

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Cherinko et al.
U.S. Patent No.: 10,392,578 Attorney Docket No. 55973-0005IP1
Issue Date: August 27, 2019
Appl. Serial No.: 13/151,014
Filing Date: June 1, 2011
Title: EXTRACTION OF LIPID FROM CELLS AND PRODUCTS
THEREFROM

DECLARATION OF DR. JONATHAN CURTIS

I currently hold the opinions set expressed in this declaration. But my analysis may continue, and I may acquire additional information and/or attain supplemental insights that may result in added observations.

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I further declare 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 the Title 18 of the United States Code.

Dated: 13th July 2023

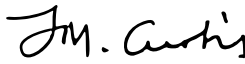
By: 
Jonathan M. Curtis, Ph.D.

TABLE OF CONTENTS

I.	EXPERIENCE AND QUALIFICATIONS.....	4
A.	Materials Considered	6
B.	Overview of Conclusions Formed.....	10
C.	Interpretation of the '578 Patent Claims.....	11
D.	Legal Standards	13
	1. Terminology.....	13
	2. Legal Standards for Anticipation.....	14
	3. Legal Standards for Obviousness.....	15
E.	Background Knowledge One of Skill in the Art Would Have Had Prior to the Priority Date of the '578 Patent.....	20
	1. Composition of Microbial Oils	20
	2. Extraction of Microbial Oils	24
II.	SUMMARY OF THE '578 PATENT	29
A.	Brief Description	29
B.	Summary of the Prosecution History of the '578 Patent	31
C.	The Effective Priority Date of the Claims of the '578 Patent.....	34
D.	Level of Ordinary Skill in the Art.....	34
III.	CLAIMS 1-7 OF THE '578 PATENT ARE UNPATENTABLE	35
A.	GROUND 1: Claim 3 is obvious over Schaap in view of Kralovec	35
	1. Overview of Schaap	35
	2. Overview of Kralovec.....	37
	3. Combination of Schaap and Kralovec	38
	4. Analysis.....	44
B.	GROUND 2: Claims 1-7 are obvious over Schaap in view of Komazawa	59
	1. Overview of Schaap	59
	2. Overview of Komazawa	59
	3. Combination of Schaap and Komazawa.....	60
	4. Analysis.....	64
C.	GROUND 3: Claims 1-7 are obvious over Bijl in view of Nichols-I ..	77
	1. Overview of Bijl.....	77
	2. Overview of Nichols-I	79
	3. Overview of Schaap	80
	4. Combination of Bijl, Nichols-I, and Schaap.....	80
	5. Analysis.....	87
D.	GROUND 4: Claim 3 is obvious over Radianingtyas and Wang	107
	1. Overview of Radianingtyas.....	107
	2. Overview of Wang.....	109

3.	Combination of Radianingtyas and Wang.....	111
4.	Analysis.....	114
IV.	CONCLUSION.....	126

I, Jonathan Curtis, declare as follows:

I. EXPERIENCE AND QUALIFICATIONS

1. I joined the Faculty of Agricultural and Environmental Sciences at the University of Alberta in Edmonton, Alberta, Canada, in 2007, where I am currently a full professor in the Department of Agricultural, Food and Nutritional Science. I will continue my association with this department as Professor Emeritus on my retirement in July 2023. The research group which I direct within this department is known as the Lipid Chemistry Group.

2. As Professor, my teaching duties within the Nutrition and Food Science program have included responsibility for a large undergraduate course in Food Chemistry and a joint 4th year undergraduate/graduate course in Analytical Chemistry. Also, as part of my university activities, I have directed a research group which has included graduate and undergraduate students, technicians, senior research associates and postdoctoral research fellows. My research activities have been focused to a considerable extent on lipid chemistry, the conversion of fats and oils to biobased chemicals and materials, lipid analysis, and the development of novel analytical methods for lipids relevant to medical sciences, the fat and oil industries, agriculture, food science and nutrition.

3. I earned a Bachelor of Science degree in Chemistry in 1981 from the University of Southampton in the U.K and a Master of Science in Analytical Chemistry in 1983 from the University of Guelph in Ontario, Canada. I earned a

Ph.D. in Analytical Chemistry in 1987 from Swansea University in Wales. My doctoral thesis was entitled “Novel Methods of Structure Determination by Mass Spectrometry.” From 1987 to 1988, I was a postdoctoral research associate in the School of Chemical Sciences at the University of Illinois, where I worked on the analysis of marine natural products.

4. Between 1992 and 1999 I was a Research Officer with the Canadian National Research Council, Institute for Marine Biosciences, in Halifax, Nova Scotia. My focus there was on the analytical chemistry of marine lipids and natural products, and the NRC certified reference material program.

5. Between 1999 and 2007 I worked in an industrial R&D position at Ocean Nutrition Canada Ltd. (now part of DSM) where my title was Director of Research: Analytical and Functional Food Technologies. There I directed analytical chemistry and food science groups involved in the processing of fish oils and omega-3 concentrates, oil quality measurements, and developing microencapsulated oil as a food ingredient and food grade oils with desirable sensory qualities. During that time, I also interacted with the group developing microalgal oils for omega-3 production.

6. I have co-authored or authored more than 180 published peer-reviewed articles in scientific journals and 7 book chapters. I have been co-inventor on 6 issued patents plus several provisional patents.

7. My curriculum vitae is attached as exhibit MARA-1004. In the past three years, I have not testified at deposition or trial.

8. I have no financial interest in the parties or in the outcome of this proceeding. I am being compensated for my work as an expert on an hourly basis. My compensation is not dependent on the outcome of these proceedings or the content of my opinions.

A. Materials Considered

9. I have been retained on behalf of Mara Renewables Corporation to offer technical opinions relating to U.S. Patent No. 10,392,578 (“the ’578 patent”) and prior art references relating to its subject matter. I have reviewed the ’578 patent, and relevant excerpts of the prosecution history of the ’578 patent. In addition to any materials cited in the present declaration, I have also reviewed the following references:

1) MARA-1005: U.S. Pat. App. Pub. No. 2005/0220958 to Schaap et al.

(“Schaap”)

2) MARA-1006: U.S. Pat. App. Pub. No. 2009/0118525 to Wang (“Wang”)

3) MARA-1007: WO 2008/129358 to Radianingtyas et al.

(“Radianingtyas”)

4) MARA-1008: WO 97/37032 to Bijl et al. (“Bijl”)

- 5) MARA-1009: Peter D. Nichols et al., *New Australian Single Cell and Crop Plant Sources of Health-Enhancing Long-Chain Omega-3 Oils*, 13:5 Australasian Biotechnology 26 (Oct./Nov. 2003) (“Nichols-I”)
- 6) MARA-1010: Single Cell Oils: Microbial and Algal Oils (2d Ed. May 1, 2010, Cohen & Ratledge, eds.)
- 7) MARA-1011: P.K. Bajpai et al., *Optimization of Production of Docosahexaenoic Acid (DHA) by Thraustochytrium aureum ATCC 34304*, 68:7 JAOCS 509 (1991).
- 8) MARA-1012: Anita N. Jakobsen et al., Accumulation of docosahexaenoic acid-rich lipid in thraustochytrid Aurantiochytrium sp. strain T66: effects of N and P starvation and O₂ limitation, 80 Appl. Microbiol. Biotechnol. 297 (2008)
- 9) MARA-1013: U.S. Pat. App. Pub. No. 2007/0141222 to Binder et al. (“Binder”)
- 10) MARA-1014: U.S. Pat. App. Pub. No. 2010/0298435 to ABRIL et al. (“Abril”)
- 11) MARA-1015: U.S. Pat. App. Pub. No. 2004/0161831 to Komazawa et al. (“Komazawa”)
- 12) MARA-1016: U.S. Pat. No. 8,148,559 to Walker et al. (“Walker”)
- 13) MARA-1017: U.S. Pat. No. 8,207,363 to Apt et al. (“Apt”)

- 14) MARA-1018: US Pat. App. Pub. No. 2005/0273885 to Singh et al.
(“Singh”)
- 15) MARA-1019: U.S. Pat. App. Pub. No. 2006/0094089 to Barclay
(“Barclay”)
- 16) MARA-1020: Peter D. Nichols et al., *Omega-3 oils down under—An update*, 15:6 Inform 383 (June 2004) (“Nichols-II”)
- 17) MARA-1022: *Long-Chain Omega-3 Specialty Oils*, H. Breivik (Ed.), The Oily Press, Bridgewater, UK (2007)
- 18) MARA-1023: *Fennema’s Food Chemistry*, S. Damodaran and K.L. Parkin (Eds.) CRC Press, Boca Raton, USA (5th ed. 2017)
- 19) MARA-1024: *Food Lipids*, C.C. Akoh and D.B. Min (Eds.), Marcel Dekker, New York, USA (2002)
- 20) MARA-1025: A.M. Burja et al., Isolation and characterization of polyunsaturated fatty acid producing *Thraustochytrium* species: screening of strains and optimization of omega-3 production, *Appl. Microbiol. Biotechnol.* 72: 1161-1169 (2006)
- 21) MARA-1026: R.D. Bowles et al., Long-chain n-3 polyunsaturated fatty acid production by members of the marine protistan group the *Thraustochytrids*: screening of isolates and optimisation of docosahexaenoic acid production. *J. Biotechnol.* 70:193–202 (1999)

- 22) MARA-1027: Edwin N. Frankel, *Lipid Oxidation*, The Oily Press (2005)
- 23) MARA-1028: J. Huang et al., Profile of Polyunsaturated Fatty Acids Produced by *Thraustochytrium* sp. KK17-3. JAOCS, Vol. 78:6 (2001)
- 24) MARA-1029: Willis A. Gortner, *An Evaluation of Micromethods for Phospholipid*, J. Biol. Chem., Vol. 159, 97-100 (June 1945)
- 25) MARA-1030: WO 2009/040676 to Kralovec et al. (“Kralovec”)
- 26) MARA-1031: T. Yaguchi et al., *Production of High Yields of Docosahexaenoic Acid by Schizochytrium sp. Strain SR21*, 74:11 JAOCS 1431 (1997).
- 27) MARA-1032: Thomas Eric Lewis, *Characterisation and application of Australian thraustochytrids*, Doctoral Thesis, University of Tasmania (June 2001)

10. I have also reviewed various supporting references and other documentation as further noted in my opinions below.

11. Counsel has informed me that I should consider these materials through the lens of one of ordinary skill in the art related to the '578 patent as of June 2010. I have done so. The '578 patent claims priority to three provisional applications: one filed on June 1, 2010; one filed on August 31, 2010; and one filed on March 15, 2011. The earliest of these is June 1, 2010 (“the Critical Date”).

Counsel has informed me that the Critical Date represents the earliest possible priority date to which the challenged claims of the '578 patent are entitled, and I have therefore used that Critical Date in my analysis below.

12. In writing this Declaration, I have considered the following: U.S. Patent No. 10,392,578 (MARA-1001), my own knowledge and experience, including my work experience in the field of high omega-3 oils, my experience in teaching food chemistry, oil processing, oleochemistry, and experience in working with others involved in this field. In addition, I have analyzed the foregoing publications and materials, in addition to any other materials I may cite in my declaration.

13. My opinions, as explained below, are based on my education, experience, and expertise in the fields relating to the '578 patent. Unless otherwise stated, my testimony below refers to the knowledge of one of ordinary skill in the art as of the Critical Date, or before. Any figures that appear within this document have been prepared with the assistance of Counsel and reflect my understanding of the '578 patent and the prior art discussed below.

B. Overview of Conclusions Formed

14. This expert Declaration explains the conclusions that I have formed based on my analysis. To summarize those conclusions:

- Based upon my knowledge and experience and my review of the

prior art publications listed above, in my opinion claim 3 of the '578 patent is obvious in light of Schaap and Kralovec;

- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion claims 1-7 of the '578 patent are obvious in light of Schaap and Komazawa;
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion claims 1-7 of the '578 patent are obvious in light of Bijl, Nichols-I, and Schaap;
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion claim 3 of the '578 patent is obvious in light of Radianingtyas and Wang.

C. Interpretation of the '578 Patent Claims

15. I understand that, for purposes of my analysis in this *inter partes* review proceeding, the terms appearing in the patent claims should be interpreted “in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent.” 37 C.F.R. § 42.100(b).

16. For purposes of this proceeding only, I have provided the construction for the term “the oil” below. All remaining terms should be given their plain

meaning. I reserve the right to opine on the construction of any other claim terms, should Patent Owner propose any other words that it contends require construction.

1. “THE OIL,” CLAIMS 1-7

17. The claim phrase “the oil” should be construed to mean “the extracted microbial lipid” in claims 1-7. The claims of the ’578 patent support this construction. For example, each of independent claims 1-3 recite “wherein the oil has an anisidine value of 0.5 to 26.” MARA-1001, 120:24-55 (cl. 1-3). But I also note that the phrase “the oil” does not appear elsewhere in those same claims. And claim 5 also refers to “the oil” only once, seemingly in reference to the “extracted microbial lipid.” I understand from counsel that this means the phrase “the oil” does not have antecedent basis. I further understand from counsel that the terms of a patent claim must have antecedent basis. In my opinion then, interpreting “extracted microbial lipid” as being synonymous with “the oil” would fix that problem in claims 1-7.

18. In my opinion, the patent specification suggests to a person of skill in the art that “lipid” and “oil” can be used interchangeably. In the section labeled “DEFINITIONS,” I note that the specification states: “As used herein, ‘lipid’ or ‘oil’ refers to one or more fatty acids.” MARA-1001, 12:31-38. It further states that “the extracted microbial lipid is a crude lipid or a crude oil.” MARA-1001, 6:14-15. And it further states that the invention “can be any lipid derived from a

microorganism, including, for example: a crude oil...a refined oil...a diluted microbial oil...or an enriched oil.” MARA-1001, 34:23-33, 38:11-21. This includes a “refined oil,” which “is a crude lipid or crude oil that has been refined.” MARA-1001, 30:45-46. In my opinion, then, the patent uses the term “extracted microbial lipid” to refer to the panoply of lipids, oils, and compositions that can be derived from microorganisms. The term “the oil,” then, should be read as “the extracted microbial lipid.”

19. I have reviewed the prosecution history of the '578 patent and it appears that the patent examiner viewed “the oil” as interchangeable with “extracted microbial lipid.” The patent owner also used the word in a similar manner.

D. Legal Standards

1. Terminology

20. I have been informed by Counsel and understand that the best indicator of claim meaning is its usage in the context of the patent specification as understood by one of ordinary skill. I further understand that the words of the claims should be given their plain meaning unless that meaning is inconsistent with the patent specification or the patent’s history of examination before the Patent Office. Counsel has also informed me, and I understand that, the words of the claims should be interpreted as they would have been interpreted by one of

ordinary skill at the time of the invention was made (not today). Because I do not know at what date the invention as claimed was made, I have used the earliest possible priority date of the '578 Patent as the point in time for claim interpretation purposes (the Critical Date).

2. Legal Standards for Anticipation

21. I have been informed by Counsel and understand that documents and materials that qualify as prior art can render a patent claim unpatentable as anticipated. I am informed by Counsel and understand that all prior art references are to be looked at from the viewpoint of a person of ordinary skill in the art.

22. I am informed by Counsel and understand that a challenged claim is unpatentable as “anticipated” under 35 U.S.C. § 102 if it is determined that all the limitations of the claim are described in a single prior art reference. I am informed by Counsel and understand that, to anticipate a claim, a prior art reference must disclose, either expressly or inherently, each and every limitation of that claim and enable one of ordinary skill in the art to make and use the invention.

23. I have been informed by Counsel and understand that in an *inter partes* review, “the petitioner shall have the burden of proving a proposition of unpatentability,” including a proposition of anticipation, “by a preponderance of the evidence.” 35 U.S.C. § 316(e).

3. Legal Standards for Obviousness

24. I have been informed by Counsel and understand that documents and materials that qualify as prior art can render a patent claim unpatentable as obvious. I am informed by Counsel and understand that all prior art references are to be looked at from the viewpoint of a person of ordinary skill in the art at the time of the invention, and that this viewpoint prevents one from using his or her own insight or hindsight in deciding whether a claim is obvious.

25. I have been informed by Counsel and understand that a claim is unpatentable for obviousness under 35 U.S.C. § 103 “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.” I am informed by Counsel and understand that obviousness may be based upon a combination of references. I am informed by Counsel and understand that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results. However, I am informed by Counsel and understand that a patent claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.

26. I am informed by Counsel and understand that when a patented invention is a combination of known elements, a court must determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue by considering the teachings of prior art references, the effects of demands known to people working in the field or present in the marketplace, and the background knowledge possessed by a person having ordinary skill in the art.

27. I am informed by Counsel and understand that a patent claim composed of several limitations is not proved obvious merely by demonstrating that each of its limitations was independently known in the prior art. I am informed by counsel and understand that identifying a reason those elements would be combined can be important because inventions in many instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known. I am informed by Counsel and understand that it is improper to use hindsight in an obviousness analysis, and that a patent's claims should not be used as a "roadmap."

28. I am informed by Counsel and understand that an obviousness inquiry requires consideration of the following factors: (1) the scope and content of the prior art; (2) the differences between the claims and the prior art; (3) the level of ordinary skill in the pertinent art; and (4) any objective indicia of non-obviousness,

such as commercial success, long-felt but unresolved need, failure of others, industry recognition, copying, and unexpected results. I understand that the foregoing factors are sometimes referred to as the “Graham factors.”

29. I have been informed by Counsel and understand that an obviousness evaluation can be based on a combination of multiple prior art references. I understand that the prior art references themselves may provide a suggestion, motivation, or reason to combine, but that the nexus linking two or more prior art references is sometimes simple common sense. I have been informed by Counsel and understand that obviousness analysis recognizes that market demand, rather than scientific literature, often drives innovation, and that a motivation to combine references may be supplied by the direction of the marketplace.

30. I have been informed by Counsel and understand that if a technique has been used to improve one device, and a person of ordinary skill at the time of invention would have recognized that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.

31. I have been informed by Counsel and understand that practical and common sense considerations should guide a proper obviousness analysis, because familiar items may have obvious uses beyond their primary purposes. I have been informed by Counsel and understand that a person of ordinary skill looking to

overcome a problem will often be able to fit together the teachings of multiple prior art references. I have been informed by Counsel and understand that obviousness analysis therefore takes into account the inferences and creative steps that a person of ordinary skill would have employed at the time of invention.

32. I have been informed by Counsel and understand that a proper obviousness analysis focuses on what was known or obvious to a person of ordinary skill at the time of invention, not just the patentee. Accordingly, I understand that any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.

33. I have been informed by Counsel and understand that a claim can be obvious in light of a single reference, without the need to combine references, if the elements of the claim that are not found explicitly or inherently in the reference can be supplied by the common sense of one of skill in the art.

34. I have been informed by Counsel and understand that secondary indicia of non-obviousness may include (1) a long felt but unmet need in the prior art that was satisfied by the invention of the patent; (2) commercial success of processes covered by the patent; (3) unexpected results achieved by the invention; (4) praise of the invention by others skilled in the art; (5) taking of licenses under the patent by others; (6) deliberate copying of the invention; (7) failure of others to

find a solution to the long felt need; and (8) skepticism by experts. I understand that evidence of secondary indicia of non-obviousness, if available, should be considered as part of the obviousness analysis.

35. I have been informed by Counsel and understand that there must be a relationship between any such secondary considerations and the invention, and that contemporaneous and independent invention by others is a secondary consideration supporting an obviousness determination.

36. In sum, my understanding is that prior art teachings are properly combined where one of ordinary skill having the understanding and knowledge reflected in the prior art and motivated by the general problem facing the inventor, would have been led to make the combination of elements recited in the claims. Under this analysis, the prior art references themselves, or any need or problem known in the field of endeavor at the time of the invention, can provide a reason for combining the elements of multiple prior art references in the claimed manner.

37. I have been informed by Counsel and understand that in an *inter partes* review, “the petitioner shall have the burden of proving a proposition of unpatentability,” including a proposition of obviousness, “by a preponderance of the evidence.” 35 U.S.C. §316(e).

E. Background Knowledge One of Skill in the Art Would Have Had Prior to the Priority Date of the '578 Patent

38. Below, I give an overview of microbial oils, including their composition, nomenclature and health benefits. I then discuss how microorganisms are sourced and processed, and fatty acids extracted and refined, to result in lipid compositions for creation of microbial oils.

1. Composition of Microbial Oils

39. It has long been recognised that the regular consumption of the polyunsaturated fat found in marine organisms is associated with a range of health benefits and disease risk reduction. Early studies pointed to its importance in cardiovascular diseases, and more recently wide-ranging benefits in infant and maternal health, inflammatory diseases, cognitive disorders and in many other areas have been described. MARA-1022, 11-18.

40. The two most important components of marine lipids that are associated with these benefits, are the n-3 fatty acids eicosapentaenoic acid (EPA) and docosohexaenoic acid (DHA). The structures of these compounds are shown in Figure 1. They are described as being omega-3 or n-3 fatty acids which refers to the position of the first double bond from the terminal methyl group with all of the remaining double bonds in the cis configuration and separated by a methylene group as shown. In contrast, n-6 fatty acids such as n-6 docosapentaenoic acid

(DPA) has the first double bond at the 6th carbon atom when counting from the terminal methyl group (Figure 1).

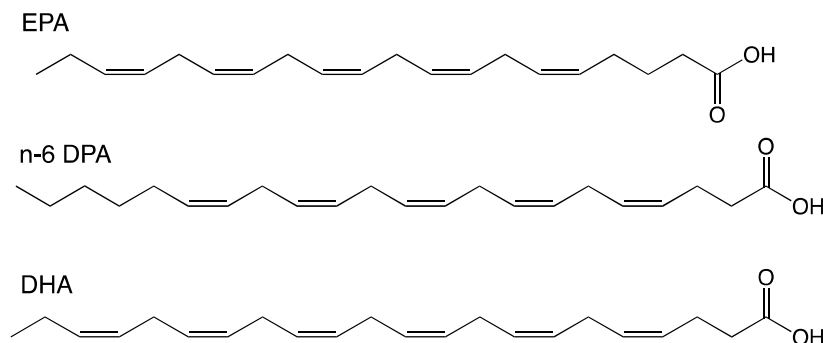


Figure 1 EPA, DHA n-6 DPA structures

41. The vast majority of fatty acids that are found in nature in fats and oils are esterified to glycerol, predominantly as triacylglycerols, meaning three ester groups are added to the glycerol molecule (Figure 2). MARA-1023, 175-176. However fatty acids may also be esterified to glycerol as mono- or di-acylglycerols or as phospholipids, and example of which (phosphatidyl choline, PC) is given in Figure 2. MARA-1023, 176-177. A phospholipid is a class of lipids where the molecule has a hydrophilic (water-loving) “head” containing a phosphate group and two hydrophobic (water-hating) “tails” derived from fatty acids. PC is the main component of lecithin obtained from soybean oil, and is also abundant in egg yolk lipids. Fatty acids can also be found esterified to other molecules such as cholesterol, sphingosine, or to fatty alcohols in wax esters. Edible oils and fats

generally contain mainly triacylglycerols with only minor amounts of these other esters and other lipids present.

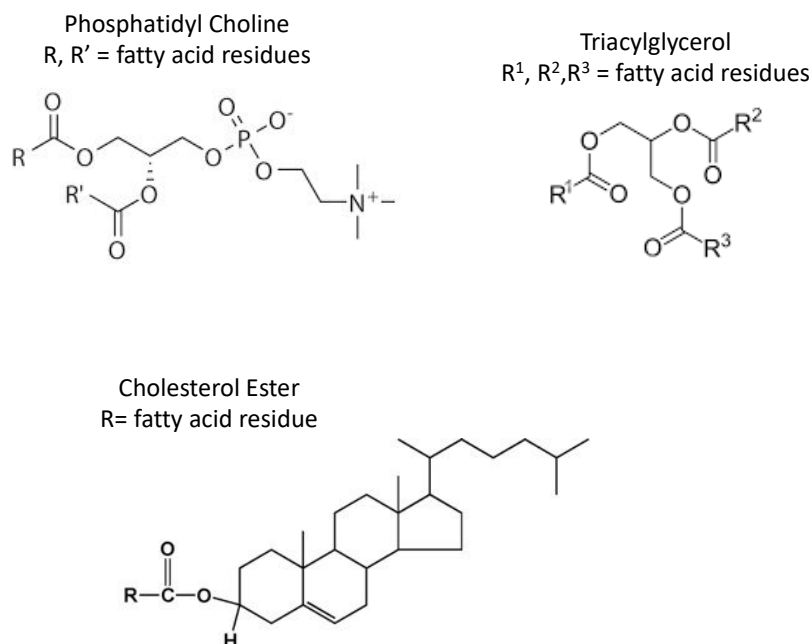


Figure 2 TAG (triacylglycerides), PC, cholesterol ester structures.

42. There are many different fatty acids that can be found in triacylglycerols (also known as TAG, triacylglycerides, or triglycerides). The fatty acid composition can be expressed as a percentage of the “total fatty acid” (TFA, the sum of all measured fatty acid abundances), or as a weight fraction (mg/g) percentage which relates the measured weight of each fatty acid to that of the oil sample.

43. Often, when a lipid is extracted from an organism, it is first mechanically disrupted such as through heating, crushing, milling, drying etc, then solvents are used to ensure that all of the lipid is extracted. MARA-1024, 224 &

Table 1, 224-245. Depending on the process used, the lipid extracted may include triacylglycerols, sterols and sterol esters, phospholipids, and other compounds soluble in that solvent. This could be described as a crude lipid extract or total lipid extract.

44. It is widely believed that most people do not have enough EPA and DHA in their diets for optimal health. MARA-1022, 14-18. As a result, a sizeable industry has been built around producing fish oils, such as from sardines, anchovies, tuna etc., and which are rich sources of these fatty acids. However, it is not sustainable to harvest increasing amounts of fish, and it is well known that fish themselves are not the primary producers of these valuable fatty acids, but rather concentrate them from the plankton they ingest. Therefore, considerable investments have been made into isolating and growing marine microbes which are the primary producers of the n-3 PUFA (polyunsaturated fatty acids) that is required for human nutrition, and can also be utilised in aquaculture.

45. Burja et al noted that “There are two groups of marine microbes that have been found to produce these oils, namely, the marine diatoms, *Cryptheconidiaceae*, and the marine protists, *Thraustochytriidae*.” MARA-1025, 1161. They further described how whilst *thraustochytrids* show particular promise for industrial production of especially DHA, “there exists a great deal of variability in biomass, lipid, and DHA yields within strains, mostly dependent upon

fermentation conditions”. MARA-1025, 1162. For instance, of the thraustochytrid strains isolated in this paper, a DHA content of 35% of total fatty acids was achieved. MARA-1025, 1166. It was noted that this was similar to commercial strains at the time but lower than reported literature values of up to 50-59% of TFA. MARA-1025, 1166. In addition, they noted that the n-6 PUFA arachidonic acid was produced as was n-6 DPA at 3-7% w/w along with variable amounts of EPA. MARA-1025, 1166. Also illustrated was that the DHA content could be manipulated through changes to the composition of the growth medium which changed over the range of 16.2-31.4 as a percentage of the TFA. MARA-1025, 1166-1167.

46. A 2007 review chapter by Wynn and Ratledge established that by 2006 two commercial microbial oils were available, both with ~40% DHA w/w of TFA which, following extensive safety studies, had been incorporated into at least 6 infant formulas. MARA-1022, 58-67. Thus, refined microbial oils are entirely suitable for human nutrition.

2. Extraction of Microbial Oils

47. In general, the steps required to produce edible oils include:
- a. Recovery of the crude lipid (fat or oil) by extraction, crushing or rendering an organism, depending on whether it is a plant, animal, or microbial source. This stage allows the crude lipid to be

separated from the remaining biomass; the latter may have other uses such as the defatted meal recovered from oilseeds. The crude lipid generally contains undesirable components such as residual solvent (often hexanes, if used in the extraction), phosphatides (gums), pigments, free fatty acids, and flavour and aroma compounds. Hence it is usual to remove these undesirables through refining to produce high quality edible oils.

- b. Various stages of refining are applied to the crude lipid. Common processes include degumming, a water wash process to remove phospholipids; caustic refining, to remove free fatty acids; bleaching, using adsorbents such as clays or carbon to remove pigments, lipid oxidation products, and other contaminants; and deodorization, using steam distillation at high temperatures and low pressures to remove residual flavours, aromas, and volatile compounds.
- c. Optionally, various types of conversions can also be applied to the crude or refined oil, such as fractional crystallization, hydrogenation, interesterification etc. which convert the oil to a form most suitable for a specific application. MARA-1024, 223-273.

48. For edible applications, refined oils must have acceptable organoleptic properties, be essentially free of residual solvent, have acceptably low levels of processing or environmental contaminants, have very low levels of lipid oxidation products, and must have suitable stability. Thus, commercially produced edible oils must go through considerable chemical and physical testing to ensure that they meet these and other required specifications. There are several types of chemical testing that are specifically described in the '578 patent claims:

- a. A test based on the use of *chromatography*, which separates one component from another. Examples include determining the amount of an individual fatty acid (such as DHA or n-6 DPA) that is present in the entire lipid, which is commonly performed by gas chromatography after conversion to fatty acid methyl esters, as explained above. Other types of chromatography could also be used to separate triacylglycerols (triglyceride) from other classes of lipids, such as polar lipids (eg phosphatidyl choline, lecithin) or free fatty acids.
- b. A test based on the accurate determination of the presence of an element in the lipid. In this case, the phosphorus content is specified, which is an indication of the phospholipid content of the lipid. There are many well-known techniques for elemental

analyses including types of atomic spectroscopy; these could accurately measure not only the level of phosphorus but also might be employed to measure levels of undesirable heavy metals.

c. Standardized tests based on a reaction, which indicate the level of a class of compound but do not specifically measure an individual compound. Two examples of these types of measurements are

- i. Peroxide value (PV). In this test, lipid hydroperoxides that are present in the oil react with potassium iodide in the presence of acetic acid to liberate iodine; the amount of iodine liberated is measured. The PV test is a measure of oxidation applicable to oils that are only oxidised to low extent, which should be the case for freshly refined oils. Since lipid hydroperoxides are the precursors to secondary oxidation products that include flavour and aroma compounds, PV is useful indication of oil quality. However, as stated by Frankel “knowledge of the history of the oil samples is important to better interpret the significance of peroxide value measurements”. MARA-1027, 105. Thus, a PV measurement alone can be misleading, for example since peroxide levels can fall following high levels of oxidation.

In addition, the PV of an oil can be lowered through the use of refining techniques like those described above; but it may also increase during storage (especially for PUFA) at a rate that depends on the presence of oxygen, antioxidants, prooxidants, temperature and other factors.

- ii. Para-Anisidine value (pAV , or AV). The AV indicates the level of carbonyl compounds that are present in oils as secondary lipid oxidation products, *ie.* that were formed by decomposition of lipid hydroperoxides, the primary oxidation products. This chemical test measures the degree of reaction of any saturated and unsaturated carbonyl compounds with the p-anisidine reagent. The AV “provides useful information on non-volatile carbonyl compounds formed in oils during processing” and hence is a widely used indication of oil quality. MARA-1027, 107. However, there is no direct correlation between AV and taste/smell since, for example since it is not sensitive to some of the low molecular weight aldehydes known to have extremely high aroma impact on the oil. The AV is also highly affected by oil refining processes (bleaching, steam deodorization) so

oils with higher AV after extraction processes can have low AV after refining.

- iii. In summary, both AV and PV are widely used specifications for edible oils and can give an indication of oil quality *at a particular point in time*, and depending on how the oil has been processed and stored. PUFA containing oils are particularly susceptible to oxidation and hence to changes in AV and PV. Hence AV and PV are not used to specify the composition of oils or lipid, but rather they are a transient indication of oil quality.

II. SUMMARY OF THE '578 PATENT

A. Brief Description

49. Generally, the '578 patent describes a process to lyse a cell and obtain a lipid by separation, as well as properties of the obtained lipids. MARA-1001, Abstract. The process of the '578 patent is described as a cost-effective, industrial-scale process that does not rely upon the use of organic solvents. MARA-1001, 1:48-2:12. "Known methods can be used to culture a thraustochytrid of the invention, to isolate a biomass from the culture, and to analyze the fatty acid profile of oils extracted from the biomass." MARA-1001, 34:16-19. Following isolation, a base is applied or the cell's pH is altered to be more basic, after which

the cell can be treated with salt, lysed and then heated to between 60 and 100 degrees Celsius, and/or agitated to obtain a composition that can be centrifuged to obtain a lipid. MARA-1001, 28:23-29:40. Afterwards, the lipid is dried to remove water, and then further refined using “caustic refining, degumming, alkali-refining, bleaching, deodorization, deacidification, or the like, and combinations thereof to remove one or more phospholipids, free fatty acids, phosphatides, color bodies, sterols, odors, and other impurities.” MARA-1001, 30:39-49. This process produces a concentrated lipid. MARA-1001, 31:42-52.

50. The lipid obtained from the process of the '578 patent possesses certain physical properties. The lipids are not “fishy” tasting and exhibit “a lower anisidine value, lower peroxide value, lower phosphorus content and/or a higher extraction yield if extraction was performed using a solvent[.]” MARA-1001, 32:4-6, 32:52-56. Additionally, lipids claimed by the invention contain varying levels of docosahexaenoic acid (DHA), (MARA-1001, 4:4-8), and docosapentaenoic acid (DPA n-6), (MARA-1001, 4:8-10), such that the triglyceride fraction of the lipid is at least 70% of the total lipid by weight, (MARA-1001, 5:46-67). Further, lipids claimed by the '578 patent have anisidine values of less than 26, (MARA-1001, 3:53-55), and less than 5% by weight organic solvent, (MARA-1001, 6:54-55).

51. The '578 patent includes seven claims. Claims 1-3 are independent. Each independent claim recites an extracted microbial lipid that contains varying concentrations of DHA, DPA n-6, anisidine values, and less than 5 wt.% of an organic solvent. The dependent claims add additional properties of the lipids, such as peroxide values, phosphorus values, and the oil's status in refinement (e.g., crude or refined).

B. Summary of the Prosecution History of the '578 Patent

52. I understand that U.S. Patent 10,392,578 issued on August 27, 2019 from U.S. Provisional Patent Application No. 61/350,363 ("the '363 application"), filed on June 1, 2010; U.S. Provisional Patent Application No. 61/378,923 ("the '923 application"), filed on August 31, 2010; and U.S. Provisional Patent Application No. 61/452,721 ("the '721 application"), filed on March 15, 2011. *See* MARA-1001.

53. My review of the prosecution history indicates that the initial application included 62 claims, of which claims 52-58 eventually issued in modified form. MARA-1002, 155-156. I understand that claims 52-54, the claims which matured into granted claims 1-3, each recited "an extracted microbial lipid," a "docosahexaenoic acid content of the triglyceride fraction is at least 50/40/60% by weight," and an "anisidine value of 26 or less." MARA-1002, 155-156. I understand specifically that claims 52 and 53, which matured into claims 1 and 2,

also recited “wherein the docsapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight.” MARA-1002, 155-156. It appears to me that each of claims 52-54 resembled the as-issued claims, but lacked the recitation of a lower anisidine value (0.5) and of “less than 5% organic solvent.” *Compare* MARA-1002, 155-156 *with* MARA-1001, cls. 1-3. It is my understanding that the patentee amended other claims several times but did not change the language of claims 52-54 during the first three years of prosecution. MARA-1002, 178-179, 205, 224. I understand that the Examiner then allowed the patent but the Applicants requested additional review. MARA-1002, 230, 246. I further understand that as a result of that additional review, the Examiner cited U.S. Pub. 2005/0115897 to Dueppen and U.S. Pub. 2008/0148433 to Metz as disclosing the invention of the ’578 patent. MARA-1002, 266-268.

54. In response to Dueppen and Metz, I understand that Applicants changed claims 52-54 to add the language “wherein the lipid has less than 5% by weight of an organic solvent.” MARA-1002, 286. I also understand that Applicants argued that Dueppen taught a suspension of DPA n-6 and DHA in a solid fraction commingled with acetone polar solvents and argued that Dueppen did not show a solvent-free solution. MARA-1002, 289-292. And Applicants argued that “Metz does not cure the deficiencies of Dueppen.” MARA-1002, 292-293.

55. I further understand that the Applicants continued to request review of the patent. MARA-1002, 326, 358. I understand that in response, the Examiner cited Dueppen and Metz again to reject the claims. MARA-1002, 498-500. I also understand that Applicants resubmitted their earlier reply, (MARA-1002, 517-520), and the Examiner subsequently re-raised the same grounds of rejection to Dueppen and Dueppen in view of Metz, (MARA-1002, 547-550). Applicants “reiterate[d] its arguments from the previous office action” in reply. MARA-1002, 575-576.

56. I understand that after this, the Examiner allowed claims 52-58 but not the rest. MARA-1002, 590. In response, I understand that Applicant cancelled the other claims. MARA-1002, 605-606. It is my understanding that the Examiner made several findings about the claims, noting that the claims “recite ingredients in the oil that need not be present, i.e., are below a particular level.” MARA-1002, 1018, 1021-1022. Based on that finding, I understand that the Examiner rejected claims 52-58 as obvious over Elswyk (U.S. Pub. 2003/0060509) and Apt (U.S. Pat. No. 8,207,363) specifically because they each did not “disclose that the oil contains any organic solvent or the anisidine value.” MARA-1002, 1022.

57. In response, I understand that Applicant agreed that the Elswyk and Apt references “do[] not disclose that the oil contains any organic solvent or the anisidine value” and amended its claims to recite an anisidine value within the

range of 0.5 to 25. MARA-1002, 1073. I understand that the '578 patent then issued without comment from the Examiner. MARA-1002, 1076-1080.

C. The Effective Priority Date of the Claims of the '578 Patent

58. I understand that the '578 patent issued from U.S. Application No. 13/151,014, filed June 1, 2011. The '578 patent claims priority to three provisional applications, the earliest of which is U.S. provisional application 61/350,363, filed on June 1, 2010.

59. Claims 1-3 of the '578 patent are independent claims. Claims 4-7 depend directly or indirectly from claim 1. I understand from counsel then that claims 4-7 cannot have an effective filing date earlier than that of claim 1. I understand then that the effective filing date is no earlier than June 1, 2010.

D. Level of Ordinary Skill in the Art

60. A person having ordinary skill in the art relating to the subject matter of the '578 patent as of June 1, 2010 (a "PHOSITA") would have had a working knowledge of lipid extraction technologies. The PHOSITA would have had a Master of Science degree in an academic discipline emphasizing chemistry, chemical engineering, or biochemistry, in combination with training or at least two to three years of related work experience with lipid chemistry technologies, including but not limited to lipid extraction technologies. Alternatively, the person

could have also had a Doctorate degree in a relevant academic discipline with one to two years of related work experience in the same discipline.

III. CLAIMS 1-7 OF THE '578 PATENT ARE UNPATENTABLE

61. I have been informed by counsel that the question of obviousness requires analyzing (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of skill in the art; and (4) objective evidence of nonobviousness. Thus I discuss each in turn below.

62. The prior art discloses each of the elements of the Challenged Claims, as well as a motivation to arrive at the Challenged Claims, thereby rendering claims 1-7 of the '578 patent invalid.

A. GROUND 1: Claim 3 is obvious over Schaap in view of Kralovec

1. Overview of Schaap

63. In my opinion, Schaap is similar to the '578 patent because it seeks to obtain quality microbial oils by using cost-effective industrial processes. MARA-1001, 1:48-61, 2:8-12; MARA-1005, [0013]. I see in both the '578 patent and Schaap that microbial oils are naturally produced by variety of microorganisms and comprise high levels of polyunsaturated fatty acids ("PUFAs"). MARA-1001, 13:16-16:3; MARA-1005, [0002]. Schaap also discusses pasteurizing microorganisms, before extracting PUFAs or microbial oils for use. MARA-1005, [0001].

64. Schaap also describes the composition of microbial oils. Schaap teaches a microbial oil “compris[ing] at least 10, 35, 40, or 45% or more of a desired PUFA” and containing “at least 90% triglycerides,” by using a pasteurization process followed by “extracting and/or isolating a PUFA from the pasteurised cells.” MARA-1005, [0041]-[0042], [0056]. The extracted PUFAs are generally composed predominantly of triglycerides, from 50 wt.% to 98 wt.% of the total oil content. MARA-1005, [0055]. Further, the resulting microbial oils will comprise PUFAs, including a preferred PUFA of docosahexaenoic acid (“DHA”). MARA-1005, [0045]-[0053]. Schaap teaches that the end product will have an anisidine value between 5 and 25, with optimal values between 7 and 12. MARA-1005, [0082]-[0083]. The end product can be “extracted from the cells by using a solvent, such as supercritical carbon dioxide, hexane or isopropanol,” (MARA-1005, [0057]), and extraction is then followed by evaporation of the solvent, (MARA-1005, [0112]).

65. In my opinion then, Schaap discloses a “better quality” oil that contains primarily triglycerides, DHA, and has a low concentration of solvent and a low anisidine value. MARA-1005, [0013], [0083]-[0085], [0112]; MARA-1001, 2:16-21, 5:46-53.

2. Overview of Kralovec

66. Kralovec relates to methods for modifying oil concentrations by concentrating polyunsaturated fatty acids. I note that Kralovec recognizes that omega-3 fatty acids like DHA have “well established” beneficial effects, such as reduction of “cardiovascular and heart disease risk” and “prevention and/or treatment of inflammation, neurogenerative diseases, and cognitive development.” MARA-1030, 1:16-2:3. Kralovec also acknowledges that omega-3 fatty acids can be found in microbial and algal oils, which “typically provide the PUFA in a triglyceride form where other undesired, fatty acids...are present along side a desired PUFA in the triglyceride molecule.” MARA-1030, 2:4-8. Kralovec is therefore directed to a modification method to produce “an oil having higher levels of a PUFA...than the starting oil composition.” MARA-1030, 5:24-28.

67. In my opinion, the Kralovec method can be broadly used with “any suitable oil composition that comprises a glyceride,” including microbial and algal oils. MARA-1030, 6:28-7:2. In fact, Kralovec specifically notes that its customizable methods allow the “specific composition of a treated oil” to be “adjusted and/or tailored to have a target ratio of various polyunsaturated fatty acids,” which can allow for “high concentrations of DHA.” MARA-1030, 6:9-13.

68. Kralovec’s method employs three steps: hydrolysis, separation, and esterification. Hydrolysis is a process that uses enzymatic lipase to contact a

starting oil, which induces hydrolysis of the oil. MARA-1030, 9:8-10. This process creates hydrolyzed glycerides and free saturated fatty acids. MARA-1030, 9:8-10. Using modifications such as atmospheric conditions, mixing/agitation, or elevated temperature, Kralovec describes that a user induces a “desired amount of hydrolysis.” MARA-1030, 11:15-28. Then, the “hydrolyzed glyceride composition...can then be separated...to separate at least a portion of the free saturated fatty acid from the glyceride.” MARA-1030, 12:13-16. In a further step, Kralovec describes that lipase is used to attach “desirable polyunsaturated fatty acid components, for example, EPA and/or DHA” to the hydrolyzed glyceride compositions. MARA-1030, 13:27-30. In this manner, Kralovec’s method provides compositions containing “triglycerides with higher DHA...contents than the starting triglycerides.” MARA-1030, 35:20-32.

3. Combination of Schaap and Kralovec

1) DHA as the preferred PUFA

69. As I discuss above in Section III.A.1, Schaap teaches a “microbial oil may comprise at least...45% or more of a desired PUFA,” in which the “[p]referred PUFAs include arachidonic acid (ARA), docosohexaenoic acid (DHA), eicosapentaenoic acid (EPA) and/or γ -linoleic acid (GLA).” MARA-1005, [0053], [0056]. In light of Schaap’s disclosure, in my opinion it would have been obvious for a PHOSITA to have sought out microbial oils having high levels of

DHA as the preferred PUFA based on its teachings as well as those of other related references available before the Critical Date. MARA-1005, [0056]; MARA-1030, 1:9-11. In particular, a PHOSITA would have been motivated to obtain DHA as the preferred PUFA in Schaap's extracted microbial oil for at least the following reasons.

70. First, a PHOSITA would have been motivated to seek DHA as the preferred PUFA in Schaap's extracted microbial oil because there were many recognized health benefits associated with DHA. For example, I note that DHA was known to reduce "cardiovascular and heart disease risk" and crucial to the "prevention and/or treatment of inflammation, neurogenerative diseases, and cognitive development." MARA-1030, 1:16-2:3. I note also that DHA was associated with "a strong insulin permeability enhancement effect" and was "viewed as a potential absorption enhancer for intestinal delivery of insulin." MARA-1030, 18:10-19:2. For example, DHA was understood to "prevent[] certain biochemical processes that originate from insulin deficiency" and "significantly increase fasting insulin levels." MARA-1030, 18:12-19:6; *see also* MARA-1010, 117 ("As well as the critical role that DHA plays in neural/eye development, there is significant (and accumulating) evidence that increased dietary DHA plays a role in the maintenance of human health and helps ward off

chronic health issues, such as heart disease, depression, and even some forms of dementia later in life.”).

71. Second, a PHOSITA would have been motivated to seek DHA as the preferred PUFA in Schaap’s microbial oil because DHA is an omega-3 fatty acid, generally known to be “vital to everyday life and function.” MARA-1030, 1:13-14. For example, consistent with what I discuss above in Section I.E.a, Kralovec notes that “[o]mega-3 fatty acids not only exhibit proven effects on lowering serum triglyceride levels, but they have strong connection to diabetes.” MARA-1030, 18:8-12. Further, omega-3 fatty acids were also “known for other cardioprotective benefits,” such as “reduc[ing] cardiovascular and heart disease risk.” MARA-1030, 1:13-2:3.

72. Specifically, it is my opinion that a PHOSITA would have been motivated to obtain DHA as the preferred PUFA in Schaap’s oil because DHA would have been an obvious choice selected from a small and finite number of preferred omega-3 fatty acids. For example, I see that Schaap discloses DHA as one of the three “suitable” n-3 PUFAs (docosahexaenoic acid (DHA, 22:6 ω 3), α -linolenic acid (ALA, 18:3 ω 3), and eicosapentaenoic acid (EPA, 20:5 ω 3)). MARA-1005, [0045]-[0053].

73. Further, I believe that a PHOSITA would have expected to be successful in pursuing DHA as the preferred PUFA in an extracted oil specifically

because Schaap identifies DHA as a preferred PUFA. MARA-1005, [0053].

Moreover, a PHOSITA would have expected success in pursuing DHA as the preferred PUFA particularly in the context of an extracted microbial oil given that it was already known that certain strains of microbes contained “very high levels of DHA (61%).” MARA-1009, 27.

2) Increased Omega-3 Fatty Acid (DHA) Concentration in Microbial Oils

74. As I discuss above in Section III.A.2, Kralovec provides methods for obtaining compositions containing “triglycerides with higher DHA...contents than the starting triglycerides.” MARA-1030, 35:20-32. Thus, it would have been obvious for a PHOSITA to combine the teachings of Schaap, which is directed to microbial oils having high levels of triglycerides (e.g., preferably at least 98%) with a preferred PUFA (DHA), with Kralovec, which is directed to methods for modifying an oil composition, in order to increase the concentration of that PUFA in the microbial oil. MARA-1005, [0056], MARA-1030, 1:9-11. More specifically, in my opinion a PHOSITA would have found it obvious to implement Kralovec’s oil modification techniques to Schaap’s extracted microbial oil to increase the amount of DHA for at least the following reasons.

75. First, a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to Schaap’s extracted microbial oil to increase its DHA content because Kralovec explains that “purifying and concentrating PUFA’s [sic]

in a triglyceride form is generally desired.” MARA-1030, 2:7-8. In my opinion, it would have been well-known to a PHOSITA as of the Critical Date that physical removal of unwanted components in a microbial oil would naturally be desirable because “[p]olyunsaturated fatty acids, such as, for example omega-3 fatty acids, are often derived from marine oils, microbial, and/or algal oils,” which “typically provide the PUFA in a triglyceride form where other undesired, fatty acids...are present along side a desired PUFA in the triglyceride molecule.” MARA-1030, 2:4-8.

76. Second, in my opinion a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to an extracted microbial oil like the oil in Schaap to increase its DHA content because the produced oils include “‘designer’ glycerides” and “often non-naturally occurring, ratios of polyunsaturated fatty acid ester” that yield triglycerides with “better color and lower levels of trans-isomers” as well as “better sensory properties.” MARA-1030, 35:21-36:2. Kralovec explains that “[s]uch oils can be incorporated into food products, nutraceuticals, pharmaceutical formulations, and microcapsules[.]” MARA-1030, 35:21-36:2.

77. Third, in my opinion a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to Schaap’s extracted microbial oil because such combinations reflect nothing more than the use of a known technology (e.g.,

enzymatic modification) to improve similar oils (e.g., extracted microbial oils) according to known methods (e.g. oil refinement methods) to yield predictable results (e.g., obtain oil with an increased concentration of a desired PUFA, e.g., DHA).

78. Additionally, it is my opinion that a PHOSITA would have expected to be successful in applying Kralovec's techniques to increase the concentration of the preferred PUFA (i.e., DHA) in Schaap's microbial oil because Schaap contemplates that its invention "can be subjected to one or more refining steps." MARA-1005, [0065]. Moreover, I believe a PHOSITA would have naturally considered Kralovec's methods for modifying the oil because Schaap specifically discloses that "the oil can be subjected to...free fatty acid removal" or to apply "winterisation...to remove saturated triglycerides[.]" MARA-1005, [0065]. Kralovec discloses that its methods remove saturated free fatty acids. MARA-1030, 2:20-24. Kralovec also discloses that its methods can be used as a "substitute for a winterization process." MARA-1030, 13:1-4. Thus, in my opinion a PHOSITA would have looked to refine Schaap's oils in accordance with Schaap's teachings by looking to Kralovec.

79. Moreover, I believe that a PHOSITA would have expected success in using Kralovec's techniques for increasing the concentration of the preferred PUFA (i.e., DHA) particularly in the context of an extracted microbial oil given

that Schaap and Kralovec note that their oils are microbial/algal in origin and thus have similar base characteristics. MARA-1005, [0063]; MARA-1030, 2:28-29. Both Schaap and Kralovec identify their oils as specifically containing omega-3 PUFAs, and even more specifically DHA. MARA-1005, [0045]-[0046]; MARA-1030, 19:7-12. Furthermore, I note that both Schaap and Kralovec acknowledge similar uses for their oils, such as human nutritional uses. Schaap, for instance, teaches that its resultant oil is “particularly suitable for nutritional purposes, and can be added to (human) foods” such as “milk, infant formula, health drinks, bread and animal feed.” MARA-1005, [0066]. Likewise, Kralovec also states that its oils “can be incorporated into food products, nutraceuticals, pharmaceutical formulations, and microcapsules.” MARA-1030, 35:31-36:2.

4. Analysis

Claim 3

[3pre] An extracted microbial lipid comprising:

80. Schaap discloses an extracted microbial lipid. Schaap generally describes an extraction process for obtaining microbial oil from microbial cells. MARA-1005, [0062]-[0063] (Section titled “PUFA Extraction Process,” describes that “[t]he PUFA (or microbial oil, usually comprising the PUFA) may...be extracted from the (pasteurised) microbial cells”), [0068] (“The microbial cells (or micro-organisms) used in the present invention....may comprise, or be able to produce, a PUFA or microbial oil, and suitably the PUFA oil may be extracted or

isolated from the cells”). In particular, Schaap describes “a PUFA-containing microbial oil.” MARA-1005, [0063] (“[PUFA] is extracted from (e.g. dried) granules (e.g. extrudates) containing the cells”), claim 5. Schaap teaches that the PUFA includes “a single PUFA or two or more different PUFAs.” MARA-1005, [0044]. Schaap describes that the “PUFA may be produced by the cells...such as a microbial cell,” including “*Mortierella, Phycomyces, Blakeslea, Aspergillus, Thraustochytrium, Pythium* or *Entomophthora*.” MARA-1005, [0054].

81. I understand Schaap’s composition comprising a lipid obtained from an extraction process to be the same thing as an “extracted microbial lipid” in the context of the claims of the ’578 patent. For example, Schaap describes that its “invention relates to a composition comprising [an] oil” obtainable through extraction: “[A] polyunsaturated fatty acid (PUFA)...or microbial oil may be extracted from the cells.”¹ MARA-1005, [0001], [0095]. Schaap’s microbial oil is an extracted composition that includes lipids in addition to unsaponifiables, solvent, fatty acids, phosphorus, silicon, and other elements. MARA-1005, [0085], table. I understand Schaap’s “microbial oil...extracted from the cells” to mean microbial *lipids* extracted from microbial cells.

¹ Emphasis has been added in this document unless otherwise noted.

[3a] a triglyceride fraction of at least about 70% by weight, of the lipid fraction

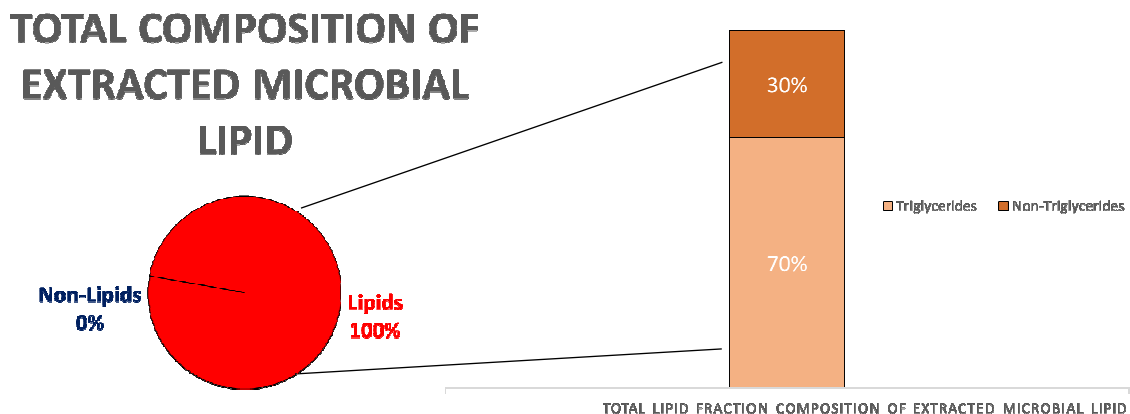
82. Schaap and Kralovec disclose and/or render obvious a triglyceride fraction of at least about 70% by weight, of the lipid fraction.

83. I note that the '578 patent does not define the term "lipid fraction." I would usually interpret that to mean the lipid portion of the extracted composition, excluding non-lipid components. That is because lipid extracts generally comprise lipid components and residual solvents. MARA-1010, 180-181. The residual solvent is not part of the "lipid fraction," and so the "lipid fraction" is less than 100 wt.% of the entire composition.

84. The '578 patent, however, claims further (in Element [3d]) that "the lipid has less than 5% by weight of an organic solvent." Since the '578 patent uses the terms "the oil" and "the lipid" interchangeably, the "less than 5% by weight of an organic solvent" element can mean that it is part of "the oil/the lipid" without being part of the "lipid fraction" because solvent is not a lipid. Alternatively, the "less than 5% by weight of an organic solvent" element can be understood to mean that it is part of the "lipid fraction" because it is found in "the oil" and therefore is part of the "extracted microbial lipid" as a whole.

85. Whether the "lipid fraction" means the same thing as "the oil" or not, Schaap discloses a triglyceride fraction that is at least 70% of that lipid fraction.

86. If the “lipid fraction” is 100 wt.% of the extracted microbial lipid, then Schaap still discloses or renders it obvious. As the figure below shows, triglycerides would constitute at least 70 wt.% of the total composition *and* of the “lipid fraction.”

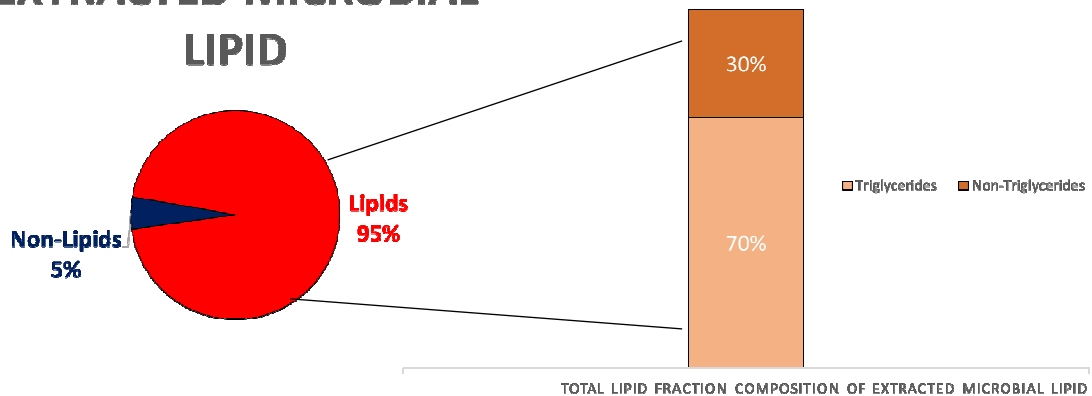


87. Schaap discloses at least 70 wt.% percent triglycerides in the total composition. For example, Schaap teaches that “optimally at least 70%” of the fatty acids are triglycerides, and may be higher, “such as at least 85%, preferably at least 90%, optimally at least 95% or 98% of the oil” are triglycerides. MARA-1005, [0055], [0056] (“Preferably, the microbial oil has a triglyceride content of from 90 to 100%”). Schaap also teaches that “[i]t is preferred that most of the PUFA is in the form of triglycerides.” MARA-1005, [0055]. Schaap also describes a microbial oil in which the percentage of triglycerides present in the entire lipid composition is greater than 70% by weight. MARA-1005, [0055].

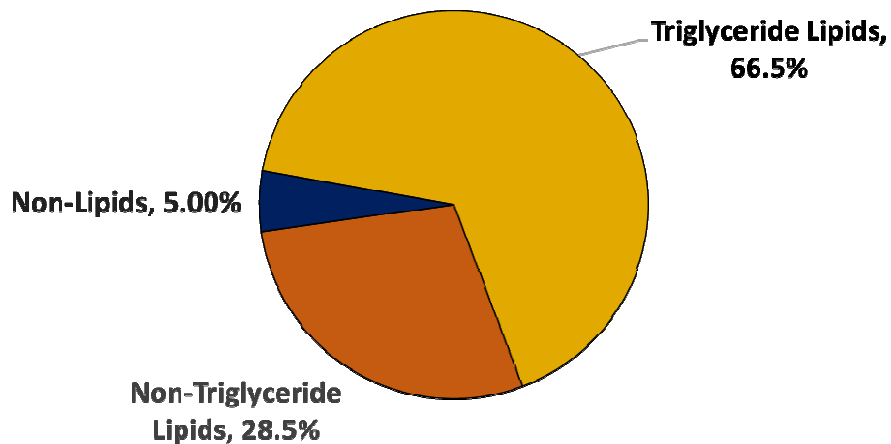
88. In the combination, in my opinion Kralovec's post-extraction methods would further increase the triglyceride fraction of the extracted microbial lipid composition. Kralovec specifically explains that its method "produce[s] triglycerides of fatty acids," which result in oils with "a high TG (triglyceride) content." MARA-1030, 35:27-28, 37:22. Thus, in my opinion, a PHOSITA starting with Schaap's starting oil, which has at least 70% triglycerides, and applying Kralovec's methods would further increase the amount and concentration of triglycerides. MARA-1030, 5:24-26 (describing formation of "a substituted triglyceride").

89. On the other hand, if the "lipid fraction" excludes solvents, then Schaap discloses that triglycerides comprise at least 70 wt.% of the lipid fraction in terms of equivalent wt.% of the total composition. As I show in the figures below, if the total composition of the extracted microbial oil is 95 wt.% lipid and 5 wt.% non-lipid components, then the lipid fraction is 95 wt.% of the total composition. Of that total composition, a minimum of 66.5 wt.% would be triglycerides (95% lipid fraction multiplied by 70 wt.% triglycerides). I understand that a composition could contain greater quantities of triglycerides as well. However, my analysis focuses on this minimum number because the '578 claims a range.

TOTAL COMPOSITION OF EXTRACTED MICROBIAL LIPID



TOTAL COMPOSITION OF EXTRACTED MICROBIAL LIPID



90. I note that Schaap discloses compositions that include triglycerides in an amount of at least 66.5 wt.% percent of the total composition. MARA-1005, [0055]-[0056]. Additionally, Schaap teaches that preferred fatty acids are generally in the form of triglycerides. MARA-1005, [0055]. And relative to the entire oil, the microbial oil is “optimally at least 70%” triglycerides, but includes when “the microbial oil has a triglyceride content of from 90 to 100%.” MARA-

1005, [0055]-[0056]. In the combination, it is my opinion that Kralovec's post-extraction methods would increase the triglyceride fraction of the extracted microbial lipid composition even further. The method "produce[s] triglycerides of fatty acids," which result in oils with "a high TG (triglyceride) content." MARA-1030, 35:27-28, 37:22. From Schaap's starting oil, which has at least 70% triglycerides, a PHOSITA applying Kralovec's methods would further increase the amount and concentration of triglycerides. MARA-1030, 5:24-26 (describing formation of "a substituted triglyceride").

91. In either interpretation, Schaap discloses a triglyceride fraction of at least 70 wt.% because it discloses triglycerides in quantities as high as 97%. That, in my opinion, means that Schaap renders this element obvious.

[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and

92. Schaap and Kralovec disclose and/or render obvious a DHA content of at least 60 wt.% of the triglyceride fraction.

93. In interpreting this element, the claims only specify a DHA content of the triglyceride fraction of the extracted microbial lipid.

94. A PHOSITA would have understood that in the Schaap microbial oil, the PUFAs would be predominately present in the triglyceride fraction. A triglyceride, by definition, is a combination of three fatty acids and glycerol that forms an ester. If a composition contains high levels of fatty acids and high levels

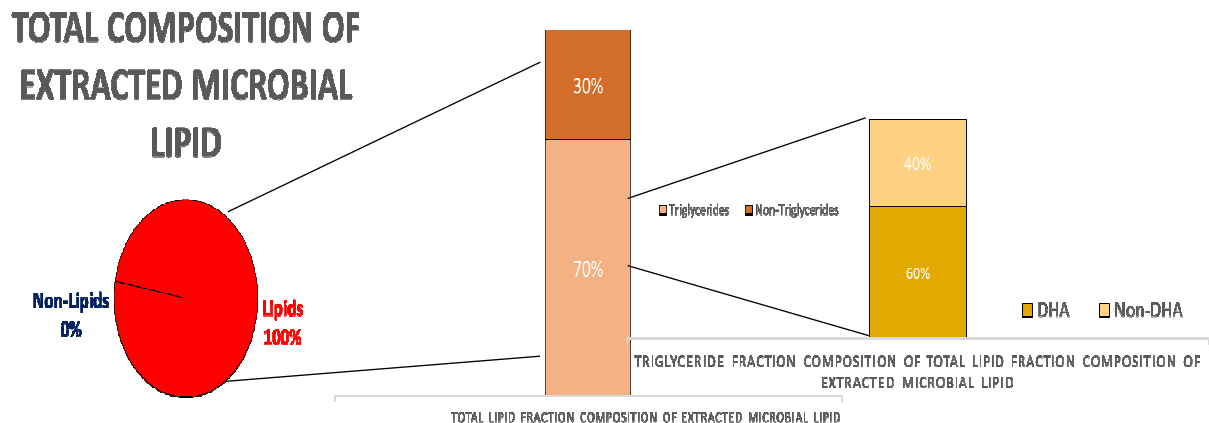
of triglycerides, then a PHOSITA would also understand that fatty acids are likely present in the triglyceride form.

95. Schaap's disclosures confirm this to be the case. If not in triglyceride form, then fatty acids are present as free fatty acids, salts, fatty acid esters, phospholipids, or as a mono- or di-glycerides. MARA-1005, [0044]. Schaap discloses that those alternative forms are not present in significant quantities. Phospholipids, for example, preferably are present in low quantities. MARA-1005, [0085], (table). Free fatty acids are also less than 0.08% in certain cases. MARA-1005, [0085], (table), [0089]. To a PHOSITA, this means that most of the fatty acids—and therefore DHA—are in triglyceride form in the lipid fraction.

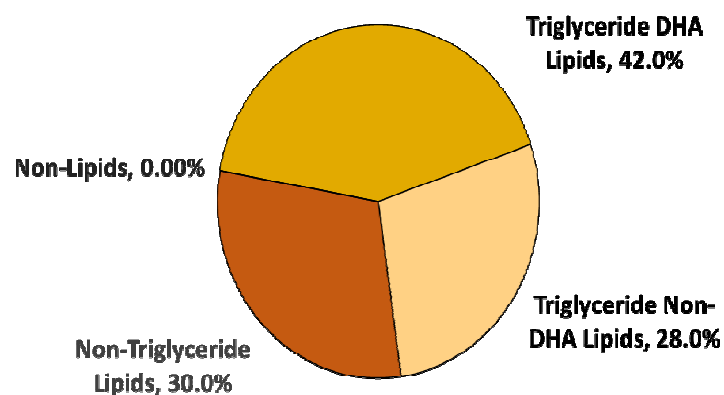
96. When a "lipid fraction" is 100% of the composition, Schaap discloses and/or renders obvious a triglyceride fraction that is at least about 70% by weight and DHA comprising at least 60 wt.% of the triglycerides. MARA-1005, [0055]-[0056]. Because I understand the claims to require DHA in the triglyceride fraction, and not in the remainder of the lipid fraction, I understand claim element [3b] to require a minimum DHA content in the lipid fraction of 42% in association with a minimum triglyceride content in the lipid fraction of 70% by weight. A PHOSITA would have understood that as well. If triglycerides comprise a minimum of 70 wt.% of the total lipid composition and DHA comprises a minimum of 60 wt.% of that triglyceride fraction, then a minimum 42 wt.% of the

“lipid fraction” (i.e., the overall composition) is DHA, as shown below. I

understand that a composition could contain greater quantities of triglyceride DHA lipids and still meet the claims of the patent; this is merely a minimum amount necessary.



TOTAL COMPOSITION OF EXTRACTED MICROBIAL LIPID



97. In my view, Schaap discloses or renders obvious this limitation. For example, Schaap teaches that “microbial oil may comprise at least...45% or more of a desired PUFA.” MARA-1005, [0053], [0056]. Schaap also teaches a PUFA-

containing microbial oil that includes “a single PUFA or two or more different PUFAs,” including docosahexaenoic acid (DHA). MARA-1005, [0045]-[0053], claim 5. Of those PUFAs, DHA is a “[p]referred” PUFA. MARA-1005, [0045], [0053], claim 9. DHA was well-known and sought after for its many benefits: “As well as the critical role that DHA plays in neural/eye development, there is significant (and accumulating) evidence that increased dietary DHA plays a role in the maintenance of human health and helps ward off chronic health issues, such as heart disease, depression, and even some forms of dementia later in life.” MARA-1010, 117.

98. Additionally, a PHOSITA would have understood that *Thraustochytrium* microbes, such as those disclosed in Schaap, would have produced oil that was “preferably 50% by weight or more” DHA. MARA-1015, [0058]; MARA-1015, [0108] (disclosing 58.8% DHA); MARA-1009, 29 (Table 2 discloses 61% DHA); MARA-1032, 27-31 (Table 2.5 discloses 67.2, 65.6, 67.8, 63.7, 64.3, 62.4 and other percentages DHA). As I explain above, Schaap discloses that its oils are microbially derived from sources, including those from *Thraustochytrium*. MARA-1005, [0045]-[0046]. Furthermore, it was well known to a PHOSITA, based on the references available before the Critical Date, that particular microbial strains (e.g., “[s]train O” of an Australian thraustochytrid) contained a high level of DHA (e.g., 61% or higher). MARA-1009, 27; *see also*

MARA-1032, 27-31 (Table 2.5 discloses 67.2, 65.6, 67.8, 63.7, 64.3, 62.4 and other percentages DHA). A PHOSITA would have understood, then, that such strains were a desirable microbial source from which to produce Schaap's extracted microbial oil. Additionally, it would have been obvious that applying Kralovec's techniques to a microbial oil derived from a high-DHA containing strain (and extracted according to Schaap's process) would be expected to produce oils having levels of DHA significantly greater than the starting amount of DHA (i.e., 61%) in the triglyceride fraction since Schaap's microbial oil generally contains a high triglyceride content (up to 100%, and preferably at least than 98%). MARA-1018, [0571]; MARA-1020, 384-385, Table 2.

99. Accordingly, a PHOSITA would have understood from Schaap's disclosure that its total composition could comprise upwards of 45% or more of DHA, a "[p]referred" PUFA. MARA-1005, [0053]. As I discuss above in Section §III.A.3, in the combination, it is my opinion that a PHOSITA would have been motivated to apply Kralovec's oil modification techniques to Schaap's extracted microbial oil to increase its DHA content. In doing so, I note that Kralovec discloses its methods can be used to produce "concentrated oils with triglycerides containing higher proportions of polyunsaturated fatty acid esters than the starting glycerides," such as "oils that comprise greater than or equal to about...80% DHA by weight of the total composition." MARA-1030, 35:20-27, 36:12-15. Thus, in

my opinion these oils would “also have a high TG (triglyceride) content” and the method would “produce triglycerides of fatty acids.” MARA-1030, 35:27-30, 37:22. The end result would be “an oil having higher levels of a PUFA...than the starting oil composition.” MARA-1030, 5:24-32.

100. In my opinion, for all of the reasons I explain above, a PHOSITA would therefore have understood and/or found it obvious to apply Kralovec’s (or similar) methods to Schaap’s microbial oil to produce a concentration of DHA in the triglyceride form of the microbial oil such that the resulting oil contains at least 50 wt.% DHA, or higher, such as at least 60 wt.% DHA in the triglyceride fraction.

[3c] wherein the oil has an anisidine value of 0.5 to 26, and

101. Schaap discloses and/or renders obvious an oil with an anisidine value of 0.5 to 26.

102. Because “the oil” means the same as “extracted microbial lipid,” this also means that a PHOSITA would have understood and/or found obvious from Schaap’s disclosure that its compositions have anisidine values between 0.5 and 26. MARA-1005, [0082]-[0083]. Anisidine values “give a measure of the aldehyde content.” MARA-1005, [0083]. Anisidine values are also a measure of the quality of processing. Schaap discloses that preferable anisidine values of its oils range from 5 to 25, which are completely encompassed within the ’578 patent’s claimed range of 0.5 to 26. MARA-1005, [0083]. Experimental results

from Schaap demonstrate that its oils have anisidine values ranging from 5 to 15. MARA-1005, [0083]. And Figure 5 demonstrates that Schaap's exemplary extracted oils have anisidine values under 25. MARA-1005, Fig. 5, [0011], [0132].

[3d] wherein the lipid has less than 5% by weight of an organic solvent.

103. As I read the '578 patent specification, solvent is optional: "the lipid optionally contains less than 5% by weight or volume of an organic solvent." *E.g.*, MARA-1001, 2:21-22, 2:54-56, 2:61-63. In other words, the lipid could contain no organic solvent. I understand from the prosecution history that the Examiner drew the same conclusion when they wrote that the claims "recite ingredients in the oil that need not be present," meaning that art that "does not disclose that the oil contains any organic solvent" reads on the patent. MARA-1002, 1018, 1021-22. I also understand that Applicant agreed that references that "do[] not disclose that the oil contains any organic solvent" read on the claim. MARA-1002, 1073.

104. It is also my opinion that if the '578 patent requires a non-zero amount of organic solvent that is less than 5 wt.% (i.e., greater than 0 wt.% but less than 5 wt.%), Schaap and Kralovec still disclose and/or render obvious that requirement. For example, Schaap discloses a crude oil obtained by extraction using a solvent (e.g., hexane) having no more than 2000 ppm of that solvent in its composition. MARA-1005, [0057], [0063]-[0065], [0085]. A PHOSITA would

have understood that 2000 ppm equates to 0.2 wt.% organic solvent based on the following conversion calculations:

$$1 \text{ ppm} = 1 \text{ part solute}/10^6 \text{ parts solution}$$

$$0.2\% = 0.2 \text{ parts in } 100 \text{ parts solution}$$

$$0.2\% = 2000/10^6 \text{ parts solution}$$

$$0.2\% = 2000 \text{ ppm}$$

Thus,

$$\mathbf{2000 \text{ ppm} = 0.2\% \text{ by wt.}}$$

105. Accordingly, a PHOSITA would have understood that Schaap's crude oil contains no more than 0.2 wt.% (i.e., 2000 ppm) of the solvent in the extracted microbial lipid composition, and therefore the organic solvent would constitute less than 5% by weight of the composition.

106. Further, a PHOSITA would have understood that residual organic solvents from extracting microbial oils were not desirable and would seek to limit or remove them from a raw or unrefined extracted oil composition (e.g., crude oil). MARA-1010, 193 (“[S]olvents are too toxic to be used for nutritional or pharmaceutical purposes” and therefore would be removed). Schaap reflects that understanding; in embodiments, Schaap describes the removal (e.g., evaporation) of organic solvents from crude oil to obtain a refined oil. MARA-1005, [0112], [0121], [0130]. I note that Schaap discloses that the “resulting oil” of its processes

is “particularly suitable for nutritional purposes,” and even includes a use as an additive for “infant formula” and “health drinks[.]” MARA-1005, [0066]. Schaap further discloses a refined oil obtained by extraction using a solvent that results in an oil with no more than 1 ppm (or undetectable levels) of that solvent in the final composition. MARA-1005, [0085] (*see also* table listing residual solvent amounts). Thus, Schaap’s refined oil would contain less than 5 wt.% of an organic solvent in total extracted microbial lipid composition. MARA-1005, [0085] (*see also* table).

107. Similarly, a PHOSITA would understand that applying Kralovec’s refinement methods would eliminate and/or reduce the quantity of solvents present. Kralovec teaches using solvents that are “compatible with human consumption,” for example, it teaches that “water is a preferred solvent.” MARA-1030, 34:17-19.

108. Water is comprised of two hydrogen molecules and one oxygen molecule. As neither of those is carbon, water is therefore not an organic solvent. Put another way, if water was used as the solvent, there would necessarily be “less than 5% by weight organic solvent” in the lipid.

109. Furthermore, I note that Kralovec also discloses that its methods steps can “be performed...in the absence of solvent.” MARA-1030, 23:15-17. Accordingly, for that additional reason, in my opinion a PHOSITA would have

understood that refining Schaap's oils according to Kralovec's methods would not introduce residual solvent, meeting Element [3d] and rendering it obvious.

110. For the reasons provided above, in my opinion Schaap discloses or renders obvious that the lipid, crude, refined or otherwise, has less than 5% by weight of an organic solvent.

B. GROUND 2: Claims 1-7 are obvious over Schaap in view of Komazawa

1. Overview of Schaap

111. I incorporate my discussion of Schaap in Section III.A.1, above.

2. Overview of Komazawa

112. Komazawa "relates to a novel microorganism having an ability of producing docosahexaenoic acid (DHA) and the use of said microorganism."

MARA-1015, [0001]. Specifically, I note that Komazawa teaches "culturing the Thraustochytrium strain having an ability of producing docosahexaenoic acid in a medium, and collecting the docosahexaenoic acid-containing fat and oil from the culture product." MARA-1015, [0012].

113. To increase the amount of DHA present, Komazawa explains that "to promote the production of docosahexaenoic acid, a docosahexaenoic acid precursor can be added to a medium." MARA-1015, [0044]. I note that Komazawa discloses that "[t]he DHA component in the triglycerides produced shows characteristic tendency." MARA-1015, [0116]. This means that

Komazawa increases DHA primarily by adjusting pH, as it discusses how the “DHA component is around 40% when culturing was carried out at pH 7.5, but DHA component becomes a high content of 80% or more, when culturing was carried out at pH 8.5.” MARA-1015, [0116].

3. Combination of Schaap and Komazawa

1) DHA as the preferred PUFA

114. As I discuss in detail above in Sections III.A.1 and III.A.3.1, it would have been obvious for a PHOSITA to have sought microbial oils having high levels of DHA as the preferred PUFA based on Schaap’s teachings and other related references available before the Critical Date. MARA-1005, [0056]; MARA-1030, 1:9-11. In my opinion, a PHOSITA would have been motivated to obtain DHA as the preferred PUFA in Schaap’s extracted microbial oil for the same reasons I discuss above.

2) Increased Omega-3 Fatty Acid (DHA) Concentration in Microbial Oils

115. Additionally, in my opinion a PHOSITA would further have been motivated by Komazawa. Komazawa provides methods for “culturing the *Thraustochytrium* strain having an ability of producing docosahexaenoic acid in a medium, and collecting docosahexaenoic acid-containing fat and oil from the culture product.” MARA-1015, [0012]. I believe that a PHOSITA would have found it obvious to combine the teachings of Schaap, directed to microbial oils having high levels of a preferred PUFA (DHA) as triglycerides, with Komazawa

because Komazawa specifically “provides a microorganism which has a high ability of producing docosahexaenoic acid.” MARA-1015, [0001]; MARA-1005, [0056]. In particular, it is my opinion that a PHOSITA would have found it obvious to implement Komazawa’s methods with Schaap’s extracted microbial oil to increase the amount of DHA for at least the following reasons.

116. First, a PHOSITA would have been motivated to apply Komazawa’s methods to Schaap’s extracted microbial oil to increase its DHA content because PHOSITAs knew that “purifying and concentrating PUFA’s [sic] in a triglyceride form is generally desired.” MARA-1030, 2:7-8. In my opinion, it would have been well-known to a PHOSITA that fewer unwanted components in a microbial oil would be desirable because if the “oil contains a variety of polyunsaturated fatty acids such as arachidonic acid or icosapentaenoic acid (EPA), it is likely to be oxidized” it would be “difficult to obtain fat and oil having stable quality.” MARA-1015, [0003]; MARA-1030, 2:4-8 (“Polyunsaturated fatty acids, such as, for example omega-3 fatty acids, are often derived from marine oils, microbial, and/or algal oils,” and “typically provide the PUFA in a triglyceride form where other undesired, fatty acids (e.g., saturated fatty acids) are present along side a desired PUFA in the triglyceride molecule.”). Furthermore, I note that Komazawa also teaches that “when docosahexaenoic acid is used in the field of medicines or the like, it is necessary that docosahexaenoic acid is separated and purified from

docosahexaenoic acid-containing fat and oil” but “a variety of polyunsaturated fatty acids having structures similar to that of docosahexaenoic acid are contained, and thus, separation and purification are difficult”—a problem that Komazawa sought to solve. MARA-1015, [0003].

117. Second, in my opinion a PHOSITA would have been motivated to apply Komazawa’s oil modification techniques to Schaap’s extracted microbial oil because “[DHA] is a useful substance, the use of which has drawn attention in the fields of medicines and food products”; as such, it would be generally desirable to have sources containing increased amounts of DHA. MARA-1015, [0002].

118. Third, it is my opinion that a PHOSITA would also have been motivated to apply Komazawa’s methods to Schaap’s extracted microbial oil because such combinations reflect nothing more than the use of a known technology (e.g., provide a desired microorganism) to improve similar oils (e.g., extracted microbial oils) according to known methods (e.g. oil extraction methods) to yield predictable results (e.g., obtain oil with an increased concentration of a desired PUFA, e.g., DHA).

119. I believe that a PHOSITA would have expected to be successful in applying Komazawa’s techniques to increase the concentration of the preferred PUFA (i.e., DHA) in Schaap’s microbial oil because Schaap contemplates that its invention “can be subjected to one or more refining steps.” MARA-1005, [0065].

Moreover, a PHOSITA would have naturally considered Komazawa's methods for providing a high DHA-producing microorganism because Schaap discloses that "microbial oil may comprise at least 10, 35, 40 or 45% *or more* of a desired PUFA." MARA-1005, [0056]. Thus, in my opinion a PHOSITA would have looked to increase the levels of the preferred PUFA in Schaap's oils by looking to references such as Komazawa.

120. Moreover, a PHOSITA would have expected success in using Komazawa's method techniques for obtaining increased amounts of the preferred PUFA (i.e., DHA) particularly in the context of an extracted microbial oil given that Schaap and Komazawa teach that their oils are microbial/algal in origin and so have similar characteristics. MARA-1005, [0046]; MARA-1015, [0001]. I note that both Schaap and Komazawa acknowledge their oils are specific to omega-3 PUFAs, and even more specifically, to DHA. MARA-1005, [0045]-[0046], [0053]; MARA-1015, [0001]. Furthermore, both Schaap and Komazawa acknowledge similar uses for their oils, such as human nutritional uses. Schaap, for example, teaches that its resultant oil is "particularly suitable for nutritional purposes, and can be added to (human) foods" such as "milk, infant formula, health drinks, bread and animal feed." MARA-1005, [0066]. Komazawa too discloses that its oils are used in "the field of functional foods such as health foods and formulas." MARA-1015, [0002].

4. Analysis

Claim 1

[1pre] An extracted microbial lipid comprising

121. The combination of Schaap and Komazawa discloses and/or renders obvious Element [1pre] for at least the same reasons I discuss above in Section III.A.4 [3pre].

[1a] a triglyceride fraction of at least 70% by weight of the lipid fraction,

122. The combination of Schaap and Komazawa discloses and/or renders obvious Element [1a] for at least the same reasons I discuss above in Section III.A.4[3a].

[1b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight,

123. The combination of Schaap and Komazawa discloses and/or renders obvious Element [1b].

124. As I explain above, Schaap teaches that “microbial oil may comprise at least...45% or more of a desired PUFA[.]” MARA-1005, [0056]. Schaap also teaches a PUFA-containing microbial oil that includes “a single PUFA or two or more different PUFAs,” including DHA. MARA-1005, [0044]-[0053]. Indeed, of those PUFAs, DHA is a “[p]referred” PUFA. MARA-1005, [0045]-[0046], [0053]. As I explain above in Section III.A.3.1, DHA was well-known and sought after for its many benefits and therefore would have been sought after as a preferred PUFA. MARA-1010, 117.

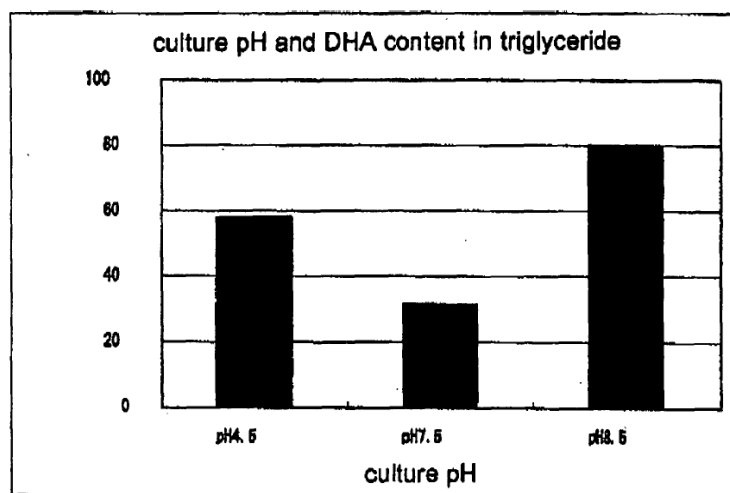
125. Moreover, in my opinion a PHOSITA would have understood that *Thraustochytrium* microbes, such as those disclosed in Schaap, would have produced oil that was “preferably 50% by weight or more” DHA. MARA-1015, [0058] (disclosing 58.8% DHA); MARA-1009, 29 (Table 2 discloses 61% DHA); MARA-1032, 27-31 (Table 2.5 discloses 67.2, 65.6, 67.8, 63.7, 64.3, 62.4 and other percentages DHA). Accordingly, a PHOSITA would have understood from Schaap’s disclosure that its composition comprises at least 50 wt.% DHA since there *is* “45% or more” of DHA, a “[p]referred” PUFA. MARA-1005, [0053].

126. Additionally, a PHOSITA would have understood that in the Schaap microbial oil, most of the PUFAs would be in triglyceride form.

127. Schaap’s disclosures confirm this to be the case, as Schaap notes that “[p]referably, the microbial oil has a triglyceride content of from 90 to 100%” which is “optimally at least 95% or 98% of the oil.” MARA-1005, [0055]-[0056]. In my opinion, a PHOSITA would have understood that since the majority of Schaap microbial oil is made up of triglycerides (e.g., up to 100%), it would have been understood that majority of the PUFAs in Schaap are in fact present in the triglyceride form.

128. Even if some fatty acids were not present in triglyceride form, and instead present as free fatty acids, salts, fatty acid esters, phospholipids, or as mono- or di-glycerides, I note that Schaap discloses that those alternative forms are

not present in significant quantities. MARA-1005, [0085] (Table). For example, Schaap discloses that phospholipids preferably are present in very low quantities. MARA-1005, [0085] (Table shows an exemplary oil with 0.05% phospholipids). Free fatty acids are also less than 0.08% in certain cases in Schaap. MARA-1005, *see also* [0089]. A PHOSITA would understand these disclosures to mean that most of the fatty acids—and therefore DHA—are in triglyceride form in the lipid fraction. As I discuss above in Section III.B.3, in the combination, a PHOSITA would have been motivated to apply Komazawa’s methods to Schaap’s extracted microbial oil to increase its DHA content. In doing so, a PHOSITA would reasonably expect Komazawa’s method to produce oils having a “DHA component *in the triglycerides*” with “a high content of 80% or more,” depending on the culturing conditions. MARA-1015, [0116], FIG. 3. This high amount of DHA in the triglycerides is also shown in Figure 3 of Komazawa:



129. Based on at least the reasons provided above, in my opinion the combination of Schaap and Komazawa discloses and/or renders obvious Element [1b].

[1c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and

130. The combination of Schaap and Komazawa discloses and/or renders obvious Element [1c].

131. In my opinion based on my knowledge and experience working in this field prior to the Critical Date, it was well known that microorganisms typically produce oil compositions containing multiple PUFAs. In particular, *thraustochytrids* were known to produce both DHA and DPA n-6. MARA-1009, 27; MARA-1018, [0571]; MARA-1020, 385. As I discuss above, Schaap discloses that its oils are microbially derived from sources, including those from *Thraustochytrium*, and it was well known before the Critical Date that preferred microbial strains containing a high level of DHA (e.g., 61%) and a low level of DPA n-6 (e.g., 3.4%) were available. MARA-1005, [0045]-[0046]; MARA-1009, 27.

132. Furthermore, as I describe in Section III.B.3 above, in the combination, a PHOSITA would have been motivated to apply Komazawa's methods to Schaap's extracted microbial oil to increase its DHA content. In doing so, a PHOSITA would have noted Komazawa's disclosure that its method can be

used to produce an exemplary oil having a DPA n-6 component of 3.2%. MARA-1015, [0108]. It would have therefore been obvious and/or well understood to a PHOSITA that the extracted microbial oil of the combination of Schaap and Komazawa would reasonably produce a concentration of oils that falls within the range of 0.5% by weight to 6% by weight DPA n-6 of the triglyceride fraction. See MARA-1009, 27, Table 2.

133. Based on at least the reasons provided above, in my opinion the combination of Schaap and Komazawa discloses and/or renders obvious Element [1c].

[1d] wherein the oil has an anisidine value of 0.5 to 26, and

134. Schaap and Komazawa disclose and/or render obvious Element [1d] for at least the same reasons I discuss above in Section III.A.4[3c].

135. As I discuss in Section I.C.1 above, the term “the oil” means “extracted microbial lipid” in the Challenged Claims because the specification and claims treat the terms “oil” and “lipid” as equivalent terms. Thus, for Element [1d], the requirement that “the oil” has an anisidine value of 0.5 to 26 is equivalent to requiring that an extracted microbial lipid has an anisidine value of 0.5 to 26.

136. A PHOSITA would have understood from Schaap’s disclosure that the combination would have anisidine values between 0.5 and 26. MARA-1005, [0082]-[0083]. Anisidine values “give a measure of the aldehyde content.”

MARA-1005, [0083]. Schaap discloses that preferable anisidine values of its oils range from 5 to 25, which are completely encompassed within the Element [3c]'s range of 0.5 to 26. MARA-1005, [0083]. I note that experimental results from Schaap demonstrate that its oils have anisidine values ranging from 5 to 15. MARA-1005, [0083]. Further, Figure 5 also demonstrates that Schaap's exemplary extracted oils have anisidine values under 25. MARA-1005, Fig. 5, [0011], [0132].

[1e] wherein the lipid has less than 5% by weight of an organic solvent.

137. Schaap-Komazawa discloses and/or renders obvious Element [1e] for at least the same reasons I discuss above in Section III.A.4[3d].

138. As I read the '578 patent specification, solvent is optional: "the lipid optionally contains less than 5% by weight or volume of an organic solvent." *E.g.*, MARA-1001, 2:21-22, 2:54-56, 2:61-63. In other words, the lipid could contain no organic solvent. I understand from the prosecution history that the Examiner drew the same conclusion when they wrote that the claims "recite ingredients in the oil that need not be present," meaning that art that "does not disclose that the oil contains any organic solvent" reads on the patent. MARA-1002, 1018, 1021-22. I also understand that Applicant agreed that references that "do[] not disclose that the oil contains any organic solvent" read on the claim. MARA-1002, 1073.

139. It is also my opinion that if the '578 patent requires a non-zero amount of organic solvent that is less than 5 wt.% (i.e., greater than 0 wt.% but less than 5 wt.%), Schaap still discloses and/or renders obvious that requirement. For example, Schaap discloses a crude oil obtained by extraction using a solvent (e.g., hexane) having no more than 2000 ppm of that solvent in its composition. MARA-1005, [0057], [0063]-[0065], [0085]. A PHOSITA would have understood that 2000 ppm equates to 0.2 wt.% organic solvent based on the following conversion calculations:

$$1 \text{ ppm} = 1 \text{ part solute}/10^6 \text{ parts solution}$$

$$0.2\% = 0.2 \text{ parts in } 100 \text{ parts solution}$$

$$0.2\% = 2000/10^6 \text{ parts solution}$$

$$0.2\% = 2000 \text{ ppm}$$

Thus,

$$\mathbf{2000 \text{ ppm} = 0.2\% \text{ by wt.}}$$

140. Accordingly, a PHOSITA would have understood that Schaap's crude oil contains no more than 0.2 wt.% (i.e., 2000 ppm) of the solvent in the extracted microbial lipid composition, and therefore the organic solvent would constitute less than 5% by weight of the composition.

141. Further, a PHOSITA would have understood that residual organic solvents from extracting microbial oils were not desirable and would seek to limit

or remove them from a raw or unrefined extracted oil composition (e.g., crude oil). MARA-1010, 193 (“[S]olvents are too toxic to be used for nutritional or pharmaceutical purposes” and therefore would be removed). Schaap reflects that understanding; in embodiments, Schaap describes the removal (e.g., evaporation) of organic solvents from crude oil to obtain a refined oil. MARA-1005, [0112], [0121], [0130]. I note that Schaap discloses that the “resulting oil” of its processes is “particularly suitable for nutritional purposes,” and even includes a use as an additive for “infant formula” and “health drinks[.]” MARA-1005, [0066]. Schaap further discloses a refined oil obtained by extraction using a solvent that results in an oil with no more than 1 ppm (or undetectable levels) of that solvent in the final composition. MARA-1005, [0085] (*see also* table listing residual solvent amounts). Thus, Schaap’s refined oil would contain less than 5 wt.% of an organic solvent in total extracted microbial lipid composition. MARA-1005, [0085] (*see also* table).

142. For the reasons provided above, in my opinion Schaap discloses or renders obvious that the lipid, crude, refined or otherwise, has less than 5% by weight of an organic solvent.

Claim 2

[2pre] An extracted microbial lipid comprising

143. The combination of Schaap and Komazawa discloses and/or renders obvious Element [2pre] for at least the same reasons I discuss above in Section III.B.4[1pre].

[2a] a triglyceride fraction of at least 70% by weight of the lipid fraction

144. The combination of Schaap and Komazawa discloses and/or renders obvious Element [2a] for at least the same reasons I discuss above in Section III.B.4[1a].

[2b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight,

145. The combination of Schaap and Komazawa discloses and/or renders obvious Element [2b] for at least the same reasons I discuss above in Section III.B.4[1b].

[2c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight,

146. The combination of Schaap and Komazawa discloses and/or renders obvious Element [2c] for at least the same reasons I discuss above in Section III.B.4[1c].

[2d] wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and

147. Schaap-Komazawa discloses and/or renders obvious Element [2d].

148. A PHOSITA would understand that any disclosure that satisfies Elements [2b]-[2c] will also satisfy Element [2d]. That is because if an extracted microbial lipid of the combination contains the DHA (40%) and the maximum amount of DPA n-6 (6%), they will be present in a ratio greater than 6:1.

149. Accordingly, in my opinion it would have been obvious to a PHOSITA that the proposed combination contains DHA and DPA n-6 in the claimed ratio of 6:1 DHA:DPA n-6.

[2e] wherein the oil has an anisidine value of 0.5 to 26, and

150. The combination of Schaap and Komazawa discloses and/or renders obvious Element [2e] for at least the same reasons I discuss above in Section III.B.4[1d].

[2f] wherein the lipid has less than 5% by weight of an organic solvent.

151. The combination of Schaap and Komazawa discloses and/or renders obvious Element [2f] for at least the same reasons I discuss above in Section III.B.4[1e].

Claim 3

[3pre] An extracted microbial lipid comprising

152. The combination of Schaap and Komazawa discloses and/or renders obvious Element [3pre] for at least the same reasons I discuss above in Section III.B.4[1pre].

[3a] a triglyceride fraction of at least 70% by weight of the lipid fraction

153. The combination of Schaap and Komazawa discloses and/or renders obvious Element [3a] for at least the same reasons I discuss above in Section III.B.4[1a].

[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and

154. The combination of Schaap and Komazawa discloses and/or renders obvious Element [3b] for at least the same reasons I discuss above in Section III.B.4[1b].

[3c] wherein the oil has an anisidine value of 0.5 to 26, and

155. The combination of Schaap and Komazawa discloses and/or renders obvious Element [3c] for at least the same reasons I discuss above in Section III.B.4[1d].

[3d] wherein the lipid has less than 5% by weight of an organic solvent.

156. The combination of Schaap and Komazawa discloses and/or renders obvious Element [3d] for at least the same reasons I discuss above in Section III.B.4[1e].

Claim 4

[4] The extracted microbial lipid of claim 1, wherein the oil has a peroxide value of 5 or less.

157. The combination of Schaap and Komazawa discloses and/or renders obvious claim 4.

158. Schaap discloses that its oils' peroxide value "ranged from 1.3 (or 0.8) to 0.4." MARA-1005, [0081]. Schaap further notes that its pasteurization method produces an oil having a peroxide value with an "acceptable value of 2.1," and other batches with peroxide values peroxide values of, for example, 2.1, 2.2, 1.1, 1.2, and 1.2. MARA-1005, [0122] (Table 3), [0123]. Accordingly, in my opinion it would have been obvious to a PHOSITA that the oil produced by the combination of Schaap and Komazawa would have peroxide values of 5 or less.

Claim 5

[5] The extracted microbial lipid of claim 1, wherein the oil has a phosphorus content of 100 ppm or less.

159. The combination of Schaap and Komazawa discloses and/or renders obvious claim 5.

160. Schaap discloses a process in which microbial oils were derived in which the resultant phospholipid concentration was less than 0.05 wt.% of the total composition. MARA-1005, [0085] (Table). In my opinion, a PHOSITA would have understood that, on a ppm basis, the entire phospholipid concentration would have encompassed less than 100ppm of phosphorus. I note further that Schaap discloses that its crude oils would contain "a phosphorus content of at least 2, 3, or 5 mg/kg." MARA-1005, [0091].

161. It is basic scientific knowledge that mg/kg is the same measurement as ppm. That is because ppm is measure of the amount of mass per each 10^6 of

solute. Because mg/kg is 0.001g/1000g, or 1×10^6 , it therefore expresses the same relationship. This would have been well known to a PHOSITA before the Critical Date.

162. Accordingly, it is my opinion that a PHOSITA would have read Schaap's disclosure of "a phosphorus content of at least 2, 3, or 5 mg/kg" as the same as "a phosphorus content of at least 2, 3, or 5 ppm."

Claim 6

[6] The extracted microbial lipid of claim 1, wherein the lipid is a crude oil.

163. The combination of Schaap and Komazawa discloses and/or renders obvious claim 6. For example, Schaap describes that its "microbial oil may be a crude oil." MARA-1005, [0057]. Furthermore, the methods in Komazawa would apply also to crude oil.

Claim 7

[7] A process for obtaining a lipid, the process comprising refining a crude lipid of claim 6.

164. The combination of Schaap and Komazawa discloses and/or further renders obvious a process for obtaining a lipid in which the process includes refining a crude lipid of claim 6. For example, I note that Schaap explains that a "crude PUFA containing oil....can be used in that state, without further processing, or it can be subjected to one or more refining steps." MARA-1005, [0065]. Accordingly, in my opinion it would have been obvious to a PHOSITA that the proposed combination includes the process of refining a crude oil.

C. GROUND 3: Claims 1-7 are obvious over Bijl in view of Nichols-I

1. Overview of Bijl

165. In my opinion, Bijl is directed to microbial oils in a similar way to the '578 patent. MARA-1001, 1:16-23, 2:16-22; MARA-1008, 2:30-36, 9:5-7. Bijl discloses that high quality microbial oils can be sourced from a variety of naturally-occurring microorganisms, such as fungi, bacteria, yeasts, and algae, just as the '578 patent does. MARA-1001, 14:16-16:3; MARA-1008, 12:22-37. Bijl discloses processes for obtaining these high quality microbial oils. MARA-1008, 2:35-3:2, 9:5-16.

166. Microbial oils disclosed by Bijl contain one or more polyunsaturated fatty acids and a triglyceride content of at least 90 wt.%. MARA-1008, 2:30-33, 4:24-28, 5:24-29. The microbial oils of Bijl also contain additional compounds, such as anti-oxidants, which remain from the sourced microorganisms. MARA-1008, 3:9-14. And the oils have low diglyceride content, low monoglyceride content, a light color, a low level of off-flavors, and a low *p-Anisidine* value. MARA-1008, 5:14-19. The microbial oils of Bijl are usable in food, nutritional supplements, and cosmetics. MARA-1008, 4:13-18.

167. Bijl also describes processes for obtaining microbial oils. One process constitutes using a mostly dry microbial biomass, granulating it into particles, drying the particles into granules, and then extracting microbial oil from

the dried granules. MARA-1008, 6:23-34. Another process begins with culturing microorganisms in fermentation broth, pasteurizing the resulting broth or biomass, and then extracting microbial oil from the microbial biomass. MARA-1008, 7:2-11. The alternate, pasteurization-based process is combinable with the described granulation process as well. MARA-1008, 8:1-6, 28:28-29:19.

168. Bijl teaches obtaining an oil from a biomass or broth that has been subjected to pasteurization. MARA-1008, Abstract. Bijl explains that its pasteurization process is used to partially inactivate one or more compound degrading substance(s), such as inactivating lipases, phospholipase, and lipoxygenases that may be present in the biomass or broth. MARA-1008, 7:12-30. The pasteurization process is attributed for removing at least some of the substances that can cause degradation of parts of the compound (such as a PUFA) and contributes to obtaining high quality PUFAs. MARA-1008, 7:12-30.

169. Use of Bijl's processes to obtaining microbial oils are cost effective because they utilize less solvent for extracting microbial oils than prior methods. MARA-1008, 9:5-19. Further, Bijl's process results in a higher quality crude oil that makes subsequent refining only minimally necessary. MARA-1008, 9:20-25, 9:30-37 (noting that the PUFA oil is suitable for nutritional purposes for humans).

2. Overview of Nichols-I

170. Nichols-I discloses newly-discovered sources of long-chain polyunsaturated fatty acids (“LC-PUFAs”) in microorganisms. Nichols-I describes strains of single cell organisms that produce high quantities of LC-PUFAs.

MARA-1009, 27. Nichols discloses that different fatty acids are obtained from different microorganisms: for example, EPA is derived from eustigmatophytes and diatoms; DHA from dinoflagellates and thraustochytrids; ARA from red algae, thraustochytrids, and fungi; and GLA from blue-green algae. MARA-1009, 27, tbl.1. And Nichols-I also reports on the relative fatty acid concentrations of strains of thraustochytrids, of which the following certain strains contain as their main omega-3 long-chain polyunsaturated fatty acids (LC-PUFA): DHA and EPA (strain A); DHA and arachidonic (AA) (strains C/H); and DHA (strain O) fatty acids. MARA-1009, 27 (Table 2). MARA-1009, 27, tbl.2. Strain O is “particularly attractive” because it “contains very high levels of DHA (61%).” MARA-1009, 27.

171. Nichols-I also discloses uses and benefits of microbially-derived oils. It teaches that omega-3 oils are beneficial to human health, and are used to treat diseases and in development and protecting of the human brain. MARA-1009, 26. Nichols-I further teaches that different omega-3 oils have different health benefits.

DHA, for example, decreases incidence of coronary heart disease. MARA-1009, 26.

172. Traditionally, omega-3 oils were derived from fish. MARA-1009, 26. As Nichols-I describes, however, “there is a need for new sources of these oils to be obtained” because “global wild fish stocks are declining.” MARA-1009, 26. So, not only does Nichols-I describe the use of microorganisms as sources of omega-3 oils, they also describes suggestions for creating transgenic plants which contain genes of these oil-producing microorganisms, to enable farmers to grow omega-3-producing plants. MARA-1009, 28.

3. Overview of Schaap

173. I incorporate my discussion of Schaap in Section III.A.1, above.

4. Combination of Bijl, Nichols-I, and Schaap

1) Preferred Microbial Strains

174. In my opinion, it would have been obvious for a PHOSITA to combine the teachings of Bijl, directed to extracted microbial oils and related processes of cultivating, processing, and extracting microbial oils from natural sources, with Nichols-I, which teaches using specific beneficial strains of microorganisms (e.g., *thraustochytrids*). A PHOSITA would have found it obvious to have used Nichols-I’s teachings of using a preferred strain of microorganisms (e.g., *thraustochytrids* of a strain O) as a microbial source for Bijl’s extracted microbial oil. In particular, a PHOSITA would have used Nichols-I’s preferred

strains, such as the strain O thraustochytrids, in producing Bijl's extracted microbial oil for at least the following reasons.

175. First, a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains (e.g., strain O thraustochytrids) as a microbial source for Bijl's extracted microbial oil. MARA-1008, 9:5-7. A PHOSITA would have understood that Nichols-I was a desired microbial source, and would have used that source as a starting point for Bijl's processes to obtain an extracted microbial oil "of a particularly high quality." MARA-1008, 9:5-7.

176. Second, a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains in as a source for Bijl's extracted microbial oil because "[t]hraustochytrids are....single cell organisms that produce both high oil and LC-PUFA." MARA-1009, 27.

177. Third, a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted oil because "a highly attractive, renewable and contaminant free, source of omega-3 oils." MARA-1009, 27. Nichols-I explains that "thraustochytrids can be grown in fermenters with an organic carbon source" and therefore provide a renewable and contaminant free microbial source. MARA-1009, 27.

178. Fourth, in my opinion a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted oil because

Nichols-I notes that “[s]train O is particularly attractive as it contains very high levels of DHA (61%).” MARA-1009, 27.

179. Fifth, a PHOSITA would have been motivated to use Nichols-I’s preferred microbial strains as a source for Bijl’s extracted oil because “DPA(6) is only a minor component in our strain O under the culture conditions employed, making this strain [O] particularly interesting.” MARA-1009, 27.

180. Sixth, a PHOSITA would have been motivated to use a preferred microbial strain (e.g., Nichols-I’s strains) as a source for Bijl’s extracted oil because a PHOSITA would have combined the teachings of Bijl and Nichols as doing so would have amounted to nothing more than the use of a known technology (e.g., identify or screen for a microbial source) to improve similar oils (e.g., extracted microbial oils) in the same way and combining prior art elements according to known methods (e.g., apply Bijl’s oil extraction methods) to yield predictable results (e.g., obtain an oil with desirable traits such as high PUFA levels).

181. For example, it was well known that thraustochytrids had fast growth rates, produced high amounts of omega-3 fatty acids as a percentage of total fatty acids, and had enhanced lipid and DHA production qualities. MARA-1010, 79-80. It was also known that certain strains of thraustochytrids were “natural over-producers of DHA,” meaning that thraustochytrids of interest could be identified

using an ecological, biochemical, and physiological approach. MARA-1010, 78-79. The proven success of these methods means that a PHOSITA would naturally seek to find thraustochytrids “with different PUFA profiles” or that produce “higher contents of specific PUFAs or lipid types,” as Nichols-I proposed, and then culture them to obtain DHA-rich oils. MARA-1010, 88-89.

182. For these reasons above, in my opinion a PHOSITA would have found it obvious to combine the teachings of Bijl with the disclosures of Nichols-I to provide an improved extracted microbial oil.

183. In my opinion, a PHOSITA would also have expected success in combining Bijl with Nichols-I. Bijl and Nichols-I both relate to the creation of microbial lipids using culturing techniques. MARA-1008, 7:2-11; MARA-1009, 27-28. Both references teach the same sources of those microbial lipids: algae, fungi, dinoflagellates, and other diatoms. MARA-1008, 12:22-37; MARA-1018, [0569-0570 & tbl.3]; MARA-1009, 27; MARA-1020, 384-385. And both teach that the desired product is one of high enough quality for use in the human diet. MARA-1008, 14:23-27; MARA-1009, 27-28. Furthermore, Bijl discloses that its processes are compatible with a wide range of natural sources, including *Cryptocodinium* and *Thraustochytrium*, which are also disclosed in Nichols-I. MARA-1008, 6:3-11; 13:19-31; MARA-1009, 27. And a PHOSITA performing culturing would have “found that extracting oil from microorganisms was no more

difficult than extracting oil from other sources of valuable oils.” MARA-1010, 179. Applying Bijl’s processes for obtaining microbial oils by using strains of Nichols-I, then, would have been well within the knowledge of a PHOSITA familiar with routine extraction processes. MARA-1010, 179-200.

2) Improved Processes for Obtaining Microbial Oil

184. Additionally, a PHOSITA would have found it obvious to combine the teachings of Bijl-Nichols-I and Schaap to yield a better oil quality by using improved techniques of pasteurizing and extracting cells, as taught by Schaap, in Bijl’s method. MARA-1005, Abstract (“An improved pasteurisation protocol for pasteurising microbial cells is disclosed.”), [0001] (“After pasteurisation, a polyunsaturated fatty acid (PUFA), such as arachidonic acid, or microbial oil may be extracted from the cells”).

185. In more detail, Schaap specifically describes already known processes that involve heating microbial cells. MARA-1005, [0007] (“Processes involving heating biomass, or microbial cells, are known”). Schaap cites to Bijl (MARA-1008), stating that it discloses that microbial cells were pasteurised prior to extraction. MARA-1005, [0007] (“It is also known, from WO-A-97/37032, that microbial cells can be pasteurised prior to extraction to a PUFA therefrom in the form of an oil.”). Schaap teaches that its new processing techniques can “improve the quality of the oil that can be extracted from the pasteurised cells,” as compared

to the already known methods, such as those disclosed by Bijl. In fact, Schaap further refers to Bijl in its disclosure as providing already known techniques for extraction as well as other oil processing related methods. MARA-1005, [0064]. I note that Albert Schaap appears to be a named inventor in both Schaap and Bijl. *Compare* MARA-1005, front page (75) and MARA-1008, front page (72). Hence, in my opinion a PHOSITA would have naturally sought to apply the improvements of Schaap to Bijl's process for obtaining an oil to advance Bijl's suggestion to obtain a high quality polyunsaturated fatty acid- (PUFA) containing oil with a high triglyceride content. MARA-1008 (Bijl), 4:2-5.

186. In my opinion, a PHOSITA would have been motivated to implement Schaap's improved technique of pasteurization to Bijl-Nichols-I for several reasons.

187. First, a PHOSITA would have been motivated to implement Schaap's pasteurization process to "improve the quality of the oil," which "oxidise[s] less, or [is] less oxidized...." MARA-1005, [0007].

188. Second, a PHOSITA would have been motivated to implement Schaap's improved pasteurization process to yield a "resulting oil" "hav[ing] a low peroxide value (POV) and a low anisidine value (AnV)," which are attributes associated with a "better quality" oil. MARA-1005, [0007], abstract ("a better quality...oil results having a peroxide value (POV) of less than 1.5 and an

anisidine value (AnV) of less than 1.0.”). A PHOSITA would have understood that having a low peroxide value is desirable because it is, in general, more stable than an oil with a higher peroxide value. A PHOSITA would have also understood that having a low anisidine value is desirable because it is an indicator that the oil has not degraded significantly, indicating stability, and also, can correspond to an oil having less of a “fishy” smell, which is also a desirable property for many oils.

189. Third, a PHOSITA would have been motivated to implement Schaap’s improved pasteurization process to Bijl’s method because the “new pasteurisation process is more efficient because it requires less energy.” MARA-1005, [0007].

190. Fourth, a PHOSITA would have been motivated to implement Schaap’s improved processing techniques for pasteurizing and extracting to Bijl-Nichols-I’s method for obtaining an oil because a PHOSITA would have combined the teachings of Bijl and Schaap as doing so would have amounted to nothing more than the use of a known technique (e.g., pasteurization) to improve similar oils in the same way and combining elements according to known methods to yield predictable results.

191. For at least the reasons I explain above, in my opinion a PHOSITA would have found it obvious to combine the teachings of Bijl and Nichols-I with the disclosures of Schaap to obtain an improved polyunsaturated fatty acid-

(PUFA) containing oil with a high triglyceride content and also to obtain an oil at reduced costs with less energy expenditure. MARA-1005, [0007].

192. A PHOSITA would also have reasonably expected success in combining Bijl-Nichols-I with Schaap. Bijl, Nichols-I, and Schaap all relate to the extraction of microbial lipids. MARA-1008, 7:2-11; MARA-1009, 27-28. All three references teach the same and/or similar sources of those microbial lipids: algae, bacteria, fungi, etc. MARA-1005, [0060]; MARA-1008, 12:22-37; MARA-1018, [0569-0570 & tbl.3]; MARA-1009, 27. And each reference teaches that the desired product is one of high enough quality for use in the human diet. MARA-1005, [0095] (“The composition may be a foodstuff and/or a food supplement for animals or humans.”), [0096]-[0097]; MARA-1008, 14:23-27; MARA-1009, 27-28. Furthermore, Schaap discloses that its processes are compatible with a wide range of natural sources, including *Crypthecodinium* and *Thraustochytrium*, which are also disclosed in Bijl and Nichols-I. MARA-1005, [0046], [0054], [0071], claims 12-13; MARA-1008, 6:3-11; 13:19-31; MARA-1009, 27.

5. Analysis

Claim 1

[1pre] An extracted microbial lipid comprising

193. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Element [1pre].

194. Bijl discloses a “pure and stable microbial oil” which comprises “at least one polyunsaturated fatty acid (PUFA), which has a triglyceride content of greater than 90%.” MARA-1008, 1:8-11; 2:30-33. Bijl discloses obtaining “microbially derived” oils by “extracting, isolating or recovering the compound from the microbial biomass.” MARA-1008, 3:3-5, 7:10-11. And like the ’578 patent, Bijl uses lipids and oils interchangeably, noting that “PUFA (oil) which has been extracted can be used” with or without further refining. MARA-1008, 9:20-22.

195. From my personal experience, I know that extracted microbial lipids were well known to a PHOSITA prior to the Critical Date. This is also noted in the literature, for example, in other prior art references such as Singh, that describe extracted microbial lipids. Singh discloses that LC-PUFA “may be present as a mixture of forms in the lipid of a cell or a purified oil or lipid extracted from cells, tissues, or organisms.” MARA-1018, [0294].

196. For the reasons above, in my opinion the proposed combination discloses and/or renders obvious Element [1pre].

[1a] a triglyceride fraction of at least 70% by weight of the lipid fraction

197. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Element [1a].

198. As I discussed above in Section III.A.2, [3a], the '578 patent does not define the term “lipid fraction.” However, a PHOSITA would have understood that the term “lipid fraction” refers to either: (1) only the lipid portion of the extracted microbial lipid, exclusive of solvent; or (2) the entire composition associated with extracted microbial lipid, including residual solvent.

199. As I discuss in more detail above, if the “lipid fraction” means the entire extracted microbial oil, then the triglyceride fraction comprises at least 70 wt.% of the total lipid composition; if instead the “lipid fraction” means only the non-solvent portion of the extracted microbial lipid, then a triglyceride fraction would constitute at least 66.5 wt.% of the total lipid composition. In my opinion, the proposed combination discloses and/or renders obvious the lipid fraction under either of the above two interpretations.

200. For example, Bijl discloses a microbial oil “which has a triglyceride content of greater than 90%.” MARA-1008, 2:30-33. Bijl discloses a triglyceride content greater than 95 wt.% and optionally higher than 97 wt.%. MARA-1008, 4:24-28. And its disclosure describes an extraction of triglyceride rich oil that has low acid values, peroxide values, and anisidine values. MARA-1008, 49:11-18.

201. Further, as I discuss above, triglyceride-rich oils would have been well known to a PHOSITA prior to the Critical Date. For example, Singh discloses triglyceride-rich oils. MARA-1018, [0294]. In preferred embodiments,

the oil comprises “at least 75% or 85% triacylglycerols” which themselves comprise the LC-PUFAs such as DHA. MARA-1018, [0294].

202. For the reasons above, the proposed combination discloses and/or renders obvious Element [1a].

[1b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight

203. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Element [1b]. Bijl discloses that its oils include DHA. MARA-1008, 13:19-31. And consistent with disclosures of other known art, I note that Bijl acknowledges that *Cryptocodinium cohnii* and *Thraustochytrium* microbes produce DHA. MARA-1008, 1:21-31, 6:3-14; MARA-1009, 27.

204. As I discuss above in Section III.C.4, in the proposed combination, a PHOSITA would have applied the teachings of using a preferred microbial strain (e.g., *thraustochytrids* strain O), as taught by Nichols-I, to Bijl’s oil to yield an improved oil composition having higher amounts of a desired PUFA (e.g., DHA). The PHOSITA would have done so because “[s]train O is particularly attractive as it contains very high levels of DHA (61%),” with DPA n-6 being “only a minor component.” MARA-1009, 27. Additionally, in my opinion it would have been obvious to a PHOSITA to combine the teachings of Bijl and Nichols-I as doing so would have amounted to nothing more than the use of a known technique to

improve similar oils in the same way and combining prior art elements according to known methods to yield predictable results.

205. Further, in my opinion a PHOSITA would have found it obvious that the DHA content of the triglyceride fraction is at least 50 wt.% in the proposed combination because Nichols-I's preferred strain O of *Thraustochytrium*, when cultured, produces an oil composition with greater than 60 wt.% DHA. MARA-1009, 27 (Table 2). Nichols-I discloses that *Thraustochytrium* strain O contains "very high levels of DHA (61%)." MARA-1009, 27; MARA-1018, [0571]; MARA-1020, 384-385, Table 2. Consistent with Nichols-I's disclosure, I note that other prior art references indicated that many microbial strains (e.g., strains in the *Thraustochytrium* family) contained DHA in amounts greater than 50 wt.%, and even greater than 60 wt.%. MARA-1032, 27-31 (Table 2.5 discloses 67.2, 65.6, 67.8, 63.7, 64.3, 62.4 and other percentages DHA); MARA-1015, [0108], [0113]-[0114], [0016], Fig. 3. For example, Komazawa is one example that discloses a *Thraustochytrium* strain (LFF1 strain) that contains DHA components ranging from 62% to 84%. MARA-1015, [0113]-[0114], [0016], Fig. 3.

206. It is my opinion that a PHOSITA would have understood that since the majority of Bijl's microbial oil is made up of triglycerides (e.g., > 97%), the majority of the DHA (61%) in the combination of Bijl and Nichols-I is likewise present in the triglyceride form. In fact, I note that Bijl discloses that DHA is a

preferred PUFA associated with the triglycerides present in its oil: the invention “preferably comprises a triglyceride, such as one of the PUFAs previously mentioned,” such as DHA. MARA-1008, 6:3-5; 7:18-19. Furthermore, a PHOSITA would have known that microbial oils having a DHA content of approx. 60 wt.% in the triglyceride fraction were consistent with teachings of other prior art: Komazawa discloses exemplary *Thraustochytrium* strains (e.g., LFF1 strain) including DHA components in the triglycerides (TAG) that range from “around 40%,” and “80% or more,” depending on the culturing conditions; and Apt discloses DHA in TAG fraction in amounts of 58.1 wt.% and 60.4 wt.%. MARA-1015, [0116], Fig. 3; MARA-1017, Table 6 (C22:6 N3*...58.1%), Table 8 (C22:6 N3*...60.4%).

207. Moreover, the PHOSITA would have understood that in Bijl’s microbial oil, most of the PUFAs (DHA) would be in triglyceride form because Bijl specifically discloses a preference for high levels of triglycerides, e.g., “a triglyceride content $\geq$ 97%.” MARA-1008, 4:24-30.

[1c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and

208. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Element [1c].

209. Since Element [1a] requires a triglyceride fraction of at least about 70 wt.% of the lipid fraction, the docosapentaenoic acid content of between 0.5 and 6

wt.% of such a triglyceride fraction (i.e., 70 wt.% of the lipid fraction) can be expressed as the docosapentaenoic acid content constituting between 0.35 and 4.2 wt.% of the lipid fraction when the triglyceride fraction is at least 70 wt.%. This means that if the “lipid fraction” constitutes the entire extracted microbial lipid, the same wt.% must be shown in the total composition. Alternatively and/or additionally, if the “lipid fraction” excludes solvents, then one must show between 0.3 wt.% and 4 wt.% DPA n-6 in the total composition.

210. Bijl discloses microbial oil compositions that “predominantly contain one particular polyunsaturated fatty acid” but “can additionally contain other polyunsaturated fatty acids in lesser amounts.” MARA-1008, 6:11-13. The invention discloses a microbial oil with “at least one polyunsaturated fatty acid,” and “microbial oils in which more than one polyunsaturated fatty acid is present.” MARA-1008, 5:24-29, 6:23-26. The PUFA can include omega-3 or omega-6 “C18, C20, or C22” polyunsaturated fatty acids. MARA-1008, 4:5-8. And DPA n-6 is a C22 omega-6 fatty acid. Bijl therefore discloses compositions that can comprise both DHA and DPA n-6.

211. In my opinion, it would have been well-known prior to the Critical Date that microorganisms typically produce an oil composition containing multiple PUFAs. Depending on the species selected, differing oil compositions result: “Green algae either do not contain or have only very low levels of EPA and DHA;

diatoms contain elevated EPA, as do eustigmatophytes; dinoflagellates contain DHA and other C18 PUFA; red algae can contain elevated proportions of 20:4 ω -6, arachidonic acid (AA).” MARA-1009, 27; MARA-1020, 384. And thraustochytrids were known to produce both DHA and DPA n-6. MARA-1018, [0571]; MARA-1009, 27; MARA-1020, 385. A PHOSITA would have known, then, that culturing thraustochytrids would have resulted in a microbial oil composition containing both DHA as well as DPA n-6.

212. As I discuss above in Section III.B.4, a PHOSITA would have implemented Nichols-I’s preferred strains (e.g., O thraustochytrids) in producing Bijl’s extracted microbial oil. In my opinion, it would have been obvious to a PHOSITA that, in the proposed combination, the extracted microbial oil includes docosapentaenoic acid n-6 content of the triglyceride fraction within the range of 0.5% by weight to 6% by weight. I note that Nichols-I explains that the DPA n-6 concentration of oils extracted from strain O thraustochytrids (3.4 wt.% DPA n-6) falls within the range of 0.5% by weight to 6% by weight of the triglyceride fraction. MARA-1009, 27 & tbl.2. Indeed, it was well known based on the prior art available before the Critical Date that “[s]train O,” a specific strain of Australian thraustochytrid that yields DPA n-6 at 3.4 wt.% of the total lipid composition. MARA-1009, 27, tbl.2; *see also* MARA-1018, [0571]; MARA-1020, 384-385, tbl.2.

213. Because a PHOSITA would have understood that “the oil of the present invention can be microbially derived,” MARA-1008, 3:3-5, they also would have understood that the implementation of Nichols-I’s Strain O thraustochytrid as a microbial source to produce Bijl’s extracted microbial oil renders obvious Elements [1c] and [2c].

214. For the reasons above, in my opinion the proposed combination discloses and/or renders obvious Element [1c].

[1d] wherein the oil has an anisidine value of 0.5 to 26, and

215. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Elements [1d].

216. Bijl generally discloses high-quality oils having low *p-Anisidine* values that are less than 26. MARA-1008, 5:20-21. A PHOSITA would have understood that *p-Anisidine* values measure the quality of an oil because “anisidine is a test for aldehydes, a product of degradation by oxidation.” MARA-1008, 5:18-19. For example, the extracted microbial oils of Bijl have a “low anisidine value” that “typically varies from 0.1 to 5” and “preferably from 0.1 to 2, more preferably from 0.1 to 1.” MARA-1008, 5:20-21. Indeed, Bijl discloses a prepared Batch A in which the anisidine value was less than 1.0, which discloses an anisidine value in the range of 0.5 to 26. MARA-1008, 49:10-17. Bijl further discloses refining

those three batches of oils, in which each had a *p*-Anisidine value of 4.1, 1.9, and 3.1, respectively. MARA-1008, 50:4-17.

217. For the reasons above, in my opinion the proposed combination discloses and/or renders obvious Elements [1d]. MARA-1008, 5:18-21, 49:10-17, 50:4-17.

[1e] wherein the lipid has less than 5% by weight of an organic solvent.

218. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Element [1e] for at least following reasons I discuss below.

1) Element [1e] is Optional

219. For the same reasons as I discussed previously in Section III.A.4, above, Element [1e] is an optional element for which prior art disclosure is not necessary to meet the claim element.² Because Elements [1e] is an optional element, in my opinion the combination of Bijl Nichols-I discloses and/or renders obvious Elements [1pre]-[1d], thus rendering claim 1 invalid regardless of whether Element [1e] is met..

2) Element [1e] is Rendered Obvious Based on Bijl's Teachings

220. In my opinion, even if the presence of the organic solvent in an amount less than 5 wt.% of the lipid was required, the proposed combination

² Elements [2f] and [3d] are also optional elements for the same reasons as provided for Element [1e].

renders this element obvious based on Bijl's disclosure. Bijl's process for obtaining an oil utilizes a solvent to perform extraction, passing it over granules of biomass. MARA-1008, 27:21-34. "[T]he PUFA is extracted from the dried granules using a solvent," which can be "[a]ny suitable solvent known to a person skilled in the art can be employed." MARA-1008, 8:36-9:4. A column is filled with dried granules and then solvent recirculated, and passed through. MARA-1008, 27:21-34.

221. Bijl uses significantly reduced amounts of solvent in its process. MARA-1008, 27:11-13. Bijl explains that "20 to 30 times less solvent" is used to perform the extraction process. MARA-1008, 27:11-13. Since less solvent is used, "significant economic saving" and minimization of "emission problems" can be attained. MARA-1008, 27:13-15. In this way, Bijl's "invention can enable a cost effective and efficient extraction of the PUFA oil, and provide an oil of a particularly high quality" in part because "the dried granules may require reduced amounts of solvent for the extraction process" and "the release of the used solvent from the biomass can be achieved more efficiently." MARA-1008, 9:5-16.

222. Furthermore, Bijl explains that "[t]he residue resulting after (solvent) extraction (and even after desolventising toasting) can be used as a feed stuff or a feed component (such as for animals)" and the "[t]he PUFA (oil) which has been extracted can be used in that state without further processing...." Any residual

organic solvent present in Bijl's crude oil is removable via evaporation. MARA-1008, 31:12-13. Based on this disclosure provided by Bijl and other related references, it would have been obvious to a PHOSITA that the lipid composition used for animal consumption, as described by Bijl, contains less than 5% by weight of an organic solvent to facilitate safe consumption. MARA-1005, [0066] ("The resulting oil is particularly suitable for nutritional purposes, and can be added to (human) foods or (animal) feedstuffs. Examples include milk, infant formula, health drinks, bread and animal feed."), [0094]-[0101], [0085] (table shows that the preferred amount of the solvent in the resulting oil is at least less than 2000 ppm).

223. Moreover, if Bijl is determined not to explicitly disclose a weight percent of organic solvent present in the extracted lipid composition, in my opinion a PHOSITA would have understood that the amount of the organic solvent would be less than 5 wt.% because Bijl's disclosure is cited to by the later-filed Schaap application for its disclosure of describing suitable techniques of extracting a microbial PUFA containing oil. MARA-1005, [0064]. Hence, a PHOSITA would have understood that Bijl's extraction techniques were applied and/or at least applicable to Schaap's process for obtaining its resulting oils. MARA-1005, [0064]. Furthermore, Schaap discloses that each of its extracted oils contain solvent (e.g., hexane) in an amount of 100-2,000 ppm (or 0.01 wt.% to 0.02 wt.% organic solvent) as a crude oil, and in undetectable or an amount of less than 1 ppm

as a refined oil. MARA-1005, [0085]-[0088]. A PHOSITA would have found obvious that that Bijl's techniques for extraction, which are the same techniques cited in Schaap, would have also resulted in oils having the same or similar organic solvent residual amounts as those of Schaap's following those same extraction processes. MARA-1005, [0064]. According, a PHOSITA would have found obvious from these disclosures that less than 5 wt.% organic solvent is present in the microbial lipid of Bijl.

3) Element [1e] is Rendered Obvious by the Proposed Combination

224. I further note that the proposed combination of Bijl, Nichol-I and Schaap would have further rendered obvious that the extracted microbial lipid contains less than 5% by weight of an organic solvent because Bijl would have implemented process techniques taught by Schaap to yield an improved oil. Schaap teaches extracting an oil from a biomass or broth that has been subjected to a pasteurization process. MARA-1008, Abstract. However, Schaap describes its processes as an improved protocol that "result[s] in a smaller energy input (and so a reduction in costs)" as well as "a better quality (and less oxidised) oil." MARA-1005, Abstract.

225. A PHOSITA would have applied the teachings of Schaap for preparing (e.g., pasteurizing) and extracting microbial oils to Bijl's process for obtaining an oil at least the following reasons: (1) to improve the quality of the

oil,” which “oxidise[s] less, or [is] less oxidized,” (MARA-1005, [0007]); (2) to produce a “resulting oil” “hav[ing] a low peroxide value (POV),” (MARA-1005, [0007]); (3) to obtain an improved quality oil having a low anisidine value (AnV), (MARA-1005, [0007]; and because its process “is more efficient” and “requires less energy” (MARA-1005, [0007]).

226. And by implementing Schaap’s processing techniques in Bijl’s method for pasteurizing and extracting oil, in my opinion it would have been obvious that the proposed combination would yield an oil having the improved characteristics disclosed by Schaap. Schaap discloses that its process “improve[s] the quality of the oil that can be extracted from the pasteurised cells.” MARA-1005, [0007]. Schaap further describes that “the crude oil in the present invention” includes features such as “a solvent (eg. hexane) content of from 10, 50 or 100 ppm up to 1000, 1500 or 2000 ppm,” which are all values that fall within the less than 5 wt.% limit of Element [1e]. MARA-1005, [0088]. In my opinion, a PHOSITA would have readily understood these disclosures to mean that less than 5 wt.% organic solvent are present in the resulting microbial lipid.

227. For the reasons provided above, in my opinion the combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious the Element [1e].

Claim 2

[2pre] An extracted microbial lipid comprising:

228. See my discussion of Section III.C.5[1pre] above.

[2a] a triglyceride fraction of at least 70% by weight of the lipid fraction

229. See my discussion of Section §III.C.5[1a] above.

[2b] wherein the docosahexaenoic acid content of the triglyceride fraction is at 40% by weight,

230. See my discussion of Element [1b] above. Since Element [2b] requires a triglyceride fraction of at least about 70 wt.% of the lipid fraction, the docosahexaenoic acid content of at least 40 wt.% of such a triglyceride fraction (i.e., 70 wt.% of the lipid fraction). For at least the reasons I provided above (e.g., §III.C.5[1b]), in my opinion the proposed combination discloses and/or renders obvious Element [2b].

[2c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight,

231. See my discussion of Section III.C.5[1c] above.

[2d] wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1,

232. The combination of Bijl, Nichols-I, and Schaap discloses and/or renders obvious Element [2d].

233. I note that any disclosure that satisfies Elements [1b]-[1c] and [2b]-[2c] will also satisfy Element [2d]. That is because if an extracted microbial lipid contains the minimum amount of DHA (40 wt.%) and the maximum amount of DPA n-6 (6 wt.%), they will be present in a ratio greater than 6:1.

234. Bijl describes that a microbial oil “predominantly (or only) contains one particular polyunsaturated fatty acid, but which may additionally contain lesser amounts of other polyunsaturated fatty acids.” MARA-1008, 5:24-27. This can include a C22 omega-3 (DHA or DPA) and a C22 omega-6 (DHA or DPA) fatty acid. MARA-1008, 4:2-7.

235. Bijl also discloses that DHA PUFAs can be isolated from *Crypthecodinium* or from *Thraustochytrium*. MARA-1008, 1:21-31, 6:3-14. In my opinion, a PHOSITA would have understood that, by culturing from *Thraustochytrium*, one would obtain a microbial lipid composition that comprises both DHA and DPA n-6.

236. As I discussed above in Section III.B.4, in the proposed combination, in my opinion a PHOSITA would have applied the teachings of using preferred microbial strains, as taught by Nichols-I, to Bijl’s oil to yield an improved oil composition. I note that Nichols-I as well as other known prior art references disclosed prior to the Critical Date that microbial compositions generally included a DHA:DPA n-6 ratio greater than 6:1 were known. For example, Nichols-I discloses that strain O, an Australian thraustochytrid, produced extracted oils contain 61% DHA and 3.4% DPA n-6, which is in a ratio greater than 6:1. MARA-1018, [0571]; MARA-1009, 27, tbl.2; MARA-1020, 384-385, tbl.2. Accordingly, in my opinion it would have been obvious to a PHOSITA that the

proposed combination produces both DHA and DPA n-6, particularly in the claimed ratio of 6:1 DHA:DPA n-6.

[2e] and wherein the oil has an anisidine value of 0.5 to 26,

237. See my discussion of Section III.C.5[1d] above.

[2f] wherein the lipid has less than 5% by weight of an organic solvent.

238. See my discussions of Sections III.A.4[3d] and §III.C.5[1e] above.

Claim 3

[3pre] An extracted microbial lipid comprising:

239. See my discussion of Section III.C.5[1pre] above.

[3a] a triglyceride fraction of at least 70% by weight of the lipid fraction

240. See my discussion of Section III.C.5[1a] above.

[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and

241. See my discussion of Section III.C.5[1b] above.

[3c] and wherein the oil has an anisidine value of 0.5 to 26, and

242. See my discussion of Section III.C.5[1d] above.

[3d] wherein the lipid has less than 5% by weight of an organic solvent.

243. See my discussions of Sections §III.A.4[3d], §III.C.5[1e] above.

Claim 4

[4] The extracted microbial lipid of claim 1, wherein the oil has a peroxide value of 5 or less.

244. The combination of Bijl, Nichols-I, and Schaap renders obvious claim

4.

245. In my opinion, a PHOSITA would have understood that peroxide value measures one aspect of the degree of oxidation during processing and storage of a microbial oil composition. I note that in Bijl, after producing crude oil according to the disclosed process, Bijl disclosed three batches of broth filter cakes obtained with different types of drying, with peroxide values of 2.7, 1.3, and 1.3 (batches a, b, and c, respectively). MARA-1008, 49:11-17.

246. Additionally, I note that Bijl further discloses refined batches, in which even lower peroxide value were observed at 1.6, 0.8, and 0.4 (batches a, b, c, respectively). MARA-1008, 50:4-16. Accordingly, in my opinion, a PHOSITA would have found obvious that the proposed combination exhibits a low degree of oxidation during processing based on the teachings of Bijl.

247. In my opinion, the proposed combination therefore renders claim 4 obvious.

Claim 5

[5] The extracted microbial lipid of claim 1, wherein the oil has a phosphorus content of 100 ppm or less.

248. The combination of Bijl, Nichols-I, and *Schaap* renders obvious the elements of claim 5.

249. I note that in microbial oil compositions, phosphorus content is largely a measure of the quantity of phospholipids in the composition. A phospholipid is a fatty acid that contains a phosphate group.

250. I further note that Bijl discloses a process in which microbial oils were derived, in which the resultant phospholipid concentration was less than 0.05 wt.% of the total composition. MARA-1008, 50:4-17. A PHOSITA would have understood that, on a ppm basis, the entire phospholipid concentration would have encompassed less than 100ppm of phosphorus.

251. A PHOSITA would have understood that a phospholipid concentration of less than 0.05 wt.% would be equivalent to less than 100 ppm of phosphorus. A 0.05 wt.% equates to ppm based on the following conversion calculations:

$$1 \text{ ppm} = 1 \text{ part}/10^6 \text{ parts solution}$$

$$0.05\% = 0.05 \text{ parts in } 100 \text{ parts solution}$$

$$0.05\% = 500 \text{ parts}/10^6 \text{ parts solution}$$

$$0.05\% = 500 \text{ ppm}$$

252. A PHOSITA would therefore have understood that the oils of Bijl disclosed phospholipids in concentrations of no more than 500 ppm. Phospholipids, however, are comprised of multiple components: glycerol, two fatty acids, and a phosphate group (PO_4). One can determine what percentage of a phospholipid comprises phosphorus by analysis of molecular weight.

253. The average phospholipid, as understood by a PHOSITA, would have a molecular weight of 744. MARA-1029, 98. The average atomic mass of

elemental phosphorus is 30.97 g/mol. In a phospholipid, then, which contains only one atom of elemental phosphorus, the percentage phosphorus is 30.974/744, or approximately 4.2%.

254. Multiplying the percentage of a phospholipid that is phosphorus by the ppm concentration of phospholipids yields the ppm concentration of phosphorus. Here, the ppm concentration of phosphorus is 4.2% of 500 ppm, or 21 ppm. Because 21 ppm of phosphorus is less than 100 ppm of phosphorus, a PHOSITA would understand that the combination of Bijl, Nichols-I, and Schaap therefore renders obvious and/or discloses claim 5.

Claim 6

[6] The extracted microbial lipid of claim 1, wherein the lipid is a crude oil.

255. The combination of Bijl, Nichols-I, and Schaap renders obvious that the lipid of claim 1 is a crude oil. For example, Bijl describes that “[b]atches of crude oil *had* been prepared by the methods described in Example 1.” MARA-1008, 49:3-5. This process includes where “a microbial species producing a lipid containing the desired polyunsaturated fatty acid(s) is cultured in a suitable medium, the biomass is then harvested and pretreated to enable subsequent extraction of lipid from the microbial biomass with a suitable solvent. The thus-extracted lipid is in a crude form and so is often subjected to several refining steps.” MARA-1008, 1:32-2:3; *see also* MARA-1008, 31:13-14 (“This resulted in a crude PUFA oil.”).

256. For at least the reasons above, in my opinion, Bijl-Nichols-I-Schaap renders obvious claim 6.

Claim 7

[7] A process for obtaining a lipid, the process comprising refining a crude lipid of claim 6.

257. The combination of Bijl, Nichols-I, and Schaap further renders obvious a process for obtaining a lipid in which the process includes refining a *crude* lipid of claim 6. For example, Bijl discloses, “[t]he thus-extracted lipid is in a crude form and so is often subjected to several refining steps.” MARA-1008, 1:32-2:3. Moreover, “for all oils destined for direct consumption,” refinement processes such as refining, bleaching, and deodorization are carried out. MARA-1010, 181. Accordingly, in my opinion a PHOSITA would have found obvious that the proposed combination includes the process of refining a crude oil.

258. For at least the reasons above, it is my opinion that the combination of Bijl-Nichols-I-Schaap renders obvious claim 7.

D. GROUND 4: Claim 3 is obvious over Radianingtyas and Wang

1. Overview of Radianingtyas

259. In my opinion, Radianingtyas is like the ’578 patent, in that it is directed to methods of culturing oil-producing microbes and extracting microbial oils rich in omega-3 and omega-6 fatty acids. MARA-1007, 1:27-2:2; MARA-1001 1:48-61, 2:8-12. Also like the ’578 patent, Radianingtyas recognizes that

fatty acids are desirable in the human diet but notes that natural sources—such as fish oils—exhibit disadvantageous features like poor flavor and issues with availability. MARA-1007, 1:13-20; MARA-1001, 31:60-32:7.

260. The method of Radianingtyas, to me, appears to include collecting cell biomass, breaking or disrupting that biomass, and then extracting the biomass with solvents such as chloroform, hexane, methanol, ethanol or via supercritical fluid extraction means. MARA-1007, 32:10-14.

261. To me, the resultant oils in Radianingtyas comprise unsaturated fatty acids in the form of approximately 96 to about 100 wt.% triglycerides. MARA-1007, 33:1-2. Constituent acids include “omega-3 fatty acids...omega-6 fatty acids, and omega-9 fatty acids, such as the (n-3) series of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and...(n-6) series of DPA.” MARA-1007, 11:4-9. As Radianingtyas discloses, naturally-occurring microorganisms produce microbial oils with “high concentrations of omega-3 fatty acids...and low concentrations of omega-6 fatty acids.” MARA-1007, 12:23-25, 13:9-10. Those fatty acids include omega-3 or omega-6 fatty acids, such as DHA, DPA n-3, and DPA n-6. MARA-1007, 17:19-20. In particular, it is my opinion that the resultant lipids comprise from about 25 wt.% to about 40 wt.% of n-3 DHA and about 6 wt.% to about 10 wt.% of n-6 DPA. MARA-1007, 40:2-4.

262. In my view, method parameters of Radianingtyas are a baseline from which different yields of DHA, EPA, and/or DPA n-6 can be achieved. MARA-1007, 27:4-8. Radianingtyas explains that modifying the “medium of an oil producing microbe with a fatty acid production antagonist” will increase the “ratio of omega n-3 and/or n-6 oils to omega n-9 oils,” as will manipulation of carbon-to-nitrogen sources and “by the addition of certain chemical compounds which impact upon fatty acid production.” MARA-1007, 27:15-33. More specifically, in my view, Radianingtyas teaches that increases in agitation, temperature, or dissolved oxygen decrease DHA content, while additions of acetate, trace elements, metal and vitamins to the production medium increase DHA, EPA, and DPA n-6 content. MARA-1007, 28:14-20, 30:15-18.

2. Overview of Wang

263. To me, Wang, also like the '578 patent, is directed to creating higher quality oils derived from marine sources through processes that remove undesirable components. MARA-1006, [0005]-[0006]. MARA-1001 1:48-61, 2:8-12. Like the '578 patent, Wang discloses a process that focuses on minimizing the cost to extract such improved oils while maintaining high yields of the same. MARA-1006, [0005]. MARA-1001 1:48-61, 2:8-12. I note that Wang also discloses the final oil products of those processes and their chemical properties. MARA-1006, [0005], [0021].

264. Wang's disclosures, to me, are largely based on processes that utilize adsorbents and temperature changes to modify the chemical composition and characteristics of derived crude oils. MARA-1006, [0075]. In particular, Wang discloses the use of silica, clay, carbon, or mixtures as adsorbents. MARA-1006, [0077-0078]. Adsorbents are either silica, clay, carbon, or a mixture thereof, which remove undesirable compounds such as cholesterol. MARA-1006, [0078], [0147-0148]. Wang teaches me that the adsorbent is vigorously mixed with the crude oil either before or after the oil is heated, and the oil is processed by exposure to elevated temperature. MARA-1006, [0082-0084]. I recognize that the temperature is elevated and is determined based on the oils present, but generally varies between 100 and 200 Celsius. MARA-1006, [0086].

265. To me, Wang discloses a process that results in an oil with reduced levels of sterols. I note that while Wang is directed primarily to fish oils, Wang does specify that the oil to be processed is sourced from a variety of plants and other animals. MARA-1006, [0021]-[0022]. The oils contain a variety of fatty acids, including the omega-3 fatty acids docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). MARA-1006, [0050]-[0052]. In particular, Wang discloses that DHA is present in approximately 0-70% wt.% of the total composition weight. MARA-1006, [0058]-[0059]. I note that no matter the

composition of individual fatty acids, the total triglyceride content is over 97 wt.% of the total composition. MARA-1006, [0072]-[0074].

266. As Wang explains, the amount of aldehydes and oxidized fatty acids in an oil can be quantified by *p*-Anisidine value. MARA-1006, [0069-0070]. I note that oils processed using Wang's method have p-Anisidine values ranging from 1 to 25. MARA-1006, [0070] ("The compositions disclosed herein, however, can have p-Anisidine values of less than or equal to about 25"), [0071] ("In other examples of the disclosed compositions, the p-Anisidine value can be from about 25 to about 1...."), [0153]-[0154tbl]. I note too that in general, employing high temperatures for longer periods of time during the methods of Wang resulted in lower quantities of DHA. MARA-1006, [0152]-[0153], [0155 Table 5], [0160].

3. Combination of Radianingtyas and Wang

267. In my opinion, a PHOSITA would have found it obvious that combining the teachings of Radianingtyas for extracting microbial oils and the teachings of Wang for applying adsorbents and temperature-based applications on extracted oils would result in an improved high quality oil. Radianingtyas teaches suitable starting oils that have not been treated, such as microbial oils from organisms that "can be used in a process for preparing a lipid or fat containing DHA, EPA and DPA." MARA-1007, 16:31-35. Wang teaches methods for refining such starting oils, where a "starting oil is meant any oil before it has been

treated according to the methods disclosed herein.” MARA-1006, [0024], [0170], [0171] (“oil qualities” shown in Table 13). In my opinion, a PHOSITA would have naturally sought Wang’s method of eliminating and/or reducing undesirable compounds and applied those methods to Radianingtyas’s oil composition to yield a higher quality oil.

268. Specifically, a PHOSITA would be motivated to apply Wang’s method of reducing undesirable compounds (e.g., sterols) to Radianingtyas’s process for extracting oil to improve the quality of the extracted oil for at least the following reasons.

269. First, doing so would improve the quality of the oil. MARA-1006, [0170], [0171] (“oil qualities” shown in Table 13; MARA-1013, [0009] (“The anisidine value test is based on the detection of carbonyl compounds...These compounds and their breakdown products affect the stability and quality of oils”). A PHOSITA would have recognized that Wang’s process yields a more stable, higher-quality oil, e.g., an oil having a lower anisidine value.

270. Second, a PHOSITA would have been motivated to apply Wang’s method of reducing undesirable compounds (e.g., cholesterol) to Radianingtyas’s process for extracting oil to produce “stable” products that are able to meet “market realities and the ability to store and transport the product at readily attainable environmental conditions.” MARA-1007, 6:34-7:2.

271. Third, in my opinion a PHOSITA would have been motivated to implement Wang's process with Radianingtyas's oil extraction process because doing so would have amounted to nothing more than the use of a known technique to improve similar oils in the same way and combining prior art elements according to known methods to yield predictable results.

272. It is also my opinion that a PHOSITA would have had a reasonable expectation of success in combining Radianingtyas's and Wang's teachings to produce an improved oil composition. Both Wang and Radianingtyas relate to the same field: obtaining and processing microbial lipids from microorganisms to yield high quality microbial lipid compositions. For example, Radianingtyas teaches that suitable microbial oils come from microbe-producing marine organisms and can be further highly refined. MARA-1007, 1:22-26. Similarly, Wang teaches that oils are isolatable from "marine life, plant life, animal life, [and] microbes." MARA-1006, [0024]. Radianingtyas also teaches that differing yields of desired fatty acids are obtainable by culturing microbe-producing marine organisms in differing conditions. MARA-1007, 27:4-8. Likewise, Wang teaches that differing yields of desired fatty acids occur by culturing microbe-producing marine organisms in differing conditions. MARA-1006, [0144], [0159].

4. Analysis

Claim 3

[3pre] An extracted microbial lipid comprising:

273. The combination of Radianingtyas and Wang discloses and/or renders obvious Element [3pre]. Radianingtyas's disclosure is directed to "compositions and methods related to oil producing microbes that can produce unsaturated fatty acids which can be purified and used." MARA-1007, Abstract. Radianingtyas discloses "methods of preparing a lipid composition" in which "the method comprising[] culturing a[n] oil producing microbe...and isolating the lipid composition." MARA-1007, 40:29-31. For example, Radianingtyas describes that "oil producing microbes, which when cultured produce quantities of unsaturated fatty acids, such as omega 3 (n- 3) and/or omega 6 (n-6) oils, such as DHA, EPA and DPA, [are] capable of being purified and used." MARA-1007, 1:27-31; *see also*, MARA-1007, 1:22-26. Radianingtyas further discloses that "[c]ellular lipids containing the (n-3) DHA, EPA and (n-6) DPA PUFAs can...be extracted from the cellular biomass...to produce a sample of concentrated lipid material." MARA-1007, 42:10-14. Radianingtyas also discloses that microbial lipids "can be obtained by breaking or disrupting the collected cell biomass, for example, via milling, ultrasonication, and then carrying out extraction with a solvent...or via supercritical fluid extraction means." MARA-1007, 32:10-14; *see also* 42:10-43:10.

274. Consistent with the '578 patent's disclosure, in my opinion a PHOSITA would have understood Radianingtyas's teachings directed to an "oil" to be referring to a "lipid," as these terms are used interchangeably in the art, as I discussed above. *See also* MARA-1001, 6:14-15, 12:31-38, 30:45-46, 34:23-33, 38:11-21.

275. As I explain above, extracted microbial lipids were well known in the art prior to the Critical Date. As another example, Wang also discloses extracted microbial lipids, which are "isolated from...microbes." MARA-1006, [0021]. Wang further provides examples of "suitable oils include crude oils from such sources disclosed herein as well as semi-refined (also called alkaline refined) and refined oils from such sources." MARA-1006, [0021].

276. Accordingly, for the reasons described above, in my opinion the combination of Radianingtyas and Wang discloses and/or renders obvious [3pre].

[3a] a triglyceride fraction of at least about 70% by weight, of the lipid fraction

277. In my opinion, the combination of Radianingtyas and Wang discloses and/or renders obvious Element [3a].

278. As I discuss above, if the "lipid fraction" refers to the entire composition being composed of the lipid obtained from an extraction process (i.e., "lipid fraction" constitutes 100 wt.% of the "extracted microbial lipid" composition), the Radianingtyas-Wang combination discloses and/or renders

obvious Element [3a]. For example, Radianingtyas generally discloses “oil producing microbes of the order *Thraustochytriales*, preferably *Thraustochytrium* or *Schizochytrium* species, having the ability to produce lipids, such as fatty acids, such as unsaturated fatty acids, such as omega-3 fatty acids, such as omega-6 fatty acids, and omega-9 fatty acids, such as the (n-3) series of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA)...” MARA-1007, 11:4-9. From Radianingtyas’s “disclosed oil producing microbes, including the oil producing microbe, ONC-T18,” compositions include triglycerides from “96 to about 100%” of the neutral lipids, which “can be at least 95% by weight of total lipids.” MARA-1007, 32:17-33:5.

279. Thus, even at the lowest range, ONC-T18 produces a triglyceride fraction of at least about 91% by weight of the lipid fraction. I calculate this by noting that 96% of the neutral lipids are triglycerides and the neutral lipids are 95% by weight of the total lipids. MARA-1007, 32:17-33:5. Thus, $0.95 \times 0.96 = 0.912$, or 91.2%, of the total weight percentage of the lipid fraction is triglycerides.

280. Accordingly, Radianingtyas’s compositions are “almost entirely triglyceride (at least 95%) in nature” and, at minimum, include a triglyceride fraction that is at least about 91 wt.% of the lipid fraction. MARA-1007, 89:24-26.

281. As I discuss at length above, microbial lipid compositions having a triglyceride fraction of at least about 70% by weight of the lipid fraction were well-

known in the prior art. As another example, Wang discloses extracted microbial lipids, which are “isolated from...microbes...as well as edible oils.” MARA-1006, [0021]. Specifically, Wang discloses compositions of greater than 97 wt.% triglycerides. MARA-1006, [0073].

282. On the other hand, if “lipid fraction” refers to the percentage of the lipids obtained from an extraction process constituting only a portion of the “extracted microbial lipid” composition, in my opinion Radianingtyas-Wang also renders obvious a microbial oil that meets such an interpretation. That is, if the total composition of the microbial oil is, for example, 95 wt.% lipids and 5 wt.% non-lipids components (e.g., organic solvent), the proposed combination would still render obvious a microbial oil wherein at least 70% by weight of the 95 wt.% lipids (i.e., 67 wt.% of the overall composition) is triglycerides. As discussed previously, it is my opinion that Radianingtyas discloses compositions of 96-100 wt.% triglycerides in the fatty acid portion of the extracted biomass. MARA-1007, 32:32-33:5, 89:24-26. Similarly, Wang discloses compositions of greater than 97 wt.% triglycerides relative to the total composition, meaning that greater than 70 wt.% of the lipid fraction is triglycerides. MARA-1006, [0073].

283. Accordingly, for the reasons described above, in my opinion the combination of Radianingtyas and Wang discloses and/or renders obvious [3a]. MARA-1006 [0021], [0073-0074]; MARA-1007, 11:4-9, 32:17-33:5, 89:24-26.

[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight

284. The combination of Radianingtyas and Wang discloses and/or renders obvious Element [3b].

285. As I discuss above in Section III.A.2, since Element [3a] requires a triglyceride fraction of at least about 70 wt.% of the lipid fraction, the docosahexaenoic acid content of at least 60 wt.% of such a triglyceride fraction (i.e., 70 wt.% of the lipid fraction) is expressed as the docosahexaenoic acid content constituting at least 42 wt.% of the lipid fraction when the triglyceride fraction is 70 wt.% of the lipid fraction.

286. I note that Radianingtyas discloses a DHA content of at least 42 wt.% of the lipid fraction (i.e., at least 60% by weight of the triglyceride fraction) because it teaches that microorganisms produce lipid compositions where DHA is present at up to 60 wt.% of the lipid composition (i.e., the lipid fraction). MARA-1007, 26:27-27:3 (“the oil producing microbe can produce a lipid composition comprising...an omega-3 fatty acid, such as n-3 DHA, (for example, at least...**45%, 50%, 55% or 60% by weight**)”).³

³ I note that this paragraph identifies “a lipid composition comprising from about 25% to about 40% of an omega-3 fatty acid, such as n-3 DHA.” MARA-1007, 26:27-27:3. Immediately thereafter, a parenthetical states “for example, at least

287. In my opinion, compositions having a docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight were well known to a PHOSITA based on the prior art that was available before the Critical Date. I note that Radianingtyas generally discloses “oil producing microbes of the order *Thraustochytriales*, preferably *Thraustochytrium* or *Schizochytrium* species, having the ability to produce lipids, such as fatty acids, such as unsaturated fatty acids, such as omega-3 fatty acids, such as omega-6 fatty acids, and omega-9 fatty acids, such as the (n-3) series of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA)....” MARA-1007, 11:4-9. It was also known prior to the Critical Date that there were microorganisms of a *Thraustochytrium* strain that had “a high ability of producing docosahexaenoic acid” and were “produc[ing] oil with at least 50% by weight DHA” and some higher than 60%. MARA-1015, Abstract, [0015] (“the fat and oil according to the present invention contains 50% by weight or more of docosahexaenoic acid, based on the total amount of fatty acid in the fat and oil.”), [0051] (“In a preferable embodiment of the present invention, the

15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% by weight[.]” Thus, this disclosure encompasses 60% by weight DHA concentration. In my opinion, the DHA disclosure, based on the parenthetical disclosure, should be read as “from about 15% to about 60% of an omega-3 fatty acid, such as n-3 DHA.”

content of docosahexaenoic acid in fatty acid components of fat and oil is as high as 50% by weight or more.”); MARA-1032, 27-31 (Table 2.5 discloses 67.2, 65.6, 67.8, 63.7, 64.3, 62.4 and other percentages of DHA); MARA-1009, 27; MARA-1015, [0116], Fig. 3. As I noted above, it was known that decreasing agitation or dissolved oxygen would result in increases of DHA. MARA-1007, 28:14-17. It was also known that changing other parameters, such as “the ratios and types of carbon to nitrogen sources, the chemical composition of salt used and by the addition of certain chemical compounds which impact upon fatty acid production, specific fatty acid profiles” can be achieved. MARA-1007, 27:13-21. In another example, “if a disclosed eukaryotic microorganism, for example, produces 35% DHA, this could be further ‘purified’ such that the final lipid composition was greater than 90% DHA.” MARA-1007, 8:2-4.

288. Accordingly, for at least the reasons described above, in my opinion the combination of Radianingtyas and Wang discloses and/or renders obvious [3b]. MARA-1006, [0058], [0093]; MARA-1007, 8:2-4, 11:4-9, 26:27-27:8, 28:14-20, 30:15-18; MARA-1015, Abs., [0015].

[3c] and wherein the oil has an anisidine value of 0.5 to 26,

289. The combination of Radianingtyas and Wang renders obvious Element [3c].

290. Before the Critical Date, a PHOSITA would have understood that “[t]he anisidine value (AV) of freshly processed oils can be used as a rough predictor of the future storage stability of that oil” and that “AV is an important quality factor for edible oils.” MARA-1013, [0009]. As I explain above, it was well-known in the art that anisidine values measure the “amount of the aldehydes and oxidized fatty acids in an oil.” MARA-1006, [0069]; *see also* MARA-1013, [0009] (“The anisidine value test is based on the detection of carbonyl compounds, primarily aldehydes, which arise through oil oxidation” and “[t]hese compounds and their breakdown products affect the stability and quality of oils.”). The “p-Anisidine values can be determined by well known methods such as the method described in ISO method number 6885:1998.” MARA-1006, [0069].

291. As I discuss above in Section III.C.1-3, a PHOSITA would have applied the teachings of Wang to Radianingtyas’s oil to obtain an oil composition with a reduced anisidine value (i.e., p-Anisidine value). In my opinion, they would have done so for multiple reasons: (1) to improve the quality of the oil, (MARA-1006, [0170], [0171]; MARA-1013); (2) to produce “stable” products that are able to meet “market realities and the ability to store and transport the product at readily attainable environmental conditions,” (MARA-1007, 6:25-2); and (3) to reduce or prevent the occurrence of “oxidative rancidity” in the composition, (MARA-1006, [0170]; MARA-1014, [0032]).

292. In my opinion, it would have been obvious to a PHOSITA that the proposed combination produces an oil with an anisidine value within the range of 0.5 to 26 because Wang teaches removing and/or reducing compounds from oils to produce a resultant oil composition having an anisidine value of less than 25. MARA-1006, [0070] (“The compositions disclosed herein, however, can have p-Anisidine values of less than or equal to about 25”), [0071] (“In other examples of the disclosed compositions, the p-Anisidine value can be from about 25 to about 1....”), [0153].

293. Accordingly, in my opinion the combination of Radianingtyas and Wang renders obvious [3c]. MARA-1006, [0069-0071], [0153], [0170-0171]; MARA-1007, 6:25-2; MARA-1013, [0009]; MARA-1014, [0032].

[3d] and wherein the lipid has less than 5% by weight of an organic solvent.

294. The combination of Radianingtyas and Wang renders obvious Element [3d].

295. For the same reasons as I discuss above in Section §III.A.2, Element [3d] is an optional element for which I understand that prior art disclosure is not necessary to meet the claim element. And since Element [3d] is an optional element and the combination of Radianingtyas and Wang discloses and/or renders obvious Elements [3pre]-[3c], in my opinion claim 3 is obvious.

296. However, even if less than 5 wt.% organic solvent is found to be a necessary element, in my opinion the combination of Radianingtyas and Wang renders obvious this element. For example, Radianingtyas describes “carrying out extraction...*via supercritical fluid extraction means*,” which uses very little or no solvent. MARA-1007, 32:10-16. It would have been obvious to a PHOSITA that using supercritical fluid extraction with supercritical CO₂ as the solvent would yield a composition having less than 5 wt.% organic solvent in the extracted microbial lipid simply because very little or no organic solvent is used. MARA-1007, 45:7-9. A PHOSITA would have been motivated to have applied a supercritical fluid extraction means using carbon dioxide instead of an organic solvent for multiple reasons, including those described below.

297. First, a PHOSITA would have been motivated to use supercritical fluid extraction with carbon dioxide as a solvent to avoid problems that “exist with conventional methods utilized for oil recovery from biomass” in that “conventional methods include organic solvent extraction, vacuum distillation, and maceration, all of which can present ecological problems.” MARA-1016, 2:24-34.

298. Second, a PHOSITA would have been motivated to use supercritical fluid extraction with carbon dioxide as a solvent to avoid “[o]ther problems concern[ing] product recovery, as natural oils such as PUFAs are susceptible to

thermal and oxidative degradation under the harsh conditions these techniques employ.” MARA-1016, 2:24-34.

299. Third, a PHOSITA would have been motivated to use supercritical fluid extraction with carbon dioxide as a solvent because “oils for human consumption should be obtained using methods that employ solvents that are acceptable in terms of toxicity, handling, safety and cost.” MARA-1016, 2:24-34.

300. Fourth, a PHOSITA would have been motivated to use supercritical fluid extraction with carbon dioxide as a solvent to because doing so would have amounted to nothing more than the use of a known technique (e.g., using supercritical fluid extraction) to improve similar oils according to known methods (e.g., extracting and refining microbial oils) to yield predictable results (e.g., resultant oil having less organic solvent residuals).

301. Although Radianingtyas further discloses an alternative method of extraction that involves “extraction with a solvent such as chloroform, hexane, methanol, ethanol,” in my opinion, “supercritical fluid extraction means” would be an obvious option considering that it is one of only *two* presented options for extraction. MARA-1007, 32:10-16 (“carrying out extraction with a solvent...or via supercritical fluid extraction mean.”), 42:10-25 (“Cellular lipids containing the (n-3) DHA, EPA and (n-6) DPA PUFAs can also be extracted from the cellular

biomass methods such as supercritical fluid extraction, or by extraction with solvents”).

302. And even if an organic solvent was used during an extraction process, methods for reducing and removing organic solvents present in an oil composition (e.g., refining methods) would have been readily known to a PHOSITA prior to the Critical Date, as I discuss above. MARA-1007, 42:10-25 (“The extracted oils can be refined by well-known processes routinely employed.... These refining processes remove impurities from extracted oils before they are used or sold as edible oils). My understanding is shown in Radianingtyas, which explicitly teaches removal of impurities, such as solvents, via filtration, centrifugation or settling, or evaporation. MARA-1007, 42:26-43:10, 104:23. A PHOSITA would have understood that residual organic solvents from extracting microbial oils were not preferred and would have naturally looked to remove them from the resulting composition because “conventional methods [that] include organic solvent extraction...can present ecological problems,” there are “[o]ther problems concern[ing] product recovery, as natural oils such as PUFAs are susceptible to thermal and oxidative degradation under the harsh conditions [that conventional] techniques employ,” and because “oils for human consumption should be obtained using methods that employ solvents that are acceptable in terms of toxicity, handling, safety and cost.” MARA-1016, 2:24-34.

303. For at least the reasons described above, in my opinion the combination of Radianingtyas and Wang discloses and/or renders obvious Element [3d].

IV. CONCLUSION

304. I reserve the right to supplement my opinions to address any information obtained, or positions taken, based on any new information introduced throughout this proceeding.

305. I declare under penalty of perjury that the foregoing is true and accurate to the best of my ability.