31-53 be withdrawn.

Rejections under 35 U.S.C. § 112

Claims 31-53 are rejected under 35 U.S.C. § 112 (pre-AIA), first paragraph, as allegedly failing to comply with the written description requirement. According to the Examiner, the claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicant respectfully traverses the rejection.

The Examiner objected to the phrase "meat-like food product" recited in claims 31-53, alleging "[w]hile the present specification is broadly directed to creating meat-like flavor in animal or non-animal based food product, including food products in the form of hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets or cubes ([0010]-[0011]), there is no description of a 'meat-like food product'". Office Action at page 3. Without conceding the appropriateness of the rejection, and solely in the interest of advancing prosecution, Applicant has amended claim 31 to recite "wherein the meat-like food product is in a form selected from the group consisting of a hotdog, a burger, ground meat, a sausage, a steak a filet, a roast, a meatball, a meatloaf, or bacon". The amended claims have written description support in the specification as filed at least on page 4, lines 26-28, among other locations throughout the specification.

As such, Applicant respectfully submits that claims 31-53, as amended, have written description support. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112.

Claims 31-50 are rejected under 35 U.S.C. § 112 (pre-AIA), second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicant regards as the invention. Applicant respectfully traverses the rejection.

The Office Action states on page 4, "[c]laims **31-50** are rejected under 35 U.S.C. § 112(b)…". Applicant believes that this was a typographical error and assumes that the Examiner intended to reject claims **31-53**.

The Examiner objected to the phrase "meat-like food product" recited in claims 31-53, alleging "[i]t is not clear what properties define a meat-like food product". Without conceding the appropriateness of the rejection, and solely in the interested of advancing prosecution, Applicant has amended claims 31-53 to recite "wherein the meat-like food product is in a form selected from the group consisting of a hotdog, a burger, ground meat, a sausage, a steak a filet, a roast, a meatball, a meatloaf, or bacon". Support for the amendment can be found in the specification as filed at, at least on page 4, lines 26-28, among other locations throughout the specification.

As such, Applicant respectfully submits that claims 31-53, as amended, particularly points out and distinctly claims the subject matter. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112.

Rejections under 35 U.S.C. § 103

Claims 31-53 are rejected under pre-AIA 35 U.S.C. § 103(a) as being allegedly unpatentable over Hsieh et al. (US 2012/0093994) in view of Proulx et al. ("Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model" - J. Agric. Food Chem." 2006, p. 1518-1522) as evidenced by Dinh et al. (Effects of USDA quality grade and cooking on water-soluble precursors of beef flavor", Meat Science, Volume 146, December 2018, pp. 122-130).

Applicant respectfully traverses the rejection. Without acceding to the merits of the rejection and solely to advance prosecution, Applicant has herein amended claim 31 to recite, in part:

> 31. A meat-like-food product produced by a method comprising:
> providing one or more plant proteins; and
> combining, with the one or more plant proteins:
> a composition comprising a heme-containing protein, **wherein the heme-containing protein is free of animal heme-containing proteins** and wherein the composition comprises at least 60% of the heme-containing protein on a dry weight basis;
> a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof;
> a compound selected from cysteine, cystine, thiamine, methionine, and mixtures of two or more thereof in to a concentration of at least 1.5 mM;
> wherein cooking the meat-like-food-product results in the production of at least two volatile compounds which have a meat-associated aroma; and
> wherein the meat-like food product is in a form selected from the group consisting of a hotdog, a burger, ground meat, a sausage, a steak a filet, a roast, a meatball, a meatloaf, or bacon.

None of the cited references, alone or in any combination, disclose or suggest this combination of features.

The Examiner has cited the Hsieh abstract, and paragraphs [0013], and [0079] as relevant to a plant containing protein, however, Hsieh fails to disclose or even suggest the limitations of the amended claims. Hsieh describes, "(a) dry ingredients that comprise: (i) a protein component that comprises a plant-derived protein material, wherein the protein component is at an amount that is no more than about 90% by weight of the dry ingredients; (ii) a carbohydrate component at an amount that is in the range of about 2 to about 50% by weight of the dry ingredients; and (iii) a lipid component at an amount that is in the range of about 0.1 to about 5% by weight of the dry ingredients; and (b) wet ingredients that comprise water". (Abstract). Hsieh further discloses that the protein component can include animal meat, including beef (paragraph [0079]). The Examiner asserts that "a structured plant protein product comprising animal meat, e.g. beef, it necessarily follows, wherein cooking the structured plant protein product, wherein cooking includes heating to a temperature of 150°C for about 3 to about 5 minutes, results in the

production of the claimed meat associated aroma compounds". (Office Action, Page 6). However, the Examiner acknowledges that Hsieh, "does not disclose the precise amount of free amino acid or reducing sugar in the added animal meat (e.g., beef)". (Office Action, page 6).

The Examiner has cited Dinh (Page 125, Table 1) and (Page 126, Table 2) as evidence to indicate that beef comprises amino acids and reducing sugars. The Examiner states, "evidenced by Dinh et al., beef is known to comprise; (a) the free amino acids, cysteine, cysteine, and methionine (p. 125/Table l); and (b) the reducing sugars, glucose, glucose-6-phosphate, fructose and ribose (p. 126/Table 2)". (Office Action, Page 6).

That is, Hsieh appears to disclose a structural plant protein product comprising animal meat (e.g., beef). The Examiner proposes that Dinh provides evidence that the structural plant protein product of Hsieh when including animal meat (e.g., beef) would include reducing sugars and amino acids. However, neither Hsieh nor Dinh disclose or suggest, "**a composition comprising a heme-containing protein, wherein the heme-containing protein is free of animal heme-containing proteins**", as recited by claim 31. At least because the structural plant protein product comprising animal meat (e.g., beef) is not free of animal heme-containing proteins.

Further, the Examiner has acknowledged that Hsieh is silent with respect to a heme protein. The Examiner states, "Hsieh et al. is silent with respect to a plant, yeast, fungal, or bacterial heme-containing protein". (Office Action, Page 6).

Proulx is cited as disclosing a heme protein in a food product (i.e., a tortilla). Proulx does not cure the deficiencies of Hsieh and Dinh. In contrast to its silence with respect to adding a heme-containing protein for any reason, let alone a heme-containing protein from a non-animal source, Hsieh affirmatively teaches the inclusion of animal meat to improve the animal flavor of its meat analogs. See, for example, paragraphs [0033] and [0079] – [0081]. In fact, Hsieh directly correlates the degree of animal flavor in the meat analog with the amount of animal meat present. See, for example, paragraph [0084], which states that:

> when a significantly vegetarian composition that has a relatively small degree of animal flavor is desired, the concentration of animal meat in a meat analog composition may be about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, about 2%, or 0% by weight. Alternatively, when a meat analog composition have a relatively high degree of

> animal meat flavor is desired, the concentration of animal meat may be about 50%, about 55%, about 60%, about 65%, about 70% or about 75%, by weight.

The teachings of Hsieh do not support the Examiner's assertion that it would be obvious to modify the meat analog of Hsieh to include the non-animal leghemoglobin of Proulx. In fact, the meat analog of Hsieh would inherently contain heme by the addition of beef to the meat analog. As such, there would be no reason for Hsieh to modify their meat analog to contain heme from a non-animal source. Further, the Examiner does not provide any evidence that a person of ordinary skill would have had a motivation to combine Hsieh, Dinh, and/or Proulx with a reasonable expectation of success.

The Proulx Thesis, which is a later reference by the same author as the primary Proulx reference used by the Office, states that:

> [f]ortification [with] *iron has a potential negative effect on organoleptic quality* of maize products, in that soluble forms of *iron can cause oxidative rancidity*

(Proulx, 2007, Diversified strategies for improving iron bioavailability of maize, Retrospective Theses and Dissertations, Iowa State University, Paper 15852, page 20, emphasis added; hereinafter the "Proulx Thesis").

One of ordinary skill would understand from Proulx's own later teachings that the inclusion of supplemental iron can cause changes in the organoleptic qualities of a food, including causing the food to taste bad through oxidative rancidity. For example, the Proulx Thesis discloses that "[i]ron compounds should be added to the level *up to which they do not change the organoleptic qualities* of the food, usually between 20 ppm and 40 ppm" (see page 29; emphasis added). In addition, the Proulx Thesis states that "[f]ortification [with] iron has *a potential negative effect on organoleptic quality* of maize products, in that soluble forms of iron can cause *oxidative rancidity*" (see page 20; emphasis added). Given the propensity of iron to cause off-flavors when added to food, Hsieh would have no reasonable expectation of success by fortifying their meat analog with additional iron.

The Examiner has pointed to nothing in Hsieh to reasonably support that a person of ordinary skill in the art would have a reason to modify the meat analogs of Hsieh by adding a heme-containing protein from a non-animal source (i.e., a heme-containing protein that is free of

anima hele-containing proteins). Instead, Hsieh teaches to those skilled in the art that in order to improve meat taste of meat analog, one would ***add meat*** to the meat analog.

The cited references do not disclose or even suggest each and every limitation of independent claim 31. The Hsieh, Dinh, and Proulx references do not disclose or suggest, "**a composition comprising a heme-containing protein, wherein the heme-containing protein is free of animal heme-containing proteins**" as recited in claim 31, as amended. Hsieh is silent as to modifying its meat analogs to include heme from non-animal sources and for at least the reasons stated above, the Proulx reference does not cure the deficiencies of the Hsieh reference.

As such, Applicant respectfully submits that Hsieh, Dinh, and Proulx, individually or in any combination, do not disclose or even suggest each and every limitation of independent claim 31, as amended. None of these references discloses or suggests a composition comprising a heme-containing protein, wherein the heme-containing protein is free of animal heme-containing proteins. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 103.

## CONCLUSION

It is believed that all of the pending claims have been addressed and the claims are in condition for allowance.

The undersigned welcomes the opportunity to further discuss by telephone any position or issue not fully addressed by the above remarks and amendments.

The fees for extension of time are being paid with this reply on the Electronic Filing System. Apply those fees and any other necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: November 12, 2020

/Kimberly M. Maize, Reg. No. 76,370/
Kimberly M. Maize
Reg. No. 76,370

Customer Number 26191
Fish & Richardson P.C.
Telephone: +1 (612) 638-5707
Facsimile: (877) 769-7945

62324678.doc

Sheet 1 of 1

| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. | Application No. |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | 38767-0015008 | 15/913,090 |
| | | Applicant: Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date: March 6, 2018 | Group Art Unit: 1796 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Filing Date If Appropriate | Class | Subclass |
|---|---|---|---|---|---|---|---|
| | 1. | 4098913 | 7/4/1978 | Baugher | | | |
| | 2. | 4197324 | 4/8/1980 | Ziminski et al. | | | |
| | 3. | 4324807 | 4/13/1982 | Kim et al. | | | |
| | 4. | 10798958 | 10/13/2020 | Varadan et al. | | | |
| | 5. | 20050112271 | 5/1/2005 | Picarski | | | |
| | 6. | 20090148558 | 6/11/2009 | Kim et al. | | | |

**Foreign Patent Documents or Published Foreign Patent Applications**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Translation | Class | Subclass |
|---|---|---|---|---|---|---|---|
| | 7. | RU 2010137628 | 3/20/2012 | RU | Abstract | | |

**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|

| 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

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1796 |
| Application No. | : | 15/913,090 | Examiner | : | Elizabeth A. Gwartney |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

This statement is being filed after a first action on the merits, but before receipt of a final action or a notice of allowance.

Apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: November 12, 2020

/Kimberly M. Maize, Reg. No. 76,370/
Kimberly M. Maize
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Customer Number 26191
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Facsimile: (877) 769-7945

62463957.doc

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 15913090 |
| **Filing Date:** | 06-Mar-2018 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Filer:** | Kimberly M. Maize/Shana Knotts |
| **Attorney Docket Number:** | 38767-0015008 |

Filed as Large Entity

**Filing Fees for Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Miscellaneous:** | | | | |
| SUBMISSION- INFORMATION DISCLOSURE STMT | 1806 | 1 | 260 | 260 |
| | | **Total in USD ($)** | | **260** |

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 41109425 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Victor Ramirez-Juarez |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 12-NOV-2020 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 16:58:33 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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| Submitted with Payment | yes |
| Payment Type | DA |
| Payment was successfully received in RAM | $260 |
| RAM confirmation Number | E2020ABG58528590 |
| Deposit Account | |
| Authorized User | |
| The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Foreign Reference | RU2010137628.pdf | 1514468<br>c50d5c9c2f983bfabb0fc636a79fa22d47ebfc9e | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Information Disclosure Statement (IDS) Form (SB08) | 0015008IDS1449.pdf | 149129<br>081a989f8aefecfbbcd553b80c7ad69c6cbb2932 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 30690<br>0a532b296327606cc28242ae77895c0dc56ada88 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 1694287 | | |

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**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**
**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**
**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Patents RU 2010137628

# Meat product analogue


## Abstract

1. An analogue product of meat containing a combination of a dry component, a liquid, a source of a monovalent cationic carbonate or bicarbonate and an acid component of a chemical baking powder, where a dry component having a protein content contains a source of a dry protein containing sulfur; the liquid contains water; a source of monovalent cationic carbonate or bicarbonate and the acidic component of the chemical baking powder of the test are present in a ratio of about 1: 2-1: 4 by weight; and the meat analogue product contains a plurality of grooved and detachable aligned fibers in the product. ! 2. The meat analogue product of claim 1, further comprising a reducing agent. ! 3. The meat analogue product according to claim 1, where the dry component contains 0.4-4.8 wt.% A source of monovalent cationic carbonate or bicarbonate relative to the total weight of the dry component. ! 4. The meat analogue product according to claim 1, wherein the source of the dry protein containing sulfur contains gluten, soy protein, pea protein, egg white, or a mixture thereof. ! 5. The meat analogue product according to claim 1, wherein the dry component contains a source of dry meat protein containing sulfur, or a dry protein, or a source of fiber obtained from whole grains, fruits, vegetables, or mixtures thereof. ! 6. The meat analogue product according to claim 1, wherein the liquid further comprises a source of fresh or frozen meat, a source of meat processed into flour, or a mixture thereof. ! 7. The meat analogue product according to claim 1, wherein the liquid, the dry component, or both additionally contain one or more humectants, a fat source, a dye, a flavor enhancer, a flavoring substance, a vitamin, a mineral, an antioxidant, dried blood plasma, salt, dextrose, sorbitol, starch, soy protein


## Classifications

- A23K50/48 Moist feed

View 8 more classifications


RU2010137628A

Russia

Download PDF Find Prior Art Similar

**Other languages:** Russian



**Inventor:** Ральф РАЙЗЕР (US), Ральф РАЙЗЕР, Винод ГУМУДАВЕЛЛИ (US), Винод ГУМУДАВЕЛЛИ, Уильям ГЭРИБИАН (US), Уильям ГЭРИБИАН, Льюис ДЖЕЙМС (US), Льюис ДЖЕЙМС, Люсио Хирошя ЙОНЕМОТО (US), Люсио Хирошя ЙОНЕМОТО



**Worldwide applications**

2009 JP GN GA EP US AU RU WO

**Application RU2010137628/13A events**

2008-02-12 Priority to US2796008P

2008-02-12 Priority to US61/027,960

2009-02-12 Application filed by Марс Инкорпорейтед (US), Марс Инкорпорейтед

2012-03-20 Publication of RU2010137628A

**Info:** Patent citations (15), Cited by (23), Legal events, Similar documents, Priority and Related Applications

**External links:** Espacenet, Global Dossier, Discuss


## Claims (20)

Hide Dependent

1. A meat analogue product comprising a combination of a dry component, a liquid, a source of a monovalent cationic carbonate or bicarbonate and an acid component of a chemical baking powder, where a dry component having a protein content contains a source of a dry protein containing sulfur; the liquid contains water; a source of monovalent cationic carbonate or bicarbonate and the acidic component of the chemical baking powder of the test are present in a ratio of about 1: 2-1: 4 by weight; and the meat analogue product contains a plurality of grooved and detachable aligned fibers in the product.

2. The meat analogue product according to claim 1, further comprising a reducing agent.

3. The meat analogue product according to claim 1, where the dry component contains 0.4-4.8 wt.% A source of monovalent cationic carbonate or bicarbonate relative to the total weight of the dry component.

4. The meat analogue product according to claim 1, wherein the source of the dry protein containing sulfur contains gluten, soy protein, pea protein, egg white, or a mixture thereof.

5. The meat analogue product according to claim 1, wherein the dry component comprises a source of dry meat protein containing sulfur or a dry protein or fiber source obtained from whole grains, fruits, vegetables, or mixtures thereof

6. The meat analogue product according to claim 1, wherein the liquid further comprises a source of fresh or frozen meat, a source of processed meat or a mixture thereof.

7. The meat analogue product according to claim 1, wherein the liquid, the dry component, or both additionally contain one or more humectants, a fat source, a dye, a flavor enhancer, a flavoring substance, a vitamin, a mineral, an antioxidant, dried blood plasma, salt, dextrose, sorbitol, starch, soy proteins, gelatin, nitrates, phosphates or a fiber source.

8. The meat analogue product according to claim 1, wherein about 30-80% by weight of the protein content in the dry component is a source of dry protein containing sulfur.

9. The meat analogue product according to claim 1, wherein the dry component further comprises a dry carbohydrate source, wherein the dry carbohydrate source includes corn starch, corn flour, pea fiber, starch, or mixtures thereof.

10. The meat analogue product according to claim 1, wherein the meat analogue product contains about 25-60 wt.% Of total protein.

РОССИЙСКАЯ ФЕДЕРАЦИЯ

(19) **RU** (11) 2010 137 628 (13) **A**

(51) МПК
*A23L 1/00* (2006.01)

ФЕДЕРАЛЬНАЯ СЛУЖБА
ПО ИНТЕЛЛЕКТУАЛЬНОЙ СОБСТВЕННОСТИ

# (12) ЗАЯВКА НА ИЗОБРЕТЕНИЕ

(21)(22) Заявка: 2010137628/13, 12.02.2009

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ДЖЕЙМС Льюис (US),
ЙОНЕМОТО Люсио Хироши (US)

## (54) ПРОДУКТ-АНАЛОГ МЯСА

### (57) Формула изобретения

1. Продукт-аналог мяса, содержащий сочетание сухого компонента, жидкости, источника одновалентного катионного карбоната или бикарбоната и кислого компонента химического разрыхлителя теста, где сухой компонент, имеющий содержание белка, содержит источник сухого белка, содержащего серу; жидкость содержит воду; источник одновалентного катионного карбоната или бикарбоната и кислый компонент химического разрыхлителя теста присутствуют в соотношении около 1:2-1:4 по массе; и продукт-аналог мяса содержит множество покрытых бороздками и отделяемых выровненных волокон в продукте.

2. Продукт-аналог мяса по п.1, дополнительно содержащий восстанавливающий агент.

3. Продукт-аналог мяса по п.1, где сухой компонент содержит 0,4-4,8 мас.% источника одновалентного катионного карбоната или бикарбоната по отношению к общей массе сухого компонента.

4. Продукт-аналог мяса по п.1, где источник сухого белка, содержащего серу, содержит глютен, соевый белок, гороховый белок, яичный белок или их смесь.

5. Продукт-аналог мяса по п.1, где сухой компонент содержит источник сухого мясного белка, содержащего серу, или сухой белок, или источник волокон, полученный из цельного зерна, фруктов, овощей или их смесей.



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6. Продукт-аналог мяса по п.1, где жидкость дополнительно содержит источник свежего или замороженного мяса, источник переработанного в муку мяса или их смеси.

7. Продукт-аналог мяса по п.1, где жидкость, сухой компонент или оба дополнительно содержат один или более увлажнитель, источник жира, краситель, усилитель вкуса, вкусовое вещество, витамин, минерал, антиоксидант, высушенную плазму крови, соль, декстрозу, сорбит, крахмал, соевые белки, желатин, нитраты, фосфаты или источник волокон.

8. Продукт-аналог мяса по п.1, где около 30-80 мас.% содержания белка в сухом компоненте составляет источник сухого белка, содержащего серу.

9. Продукт-аналог мяса по п.1, где сухой компонент дополнительно содержит сухой источник углеводов, где сухой источник углеводов включает кукурузный крахмал, кукурузную муку, гороховые волокна, крахмал или их смеси.

10. Продукт-аналог мяса по п.1, где продукт-аналог мяса содержит около 25-60 мас.% общего белка.

11. Продукт-аналог мяса по п.1, где продукт-аналог мяса имеет pH около 6,0-7,8.

12. Продукт-аналог мяса по п.1, где продукт-аналог мяса содержит жир в количестве 0,2-20 мас.%.

13. Продукт-аналог мяса по п.1, где продукт-аналог мяса содержит около 25-65 мас.% влаги.

14. Способ получения продукта-аналога мяса, где способ предусматривает:

a) объединение источника одновалентного катионного бикарбоната или карбоната, воды и сухого компонента, содержащего источник белка, содержащего серу, в условиях смешивания с низким сдвигом с получением тестообразной массы;

b) нагревание тестообразной массы до температуры около 100-150°C с получением нагретой тестообразной массы;

c) подача нагретой тестообразной массы на входное отверстие охлаждающего устройства;

d) перемещение нагретой тестообразной массы через охлаждающее устройство под давлением 50-900 фунтов/дюйм$^2$ к выходному отверстию охлаждающего устройства с получением продукта-аналога мяса, где продукт-аналог мяса имеет температуру обработки 100°C или менее на выходном отверстии.

15. Способ по п.14, где тестообразная масса дополнительно содержит восстанавливающий агент.

16. Способ по п.14, где продукт-аналог мяса содержит множество покрытых бороздками и отделяемых выровненных волокон.

17. Способ по п.14, где нагретую тестообразную массу формируют и нагревают в устройстве обработки под давлением с нагревом.

18. Способ по п.17, где устройством для обработки под давлением с нагревом является поршневый насос, двушнековый экструдер, одношнековый экструдер, экструзионный измельчитель или перемешивающий экструдер.

19. Способ по п.18, где устройством для обработки под давлением с нагревом является одношнековый экструдер; и тестообразную массу формируют в устройстве для обработки под давлением с нагревом, подавая жидкость и сухой компонент в устройство для обработки под давлением с нагревом.

20. Продукт-аналог мяса, полученный способом по п.14.

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RU 2013762 8 A

| **Doc Code: DIST.E.FILE**<br>**Document Description: Electronic Terminal Disclaimer - Filed** | PTO/SB/25<br>PTO/SB/26<br>U.S. Patent and Trademark Office<br>Department of Commerce |
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| | |
|---|---|
| Electronic Petition Request | **TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION AND TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT** |
| Application Number | 15913090 |
| Filing Date | 06-Mar-2018 |
| First Named Inventor | Rachel Fraser |
| Attorney Docket Number | 38767-0015008 |
| Title of Invention | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

| Owner | Percent Interest |
|---|---|
| Impossible Foods Inc. | 100 % |

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

16238802 filed on 01/03/2019

15913018 filed on 03/06/2018

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

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as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:
- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

◉ Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.

○ I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.

Applicants claims the following fee status:

○ Small Entity

○ Micro Entity

◉ Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

- (●) An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application

  Registration Number 76370

- ( ) A sole inventor
- ( ) A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application
- ( ) A joint inventor; all of whom are signing this request

| | |
|---|---|
| Signature | /Kimberly M. Maize, Reg. No. 76,370/ |
| Name | Kimberly M. Maize |

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 15913090 |
| **Filing Date:** | 06-Mar-2018 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Filer:** | Kimberly M. Maize/Emily Palmer |
| **Attorney Docket Number:** | 38767-0015008 |

Filed as Large Entity

**Filing Fees for Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| STATUTORY OR TERMINAL DISCLAIMER | 1814 | 1 | 170 | 170 |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **170** |

Doc Code: DISQ.E.FILE
Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 15913090

Filing Date: 06-Mar-2018

Applicant/Patent under Reexamination: Fraser

Electronic Terminal Disclaimer filed on November 12, 2020

☒ APPROVED

**This patent is subject to a terminal disclaimer**

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 41109833 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Kimberly M. Maize/Emily Palmer |
| **Filer Authorized By:** | Kimberly M. Maize |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 12-NOV-2020 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 17:26:30 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | DA |
| Payment was successfully received in RAM | $170 |
| RAM confirmation Number | E2020ABH26279122 |
| Deposit Account | |
| Authorized User | |
| The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Terminal Disclaimer-Filed (Electronic) | eTerminal-Disclaimer.pdf | 38133<br>6a14d9ffb5bbdb890676d0063585df72621e7423 | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Fee Worksheet (SB06) | fee-info.pdf | 30595<br>297d6b0577e033c31081c7c98b1c203305d6b1df | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 68728 | | |

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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**
**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**
**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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| PATENT APPLICATION FEE DETERMINATION RECORD<br>Substitute for Form PTO-875 | Application or Docket Number<br>15/913,090 | Filing Date<br>03/06/2018 | ☐ To be Mailed |
|---|---|---|---|

**ENTITY:** ☑ LARGE ☐ SMALL ☐ MICRO

## APPLICATION AS FILED - PART I

| | (Column 1) | (Column 2) | | | |
|---|---|---|---|---|---|
| FOR | NUMBER FILED | NUMBER EXTRA | | RATE ($) | FEE ($) |
| ☐ BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | | x $100 = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | | x $460 = | |
| ☐ APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | |
| ☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | | TOTAL | |

## APPLICATION AS AMENDED - PART II

| | | (Column 1) | | (Column 2) | (Column 3) | | | |
|---|---|---|---|---|---|---|---|---|
| AMENDMENT | 11/12/2020 | CLAIMS REMAINING AFTER AMENDMENT | | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | | RATE ($) | ADDITIONAL FEE ($) |
| | Total (37 CFR 1.16(i)) | * 23 | Minus | ** 23 | = 0 | | x $100 = | 0 |
| | Independent (37 CFR 1.16(h)) | * 1 | Minus | *** 3 | = 0 | | x $480 = | 0 |
| | ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | | |
| | ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | |
| | | | | | | | TOTAL ADD'L FEE | 0 |

| | | (Column 1) | | (Column 2) | (Column 3) | | | |
|---|---|---|---|---|---|---|---|---|
| AMENDMENT | | CLAIMS REMAINING AFTER AMENDMENT | | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | | RATE ($) | ADDITIONAL FEE ($) |
| | Total (37 CFR 1.16(i)) | * | Minus | ** | = | | x $0 = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | | x $0 = | |
| | ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | | |
| | ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | |
| | | | | | | | TOTAL ADD'L FEE | |

| | |
|---|---|
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3. | SLIE |
| ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". | /TAMMY ACREE/ |
| *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". | |
| The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1. | |

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 15/913,090 | 03/06/2018 | Rachel Fraser | 38767-0015008 | 9878 |

26191 7590 02/08/2021
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

| EXAMINER | |
|---|---|
| GWARTNEY, ELIZABETH A | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1796 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 02/08/2021 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com

PTOL-90A (Rev. 04/07)

| *Office Action Summary* | **Application No.** 15/913,090 | **Applicant(s)** Fraser et al. | |
|---|---|---|---|
| | **Examiner** ELIZABETH A GWARTNEY | **Art Unit** 1796 | **AIA (FITF) Status** No |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☑ Responsive to communication(s) filed on 12 November 2020.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims***

5) ☑ Claim(s) 31-53 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 31-53 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

## DETAILED ACTION

1. The present application is being examined under the pre-AIA first to invent provisions.

2. The Amendment filed 12 November 2020 has been entered. **Claims 31-53 are pending.**

3. The objection to the drawings has been withdrawn in light of Applications submission of a replacement sheet for FIG. 2 filed 12 November 2020.

4. The previous rejections under 35 U.S.C. 112 first and second paragraph have been withdrawn in light of Applicants' amendment and remarks filed 12 November 2020.

### *Terminal Disclaimer*

5. The terminal disclaimers filed on 13 November 2020 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of **U.S. Patent No. 10,327,464, 9,826,772, 9,808,029 and 9,700,067** and any patent granted on **U.S. Application No. 15/913,090 and 16/238,802** has been reviewed and are accepted.

### *Claim Rejections - 35 USC § 103*

6. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

8. This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

9. **Claims 31-53 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Hsieh et al. (US 2012/0093994) in view of Proulx et al. ("Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model" - J. Agric. Food Chem." 2006, p. 1518-1522) as evidenced by Dinh et al. ("Effects of USDA quality grade and cooking on water-soluble precursors of beef flavor", Meat Science, Volume 146, December 2018, pp. 122-130).**

Regarding **claims 31, 34-36, 38-41, 46 and 49-53,** Hsieh et al. disclose a structured plant protein product having protein fibers that are substantially aligned and has meat-like moisture, texture, mouthfeel, flavor and color (i.e. meat-like product) comprising: (a) dry ingredients that comprise: (i) a protein component that comprises a plant-derived protein material, wherein the protein component is at an amount that is no more than about 90% by weight of the dry ingredients; (ii) a carbohydrate component at an amount that is in the range of about 2 to about 50% by weight of the dry ingredients; and (iii) a lipid component at an amount that is in the

range of about 0.1 to about 5% by weight of the dry ingredients; and (b) wet ingredients that comprise water (Abstract, [0013]). Hsieh et al. disclose wherein the protein component may also include animal meat, including beef ([0079]). Hsieh et al. disclose wherein the meat analog composition may be in the form of a chicken cutlet, ground meat, steak, sirloin tip, kebab, shredded product, chunk meat product, a strip or a nugget product ([0093]).

As evidenced by Dinh et al., beef is known to comprise; (a) the free amino acids, cysteine, cysteine, and methionine (p. 125/Table 1); and (b) the reducing sugars, glucose, glucose-6-phosphate, fructose and ribose (p. 126/Table 2).

Given Hsieh et al. disclose a structured plant protein product comprising animal meat, e.g. beef, it necessarily follows, cooking the structured plant protein product, wherein cooking includes heating to a temperature of 150ºC for about 3 to about 5 minutes, results in the production of the claimed meat associated aroma compounds.

While Hsieh et al. does not disclose the precise amount of free amino acid or reducing sugar in the added animal meat (e.g. beef) and thus the final structured plant protein product, given beef is known to comprise free amino acids including cysteine, cysteine and methionine and reducing sugars including glucose, glucose-6-phosphate, fructose and ribose, since beef would necessarily exhibit beef flavor when cooked, one of ordinary skill in the art would have adjusted to the amount of added animal meat, and therefore the amount of free amino acid and reducing sugar, to obtain the desired beef or meat-like flavor.

Hsieh et al. is silent with respect to a non-animal heme-containing protein.

Proulx et al. teach that iron deficiency is a major nutritional problem and fortification of foods with iron has been a successful strategy for improving iron content of foods (p. 1518/Introduction). Proulx et al. teach that iron bioavailability is higher from heme iron sources

because of lack of inhibition from chelating compounds (p. 1518/Introduction). Proulx et al. teach that heme iron in the human diet is almost exclusively from animal sources and its intake has been shown to have a positive correlation with iron status (p. 1520/Results and Discussion). Proulx et al. teach that the incorporation of animal sourced heme iron is often unfeasible because of economic costs and because cultural and religious barrier forbid the consumption of meat (p. 1520-1521/Results and Discussion).

Proulx et al. teach that heme iron in the human diet is generally present in animal sources as part of hemoglobin and myoglobin but is also found in many invertebrates, bacteria, fungi and in the plant kingdom (p. 1518/Introduction). Proulx et al. teach ***leghemoglobin*** is a heme protein identified in soybean root nodules (p.1518/Introduction). Proulx et al. disclose a purified leghemoglobin protein (i.e. 73% by weight in the extract – p. 1520/Results and Discussion). Proulx et al. teach that the bioavailability of hemoglobin from soy root nodules is similar to that of heme iron from animal sources (p. 1521/Results and Discussion). Proulx et al. teach using plant hemoglobin, i.e. leghemoglobin, as a heme iron source in diets that are consumed by humans (p. 1518/Introduction).

Given Proulx et al. teach that iron deficiency is a major nutritional problem and that fortification of foods with iron has been a successful strategy from improving iron content, since Proulx et al. teach that bioavailability is higher from heme iron sources including, leghemoglobin from soy root nodules, it would have been obvious to one of ordinary skill in the art to have fortified the structured plant protein product of Hsieh et al., with leghemoglobin from soy root nodules, to obtain a product with improved nutritional status, i.e. bioavailable source of iron. One of ordinary skill in the art would have added an amount of the leghemoglobin depending on the level of iron fortification desired.

Note, while **claim 31** requires "a composition comprising a heme-containing protein, wherein the heme-containing protein is free of animal heme-containing protiens" this limitation does not exclude animal heme-containing protein from the meat-like-food product.

Regarding **claims 32 and 33**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Hsieh et al. disclose that the protein component is texturized in an extruder ([0006], [0009]), since Hsieh et al. disclose the protein component includes plant protein, it follows that the structured plant protein product would comprise textured plant (i.e. vegetable) protein.

Regarding **claim 37**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Proulx et al. teach leghemoglobin from soybean, intrinsically the plant heme-containing protein would comprise an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NO: 1-26.

Regarding **claims 42-45**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Hsieh et al. also disclose wherein the structured plant protein product may comprise flavoring agents such as an animal meat flavor, yeast extract, hydrolyzed proteins, glutamic acid salts, guanylic acid salts (e.g. guanosine monophosphate – GMP) and inosinic acid salts (e.g. inosine monophosphate- IMP) ([0090]) and lipids such as corn oil, palm oil, and cottonseed oil ([0052]).

Hsieh et al. disclose wherein the structured plant protein product may comprise coloring agents, including for example, beta carotene ([0086]).

Hsieh et al. disclose wherein the structured plant protein product may comprise antioxidants, including for example, propyl gallate, alpha tocopherol and caffeic acid ([0089]).

Hsieh et al. disclose where in the structured plant protein product may comprise a pH-lowering agent, including for example, citric acid and tartaric acid ([0058]).

Regarding **claims 47 and 48**, modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Hsieh et al. disclose a structured plant protein product comprising animal meat, including beef, it necessarily follows that the product would display the raw and cooked color properties as presently claimed.

***Response to Arguments***

10. **Applicants' arguments filed 12 November 2020 have been fully considered but they are not persuasive.**

Applicants submit neither Hsieh et al. or Dinh et al. disclose or suggest, "[a] food product, comprising: a) 0.01%-5% by weight of a plant, yeast, fungal, algal, ciliate, archaeal, or bacterial heme-containing protein, **wherein the heme-containing proteins are free of animal heme-containing proteins."**

While Hsieh et al. require a meat component which would necessarily comprise an animal heme-containing protein, claim 31 does not exclude animal heme-containing protein. The recitation "wherein the heme-containing protein is free of animal heme-containing proteins" limits the requirement for a composition comprising a heme-containing protein and does not limit the claimed meat-like-food product as claimed. Note, the transitional term "comprising" is inclusive or open-ended and does not exclude additional, unrecited elements or method steps (MPEP §2111.03).

Applicants submit "Proulx does not cure the deficiencies of Hsieh and Dinh." Applicants note, "Hsieh affirmatively teaches the inclusion of animal meat to improve the animal flavor of its meat analogs." Applicants submit "the teachings of Hsieh do not support the Examiner's

assertion that it would be obvious to modify the meat analog of Hsieh to include the non-animal leghemoglobin of Proulx." Applicants argue "there would be no reason for Hsieh to modify their meat analog to contain heme from a non-animal source."

Hsieh et al. is directed to meat analog compositions ([0002]). Hsieh et al. disclose that there are people who either do not eat meat or prefer to eat less meat for health or religious reasons ([0003]). Hsieh et al. explains that vegetable proteins, therefore, play an important role in meeting recommended daily dietary requirements for protein ([0003]). Hsieh et al. disclose embodiments wherein the meat analog composition comprise both the structured protein product made from vegetable protein and an animal meat component ([0079]). Here, the motivation to add a non-animal heme protein, e.g. leghemoglobin as taught by Proulx et la., to the meat analog product of Hsieh et al. would be to maintain or elevate the iron content of the meat analog to be similar to that of meat while producing a product with less meat and more vegetable protein.

Applicants cite "Proulx Thesis" and submit "[o]ne of ordinary skill would understand from Proulx's own later teachings that the inclusion of supplemental iron can cause changes in the organoleptic qualities of a food, including causing the food to taste bed through oxidative rancidity."

While Proulx notes "[f]ortification [with] iron has a potential negative effect on organoleptic quality of maize products, in that soluble forms of iron can cause oxidative randicity", this statement is directed to maize products. Proulx acknowledges that "[i]ron should be added to the level up to which they do not change the organoleptic qualities of a food." The person of ordinary skill in the art would know how to experiment with different iron sources, including non-animal heme-containing proteins such as leghemoglobin, to determine the

optimum amount to add in a specific matrix, including the meat analog disclosed by Hsieh et al., to obtain satisfactory iron supplementation while maintaining the sensory profile of the product.

***Conclusion***

11. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELIZABETH A GWARTNEY whose telephone number is (571)270-3874. The examiner can normally be reached on M-F: 8 a.m. - 4 p.m..

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Donald (Larry) Tarazano can be reached on 571-272-1515. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ELIZABETH A. GWARTNEY
Primary Examiner
Art Unit 1796

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1796

| Index of Claims | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | Examiner | Art Unit |
| | ELIZABETH A GWARTNEY | 1796 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ✓ | Rejected | - | Cancelled | N | Non-Elected | A | Appeal |
| = | Allowed | ÷ | Restricted | I | Interference | O | Objected |

**CLAIMS**

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

| CLAIM | | DATE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Final | Original | 05/07/2020 | 02/02/2021 | | | | | | | |
| | 1 | - | - | | | | | | | |
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| | 25 | - | - | | | | | | | |
| | 26 | - | - | | | | | | | |
| | 27 | - | - | | | | | | | |
| | 28 | - | - | | | | | | | |
| | 29 | - | - | | | | | | | |
| | 30 | - | - | | | | | | | |
| | 31 | ✓ | ✓ | | | | | | | |
| | 32 | ✓ | ✓ | | | | | | | |
| | 33 | ✓ | ✓ | | | | | | | |
| | 34 | ✓ | ✓ | | | | | | | |
| | 35 | ✓ | ✓ | | | | | | | |
| | 36 | ✓ | ✓ | | | | | | | |
| | 37 | ✓ | ✓ | | | | | | | |
| | 38 | ✓ | ✓ | | | | | | | |
| | 39 | ✓ | ✓ | | | | | | | |
| | 40 | ✓ | ✓ | | | | | | | |
| | 41 | ✓ | ✓ | | | | | | | |
| | 42 | ✓ | ✓ | | | | | | | |

| Index of Claims | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | **Examiner** | **Art Unit** |
| | ELIZABETH A GWARTNEY | 1796 |

| CLAIM | | DATE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Final | Original | 05/07/2020 | 02/02/2021 | | | | | | | |
| | 43 | ✓ | ✓ | | | | | | | |
| | 44 | ✓ | ✓ | | | | | | | |
| | 45 | ✓ | ✓ | | | | | | | |
| | 46 | ✓ | ✓ | | | | | | | |
| | 47 | ✓ | ✓ | | | | | | | |
| | 48 | ✓ | ✓ | | | | | | | |
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| | 50 | ✓ | ✓ | | | | | | | |
| | 51 | ✓ | ✓ | | | | | | | |
| | 52 | ✓ | ✓ | | | | | | | |
| | 53 | ✓ | ✓ | | | | | | | |

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | Examiner | Art Unit |
| | ELIZABETH A GWARTNEY | 1796 |

| CPC - Searched* | | |
|---|---|---|
| **Symbol** | **Date** | **Examiner** |
| A23J3/227 | 05/06/2020 | EG |
| A23J3/227 | 02/02/2021 | EG |

| CPC Combination Sets - Searched* | | |
|---|---|---|
| **Symbol** | **Date** | **Examiner** |
| | | |

| US Classification - Searched* | | | |
|---|---|---|---|
| **Class** | **Subclass** | **Date** | **Examiner** |
| | | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| **Search Notes** | **Date** | **Examiner** |
| Inventor search completed | 05/05/2020 | EG |
| Review of prosecution history for US App 14797006 and 15913018 | 05/05/2020 | EG |
| A23J3/227 (text limited by heme) | 05/06/2020 | EG |
| A23J3/227 (text limited by heme) | 02/02/2021 | EG |

| Interference Search | | | |
|---|---|---|---|
| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
| | | | |

Receipt date: 11/12/2020

Sheet 1 of 1

| Substitute Disclosure Form | U.S. Department of Commerce Patent and Trademark Office | Attorney Docket No. | Application No. |
|---|---|---|---|
| **Information Disclosure Statement by Applicant** (Use several sheets if necessary) | | 38767-0015008 | 15/913,090 |
| | | Applicant: Impossible Foods Inc. | |
| (37 CFR §1.98(b)) | | Filing Date: March 6, 2018 | Group Art Unit: 1796 |

**U.S. Patent Documents**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Patentee | Filing Date If Appropriate | Class | Subclass |
|---|---|---|---|---|---|---|---|
| /E.G./ | 1. | 4098913 | 7/4/1978 | Baugher | | | |
| /E.G./ | 2. | 4197324 | 4/8/1980 | Ziminski et al. | | | |
| /E.G./ | 3. | 4324807 | 4/13/1982 | Kim et al. | | | |
| /E.G./ | 4. | 10798958 | 10/13/2020 | Varadan et al. | | | |
| /E.G./ | 5. | 20050112271 | 5/1/2005 | Picarski | | | |
| /E.G./ | 6. | 20090148558 | 6/11/2009 | Kim et al. | | | |

**Foreign Patent Documents or Published Foreign Patent Applications**

| Examiner Initial | Desig. ID | Document Number | Publication Date | Country or Patent Office | Translation | Class | Subclass |
|---|---|---|---|---|---|---|---|
| /E.G./ | 7. | RU 2010137628 | 3/20/2012 | RU | Abstract | | |

**Other Documents (include Author, Title, Date, and Place of Publication)**

| Examiner Initial | Desig. ID | Document |
|---|---|---|

| Examiner Signature | Date Considered |
|---|---|
| /ELIZABETH GWARTNEY/ | 02/02/2021 |

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

Receipt date: 11/12/2020

Attorney Docket No.: 38767-0015008

/E.G./

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1796 |
| Application No. | : | 15/913,090 | Examiner | : | Elizabeth A. Gwartney |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| 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 documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

This statement is being filed after a first action on the merits, but before receipt of a final action or a notice of allowance.

Apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) 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: November 12, 2020

/Kimberly M. Maize, Reg. No. 76,370/
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Reg. No. 76,370

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Fish & Richardson P.C.
Telephone: +1 (612) 638-5707
Facsimile: (877) 769-7945

62463957.doc

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| S1 | 1 | "15913090" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2020/05/05 12:26 |
| S2 | 9 | "20120093994" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2020/05/06 10:51 |
| S3 | 9 | "20120093994" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2020/05/06 12:27 |
| S4 | 46 | A23J3/227.cpc. AND heme | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2020/05/06 14:40 |
| S5 | 9 | "20120093994" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2021/02/01 12:50 |
| S6 | 10 | "2015134865" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2021/02/02 11:32 |
| S7 | 61 | A23J3/227.cpc. AND heme | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | OFF | 2021/02/02 12:57 |
| S8 | 61 | A23J3/227.cpc. AND heme | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/02/02 12:57 |

**EAST Search History (Interference)**

<This search history is empty>

2/2/2021 6:54:13 PM
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PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

| PATENT APPLICATION FEE DETERMINATION RECORD<br>Substitute for Form PTO-875 | Application or Docket Number<br>15/913,090 | Filing Date<br>03/06/2018 | ☐ To be Mailed |
|---|---|---|---|

**ENTITY:** ☑ LARGE ☐ SMALL ☐ MICRO

## APPLICATION AS FILED - PART I

| | (Column 1) | (Column 2) | | | |
|---|---|---|---|---|---|
| FOR | NUMBER FILED | NUMBER EXTRA | | RATE ($) | FEE ($) |
| ☐ BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | | x $100 = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | | x $460 = | |
| ☐ APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | |
| ☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | | TOTAL | |

## APPLICATION AS AMENDED - PART II

| | | (Column 1) | | (Column 2) | (Column 3) | | | |
|---|---|---|---|---|---|---|---|---|
| AMENDMENT | 04/08/2021 | CLAIMS REMAINING AFTER AMENDMENT | | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | | RATE ($) | ADDITIONAL FEE ($) |
| | Total (37 CFR 1.16(i)) | * 23 | Minus | ** 23 | = 0 | | x $100 = | 0 |
| | Independent (37 CFR 1.16(h)) | * 2 | Minus | *** 3 | = 0 | | x $480 = | 0 |
| | ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | | |
| | ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | |
| | | | | | | | TOTAL ADD'L FEE | 0 |

| | | (Column 1) | | (Column 2) | (Column 3) | | | |
|---|---|---|---|---|---|---|---|---|
| AMENDMENT | | CLAIMS REMAINING AFTER AMENDMENT | | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | | RATE ($) | ADDITIONAL FEE ($) |
| | Total (37 CFR 1.16(i)) | * | Minus | ** | = | | x $0 = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | | x $0 = | |
| | ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | | |
| | ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | |
| | | | | | | | TOTAL ADD'L FEE | |

| | |
|---|---|
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3. | SLIE |
| ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". | /TONYA Q MCBRIDE/ |
| *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". | |
| The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1. | |

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

Attorney Docket No.: 38767-0015008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | | |
|---|---|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Art Unit | : | 1796 |
| Application No. | : | 15/913,090 | Examiner | : | Elizabeth A. Gwartney |
| Filed | : | March 6, 2018 | Conf. No. | : | 9878 |
| Title | : | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES | | | |

**MAIL STOP AF**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND REPLY TO ACTION OF FEBRUARY 8, 2021
FILED WITH REQUEST UNDER AFCP 2.0

This reply is in response to the Office Action dated February 8, 2021, with a three-month shortened statutory period set to expire May 8, 2021.

AMENDMENTS TO THE CLAIMS

Listing of Claims

1-30. (Cancelled)

31. (Currently Amended) A meat-like-food product produced by a method comprising:

providing one or more plant proteins; and

combining, with the one or more plant proteins:

a composition comprising a heme-containing protein, ~~wherein the heme-containing protein is free of animal heme-containing proteins and~~ wherein the composition comprises at least 60% of the heme-containing protein on a dry weight basis;

a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof; and

a compound selected from cysteine, cystine, thiamine, methionine, and mixtures of two or more thereof in to a concentration of at least 1.5 mM;

wherein the meat-like food product is free of animal heme-containing protein;

wherein cooking the meat-like-food-product results in the production of at least two volatile compounds which have a meat-associated aroma; and

wherein the meat-like food product is in a form selected from the group consisting of a hotdog, a burger, ground meat, a sausage, a steak, a filet, a roast, a meatball, a meatloaf, or bacon.

32. (Currently Amended) The meat-like-food product of claim 31, wherein the one or more plant proteins ~~comprises~~ comprise a textured vegetable protein.

33. (Currently Amended) The meat-like-food product of claim [[31]] 32, wherein the ~~texture~~ textured vegetable protein is textured soy protein.

34. (Previously Presented) The meat-like-food product of claim 31, wherein the source of the heme-containing protein comprises at least 70% of the heme-containing protein on a dry weight basis.

35. (Previously Presented) The meat-like-food product of claim 31, wherein the source of the heme-containing protein comprises at least 80% of the heme-containing protein on a dry weight basis.

36. (Previously Presented) The meat-like-food product of claim 31, wherein the heme-containing protein is a globin.

37. (Previously Presented) The meat-like-food product of claim 31, wherein the heme-containing protein comprises an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs. 1-26.

38. (Cancelled)

39. (Cancelled)

40. (Previously Presented) The meat-like-food product of claim 31, wherein the cysteine is added to a concentration of about 0.8 mM to about 10 mM.

41. (Previously Presented) The meat-like-food product of claim 31, wherein the thiamine is added to a concentration of about 0.1 mM to about 2 mM.

42. (Currently Amended) The meat-like-food product of claim 31, further comprising ~~adding~~ combining, with the one or more plant proteins, one or more of inosine, inosine monophosphate (IMP), guanosine, guanosine monophosphate (GMP), and adenosine monophosphate (AMP).

43. (Currently Amended) The meat-like-food product of claim 31, further comprising ~~adding~~ combining with the one more plant proteins, one or more of beta-carotene, alpha-tocopherol, caffeic acid, propyl gallate, and epigallocatechin gallate.

44. (Currently Amended) The meat-like-food product of claim 31, further comprising ~~adding~~ combining with the one more plant proteins, one or more of a vegetable oil, an 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, canola oil, flaxseed oil, coconut oil, and mango oil.

45. (Currently Amended) The meat-like-food product of claim 31, further comprising ~~adding~~ combining with the one more plant proteins, one or more of acetic acid, lactic acid, glycolic acid, citric acid, succinic acid, tartaric acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, and erucic acid.

46. (Previously Presented) The meat-like-food product of claim 31, wherein cooking comprises heating the meat-like food product at 150°C for about 3 to about 5 minutes.

47. (Previously Presented) The meat-like-food product of claim 31, wherein the meat-like food product has a pink to red color before cooking to indicate a raw or uncooked state.

48. (Previously Presented) The meat-like-food product of claim 31, wherein at least a portion of the meat-like food product, upon cooking, transitions in color from a pink to red color in a raw or uncooked state to a lighter pink to brown color in a partially cooked to fully cooked state.

49. (Currently Amended) The meat-like-food product of claim 31, wherein cooking the meat-like-food product results in the production of at least five volatile compounds selected from the group consisting of (1-ethyl-1-propenyl)-benzene, (E)-2-decenal, (E)-2-heptenal, (E)-2-

hexenal, (E)-2-nonenal, (E)-2-octen-1-ol, (E)-2-octenal, (E)-2-tridecen-1-ol, (E)-3-penten-2-one, (E)-4-octene, (E)-5-decene, (E,E)-3,5-octadien-2-one, (Z)-2-decenal, (Z)-2-heptenal, (Z)-3-decen-1-ol, acetate, [(methylsulfonyl)methyl]-benzene, 1-(ethylthio)-2-(methylthio)-buta-1,3-diene, 1-(methylthio)-propane, 1,1'-(1,2-cyclobutanediyl)bis-cis-benzene, 1,10-undecadiene, 1,3,5-cycloheptatriene, 1,3-bis(1,1-dimethylethyl)-benzene, 1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane, 1,3-dimethyl-benzene, 1-decen-3-one, 1-heptanol, 1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene, 1-hexanol, 1-hydroxy-2-propanone, 1-nonanol, 1-octanol, 1-octen-3-ol, 1-octen-3-one, 1-octene, 1-pentanol, 1-penten-3-one, 1-undecanol, 2,2,4,6,6-pentamethyl-heptane, 2,3,4-trimethyl-pentane, 2,3-butanedione, 2,3-dihydro-5-methyl-furan, 2,3-dimethyl-pyrazine, 2,4-decadienal, 2,4-dimethyl-1-heptene, 2,5-dimethyl-pyrazine, 2-acetatyl-1-propene, 2-acetyl-propen-2-ol, 2-acetylthiazole, 2-butanone, 2-butenal, 2-butyl-1-decene, 2-decanone, 2-decenal, 2-ethyl-5-methyl-pyrazine, 2-ethylacrolein, 2-ethyl-furan, 2-heptanone, 2-heptenal, 2-hexyl furan, 2-methyl-1(H)-pyrrole, 2-methyl-1-heptene, 2-methyl-1-penten-1-one, 2-methyl-3-(methylthio) furan, 2-methyl-3-furanthiol, 2-methyl-5-(methylthio)-furan, 2-methyl-butanal, 2-methyl-cyclopentanone, 2-methyl-furan, 2-methyl-heptane, 2-methyl-pentanal, 2-methyl-propanal, 2-methyl-thiazole, 2-n-octylfuran, 2-nonanone, 2-nonen-4-one, 2-octanone, 2-octenal, 2-pentanone, 2-pentyl-furan, 2-pentyl-thiophene, 2-phenylpropenal, 2-propenal, 2-propenoic acid, methyl ester, 2-thiophenecarboxaldehyde, 2-tridecen-1-ol, 2-undecanone, 2-undecenal, 3-(methylthio)-propanenitrile, 3-(phenylmethyl)-2,5-piperazinedione, 3,3'-dithiobis-2-methyl-furan, 3,5-bis(1,1-dimethylethyl)-phenol, 3,5-di-tert-butylbenzoic acid, 3-decen-2-one, 3-ethylcyclopentanone, 3-ethyl-pentane, 3-methyl-2-butenal, 3-methyl-2-thiophenecarboxaldehyde, 3-methyl-3-buten-2-one, 3-methyl-butanal, 3-methyl-furan, 3-methylheptyl acetate, 3-methyl-pentanal, 3-pentyl-furan, 3-phenyl-furan, 3-thiophenecarboxaldehyde, 3-thiophenemethanol, 4,7-dimethyl-undecane, 4-chloro-2,6-bis(1,1-dimethylethyl)-phenol, 4-ethoxy-benzoic acid, ethyl ester, 4-ethyl-2-methyl-pyrrole, 4-ethyl-benzaldehyde, 4-methyl-2-heptanone, 4-methyl-5-thiazoleethanol, 4-methylthiazole, 4-nitrophenyl 2-thiophenecarboxylic acid ester, 4-penten-2-one, 4-quinolinecarboxaldehyde, 5-(2-chloroethyl)-4-methylthiazole, 5-ethenyl-4-methyl-thiazole, 5-ethyldihydro-2(3H)-furanone, 5-formyl-4-methylthiazole, 5-methyl-1-undecene, 6-methyl-2-heptanone, 7-methyl-(E)-5-undecene, 9-octadecenal, acetaldehyde, acetic acid, acetone, acetonitrile, acetophenone, acetyl

valeryl, aminophenylacetylene, benzaldehyde, benzene, benzoic acid, benzyl alcohol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, butanal, butanone, butyrolactone, carbon disulfide, decanal, dihydro-3-(2H)-thiophenone, dihydro-5-pentyl-2(3H)-furanone, dimethyl selenone, dimethyl sulfide, dimethyl trisulfide, dodecanal, ethanol, ethyl acetate, formic acid, heptyl ester, furan, furfural, heptanal, hexanoic acid, isopropyl alcohol, isothiazole, isothiocyanato-methane, m-aminophenylacetylene, methacrolein, methanethiol, methional, methyl (methylthio)methyl disulfide, methyl isopropyl disulphide, methyl thiolacetate, methyl vinyl ketone, methyl-pyrazine, methyl-thiirane, nonanal, octanal, octane, oxalic acid, diallyl ester, oxalic acid, isobutyl pentyl ester, ~~p-Cresol,~~ p-cresol, p-dithiane-2,5-diol, pentanal, pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester, phenol, phenylacetaldehyde, propanal, propyl-cyclopropane, p-xylene, pyrazine, pyrrole, styrene, thiazole, thiocyanic acid, methyl ester, thiophene, toluene, trans-2-(2-pentenyl)furan, and tridecane.

50. (Currently Amended) The meat-like food product of claim 31, wherein cooking the meat-like food product results in the production of at least ten volatile compounds selected from the group consisting of (1-ethyl-1-propenyl)-benzene, (E)-2-decenal, (E)-2-heptenal, (E)-2-hexenal, (E)-2-nonenal, (E)-2-octen-1-ol, (E)-2-octenal, (E)-2-tridecen-1-ol, (E)-3-penten-2-one, (E)-4-octene, (E)-5-decene, (E,E)-3,5-octadien-2-one, (Z)-2-decenal, (Z)-2-heptenal, (Z)-3-decen-1-ol, acetate, [(methylsulfonyl)methyl]-benzene, 1-(ethylthio)-2-(methylthio)-buta-1,3-diene, 1-(methylthio)-propane, 1,1'-(1,2-cyclobutanediyl)bis-cis-benzene, 1,10-undecadiene, 1,3,5-cycloheptatriene, 1,3-bis(1,1-dimethylethyl)-benzene, 1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane, 1,3-dimethyl-benzene, 1-decen-3-one, 1-heptanol, 1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene, 1-hexanol, 1-hydroxy-2-propanone, 1-nonanol, 1-octanol, 1-octen-3-ol, 1-octen-3-one, 1-octene, 1-pentanol, 1-penten-3-one, 1-undecanol, 2,2,4,6,6-pentamethyl-heptane, 2,3,4-trimethyl-pentane, 2,3-butanedione, 2,3-dihydro-5-methyl-furan, 2,3-dimethyl-pyrazine, 2,4-decadienal, 2,4-dimethyl-1-heptene, 2,5-dimethyl-pyrazine, 2-acetatyl-1-propene, 2-acetyl-propen-2-ol, 2-acetylthiazole, 2-butanone, 2-butenal, 2-butyl-1-decene, 2-decanone, 2-decenal, 2-ethyl-5-methyl-pyrazine, 2-ethylacrolein, 2-ethyl-furan, 2-heptanone, 2-heptenal, 2-hexyl furan, 2-methyl-1(H)-pyrrole, 2-methyl-1-heptene, 2-methyl-1-penten-1-one, 2-methyl-3-(methylthio) furan, 2-methyl-3-furanthiol, 2-methyl-5-(methylthio)-furan, 2-methyl-butanal, 2-

methyl-cyclopentanone, 2-methyl-furan, 2-methyl-heptane, 2-methyl-pentanal, 2-methyl-propanal, 2-methyl-thiazole, 2-n-octylfuran, 2-nonanone, 2-nonen-4-one, 2-octanone, 2-octenal, 2-pentanone, 2-pentyl-furan, 2-pentyl-thiophene, 2-phenylpropenal, 2-propenal, 2-propenoic acid, methyl ester, 2-thiophenecarboxaldehyde, 2-tridecen-1-ol, 2-undecanone, 2-undecenal, 3-(methylthio)-propanenitrile, 3-(phenylmethyl)-2,5-piperazinedione, 3,3'-dithiobis-2-methyl-furan, 3,5-bis(1,1-dimethylethyl)-phenol, 3,5-di-tert-butylbenzoic acid, 3-decen-2-one, 3-ethylcyclopentanone, 3-ethyl-pentane, 3-methyl-2-butenal, 3-methyl-2-thiophenecarboxaldehyde, 3-methyl-3-buten-2-one, 3-methyl-butanal, 3-methyl-furan, 3-methylheptyl acetate, 3-methyl-pentanal, 3-pentyl-furan, 3-phenyl-furan, 3-thiophenecarboxaldehyde, 3-thiophenemethanol, 4,7-dimethyl-undecane, 4-chloro-2,6-bis(1,1-dimethylethyl)-phenol, 4-ethoxy-benzoic acid, ethyl ester, 4-ethyl-2-methyl-pyrrole, 4-ethyl-benzaldehyde, 4-methyl-2-heptanone, 4-methyl-5-thiazoleethanol, 4-methylthiazole, 4-nitrophenyl 2-thiophenecarboxylic acid ester, 4-penten-2-one, 4-quinolinecarboxaldehyde, 5-(2-chloroethyl)-4-methylthiazole, 5-ethenyl-4-methyl-thiazole, 5-ethyldihydro-2(3H)-furanone, 5-formyl-4-methylthiazole, 5-methyl-1-undecene, 6-methyl-2-heptanone, 7-methyl-(E)-5-undecene, 9-octadecenal, acetaldehyde, acetic acid, acetone, acetonitrile, acetophenone, acetyl valeryl, aminophenylacetylene, benzaldehyde, benzene, benzoic acid, benzyl alcohol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, butanal, butanone, butyrolactone, carbon disulfide, decanal, dihydro-3-(2H)-thiophenone, dihydro-5-pentyl-2(3H)-furanone, dimethyl selenone, dimethyl sulfide, dimethyl trisulfide, dodecanal, ethanol, ethyl acetate, formic acid, heptyl ester, furan, furfural, heptanal, hexanoic acid, isopropyl alcohol, isothiazole, isothiocyanato-methane, m-aminophenylacetylene, methacrolein, methanethiol, methional, methyl (methylthio)methyl disulfide, methyl isopropyl disulphide, methyl thiolacetate, methyl vinyl ketone, methyl-pyrazine, methyl-thiirane, nonanal, octanal, octane, oxalic acid, diallyl ester, oxalic acid, isobutyl pentyl ester, ~~p-Cresol,~~ p-cresol, p-dithiane-2,5-diol, pentanal, pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester, phenol, phenylacetaldehyde, propanal, propyl-cyclopropane, p-xylene, pyrazine, pyrrole, styrene, thiazole, thiocyanic acid, methyl ester, thiophene, toluene, trans-2-(2-pentenyl)furan, and tridecane.

51. (Currently Amended) The meat-like-food product of claim 31, wherein cooking the meat-like-food product results in the production of at least twenty volatile compounds selected from the group consisting of (1-ethyl-1-propenyl)-benzene, (E)-2-decenal, (E)-2-heptenal, (E)-2-hexenal, (E)-2-nonenal, (E)-2-octen-1-ol, (E)-2-octenal, (E)-2-tridecen-1-ol, (E)-3-penten-2-one, (E)-4-octene, (E)-5-decene, (E,E)-3,5-octadien-2-one, (Z)-2-decenal, (Z)-2-heptenal, (Z)-3-decen-1-ol, acetate, [(methylsulfonyl)methyl]-benzene, 1-(ethylthio)-2-(methylthio)-buta-1,3-diene, 1-(methylthio)-propane, 1,1'-(1,2-cyclobutanediyl)bis-cis-benzene, 1,10-undecadiene, 1,3,5-cycloheptatriene, 1,3-bis(1,1-dimethylethyl)-benzene, 1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane, 1,3-dimethyl-benzene, 1-decen-3-one, 1-heptanol, 1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene, 1-hexanol, 1-hydroxy-2-propanone, 1-nonanol, 1-octanol, 1-octen-3-ol, 1-octen-3-one, 1-octene, 1-pentanol, 1-penten-3-one, 1-undecanol, 2,2,4,6,6-pentamethyl-heptane, 2,3,4-trimethyl-pentane, 2,3-butanedione, 2,3-dihydro-5-methyl-furan, 2,3-dimethyl-pyrazine, 2,4-decadienal, 2,4-dimethyl-1-heptene, 2,5-dimethyl-pyrazine, 2-acetatyl-1-propene, 2-acetyl-propen-2-ol, 2-acetylthiazole, 2-butanone, 2-butenal, 2-butyl-1-decene, 2-decanone, 2-decenal, 2-ethyl-5-methyl-pyrazine, 2-ethylacrolein, 2-ethyl-furan, 2-heptanone, 2-heptenal, 2-hexyl furan, 2-methyl-1(H)-pyrrole, 2-methyl-1-heptene, 2-methyl-1-penten-1-one, 2-methyl-3-(methylthio) furan, 2-methyl-3-furanthiol, 2-methyl-5-(methylthio)-furan, 2-methyl-butanal, 2-methyl-cyclopentanone, 2-methyl-furan, 2-methyl-heptane, 2-methyl-pentanal, 2-methyl-propanal, 2-methyl-thiazole, 2-n-octylfuran, 2-nonanone, 2-nonen-4-one, 2-octanone, 2-octenal, 2-pentanone, 2-pentyl-furan, 2-pentyl-thiophene, 2-phenylpropenal, 2-propenal, 2-propenoic acid, methyl ester, 2-thiophenecarboxaldehyde, 2-tridecen-1-ol, 2-undecanone, 2-undecenal, 3-(methylthio)-propanenitrile, 3-(phenylmethyl)-2,5-piperazinedione, 3,3'-dithiobis-2-methyl-furan, 3,5-bis(1,1-dimethylethyl)-phenol, 3,5-di-tert-butylbenzoic acid, 3-decen-2-one, 3-ethylcyclopentanone, 3-ethyl-pentane, 3-methyl-2-butenal, 3-methyl-2-thiophenecarboxaldehyde, 3-methyl-3-buten-2-one, 3-methyl-butanal, 3-methyl-furan, 3-methylheptyl acetate, 3-methyl-pentanal, 3-pentyl-furan, 3-phenyl-furan, 3-thiophenecarboxaldehyde, 3-thiophenemethanol, 4,7-dimethyl-undecane, 4-chloro-2,6-bis(1,1-dimethylethyl)-phenol, 4-ethoxy-benzoic acid, ethyl ester, 4-ethyl-2-methyl-pyrrole, 4-ethyl-benzaldehyde, 4-methyl-2-heptanone, 4-methyl-5-thiazoleethanol, 4-methylthiazole, 4-nitrophenyl 2-thiophenecarboxylic acid ester, 4-penten-2-one, 4-quinolinecarboxaldehyde, 5-(2-

chloroethyl)-4-methylthiazole, 5-ethenyl-4-methyl-thiazole, 5-ethyldihydro-2(3H)-furanone, 5-formyl-4-methylthiazole, 5-methyl-1-undecene, 6-methyl-2-heptanone, 7-methyl-(E)-5-undecene, 9-octadecenal, acetaldehyde, acetic acid, acetone, acetonitrile, acetophenone, acetyl valeryl, aminophenylacetylene, benzaldehyde, benzene, benzoic acid, benzyl alcohol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, butanal, butanone, butyrolactone, carbon disulfide, decanal, dihydro-3-(2H)-thiophenone, dihydro-5-pentyl-2(3H)-furanone, dimethyl selenone, dimethyl sulfide, dimethyl trisulfide, dodecanal, ethanol, ethyl acetate, formic acid, heptyl ester, furan, furfural, heptanal, hexanoic acid, isopropyl alcohol, isothiazole, isothiocyanato-methane, m-aminophenylacetylene, methacrolein, methanethiol, methional, methyl (methylthio)methyl disulfide, methyl isopropyl disulphide, methyl thiolacetate, methyl vinyl ketone, methyl-pyrazine, methyl-thiirane, nonanal, octanal, octane, oxalic acid, diallyl ester, oxalic acid, isobutyl pentyl ester, ~~p-Cresol,~~ p-cresol, p-dithiane-2,5-diol, pentanal, pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester, phenol, phenylacetaldehyde, propanal, propyl-cyclopropane, p-xylene, pyrazine, pyrrole, styrene, thiazole, thiocyanic acid, methyl ester, thiophene, toluene, trans-2-(2-pentenyl)furan, and tridecane.

52. (Currently Amended) The meat-like-food product of claim 31, wherein the at least two volatile compounds are selected from 2-methyl-furan, bis(2-methyl-3-furyl)disulfide, 2-pentyl- furan, 3,3'-dithiobis-2-methyl-furan, 2,5-dimethyl-pyrazine, 2-methyl-3-furanthiol, dihydro-3- (2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl sulfide, decanal, 5-ethyldihydro-2(3H)-furanone, dihydro-5-pentyl- 2(3H)-furanone, 2-octanone, 3,5-octadien-2-one, ~~p-Cresol,~~ p-cresol, and hexanoic acid.

53. (Previously Presented) The meat-like-food product of claim 31, wherein the at least two volatile compounds are 2-methyl-furan and bis(2-methyl-3-furyl)disulfide.

54. (New) A meat-like-food product produced by a method comprising:

providing one or more plant proteins; and

combining, with the one or more plant proteins:

a heme-containing protein;

one or more of glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose; and

one or more of cysteine, cystine, thiamine, and methionine;

wherein the meat-like food product is free of animal heme-containing protein;

wherein cooking the meat-like-food-product results in the production of at least two volatile compounds which have a meat-associated aroma; and

wherein the meat-like food product is in a form selected from the group consisting of a hotdog, a burger, ground meat, a sausage, a steak, a filet, a roast, a meatball, a meatloaf, or bacon.

55. (New) The meat-like food product of claim 54, wherein the meat-like food product is free of animal products.

## REMARKS

Claims 31-37, and 40-55 are pending, with claims 31 and 54 being independent. Claims 31-33, 42-45, and 49-52 have been amended, and claims 54 and 55 have been added. Support for the amendment to claim 31 is found at least at paragraph [0068] of the published application (US 2018/0199606). Claims 32, 33, and 42-45 have been amended for consistency with claim 31, and claims 49-52 have been amended to correct minor errors. Support for new claims 54 and 55 is found at least in claim 31 and paragraph [0068] of the published application.

A Request for Consideration under AFCP 2.0 is being filed with this paper. Applicant requests entry of these amendments and new claims, which introduce no new matter.

### *Rejections under 35 U.S.C § 103*

Claims 31-53 were rejected under pre-AIA 35 U.S.C. § 103(a) over US 2012/0093994 (Hsieh) in view of J. Agric. Food Chem. 2006, p. 1518-1522 (Proulx), as evidenced by Meat Science, Volume 146, December 2018, pp. 122-130 (Dinh).

Applicant requests withdrawal of this rejection because the proposed modification of Hsieh with Proulx as evidenced by Dinh would not yield a meat-like food product that is "free of animal heme-containing protein," as recited by amended claim 31.

Hsieh describes a meat analog composition that optionally comprises animal meat. Hsieh, [0079]. The animal meat can be "included in the protein components used in the formation of the structure protein product and/or as a constituent of the meat analog compositions in addition the structure protein product." *Id.* Although animal meat is optional in the meat analog composition of Hsieh, the Office Action predicates this rejection on the compositions of Hsieh that include meat (i.e., beef), and relies on Dinh as evidence that beef comprises free amino acids, cysteine, and methionine, as well as reducing sugars, glucose, glucose-6-phosphate, fructose, and ribose. Office Action, p. 4. Thus, while some of Hsieh's compositions do not include animal meat, reliance on the meat-free compositions of Hsieh would render moot the rejection over the meat-containing composition of Hsieh as evidenced by Dinh. Moreover, modification of Hsieh as evidenced by Proulx, relied on by the Office Action does not cure the deficiency of Hsieh as evidenced by Dinh. As such, claim 31 does not read on Hsieh in view of Proulx as evidenced by Dinh, and this rejection should be withdrawn.

Claims dependent from claim 31 are separately patentable. For example, as to claim 46, the Office Action states: "Given Hsieh et al. disclose a structured protein product comprising animal meat, e.g. beef, it necessarily follows, cooking the structured plant protein product, wherein cooking includes heating to a temperature of 150°C for about 3 to 5 minutes, results in the production of the claimed meat associated aroma compounds." Office Action, p. 4. As to claims 47 and 48, the Office Action states: "modified Hsieh et al. disclose all of the claim limitations as set forth above. Given Hsieh et al. discloses a structured plant protein product comprising animal meat, including beef, it necessarily follows that the product would display the raw and cooked color properties as presently claimed." Office Action, p. 7. Here, the claims appear to be rejected as inherently obvious. To be rejected as inherently obvious, the "inherent" limitation must necessarily be present in the combination of references. However, the Office Action provides no reasoning as to why these features would necessarily be present, and thus has not satisfied the strict standards of inherent obviousness. Absent a showing that the claimed features are necessarily present in modified Hsieh, claims 46-48 are allowable for at least this additional reason.

*New Claims*

New independent claim 54 is based on amended claim 31, with some omissions and minor edits. New claim 55 depends from claim 54 and recites: "wherein the meat-like food product is free of animal products." Claims 54 and 55 are allowable for at least the reasons discussed above with respect to claim 31.

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|---|---|---|---|
| First Named Inventor | : | Rachel Fraser | Attorney Docket: 38767-0015008 |
| Application No. | : | 15/913,090 | |
| Filed | : | March 6, 2018 | |
| Page | : | 13 of 13 | |

All claims are in condition for allowance. No fees are believed to be due. Apply any necessary charges or credits to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: April 8, 2021

/Heather L. Flanagan/
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PTO/SB/434 (05-13)

## CERTIFICATION AND REQUEST FOR CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0

| Practitioner Docket No.: | Application No.: | Filing Date: |
|---|---|---|
| 38767-0015008 | 15/913,090 | March 6, 2018 |

| First Named Inventor: | Title: |
|---|---|
| Rachel Fraser | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0 (AFCP 2.0) OF THE ACCOMPANYING RESPONSE UNDER 37 CFR 1.116.

1. The above-identified application is (i) an original utility, plant, or design nonprovisional application filed under 35 U.S.C. 111(a) [a continuing application (*e.g.*, a continuation or divisional application) is filed under 35 U.S.C. 111(a) and is eligible under (i)], or (ii) an international application that has entered the national stage in compliance with 35 U.S.C. 371(c).
2. The above-identified application contains an outstanding final rejection.
3. Submitted herewith is a response under 37 CFR 1.116 to the outstanding final rejection. The response includes an amendment to at least one independent claim, and the amendment does not broaden the scope of the independent claim in any aspect.
4. This certification and request for consideration under AFCP 2.0 is the only AFCP 2.0 certification and request filed in response to the outstanding final rejection.
5. Applicant is willing and available to participate in any interview requested by the examiner concerning the present response.
6. This certification and request is being filed electronically using the Office's electronic filing system (EFS-Web).
7. Any fees that would be necessary consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, extension of time fees, are being concurrently filed herewith. [There is no additional fee required to request consideration under AFCP 2.0.]
8. By filing this certification and request, applicant acknowledges the following:
    - Reissue applications and reexamination proceedings are not eligible to participate in AFCP 2.0.
    - The examiner will verify that the AFCP 2.0 submission is compliant, *i.e.*, that the requirements of the program have been met (see items 1 to 7 above). For compliant submissions:
        - The examiner will review the response under 37 CFR 1.116 to determine if additional search and/or consideration (i) is necessitated by the amendment and (ii) could be completed within the time allotted under AFCP 2.0. If additional search and/or consideration is required but cannot be completed within the allotted time, the examiner will process the submission consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, by mailing an advisory action.
        - If the examiner determines that the amendment does not necessitate additional search and/or consideration, or if the examiner determines that additional search and/or consideration is required and could be completed within the allotted time, then the examiner will consider whether the amendment places the application in condition for allowance (after completing the additional search and/or consideration, if required). If the examiner determines that the amendment does not place the application in condition for allowance, then the examiner will contact the applicant and request an interview.
            - The interview will be conducted by the examiner, and if the examiner does not have negotiation authority, a primary examiner and/or supervisory patent examiner will also participate.
            - If the applicant declines the interview, or if the interview cannot be scheduled within ten (10) calendar days from the date that the examiner first contacts the applicant, then the examiner will proceed consistent with current practice concerning responses after final rejection under 37 CFR 1.116.

| Signature | Date |
|---|---|
| /Heather L. Flanagan/ | April 8, 2021 |
| Name (Print/Typed) Heather L. Flanagan | Practitioner Registration No. 54,101 |

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.*

☐ * Total of _____ forms are submitted.

# Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 42407197 |
| **Application Number:** | 15913090 |
| **International Application Number:** | |
| **Confirmation Number:** | 9878 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES |
| **First Named Inventor/Applicant Name:** | Rachel Fraser |
| **Customer Number:** | 26191 |
| **Filer:** | Heather L. Flanagan/Lisa Gray |
| **Filer Authorized By:** | Heather L. Flanagan |
| **Attorney Docket Number:** | 38767-0015008 |
| **Receipt Date:** | 08-APR-2021 |
| **Filing Date:** | 06-MAR-2018 |
| **Time Stamp:** | 18:37:22 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | | 38767-0015008response.pdf | 128445<br>7b6d9178b9b8ded40845fc00124a657aa1d afdf4 | yes | 13 |

| | Multipart Description/PDF files in .zip description | | |
|---|---|---|---|
| | **Document Description** | **Start** | **End** |
| | Response After Final Action | 1 | 1 |
| | Claims | 2 | 10 |
| | Applicant Arguments/Remarks Made in an Amendment | 11 | 13 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | After Final Consideration Program Request | 38767-0015008afcp.pdf | 124632<br>b955914c36196bafac9cf4387db42ab351dcd1fe | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 253077 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**
**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**
**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

# NOTICE OF ALLOWANCE AND FEE(S) DUE

26191 7590 04/27/2021
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

| EXAMINER | |
|---|---|
| GWARTNEY, ELIZABETH A | |
| ART UNIT | PAPER NUMBER |
| 1796 | |

DATE MAILED: 04/27/2021

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 15/913,090 | 03/06/2018 | Rachel Fraser | 38767-0015008 | 9878 |

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

| APPLN. TYPE | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | UNDISCOUNTED | $1200 | $0.00 | $0.00 | $1200 | 07/27/2021 |

**THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.**

**THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.**

**HOW TO REPLY TO THIS NOTICE:**

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

**IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.**

# PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

26191 7590 04/27/2021
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

**Certificate of Mailing or Transmission**

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

| (Typed or printed name) |
|---|
| (Signature) |
| (Date) |

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 15/913,090 | 03/06/2018 | Rachel Fraser | 38767-0015008 | 9878 |

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

| APPLN. TYPE | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | UNDISCOUNTED | $1200 | $0.00 | $0.00 | $1200 | 07/27/2021 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---|---|---|
| GWARTNEY, ELIZABETH A | 1796 | 426-533000 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required) ❑Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 15/913,090 | 03/06/2018 | Rachel Fraser | 38767-0015008 | 9878 |

26191 7590 04/27/2021

FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

| EXAMINER | |
|---|---|
| GWARTNEY, ELIZABETH A | |
| ART UNIT | PAPER NUMBER |
| 1796 | |

DATE MAILED: 04/27/2021

**Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)**
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

## OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## Privacy Act Statement

**The Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

| Notice of Allowability | Application No. 15/913,090 | Applicant(s) Fraser et al. | |
|---|---|---|---|
| | Examiner ELIZABETH A GWARTNEY | Art Unit 1796 | AIA (FITF) Status No |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--***

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to After Final Amendment filed 8 April 2021.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 31-37 and 40-54 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   **Certified copies:**

   a) ☐All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   **Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

1. ☑ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☑ Other PTO-2323, A.NE.

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1796

***Notice of Allowability***

1. The present application is being examined under the pre-AIA first to invent provisions.

2. The Amendment filed After Final, 8 April 2021, has been entered. **Claims 38 and 39** have been cancelled and **claims 54 and 55** are new.

3. The rejection of claims 31-53 under 35 U.S.C. 103(a) as being unpatentable over Hsieh et al. (US 2012/0093994) in view of Proulx et al. ("Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model" - J. Agric. Food Chem.“ 2006, p. 1518-1522) as evidenced by Dinh et al. (“Effects of USD A quality grade and cooking on water-soluble precursors of beef flavor”, Meat Science, Volume 146, December 2018, pp. 122-130) has been withdrawn in light of Applicants’ amendment and remarks filed 8 April 2021.

4. **Claims 31-37 and 40-55 are allowed.**

5. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

- McConnell et al. (WO 2019/079135) teaches producing heme-containing protein from cyanobacteria for use in food products.

***Reasons for Allowance***

6. The following is an examiner’s statement of reasons for allowance:

The closest prior art, Hsieh et al. (US 2012/0093994) in combination with Proulx et al. ("Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model" - J. Agric. Food Chem.“ 2006, p. 1518-1522) teach the concept of adding leghemoglobin to a structured plant protein product to fortify with iron. While Hsieh et al. disclose that the plant protein product can comprise beef, and beef is known to comprise (a) the amino acids, cysteine, cysteine and methionine; and (b) the reducing sugars, glucose, glucose-6-phosphate, fructose and

ribose, the reference does not disclose a similar product free of animal heme-containing protein or animal products.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

***Conclusion***

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELIZABETH A GWARTNEY whose telephone number is (571)270-3874. The examiner can normally be reached on M-F: 8 a.m. - 4 p.m..

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Donald (Larry) Tarazano can be reached on 571-272-1515. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-

free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ELIZABETH A. GWARTNEY
Primary Examiner
Art Unit 1796

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1796

| ***Notice of References Cited*** | Application/Control No.<br>15/913,090 | Applicant(s)/Patent Under Reexamination<br>Fraser et al. | |
|---|---|---|---|
| | Examiner<br>ELIZABETH A GWARTNEY | Art Unit<br>1796 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number<br>Country Code-Number-Kind Code | Date<br>MM-YYYY | Name | CPC Classification | US Classification |
|---|---|---|---|---|---|---|
| | A | | | | | |
| | B | | | | | |
| | C | | | | | |
| | D | | | | | |
| | E | | | | | |
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**FOREIGN PATENT DOCUMENTS**

| * | | Document Number<br>Country Code-Number-Kind Code | Date<br>MM-YYYY | Country | Name | CPC Classification |
|---|---|---|---|---|---|---|
| | N | WO 2019079135 | 04-2019 | WIPO | McConnell et al. | |
| | O | | | | | |
| | P | | | | | |
| | Q | | | | | |
| | R | | | | | |
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**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
|---|---|---|
| | U | |
| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

| Issue Classification | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | **Examiner** | **Art Unit** |
| | ELIZABETH A GWARTNEY | 1796 |

| CPC | | | | | | |
|---|---|---|---|---|---|---|
| **Symbol** | | | | | **Type** | **Version** |
| A23L | / | 13 | / | 426 | F | 2016-08-01 |
| A23D | / | 7 | / | 0053 | I | 2013-01-01 |
| A23D | / | 7 | / | 0056 | I | 2013-01-01 |
| A23L | / | 2 | / | 66 | I | 2013-01-01 |
| A23J | / | 3 | / | 227 | I | 2013-01-01 |
| A23L | / | 29 | / | 238 | I | 2016-08-01 |
| A23L | / | 7 | / | 109 | I | 2016-08-01 |
| A23L | / | 27 | / | 10 | I | 2016-08-01 |
| A23L | / | 5 | / | 41 | I | 2016-08-01 |
| A23L | / | 33 | / | 185 | I | 2016-08-01 |
| A23J | / | 3 | / | 14 | I | 2013-01-01 |
| A23L | / | 27 | / | 26 | I | 2016-08-01 |
| A23L | / | 27 | / | 00 | I | 2016-08-01 |
| A23L | / | 13 | / | 424 | I | 2016-08-01 |
| A23L | / | 27 | / | 20 | I | 2016-08-01 |
| A23L | / | 13 | / | 42 | I | 2016-08-01 |

| CPC Combination Sets | | | | | | | | |
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| **Symbol** | | | | | **Type** | **Set** | **Ranking** | **Version** |
| A23V | / | 2002 | / | 00 | A | 1 | 1 | 2013-01-01 |
| A23V | / | 2200 | / | 15 | A | 1 | 2 | 2013-01-01 |

| NONE | | **Total Claims Allowed:** | |
|---|---|---|---|
| (Assistant Examiner) | (Date) | 23 | |
| /ELIZABETH GWARTNEY/<br>Primary Examiner, Art Unit 1796 | 21 April 2021 | O.G. Print Claim(s) | O.G. Print Figure |
| (Primary Examiner) | (Date) | 1 | 1 |

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| | 15/913,090 | Fraser et al. |
| | **Examiner** | **Art Unit** |
| | ELIZABETH A GWARTNEY | 1796 |

| INTERNATIONAL CLASSIFICATION | | | | |
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| **CLAIMED** | | | | |
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| US ORIGINAL CLASSIFICATION | |
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| **CLASS** | **SUBCLASS** |
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| CROSS REFERENCES(S) | | | | | | |
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| **CLASS** | **SUBCLASS (ONE SUBCLASS PER BLOCK)** | | | | | |
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| NONE | | **Total Claims Allowed:** | |
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| (Assistant Examiner) | (Date) | 23 | |
| /ELIZABETH GWARTNEY/<br>Primary Examiner, Art Unit 1796 | 21 April 2021 | O.G. Print Claim(s) | O.G. Print Figure |
| (Primary Examiner) | (Date) | 1 | 1 |

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| Issue Classification | Application/Control No. | Applicant(s)/Patent Under Reexamination |
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| | 15/913,090 | Fraser et al. |
| | **Examiner** | **Art Unit** |
| | ELIZABETH A GWARTNEY | 1796 |

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

**CLAIMS**

| Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| - | 1 | - | 10 | - | 19 | - | 28 | 7 | 37 | 14 | 46 | 23 | 55 | | |
| - | 2 | - | 11 | - | 20 | - | 29 | - | 38 | 15 | 47 | | | | |
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| - | 5 | - | 14 | - | 23 | 2 | 32 | 9 | 41 | 18 | 50 | | | | |
| - | 6 | - | 15 | - | 24 | 3 | 33 | 10 | 42 | 19 | 51 | | | | |
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| NONE<br>(Assistant Examiner) | (Date) | **Total Claims Allowed:**<br>23 | |
| /ELIZABETH GWARTNEY/<br>Primary Examiner, Art Unit 1796<br>(Primary Examiner) | 21 April 2021<br>(Date) | O.G. Print Claim(s)<br>1 | O.G. Print Figure<br>1 |

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| AFCP 2.0 Decision | Application No. | Applicant(s) | |
|---|---|---|---|
| | 15/913,090 | Fraser et al. | |
| | Examiner | Art Unit | AIA (FITF) Status |
| | ELIZABETH A GWARTNEY | 1796 | No |

This is in response to the After Final Consideration Pilot request filed 08 April 2021.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

   ☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

   ☐ A non-broadening amendment to at least one independent claim was not submitted.

   ☐ The request is not the first proper AFCP 2.0 request submitted in response to the most recent final rejection.

   ☐ Other: _____

2. **Proper Request**

   **A.** After final amendment submitted with the request will not be treated under AFCP 2.0.
   The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

   ☐ The after final amendment will be treated under pre-pilot procedure.

   **B.** Updated search and/or completed additional consideration.
   The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

   ☑ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

   ☐ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action . See attached interview summary for further details.

   ☐ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

   ☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

   ☐ 5. Other: _____

   Examiner Note: Please attach an interview summary when necessary as described above.

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | Examiner | Art Unit |
| | ELIZABETH A GWARTNEY | 1796 |

| CPC - Searched* | | |
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| **Symbol** | **Date** | **Examiner** |
| A23J3/227 | 05/06/2020 | EG |
| A23J3/227 | 02/02/2021 | EG |

| CPC Combination Sets - Searched* | | |
|---|---|---|
| **Symbol** | **Date** | **Examiner** |
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| US Classification - Searched* | | | |
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| **Class** | **Subclass** | **Date** | **Examiner** |
| | | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| **Search Notes** | **Date** | **Examiner** |
| Inventor search completed | 05/05/2020 | EG |
| Review of prosecution history for US App 14797006 and 15913018 | 05/05/2020 | EG |
| A23J3/227 (text limited by heme) | 05/06/2020 | EG |
| A23J3/227 (text limited by heme) | 02/02/2021 | EG |
| Google/Google Scholar search - heme containing protein or hemoglobin from bacteria, algae and fungus | 04/19/2021 | EG |

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 15/913,090 | Fraser et al. |
| | Examiner | Art Unit |
| | ELIZABETH A GWARTNEY | 1796 |

| Interference Search | | | |
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| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
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(74) Agent: **JEPSON, Suzanne**; c/o Algenol Biotech LLC, 16121 Lee Road, Suite 110, Fort Myers, FL 33912 (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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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.

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(54) Title: PRODUCTION OF HEME-CONTAINING PROTEINS IN CYANOBACTERIA

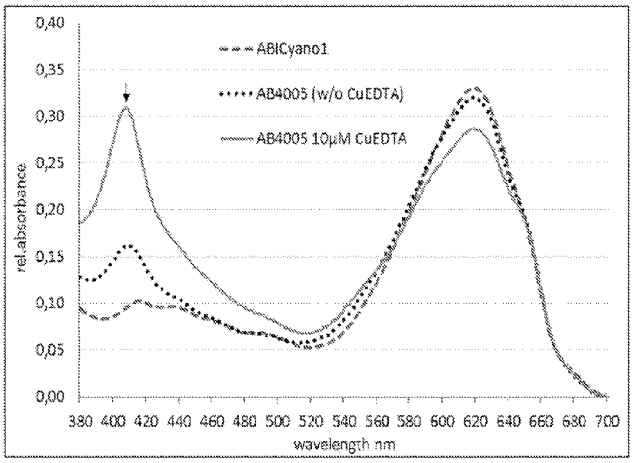



FIG. 25

(57) Abstract: Modified cyanobacterial cells that are capable of producing various heme-containing proteins (such as cyanoglobin or leghemoglobin) are described, as well as methods of producing the heme-containing proteins from the cells, and methods of use for protein isolates and food products. Further disclosed are cyanobacterial genera that can be used for producing various heme-containing proteins.

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[Continued on next page]

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).

**Published:**

— *with international search report (Art. 21(3))*
— *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))*
— *with sequence listing part of description (Rule 5.2(a))*

# TITLE

# PRODUCTION OF HEME-CONTAINING PROTEINS IN CYANOBACTERIA

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application No. 62/573,088, filed on October 16, 2017, the disclosure of which is hereby incorporated by reference.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

**[0002]** Not Applicable.

## REFERENCE TO SEQUENCE LISTING

**[0003]** This application contains a sequence listing submitted by EFS-Web, thereby satisfying the requirements of 37 C.F.R. §§ 1.821-1.825. The sequence listing, created on October 9, 2018, contains 60 sequences and is 195 KB in size.

## FIELD OF THE INVENTION

**[0004]** The present invention relates generally to the recombinant production of heme-containing proteins in cyanobacterial host cells. The heme-containing proteins can be used, for example, in meat substitute products.

## BACKGROUND OF THE INVENTION

**[0005]** A vegetarian diet is preferred by many consumers. Non-animal derived meat-like material, that tastes and looks like red meat but is made entirely of non-animal ingredients, would be a valuable consumer product. However, many vegetarian meat-like products are lacking in taste and color. Ideally, a red meat-like product would have a pleasing taste and would look and process like an actual meat product. For example, a vegetarian-based ground meat or sausage meat that is similar to real meat would be desirable.

**[0006]** Modified cyanobacteria can be used to produce many types of proteins and other products in an environmentally friendly manner. The transformation of the cyanobacterial genus *Synechococcus* with genes of interest has been described (U.S. Patent Nos. 6,699,696 and 6,306,639, both to Woods *et al.*). The transformation of the cyanobacterial genus *Synechocystis*

has been described, for example, in PCT/EP2009/000892 and in PCT/EP2009/060526. The transformation of the cyanobacterial genus *Cyanobacterium* sp. has been described (U.S. Patent No. 8,846,369, U.S. Patent No. 9,315,832, and PCT/US2013/077364).

## SUMMARY OF THE INVENTION

**[0007]** In an embodiment of the invention, a genetically modified cyanobacterium having a polynucleotide encoding a heterologous heme-containing protein is provided. The protein can be, for example, a leghemoglobin or a cyanoglobin. The polynucleotide can be, for example, codon optimized for optimal expression in the cyanobacterium, and can be linked to a constitutive or inducible (regulatable) promoter. The polynucleotide can be located on an endogenous or heterologous extrachromosomal plasmid, or can be integrated into the chromosome.

**[0008]** In an embodiment of the invention, the cyanobacterium can also have a modification that increases heme levels. This can be accomplished, for example, by a knockdown of endogenous heme oxygenase HO1. This can also be accomplished by a modification that increases the flux through ferrochelatase, such as by a knockdown of the gene encoding magnesium chelatase (MgCh), the overexpression of ferrochelatase (FeCh), the knockdown of gun4 (MgCh activator), a gun4(W192A) mutation, magnesium-starvation, iron supplementation, or nitrogen-starvation.

**[0009]** In another embodiment, the cyanobacterium can also have a modification that results in a deficiency in phycocyanin.

**[0010]** In yet another embodiment of the invention, a method of producing a heme-containing protein in a cyanobacterium is provided, by growing the genetically modified cyanobacterium under conditions to produce the heme-containing protein; and obtaining the heme-containing protein from the cyanobacterium.

**[0011]** In another embodiment of the invention, a method of producing a heme-containing protein in a cyanobacterium is provided, by growing the genetically modified cyanobacterium under conditions to produce the heme-containing protein and additionally under conditions to increase heme production in the cyanobacterium, then obtaining the heme-containing protein from the cyanobacterium.

**[0012]** In yet another embodiment of the invention, a biomass containing the genetically modified cyanobacterium is provided. In yet another embodiment of the invention, a dried meal (or bulk protein) obtained from the genetically modified cyanobacterium is provided. In a further embodiment of the invention, a partially purified heme-containing protein obtained from the genetically modified cyanobacterium is provided.

**[0013]** In a further embodiment of the invention, a food product prepared from the heme-containing protein made from the genetically modified cyanobacterium is provided. The food product can be, for example, a protein-rich product such as a meat substitute.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0014]** FIG. 1 is a map of the endogenous 6.8 kb plasmid isolated from *Cyanobacterium* sp. ABICyano1 (SEQ ID NO: 17).

**[0015]** FIG. 2 depicts a map of the 10403 BP circular plasmid construct and sequence annotation of plasmid #2884 (pAB1_6.8::PsmtA-legH_Gm(AB1opt)-TpsaB) (SEQ ID NO: 18).

**[0016]** FIG. 3 depicts a map of the 10412 BP circular plasmid construct and sequence annotation of plasmid #2885 (pAB1_6.8::PsmtA-legH_As(AB1opt)-TpsaB) (SEQ ID NO: 19).

**[0017]** FIG. 4 depicts a map of the 10406 BP circular plasmid construct and sequence annotation of plasmid # #2886 (pAB1_6.8::PsmtA-legH_Cc(AB1opt)-TpsaB) (SEQ ID NO: 20).

**[0018]** FIG. 5 depicts a map of the 10340 BP circular plasmid construct and sequence annotation of plasmid # 2887 (pAB1_6.8::PsmtA-glbN_S6803(AB1opt)-TpsaB) (SEQ ID NO: 21).

**[0019]** FIG. 6 depicts a map of the 10340 BP circular plasmid construct and sequence annotation of plasmid #2888 (pAB1_6.8::PsmtA-glbN_Ap8005(AB1opt)-TpsaB) (SEQ ID NO: 22).

**[0020]** FIG. 7 depicts a map of the 10456 BP circular plasmid construct and sequence annotation of plasmid #2889 (pAB1_6.8::PsmtA-legH_Gm(AB1opt)-TpsaB-PnirA'-anti_HO1_v1) (SEQ ID NO: 23).

**[0021]** FIG. 8 depicts a map of the 10393 BP circular plasmid construct and sequence annotation of plasmid #2890 (pAB1_6.8::PsmtA-glbN_S6803(AB1opt)-TpsaB-PnirA'-anti_HO1_v1) (SEQ ID NO: 24).

**[0022]** FIG. 9 depicts a map of the 10634 BP circular plasmid construct and sequence annotation of plasmid #2942 (pABICyano1_6.8::PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-Porf0316'-anti_HO1_v1) (SEQ ID NO: 56).

**[0023]** FIG. 10 depicts a map of the 10491 BP circular plasmid construct and sequence annotation of plasmid #2994 (pAB1_6.8::PcpcB-glbN_Ap8005(AB1opt)-TpsaB) (SEQ ID NO: 57).

**[0024]** FIG. 11 depicts a map of the 11432 BP circular plasmid construct and sequence annotation of plasmid #3022 (pABICyano1_6.8::PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-Porf0316'-anti_HO1_v1) (SEQ ID NO: 58).

**[0025]** FIG. 12 depicts a map of the 7033 BP circular plasmid construct and sequence annotation of plasmid #2949 (nblA2::PcpcB1918-glbN_Ap8005-TpsaB-PrbcL2293-ble-TB1002) (SEQ ID NO: 59).

**[0026]** FIG. 13 depicts a map of the 8060 BP circular plasmid construct and sequence annotation of plasmid #3057 (nblA2::PcpcB1918-glbN_Ap8005-PcpcB1918_glbN_Ap8005-TpsaB-PrbcL2293-ble-TB1002) (SEQ ID NO: 60).

**[0027]** FIG. 14 is a diagram of the biosynthetic pathway from glutamate to protoporphyrin, heme, chlorophyll, biliverdin, phycobilin, and leghemoglobin. Also shown is the location of inhibition of heme oxygenase (HO1) using an inducible sRNA for gene knock-down.

**[0028]** FIG. 15 is a stained protein gel showing the expression of the cyanoglobin (GlbN) protein from *Synechocystis* strain PCC 6803 in *Cyanobacterium* sp. ABICyano1. Expression of the cyanoglobin (black arrow) was induced with 20 μM zinc sulfate. Protein extracts were separated on a 16% native PAGE and stained with Coomassie Brilliant Blue.

**[0029]** FIG. 16 is a photograph of a stained protein gel showing the expression of the cyanoglobin (GlbN) protein from *Synechocystis* strain PCC 6803 in *Cyanobacterium* sp. ABICyano1 in the presence and absence of the anti-HO1 sRNA. Expression of the cyanoglobin was induced with 20 μM zinc sulfate (black arrow). Expression of the anti-HO sRNA was induced in the presence of nitrate and repressed in the presence of urea. Protein extracts were separated on a 16% native PAGE and stained with Coomassie Brilliant Blue.

**[0030]** FIG. 17 is a photograph of a stained protein gel showing the expression of the leghemoglobin C2 protein (black arrow) in *Cyanobacterium* sp. ABICyano1. Expression of leghemoglobin C2 was induced with different zinc concentrations in the presence of nitrate to knockdown expression of the heme oxygenase ABICyano1.1_orf29000 via sRNA. Protein extracts were separated by denaturing electrophoresis on a 16% Tricine-SDS-PAGE including 6 M urea and subjected to a Coomassie Brilliant Blue staining.

**[0031]** FIG. 18A and FIG. 18B show the constitutive expression of the cyanoglobin (GlbN) protein (black arrows) from *Synechocystis* strain PCC 6803 in *Cyanobacterium* sp. ABICyano1 in the presence and absence of the anti-HO1 sRNA, induced with 10 μM copper-EDTA. AB1336 has a single copy of the PcpcB-glbN_S6803, whereas AB4005 exhibits two PcpcB-glbN_S6803 copies on the same plasmid. Protein extracts were separated on a 16% native PAGE (FIG. 18A, unstained) and stained with Coomassie Brilliant Blue (FIG. 18B).

**[0032]** FIG. 19 is a line graph showing the relative absorbance spectra of *Cyanobacterium* sp. ABICyano1 and a Phycocyanin-deficient strain AB0492 (ΔcpcA).

**[0033]** FIG. 20 is a photograph of a stained protein gel showing the constitutive expression of the cyanoglobin (GlbN) protein from *Synechocystis* strain PCC 6803 in the *Cyanobacterium* sp. ABICyano1 and in a phycocyanin-deficient derivative AB0492 (ΔcpcA). The strains were grown in the absence and presence of 10 μM copper-EDTA to induce the knockdown of the heme oxygenase via anti-HO1 sRNA. AB4011 has a single copy of the PcpcB-glbN_S6803, whereas AB4012 comprises two PcpcB-glbN_S6803 copies on the same plasmid. Protein extracts were separated on a 16% native PAGE and stained with Coomassie Brilliant Blue.

**[0034]** FIG. 21 is a photograph of a stained protein gel showing the constitutive expression of the cyanoglobin (GlbN) protein from *Arthrospira platensis* PCC 8005 in *Cyanobacterium* sp. ABICyano1. Protein extracts were separated on a 16% native PAGE and stained with Coomassie Brilliant Blue.

**[0035]** FIG. 22A and FIG. 22B are photographs of a protein gel showing the expression of the cyanoglobin (GlbN) protein from *Arthrospira platensis* PCC 8005 in *Arthrospira platensis* PCC9108 (AB4041, AB4065) and the derivative strain *Arthrospira platensis* AB1298 (AB4066). AB4041 has a single copy of the PcpcB-glbN_Ap8005, whereas AB4065 and AB4066 comprise two PcpcB-glbN_Ap8005 copies on the same plasmid. Protein extracts were separated on a 16% native PAGE (FIG. 22A, unstained) and stained with Coomassie Brilliant Blue (FIG. 22B).

**[0036]** FIG. 23A is a photograph of a purification column showing the purification of the cyanoglobin (GlbN) protein from *Synechocystis* strain PCC 6803 expressed in *Cyanobacterium* sp. ABICyano1 strain AB4005 via gel filtration with a Sephacryl 100-HR column.

**[0037]** FIG. 23B is a photograph of several of the elution fractions (E1 to E14) which were collected for further analysis. The reddish heme coloration can be seen in the samples.

**[0038]** FIG. 24A and FIG. 24B are photpgraphs of protein gels showing the purification of the cyanoglobin (GlbN) protein from *Synechocystis* strain PCC 6803 expressed in *Cyanobacterium* sp. ABICyano1 strain AB4005 via gel filtration with a Sephacryl 100-HR column. Elution fractions were collected and separated on a 16% native PAGE (FIG. 24A, unstained) and stained with Coomassie Brilliant Blue (FIG. 24B). CE water-soluble cell extract, E2 – E14 elution fractions.

**[0039]** FIG. 25 is a line graph depicting the relative absorbance spectra of cell extracts from *Cyanobacterium* sp. ABICyano1 expressing the cyanoglobin (GlbN) protein from *Synechocystis* strain PCC 6803. AB4005 exhibits 2 PcpcB-glbN copies in tandem and an inducible sRNA to knockdown expression of the heme oxygenase ABICyano1.1_orf29000. AB4005 was grown in the absence and presence of 10 μM copper-EDTA to induce the anti-HO1 sRNA. The ferric form of GlbN from *Synechocystis* strain PCC 6803 has a main absorbance peak at 410 nm.

## DETAILED DESCRIPTION

**[0040]** Disclosed herein is a cyanobacterium that is capable of producing a heterologous heme-containing protein. The biomass produced from the modified cyanobacterium can be used to produce various valuable products, such as a protein-rich meal, iron-rich meal, nutritional supplements, food ingredients, or food supplements. In an embodiment, the heme-containing protein can be processed to form a meat-like material. The cyanobacterium can be grown outdoors using light, $CO_2$, and nutrients, and can then be processed as needed to produce a red meat-like material which contains a heme-containing protein. The nucleic acid encoding the heme-containing protein can be derived from, for example, an animal sequence, a plant sequence, a bacterial sequence, or a cyanobacterial sequence. The nucleic acid encoding the heme-containing protein can also be a synthetic nucleic acid sequence.

**[0041]** Aspects of the invention utilize techniques and methods common to the fields of molecular biology, microbiology and cell culture. Useful laboratory references for these types of methodologies are readily available to those skilled in the art. See, for example, Molecular Cloning: A Laboratory Manual (Third Edition), Sambrook, J., et al. (2001) Cold Spring Harbor Laboratory Press; Current Protocols in Microbiology (2007) Edited by Coico, R, et al., John Wiley and Sons, Inc.; The Molecular Biology of Cyanobacteria (1994) Donald Bryant (Ed.), Springer Netherlands; Handbook Of Microalgal Culture Biotechnology And Applied Phycology

(2003) Richmond, A.; (ed.), Blackwell Publishing; and "The cyanobacteria, molecular Biology, Genomics and Evolution", Edited by Antonia Herrero and Enrique Flores, Caister Academic Press, Norfolk, UK, 2008.

**[0042]** All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

**Definitions**

**[0043]** Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

**[0044]** The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical value/range, it modifies that value/range by extending the boundaries above and below the numerical value(s) set forth. In general, the term "about" is used herein to modify a numerical value(s) above and below the stated value(s) by a variance of 20%.

**[0045]** The term "cyanobacterium" refers to a member from the group of photoautotrophic prokaryotic microorganisms which can utilize solar energy and fix carbon dioxide. Cyanobacteria are also referred to as blue-green algae.

**[0046]** The terms "host cell" and "recombinant host cell" are intended to include a cell suitable for metabolic manipulation, e.g., which can incorporate heterologous polynucleotide sequences, e.g., which can be transformed. The term is intended to include progeny of the cell originally transformed. In particular embodiments, the cell is a prokaryotic cell, e.g., a cyanobacterial cell. The term recombinant host cell is intended to include a cell that has already been selected or engineered to have certain desirable properties and to be suitable for further genetic enhancement.

**[0047]** "Competent to express" refers to a host cell that provides a sufficient cellular environment for expression of endogenous and/or exogenous polynucleotides.

**[0048]** As used herein, the term "genetically modified" refers to any change in the endogenous genome of a wild type cell or to the addition of non-endogenous genetic code to a wild type cell, e.g., the introduction of a heterologous gene. More specifically, such changes are made by the

hand of man through the use of recombinant DNA technology or mutagenesis. The changes can involve protein coding sequences or non-protein coding sequences, including regulatory sequences such as promoters or enhancers.

**[0049]** The terms "polynucleotide" and "nucleic acid" also refer to a polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs. It will be understood that, where required by context, when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

**[0050]** The nucleic acids of this present invention may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages, charged linkages, alkylators, intercalators, pendent moieties, modified linkages, and chelators. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions.

**[0051]** The term "nucleic acid" (also referred to as polynucleotide) is also intended to include nucleic acid molecules having an open reading frame encoding a polypeptide, and can further include non-coding regulatory sequences and introns. In addition, the terms are intended to include one or more genes that map to a functional locus. In addition, the terms are intended to include a specific gene for a selected purpose. The gene can be endogenous to the host cell or can be recombinantly introduced into the host cell.

**[0052]** In one aspect the invention also provides nucleic acids which are at least 60%, 70%, 80% 90%, 95%, 99%, or 99.5% identical to the nucleic acids disclosed herein.

**[0053]** The percentage of identity of two nucleic acid sequences or two amino acid sequences can be determined using the algorithm of Thompson et al. (CLUSTALW, 1994, Nucleic Acids Research 22: 4673-4680). A nucleotide sequence or an amino acid sequence can also be used as a so-called "query sequence" to perform a search against public nucleic acid or protein sequence

databases in order, for example, to identify further unknown homologous promoters, which can also be used in embodiments of this invention. In addition, any nucleic acid sequences or protein sequences disclosed in this patent application can also be used as a "query sequence" in order to identify yet unknown sequences in public databases, which can encode for example new enzymes, which could be useful in this invention. Such searches can be performed using the algorithm of Karlin and Altschul (1990, Proceedings of the National Academy of Sciences U.S.A. 87: 2,264 to 2,268), modified as in Karlin and Altschul (1993, Proceedings of the National Academy of Sciences U.S.A. 90: 5,873 to 5,877). Such an algorithm is incorporated in the NBLAST and XBLAST programs of Altschul et al. (1990, Journal of Molecular Biology 215: 403 to 410). Suitable parameters for these database searches with these programs are, for example, a score of 100 and a word length of 12 for BLAST nucleotide searches as performed with the NBLAST program. BLAST protein searches are performed with the XBLAST program with a score of 50 and a word length of 3. Where gaps exist between two sequences, gapped BLAST is utilized as described in Altschul et al. (1997, Nucleic Acids Research, 25: 3,389 to 3,402).

**[0054]** In one aspect the invention also provides peptides, polypeptides, and proteins having an amino acid sequence that is at least 50%, 55%, 60%, 70%, 80% 90%, 95%, 99%, or 99.5% or more identical to the amino acid sequence of a protein disclosed herein.

**[0055]** Database entry numbers given in the following are for the CyanoBase, the genome database for cyanobacteria (available on the world wide web at genome.microbedb.jp/cyanobase/) Fujisawa et al. "CyanoBase: a large-scale update on its 20th anniversary. *Nucleic Acids Research*. 2017, Vol. 45, p. D551.

**[0056]** The enzyme commission numbers (EC numbers) cited throughout this patent application are numbers which are a numerical classification scheme for enzymes based on the chemical reactions which are catalyzed by the enzymes.

**[0057]** "Recombinant" refers to polynucleotides synthesized or otherwise manipulated in vitro ("recombinant polynucleotides") and to methods of using recombinant polynucleotides to produce gene products encoded by those polynucleotides in cells or other biological systems. For example, a cloned polynucleotide may be inserted into a suitable expression vector, such as a bacterial plasmid, and the plasmid can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell" or a

"recombinant bacterium" or a "recombinant cyanobacterium." The gene is then expressed in the recombinant host cell to produce, e.g., a "recombinant protein." A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

**[0058]** The term "homologous recombination" refers to the process of recombination between two nucleic acid molecules based on nucleic acid sequence similarity. The term embraces both reciprocal and nonreciprocal recombination (also referred to as gene conversion). In addition, the recombination can be the result of equivalent or non- equivalent cross-over events. Equivalent crossing over occurs between two equivalent sequences or chromosome regions, whereas nonequivalent crossing over occurs between identical (or substantially identical) segments of nonequivalent sequences or chromosome regions. Unequal crossing over typically results in gene duplications and deletions. For a description of the enzymes and mechanisms involved in homologous recombination see Court et al., "Genetic engineering using homologous recombination," Annual Review of Genetics 36:361-388; 2002.

**[0059]** The term "non-homologous or random integration" refers to any process by which DNA is integrated into the genome that does not involve homologous recombination. It appears to be a random process in which incorporation can occur at any of a large number of genomic locations.

**[0060]** The term "expressed endogenously" refers to polynucleotides that are native to the host cell and are naturally expressed in the host cell.

**[0061]** The term "operably linked" refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Thus, a polynucleotide is "operably linked to a promoter" when there is a functional linkage between a polynucleotide expression control sequence (such as a promoter or other transcription regulation sequences) and a second polynucleotide sequence (e.g., a native or a heterologous polynucleotide), where the expression control sequence directs transcription of the polynucleotide.

**[0062]** The term "vector" as used herein is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which generally refers to a circular double stranded DNA molecule into which additional DNA segments may be ligated, but also includes linear double-stranded molecules

such as those resulting from amplification by the polymerase chain reaction (PCR) or from treatment of a circular plasmid with a restriction enzyme.

**[0063]** Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., vectors having an origin of replication which functions in the host cell). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and are thereby replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply "expression vectors").

**[0064]** A "promoter" is an array of nucleic acid control sequences that direct transcription of an associated polynucleotide, which may be a heterologous or native polynucleotide. A promoter includes nucleic acid sequences near the start site of transcription, such as a polymerase binding site. The promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. The term "promoter" is intended to include a polynucleotide segment that can transcriptionally control a gene of interest, e.g., a pyruvate decarboxylase gene that it does or does not transcriptionally control in nature. In one embodiment, the transcriptional control of a promoter results in an increase in expression of the gene of interest. In an embodiment, a promoter is placed 5' to the gene of interest. A heterologous promoter can be used to replace the natural promoter, or can be used in addition to the natural promoter. A promoter can be endogenous with regard to the host cell in which it is used or it can be a heterologous polynucleotide sequence introduced into the host cell, e.g., exogenous with regard to the host cell in which it is used. Promoters of the invention may also be inducible, meaning that certain exogenous stimuli (e.g., nutrient starvation, heat shock, mechanical stress, light exposure, etc.) will induce the promoter leading to the transcription of the gene.

**[0065]** The term "recombinant nucleic acid molecule" includes a nucleic acid molecule (e.g., a DNA molecule) that has been altered, modified or engineered such that it differs in nucleotide sequence from the native or natural nucleic acid molecule from which the recombinant nucleic acid molecule was derived (e.g., by addition, deletion or substitution of one or more nucleotides). The recombinant nucleic acid molecule (e.g., a recombinant DNA molecule) also includes an isolated nucleic acid molecule or gene of the present invention.

**[0066]** The term "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

**[0067]** The term "endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

**[0068]** The term "fragment" refers to a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence substantially identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in length from at least about 6, 50, 100, 200, 500, 1,000, to about 1,500 or more consecutive nucleotides of a polynucleotide according to the invention.

**[0069]** The term "open reading frame," abbreviated as "ORF," refers to a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

**[0070]** The term "upstream" refers to a nucleotide sequence that is located 5' to reference nucleotide sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on the 5' side of a coding sequence or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

**[0071]** The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

**[0072]** The term "homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a

direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the digested fragments.

**[0073]** As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence.

**[0074]** The term "substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript.

**[0075]** The terms "restriction endonuclease" and "restriction enzyme" refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

**[0076]** The term "expression", as used herein, refers to the transcription and stable accumulation mRNA derived from a nucleic acid or polynucleotide. Expression may also refer to translation of mRNA into a protein or polypeptide.

**[0077]** An "expression cassette" or "construct" refers to a series of polynucleotide elements that permit transcription of a gene in a host cell. Typically, the expression cassette includes a promoter and a heterologous or native polynucleotide sequence that is transcribed. Expression cassettes or constructs may also include, e.g., transcription termination signals, polyadenylation signals, and enhancer elements.

**[0078]** The term "codon" refers to a triplet of nucleotides coding for a single amino acid.

**[0079]** The term "codon–anticodon recognition" refers to the interaction between a codon on an mRNA molecule and the corresponding anticodon on a tRNA molecule.

**[0080]** The term "codon bias" refers to the fact that different organisms use different codon frequencies.

**[0081]** The term "codon optimization" refers to the modification of at least some of the codons present in a heterologous gene sequence from a triplet code that is infrequently used in the host organism to a triplet code that is more common in the particular host organism. This can result in a higher expression level of the gene of interest.

**[0082]** The term "transformation" is used herein to mean the insertion of heterologous genetic material into the host cell. Typically, the genetic material is DNA on a plasmid vector, but other means can also be employed. General transformation methods and selectable markers for bacteria and cyanobacteria are known in the art (Wirth, Mol Gen Genet. 216:175-177 (1989); Koksharova, Appl Microbiol Biotechnol 58:123-137 (2002). Additionally, transformation methods and selectable markers for use in bacteria are well known (see, e.g., Sambrook et al, supra).

**[0083]** The term "selectable marker" means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, i.e., resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, spectinomycin, kanamycin, zeocin, chloramphenicol, hygromycin, and the like.

**[0084]** A "polypeptide" is a polymeric compound comprised of covalently linked amino acid residues. A "protein" is a polypeptide that performs a structural or functional role in a living cell.

**[0085]** A "heterologous protein" refers to a protein not naturally produced in the cell.

**[0086]** An "isolated polypeptide" or "isolated protein" is a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (e.g., other proteins or polypeptides, nucleic acids, carbohydrates, lipids).

**[0087]** The term "fragment" of a polypeptide refers to a polypeptide whose amino acid sequence is shorter than that of the reference polypeptide. Such fragments of a polypeptide according to the invention may have a length of at least about 2, 50, 100, 200, or 300 or more amino acids.

**[0088]** A "variant" of a polypeptide or protein is any analogue, fragment, derivative, or mutant which is derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or

post-translational modification. The skilled artisan can produce variants having single or multiple amino acid substitutions, deletions, additions, or replacements.

**[0089]** The term "primer" is an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction.

**[0090]** The term "polymerase chain reaction," also termed "PCR," refers to an in vitro method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

**[0091]** The term "sRNA" refers to small non-coding regulatory RNA (typically < 200bp), which can form a stable secondary structure and is able to bind and affect stability and translation of specific mRNAs in a targeted manner.

**[0092]** The term "globin" refers to a type of heme-containing globular protein involved in reversible binding and transporting of oxygen utilizing a heme prosthetic group. Globins incorporate a series of alpha helical segments known as globin folds.

**[0093]** The terms "heme-containing protein" or "heme-binding protein" refer to a polypeptide that can bind to a heme group. Examples of heme-containing proteins include but are not limited to hemoglobin, cyanoglobin, and leghemoglobin.

**[0094]** The term "heme" refers to a heterocyclic organic compound cofactor made up of four joined pyrrolic groups, having an iron ($Fe^{2+}$) group located in the center of the molecule. The heme iron often acts as an electron source or sink during electron transfer or redox chemistry.

**[0095]** The term "heme oxygenase" or "HO1" refers to an enzyme that catalyzes the degradation of the heme molecule to form other cellular components, such as biliverdin and phycobilins.

**[0096]** The term "selection-free" refers to a growth medium that does not include a selection agent, such as an antibiotic, that would allow only cells having a functional selectable marker gene to survive.

**[0097]** The term "plurality" means more than one.

**Heterologous Production of Heme-containing Proteins**

**[0098]** In an embodiment of the invention, cyanobacterial cells can be modified to express a heterologous heme-containing protein. The modified cells can then be cultured at a large scale, such as in outdoor photobioreactors, using $CO_2$, light, and nutrients, in order to produce large quantities of the heme-containing protein. By use of this method, heme-containing protein can be made without being obtained from animal sources.

**[0099]** Leghemoglobin is naturally synthesized in the root nodules of certain types of plants, such as leguminous plants that utilize the root nodules for nitrogen fixation. One such plant is soybean. U.S. Patent No. 8,021,695, to Gruber, describes a personal care composition (soaps, shampoos, skin care medicaments, cosmetics, and therapeutic formulations) that contains, in part, leghemoglobin extracted from nitrogen fixing root nodules. U.S. Patent Application No. 20110286992A1 describes methods of isolating the leghemoglobin from root nodules in order to prepare such compositions. The nodules have a reddish color due to the concentration of leghemoglobin. This extraction method is time consuming and expensive, however. The crop is planted and grown for a period of time, then the root nodules are collected and washed. The leghemoglobin is then isolated and purified from the washed nodules. This is a time consuming process.

**[00100]** International Patent Application Publication No. WO1998012913A1 discloses plants that have been transformed with a nucleic acid sequence that encodes a globin protein. The patent application also discloses that certain globin proteins can be produced from a host cell that is a bacterium or an algae. However, the patent application does not teach the production the protein from cyanobacteria.

**[00101]** WO/2015/153666 discloses the production of ground meat replicas that include a certain amount of a heme-containing protein, such as a leghemoglobin from soybean, pea, or cowpea. The leghemoglobin can be recombinantly produced, for example, in *E. coli* or yeast. However, there is no discussion of the recombinant production of leghemoglobin (or other globins or heme-containing proteins) in cyanobacteria.

**Production of Heme-containing Proteins in Cyanobacterial Cells**

**[00102]** In an embodiment of the invention, cyanobacterial cells can be modified to produce the heme-containing protein. In another embodiment, the cyanobacterial cell is modified with a gene encoding the heme-containing protein, as well genes encoding other enzymes involved in assembling the heme-protein complex.

**[00103]** In an embodiment of the invention, any suitable cyanobacterial strain can be modified to produce a heme-containing protein.

**[00104]** In an embodiment of the invention, the cyanobacterial host cell is *Cyanobacterium* sp. ATCC accession number PTA-13311 (herein termed "ABICyano1" or "AB1"). This strain has been found to grow well under various outdoor conditions, and can be genetically modified to produce a compound of interest. The strain, as well as its endogenous plasmid p6.8 (FIG. 1, SEQ ID NO: 17), has been described, for example, in U.S. Patent No. 8,846,369, U.S. 9,315,832, and U.S. Patent No. 9,157,101, all of which are hereby incorporated by reference in their entireties.

**[00105]** In another embodiment of the invention, the cyanobacterial host cell for production of a heme-containing protein is another cyanobacterial strain, such as *Arthrospira* sp., *Synechocystis* sp., or *Synechococcus* sp.

**[00106]** In an embodiment, a culture of the cyanobacterial cell is grown in an outdoor photobioreactor system, and the resulting cellular material is collected. The entire protein can be separated from the remaining cell contents, or the heme-containing protein itself can be separated from the cellular material. The separated protein can then be dried, frozen, or further processed as desired.

**[00107]** In an embodiment, leghemoglobin (or other heme-containing proteins) can be recombinantly produced in cyanobacteria. Using this method, $CO_2$ and inorganic nutrients can be used to convert light to heme-like proteins that can then be further processed, for example, to a meat-like material. One benefit of using cyanobacteria is that $CO_2$, rather than organic carbon-based materials, can be used as the starting material. This lowers the cost of producing the protein, as organic carbon sources are not needed.

**[00108]** Many types of heme-containing proteins can be heterologously produced in cyanobacteria. Many heme-containing proteins are found in plants.

**[00109]** One exemplary heme-containing protein that can be expressed in cyanobacteria is leghemoglobin C2 from soybean (*Glycine max*) GenBank No. AAA33980.1. The native DNA sequence is shown in SEQ ID NO: 1. The codon optimized gene sequence (codon optimized for optimal expression in *Cyanobacterium* sp. ABICyano1) is SEQ ID NO: 2. The protein sequence is SEQ ID NO: 3.

**[00110]** Another exemplary heme-containing protein that can be expressed in cyanobacteria is Leghemoglobin from *Astragalus sinicus* GenBank No. ABB13622.1. The native DNA sequence is shown in SEQ ID NO: 4. The codon optimized gene sequence (codon optimized for optimal expression in *Cyanobacterium* sp. ABICyano1) is SEQ ID NO: 5. The protein sequence is SEQ ID NO: 6.

**[00111]** Yet another exemplary heme-containing protein that can be expressed in cyanobacteria is Leghemoglobin from *Cajanus cajan* GenBank No. KYP60668.1. The native DNA sequence is shown in SEQ ID NO: 7. The codon optimized gene sequence (codon optimized for optimal expression in *Cyanobacterium* sp. ABICyano1) is SEQ ID NO: 8. The protein sequence is SEQ ID NO: 9.

**[00112]** Cyanobacterial organisms also naturally produce certain types of heme-containing proteins. Native heme-containing proteins present in cyanobacteria include, for example, leghemoglobin and cyanoglobin.

**[00113]** An example of a native cyanobacterial heme-containing protein is cyanoglobin from *Synechocystis* sp. PCC6803 (GenBank No. BAA17991.1). The native DNA sequence is shown in SEQ ID NO: 10. The codon optimized gene sequence (codon optimized for optimal expression in *Cyanobacterium* sp. ABICyano1) is SEQ ID NO: 11. The protein sequence is SEQ ID NO: 12.

**[00114]** Another example of a native cyanobacterial heme-containing protein is cyanoglobin from *Arthrospira* sp. PCC 8005 (GenBank No. CCE20200.1). The native DNA sequence is shown in SEQ ID NO: 13. The codon optimized gene sequence (codon optimized for optimal expression in *Cyanobacterium* sp. ABICyano1) is SEQ ID NO: 14. The protein sequence is SEQ ID NO: 15.

**[00115]** Cyanobacteria can be modified to add genes of interest as shown herein in order to produce heme-containing proteins. The DNA sequences encoding the genes can be amplified by polymerase chain reaction (PCR) using specific primers. The amplified PCR fragments were

digested with the appropriate restriction enzymes and cloned into either a self-replicating plasmid or an integrative plasmid. An antibiotic resistance cassette for selection of positive clones can be present on the appropriate plasmid.

**[00116]** In an embodiment, the nucleic acids of interest can be amplified from nucleic acid samples using amplification techniques. PCR can be used to amplify the sequences of the genes directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other in vitro amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, and for nucleic acid sequencing.

**[00117]** To use isolated sequences in the above techniques, recombinant DNA vectors suitable for transformation of cyanobacteria can be prepared. Techniques for transformation are well known and described in the technical and scientific literature. For example, a DNA sequence encoding one or more of the genes described herein can be combined with transcriptional and other regulatory sequences which will direct the transcription of the sequence from the gene in the transformed cyanobacteria.

**[00118]** In an embodiment, the genes of interest are inserted into the cyanobacterial chromosome. When the cell is polyploid, the gene insertions can be present in all of the copies of the chromosome, or in some of the copies of the chromosome.

**[00119]** In another embodiment, the inserted genes are present on an extrachromosomal plasmid. The extrachromosomal plasmid can be derived from an outside source, such as, for example, RSF1010-based plasmid vectors, or it can be derived from an endogenous plasmid from the cyanobacterial cell or from another species of cyanobacteria.

**[00120]** In an embodiment, the inserted genes are present on an extrachromosomal plasmid, wherein the plasmid has multiple copies per cell. The plasmid can be present, for example, at about 1, 3, 5, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or more copies per host cyanobacterial cell. In an embodiment, the plasmid containing the inserted gene is fully segregated in the cell.

**[00121]** In another embodiment, the inserted genes are present on one cassette driven by one promoter. In another embodiment, the inserted genes are present on separate plasmids, or on multiple cassettes on one plasmid.

**[00122]** In another embodiment, the inserted genes are modified for optimal expression by modifying the nucleic acid sequence to accommodate the cyanobacterial cell's protein translation system. Modifying the nucleic acid sequences in this manner can result in an increased expression of the genes.

**[00123]** Methods to modify cyanobacterial cells with genetic modifications to produce compounds of interest are also described in U.S. Patent No. 9,315,820, U.S. Patent No. 9,965,364, U.S. Patent No. 9,551,014, U.S. Patent No. 9,476,067, U.S. Patent No. 9,493,794, and U.S. Patent No. 9,353,400, all of which are incorporated by reference herein in their entireties.

**Codon Improvement of Recombinant Genes**

**[00124]** At least some of the heterologous nucleic acid sequences to be expressed in cyanobacterial host cells can be codon improved for optimal expression in the target cyanobacterial strain. The underlying rationale is that the codon usage frequency of highly expressed genes is generally correlated to the host cognate tRNA abundance. (Bulmer, Nature 325:728–730; 1987). Codon improvement (sometimes referred to as codon optimization or codon adaptation) can be performed to increase the expression level of foreign genes.

**[00125]** Codon improvement of the gene encoding the heme-containing protein can be performed for improved expression in the cyanobacterial host cell. Codon improvement can also be performed by adapting the codon usage of the at least one recombinant gene to the codon usage in *Cyanobacterium sp.*, in particular ABICyano1. In an embodiment, the G and/or C wobble bases in the codons for the amino acids in the at least one recombinant gene can be replaced by A and/or T because the G+C content of the genome of ABICyano1 is relatively low at about 36%.

**[00126]** In an embodiment, only 2% to 6% or 1% to 10% of the codons of variants of recombinant genes are codon improved. In another embodiment, highly codon improved variants of recombinant genes, at least 25%, to at least 50%, 65% or even at least 70% of the codons have been changed. In another embodiment, recombinant genes are used which are not codon improved.

**[00127]** Codon improvement of heterologously derived genes (such as genes encoding antibiotic resistance genes, and the recombinant production genes) was guided by the ABICyano1 codon usage table derived from ribosomal proteins and highly expressed genes

(such as photosynthesis genes). To improve heterologous gene expression, original sequences of interest were assessed with the online software OPTIMIZER (Puigbò P, Guzmán E, Romeu A, & Garcia-Vallvé S (2007) OPTIMIZER: a web server for optimizing the codon usage of DNA sequences *Nucleic Acids Research* 35(suppl 2):W126-W131) based on the codon-usage table derived from ABICyano1 genome.

**[00128]** The codon adaptation index is a measure of directional synonymous codon usage bias, and its potential applications, (see Nucleic Acids Research 15(3):1281-1295). The effective number of codons (see, Wright F (1990) Gene 87(1):23-29) are designed match those of highly expressed genes (such as ribosomal proteins) in the ABICyano1 genome. The resulting polynucleotides using improved codons were further modified and optimized to avoid the presence of any known or predicted putative ABICyano1 endonuclease restriction sites (*Ava*I, *Bsa*HI, *Kas*I, *Xho*I, etc.); internal Shine-Dalgarno sequence and RNA destabilizing sequences; an internal terminator sequence; and a repeat sequence of greater than about 10 bp (see, Welch et al., PLOS One 4, e7002; 2009; and Welch et al., Journal of the Royal Society; Interface 6 (Suppl 4), S467–S476; 2009).

**[00129]** In an embodiment, the selectable marker genes are also modified so that they will have improved expression in cyanobacteria. For example, the selectable marker gene that confers gentamycin or kanamycin resistance was codon optimized for higher expression in cyanobacteria. In an embodiment, as a result of codon improvement, the G+C % of the antibiotic resistance genes decreased from 40-53% to 33-40%, which is similar to that of ABICyano1 coding genes (about 36% on average). The codon adaptation index of the codon improved antibiotic resistance genes is significantly improved from less than 0.4 to greater than 0.8, which is similar to that of ABICyano1 endogenous genes. Table 1, below, depicts the codon usage statistics within the cyanobacterial strain ABICyano1.

**Table 1: Codon Usage – ABICyano1**

| Amino Acid | Codon | Fraction | Number | Frequency (/1000) |
|---|---|---|---|---|
| Ala | GCA | 0.293 | 20724 | 18.356 |
| Ala | GCC | 0.214 | 15144 | 13.414 |

| | | | | |
|---|---|---|---|---|
| Ala | GCG | 0.14 | 9870 | 8.742 |
| Ala | GCT | 0.353 | 24915 | 22.068 |
| Arg | AGA | 0.347 | 16040 | 14.207 |
| Arg | AGG | 0.09 | 4158 | 3.683 |
| Arg | CGA | 0.106 | 4886 | 4.328 |
| Arg | CGC | 0.131 | 6043 | 5.353 |
| Arg | CGG | 0.039 | 1813 | 1.606 |
| Arg | CGT | 0.288 | 13329 | 11.806 |
| Asn | AAC | 0.22 | 14609 | 12.94 |
| Asn | AAT | 0.78 | 51712 | 45.804 |
| Asp | GAC | 0.193 | 11063 | 9.799 |
| Asp | GAT | 0.807 | 46399 | 41.098 |
| Cys | TGC | 0.218 | 2501 | 2.215 |
| Cys | TGT | 0.782 | 8976 | 7.95 |
| Gln | CAA | 0.806 | 43747 | 38.749 |
| Gln | CAG | 0.194 | 10554 | 9.348 |
| Glu | GAA | 0.787 | 60690 | 53.756 |
| Glu | GAG | 0.213 | 16451 | 14.571 |
| Gly | GGA | 0.324 | 22709 | 20.114 |
| Gly | GGC | 0.125 | 8720 | 7.724 |
| Gly | GGG | 0.151 | 10542 | 9.338 |
| Gly | GGT | 0.401 | 28065 | 24.859 |
| His | CAC | 0.251 | 4859 | 4.304 |
| His | CAT | 0.749 | 14516 | 12.858 |
| Ile | ATA | 0.195 | 18334 | 16.239 |
| Ile | ATC | 0.19 | 17872 | 15.83 |

| | | | | |
|---|---|---|---|---|
| Ile | ATT | 0.616 | 57964 | 51.342 |
| Leu | CTA | 0.088 | 10776 | 9.545 |
| Leu | CTC | 0.058 | 7129 | 6.314 |
| Leu | CTG | 0.033 | 4040 | 3.578 |
| Leu | CTT | 0.116 | 14162 | 12.544 |
| Leu | TTA | 0.571 | 69559 | 61.612 |
| Leu | TTG | 0.133 | 16235 | 14.38 |
| Lys | AAA | 0.836 | 59396 | 52.61 |
| Lys | AAG | 0.164 | 11694 | 10.358 |
| Met | ATG | 1 | 20093 | 17.797 |
| Phe | TTC | 0.172 | 8420 | 7.458 |
| Phe | TTT | 0.828 | 40450 | 35.829 |
| Pro | CCA | 0.169 | 7746 | 6.861 |
| Pro | CCC | 0.275 | 12613 | 11.172 |
| Pro | CCG | 0.066 | 3012 | 2.668 |
| Pro | CCT | 0.491 | 22560 | 19.982 |
| Ser | AGC | 0.088 | 6435 | 5.7 |
| Ser | AGT | 0.306 | 22393 | 19.835 |
| Ser | TCA | 0.14 | 10217 | 9.05 |
| Ser | TCC | 0.102 | 7465 | 6.612 |
| Ser | TCG | 0.044 | 3196 | 2.831 |
| Ser | TCT | 0.321 | 23473 | 20.791 |
| Thr | ACA | 0.26 | 15649 | 13.861 |
| Thr | ACC | 0.236 | 14251 | 12.623 |
| Thr | ACG | 0.083 | 5024 | 4.45 |
| Thr | ACT | 0.42 | 25340 | 22.445 |

| | | | | |
|---|---|---|---|---|
| Trp | TGG | 1 | 14964 | 13.254 |
| Tyr | TAC | 0.187 | 7364 | 6.523 |
| Tyr | TAT | 0.813 | 31912 | 28.266 |
| Val | GTA | 0.28 | 18541 | 16.423 |
| Val | GTC | 0.117 | 7778 | 6.889 |
| Val | GTG | 0.184 | 12184 | 10.792 |
| Val | GTT | 0.419 | 27713 | 24.547 |
| End | TAA | 0.63 | 2495 | 2.23 |
| End | TAG | 0.22 | 848 | 0.76 |
| End | TGA | 0.15 | 591 | 0.53 |

**Choice of Promoters**

**[00130]** The inserted genes can be controlled by one promoter, or they can be controlled by different individual promoters. The promoters can be constitutive or regulatable. The promoters can be, for example, inducible. The promoter sequences can be derived, for example, from the host cell, from another organism, or can be synthetically derived.

**[00131]** Any desired promoter can be used to regulate the expression of the inserted genes. Exemplary promoter types include but are not limited to, for example, constitutive promoters, regulatable promoters such as inducible promoters (e.g., by nutrient source, nutrient starvation, heat shock, mechanical stress, environmental stress, metal concentration, specific metabolites, light exposure, etc.), endogenous promoters, heterologous promoters, and the like. Suitable promoter sequences are also disclosed, for example, in U.S. Patent No. 9,315, 820, U.S. Patent No. 9,551,014, PCT/EP2012/067534, U.S. Patent No. 9,476,067, U.S. Patent No. 9,157,101, PCT/US2013/077364, U.S. Patent No. 9,493,794, and PCT/US2015/000210, all of which are hereby incorporated by reference in their entireties.

**[00132]** The recombinant gene(s) can be under the transcriptional control of a constitutive promoter. In this way, a sustained level of transcription and, therefore, enzymatic activity of the corresponding protein can be maintained during the whole period of cultivation. For example, the constitutive promoter can be endogenous to the cyanobacterial cell. This has the advantage that no recombinant transcription factor has to be present in the host cell. The endogenous

promoter is usually well-recognized by the metabolically enhanced cyanobacterial cell without the need to introduce further genetic modifications.

**[00133]** Suitable constitutive promoters include, without limitation, the PrpsL promoter (Gene ID: ABICyano1_orf1758), PpsaA promoter (ABICyano1_orf3243), PpsbB (ABICyano1_orf2107), PcpcB promoter (ABICyano1_orf2472), PatpG (ABICyano1_orf1814), PrbcL promoter (ABICyano1_orf1369), and variations thereof. Further suitable endogenous constitutive promoters from genes with unknown function exhibiting appropriate transcriptional activity include, without limitation, the promoters of Gene IDs ABICyano_orf1924, ABICyano_orf1997, ABICyano_orf3446, ABICyano_orf0865, ABICyano_orf1919, ABICyano_orf3278, ABICyano_orf1181, ABICyano_orf1627, ABICyano_orf0265 and ABICyano_orf2536, ABICyano_orf0615, and variants thereof.

**[00134]** In an embodiment, the promoters can be derived from the cyanobacterial strain Cyanobacterium sp. PTA-13311, or they can be derived from another cyanobacterium or from another organism. In an embodiment, the promoters can be about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100 % identical to the promoter sequences described herein.

**[00135]** The promoters can be regulatable promoters, such as inducible promoters. For example, certain promoters are up-regulated by the presence of a compound, while other promoters can be up-regulated by the absence of a compound (also termed "repressible").

**[00136]** Various promoters that can be used include promoters that are regulatable by the presence (or in other promoters, by the absence) of inductors such as different metal ions, different nutrient sources, different metabolites, different external stimuli such as heat, cold, salinity or light. In some embodiments, the regulatable or inducible promoters are induced under conditions such as nutrient starvation, stationary growth phase, heat shock, cold shock, oxidative stress, salt stress, light, darkness, metal ions, organic chemical compounds, and combinations thereof. For example, a particularly tight control of the expression of gene can be achieved if a gene is under the transcriptional control of a Zn-, Ni-, or Co-inducible promoter. Exemplary Zn-regulatable promoters and their variants are described, for example, in International Application No. PCT/EP2013/077496. Exemplary Zn, Ni, and Co-regulatable promoters are described, for example, in International Application No. PCT/2012/076790, both of which are incorporated by reference herein in their entireties.

**[00137]** In a further embodiment, the regulatable or inducible promoter is inducible by a change of a metal-ion concentration. Such a change of metal-ion concentration includes for instance the addition or depletion of certain metal ions. Suitable inducible promoters include, without limitation, the PziaA promoter, the PsmtA promoter, PaztA promoter, the PcorT promoter, the PnrsB promoter, the PpetJ promoter, PpetE promoter, the Porf0316 promoter, the Porf0221 promoter, the Porf0223 promoter, the Porf3126 promoter, the PmntC promoter, and variations thereof.

**[00138]** Preferably, the regulatable or inducible promoter is endogenous to the cyanobacterial cell. An endogenous inducible promoter is usually well-recognized by the metabolically enhanced cyanobacterial cell without the need to introduce further genetic modifications.

**[00139]** In further embodiments, the choice of regulatable or inducible promoters can include, but are not limited to, PntcA, PnblA, PisiA, PpetJ, PggpS, PpsbA2, PsigB, PlrtA, PhtpG, PnirA, PnarB, PnrtA, PhspA, PclpB1, PhliB, PcrhC, PziaA, PsmtA, PcorT, PnrsB, PnrsB916, PaztA, PbmtA, Pbxa1, PzntA, PczrB, PnmtA, PpstS, and the like.

**[00140]** The regulatable or inducible promoter can, for instance, also be a nitrate inducible promoter. Suitable nitrate inducible promoters include, without limitation, the PnirA promoter, the PnrtA promoter, the PnarB promoter, and variations thereof.

**[00141]** In certain other preferred embodiments, truncated or partially truncated versions of these promoters including only a small portion of the native promoters upstream of the transcription start point, such as the region ranging from -35 to the transcription start can often be used. Furthermore, introducing nucleotide changes into the promoter sequence, e.g. into the TATA box, the operator sequence, 5'-untranslated region and/or the ribosomal binding site (RBS) can be used to tailor or optimize the promoter strength and/or its induction conditions, e.g. the concentration of inductor required for induction. In some preferred variants, the different inducible promoters are inducible by different metal ions.

**[00142]** The promoters hspA, clpBl, and hliB can be induced by heat shock (raising the growth temperature of the host cell culture from 30°C to 40°C), cold shock (such as, for example, reducing the growth temperature of the cell culture from 30°C to 20°C), oxidative stress (for example by adding oxidants such as hydrogen peroxide to the culture), or osmotic stress (for example by increasing the salinity). The promoter sigB can be induced by stationary

growth, heat shock, and osmotic stress. The promoters ntcA and nblA can be induced by decreasing the concentration of nitrogen in the growth medium and the promoters psaA and psbA2 can be induced by low light or high light conditions. The promoter htpG can be induced by osmotic stress and heat shock. The promoter crhC can be induced by cold shock. An increase in copper concentration can be used in order to induce the promoter petE, whereas the promoter petJ is induced by decreasing the copper concentration. Additional details of these promoters can be found, for example, in PCT/EP2009/060526, which is incorporated by reference herein in its entirety.

**[00143]** In an embodiment, the promoters of any of the above embodiments may be selected from the endogenous inducible promoters identified in *Cyanobacterium* sp. with the ATCC accession number PTA-13311 ("ABICyano1") as listed in Table 2 below, and variants thereof.

**Table 2: *Cyanobacterium* sp. ABICyano1 endogenous promoter sequences**

| Promoter | SEQ ID NO: |
|---|---|
| PnirA | 25 |
| Porf3126 (PsmtA) | 26 |
| PnrtA | 27 |
| Porf3461 (PpetJ) | 28 |
| PnarB | 29 |
| Porf1071 (PmntC) | 30 |
| Porf0221 | 31 |
| Porf0223 | 32 |
| Porf0316 | 33 |
| Porf0128 | 34 |
| Porf1486 | 35 |
| Porf3293 | 36 |
| Porf3621 | 37 |
| Porf3635 | 38 |
| Porf3164 | 39 |
| Porf1072 | 40 |
| Porf1074 | 41 |
| Porf1075 | 42 |
| Porf1542 | 43 |
| Porf1823 | 44 |
| Porf0222 | 45 |
| Porf3232 | 46 |
| Porf3749 | 47 |
| PrbcL (Prbc) | 48 |

| | |
|---|---|
| PrnpA | 49 |
| PrpsL | 50 |
| PrpoA | 51 |
| PpsaA | 52 |
| PpsbA2 | 53 |
| PpsbD | 54 |
| PcpcB | 55 |

**[00144]** In certain other preferred embodiments, truncated or partially truncated versions of these promoters including only a small portion of the native promoters upstream of the transcription start point, such as the region ranging from -35 to the transcription start can often be used. Furthermore, introducing nucleotide changes into the promoter sequence, e.g. into the TATA box, the operator sequence and/or the ribosomal binding site (RBS) can be used to tailor or optimize the promoter strength and/or its induction conditions, e.g. the concentration of inductor required for induction.

**Plasmid Vector Construction**

**[00145]** In an embodiment, the cyanobacterial strain ABICyano1 is transformed to produce the heme-containing protein. Methods for producing a genetically enhanced, non-naturally occurring cyanobacterial host cells are disclosed herein. In an embodiment, methods include introducing a recombinant nucleic acid sequence into a cyanobacterial host cell. At least one recombinant gene can be introduced into the host cells through the transformation of the host cell by an extrachromosomal plasmid. In an embodiment, the extrachromosomal plasmid can independently replicate in the host cell. In another embodiment, at least one recombinant gene can be introduced into the genome of the host cell. In yet another embodiment, at least one recombinant gene is introduced into the genome of the host cell by homologous recombination.

**[00146]** Genetically enhanced cyanobacterial cells can include further genetic enhancements such as partial deletions of endogenous genes or other recombinant genes which can increase the overall yield of the compound being produced by the host cells. Examples of such genetic enhancements are described in PCT patent publication number WO 2009/098089 A2, which is hereby incorporated by reference in its entirety.

**[00147]** In another embodiment, genetic enhancements of the genes encoding enzymes of the carbon fixation and subsequent carbohydrate metabolism can be genetically enhanced to

further increase the production of a compound of interest. Genetic enhancement targets include, but are not limited to, components of the photosystems (antennas and pigment modification), and components of the photosynthetic and respiratory electron transport systems. Genetic enhancement targets include local and global regulatory factors including, but not limited to, the two component system, sigma factors, small regulating RNAs and antisense RNAs.

**[00148]** In an embodiment, a recombinant nucleic acid sequence can be provided as part of an extrachromosomal plasmid containing cyanobacterial nucleic acid sequences in order to increase the likelihood of success for the transformation.

**[00149]** In another embodiment, the method for producing a genetically enhanced cyanobacterial cell uses an extrachromosomal plasmid derived from an endogenous plasmid of the host cell to introduce a recombinant nucleic acid sequence into the host cell. This endogenous plasmid can be, for example, an extrachromosomal plasmid derived from the 6.8 kb endogenous plasmid of ABICyano1.

**[00150]** In an embodiment, the cyanobacterial strain ABICyano1 is transformed to produce the heme-containing protein. It has been found that the use of modified endogenous plasmids improves the stability of the plasmid in the host cell. The cyanobacterial strain ABICyano1 contains three endogenous plasmids. In combination with other genotypic and phenotypic attributes, these endogenous plasmids differentiate ABICyano1 from other cyanobacterial species. One plasmid is 6,826 base pairs, another is 39,702 base pairs, and a third plasmid is 28,554 base pairs. The 6,826 bp endogenous plasmid is alternatively referred to herein as pABICyano1, p6.8 or 6.8. As disclosed herein, plasmid 6.8 has been modified *in vivo* and *in vitro* for use as a plasmid vector containing genes of interest for the production of compounds of interest. In an embodiment, a modified endogenous vector derived from p6.8 from ABICyano1was developed. The modified endogenous vector from ABICyano1 can be used to transform cyanobacteria from a broad range of genera, including ABICyano1 itself.

**[00151]** In certain embodiments, the present invention includes the p6.8 plasmid and modified vectors comprising sequences of the p6.8 plasmid. In an embodiment, the modified endogenous vector contains at least one of the following: a recombinant gene that encodes a heme-containing protein; and an origin of replication suitable for replication in ABICyano1.

**[00152]** Other suitable plasmids that can be used to carry genes of interest in several strains of cyanobacteria (such as, for example, *Synechocystis, Synechococcus* 7002 and

*Anabaena*), are the RSF1010-based shuttle plasmids (extrachromosomal broad-host vector) such as pVZ321-6 series (described, for example, in PCT/EP2009/060526 and in PCT/EP2009/000892, both of which are incorporated herein by reference in their entireties). Further, the plasmid vector described in U.S. Patent No. 9,476,067 can be utilized to carry heterologous genes of interest in many cyanobacterial species.

**[00153]** In certain embodiments, a gene coding for a replication initiation factor that binds to the origin of replication can either be present on the modified endogenous vector or can be present in the chromosomes or other extrachromosomal plasmids of ABICyano1. An origin of replication suitable for replication in ABICyano1 and the gene coding for the replication initiation factor binding to that origin of replication ensure that the modified endogenous vector can be replicated in ABICyano1.

**[00154]** In an embodiment, the nucleotide sequence of an origin of replication of the modified endogenous plasmid vector can have at least 80%, 90%, and 95% identity or can be identical to the nucleotides 3375 to 3408 of the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 17).

**[00155]** In an embodiment, the sequence of the gene coding for the replication initiation factor has at least 80%, 90%, and 95% identity or is identical to nucleotides 594 to 3779 of the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 17). In an embodiment, the gene coding for the replication initiation factor codes for a protein having at least 80%, 90%, and 95% sequence identity or is identical to the protein coded by nucleotides 594 to 3779 of the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 17) of ABICyano1. This putative initiation replication factor is thought to bind to the putative origin of replication, thereby ensuring the replication of a plasmid containing the initiation factor in ABICyano1.

**[00156]** In an embodiment, a modified endogenous plasmid vector can contain a sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% identity to the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 17). In another embodiment, the modified endogenous vector contains the entire p6.8 endogenous plasmid from ABICyano1.

**[00157]** In another embodiment, gene delivery vehicles that are developed using the endogenous 6.8 kb plasmid (or a portion of the plasmid) containing characteristic portions of the endogenous 6.8 kb plasmid may be able to be efficiently transformed into a wide range of

cyanobacteria. In an embodiment, characteristic portions of the 6.8 kb endogenous plasmid from ABICyano1 include portions that enable it to replicate in a host cell (origin of replication and replication initiation factor, for example) and can be referred to as the backbone of the endogenous 6.8 kb plasmid. Such vectors may also be able to efficiently produce heterologous proteins and other compounds of interest in cyanobacterial cultures.

**[00158]** In another embodiment, modifications starting with the backbone of the 6.8 kb endogenous plasmid from ABICyano1 are performed individually or together to increase transformation efficiency, increase the replication rate within the cell, and to increase the production of a desired product from the cyanobacterial cell. Suitable modifications include, for example, insertion of selection markers (such as antibiotic resistance genes), recombinant genes or cassettes for the production of a desired compound, and other modifications to increase the expression or stability of the plasmid in the cyanobacterial cell. In an embodiment, the invention includes cyanobacteria, e.g. ABICyano1, comprising a modified p6.8 plasmid having any of these improved characteristics.

**[00159]** In an embodiment, the modular design of the p6.8 derived vector allows complex sequence manipulation in cyanobacteria.

**[00160]** Several exemplary strains and plasmids carrying genes encoding heme-containing proteins are listed below in Table 3. The plasmid maps and SEQ ID NOs of these constructs are shown below.

**Table 3: Cyanobacterial Strains and Plasmids for Production of Heme-containing Proteins**

| Strain | Plasmid No. | Genotype | Construct Details | SEQ ID NO: | FIG. # |
|---|---|---|---|---|---|
| ABICyano 1 | p6.8 | wild-type (control) | **p6.8 endogenous plasmid from ABICyano1** | 17 | 1 |
| AB1304 | #2884 | pAB1_6.8::PsmtA-legH_Gm(AB1opt)-TpsaB | Zinc inducible expression of codon-optimized leghemoglobin C2 from *Glycine max* | 18 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| AB1305 | #2885 | pAB1_6.8::PsmtA-legH_As(AB1opt)-TpsaB | Zinc inducible expression of codon-optimized leghemoglobin from *Astragalus sinicus* | 19 | 3 |
| AB1306 | #2886 | pAB1_6.8::PsmtA-legH_Cc(AB1opt)-TpsaB | Zinc inducible expression of codon-optimized leghemoglobin from *Cajanus cajan* | 20 | 4 |
| AB1307 | #2887 | pAB1_6.8::PsmtA-glbN_S6803(AB1opt)-TpsaB | Zinc inducible expression of codon-optimized cyanoglobin from *Synechocystis* sp. PCC 6803 | 21 | 5 |
| AB1308 | #2888 | pAB1_6.8::PsmtA-glbN_Ap8005(AB1opt)-TpsaB | Zinc inducible expression of codon-optimized cyanoglobin from *Arthrospira platensis* PCC 8005 | 22 | 6 |
| AB1309 | #2889 | pAB1_6.8::PsmtA-legH_Gm(AB1opt)-TpsaB-PnirA'-anti_HO1_v1 | Zinc inducible expression of codon-optimized leghemoglobin C2 from *Glycine max* and nitrate inducible sRNA knockdown of heme oxygenase AB1.1_orf29000 | 23 | 7 |
| AB1310 | #2890 | pAB1_6.8::PsmtA-glbN_S6803(AB1opt)-TpsaB-PnirA'-anti_HO1_v1 | Zinc inducible expression of codon-optimized cyanoglobin from *Synechocystis sp.* PCC6803 and nitrate inducible sRNA knockdown of heme oxygenase AB1.1_orf29000 | 24 | 8 |
| AB0492 (UD3) | NA | ΔcpcA | PC less strain (control) | -- | -- |
| AB1336 | #2942 | pABICyano1_6.8::PcpcB-glbN_S6803(ABICyano1opt)-TpsaB- | Constitutive expression of codon-optimized cyanoglobin from *Synechocystis* sp. PCC6803 and copper inducible sRNA | 56 | 9 |

| | | | | | |
|---|---|---|---|---|---|
| | | Porf0316'-anti_HO1_v1 | knockdown of heme oxygenase ABICyano1.1_orf29000 | | |
| AB1364 | #2994 | pAB1_6.8::PcpcB-glbN_Ap8005(AB1opt)-TpsaB | Constitutive expression of codon-optimized cyanoglobin from *Arthrospira* sp. PCC 8005 | 57 | 10 |
| AB4005 | #3022 | pABICyano1_6.8::PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-Porf0316'-anti_HO1_v1 | Constitutive expression of codon-optimized cyanoglobin from *Synechocystis* sp. PCC6803 (2 copies in tandem) and copper inducible sRNA knockdown of heme oxygenase ABICyano1.1_orf29000 | 58 | 11 |
| AB4011 | #2942 | ΔcpcA pABICyano1_6.8::PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-Porf0316'-anti_HO1_v1 (in PC-deficient background) | Constitutive expression of codon-optimized cyanoglobin from *Synechocystis* sp. PCC6803 and copper inducible sRNA knockdown of heme oxygenase ABICyano1.1_orf29000 (in a PC-deficient background) | -- | -- |
| AB4012 | #3022 | ΔcpcA pABICyano1_6.8::PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-PcpcB-glbN_S6803(ABICyano1opt)-TpsaB-Porf0316'-anti_HO1_v1 (in PC-deficient background) | Constitutive expression of codon-optimized cyanoglobin from *Synechocystis* sp. PCC6803 (2 copies in tandem) and copper inducible sRNA knockdown of heme oxygenase ABICyano1.1_orf29000 (in a PC-deficient background) | -- | -- |
| AB4041 | #2949 | nblA2::PcpcB1918-glbN_Ap8005-TpsaB- | Constitutive expression of glbN from *Arthrospira* sp. PCC | 59 | 12 |

| | | | | | |
|---|---|---|---|---|---|
| | | PrbcL2293-ble-TB1002 | 8005 in *Arthrospira platensis* PCC9108 | | |
| AB4065 | #3057 | nblA2::PcpcB1918-glbN_Ap8005-PcpcB1918_glbN_Ap8005-TpsaB-PrbcL2293-ble-TB1002 | Constitutive expression of glbN (2 copies in tandem) from *Arthrospira* sp. PCC 8005 in *Arthrospira platensis* PCC9108 | 60 | 13 |
| AB4066 | #3057 | nblA2::PcpcB1918-glbN_Ap8005-PcpcB1918_glbN_Ap8005-TpsaB-PrbcL2293-ble-TB1002 | Constitutive expression of glbN (2 copies in tandem) from *Arthrospira* sp. PCC 8005 in a derivative strain of *Arthrospira platensis* PCC9108 | -- | -- |

**[00161]** In yet another embodiment, the method for producing a genetically enhanced cyanobacterial cell involves protecting the recombinant nucleic acid sequence, for example a plasmid, against endogenous restriction endonucleases of the host cell by methylating at least a part of the recombinant nucleic acid sequence or modifying and/or eliminating the recognition sequences of the endogenous restriction endonucleases. By changing the nucleic acid sequence of potential recognition sites of restriction endonucleases, a digest of the recombinant nucleic acid sequence can be avoided. It was discovered that endogenous restriction endonucleases of ABICyano1, for example, can cut an extrachromosomal plasmid carrying recombinant genes, thereby preventing a genetic transformation event of this host cell.

**[00162]** In an embodiment, methyltransferases, for example *Ava*I and *Acy*I, can be used to protect recombinant vector extrachromosomal plasmids. The plasmids can either be incubated with the methyltransferases *in vitro* or a helper plasmid can be present in a helper *E. coli* strain in order to methylate the extrachromosomal plasmids *in vivo* before conjugation takes place during the transformation of ABICyano1. In another embodiment, recognition sequences for the restriction enzymes can be modified or deleted. As described by Elhai and Wolk (Conjugal Transfer of DNA to Cyanobacteria in Methods in Enzymology 02/1988; 167:747-54, herein incorporated by reference), plasmid pRL528 can be used as a helper plasmid for conjugal transfer. The indicated genes are M. *Ava*I coding for the methyltransferase protecting against the restriction endonuclease *Ava*I and the respective gene coding for M. *Ava*II. The latter is not required for transformation of ABICyano1, as it lacks any endonuclease activity of *Ava*II.

**[00163]** In an embodiment, the vector to be transformed into cyanobacterial host cells can be modified to integrate into the cyanobacterial chromosome by adding an appropriate DNA sequence homologous to the target region of the host genome. In another embodiment, the vector to be transformed can be modified to integrate into the cyanobacterial chromosome through *in vivo* transposition by introducing mosaic ends to the vector. Once the plasmid is established in the host cell, it can be present, for example, at a range of from 1 to many copies per cell.

**[00164]** In an embodiment, an endogenous plasmid derived from ABICyano1 can be modified, either *in vivo* or *in vitro*, to be a plasmid vector capable of introducing exogenous genes encoding enzymes for the production of a compound or compounds of interest into a wide range of host cyanobacterial cells such as ABICyano1, *Cyanobacterium* sp., or other cyanobacterial genera such as *Synechocystis* and *Synechococcus*.

**[00165]** In an embodiment, the ABICyano1 6.8 kb endogenous plasmid is used as a backbone for a plasmid vector used for transformation of *Cyanobacterium* sp. Since this is the endogenous vector from the species, it is likely to be more stable when transformed into the cell than plasmids derived from completely different organisms. In an embodiment, the entire p6.8 endogenous plasmid is inserted into a vector used for transformation. In another embodiment, a sequence of about 50%, 70%, 75%, 80% 85%, 90%, 95%, 98%, 99%, or 99.5% identity to the entire endogenous plasmid sequence is inserted into the extrachromosomal plasmid vector.

**[00166]** In an embodiment, a modified p6.8 vector is designed to have several modular units that can be swapped out using specific restriction enzymes. Promoters, genes of interest, selectable markers, and other desired sequences can be moved in and out of the vector as desired. This modular design makes genetic experiments faster and more efficient.

**[00167]** The modified p6.8 vector, according to certain embodiments of the disclosure, can replicate in both cyanobacteria and in *E. coli*. The vector contains a replication unit that can function in a broad range of cyanobacterial genera. The vector also contains a replicon for propagation in *E. coli* for ease of cloning and genetic manipulation using *E. coli*.

**[00168]** In an embodiment, a plasmid shuttle vector is provided which is characterized by being replicable in both *E. coli* and cyanobacterial species. The plasmid comprises a promoter capable of functioning in cyanobacteria and *E. coli* and a DNA sequence encoding a sequence capable of functioning as a selective marker for both *E. coli* and cyanobacteria. In another embodiment, the shuttle vector includes two different promoter systems, one functioning in

cyanobacteria and the other one functional in *E. coli*. In an embodiment, the plasmid shuttle vector contains at least 50% of the p6.8 plasmid. The plasmid shuttle vector enables the efficient transformation of cyanobacteria and the expression of recombinant genes of interest.

**[00169]** In another embodiment, the p6.8 derived plasmid vector also contains an origin of transfer (oriT) which is suitable for conjugation. In particular, the plasmid vector can contain a combined origin of replication and an origin of transfer (oriVT), which enables replication in *Enterobacteriaceae*, in particular *E. coli*, and which also enables conjugation with, for example, an *E. coli* donor strain and *Cyanobacterium* sp., in particular ABICyano1 as a recipient strain. Such an plasmid vector can be used for triparental mating wherein a conjugative plasmid present in one bacterial strain assists the transfer of a mobilizable plasmid, for example a plasmid vector disclosed herein, present in a second bacterial strain into a third recipient bacterial strain, which can be ABICyano1.

**[00170]** Also disclosed herein is a non-naturally occurring plasmid vector in which a gene encoding a heme-containing protein of interest is operably linked to a shuttle vector. In an embodiment, cyanobacterial cells are transformed with the recombinant shuttle vector. The recombinant shuttle vector is relatively small in size, relatively stable in a cyanobacterial host cell, and can replicate in a variety of cyanobacterial species. This recombinant vector is useful for expressing a variety of heterologous genes in cyanobacteria.

**[00171]** In an embodiment for transforming host cells with modified plasmid vectors, a shuttle vector expresses a codon-optimized antibiotic resistance gene ($Ab^R$), such as codon improved kanamycin or gentamycin resistance genes. In an embodiment, the shuttle vector is constructed based on a modular basis so that all of the key elements (replication ori, $Ab^R$ gene and reporter gene) are exchangeable via unique restriction sites thus providing versatile cloning options and facilitating the delivery of genes of interest to target organisms. Other antibiotic resistance genes can be used if desired. For example, genes conferring resistance to ampicillin, chloramphenicol, erythromycin, zeocin, kanamycin, gentamycin, spectinomycin or other antibiotics can be inserted into the vector, under the control of a suitable promoter. In some embodiments, the vector contains more than one antibiotic resistance gene.

**[00172]** In yet another embodiment, the plasmid is modified by several factors so that it is capable of efficient replication in multiple types of cyanobacterial species. The vector has also been organized so that various sequences can be easily replaced with other desired sequences as

needed. Thus, a construct having a different gene (or genes) of interest, a different antibiotic, a different promoter, etc. can be made with relative ease. The modified vector allows for rapid testing of various heterologous constructs in a cyanobacterial cell.

**[00173]** The transfer of exogenous genes into cyanobacteria often involves the construction of vectors having a backbone from a broad-host range bacterial plasmid, such as RSF1010. The RSF1010-based vector has been widely used as a conjugation vector for transforming bacteria, including cyanobacteria (Mermet-Bouvier et al. (1993) "Transfer and Replication of RSF1010-derived Plasmids in Several Cyanobacteria of the Genera *Synechocystis* and *Synechococcus*" Current Microbiology 27:323-327). RSF1010 has an *E. coli* origin of replication, but does not have a cyanobacterial origin of replication.

**[00174]** As an example of additional tools used to transfer exogenous genes into cyanobacteria, several endogenous plasmids from *Synechococcus* sp. PCC 7002 were used as the backbone portions for plasmids to prepare vectors for heterologous gene expression, see Xu et al., Photosynthesis Research Protocols 684:273-293 (2011). Other vectors for transformation of cyanobacteria include the pDU1-based vectors. The pDU1 origin of replication is best suited for filamentous cyanobacteria. Attempts to transform ABICyano1, with either RSF1010 or pDU1-based shuttle vectors were unsuccessful using the techniques as described in the art.

**[00175]** In an embodiment, cyanobacteria disclosed herein can be transformed to add biochemical pathways to produce heme-containing proteins of interest. In an embodiment, the recombinant nucleic acids of interest can be amplified from nucleic acid samples using known amplification techniques. PCR can be used to amplify the sequences of the genes directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, and for nucleic acid sequencing.

**[00176]** In an embodiment, recombinant genes are present on an extrachromosomal plasmid having multiple copies per cell. The plasmid can be present, for example, at about 1, 3, 5, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or more copies per cyanobacterial host cell. In an embodiment, the recombinant plasmids are fully segregated from the non-recombinant plasmids.

**[00177]** In another embodiment, recombinant genes are present on one cassette driven by one promoter. In another embodiment, the recombinant genes are present on separate plasmids, or on different cassettes.

**[00178]** In yet another embodiment, recombinant genes are modified for optimal expression by modifying the nucleic acid sequence to accommodate the cyanobacterial cell's protein translation system. Modifying the nucleic acid sequences in this manner can result in an increased expression of the genes.

**Transformation of Cyanobacterial Cells**

**[00179]** Some strains of cyanobacteria can be transformed through natural uptake of exogenous DNA. Other cyanobacterial strains can be transformed, for example, by the use of conjugation or electroporation. Some cyanobacterial strains are difficult to transform by any known means. For many of these types of difficult to transform strains, specific methods of preparing the cells for transformation, as well as specific methods of allowing entry of the foreign DNA into the cells, need to be designed *de novo*.

**[00180]** Exemplary cyanobacteria that can be transformed include, but are not limited to, *Synechocystis*, *Synechococcus*, *Acaryochloris*, *Anabaena*, *Arthrospira, Thermosynechococcus*, *Chamaesiphon*, *Chroococcus*, *Cyanobacterium*, *Cyanobium*, *Dactylococcopsis*, *Gloeobacter*, *Gloeocapsa*, *Gloeothece*, *Microcystis*, *Prochlorococcus*, *Prochloron*, *Chroococcidiopsis*, *Cyanocystis*, *Dermocarpella*, *Myxosarcina*, *Pleurocapsa*, *Stanieria, Xenococcus*, *Borzia*, *Crinalium*, *Geitlerinema*, *Halospirulina*, *Leptolyngbya*, *Limnothrix, Lyngbya, Microcoleus, Cyanodictyon, Aphanocapsa, Oscillatoria, Planktothrix, Prochlorothrix, Pseudanabaena, Spirulina, Starria, Symploca, Trichodesmium, Tychonema, Anabaenopsis, Aphanizomenon, Calothrix, Cyanospira, Cylindrospermopsis, Cylindrospermum, Nodularia, Nostoc, Chlorogloeopsis, Fischerella, Geitleria, Nostochopsis, Iyengariella, Stigonema, Rivularia, Scytonema, Tolypothrix, Cyanothece, Phormidium, Adrianema,* and the like.

**[00181]** Exemplary methods suitable for transformation of Cyanobacteria, include, as nonlimiting examples, natural DNA uptake (Chung, et al. (1998) FEMS Microbiol. Lett. 164: 353-361; Frigaard, et al. (2004) Methods Mol. Biol. 274: 325-40; Zang, et al. (2007) J. Microbiol. 45: 241-245), conjugation, transduction, glass bead transformation (Kindle, et al. (1989) J. Cell Biol. 109: 2589-601; Feng, et al. (2009) Mol. Biol. Rep. 36: 1433-9; U.S. Pat. No.

5,661,017), silicon carbide whisker transformation (Dunahay, et al. (1997) Methods Mol. Biol. (1997) 62: 503-9), biolistics (Dawson, et al. (1997) Curr. Microbiol. 35: 356-62; Hallmann, et al. (1997) Proc. Natl. Acad. USA 94: 7469-7474; Jakobiak, et al. (2004) Protist 155:381-93; Tan, et al. (2005) J. Microbiol. 43: 361-365; Steinbrenner, et al. (2006) Appl Environ. Microbiol. 72: 7477-7484; Kroth (2007) Methods Mol. Biol. 390: 257-267; U.S. Pat. No. 5,661,017) electroporation (Kjaerulff, et al. (1994) Photosynth. Res. 41: 277-283; Iwai, et al. (2004) Plant Cell Physiol. 45: 171-5; Ravindran, et al. (2006) J. Microbiol. Methods 66: 174-6; Sun, et al. (2006) Gene 377: 140-149; Wang, et al. (2007) Appl. Microbiol. Biotechnol. 76: 651-657; Chaurasia, et al. (2008) J. Microbiol. Methods 73: 133-141; Ludwig, et al. (2008) Appl. Microbiol. Biotechnol. 78: 729-35), laser-mediated transformation, or incubation with DNA in the presence of or after pre-treatment with any of poly(amidoamine) dendrimers (Pasupathy, et al. (2008) Biotechnol. J. 3: 1078-82), polyethylene glycol (Ohnuma, et al. (2008) Plant Cell Physiol. 49: 117-120), cationic lipids (Muradawa, et al. (2008) J. Biosci. Bioeng. 105: 77-80), dextran, calcium phosphate, or calcium chloride (Mendez-Alvarez, et al. (1994) J. Bacteriol. 176: 7395-7397), optionally after treatment of the cells with cell wall-degrading enzymes (Perrone, et al. (1998) Mol. Biol. Cell 9: 3351-3365). Biolistic methods (see, for example, Ramesh, et al. (2004) Methods Mol. Biol. 274: 355-307; Doestch, et al. (2001) Curr. Genet. 39: 49-60; all incorporated herein by reference in their entireties).

**[00182]** In an embodiment, the cyanobacterial strain ABICyano1 is used as the host cell for production of the heme-containing protein. In addition to the conjugation methods described below in the example section, electroporation can also be used for successful transformation of ABICyano1, although at a somewhat lower efficiency. Strain-specific adaptations of standard electroporation protocols may be made to avoid DNA digestion by endogenous restriction enzymes and to allow DNA entry through the EPS layer of ABICyano1 host cells. To achieve successful electroporation, DNA may be protected against endogenous restriction enzymes by methylation. Prior to electroporation using techniques well known in the art, ABICyano1 cells may be pretreated with positively charged polyaminoacids such as poly-L-lysine hydrobromide or poly-L-ornithine hydrochloride or combinations thereof (in particular poly-L-lysine hydrobromide) in order to increase the DNA uptake efficiency.

**Selecting for Successful Transformation**

**[00183]** In an embodiment, the presence of a foreign gene encoding antibiotic resistance in a cell is selected by placing putatively transformed cells into a media containing an amount of the corresponding antibiotic and selecting cells that survive. The selected cells are then grown in the appropriate culture medium to allow for further testing.

**[00184]** In another embodiment, colony PCR methods are used to confirm transformants. In certain embodiments of this procedure, three primer sets are used and are directed against parts of the shuttle vectors to detect specific fragments of the shuttle vector. Transformants exhibiting the predicted PCR products are analyzed further by plasmid rescue. In one embodiment for plasmid rescue, a 25 mL liquid culture is subjected to DNA isolation.

**[00185]** In a non-limiting example of plasmid rescue, 500 ng to 1μg of isolated DNA from transformants containing the transformed plasmids is re-transformed into *E. coli* and usually results in approximately 10-20 transformants per transformation implemented. Plasmid DNA of ten *E. coli* colonies is isolated and analyzed by PCR using specific primers for the transformed plasmids. The plasmid DNA is further analyzed with specific restriction enzymes and then sequenced.

**Further Modifications to Improve Heme-containing Protein Production**

**[00186]** Several additional modifications can be made to improve the levels of heme-containing protein present in a host cell. One suitable example is a modification that results in a lower level of heme oxygenase in the host cell. This can be achieved, for example, by use of an inducible sRNA designed to target the heme oxygenase. Examples 9 and 10 provide further details of this procedure. As shown in FIG. 16, this combined procedure results in a much higher level of heme-containing protein.

**[00187]** Another method of increasing the heme-containing protein level is by additional modifications that result in an increase in the flux through ferrochelatase. This can be accomplished, for example, by a knockdown of the gene encoding magnesium chelatase (MgCh), the overexpression of ferrochelatase (FeCh), the knockdown of gun4 (MgCh activator), or a gun4(W192A) mutation. FIG. 14 provides a schematic diagram showing the pathway interactions that can affect the levels of production of the heme-containing protein. Culture

medium modifications can also be used to increase the level of the heme-containing protein produced in the cyanobacterial cells. Examples of these modifications include but are not limited to magnesium-starvation, iron supplementation, and nitrogen-starvation. A combination of these methods and genetic modifications can also be used.

**Phycocyanin Deficiency in the Host Cell Improves Heme Production Levels**

**[00188]** Heme-containing proteins can be produced at even higher levels when the host cells have a phycocyanin-deficient background. This is demonstrated in Examples 13 and 14. The phycocyanin deficiency can be obtained, for example, by a knockout of the phycocyanin gene. The phycocyanin deficiency can also be obtained, for example, by an inducible or constitutive knockdown of the phycocyanin gene, such as by the use of a sRNA or antisense molecule that targets phycocyanin levels.

**Cultivation of Cyanobacterial Cells to Produce the Heme-containing Protein**

**[00189]** In an embodiment, a heme-containing protein is synthesized in cyanobacterial cultures by preparing host cyanobacterial cells having the gene constructs discussed herein, and growing cultures of the cells.

**[00190]** Methods of growing cyanobacteria in liquid media and on agarose-containing plates are well known to those skilled in the art (see, e.g., websites associated with ATCC). Any of these methods or media maybe used to culture the cyanobacterial cells. A number of known recipes for cyanobacterial growth medium can be used. In an embodiment, BG11 medium is used, see Stanier, R.Y., et al., Bacteriol. Rev. 1971, 35: 171-205, which is hereby incorporated by reference.

**[00191]** In an embodiment, the cyanobacterial strain is a fresh water strain, and BG11 is used. In another embodiment, the cyanobacteria culture grows best in a marine (salt water) medium, by adding an amount of salt to the BG11 medium. In an embodiment, marine BG11 (mBG11) contains about 35 practical salinity units (psu), see Unesco, The Practical Salinity Scale 1978 and the International Equation of State of Seawater 1980. Tech. Pap. Mar. Sci., 1981, 36: 25 which is hereby incorporated by reference.

**[00192]** In an embodiment, the cells are grown autotrophically, and the only carbon source is $CO_2$. In another embodiment, the cells are grown mixotrophically, for example with the addition of another carbon source such as glycerol or glucose.

**[00193]** The cultures can be grown indoors or outdoors. The light cycle can be set as for continuous light, or for periodic exposure to light, e.g., 16 hours on and 8 hours off, or 14 hours on and 10 hours off, or 12 hours on and 2 hours off, or any alternative variation of on and off hours of light comprising about a day.

**[00194]** The cultures can be axenic, or the cultures can also contain other contaminating species.

**[00195]** In an embodiment, the cyanobacteria are grown in enclosed bioreactors in quantities of at least about 1 L, 20L, 50L, 100 L, 500 L, 1,000 L, 2,000 L, 5,000 L, or more. In a preferred embodiment the bioreactors are about 20 L to about 100 L. In an embodiment, the cyanobacterial cell cultures are grown in disposable, flexible, and tubular photobioreactors made of a clear plastic material.

**[00196]** In another embodiment, cultures are grown indoors or outdoors with continuous light, in a sterile environment. In another embodiment, the cultures are grown outdoors in an open pond type of photobioreactor.

**[00197]** The choice of culture medium can depend on the cyanobacterial species. In an embodiment of the invention, the following BG11 medium for growing cyanobacteria can be used. When salt water species are grown, NaCl is added to the culture medium.

**Table 4: Culture Medium**

| **BG11 Medium** | |
|---|---|
| **Compound** | **Amount** |
| $NaNO_3$ | 1.5 g |
| $K_2HPO_4$ | 0.04 g |
| $MgSO_4 \cdot 7H_2O$ | 0.075 g |
| $CaCl_2 \cdot 2H_2O$ | 0.036 g |
| Citric acid | 0.006 g |
| Ferric ammonium citrate | 0.006 g |
| EDTA (disodium salt) | 0.001 g |

| | |
|---|---|
| $NaCO_3$ | 0.02 g |
| Trace metal mix A5 | 1.0 ml |
| Distilled water | 1.0 L |
| ---------- | ---------- |
| **Composition of Trace Metal Mix** | |
| **Compound** | **Amount** |
| $H_3BO_3$ | 2.86 g |
| $MnCl_2 \cdot 4H_2O$ | 1.81 g |
| $ZnSO_4 \cdot 7H_2O$ | 0.222 g |
| $NaMoO_4 \cdot 2H_2O$ | 0.39 g |
| $CuSO_4 \cdot 5H_2O$ | 0.079 g |
| $Co(NO_3)_2 \cdot 6H_2O$ | 49.4 mg |
| Distilled water | 1.0 L |

**[00198]** The present disclosure is further described by the following non-limiting examples. However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present disclosure.

## EXAMPLES

### Example 1

### General Methods

**[00199]** Restriction endonucleases were purchased from New England Biolabs (NEB; Ipswich, MA), unless otherwise noted. PCR was performed using an Eppendorf Mastercycler thermocycler (Hauppauge, NY), using Phire II Hot Start polymerase or Taq DNA polymerase (NEB) for diagnostic amplifications, and Phusion polymerase or Crimson LongAmp Taq Polymerase (NEB) for high fidelity amplifications. PCR temperature profiles were set up as recommended by the Taq manufacturer. Cloning was performed in *E. coli* using XL10-Gold Ultracompetent cells (Agilent Technologies, Santa Clara, CA) following the manufacturer's

protocol. TOPO cloning kits (Zero Blunt TOPO PCR Cloning kit) were purchased from Invitrogen (Carlsbad, CA), and were used according to the manufacturer's protocol.

**[00200]** *E. coli* strains HB101 (Promega, Madison, WI), XL10-Gold (Stratagene, San Diego, CA), and α-select (Bioline) were grown in Luria-Bertani (LB) medium at 37 °C. Ampicillin (50 μg/mL), kanamycin (50 μg/mL), and chloramphenicol (34 μg/mL) were used when appropriate. Cultures were continuously shaken overnight at 200 rpm and at 100 rpm when used for conjugation. ABICyano1 was cultured at from 28 °C to 37 °C in liquid BG11 fresh water medium on a reciprocal shaker at 150 rpm under continuous illumination of approximately 30 – 40 μmol photons*$m^{-2}$ *$sec^{-1}$.

**[00201]** Plasmid DNA from *E. coli* strains was isolated using a GeneJet Plasmid Miniprep Kit (Fermentas) according to the manufacture's protocol. For plasmid isolation from putative ABICyano1 transformants, total DNA was prepared according to Saha et al. (2005), World Jour. Microbiol Biotechnol 21:877-881.

## Example 2

## Preparation of Cyanobacterial Culture Medium

**[00202]** For cyanobacterial growth, BG-11 stock solution was purchased from Sigma Aldrich (St. Louis, MO). Marine BG-11 (MBG-11) was prepared by dissolving 35 g Instant Ocean (United Pet Group, Inc, Cincinnati, OH) in 1 L water and supplementing with BG-11 stock solution. Vitamin B12 (Sigma Aldrich) was supplemented to MBG-11 to achieve a final concentration of 1 μg/L, as needed. Stock solutions of the antibiotics spectinomycin (100 mg/ml) and kanamycin (50 mg/ml) were purchased from Teknova (Hollister, CA). Stock solution of the antibiotic gentamycin (10 mg/ml) was purchased from MP Biomedicals (Solon, OH).

**[00203]** Unless otherwise noted, the ABICyano1 transformants were selected on solid BG11 medium containing 10 - 20 μg/mL kanamycin and were maintained on BG11 plates containing 40 μg/mL kanamycin. For growth in liquid freshwater BG11 medium, 30-40 μg/mL of kanamycin was applied.