Preferably the solvent used in the process of the present invention comprises 95% aqueous ethanol.

Preferably the mass fraction of the co-solvent in CO_2 is between 5% and 60%. More preferably the mass fraction is between 20% and 50%. Most preferably the mass fraction is between 25% and 30%.

Preferably the contacting temperature between the feed material and solvent is between 10°C and 80°C. More preferably the contacting temperature is between 55°C and 65°C. Most preferably the contacting pressure is between 100 bar and 500 bar.

Preferably the contacting pressure is between 200 bar and 300 bar. More preferably the ratio of the co-solvent to feed material is in the range 10:1 to 200:1. Most preferably the ratio of the co-solvent to feed material is in the range 15:1 to 50:1.

Preferably the separating pressure is between atmospheric pressure and 90 bar. More preferably the separating pressure is between 40 bar and 60 bar.

Preferably the co-solvent is recycled for further use.

Preferably the CO_2 is recycled for further use.

The co-solvent may be removed by evaporation under vacuum.

Preferably the feed material is contacted with a continuous flow of solvent.

Preferably the feed material is contacted with one or more batches of solvent.

The lipid and solvent streams may be fed continuously.

Optionally, the feed material and co-solvent streams may be mixed prior to contacting with CO_2.

The invention also provides products produced by the process of the invention, both the insoluble components remaining after contact with the solvent (also referred to herein as the "residue"); and the soluble components that are dissolved in the solvent after contact with the feed material (also referred to herein as the "extract"). Where the feed material is contacted with more than one batch of solvent, or the solvent is cooled in a number of steps, there will be multiple "extract" products.

Preferably the product contains more sphingomyelin than the feed material. More preferably the product comprises greater than 3% sphingomyelin. Even more preferably the product comprises greater than 10% sphingomyelin. Most preferably the product comprises greater than 15% sphingomyelin.

Preferably the product contains more phosphatidyl serine than the feed material. More preferably the product comprises greater than 5% phosphatidyl serine. Even more preferably the product comprises greater than 30% phosphatidyl serine. Most preferably the product comprises greater than 70% phosphatidyl serine.

Preferably the product contains more gangliosides than the feed material. More preferably the product comprises greater than 2% gangliosides. Even more preferably the product comprises greater than 4% gangliosides. Most preferably the product comprises greater than 6% gangliosides.

Preferably the product contains more cardiolipin than the feed material. More preferably the product comprises greater than 5% cardiolipin. Even more preferably the product comprises greater than 10% cardiolipin. Most preferably the product comprises greater than 25% cardiolipin.

Preferably the product contains more acylalkyphospholipids and/or plasmalogens than the feed material. More preferably the product comprises greater than 5% acylalkyphospholipids and/or plasmalogens. Even more preferably the product comprises greater than 10% acylalkyphospholipids and/or plasmalogens. Most preferably the product comprises greater than 25% acylalkyphospholipids and/or plasmalogens.

Preferably the product contains more aminoethylphosphonate and/or other phosphonolipids than the feed material. More preferably the product comprises greater than 5% aminoethylphosphonate and/or other phosphonolipids. Even more preferably the product comprises greater than 10% aminoethylphosphonate and/or other phosphonolipids. Most preferably the product comprises greater than 25% aminoethylphosphonate and/or other phosphonolipids.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention may be more fully understood by having reference to the accompanying drawings wherein:

Figure 1 is scheme drawing illustrating a preferred process of the current invention.

Figure 2 is a scheme drawing illustrating a second preferred process of the current invention

Figure 3 is a scheme drawing illustrating a third preferred process of the current invention

Figure 4 is a scheme drawing illustrating a fourth preferred process of the current invention

ABBREVIATIONS AND ACRONYMS

In this specification the following are the meanings of the abbreviations or acronyms used.

"CL" means cardiolipin

"PC" means phosphatidyl choline

"PI" means phosphatidyl inositol

"PS" means phosphatidyl serine

"PE" means phosphatidyl ethanolamine

"PA" means phosphatidic acid

"PL" means plasmalogen

"PP" means phosphonolipid

"ALP" means alkylacylphospholipid

"SM" means sphingomyelin

"CAEP" means ceramide aminoethylphosphonate

"GS" means ganglioside

"N/D" means not detected

"CO_2" means carbon dioxide

GENERAL DESCRIPTION OF THE INVENTION

As discussed in the Background, it is known that supercritical CO_2 with up to 12.5% ethanol as a co-solvent can extract the phospholipids PC, and to a much lesser extent, PE and PI from soy or egg. Surprisingly, we have found that the phospholipids PS, CAEP and CL; and gangliosides are virtually insoluble in CO_2 and a C_1-C_3 monohydric alcohol co-solvent, and that SM, ALP, PL and PP are soluble. Therefore it is possible to separate the soluble phospholipids from the insoluble phospholipids and gangliosides to achieve fractions enriched in one or other of the desired components.

There are a number of factors affecting the operation of the process:

- Feed material and feed preparation
- Extraction temperature and pressure
- Co-solvent concentration
- Total solvent throughput
- Solvent flow rate and contacting conditions

It is advantageous to start with a feed material containing at least 5 % by mass of lipids, and ideally at least 2 % by mass of phospholipids, particularly PS, SM, CL, ALP, PL, PP, CAEP and/or gangliosides.

The feed material can be processed using pure CO_2 before the co-solvent is introduced to remove much or all of neutral lipids. This reduces the neutral lipid content in the CO_2+co-solvent extract leading to an extract enriched in soluble phospholipids and/or gangliosides.

The form of the feed material depends on the source of the lipids and its lipid composition. For example dairy lipid extracts high in phospholipids may be substantially solid even at elevated temperatures. Egg yolk and marine lipids in comparison have a lower melting point. The presence of neutral lipids also tends to produce a more fluid feed material. To promote good contacting it may be beneficial to prepare the feed material. Solid materials containing lipids may be able to be cryomilled. Lipid feed materials can also be made more fluid by the inclusion of some ethanol or water.

Changing the processing conditions of temperature, pressure, co-solvent concentration, and total solvent usage, influences the amount of material extracted, the purity of the final product, and the recovery (or efficiency) of the process. For example, the virtually insoluble lipids such as PS, GS, CAEP and CL, have very slight solubilities so that excessive use of solvent, or very favourable extraction conditions, can result in small losses of PS, GS and CL from the residual fraction. A high purity product may be achieved, but with a reduced yield. Conversely the enrichment of soluble lipids will be greater if smaller amounts of the other lipids are co-extracted, but the total yield will be lower. Processing economics, and the relative values of the products, will determine where this balance lies. A further option to obtain multiple enriched fractions is to carry out extractions under progressively more favourable extraction conditions, such as increasing the temperature.

We have found that co-solvent concentrations below about 10% produce very little extract of phospholipids and/or gangliosides. At higher concentrations the rate of material extracted increases rapidly. We have found the co-solvent concentrations of at least 20%, and more preferably 30% achieve high levels of extraction of PC, PE, SM, ALP, PL, PP and PI, while the lipids PS, CL and GS remain virtually insoluble.

Every substance has its own "critical" point at which the liquid and vapour state of the substance become identical. Above but close to the critical point of a substance, the substance is in a fluid state that has properties of both liquids and gases. The fluid has a density similar to a liquid, and viscosity and diffusivity similar to a gas. The term "supercritical" as used herein refers to the pressure-temperature region above the critical point of a substance. The term "subcritical" as used herein refers to the pressure-temperature region equal to or above the vapour pressure for the liquid, but below the critical temperature. The term "near-critical" as used herein encompasses both "supercritical" and "subcritical" regions, and refers to pressures and temperatures near the critical point.

Percentages unless otherwise indicated are on a w/w solids basis.

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

The invention consists in the foregoing and also envisages constructions of which the following gives examples only.

EXAMPLES

The experimental process is described, with reference to figure 1, as follows.

A measured mass of feed material containing lipids to be fractionated was placed in basket BK1 with a porous sintered steel plate on the bottom. Basket BK1 was placed in a 300 mL extraction vessel EX1. The apparatus was suspended in heated water bath WB1 and maintained at a constant temperature through use of a thermostat and electric heater.

In the continuous extraction mode of operation, liquid CO_2 from supply bottle B1 was pumped using pump P1 into extraction vessel EX1 until the pressure reached the desired operating pressure, after which valve V1 was operated to maintain a constant pressure in the extraction vessel. After passing through valve V1, the pressure was reduced to the supply cylinder pressure of 40 to 60 bar, which caused the CO_2 to be converted to a lower density fluid and lose its solvent strength. Precipitated material was captured in separation vessel SEP1, and the CO_2 exited from the top of separator SEP1 and was recycled back to the feed pump through coriolis mass flow meter FM1 and cold trap CT1 operated at -5°C. Extracted material was collected periodically from separator SEP1 by opening valve V2. The extraction was optionally carried out using CO_2 only until all of the compounds soluble in CO_2 only, such as neutral lipids, were extracted. When no further extract was produced by CO_2 extraction, ethanol co-solvent with or without added water was added to the CO_2 at the desired flow ratio from supply bottle B2 using pump P2. Ethanol and further extracted material were separated from the CO_2 in separator SEP1 and periodically removed through valve V2. After the desired amount of ethanol had been added the ethanol flow was stopped and the CO_2 flow continued alone until all the ethanol had been recovered from the system. The remaining CO_2 was vented and the residual material in basket BK1 was removed and dried under vacuum. The extract fraction was evaporated to dryness by rotary evaporation.

In the batch extraction mode of operation CO_2 alone was optionally passed continuously through the apparatus, as for the continuous flow mode of operation, until all CO_2 alone extractable material was removed. The CO_2 flow was then stopped and valve V1 closed to maintain the pressure. Approximately 140g of ethanol was pumped from supply bottle B2

through pump P2 into extraction vessel EX1. The system was left for 15 minutes to allow the system to equilibrate, after which time the CO_2 flow was started and valve V1 opened to maintain a constant pressure and allow ethanol and dissolved compounds to flow through to separator SEP1. This process was repeated twice more, after which the CO_2 was vented and the residual material in basket BK1 was removed and dried under vacuum.

Extract and residue fractions were analysed for phospholipid content and profile by ^{31}P-NMR. The phospholipid mass fractions reported here are for phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), plasmalogens (PL), phosphonolipids (PP), alkylacylphospholipids (ALP), sphingomyelin (SM), ceramide aminoethylphosphonate (CAEP), phosphatidylserine (PS), and cardiolipin (CL).

The process option illustrated in Figure 1 is for a batch process while the processing options illustrated in Figures 2-4 are for a continuous flow process.

Example 1: Fractionation of dairy lipid extract A, ethanol mass fraction 25%

Lipid extract A is a total lipid extract obtained by a processes disclosed in PCT international applications PCT/NZ2005/000262 (published as WO 2006/041316).

40g of dairy lipid extract A, with composition shown in Table 1 (feed), was extracted using the continuous extraction mode of operation at 60°C and 300 bar. The 'other compounds' consist mainly of neutral lipids. 44% of the feed material was extracted (extract 1) using CO_2 only. This extract contained no phospholipids, and was entirely neutral lipids. A further 31% of the feed material (extract 2) was extracted using 95% aqueous ethanol at a concentration in CO_2 of 25%. The total ethanol and water added was 880g. The composition of the fraction extracted with CO_2 and ethanol (extract 2), and the composition of the residual fraction are shown in Table 1. The extract is enriched in phosphatidylcholine (PC) and sphingomyelin (SM) which are more soluble in CO_2 and ethanol, while the residual fraction is substantially enriched in phosphatidylserine (PS). Phosphatidylserine levels are virtually undetectable in the extract phase indicating very low solubility in CO_2 and ethanol, and almost complete recovery of phosphatidylserine in the residue phase.

Table 1

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		11.2	2.8	4.3	13.2	7.8	2.2	58.3
Extract 2	31	28.2	0.0	0.2	14.4	15.4	4.9	37.0
Residue	25	6.5	10.5	15.6	30.8	10.2	3.6	22.8

Example 2: Fractionation of dairy lipid extract A, ethanol mass fraction 31%

41g of dairy lipid extract A, with composition as for example 1 was extracted using the continuous extraction mode of operation at 60°C and 300 bar as for example 1, using firstly CO_2 alone to extract 50 % of the feed material (extract 1), which is neutral lipids only, and then using 95% aqueous ethanol at a concentration in CO_2 of 31%. 33% of the feed material was extracted (extract 2). The total ethanol and water added was 1150g. The composition of the residual fraction is shown in Table 2. The higher ethanol concentration gives a more complete extraction of lipids and the concentration of phosphatidylserine in the residue fraction is higher than found in example 1 at 19.3 %.

Table 2

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		11.2	2.8	4.3	13.2	7.8	2.2	58.3
Extract 2	33	-	-	-	-	-	-	-
Residue	17	4.4	12.6	19.3	27.1	8.5	2.5	25.5

Example 3: Fractionation of dairy lipid extract A, ethanol mass fraction 43%

40g of dairy lipid extract A, with composition as for example 1 was extracted using the continuous extraction mode of operation at 60°C and 300 bar as for example 1, using firstly CO_2 alone to extract 41 % of the feed material (extract 1), which is neutral lipids only, and then using 95% aqueous ethanol at a concentration in CO_2 of 43% to extract 32 % of the feed (extract 2). The total ethanol and water added was 960g. The composition of extract 2 and residual fractions are shown in Table 3. The concentration of phosphatidylserine in the residue fraction is higher than found in example 1 and example 2 at 20.7 %. The

concentration of SM in the extract, at 12.5 % by mass, is enriched relative to the feed, at 7.8 % by mass, even though it also contains a high level of neutral lipids.

Table 3

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		11.2	2.8	4.3	13.2	7.8	2.2	58.3
Extract 2	32	21.1	0.0	0.5	13.3	12.5	3.5	49.1
Residue	27	4.2	13.6	20.7	26.7	7.8	1.9	25.0

Example 4: Fractionation of dairy lipid extract A, 40°C

39g of dairy lipid extract A, with composition as for example 1 was extracted using the continuous extraction mode of operation at 300 bar using firstly CO_2 alone to extract 54 % of the feed material (extract 1), which is neutral lipids only, and then using 95% aqueous ethanol at a concentration in CO_2 of 30 % to extract 12 % of the feed (extract 2). The temperature in this example was 40°C. The total ethanol and water added was 975g. The composition of the extracted and residual fractions are shown in Table 5. The degree of extraction of SM is lower than for examples 1 to 3 at 60°C, but the concentration in the extract is higher. The concentration of PS in the residue, at 12.4 %, is lower than examples 1 to 3.

Table 4

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		11.2	2.8	4.3	13.2	7.8	2.2	58.3
Extract 2	12	27.9	0.0	0.3	16.7	15.3	4.9	34.9
Residue	34	9.9	8.1	12.4	25.3	12.2	3.6	28.5

Example 5: Fractionation of dairy phospholipid concentrate

40g of a dairy phospholipid concentrate with composition as shown in Table 5 (feed) was extracted using the continuous extraction mode of operation at 300 bar and 60°C without the prior CO_2 only extraction step. The ethanol (95% aqueous ethanol) mass fraction in CO_2 was 30%. The total ethanol and water added was 1026g. The composition of the extracted and residual fractions are shown in Table 5. Only 11% of the feed lipid was extracted, so the enrichment of phosphatidylserine in the residue is not significant, but the concentration did increase from 8% to 8.8%. The poor degree of extraction in this example is due to the

physical properties of the solid feed material limiting mass transfer. In comparison, the dairy lipid extract in examples 1 through 4, is liquid at the processing temperature and better extraction rates are observed.

Different feed preparation methods and/or longer equilibration times and/or greater solvent quantities are expected to increase the amount of extractable material.

Table 5

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		15.4	5.3	8.0	21.6	15.1	0.3	34.3
Extract	11							
Residue	89	13.0	5.9	8.8	21.4	10.9	2.8	37.2

Example 6: Fractionation of dairy phospholipid concentrate using the batch extraction process

19g of a dairy phospholipid concentrate with composition as described in example 5 was extracted using the batch extraction mode of operation at 300 bar and 60°C. A total of 22% of the feed mass was extracted in three sequential extractions each consisting of 140g of ethanol (95% aqueous ethanol) in 300mL of CO_2. The composition of the extracted and final residual fractions are shown in Table 6. In this example 22% of the feed lipid was extracted, significantly higher than that obtained in the continuous extraction example (example 5) and using a lower total quantity of ethanol co-solvent. The phosphatidylserine concentration in the residue has increased from 8% to 11.2%; and the sphingomyelin concentration in the extract has increased from 15.1 to 16.7 %. This example shows the increase in total extracted material by allowing a greater contacting time to more completely dissolve the soluble fraction.

Table 6

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		15.4	5.3	8.0	21.6	15.1	0.3	34.3
Extract	22	32.4	0.5	0.4	17.7	16.7	4.7	27.5
Residue	78	13.6	7.4	11.2	26.6	13.3	2.9	25.0

Example 7: Fractionation of dairy lipid extract B, ethanol mass fraction 10%

This example relates to extraction of dairy lipid extract B, a total lipid extract obtained from high fat whey protein concentrate processes disclosed in PCT international applications PCT/NZ2004/000014 (published as WO WO2004/066744).

with composition shown in Table 7 (feed). The 'other compounds' listed include 2-3% gangliosides and about 3% lactose, both absent in dairy lipid extract A. In this example 42g of dairy lipid extract B was extracted using the continuous extraction mode of operation at 300 bar and 60°C. 52% of the feed mass was extracted using CO_2 alone (extract 1). Only 3% of the feed lipid was further extracted using 460g of 95% aqueous ethanol (extract 2), and the extract contained less than 10% phospholipids. The extraction of phospholipids does not occur to any significant extent for ethanol mass fractions of 10% or lower. The ethanol does however extract some additional neutral lipid that is not extracted using CO_2 alone. In this case, both the PS and SM are enriched in the residue.

Table 7

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		7.4	2.5	3.9	10.3	5.7	1.3	69.0
Extract 2	3	4.5	0.0	0.0	1.6	1.0	0.3	92.6
Residue	45	15.0	6.1	8.7	21.8	12.0	5.9	30.7

Example 8: Fractionation of dairy lipid extract B, ethanol mass fraction 30%

In this example 40g of dairy lipid extract B was extracted using the continuous extraction mode of operation at 300 bar and 60°C. 51% of the feed mass was extracted using CO_2 alone (extract 1). A further 7% of the feed material was extracted using 760g of 95% aqueous ethanol at a mass concentration of 30% in CO_2 (extract 2). Phospholipid profiles for the extract and residual fractions are shown in Table 8. Both PS and SM are enriched in the residue

Table 8

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		7.4	2.5	3.9	10.3	5.7	1.3	69.0
Extract (2)	7	22.5	0.5	0.4	14.0	11.2	3.3	48.2
Residue	41	12.0	5.5	8.5	20.2	10.0	2.4	41.5

Example 9: Fractionation of dairy lipid extract A, ethanol mass fraction 3%

This example shows that when the co-solvent concentration is below 10% by mass, no phospholipids are extracted.

In this example 27g of dairy lipid extract A, as described in example 1, was extracted using the continuous extraction mode of operation at 300 bar and 60°C, using 98% ethanol at 3 % by mass ratio with CO_2, without the CO_2 only extraction step. 62% of the feed mass was extracted. No detectable phospholipids were extracted. This extract represents 90% of the neutral lipid present in the feed material. The rate of extraction of neutral lipid from the feed material was substantially faster using the ethanol co-solvent than using CO_2 only. The extract material was substantially extracted using less than the total of 150g of ethanol in 4850g of CO_2 used, while typically 10 kg of CO_2 alone is required for extraction of neutral lipids, as in example 1.

Example 10: Fractionation of egg yolk lecithin

This example relates to fractionation of a commercially available egg yolk lecithin, with phospholipid profile shown in Table 9. No phosphatidylserine was detected in the feed lipid, indicating concentration levels <0.5%. In this example 34g of the feed material was extracted using the continuous extraction mode of operation at 300 bar and 60°C, and 95% aqueous ethanol at a concentration of 25%. 45% of the feed mass was extracted as neutral lipids using CO_2 alone. A further 49% of the feed material was extracted using ethanol and CO_2 with a total ethanol flow of 640g. Phospholipid profiles for the extract and residual fractions are shown in Table 9. In this example, the phosphatidylserine levels in the residual material are substantially enriched compared with non-detectable levels in the feed material.

Table 9

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		56.4	N/D	N/D	6.4	2.0	5.7	29.4
Extract	49	43.5	N/D	N/D	9.2	2.6	2.1	42.5
Residue	6	17.4	8.0	5.9	19.1	3.8	3.8	42.0

Example 11: Fractionation of egg yolk phospholipid extract

This example relates to fractionation of an egg yolk phospholipid fraction with phospholipid profile shown in Table 9. In this example 40g of the feed material was extracted using the continuous extraction mode of operation at 300 bar and 60°C, and 95% aqueous ethanol at a concentration of 28%. 50% of the feed mass was extracted as neutral lipids using CO_2 alone. A further 46% of the feed material was extracted using ethanol and CO_2 with a total ethanol flow of 800g. Phospholipid profiles for the extract and residual fractions are shown in Table 10. In this example, the phosphatidylserine levels in the residual material are substantially enriched compared with levels in the feed material, while sphingomyelin is enriched in the extract relative to the feed.

Table 10

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		21.2	0.6	0.4	5.2	1.6	0.9	70.1
Extract	46	65.6	0.3	N/D	6.3	2.8	2.3	22.8
Residue	4	12.9	11.2	8.2	27.6	2.8	8.2	29.2

Example 12: Fractionation of Hoki head lipid extract

This example relates to fractionation of a Hoki head lipid extract with phospholipid profile shown in Table 11. In this example 25g of the feed material was extracted using the continuous extraction mode of operation at 300 bar and 60°C, and 95% aqueous ethanol at a concentration of 31%. 1% of the feed mass was extracted as neutral lipids using CO_2 alone. A further 72% of the feed material was extracted using ethanol and CO_2 with a total ethanol flow of 940g. Phospholipid profiles for the extract and residual fractions are shown in Table 11. In this example, the phosphatidylserine levels in the residual material are substantially enriched compared with levels in the feed material. Some PS is also observed in the extract phase. The alkylacylphosphatidylcholine (AAPC), a type of alkylacylphospholipid, is completely extracted.

Table 11

	Yield % of feed	Composition, %							
		PC	PI	PS	PE	SM	AAPC	Other phosph	Other compounds
Feed	1	9.2	1.1	1.4	4.8	0.5	1.1	1.8	80.8
Extract	72	14.2	0.0	0.7	5.3	0.5	1.6	0.6	71.2
Residue	27	14.3	7.1	7.6	13.9	0.0	0.0	6.2	47.7

Example 13: Fractionation of bovine heart lipid extract

This example relates to fractionation of a bovine heart phospholipid lipid extract with phospholipid profile shown in Table 9. In this example 40g of the feed material was extracted using the continuous extraction mode of operation at 300 bar and 60°C, and 95% aqueous ethanol at a concentration of 33% in CO_2. No lipid was extracted using CO_2 alone. 79% of the feed material was extracted using ethanol and CO_2 with a total ethanol flow of 960g. Phospholipid profiles for the extract and residual fractions are shown in Table 12. The phosphatidylserine levels in the residual material are substantially enriched compared with levels in the feed material. Cardiolipin is also significantly enriched in the residue.

Table 12

	Yield wt% of feed	Composition, wt%							
		CL	PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		16.8	13.4	3.2	1.5	12.3	3.6	15.3	33.9
Extract	79	8.2	18.6	0.8	0.4	8.6	3.5	13.1	46.7
Residue	21	42.2	2.8	14.1	4.7	23.4		12.8	0.0

Example 14: Fractionation of dairy lipid extract A with propan-2-ol co-solvent

In this example 39g of the dairy lipid extract A, as described in example 1, was extracted using the continuous extraction mode of operation at 300 bar and 60°C, and 95% aqueous propan-2-ol at a mass concentration of 35% in CO_2. 48% of the feed material was extracted as neutral lipids using CO_2 alone. 23% of the feed material was further extracted using the propan-2-ol co-solvent and CO_2 with a total propanol mass of 810g. Phospholipid profiles for the extract and residual fractions are shown in Table 13. The phosphatidylserine levels in the residual material are substantially enriched, and the result is comparable to results for examples 1 and 2. A slightly lower total PS level is achieved than for example 2 using a

comparable concentration of ethanol. The levels of PS observed in the extracted fraction is also higher suggesting the propan-2-ol is not as selective as ethanol. On this basis alone ethanol would be the preferred co-solvent.

Table 13

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		11.2	2.8	4.3	13.2	7.8	2.2	58.3
Extract	23	27.9	0.8	1.3	19.5	14.0	4.2	32.4
Residue	29	10.7	8.6	13.0	23.8	15.5	3.4	25.0

Example 15: Fractionation of soy lecithin

This example relates to fractionation of a soy lecithin (Healtheries Lecithin natural dietary supplement, Healtheries of New Zealand Limited) with composition shown in Table 9 . In this example 42g of feed material was extracted using the continuous extraction mode of operation at 300 bar and 60°C, and 95% aqueous ethanol at a concentration of 33% in CO_2. No lipid was extracted using CO_2 alone. 91% of the feed material was extracted using ethanol and CO_2 with a total ethanol flow of 520g. Phospholipid profiles for the extract and residual fractions are shown in Table 14. PC and PE are preferentially extracted and are significantly enriched in the extract. There are no detectable levels of PS or SM in this example.

Table 14

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		22.2	12.3	0.0	17.4	0.0	11.7	36.4
Extract	9	31.9	0.7	0.0	9.9	0.0	6.1	51.4
Residue	91	20.7	13.2	0.0	18.4	0.0	12.4	35.2

Example 16: Continuous fractionation of egg yolk lipids

This example relates to fractionation of an egg yolk lipid extract containing 15% phospholipids and the balance mostly neutral lipids by HPLC analysis. The phospholipid fraction contains 55% PC, 29% PE, and 14% PI . The feed lipid was pumped into the top of a 10L pressure vessel, and contacted with CO_2 containing 8.7 % of 98% aqueous ethanol flowing upwards through the vessel at 300 bar pressure and temperature of 60°C. An extract phase was continuously taken from the top of the contacting vessel, and a raffinate phase

was periodically withdrawn from the bottom of the vessel. The lipid feed rate was 1.5 kg/hr. The CO_2+ co-solvent flow rate was 27 kg/hr.

The extract phase was predominantly neutral lipids but contained 20% of the phospholipids present in the feed stream. The phospholipids in the extract fraction consisted of between 70% and 100% PC, with the balance mostly PE. This represents a preferential extraction of PC over other phospholipids.

In a second experiment, feed lipid was premixed with 98% ethanol (with 2 % water) at a concentration of 10.2% lipid. This mixture was pumped into the top of the pressure vessel and contacted with CO_2 in upflow. The overall concentration of ethanol in CO_2 under steady state processing conditions was 5.9%. In this case 50% of the mass of phospholipids in the feed were extracted. The composition of the extract phase consisted of between 60% and 70% PC, with the balance mostly PE. The presence of PI and other phospholipids in the extract was not detectable by the HPLC method used.

Example 17: Fractionation of green-lipped mussel lipid extract

This example relates to fractionation of a green-lipped mussel lipid extract with phospholipid profile shown in Table 11. In this example 12.2g of the feed material was extracted using a batch stirred tank method at 250 bar and 60°C using CO_2 and ethanol (containing 5 % water) at a concentration of 30.5 %. The lipid was placed in the stirred tank, CO_2 was added to give the desired pressure and then the 95 % ethanol was added in during constant stirring. 65 % of the feed material was then extracted using CO_2 and ethanol after stirring for 1 hour by sampling the extract phase at constant pressure. Phospholipid profiles for the extract and residual fractions are shown in Table 15. In this example, the CAEP levels in the residual material are substantially enriched compared with levels in the feed material. The alkylacylphosphatidylcholine (AAPC), a type of alkylacylphospholipid, is partially extracted.

Table 15

	Yield % of feed	Composition, %							
		PC	PI	PS	PE	SM	AAPC	CAEP	Other compounds
Feed		1.89	0.0	0.0	1.60	0.0	0.8	2.0	92.2
Extract	65.3	1.97	0.0	0.0	0.0	0.0	0.9	0.71	96.0
Residue	34.7	3.77	0.0	0.0	3.67	0.0	1.3	3.22	84.0

Example 18: Fractionation of krill lipids

This example shows the fractionation of krill lipids from krill powder and demonstrates concentration of AAPC in the extract, and AAPE in the residue. 5619.9 g of freeze-dried krill powder containing 21.4 % lipid and corresponding phospholipids concentrations shown in table 16 was extracted continuously with supercritical CO_2 at 300 bar and 313 K until no further extract was obtained. This extract (extract 1) contained no phospholipids, and was substantially all neutral lipids. A total of 650 g of this extract was obtained, and 66.41 kg of CO_2 was used. The residual powder was then extracted with CO_2 and absolute ethanol, using a mass ratio of ethanol to CO_2 of 11 %. The CO_2 and ethanol extract phase was passed through two sequential separators in which the pressure was 95 and 60 bar respectively. The bulk of the phospholipids-rich extract (extract 2) was obtained in the first separator, and the bulk of the co-solvent in the second separator (extract 3). The composition of extract 2 and residual powder are shown in table 16. The alkylacylphosphatidylcholine (AAPC), a type of alkylacylphospholipid, is highly enriched in the concentrated phospholipids-rich extract, whilst alkylacylphosphatidylethanolamine (AAPE), another type of alkylacylphospholipid, is not extracted to any great degree.

Table 16

	Yield % of feed	Composition, %							
		PC	PI	PS	PE	CL	AAPC	AAPE	Other compounds
Feed		6.6	0.0	0.0	0.4	0.1	0.6	0.1	78.6
Extract 2	4.3	39.8	0.0	0.0	0.3	0.2	4.6	0.2	53.7
Residue	79.2	3.6	0.0	0.0	0.3	0.2	0.5	0.1	93.4

Example 18: Fractionation of dairy lipids from beta-serum powder

This example shows the fractionation of dairy lipids from beta-serum powder (a milk fat globular membrane concentrate powder) and demonstrates concentration of PS in the residual powder, and concentration of SM in the extract obtained using supercritical CO_2 + ethanol. 5835.3 grams of beta-serum powder containing phospholipids in the concentrations shown in table 17, was extracted continuously with supercritical CO_2 at 300 bar and 313 K until no further extract was obtained. This extract contained no phospholipids, and was substantially all neutral lipids. 1085.6 g of this extract (extract 1) was obtained using 94.42 kg of CO_2. 2906.3 grams of the residual powder was then re-extracted with CO_2 and anhydrous ethanol at 300 bar and 323 K, using a mass ratio of ethanol to CO_2 of 25 %. The powder was extracted with this mixture for 90 minutes (7.82 kg ethanol). The CO_2 and

ethanol extract phase was passed through two sequential separators in which the pressure was 100 (extract 2) and 54 bar (extract 3) respectively. The extract was split between both separators. A total of 262.2 g of extract was obtained. The composition of the combined extract (extract 2 and 3) and residual powder are shown in table 17. The extract is highly enriched in sphingomyelin, whilst the residue is enriched in phosphatidylserine.

Table 17

	Yield % of feed	Composition, %						
		PC	PI	PS	PE	SM	Other Phospholipids	Other compounds
Feed		4.9	1.5	2.3	5.6	4.3	0.1	81.3
Extract 2+3	9.02	49.6	0.0	0.0	12.4	30.1	0.7	7.1
Residue	71.14	0.3	2.0	3.0	3.0	0.5	0.1	91.1

INDUSTRIAL APPLICATION

The present invention has utility in providing products with high levels of particular phospholipids and/or glycolipids including cardiolipin and phosphatidyl serine, and sphingomyelin. The described compositions and methods of the invention may be employed in a number of applications, including infant formulas, brain health, sports nutrition and dermatological compositions.

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WHAT WE CLAIM IS:

1. A process for separating a feed material into soluble and insoluble components, comprising

(e) providing a feed material comprising one or more of:

(i) at least 1% by mass phosphatidyl serine

(ii) at least 1% by mass sphingomyelin

(iii) at least 0.3 % by mass acylalkylphospholipids and/or plasmalogens

(iv)at least 0.5 % by mass aminoethylphosphonate and/or other phosphonolipids

(v) at least 1% by mass cardiolipin

(vi) at least 0.3% by mass gangliosides

(f) providing a solvent comprising:

(i) supercritical or near-critical CO_2, and

(ii) a co-solvent comprising one or more C_1-C_3 monohydric alcohols, and water

wherein the co-solvent makes up at least 10% by mass of the CO_2, and the water content of the co-solvent is 0 to 40 % by mass

(g) contacting the feed material and the solvent and subsequently separating the solvent containing the soluble components from the insoluble components

(h) optionally separating the soluble components and the solvent.

2. The process of claim 1 wherein the feed material comprises greater than 1% phosphatidyl serine.

3. The process of claim 1 wherein the feed material comprises greater than 2% phosphatidyl serine.

4. The process of claim 1 wherein the feed material comprises greater than 5% phosphatidyl serine.

5. The process of claim 1 wherein the feed material comprises greater than 1% sphingomyelin.

6. The process of claim 1 wherein the feed material comprises greater than 5% sphingomyelin.

7. The process of claim 1 wherein the feed material comprises greater than 15% sphingomyelin.

8. The process of claim 1 wherein the feed material comprises greater than 1% cardiolipin.

9. The process of claim 1 wherein the feed material comprises greater than 2% cardiolipin.

10. The process of claim 1 wherein the feed material comprises greater than 5% cardiolipin.

11. The process of claim 1 wherein the feed material comprises greater than 0.3% gangliosides.

12. The process of claim 1 wherein the feed material comprises greater than 1% gangliosides.

13. The process of claim 1 wherein the feed material comprises greater than 2% gangliosides.

14. The process of claim 1 wherein the feed material comprises greater than 0.5% acylalkyphospholipids and/or plasmalogens.

15. The process of claim 1 wherein the feed material comprises greater than 2% acylalkyphospholipids and/or plasmalogens.

16. The process of claim 1 wherein the feed material comprises greater than 10% acylalkyphospholipids and/or plasmalogens.

17. The process of claim 1 wherein the feed material comprises greater than 0.5% aminoethylphosphonate and/or other phosphonolipids.

18. The process of claim 1 wherein the feed material comprises greater than 5% aminoethylphosphonate and/or other phosphonolipids.

19. The process of claim 1 wherein the feed material comprises greater than 20% aminoethylphosphonate and/or other phosphonolipids.

20. A process for separating a feed material into soluble and insoluble components, comprising

(h) providing a feed material comprising one or more of:

(i) at least 1% by mass phosphatidyl serine,

(ii) at least 1% by mass sphingomyelin,

(iii) at least 0.3 % by mass acylalkylphospholipids and/or plasmalogens

(iv) at least 0.5 % by mass aminoethylphosphonate and/or other phosphonolipids

(v) at least 1% by mass cardiolipin, or

(vi) at least 0.3% by mass gangliosides

(i) providing a first solvent comprising supercritical or near-critical CO_2

(j) contacting the feed material and the first solvent and subsequently separating the first solvent containing the first soluble components from the first insoluble components

(k) optionally separating the first soluble components and the first solvent

(l) providing a second solvent comprising:

(iii) supercritical or near-critical CO_2, and

(iv)a co-solvent comprising one or more C_1-C_3 monohydric alcohols, and water

wherein the co-solvent makes up at least 10% by mass of the CO_2, and the water content of the co-solvent is 0 to 40% by mass

(m)contacting the first insoluble components and the second solvent and subsequently separating the second solvent containing the second soluble components from the second insoluble components

(n) optionally separating the second soluble components and the second solvent.

21. The process of claim 20 wherein the first solvent comprises a mixture of supercritical or near-critical CO_2 and less than 10% C_1-C_3 monohydric alcohol.

22. The process of claim 20 or claim 21 wherein the feed material comprises greater than 1% phosphatidyl serine.

23. The process of claim 20 or claim 21 wherein the feed material comprises greater than 2% phosphatidyl serine.

24. The process of claim 20 or claim 21 wherein the feed material comprises greater than 5% phosphatidyl serine.

25. The process of claim 20 or claim 21 wherein the feed material comprises greater than 1% sphingomyelin.

26. The process of claim 20 or claim 21 wherein the feed material comprises greater than 5% sphingomyelin.

27. The process of claim 20 or claim 21 wherein the feed material comprises greater than 15% sphingomyelin.

28. The process of claim 20 or claim 21 wherein the feed material comprises greater than 1% cardiolipin.

29. The process of claim 20 or claim 21 wherein the feed material comprises greater than 2% cardiolipin.

30. The process of claim 20 or claim 21 wherein the feed material comprises greater than 5% cardiolipin.

31. The process of claim 20 or claim 21 wherein the feed material comprises greater than 0.3% gangliosides.

32. The process of claim 20 or claim 21 wherein the feed material comprises greater than 1% gangliosides.

33. The process of claim 20 or claim 21 wherein the feed material comprises greater than 2% gangliosides.

34. The process of claim 20 or claim 21 wherein the feed material comprises greater than 0.5% acylalkyphospholipids and/or plasmalogens.

35. The process of claim 20 or claim 21 wherein the feed material comprises greater than 2% acylalkyphospholipids and/or plasmalogens.

36. The process of claim 20 or claim 21 wherein the feed material comprises greater than 10% acylalkyphospholipids and/or plasmalogens.

37. The process of claim 20 or claim 21 wherein the feed material comprises greater than 0.5% aminoethylphosphonate and/or other phosphonolipids.

38. The process of claim 20 or claim 21 wherein the feed material comprises greater than 5% aminoethylphosphonate and/or other phosphonolipids.

39. The process of claim 20 or claim 21 wherein the feed material comprises greater than 20% aminoethylphosphonate and/or other phosphonolipids.

40. The process of any one of claim 1 to 39 wherein the feed material is derived from terrestrial animals, marine animals, terrestrial plants, marine plants, or micro-organisms such as microalgae, yeast and bacteria.

41. The process of claim 40 wherein the feed material is derived from sheep, goat, pig, mouse, water buffalo, camel, yak, horse, donkey, llama, bovine or human.

42. The process of claim 40 or claim 41 wherein the feed material is selected from: tissue, a tissue fraction, organ, an organ fraction, milk, a milk fraction, colostrum, a colostrum fraction, blood and a blood fraction.

43. The process of claim 40 wherein the feed material is derived from dairy material, soy material, eggs, animal tissue, animal organ or animal blood.

44. The process of claim 40 wherein the feed material is selected from: a composition comprising dairy lipids, a composition comprising egg lipids, and a composition comprising marine lipids.

45. The process of any one of claims 1 to 44 wherein the feed material is a bovine milk fraction.

46. The process of claim 45 wherein the feed material is selected from: buttermilk, a buttermilk fraction, beta serum, a beta serum fraction, butter serum, a butter serum fraction, whey, a whey fraction, colostrum, and a colostrum fraction.

47. The process of any one of claims 1 to 46 wherein the feed material comprises milk fat globule membrane.

48. The process of any one of claims 1 to 47 wherein the feed material comprises at least:

(a) 1% phosphatidyl serine, and

(b) 0.3% gangliosides.

49. The process of claim 48 wherein the feed material comprises at least:

(a) 1% phosphatidyl serine,

(b) 1% sphingomyelin, and

(c) 0.3% gangliosides.

50. The process of claim 48 wherein the feed material comprises at least:

(a) 1% phosphatidyl serine,

(b) 1% sphingomyelin,

(c) 1% cardiolipin, and

(d) 0.3% gangliosides.

51. The process of any one of claims 1 to 50 wherein the feed material has been genetically modified.

52. The process of any one of claims 1 to 51 wherein the feed material is in solid form.

53. The process of claim 52 wherein the feed material is cryomilled before contact with a solvent.

54. The process of any one of claims 1 to 53 wherein the co-solvent comprises:

(a) an alcohol selected from: methanol, ethanol, n-propanol, isopropanol and mixtures thereof; and

(b) 0-40% by mass water.

55. The process of claim 54 wherein the co-solvent comprises between 0 and 20% by mass water.

56. The process of claim 54 wherein the co-solvent comprises between 1 and 10% by mass water.

57. The process of any one of claims 54 to 56 wherein the alcohol is ethanol.

58. The process of any one of claims 1 to 57 wherein the co-solvent is 95% aqueous ethanol.

59. The process of any one of claims 1 to 58 wherein the mass fraction of the co-solvent in CO_2 is between 5% and 60%.

60. The process of claim 59 wherein the mass fraction is between 20% and 50%.

61. The process of claim 59 wherein the mass fraction is between 25% and 30%.

62. The process of any one of claims 1 to 61 wherein the contacting temperature between the feed material and solvent is between 10°C and 80°C.

63. The process of claim 62 wherein the contacting temperature is between 55°C and 65°C.

64. The process of any one of claims 1 to 63 wherein the contacting pressure is between 100 bar and 500 bar.

65. The process of claim 64 wherein the contacting pressure is between 200 bar and 300 bar.

66. The process of any one of claims 1 to 65 wherein the ratio of the co-solvent to feed material is in the range 10:1 to 200:1.

67. The process of claim 66 wherein the ratio of the co-solvent to feed material is in the range 15:1 to 50:1.

68. The process of any one of claims 1 to 67 wherein the separating pressure is between atmospheric pressure and 90 bar.

69. The process of claim 68 wherein the separating pressure is between 40 bar and 60 bar.

70. The process of any one of claims 1 to 69 wherein the co-solvent is recycled for further use.

71. The process of any one of claims 1 to 70 wherein the CO_2 is recycled for further use.

72. The process of any one of claims 1 to 71 wherein the co-solvent is removed by evaporation under vacuum.

73. The process of any one of claims 1 to 72 wherein the feed material is contacted with a continuous flow of solvent.

74. The process of any one of claims 1 to 72 wherein the feed material is contacted with one or more batches of solvent.

75. The process of any one of claims 1 to 73 wherein the lipid and solvent streams are fed continuously.

76. The process of any one of claims 1 to 75 wherein the feed material and co-solvent streams are mixed prior to contacting with CO_2.

77. A product produced by the process of any one of claims 1 to 76.

78. The product of claim 77 wherein the product contains more sphingomyelin than the feed material.

79. The product of claim 77 wherein the product comprises greater than 3% sphingomyelin.

80. The product of claim 77 wherein the product comprises greater than 10% sphingomyelin.

81. The product of claim 77 wherein the product comprises greater than 15% sphingomyelin.

82. The product of claim 77 wherein the product contains more phosphatidyl serine than the feed material.

83. The product of claim 77 wherein the product comprises greater than 5% phosphatidyl serine.

84. The product of claim 77 wherein the product comprises greater than 30% phosphatidyl serine.

85. The product of claim 77 wherein the product comprises greater than 70% phosphatidyl serine.

86. The product of claim 77 wherein the product contains more gangliosides than the feed material.

87. The product of claim 77 wherein the product comprises greater than 2% gangliosides.

88. The product of claim 77 wherein the product comprises greater than 4% gangliosides.

89. The product of claim 77 wherein the product comprises greater than 6% gangliosides.

90. The product of claim 77 wherein the product contains more cardiolipin than the feed material.

91. The product of claim 77 wherein the product comprises greater than 5% cardiolipin.

92. The product of claim 77 wherein the product comprises greater than 10% cardiolipin.

93. The product of claim 77 wherein the product comprises greater than 25% cardiolipin.

94. The product of claim 77 wherein the product contains more acylalkyphospholipids and/or plasmalogens than the feed material.

95. The product of claim 77 wherein the product comprises greater than 5% acylalkyphospholipids and/or plasmalogens.

96. The product of claim 77 wherein the product comprises greater than 10% acylalkyphospholipids and/or plasmalogens.

97. The product of claim 77 wherein the product comprises greater than 25% acylalkyphospholipids and/or plasmalogens.

98. The product of claim 77 wherein the product contains more aminoethylphosphonate and/or other phosphonolipids than the feed material.

99. The product of claim 77 wherein the product comprises greater than 5% aminoethylphosphonate and/or other phosphonolipids.

100. The product of claim 77 wherein the product comprises greater than 10% aminoethylphosphonate and/or other phosphonolipids.

101. The product of claim 77 wherein the product comprises greater than 25% aminoethylphosphonate and/or other phosphonolipids.

Figure 1

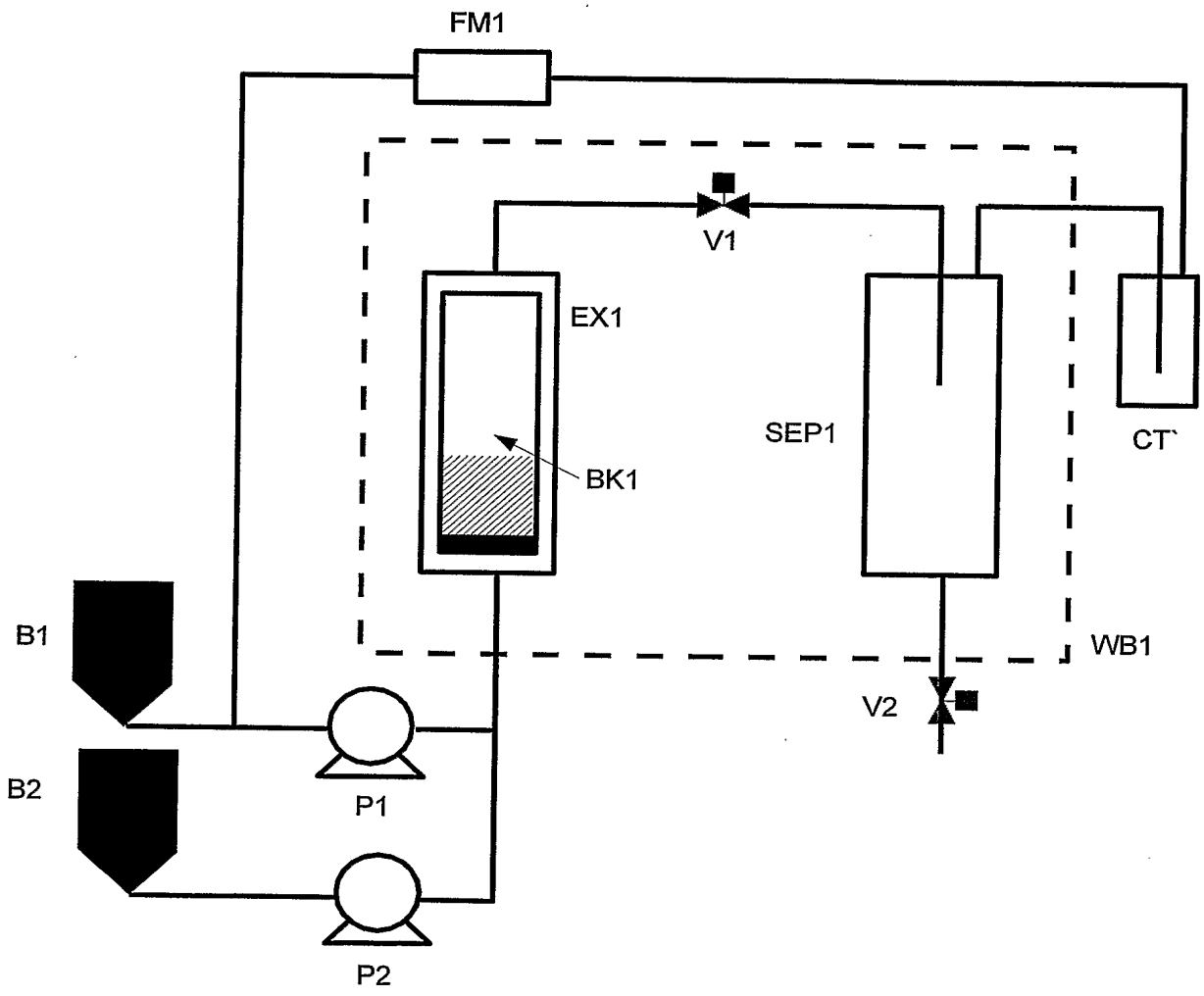


Figure 2

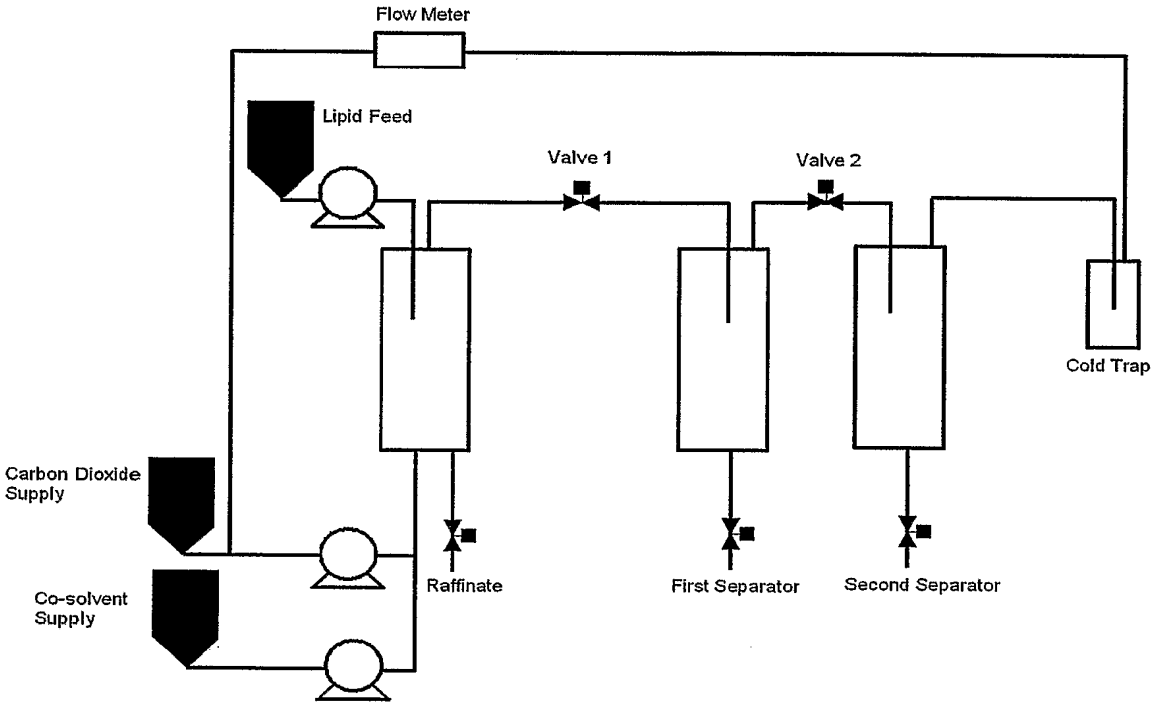


Figure 3

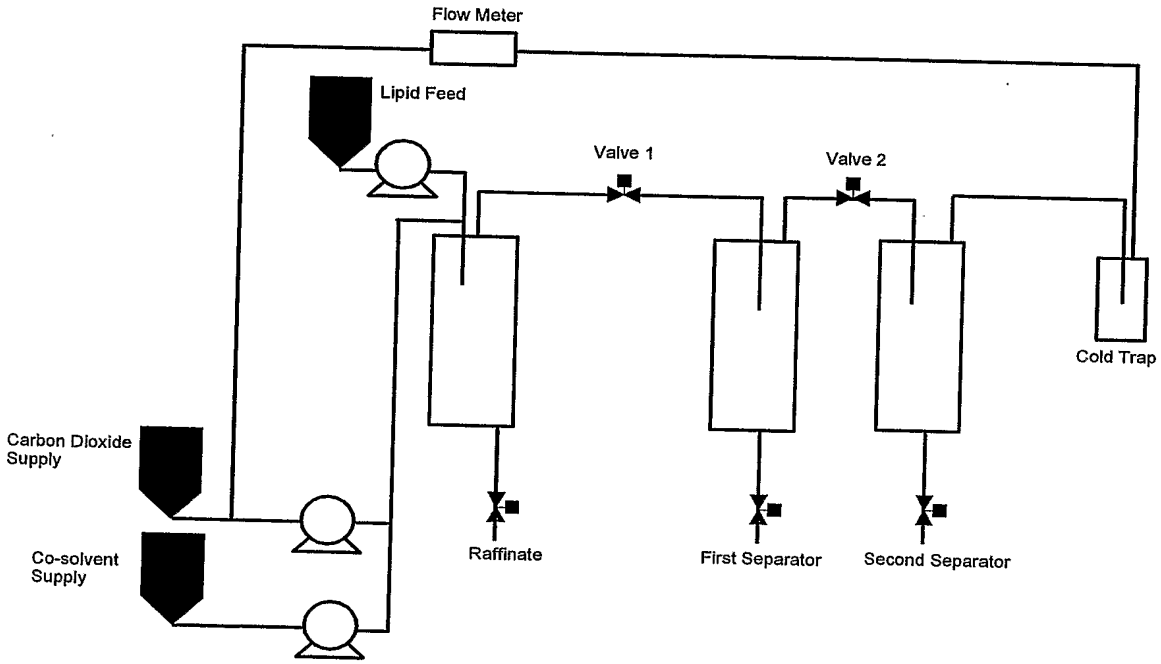
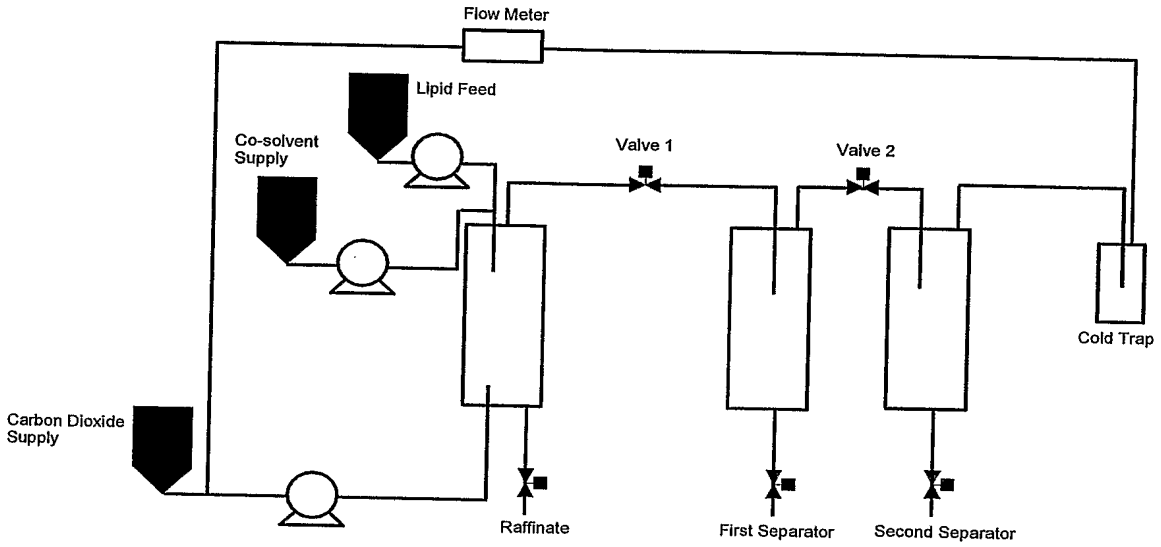


Figure 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2007/000087

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C11B 7/00 (2006.01) ***C11B 1/10*** (2006.01) ***A23L 1/48*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, JAPIO, FSTA, ESPACE, USPTO (phospholipid+, phosphatidyl?serine, carbon dioxide)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0170063 A1 (CHORDIA et al.) 4 August 2005 See entire document.	1-101
A	Derwent abstract accession no. 2000-500083/45, Class D23 E11, EP 1004245 A2 (KRUPP UHDE GMBH) 31 May 2000. See abstract.	1, 20
A	Derwent abstract accession no. 2003-117963/11, Class B04 D13, KR 2002064645 A (GOSHENCRITEC CO LTD) 9 August 2002. See abstract.	1, 20
A	Patent Abstracts of Japan, JP 03-133991 (GREEN CROSS CORP: THE CHLORINE ENG CORP LTD) 7 June 1991 See abstract.	1, 20

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

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Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ2007/000087

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	2005170063						
EP	1004245	AU	61700/99	CA	2288469	CN	1257660
		DE	19854807				
KR	2002064645						
JP	3133991						

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

(12) 特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局

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2008 年6 月19 日 (19.06.2008)

PCT

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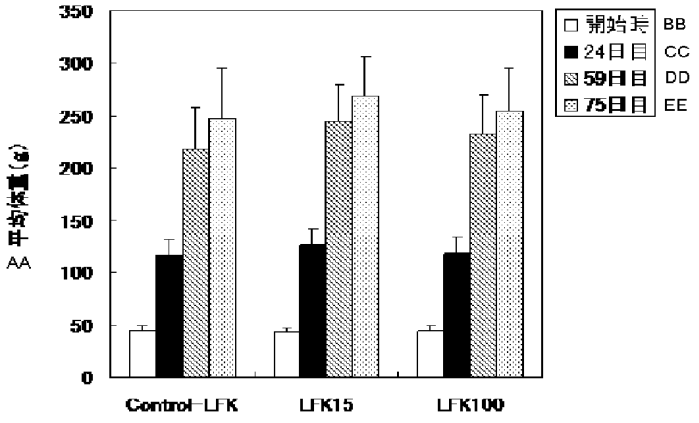
添付公開書類:
— 国際調査報告書

(54) **Title:** FEED USING PEELED KRILL AS THE STARTING MATERIAL AND METHOD OF PREVENTING DECREASE IN FISH GROWTH RATE BY USING THE SAME

(54) 発明の名称: 原料として外殻を除去したオキアミを用いた飼料及びそれを用いることによる魚類の成長率の低下の抑制方法



AA. AVERAGE BODY WEIGHT (g)
BB. STARTING
CC. DAY 24
DD. DAY 59
EE. DAY 75

(57) **Abstract:** It is intended to provide a substitute for fish meal that has been used as a protein source in feeds for cultured fishes. It is also intended to effectively utilize krill as a feed material which has been employed as a feed material only for limited purposes. Specifically, a feed using krill as a part or the whole of the protein source, characterized in that peeled krill are used as the krill. It is preferable that the fluorine content of the krill has been lowered to 250 mg/kg or less on the basis of dry weight by peeling. A method of preventing a decrease in the growth rate of fish by using krill as a starting material of a feed characterized in that peeled krill are employed as the protein source of the feed for cultured fish.

(57) 要約: 養魚用飼料のタンパク源である魚粉の代替物を提供するものであり、また、飼料原料としての利用が限定されていたオキアミの飼料原料としての活用を可能とするものである。具体的には、飼料のタンパク源の全量または一部としてオキアミを用いた飼料であって、オキアミとして外殻を除去したオキアミを用いたことを特徴とする飼料であり、外殻を除去したことによりフッ素含有量が２５０ｍｇ／ｋｇ乾燥重量以下に減少したものが好ましい。外殻を除去したオキアミを養魚用飼料のタンパク源として用いることを特徴とする、オキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法である。

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明 細 書

原料として外殻を除去したオキアミを用いた飼料及びそれを用いることによる魚類の成長率の低下の抑制方法

技術分野

[0001] 本発明は、飼料のタンパク原料である魚粉の代替物に関する。

背景技術

[0002] Food and Agriculture Organizationによれば、全世界における肉食性の養殖魚の生産量は1990年代に大きく増加し、この成長は今後も続くと予想されている。水産養殖業界で使用される飼料は通常イワシ、カタクチイワシ、アジ、マアジなどの多穫魚の魚粉及び魚油を主原料としている。しかし、これらの資源は利用しつくされており、かつ、生産量増加の見込みは少ない。これが魚類飼料の水産原料の代替物の研究に推進力を与えている。魚粉の代替としては水産物由来のタンパク質よりも安く入手しやすい植物由来のものがあるが、必須アミノ酸とミネラルが不十分であり、反栄養的因子や炭水化物複合物の存在などのためそれらの使用は限られている。畜肉ミール、骨粉、ブラッドミール、フェザーミールなどの動物由来タンパク質が魚粉の一部代替として用いられていたが、狂牛病、鳥インフルエンザのために養魚用飼料への使用は制限された。その結果、現在、魚粉の代替として用いられている動物性タンパク質は存在しない。

[0003] 水産資源には未利用あるいは低利用でありかつ大量のバイオマスがあるものがある。オキアミはそれらのうちもっとも期待できる資源のひとつである。オキアミは「euphausiids」に対して一般的に用いられている用語である。世界では、およそ85種類のオキアミが報告されている。特に南極オキアミ(Euphausia superba)は南洋の生態系において重要な種である。なぜならオキアミは鯨、あざらし、海鳥など多くの上位捕捉者の主要な食料であり、植物性プランクトンの主要な消費者である。オキアミは1960年代以降商業的に漁獲されてきており、今日では数カ国による活発な漁獲の対象となっている。南極オキアミの現在の資源量は5億トンと推定されている。2004年12月から2005年11月の漁獲量は約12万7千トンであった。Convention on the

Conservation of Antarctic Marine Living resourcesにより設定されている南極オキアミに対する漁獲制限量は音波調査によるバイオマスから推定している;南大西洋の漁獲制限量は2000年には400万トンであり、実際の漁獲量のおよそ40倍である。

大量のバイオマスの存在にもかかわらず、需要がないために現在の世界のオキアミの漁獲量は限られている。オキアミ製品のうち圧倒的に多いのは船上で凍結されたオキアミであり、ほとんどが水産養殖又は釣り用に使用されている。オキアミ、特に南極オキアミについてはヒトの食料に適した製品の開発にかなりの努力が払われてきたが、ヒトの食料用の市場サイズはいまだ小さい。

[0004] オキアミの飼料原料としての適性は1980年代のいくつかの論文の題材となっており、オキアミは魚類用飼料に用いることができると結論された(非特許文献1～3)が、それ以上研究は進まなかった。そのひとつの理由は、オキアミのフッ素含有量が高いこと(南極オキアミでは1000-2500mg/kg)及び欧州連合が飼料中のフッ素の最大量を150mg/kg dry feedと規定したことによる。

オキアミミールを含有する試験試料を給餌された魚Barramundi (Lates calcarifer) の幼生は成長性の減少と甲状腺ホルモン(T4)の減少を示したことが報告されている(非特許文献4)。サケ科魚類の飼料の原料の魚粉の一部または全部をオキアミミールで置換した試験が報告されている(非特許文献3)。多くの場合、飼料中の魚粉をすべてオキアミで代替すると成長率と飼料効率が低下し、魚粉の一部をオキアミで代替すると同等あるいは優れた結果が得られる(非特許文献5)。飼料中の魚粉の全量をオキアミで代替することは行われていない。

Julshamn ら(非特許文献6)は海水のアトランティックサーモン(Salmo salar)に市販の魚粉飼料の魚粉をオキアミミールで10－30％置換した飼料を12週間摂餌させる試験を行い、サケの飼料においては、魚粉の48％までオキアミミールで置換しても成長性や生存率に影響を与えないと報告している。

オキアミのフッ素が外殻に高濃度で存在することは知られている(非特許文献7)。

[0005] 非特許文献1:Aquaculture 24, p191-196 (1981), Grave, H., "Fluoride content ofsalmonids fed on Antarctic krill."

非特許文献2:Diet. Bull. Japan Soc. Sci. Fish 50, p815-820 (1984), Allahpichay, Ian

d Shimizu, C., "Supplemental effect of the whole body krill meal and thenon-muscle krill meal of Euphausia superba in fish diet."

非特許文献3:Aquaculture 70, p193-205 (1988), Storebakken, T., "Krill as apotenti al feed source for Salmonids."

非特許文献4:Aquaculture 257, p453-464 (2006), Nankervis, L. and Southgate, P., "An integrated assessment of gross marine protein sources used in formulatedmicrob ound diets for barramandi (Lates calcarifer) larvae."

非特許文献5:Proc. World Symp. Finfish Nutrition and Fishfeed Technology. Hambu rg, 20-23 June 1978. Vol. II. Heenemann, Berlin, p281-292 (1979), Koops et al., " Krillin trout diets."

非特許文献6:Aquaculture Nutr. 10, p9-13 (2004), Julshamn, K. et al., "Fluorideret ention of Antarctic salmon (Salmo salar) fed krill meal."

非特許文献7:J. Fish. Res. Bd. Can. 36, p1414-1416 (1979), Soevik, T. andBraekka n, O. R., "Fluoride in Antarctic krill (Euphausia superba) and Atlantickrill (Meganoc ytiphanes norvegica)."

発明の開示

発明が解決しようとする課題

[0006] 本発明は、今後不足が予想される、養魚用飼料のタンパク源である魚粉の代替物を提供することを課題とする。また、飼料原料としての利用が限定されていたオキアミの飼料原料としての活用を可能とすることを課題とする。

課題を解決するための手段

[0007] オキアミは魚類飼料に一定割合以上使用すると、魚類の成長率が低下することが知られている。発明者は、その原因がオキアミの高いフッ素含量によるのではないかと考え、本発明を完成させた。オキアミのフッ素含量が高いことからヒトへの影響を考慮して飼料への使用が制限されている。したがって、オキアミを一定以上飼料原料として用いることを誰も試みることがなかった。しかし、オキアミのうちフッ素含量が最も高い外殻を除去することによりオキアミのフッ素含量を1／4以下に減少させることができることを確認し、それを飼料に添加し飼育試験を行うことにより、飼料原料のタン

パク質として100％オキアミを使用しても魚類の成長率に悪影響を及ぼさないことを確認し、本発明を完成させた。

[0008] 本発明は、(1)～(6)の飼料を要旨とする。

(1)飼料のタンパク源の全量または一部としてオキアミを用いた飼料であって、オキアミとして外殻を除去したオキアミを用いたことを特徴とする飼料。

(2)飼料のタンパク源である魚粉の全量または一部をオキアミで代替した飼料であって、オキアミとして外殻を除去したオキアミを用いたことを特徴とする(1)の飼料。

(3)外殻を除去したオキアミが、オキアミを加熱乾燥し、得られたオキアミ乾燥物を裁断し、外殻等の軽量画分を風力分級機により除いて得られたものである(1)又は(2)の飼料。

(4)外殻を除去したオキアミが、外殻を除去したことによりフッ素含有量が250mg／kg乾燥重量以下に減少したものである(1)ないし(3)いずれかの飼料。

(5)外殻を除去したオキアミを飼料のタンパク源の50重量％以上用いることを特徴とする(1)ないし(4)いずれかの飼料。

(6)飼料が養魚用飼料である(1)ないし(5)いずれかの飼料。

[0009] 本発明は、(7)～(11)のオキアミを飼料に添加することによる魚類の成長率の低下を抑制する方法を要旨とする。

(7)外殻を除去したオキアミを養魚用飼料のタンパク源として用いることを特徴とする、オキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

(8)外殻を除去したオキアミを魚粉の代替として用いることを特徴とする、(7)のオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

(9)外殻を除去したオキアミが、オキアミを加熱乾燥し、得られたオキアミ乾燥物を裁断し、外殻等の軽量画分を風力分級機により除いて得られたものである(7)又は(8)のオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

(10)外殻を除去したオキアミが、外殻を除去したことによりフッ素含有量が250mg／kg乾燥重量以下に減少したものである(7)ないし(9)いずれかのオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

(11)外殻を除去したオキアミを飼料のタンパク源の50重量％以上用いることを特徴

とする(7)ないし(10)のオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

発明の効果

[0010] 本発明の飼料は、フッ素による悪影響を発現することなく、飼料のタンパク源として用いることができる。養魚用飼料として用いた場合、魚類の成長性に悪影響を与えることなく、魚粉を原料とする養魚用飼料と同等に用いることができる。従来、魚粉の一部の代替としてしか用いることができなかったオキアミを高い割合で、あるいは、全部の代替として用いることができる。

図面の簡単な説明

[0011] [図1]95日間の試験期間中の魚のフッ素取り込みと脊椎骨のフッ素濃度の関係を示した図である。魚のフッ素取り込みは、「各魚の飼料摂取量×飼料中のフッ素濃度」で計算した。

[図2]試験試料を95日間摂取したニジマスの肝臓切片。図の符号は表2と同じ。写真中の線分は20μm。

[図3]比較例1のニジマスの成長率を示す図である。

[図4]比較例2のブリの成長率を示す図である。

[図5]実施例3のブリの成長率を示す図である。

発明を実施するための最良の形態

[0012] 本発明において飼料とは、主に魚粉を原料とする水産養殖、畜産、養鶏、ペット用などの飼料である。通常、これらの飼料は魚粉等の動物質性飼料原料に、穀類、そうこう類、植物性油かす類、植物性蛋白質類、ビタミン類、ミネラル類、さらに色素等を加えてペレット、顆粒、粉末状等の製品とされる。本発明は、特に魚粉を多く用いる養魚用、甲殻類用の水産養殖用飼料に適している。養魚、甲殻類としてはマダイなどのタイ類、ニジマス、サケ、マス等のサケ類、ハマチ、シマアジ、マス、ヒラメ、ウナギ、エビ等の養殖対象魚が例示される。

本発明において使用するオキアミは、資源量が豊富な南極オキアミが好ましいが、その他のオキアミでも同様に使用できる。

従来、オキアミは外殻も含んだ全体をボイルして乾燥したものがオキアミミールと称

されて用いられてきた。

本発明において、外殻を除去したオキアミとは、オキアミの触角、顎脚、脚、頭部外殻、胴部外殻、尾部外殻部分を除去したものである。100%除去するに越したことはないが、実質的には、75%程度以上除去したものを使用することができる。フッ素含有量で表現すれば、外殻を除去したことによりフッ素含有量が250mg／kg以下に減少したものが好ましい。通常の魚粉でもフッ素含有量は100～200mg／kg程度含まれる。

外殻を除去する方法は、オキアミを煮熟、蒸煮、誘電加熱、マイクロ波等の方法により加熱し後、もしくは加熱を行わず直接、乾燥し、水分を15%以下にする。乾燥方法は熱風乾燥、真空凍結乾燥などが例示されるが、どんな方法でもよい。得られたオキアミ乾燥物を約5mm程度の断片に裁断し、風力分級機に供し、外殻等の軽量画分と主にオキアミ筋肉等の重量画分とに分級する。重量画分に外殻を除去したオキアミ画分を得ることができる。あるいは、オキアミの外殻をロール式むき身機等（特公昭57－1207、特公平7－40868等）で除去することもできる。

タンパク源の全量又は一部としてオキアミを用いるとは、通常飼料の原料として用いられる、魚粉（フィッシュミール）他の動物タンパク質、又は、大豆粕他の植物タンパク質等の合計重量の全部あるいは一部分をオキアミで置換する意味である。外殻を除去したオキアミを飼料のタンパク源の30重量%以上用いるとは、これらタンパク質の合計重量の30重量%以上を外殻を除いたオキアミで置換する意味である。

[0013] 以下に本発明の実施例を記載するが、本発明はこれらに何ら限定されるものではない。

実施例

[0014] ＜製造例1＞外殻を除去したオキアミミール（以下、低フッ素オキアミミール、又は、LFKとも表記する。）の製造

凍結した生の南極オキアミ（日本水産株式会社製）を室温で解凍し、95℃で10分間煮てから、連続ベルト式乾燥機(DC-200, Samson Co., Ltd., Kagawa, Japan)を用いて120℃（温風温度）でオキアミの水分量が10%以下になるまで乾燥した。乾燥したオキアミ全体をハンマーミル(D-3, Dalton

Co., Ltd, Tokyo, Japan)と5mmのメッシュのふるいを用いて粉砕した。粉砕したオキアミは風力分級機 (VS10, Hattori Seisakusho Co., Ltd., Kyoto, Japan)にかけ、低フッ素オキアミミール(LFK)と外殻とに分離した。

表1にオキアミ全体の乾燥物、LFKおよび外殻の組成を示した。外殻を除去することにより、粗タンパクはオキアミ全体の乾燥物では66.04%であるのに対し、LFKでは76.76%と約10%高くなった。除去した外殻の粗タンパクは56.74%であった。LFKの粗脂肪、灰分及びアスタキサンチンはオキアミ全体の乾燥物や除去した外殻よりも低かった。これはアスタキサンチンを含有するオキアミの脂肪組織が除去した外殻にしっかりと付着していたからである。

LFKのフッ素含有量は210mg/kgであり、オキアミ全体の乾燥物の870mg/kgと比較すると約1/4であった。除去した外殻には1800mg/kgのフッ素が含まれていた。オキアミ全体の乾燥物、LFK、及び除去した外殻の脂肪酸組成はほとんど同じであった。脂質種類のうち、トリアシルグリセロールは、LFKでは20.6%で、オキアミ全体の乾燥物では26.2%とLFKのほうが低く、リン脂質では、LFKでは77.3%、オキアミ全体の乾燥物では72.0%とLFKのほうが高かった。

[0015] [表1]

オキアミミールと低フッ素オキアミミールの比較(乾燥物ベース(重量%))

	オキアミ全体の乾燥物	低フッ素オキアミミール	除去した外殻
粗タンパク	66.04	76.76	56.74
粗脂肪	19.87	13.27	21.51
粗繊維	2.44	0.11	4.46
灰分	11.65	9.18	14.83
可溶性無窒素物	0	0.79	2.45
ｱｽﾀｷｻﾝﾁﾝ(mg/kg)	90	38	96
フッ素 (mg/kg)	870	210	1800
脂肪酸組成			
14:00	11.33	10.81	11.45
16:00	21.67	22.11	21.82
16:1n-7	8.07	7.73	7.78
18:1n-9	19.1	19.28	19.33
18:2n-6	1.39	1.48	1.5
18:3n-3	0.71	0.77	0.75
18:4n-3	2.38	2.24	2.54
20:1n-9	1.04	1	1.04
20:4n-3	0.36	0.37	0.36
20:4n-6	0.29	0.3	0.31
20:5n-3	14.21	14.31	13.55
22:5n-3	0.37	0.37	0.36
22:6n-3	7.12	8.11	6.87
脂質分類			
炭化水素	0	0	0
ワックスエステル	微量	微量	微量
ﾄﾘｱｼﾙｸﾞﾘｾﾛｰﾙ	26.2	20.6	26.1
遊離脂肪酸	1	1.4	1.3
ステロール	0.8	0.7	0.8
リン脂質	72	77.3	71.8

[0016] ＜実施例1＞低フッ素オキアミミール(LFK)を使用した飼料の製造

表2に示した配合で試験飼料を製造した。各飼料の組成を表2に示した。2軸エクストルーダ(α-50, Suehiro EPM Corporation, Mie, Japan)を用いて、LFKで魚粉の一定量を代替した5種類の飼料とコントロールとして、100%魚粉を用いた飼料を製造した。飼料の主要蛋白源としては、褐色魚粉を用い、魚粉のうち、7.69% (LFK7)、15.39% (LFK15)、30.77% (LFK30)、46.16% (LFK46)、100.00% (LFK100) をLFKで置換する配合とした。魚粉をLFKで代替する割合が増加すると、飼料中の粗タンパクは52.05% (Control)から54.18% (LFK100)と増加し、フッ素量は89mg/kg (Control)から220 mg/kg (LFK100)と増加した。しかし、灰分は12.38% (Control)から8.24% (LFK100)と減少した。精製魚油(日本水産株式会社製)を主脂質源として添加した。飼料の粗脂肪は16.51%から17.21%であった。

表3に試験飼料の必須アミノ酸含有量を示した。飼料中のLFKによる代替率が高まるほど、ヒスチジン以外の全アミノ酸が増加した。

表4に飼料の脂肪酸組成と脂質分類を示した。n-3系高度不飽和脂肪酸は総脂肪酸のうちの26.13%から30.45%であった。エイコサペンタエン酸 (EPA; 20:5 n-3)は飼料中のLFK割合の増加に伴い、コントロール8.92%からLFK100の12.42%と増加し、ドコサヘキサエン酸 (DHA; 22:6 n-3)はコントロール13.84% からLFK100の10.56%へと減少した。飼料中の脂質分類では、飼料中の魚粉がLFKで代替される率が高くなると、トリアシルグリセロールはコントロールの70.9%からLFK100の42.0%へと減少し、リン脂質 はコントロールの27.9%からLFK100の56.8%へと増加した。

[0017] [表2]

試験飼料の配合と化学組成（乾燥物ベース（重量%））

	飼料					
	コントロール	LFK7	LFK15	LMK30	LFK46	LFK100
褐色魚粉	57.98	53.52	49.06	40.14	31.22	0
低フッ素オキアミミール (LFK)	0	4.46	8.92	17.84	26.76	57.98
小麦粉 (Off grade)	11.6	11.6	11.6	11.6	11.6	11.6
脱脂大豆ミール	8.92	8.92	8.92	8.92	8.92	8.92
キャツサバ澱粉	7.14	7.14	7.14	7.14	7.14	7.14
ビタミンプレミックス	1.83	1.83	1.83	1.83	1.83	1.83
ミネラルプレミックス	1.83	1.83	1.83	1.83	1.83	1.83
飼料用魚油	10.7	10.7	10.7	10.7	10.7	10.7
合計	100	100	100	100	100	100
ＬＦＫによる魚粉の代替率（%）	0	7.69	15.39	30.77	46.16	100
粗タンパク	52.05	52.56	52.32	52.59	53.06	54.18
粗脂肪	17.21	16.51	16.98	17.14	17.19	17.02
粗繊維	0.42	0.63	0.63	0.53	0.53	0.64
灰分	12.38	12.23	11.81	11.22	10.55	8.24
可溶性無窒素物	17.94	18.08	18.35	18.62	18.67	19.91
アスタキサンチン (mg/kg)	ND	3.2	5.5	7.5	7.6	8.6
フッ素 (mg/kg)	89	100	110	130	150	220

Vitamin premix and mineral premix
according to National Research Council (NRC, 1993) recommendations.
ND: Not detectable (検出限界 0.5mg/kg)

[0018] [表3]

試験飼料の必須アミノ酸含有量 (g/100g diet, wet basis)

	Diets					
	コントロール	LFK7	LFK15	LFK30	LFK46	LFK100
アルギニン	2.774	2.843	2.945	2.922	2.925	3.231
ヒスチジン	1.308	1.279	1.279	1.101	1.133	1.013
イソロイシン	2.058	2.083	2.19	2.2	2.181	2.461
ロイシン	3.426	3.489	3.659	3.588	3.593	3.901
リジン	3.449	3.501	3.663	3.642	3.565	3.963
メチオニン	1.081	1.094	1.169	1.146	1.146	1.337
シスチン	0.218	0.25	0.257	0.244	0.261	0.236
フェニルアラニン	1.919	1.963	2.068	2.069	2.051	2.306
チロシン	1.284	1.33	1.414	1.444	1.429	1.584
スレオニン	1.783	1.801	1.876	1.793	1.816	1.849
バリン	2.359	2.37	2.45	2.347	2.377	2.431

[0019] [表4]

試験飼料の脂肪酸組成 (総脂肪酸中の重量%) 及び脂質分類 (総脂質中の重量%)

	Diets					
	コントロール	LFK7	LFK15	LFK30	LFK46	LFK100
14:00	4.75	5	4.51	5.83	5.14	6.69
16:00	14.41	15.01	12.63	16.36	13.3	15.06
16:1*n*-7	5.51	5.55	6.09	6.19	6.36	7.66
18:1*n*-9	15.59	15.73	16.48	17.37	16.55	19.85
18:2*n*-6	4.55	4.44	4.67	4.59	5.05	5.04
18:3*n*-3	1.16	1.14	1.24	1.11	1.3	1.2
18:4*n*-3	1.89	1.86	2.13	2.02	2.32	2.22
20:1*n*-9	6.34	6.13	5.93	5.92	4.76	3.53
20:4*n*-3	0.68	0.66	0.73	0.66	0.7	0.54
20:4*n*-6	0.95	0.92	0.99	0.88	0.91	0.63
20:5*n*-3 (EPA)	8.92	9.08	10.39	10.25	12.08	12.42
22:5*n*-3	1.6	1.54	1.67	1.47	1.51	1
22:6*n*-3 (DHA)	13.84	13.39	14.43	13.2	14.05	10.56
炭化水素	0	0	0	0	0	0
ワックスエステル	微量	微量	微量	微量	微量	微量
トリアシルグリセロール	70.9	67.1	62.5	60.5	60.3	42
遊離脂肪酸	0.7	0.8	1.1	1.2	1	1.2
ステロール	0.5	0	0	0	0.7	0
リン脂質	27.9	32.1	36.4	38.3	38	56.8

[0020] ＜実施例2＞

1．試験方法

95日間の給餌試験を高知大学農学部の淡水試験施設にて行った。試験魚として、愛媛県の藤岡養鱒場から入手したニジマスを用いた。試験開始前、魚には市販の飼料(初期餌料 D-2、日本水産株式会社製)を給餌し、無作為に2×100尾ずつの6群に分けた。各群の魚は約4．1gであった。全群とも屋内の200L円形ポリカーボネートの水槽に保持し、各水槽には100L/hの速度で井戸水を供給した。試験期間中の平

均水温は18.4±0.3℃であり、すべての水槽を曝気した。試験期間中(2005年10月－2006年1月)の日照時間は現地の自然条件のままである(33° 34' N, 133° 39' E)。

試験飼料として、実施例1で製造した6種類の飼料を用いた。試験飼料は人手により1日2回(9時と15時)飽食給餌し、飼料消費量を毎日記録した。

[0021] 試験開始時、摂餌開始後36、64、95日目に、各群の全魚の体重をフェノキシエタノール麻酔下で測定した。魚はサンプリング前24時間絶食させた。試験終了時に各水槽から15尾の魚を無作為にサンプリングした。それらの体重と摘出した肝臓の重量を測定し、肝重量比(HSI)を計算し、肝臓のパラフィン切片を調整した。皮を除いた背部の筋肉と脊椎骨を用いて化学組成とフッ素の解析を行った。脊椎骨は95℃で60分間煮た後、0.1%濃度のアルカラーゼ2.4L FG (Novozymes A/S, Bagsvaerd, Denmark)中、室温下で一晩撹拌した。骨を蒸留水で洗浄し、85℃で3時間乾燥させた。分析するまで、すべてのサンプルを－25℃で保存した。

[0022] 2. 分析方法

分析は財団法人日本食品分析センター、又は、日本水産株式会社食品分析センターにて行った。水分含量は105℃で2時間乾燥後の重量の減少から計算した。粗タンパク量はケールダール法(窒素係数6. 25)により測定した。粗脂肪量はジエチルエーテルで抽出後、重量測定法に測定した。灰分量は550℃で燃焼後測定した。可溶性無窒素物は次式により計算した:総湿重量－(水分含量＋粗タンパク量＋粗脂肪量＋粗繊維＋灰分量)。フッ素は、H2SiF6としてアリザリンコンプレキソン－ランタン試薬で発色させ、620nmの波長で吸光度を測定した。

アミノ酸組成は高速液体クロマトグラフとアミノ酸分析機 (L-8500, 日立 High-Technologies Corporation)を用いた常法であるニンヒドリン反応法により測定した。トリプトファンは測定しなかった。

総脂質はBligh and Dyerの変法(Bligh and Dyer, 1959)により抽出した。脂肪酸組成はDB-WAX fused silica capillary column付きのガスクロマトグラフ(HP-6800, Hewlett-Packard, Yokogawa Electric, Tokyo, Japan)を用いて測定した。総脂質の組成は薄層クロマトグラフと水素イオン化検出器 (Iatroscan TH-10 TLC-FLD Analyzer, Iatron laboratories Inc., Tokyo,

Japan)を用いて解析した。

試験終了後、各群から15尾の魚を無作為にサンプリングし、肝臓の組織標本を切り出し、中性10%ホルマリンで固定し、パラフィン包埋した。4μmの切片とし、ヘマトキシリン及びエオジンで染色した。

[0023] 3. 統計解析

各水槽の魚体重はPearsonのカイ二乗検定により正規分布していることを確認した。各水槽の魚体重の差を検定するために一元配置分散分析(ANOVA)を行った。群間の差を比較するときはデータを、重複した水槽を偶然要因とする多重比較検定(Scheffe's F)を用いて検定した。摂餌量(FI)の差、特殊成長率(SGR)、飼料効率(FE)、肝臓重量比(HSI)は一元配置分散分析を用いて検定した。生残率はLogrank検定を用いて比較した。各魚のフッ素摂取量と脊椎骨中のフッ素濃度の相関直線は、マイクロソフトエクセル(Microsoft Cooperation, Redmond, WA)を用いて、作図した。すべての統計的データ処理はマッキントッシュ用Statcel™ (OMS-Publishing, Saitama, Japan)を用いて行った。

P<0.05を有意差ありとした。

[0024] 4. 結果

(1)成長性

表5に6種の試験飼料を摂餌した魚の成長性を示す。0、36、64及び95日目に各群の体重を測定したが、どの中間時点においても平均体重から有意差はなかった(P<0.05)。95日目の成長性についてもいずれの試験群のFI, SGR, FE, HSI及び生存率においても顕著な差は認められなかった(P<0.05)。表6は95日目の背部の筋肉の化学組成を示す。コントロール群と各試験群間に、水分量(75.5-76.0%)、粗タンパク(20.1-20.9%)、粗脂肪(2.5-2.9%)、灰分(1.4-2.1%)においても顕著な差は認められなかった。

[0025] [表5]

	試験飼料					
	コントロール	LFK7	LFK15	LFK30	LFK46	LFK100
試験前体重 (g)	4.19±1.37[a]	4.00±1.27[a]	4.20±1.44[a]	4.04±1.25[a]	4.26±1.24[a]	4.10±1.23[a]
36日目の体重 (g)	18.35±6.72[a]	17.98±6.36[a]	17.83±6.30[a]	17.94±5.74[a]	18.86±6.14[a]	17.27±5.74[a]
64日目の体重 (g)	39.97±13.86[a]	41.10±13.68[a]	41.34±14.37[a]	40.01±12.42[a]	41.95±13.53[a]	39.17±12.20[a]
95日目の最終体重 (g)	73.94±22.49[a]	72.75±21.21[a]	72.64±25.37[a]	72.79±20.83[a]	73.31±22.76[a]	72.90±20.31[a]
飼料摂取量 (FI) (g /fish)	64.35±0.60[a]	62.91±1.01[a]	62.77±1.43[a]	64.50±3.88[a]	61.10±1.58[a]	64.35±0.06[a]
比増重速度(SGR) (%)	3.06±0.06[a]	3.01±0.06[a]	2.99±0.01[a]	3.06±0.03[a]	3.01±0.02[a]	3.01±0.00[a]
飼料効率 (FE)	0.87±0.03[a]	0.87±0.02[a]	0.87±0.02[a]	0.86±0.03[a]	0.88±0.02[a]	0.86±0.01[a]
肝臓重量比(HSI)	0.90±0.15[a]	0.95±0.11[a]	0.88±0.13[a]	0.87±0.11[a]	0.97±0.14[a]	0.93±0.10[a]
生残率 (%)	98.50±0.71[a]	99.50±0.71[a]	97.50±0.71[a]	98.00±0.00[a]	97.00±1.41[a]	99.50±0.71[a]

飼料摂取量(FI) = 乾飼料摂取量/魚数
比増重速度(SGR) = [ln(最終魚体重)-ln(試験前魚体重)]/日数×100
飼料効率(FE) = 湿増加体重量/乾飼料摂取量
肝臓重量比(HSI) = 湿肝臓重量/湿体重×100
数値は平均値±標準偏差
同列において同じ文字がついていない数値間には有意差あり ($P<0.05$)

[0026] (2)フッ素濃度

95日目の背部の筋肉中及び脊椎骨のフッ素濃度を解析した(表6)。LFK100群を除く各試験群の背部の筋肉中のフッ素濃度は検出限界(1mg/kg)以下であり、LFK100群を除く各試験群の脊椎骨中のフッ素濃度は340mg/kg～420mg/kgであった。LFK100群の背部の筋肉及び脊椎骨のフッ素濃度はそれぞれ1mg/kg、1800mg/kgであり、コントロール群の脊椎骨中では、他の群より低く220mg/kgであった。

図1には、総フッ素摂取量と脊椎骨中のフッ素濃度間の相関直線を示す。フッ素の摂取量と濃度は正の相関を示した。

(3)組織学的研究

肝臓組織は6つの試験群においてほとんど同じであり、組織病理学的変化は認められなかった(図2)。これは、飼料由来のフッ素が肝臓組織に影響を与えなかったことを示唆するものである。

[0027] [表6]

試験終了時の背部筋肉の化学組成(%)及び背部筋肉と脊椎骨のフッ素濃度(mg/kg, dry basis)

	試験飼料					
	コントロール	LFK7	LFK15	LFK30	LFK46	LFK100
水分量	75.5[a]	75.8[a]	75.5[a]	75.6[a]	75.5[a]	76.0[a]
粗タンパク	20.4[a]	20.6[a]	20.6[a]	20.9[a]	20.8[a]	20.1[a]
粗脂肪	2.9[a]	2.5[a]	2.5[a]	2.6[a]	2.7[a]	2.5[a]
灰分	2.1[a]	2.1[a]	1.7[a]	2.0[a]	1.9[a]	1.4[a]
	フッ素濃度					
背部筋肉	ND	ND	ND	ND	ND	1
脊椎骨	220[a]	420[b]	340[b]	380[b]	350[b]	1800[c]

数値は平均値 (n = two dietary groups, each containing pooled sample of 15 individuals)
同列において同じ文字がついていない数値間には有意差あり(P<0.05)
フッ素分析方法の検出限界は1 mg/kg
ND：検出せず

[0028] ＜比較例1＞

淡水中のニジマスに魚粉を主原料とする飼料の魚粉を0%、7%、15%、30%オキアミミール(外殻を含む従来のもの)で置換した飼料を92日間摂餌させる試験を行った。これらの飼料のフッ素濃度はそれぞれ105、184、238、444mg/kgであった。試験終了時、各試験群の背部の筋肉のフッ素濃度は検出限界(1mg/kg)以下であったが、脊椎骨ではオキアミミールの添加量の増加に伴って490mg/kg ～2400mg/kgに増加し、成長率(図3)も低下した。

[0029] ＜比較例2＞

海水中のブリに魚粉を主原料とする飼料の魚粉を0%、15%、100%オキアミミール(外殻を含む従来のもの)で置換した飼料を95日間摂餌させる試験を行った。ブリでは、15%置換では成長率に影響は認められなかったが、100%置換した飼料では大幅な成長率の低下が認められた(図4)。

[0030] ＜実施例3＞

一方、海水中のブリに対し、魚粉を主原料とする飼料中の魚粉を0%、15%、そして100%低フッ素オキアミミール(LFK)で置換した飼料を75日間説示させる試験を行った。この結果、いずれの試験区でも成長に差が見られず、オキアミ外殻を除去することにより得られた低フッ素オキアミミール(LFK)と魚粉は飼料原料として同等の性能を有することが判った(図5)。

産業上の利用可能性

[0031] 本発明により、魚粉の代替タンパク源として制限なく使用することができる、新しい

動物性タンパク源を提供することができる。従来オキアミミールを飼料に一定割合以上添加すると、魚類の成長率の低下が認められたが、本発明によりオキアミを添加しても成長率を低下させない方法を提供することができる。

請求の範囲

[1] 飼料のタンパク源の全量または一部としてオキアミを用いた飼料であって、オキアミとして外殻を除去したオキアミを用いたことを特徴とする飼料。

[2] 飼料のタンパク源である魚粉の全量または一部をオキアミで代替した飼料であって、オキアミとして外殻を除去したオキアミを用いたことを特徴とする請求項1の飼料。

[3] 外殻を除去したオキアミが、オキアミを加熱乾燥し、得られたオキアミ乾燥物を裁断し、外殻等の軽量画分を風力分級機により除いて得られたものである請求項1又は2の飼料。

[4] 外殻を除去したオキアミが、外殻を除去したことによりフッ素含有量が250mg／kg乾燥重量以下に減少したものである請求項1、2又は3の飼料。

[5] 外殻を除去したオキアミを飼料のタンパク源の50重量%以上用いることを特徴とする請求項1ないし4いずれかの飼料。

[6] 飼料が養魚用飼料である請求項1ないし5いずれかの飼料。

[7] 外殻を除去したオキアミを養魚用飼料のタンパク源として用いることを特徴とする、オキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

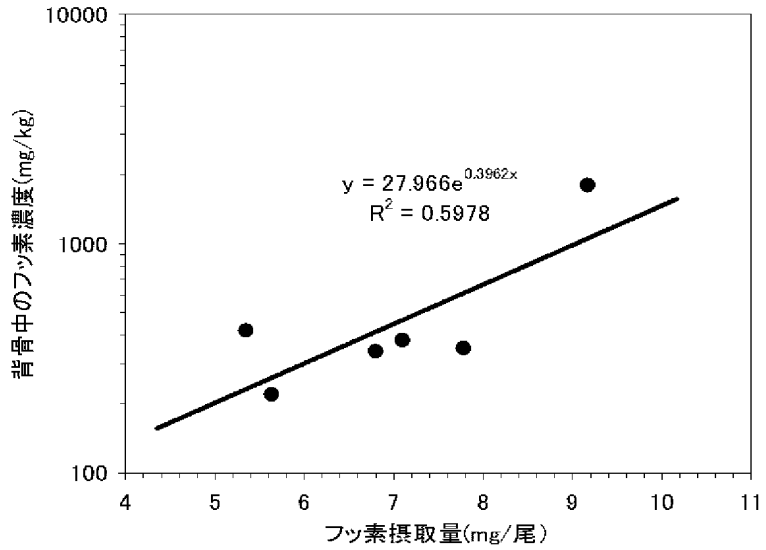
[8] 外殻を除去したオキアミを魚粉の代替として用いることを特徴とする、請求項7のオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

[9] 外殻を除去したオキアミが、オキアミを加熱乾燥し、得られたオキアミ乾燥物を裁断し、外殻等の軽量画分を風力分級機により除いて得られたものである請求項7又は8のオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

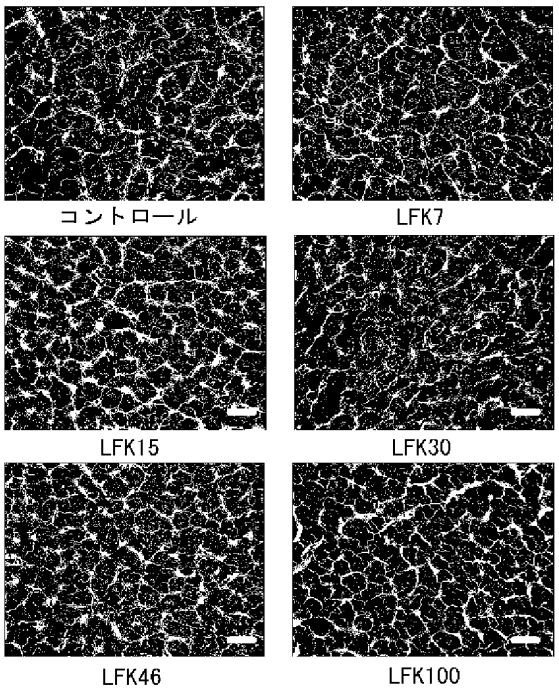
[10] 外殻を除去したオキアミが、外殻を除去したことによりフッ素含有量が250mg／kg乾燥重量以下に減少したものである請求項7、8又は9の飼料。

[11] 外殻を除去したオキアミを飼料のタンパク源の50重量%以上用いることを特徴とする請求項7ないし10いずれかのオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

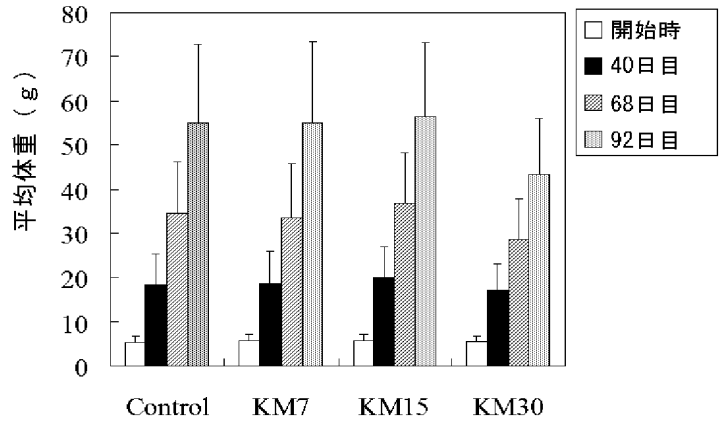
[図1]


10000
1000
100
背骨中のフッ素濃度(mg/kg)
y = 27.966e^0.3962x
R^2 = 0.5978
4
5
6
7
8
9
10
11
フッ素摂取量(mg/尾)

[図2]


コントロール
LFK7
LFK15
LFK30
LFK46
LFK100

[図3]


80
70
60
50
40
30
20
10
0
平均体重（g）
Control
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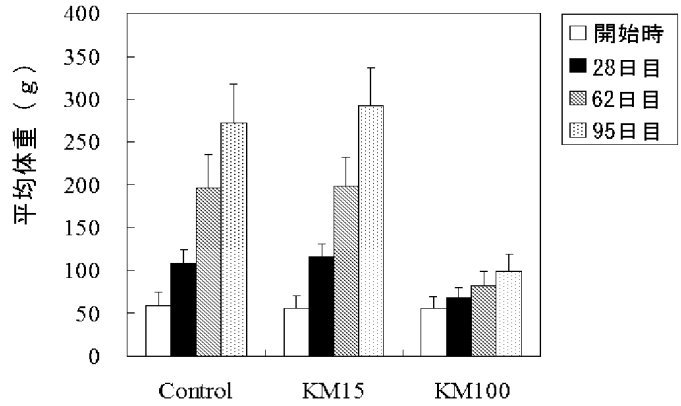
KM15
KM30
開始時
40日目
68日目
92日目

[図4]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350
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250
200
150
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50
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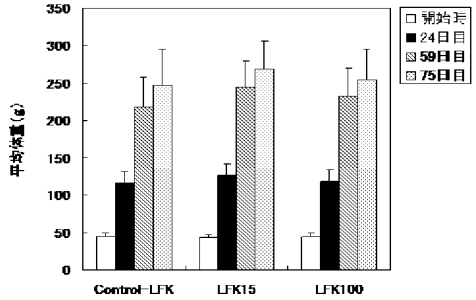
平均体重（g）
Control
KM15
KM100
開始時
28日目
62日目
95日目

[図5]


350
300
250
200
150
100
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0
Control-LFK
LFK15
LFK100
24日目
59日目
75日目

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2007/073669

A. CLASSIFICATION OF SUBJECT MATTER
A23K1/10(2006.01)i, *A23K1/18*(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K1/00-3/04, A23L1/33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2008
Kokai Jitsuyo Shinan Koho 1971-2008 Toroku Jitsuyo Shinan Koho 1994-2008

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	JP 61-274653 A (Nippon Nosan Kogyo Kabushiki Kaisha), 04 December, 1986 (04.12.86), Full text (Family: none)	1-8,10,11 9
A	JP 8-322474 A (Mitsuyoshi TOKUMOTO), 10 December, 1996 (10.12.96), Full text (Family: none)	1-11
A	JP 56-64767 A (Alfa-Laval AB.), 02 June, 1981 (02.06.81), Full text & DE 3038190 A & NO 803050 A & SE 7908433 A	1-11

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February, 2008 (19.02.08)	26 February, 2008 (26.02.08)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2007/073669

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 3-240448 A (Korea Food Research Institute), 25 October, 1991 (25.10.91), Full text & GB 2240786 A & KR 9201478 B	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2007/073669

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

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

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

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

Although the matter common to the inventions according to claims 1 to 11 resides in "using peeled krill as the protein source in a feed", this matter is not novel because of having been disclosed by document JP 61-274653 A (Nippon Nosan Kogyo Kabushiki Kaisha), 4 December, 1986 (04.12.86) and, therefore, cannot be recognized as "a special technical feature" in the meaning within the second sentence of PCT Rule 13.2., Such being the case, it does not appear that there is a technical relationship among the inventions according to claims 1 to 11 involving one or more of the same or corresponding special technical features and, therefore, these inventions are not so linked as to form a single general inventive concept.

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

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

the

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２００７／０７３６６９

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl.　A23K1/10(2006.01)i, A23K1/18(2006.01)i

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　A23K1/00-3/04,A23L1/33

最小限資料以外の資料で調査を行った分野に含まれるもの

日本国実用新案公報	１９２２－１９９６年
日本国公開実用新案公報	１９７１－２００８年
日本国実用新案登録公報	１９９６－２００８年
日本国登録実用新案公報	１９９４－２００８年

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

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X A	JP 61-274653 A（日本農産工業株式会社）1986.12.04, 全文（ファミリーなし）	1-8,10,11 9
A	JP 8-322474 A（徳元　光義）1996.12.10, 全文（ファミリーなし）	1-11
A	JP 56-64767 A（アルファーラヴアル・アクツイエボラーグ）1981.06.02, 全文 & DE 3038190 A & NO 803050 A & SE 7908433 A	1-11

☑　Ｃ欄の続きにも文献が列挙されている。

☐　パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「Ｌ」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「Ｏ」口頭による開示、使用、展示等に言及する文献
「Ｐ」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「Ｔ」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「Ｘ」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Ｙ」特に関連のある文献であって、当該文献と他の１以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「＆」同一パテントファミリー文献

国際調査を完了した日 １９．０２．２００８	国際調査報告の発送日 ２６．０２．２００８
国際調査機関の名称及びあて先 日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	特許庁審査官（権限のある職員）　２Ｂ　３３０１ 木村　隆一 電話番号　０３－３５８１－１１０１　内線　３２３７

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（２００７年４月）

Ｃ（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
A	JP 3-240448 A (財団法人韓国食品開発研究院) 1991.10.25, 全文 & GB 2240786 A & KR 9201478 B	1-11

第Ⅱ欄　請求の範囲の一部の調査ができないときの意見（第１ページの２の続き）

法第8条第3項（ＰＣＴ17条(2)(a)）の規定により、この国際調査報告は次の理由により請求の範囲の一部について作成しなかった。

１．☐ 請求の範囲＿＿＿＿＿＿＿＿＿＿は、この国際調査機関が調査をすることを要しない対象に係るものである。
つまり、

２．☐ 請求の範囲＿＿＿＿＿＿＿＿＿＿は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、

３．☐ 請求の範囲＿＿＿＿＿＿＿＿＿＿は、従属請求の範囲であってＰＣＴ規則6.4(a)の第２文及び第3文の規定に従って記載されていない。

第Ⅲ欄　発明の単一性が欠如しているときの意見（第１ページの３の続き）

次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。

請求の範囲１－１１に係る発明に共通する事項は、「外殻を除去したオキアミを飼料のタンパク源として用いる」という点であるが、かかる事項は、文献ＪＰ６１－２７４６５３　Ａ（日本農産工業株式会社）１９８６．１２．４に開示されているように新規なものではなく、ＰＣＴ規則１３．２の第２文でいう「特別な技術的特徴」とは認められない。よって、請求の範囲１－１１に係る発明は、一又は二以上の同一又は対応する特別な技術的特徴を含む技術的な関係になく、単一の一般的発明概念を形成するように連関していない。

１．☐ 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な請求の範囲について作成した。

２．☑ 追加調査手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、追加調査手数料の納付を求めなかった。

３．☐ 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の納付のあった次の請求の範囲のみについて作成した。

４．☐ 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に記載されている発明に係る次の請求の範囲について作成した。

追加調査手数料の異議の申立てに関する注意

☐ 追加調査手数料及び、該当する場合には、異議申立手数料の納付と共に、出願人から異議申立てがあった。

☐ 追加調査手数料の納付と共に出願人から異議申立てがあったが、異議申立手数料が納付命令書に示した期間内に支払われなかった。

☐ 追加調査手数料の納付はあったが、異議申立てはなかった。

Electronic Acknowledgement Receipt

EFS ID:	20979869
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Vickie Hoeft
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON
Receipt Date:	17-DEC-2014
Filing Date:	06-SEP-2013
Time Stamp:	17:31:40
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	14409US6IDSLetter121614.pdf	80636 ebf74217f48117e5994fade048366e078f497984	no	1

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	14409_US6_IDs.pdf	614322 7f7ae4592ad8a5a6f57c7ac87dca27581e40b518	no	7
Warnings:					
Information:					
3	Foreign Reference	CL_40348_App102_1995_patente_EnglishTrans.pdf	1977383 885ace055996975c639f26eb30f3c3bb2d3e186f	no	76
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
4	Foreign Reference	WO001031A1.pdf	2443714 59cc3f6ddbbe4d8b438fd9818e42a988518a175f	no	20
Warnings:					
Information:					
5	Foreign Reference	WO010960A1.pdf	4457079 73fa13a7c452be58aa0da6d7f5427d57c0a367bb	no	36
Warnings:					
Information:					
6	Foreign Reference	WO1997038585A1.pdf	499494 c70abd9354e0fd615f0a0a3c64b51f40a2ec9e0e	no	19
Warnings:					
Information:					
7	Foreign Reference	WO1998034498A1.pdf	1088473 d878be3a1f2ad4cf4828d431c7723b748213579d	no	59
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Information:					
8	Foreign Reference	WO1999039589A1.pdf	1149668 9bf1ac2abcbabde04358ac34952ac43114217310	no	59
Warnings:					
Information:					
9	Foreign Reference	WO2006111633A3.pdf	147703 064a2f4ffc7be7d0c1769cd6e56deb9305aaa75c	no	7
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10	Foreign Reference	WO2007123424A1.pdf	1133186 a072a421fcfc9ed84d7ce845e19405dd1991668	no	42

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Information:					
11	Foreign Reference	WO2008072563A1.pdf	386379 b480125948fca9fe6a8cdde7db2edfc8f65379cf	no	25
Warnings:					
Information:					
12	Other Reference-Patent/App/Search documents	14409EP1PCT_NoticeofOppositionFiled02-14-2014.pdf	15222775 47f9023abc353c46c7fb262fbe1bb27220623db3	no	131
Warnings:					
Information:					
13	Non Patent Literature	BrzustowiczBiochemistry2002.pdf	3329266 ffe2d2067245992f4b093f68af56161ef7be2aaf	no	13
Warnings:					
Information:					
14	Non Patent Literature	Jong_Ho_Lee_D1_cute.pdf	1105425 70c8335c47f80c2a45355281e5b4f3eb35b883dc	no	8
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Information:					
15	Non Patent Literature	Ki_woong_Cho_D2_cute.pdf	1245361 3d1649e40ba0f981b85a2ebadfd531f03374e700	no	8
Warnings:					
Information:					
16	Non Patent Literature	HvattumJournalofChromatography2000.pdf	1888912 1786ba65bcbbe031cfc31aa3934eb7f17f5789c4	no	15
Warnings:					
Information:					
17	Non Patent Literature	IGARASHI2001_English.pdf	429031 53b1ca1ba56a69d280d913a69ed5bde80dc42a85	no	12
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Information:					
18	Non Patent Literature	TOCHIZAWAJpnOilChemSoc1997.pdf	609363 76bb7f5bd1ae2944be3f540162b569131cdc7f9e	no	9
Warnings:					
Information:					
19	Non Patent Literature	Zerouga1995.pdf	1057697 e7fb4e1e888a02b223d6e1604ce2b2b6304f607e	no	9

Warnings:					
Information:					
20	Non Patent Literature	Eung_Ho_Lee_D8cute.pdf	1465563 93e7e748bd7215dca4c38f9dfd4246f48a6d7a39	no	10
Warnings:					
Information:					
21	Non Patent Literature	Hyun_ku_Kim_D5_cute.pdf	910799 69dd8dca935835d6eb6f6b5ab3cb59204f3877de	no	6
Warnings:					
Information:					
22	Non Patent Literature	Mi_Yae_Shon_D3_cute.pdf	571990 593230ffc0d00efdc719f755fa8450b73e362840	no	6
Warnings:					
Information:					
23	Other Reference-Patent/App/Search documents	SummonsMaterials_EP08718910_6.pdf	428722 ee9960446697a4c01871a6dd2d6fb727f7989b39	no	12
Warnings:					
Information:					
		Total Files Size (in bytes):	42242941		

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bruheim et al.	Group No.:	1651
Serial No.:	14/020,162	Examiner:	D.K. Ware
Filed:	06 September 2013		
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

INFORMATION DISCLOSURE STATEMENT LETTER

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

Sir or Madam:

The citations listed in the attached **IDS Form SB08A** may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97.

Applicants wish to bring to the Examiner's attention that we are not providing copies of US Patents as instructed under 37 CFR 1.98(a)(2). The Examiner is requested to make these citations of official record in this application.

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

The Commissioner is hereby authorized to charge any required fees or credit any overpayments to Attorney Deposit Account No.: **50-4302**, referencing Attorney Docket No.: AKBM-14409/US-6/CON.

Dated: December 16, 2014

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PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-14409/US-6/CON

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	2652235		1953-09-15	Samuelsen	
	2	5006281		1991-04-09	Rubin et al.	
	3	4251557		1981-02-17	Shimose et al.	
	4	4505936		1985-03-19	Meyers et al.	
	5	6214396		2001-04-10	Barrier	
	6	4036993		1977-07-19	Ikeda	
	7	6346276		2002-02-12	Tanouchi et al.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-14409/US-6/CON

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	1098900	CA		1981-04-07	Inst. Elementoorganicheskikh So, et al.		☐

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	1	VALERI, D., et al., "Visocities of Fatty acids, triglycerides and their binary mixtures," JAOCS 74 (1997) pp. 1221-1226	☐
	2	CRC 2013-2014, 94th ed., pp. 6-231-6-235	☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14020162
	Filing Date	2013-09-06
	First Named Inventor	Bruheim
	Art Unit	1651
	Examiner Name	D.K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-14409/US-6/CON

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☒ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2014-06-12
Name/Print	J. Mitchell Jones	Registration Number	44174

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bruheim et al.	Group No.:	1651
Serial No.:	14/020,162	Examiner:	D.K. Ware
Filed:	06 September 2013		
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

INFORMATION DISCLOSURE STATEMENT LETTER

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

Sir or Madam:

The citations listed in the attached **IDS Form SB08A** may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97.

Applicants wish to bring to the Examiner's attention that we are not providing copies of US Patents as instructed under 37 CFR 1.98(a)(2). The Examiner is requested to make these citations of official record in this application.

CERTIFICATION STATEMENT

Applicants wish to bring to the Examiner's attention that the references supplied in this IDS are from a May 23, 2014 AU Examination Report from related AU Patent Application No. 2013202260. Also, the VALERI and CRC references are from the March 26, 2014 Office Action from corresponding U.S. Patent Application No. 12/711,553. This IDS is filed within three months of the mailing of the Search Report and U.S. Office Action; therefore, applicants believe that no fees are due.

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

The Commissioner is hereby authorized to charge any required fees or credit any overpayments to Attorney Deposit Account No.: **50-4302**, referencing Attorney Docket No.: AKBM-14409/US-6/CON.

Dated: June 12, 2014

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Electronic Acknowledgement Receipt

EFS ID:	19290807
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Amanda Jones
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON
Receipt Date:	12-JUN-2014
Filing Date:	06-SEP-2013
Time Stamp:	17:14:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	VALERI.pdf	360428 0fd6bc3596f268ee25b716c9ad89f2934bab79ec	no	6

Warnings:	
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2	Non Patent Literature	CRC.pdf	261666 b0e6f92385faf3c61201f41ac79428bcff150b56	no	5
Warnings:					
Information:					
3	Foreign Reference	CA1098900.pdf	445086 f00e547133aaaf3cc4adea4f3843b70636d720ab	no	11
Warnings:					
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4	Information Disclosure Statement (IDS) Form (SB08)	30102US4_IDS_June2014.pdf	612659 fcceecab056c51f7ebc961c7824164e9c2e902d4	no	5
Warnings:					
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5	Transmittal Letter	14409US6_IDSLetterJune2014.pdf	81886 a95a1822ce6a85070867cc2596f512e3051f93d1	no	1
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

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New International Application Filed with the USPTO as a Receiving Office

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

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(52) CLASS 260-129
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(51) INT. CL.[2] C07G 7/00, C08B 37/08

(19) (CA) **CANADIAN PATENT** (12)

(54) METHOD FOR THE PROCESSING OF KRILL TO PRODUCE PROTEIN, LIPIDS AND CHITIN

(72) Rogozhin, Sergei V.;
Vainerman, Efim S.;
Burmistrova, Ljubov M.;
Davidovich, Jury A.;
Ryashentsev, Vladimir J.;
Kulakova, Valentina K.;
Lagunov, Lev L.;
Bykov, Vladimir P.,
USSR

(73) Granted to Institut Elementoorganicheskikh Soedineny Akademii Nauk SSSR, USSR Vsesojuzny Nauchno-Issledovatelsky Institut Rybnogo Khozyaistva I Okeanografii, VNIRO, USSR

(21) APPLICATION No. 293,095

(22) FILED 771214

No. OF CLAIMS 3 - NO DRAWING

X93095

1098900 ABSTRACT OF THE DISCLOSURE APR 7 1981

A1

The method for the processing of ~~kriol~~ krill to produce protein, lipids and chitin comprises emulsification of lipids by intensively stirring ~~kriol~~ krill in an aqueous medium. The resultant emulsion of lipids is separated from the ~~kriol~~ krill mass and from the ~~kriol~~ krill mass proteins are extracted at a pH of 10 to 12. The alkaline extract of proteins is separated from chitin integuments and protein is separated therefrom.

METHOD FOR THE PROCESSING OF KRILL TO PRODUCE PROTEIN, LIPIDS AND CHITIN

The present invention relates to methods for the processing of krill to produce protein, lipids and chitin. Krill is a prospective source of food protein and other practically useful products such as chitin and lipids which find wide application in different branches of the national economy -- the food industry, textile and paint and varnish industry, in agriculture and medicine.

Known in the art is a method for the production of a proteinaceous nutritive substance from krill residing in comminuting and pressing fresh or frozen and then defrosted krill. The liquid separated during pressing is heated for 10 to 15 minutes at a temperature of 90 to 95°C for coagulation of proteins contained therein. The proteinaceous coagulate is separated from the broth by filtration or centrifugation to produce a mass which is used in the USSR under a trade name of the Okean protein paste.

A disadvantage of said method for the processing of krill is loss of nutritive substances, particularly protein, and an insufficiently full utilization of other components of krill. The broth containing a considerable amount of nutritive substances is not processed and is poured off. The yield of protein is 35 to 40%. It should be pointed out that the Okean paste is a perishable product and should be stored only when frozen at a temperature not exceeding -18°C for not more than 12 to 14 months. The thermally denatured protein contained in the Okean paste possesses low functional properties (foam-forming and gel-forming properties, a water-holding capacity, etc.) which makes its processing and use difficult. The cake formed after pressing comprising a portion of the starting

proteins, lipids and chitin integuments can at present be processed and used only as feed meal.

Known in the art is a method for the production of a protein concentrate from frozen krill kept at a temperature of -20°C comprising defrostation, comminution of krill, extraction with isopropanol with subsequent removal of the solvent, and drying under vacuum at 70°C.

Using the present method a proteinaceous concentrate is produced with a content of protein of 710 to 775%, lipids of 0.3%, and chitin of 5.8 to 6.4% (as calculated for dry substance). Said method has the following disadvantages. The use of organic solvent makes the production more difficult. In addition, the solvent itself and the process for the removal thereof may deteriorate the quality of the protein. The proteinaceous concentrate has a comparatively low content of protein and a high chitin content.

It is an object of the present invention to develop such a method for the processing of krill which would make it possible to produce protein, lipids and chitin with a high yield and quality.

The method for the processing krill to produce protein, lipids and chitin, according to the invention, is characterized in comprising emulsification of lipids of krill in an aqueous medium; separation of the emulsion of lipids from the krill mass; alkaline extraction of proteins from the krill mass at a pH of 10 to 12; separation of the protein extract produced from chitin integuments; separation of protein from the protein extract.

The invention makes it possible to obtain a protein product with a content of protein of up to 95% by weight as calculated for dry substance.

According to the invention, the first stage of the

processing of krill provides for extraction of lipids. This extraction of lipids is effected by emulsification using various techniques such as intensive stirring in an apparatus with a stirrer, or an ultrasonic method. Used as a medium in which emulsification is conducted is water or aqueous solutions of salts. To reduce losses of protein in the process of emulsification the pH of the emulsifying medium should be maintained within 4.5 to 5.0. In emulsification lipids are separated with a yield of up to 95% by weight.

The krill after separation of lipids therefrom is treated with an alkaline solution with a pH of 10 to 12 for extraction of proteins therefrom. A two-phase system is formed comprising an aqueous-alkaline solution containing protein, and a solid residue containing chitin integuments and other insoluble substances. The aqueous-alkaline solution containing protein is separated from the solid residue by filtration or centrifugation. Protein is separated from the resultant aqueous-alkaline solution by various mehtods, for example, by precipitation with alcohol or ultrafiltration, precipitation in the isoelectric point, or thermal coagulation. The isoelectric precipitation is carried out by food acids at a pH of 4 to 5. A curdled, easily settling precipitate of protein is formed which is separated and washed with 2 to 5 volumes of water. The washed precipiate is dried. As a result a product is obtained with a protein content of up to 95% by weight as calculated for dry substance.

Thus, the proposed method for the processing of krill makes it possible to produce such valuable substances as protein, lipids and chitin.

The simple technology and the availability of the reactants used make the process commercially profitable.

For a better understanding of the present invention

examples are presented below.

Example 1

In an apparatus with a capacity of 10 l provided with a stirrer there is placed 1 kg of krill which is filled with water and stirred at 1,000 rpm for 0.3 hour. The resultant emulsion of lipids is separated from the krill mass by filtration through a stainless steel screen having a mesh size of 1x1 mm. The krill mass is transferred to the vessel with a stirrer into which there is added 3 l of an aqueous solution of NaOH of such a concentration as to reach a pH of the mixture of 10 and stirred for half an hour. When krill is treated with alkali extraction of proteins takes place. The resultant extract of proteins is separated from the insoluble residue of chitin integuments by filtration through a metal screen with a mesh size of 1x1 mm and centrifuged at 25,000 rpm for 0.15 hour to remove impurities. To the purified extract of proteins there is added while stirring a 1-mole solution of HCl to reach a pH of 4.5, protein being precipitated. The precipitate is left to settle for 3 hours, thereafter it is separated from the liquid, washed with 3 liters of water and dried lyophilically. The protein product obtained in an amount of 50 g is a pale-pink odorless powder, having a moisture content of 10% by weight and comprising 85% by weight of protein and 2% by weight of lipids.

The residue of krill produced after separation of the extract of proteins is pressed to remove moisture and dried to produce 17 g of chitin integuments.

Example 2

The processing of krill is carried out in the same manner as in Example 1, except that emulsification of lipids is conducted in a 0.15 mole aqueous solution of sodium chloride at a pH of 4.5. The protein product obtained in an amount of 54 g has a moisture content of 12% and comprises 80% by weight of protein and 3% by weight of lipids, and 20 g of

chitin integuments.

Examples 3

The processing of krill is conducted in the same manner as in Example 1, except that emulsification of lipids is carried out for half an hour, and protein is precipitated from the alkaline extract by adding thereto a 1 mole solution of acetic acid. The resultant protein precipitate is washed with 5 volumes of water to produce 60 g of a protein product having a moisture content of 8% and comprising 85% by weight of protein, 5% by weight of lipids and 18 g of chitin integuments.

Example 4

The processing of krill is carried out in the same manner as in Example 1, except that precipitation of protein from the alkaline extract is conducted by adding thereto a 0.8 mole solution of sulfuric acid. The resultant precipitate of protein is washed with 3 volumes of water to produce 54 g of a protein product having a moisture content of 11% and comprising 80% by weight of protein and 5% by weight of lipids, and 24 g of chitin integuments.

Example 5

The processing of krill is conducted in the same manner as in Example 1, except that emulsification of lipids is conducted in an aqueous solution of salts -- 0.2 mole of NaCl; 0.03 mole of $MgCl_2$; 0.01 mole of $MgSO_4$; and 0.005 mole of $CaSO_4$.

The protein product obtained in an amount of 60 g has a moisture content of 10% and comprises 82% by weight of protein, 4.2% by weight of lipids, and 20 g of chitin integuments.

liquid, washed with 3 liters of water and dried lyophilically. The protein product obtained in an amount of 50 g is a pale-pink odorless powder, having a moisture content of 10% by weight and comprising 85% by weight of protein and 2% by weight of lipids.

A The residue of ~~kriol~~ krill produced after separation of the extract of proteins is pressed to remove moisture and dried to produce 17 g of chitin integuments.

Example 2

The processing of ~~kriol~~ krill is carried out in the same manner as in Example 1, except that emulsification of lipids is conducted in a 0.15 mole aqueous solution of sodium chloride at a pH of 4.5. The protein product obtained in an amount of 54 g has a moisture content of 12% and comprises 80% by weight of protein and 3% by weight of lipids, and 20 g of chitin integuments.

Example 3

The processing of ~~kriol~~ krill is conducted in the same manner as in Example 1, except that emulsification of lipids is carried out for half an hour, and protein is precipitated from the alkaline extract by adding thereto a 1 mole solution of acetic acid. The resultant protein precipitate is washed with 5 volumes of water to produce 60 g of a protein product having a moisture content of 8% and comprising 85% by weight of protein, 5% by weight of lipids and 18 g

of chitin integuments.

Example 4

A The processing of ~~kriol~~ krill is carried out in the same manner as in Example 1, except that precipitation of protein from the alkaline extract is conducted by adding thereto a 0.8 mole solution of sulfuric acid. The resultant precipitate of protein is washed with 3 volumes of water to produce 54 g of a protein product having a moisture content of 11% and comprising 80% by weight of protein and 5% by weight of lipids, and 24 g of chitin integuments.

Example 5

The processing of ~~kriol~~ krill is conducted in the same manner as in Example 1, except that emulsification of lipids is conducted in an aqueous solution of salts -- 0.2 mole of NaCl; 0.03 mole of $MgCl_2$; 0.01 mole of $MgSO_4$; and 0.005 mole of $CaSO_4$.

The protein product obtained in an amount of 60 g has a moisture content of 10% and comprises 82% by weight of protein, 4.2% by weight of lipids, and 20 g of chitin integuments.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method for the processing of krill to produce protein, lipids and chitin which comprises emulsification of lipids of krill in an aqueous medium; separation of the resultant emulsion of lipids from the krill mass; alkaline extraction of proteins from the krill mass at a pH of 10 to 12; separation of the alkaline extract of proteins from chitin integuments; separation of protein from the alkaline extract.

2. A method as claimed in claim 1, wherein emulsification is carried out in the presence of mineral salts.

3. A method as claimed in claim 1, wherein emulsification is carried out at a pH of the medium of 4.5 to 5.0.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente

Electronic Acknowledgement Receipt

EFS ID:	17913143
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Thomas Vita
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON
Receipt Date:	15-JAN-2014
Filing Date:	06-SEP-2013
Time Stamp:	15:00:11
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Grit_1989_pp1-6.pdf	641658 bfd7ae9bee58e13c6381186f015bca31efd57441	no	6

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2	Non Patent Literature	Henderson_1994_pp311-317.pdf	6645569 c65db91ec3d35a2aff33c1817732b17747afe22d	no	7
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3	Non Patent Literature	Herman-Groves_1993_pp774-776.pdf	244315 e2f691b02deaa18b8980e60dd40d1d87e280abf4	no	3
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7	Non Patent Literature	Lin_1994_pp794-803.pdf	1329895 f96890bfa276072d6851e8bdd0562c3058e03380	no	10
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10	Non Patent Literature	OfficeAction-Jan-5-2012-351-patent.pdf	543851 2e0c04a3848be427a36a60cd25f5004ac22612df	no	14
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11	Non Patent Literature	Provisional-Application-60307842.pdf	1580888 42b2ae5ee9877fe7e1daf7de750a00e5adcfb3a4	no	37
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12	Non Patent Literature	Supplemental-Declaration-Bjorn-Ole-Haugsgjerd-8030348.pdf	948103 c9ae46045ddbc460a4ab1f5c767787857b00f006	no	4
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13	Non Patent Literature	Supplemental-Declaration-Earl-White-8030348.pdf	1503773 85940899180baf6d3b6a07423db92247480a5341	no	19
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14	Non Patent Literature	Supplemental-Declaration-Earl-White-8278351.pdf	487925 a2c3da8c882191cfddd2af9cc427507b8f443e52	no	7
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15	Non Patent Literature	Supplemental-Declaration-Thomas-Gundersen-8030348.pdf	3042898 7d1c0f18a6a48056f8b479e72e983ca2afcbf13e	no	42
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17	Non Patent Literature	Takahashi_1986_pp80-84.pdf	299970 fcc068b9d51aeb13777b4e2d2b91e92a022f434d	no	6
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22	Non Patent Literature	Watanabe_1991_pp681-694.pdf	788087 1203c5f19a27e567b8bf41614d0697fccbc69756	no	14
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23	Non Patent Literature	WHO-News_1995_pp547-51.pdf	4533905 ba3db6f08be4d5c0db86ebe4d7e82e6e98cea3e1	no	11
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D. K. Ware
Attorney Docket Number	AKBM-14409/US-6/CON

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	1	4714571		1987-12-22	Kearns et al.	
	2	8278351		2012-10-02	Sampalis	
	3	8383675		2013-02-26	Sampalis	

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	1	2251265	CA		2000-04-21	Beaudoin		☐

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	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

	2	60-153779	JP		1985-08-13	Honen Seiyu Co. Ltd.		☐
	3	H08-231391	JP		1996-09-10	Kanagawa Kagaku Kenkyuujo Co., Ltd. Et al.		☐

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	1	"Neptune Technologies & Bioressources Soon to Obtain a Major Patent in Over 30 Countries" ("2001 Press Release,")	☐
	2	Action Closing Prosecution, '348 patent	☐
	3	April 2, 2012 Response to Office Action, '351 patent	☐
	4	Balassa et al., Microencapsulation in the Food Industry, Critical Reviews in Food Technology, 2:2, 245-265 (1971) ("Balassa")	☐
	5	Bell and Dick, Molecular Species Composition of the Major Diacyl Glycerophospholipids from Muscle, Liver, Retina and Brain of Cod (Gadus morhua), Lipids, Vol. 26, No. 8, pp. 565-573 (1991) ("Bell and Dick")	☐
	6	Bell, Molecular Species Analysis of Phosphoglycerides from the Ripe Roes of Cod, Lipids, Vol. 24, No. 7 (1989)	☐
	7	Bell, Molecular Species Composition of Phosphatidylcholine from Crypthecodinium cohnii in Relation to Growth Temperature Lipids 25, 115-118 (1990)	☐

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	Art Unit	1651
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	Attorney Docket Number	AKBM-14409/US-6/CON

	8	Bergelson (ed.), Lipid Biochemical Preparations, Chapter I.1, pp. 1-13 (1980) ("Bergelson")	☐
	9	Bottino, N.R., "Lipid Composition of Two Species of Antarctic Krill: Euphausia Superba and E. Crystallorophias," Comp. Biochem. Physiol., 1975, Vol. 50B, pp. 479-484 ("Bottino")	☐
	10	Buchi R-220 Rotovapor® Manual	☐
	11	Buda, Structural order of membranes and composition of phospholipids in fish brain cells during thermal acclimatization, Proc. Natl. Acad. Sci. USA Vol. 91, pp. 8234-8238, August 1994	☐
	12	Certificate of translation of Ex. 1072: Fisheries Agency, General Report on Research and Development of Techniques in Processing and Utilization of Marine Products, Chapter 6, Development of technology for recovery of valuable substances (astaxanthin) from krill, by Takao Fujita, pp. 273-307 (March 1985); Japanese language document	☐
	13	Certificate of translation of Ex. 1074: Japanese Patent No. 60-153779, entitled "Nutritional Supplement"	☐
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	15	Certification of translation of Ex. 1070: Japanese Unexamined Patent Application Publication No. 02-215351	☐
	16	Certified translation of Ex. 1070: Japanese Unexamined Patent Application Publication No. 02-215351, titled Krill Phospholipids Fractioning Method ("Maruyama,"); Certificate of Translation provided as Ex. 1071.	☐
	17	Certified translation of Ex. 1072: Fisheries Agency, General Report on Research and Development of Techniques in Processing and Utilization of Marine Products, Chapter 6, Development of technology for recovery of valuable substances (astaxanthin) from krill, by Takao Fujita, pp. 273-307 (March 1985) ("Fujita") ; Certificate of Translation provided as Ex. 1073.	☐
	18	Certified translation of Ex. 1074: Japanese Patent No. 60-153779, entitled "Nutritional Supplement" ("Fukuoka "); Certificate of Translation provided as Ex. 1075	☐

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	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

	19	Certified translation of Ex. 1076: Japanese Patent Publication No. H08-231391, entitled "Medicine for Improvement of Dementia Symptoms" ("Yasawa"); Certificate of Translation provided as Ex. 1077.	☐
	20	Declaration of Bjorn Ole Haugsgjerd in support of Inter Partes Review of U.S. Pat. No. 8,278,351 ("Haugsgjerd")	☐
	21	Declaration of Bjorn Ole Haugsgjerd submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("Haugsgjerd '348 Decl.")	☐
	22	Declaration of Dr. Albert Lee in Support of Inter Partes Review of U.S. Pat. No. 8,278,351 ("Lee")	☐
	23	Declaration of Dr. Albert Lee in Support of Inter Partes Review of U.S. Pat. No. 8,383,675 ("Lee")	☐
	24	Declaration of Dr. Chong Lee submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("Yeboah Reexam Decl.")	☐
	25	Declaration of Dr. Earl White submitted during prosecution of parent patent U.S. 8,030,348 ("2011 White Decl.")	☐
	26	Declaration of Dr. Ivar Storrø in support of Inter Partes Review of U.S. Pat. No. 8,278,351 ("Storrø")	☐
	27	Declaration of Dr. Ivar Storrø in support of Inter Partes Review of U.S. Pat. No. 8,383,675 ("Storrø")	☐
	28	Declaration of Dr. Jacek Jaczynski from inter partes reexamination of the parent patent U.S. 8,030,348 ("Jaczynski Reexam. Decl.")	☐
	29	Declaration of Dr. Jaczynski submitted during prosecution of parent patent U.S. 8,278,351 (Jaczynski '351 Decl.")	☐

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	First Named Inventor	Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

	30	Declaration of Dr. Jeff Moore in Support of Inter Partes Review of U.S. Pat. No. 8,278,351 ("Moore")	☐
	31	Declaration of Dr. Jeff Moore in Support of Inter Partes Review of U.S. Pat. No. 8,383,675 ("Moore")	☐
	32	Declaration of Dr. Richard van Breemen in Support of Inter Partes Review of U.S. Pat. No. 8,278,351 ("Van Breemen")	☐
	33	Declaration of Dr. Richard van Breemen in Support of Inter Partes Review of U.S. Pat. No. 8,383,675 ("Van Breemen")	☐
	34	Declaration of Dr. Shahidi submitted during inter partes reexamination of parent patent U.S. 8,030,348 (Shahidi Reexam. Decl.")	☐
	35	Declaration of Dr. Shahidi submitted during prosecution of parent patent U.S. 8,278,351 (Shahidi '351 Decl.")	☐
	36	Declaration of Dr. Suzanne Budge in Support of Inter Partes Review of U.S. Pat. No. 8,278,351 ("Budge")	☐
	37	Declaration of Dr. Suzanne Budge in Support of Inter Partes Review of U.S. Pat. No. 8,383,675 ("Budge")	☐
	38	Declaration of Dr. Thomas Brenna in support of Inter Partes Review of U.S. Pat. No. 8,278,351	☐
	39	Declaration of Dr. Thomas Brenna in support of Inter Partes Review of U.S. Pat. No. 8,383,675	☐
	40	Declaration of Dr. Thomas Gundersen submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("Gundersen Decl.")	☐

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	First Named Inventor	Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

	41	Declaration of Dr. Tina Sampalis submitted during inter partes reexamination of parent patent U.S. 8,030,348 (Sampalis")	☐
	42	Declaration of Dr. Van Breemen submitted during Ex parte Reexamination of the '351 patent (Van Breemen '351 Reexam. Decl."	☐
	43	Declaration of Dr. Van Breemen submitted during Inter partes Reexamination of the '348 patent (Van Breemen '348 Reexam Decl."	☐
	44	Declaration of Dr. Yeboah submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("Yeboah Reexam Decl.")	☐
	45	Declaration of Dr. Yeboah submitted during prosecution of parent patent U.S. 8,278,351 ("Yeboah '351 Decl.")	☐
	46	Eichberg, "Lecithin – It Manufacture and Use in the Fat and Oil Industry," Oils and Soap 51-54, 1939 ("Eichberg")	☐
	47	Expert Witness Report of Dr. Theodore Welch submitted in relation to ITC Investigation No. 337-TA-877 ("Welch")	☐
	48	Farkas, Composition and Physical State of Phospholipids in Calanoid Copepods from India and Norway, LIPIDS, Vol. 23, No. 6 (1988)	☐
	49	Final Prospectus dated May 11, 2001 ("Final Prospectus")	☐
	50	Fisheries Agency, General Report on Research and Development of Techniques in Processing and Utilization of Marine Products, Chapter 6, Development of technology for recovery of valuable substances (astaxanthin) from krill, by Takao Fujita, pp. 273-307 (March 1985); Japanese language document	☐

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	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

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	Examiner Name	D. K. Ware
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Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2014-01-14
Name/Print	J. Mitchell Jones	Registration Number	44174

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	Attorney Docket Number	AKBM-14409/US-6/CON

	1	Folch, et al., A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. J. Biol. Chem., 226, 497-509 (1957)	☐
	2	Grant of Request for Ex parte Reexamination of the '351 patent	☐
	3	Grit et al., Hydrolysis of phosphatidylcholine in aqueous liposome dispersions, Int. J. Pharmaceutics 50:1-6 (1989)	☐
	4	Henderson et al., Lipid Composition of the Pineal Organ from Rainbow Trout (Oncorhynchus mykiss), Lipids, Vol. 29, No. 5, pp. 311-317 (1994) ("Henderson ")	☐
	5	Herman and Groves, The Influence of Free Fatty Acid Formation on the pH of Phospholipid-Stabilized Triglyceride Emulsions, Pharmaceutical Research 10(5):774-776 (1993)	☐
	6	Itano Refrigerated Food Co., Ltd., Bio & High Technology Announcement and Natural Astaxanthin & Krill Lecithin, pp. 1-16 (on or before December 28, 1994) ("Itano")	☐
	7	Johnson and Lucas, Comparison of Alternative Solvents for Oils Extraction, JAOCS 60(2):229-242 (1983)	☐
	8	Le Grandois et al., Investigation of Natural Phosphatidylholine Sources: Separation and Identification by Liquid Chromatography -Electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS2) of Molecular Species, J. Agric. Food Chem., 57, 6014-20 (2009) ("Le Grandois")	☐
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	10	Medina et al., C Nuclear Magnetic Resonance Monitoring of Free Fatty Acid Release After Fish Thermal Processing, J. Amer. Oil Chem. Soc. 71(5):479-82 (1994)	☐
	11	October 24, 2012 Office Action, '675 patent	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14020162
	Filing Date	2013-09-06
	First Named Inventor	Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-6/CON

	12	Office Action dated January 5, 2012, '351 patent	☐
	13	Provisional Application No. 60/307,842 (Priority document for the '351 patent)	☐
	14	Supplemental Declaration of Bjorn Ole Haugsgjerd submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("Haugsgjerd '348 Supp. Decl.")	☐
	15	Supplemental Declaration of Dr. Earl White submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("White Supp. Reexam. Decl.")	☐
	16	Supplemental Declaration of Dr. Earl White submitted during prosecution of parent patent U.S. 8,278,351 ("White Supp. Decl.")	☐
	17	Supplemental Declaration of Dr. Thomas Gundersen submitted during inter partes reexamination of parent patent U.S. 8,030,348 ("Gundersen Supp. Decl.")	☐
	18	Suzuki, T. and Shibata, N., "The utilization of Antarctic krill for human food," Food Rev. Int'l, 6:1, 119-147 (1990) ("Suzuki")	☐
	19	Takahashi et al., Compositional Changes in Molecular Species of Fish Muscle Phosphatidylcholine During Storage, Bull. Fac. Fish. Hokkaido Univ. 37(1), 80-84 1986.	☐
	20	Takahashi et al., Molecular Species of Fish Muscle Lecithin, Bulletin of the Japanese Society of Scientific Fisheries 48 (12), 1803-1814 (1982)	☐
	21	Takahashi et al., Prediction of Relative Retention Value of the Individual Molecular Species of Diacyl Glycerolipid on High Performance Liquid Chromatography, Bull. Fac. Fish. Hokkaido Univ. 38(4), 398-404. 1987	☐
	22	Tanaka, Biosynthesis of 1,2-dieicosapentaenoyl-sn-glycero-3-phosphocholine in Caenorhabditis elegans, Eur. J. Biochem. 263, 189±194 (1999)	☐

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	Attorney Docket Number	AKBM-14409/US-6/CON

	23	Tocher, Chapter 6, Glycerophospholipid metabolism, Biochemistry and molecular biology of fishes, vol. 4, Hochachka and Mommsen (eds.)(1995)	☐
	24	Watanabe et al., Effective Components in Cuttlefish Meal and Raw Krill for Improvement of Quality of Red Seabream Pagrus major Eggs, Nippon Suisan Gakkaishi 57(4):681-694 (1991)("Watanabe")	☐
	25	WHO News and Activities, Bulletin of the World Health Organization, 73(4), pp. 547-51 (1995) ("WHO Bulletin")	☐

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

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Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2014-01-14
Name/Print	J. Mitchell Jones	Registration Number	44174

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(54) **PROCEDE D'EXTRACTION DES LIPIDES DE TISSUS D'ANIMAUX AQUATIQUES PRODUISANT UN RESIDU DESHYDRATE**

(54) **PROCESS FOR LIPID EXTRACTION OF AQUATIC ANIMAL TISSUES PRODUCING A DEHYDRATED RESIDUE**

(57) The procedure includes the suspension of freshly collected material in an equal volume of acetone under inert gas atmosphere. Lipids are extracted by successive acetone and ethanol treatments. The procedure produces two lipid fractions and a dry residue enriched in protein and other material insoluble in organic solvents. Recovery of total lipids is comparable or superior to the Folch et al. (1957) procedure. It has been tested with krill, Calanus and fish tissues.

TITLE OF THE INVENTION

PROCESS FOR LIPID EXTRACTION OF AQUATIC ANIMAL TISSUES PRODUCING A DEHYDRATED RESIDUE

FIELD OF THE INVENTION

The present invention relates to a method for lipid extraction of animal tissues and to the lipid and dry residue fractions obtained therefrom. More particularly, the present invention relates to a lipid extraction method using krill, *Calanus* and fish tissues as starting material.

SUMMARY OF THE INVENTION

Extraction process

Fresh (or frozen) material (*Euphausia pacifica* and other species) is suspended in cold acetone for a given period of time at low temperature (5°C or lower). A ratio of krill-acetone 1:6 (w/v) and an incubation time of 2 h in acetone were found to be optimal. Alternatively the material can be kept in an equal volume of acetone at low temperature for long periods of time (months) under inert atmosphere. The size of the material is an important factor for the penetration of acetone. Indeed, it is preferable to grind material with dimensions superior to 5 mm before getting it in contact with acetone. The suspension is swirled for a short period of time (about 20 min) after acetone addition. After filtration on an organic solvent resistant filter (metal, glass or paper) the residue is washed with two volumes of pure acetone. The combined filtrates are evaporated under reduced pressure. The water residue obtained after evaporation is allowed to separate from the oil phase (fraction I) at low temperature. The solid residue collected on the filter is suspended and extracted with two volumes (original volume of frozen material) of 100% ethanol. The ethanol filtrate is evaporated leaving a second fraction of lipids (identified as fraction II).

Variations of the process

Variable volumes of acetone relative to the levels of sample can be used. It is also applicable to the volume of acetone used to wash and to the volume of ethanol used to extract. Incubation times in solvents may vary. Particle size affect the recovery of lipids and the material could be ground in various sizes of particles, depending on the grinder used. Temperature of the organic solvents and temperature of the sample are not critical parameters, but it is preferable to be as cold as possible.

Methods

To compare the efficiency of the extraction process, a classical technique (Folch et al. 1957) implying chloroform and methanol was applied to krill. This is the standard of reference for the efficiency of the extraction process. Lipid recovery was estimated by suspending lipid fractions in small volumes of their original solvents and measuring by gravimetry small aliquots after evaporation.

To analyze lipid composition, small aliquots of the various extracts were loaded on silica-gel plates and fractionated by thin layer chromatography, TLC (Bowyer et al. 1962) with the following solvents. Neutral lipids: hexane, ethyl ether, acetic acid (90:10:1 v/v) and phospholipids: chloroform, methanol, water (80:25:2 v/v). Fatty acid composition of *E. pacifica* was analyzed by gas liquid chromatography, GLC (Bowyer et al. 1962) including some modifications to the original technique: 1h at 65°C instead of 2h at 80°C, three washes with hexane instead of two and no wash with water.

The dry residue is wetted with ethanol to facilitate a progressive rehydratation of the proteins.

To get rid of traces of organic solvents, lipid fraction I and II are warmed (60°C for fraction I and 70°C for fraction II) for 5 min under inert atmosphere.

Applications

The different fractions (oil, proteins, and others) of aquatic animal biomass extracted by the current procedure could be used in many fields:

1-Aquaculture

As mentioned in results, fatty acids 20:5 (eicosapentaenoic acid) and 22:6 (docosahexaenoic acid) are found in high concentrations in krill, *Calanus*, and fish. Farming fish on high quality marine oils rich in docosahexaenoic and eicosapentaenoic (EPA) acids is an efficient means of delivering these essential nutrients in human diets and also efficiently exploiting a strictly limited marine bioresource (Sargent 1997). Krill may be used as food supplement for fish and shrimp (Sargent 1997) because of its capacity to improve growth and survival capacity against diseases (Runge 1994), as pigmentation enhancer for ornamental fish species and as starter diet for marine and fresh water species (Prawn Hatchery Food 1997).

2-Nutraceuticals

Considering the beneficial effects of omega-3 fatty acids, the marine oils from krill, *Calanus* and fish could be used as dietary supplements to human diet. 22:6 *n*-3 fatty acid is essential for proper development of the brain and the eye (Sargent 1997).The beneficial effects of *n*-3 polyunsaturated fatty acids in reducing the incidence of cardiovascular disease by lowering plasma triacylglycerol level and altering platelet function towards a more anti-atherogenic state has been reviewed (Christensen 1994). Also, dietary krill oil, like fish oil, can suppress the development of autoimmune murine lupus: EPA substitutes for arachidonic acid, a substrate for cycloxygenase thereby reducing the production of prostaglandins (Chandrasekar 1996). The effects of dietary supplementation with w-3 lipid-rich krill oil includes decreased expression of TGF_{β} in kidneys and of the oncogene–c-*ras* in splenocytes (Chandrasekar 1996). Krill oil has beneficial effects on life span and amelioration of renal disease similar to those previously described in studies with fish oil (Chandrasekar 1996).

3-Animal food

Feeding the animals with omega-3 fatty acids may increase the level of unsaturated fatty acids and decrease cholesterol levels of meat. This property is exploited in the poultry industry to improve the quality of eggs. *Calanus*, in particular, is a full of promise ingredient of domestic animal's food (Runge 1994).

4-Cosmetic industry

Calanus is used for the production of moisturizing creams (Runge 1994).

5-Medical applications

Krill may be used as a source of enzymes for medical application like the debridement of ulcers and wounds (Hellgren 1991) or to facilitate food digestion.

Finally, these marine products are also rich in liposoluble vitamins A, D, E and K and carotenoids that are extracted with lipids. The chitin of krill and *Calanus* could be exploited to protect plants against fungi. Also, marine oils contain unidentified antioxidants which may have potential therapeutic properties.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawing which is exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Results

Note on experimental conditions

The lipid extraction with acetone, then ethanol is practicable under different experimental conditions, as mentioned on page 1 of this document (variation of the process). Moreover, the majority of data shown in this document are from experiments made with sample-acetone ratio of 1:9 (w/v) incubated overnight at 4°C and with sample-ethanol ratio of 1:4 (w/v) incubated 1h at 4°C. In addition, no material has been ground in most experiments. Only later, tests have been made to standardize the method for extraction of lipids with acetone, then ethanol. As shown in Figure 9 and 11, it appears that optimal ratios of sample-solvent are 1:6 (w/v) for acetone and 1:2 (w/v) for ethanol. Figure 10 and Figure 12 show that optimal incubation times are 2 h for the first solvent and 30 min for the second. Grinding has been experimented and it is clear that solvents have a better impact on ground material, as shown in Table 5. Then, experimental conditions are specified for each experiment.

Diagram 1 illustrates the procedure of lipid extraction from frozen krill which is the same used with dry krill and other fresh species as *Calanus*, mackerel, trout and herring.

Interpretation of results

Table 1 shows that higher levels of lipids are extracted by acetone followed by ethanol as compared to the classical procedure of Folch et al. (1957). The same information is found in Table 5 concerning another krill species (*Megayctiphanes norvegica*). Back to Table 1, one can see that the combination of acetone and ethanol as a single step did not improve the extraction process.

Table 2 shows the results of lipid extraction from frozen *Euphausia pacifica*, a species of krill from Pacific Ocean. Assuming an eighty percent content of water, the lipid content is comparable to dry krill as shown in Table 1. Samples of *E. pacifica* incubated in different ratios of acetone at 4°C for 112 days have been inoculated on NA medium containing Bacto beef extract 0,3%, Bacto peptone 0,5% and Bacto agar 1,5% (Difco 1984) then incubated at room temperature or 4°C for 18 days. No significant bacterial growth was observed at a ratio of 1 volume of acetone per gram of krill. At higher proportions of acetone (2 volumes and 5 volumes), there was no bacterial growth at all, which means that acetone preserves krill samples. Acetone is known as an efficient bactericidal and viricidal agent (Goodman et al. 1980).

Table 3 shows the yield of lipids from *M. norvegica*. The percentage of lipids is lower (3,67 %) than for *E. pacifica* (4,04 %) shown in Table 2. These variations can be attributable to the season of catch.

Table 4 shows the krill composition obtained from experiments 3 and 4 with frozen *E. pacifica* (Table 2). One finds about 83% of water, 4% of lipids and 12% of dry residue.

Table 5 shows the influence of grinding on the efficiency of extraction of *M. norvegica* lipids. These extractions were carried out under optimal conditions and show the definite advantage of the procedure over the classical method (4,46 % versus 3,30 %). It also shows that grinding may be an important factor when the species is large (4,46% versus 3,53 %).

Considerable quantity of lipids were obtained from *Calanus* (Table 6). Some variations in *Calanus* species composition may explain the variations between experiments 1 and 2 (8,22 % and 10,90 % of fresh weight).

When the technique was applied to fish (mackerel) peripheral tissues (mainly muscles) or viscera, an amount of lipids was extracted (Table 7) but it appeared less efficient than the classical method since extractions of the residue with the latter technique allowed us to recover less lipid. Overall, our technique would allow us to exploit parts of fish that are usually wasted after the withdrawal of fillets of the fish or lipid extracts from fishes not used for human consumption. Those fish tissues not used after the transformation of the fish for human consumption could be stored in acetone, then lipids could be extracted with our process. Extraction of lipids from trout and herring were carried out in parallel with the classical method. Results appear in Table 8 and 9. The yield is not significantly different for the viscera whereas with peripheral tissues (muscles) the classical technique is superior (14,93 % versus 6,70 %). Technique using acetone followed by ethanol for trout and herring (and maybe for other species) seems applicable as well as for mackerel. Table 11 shows the suggested procedure for lipid extraction of aquatic animal tissues.

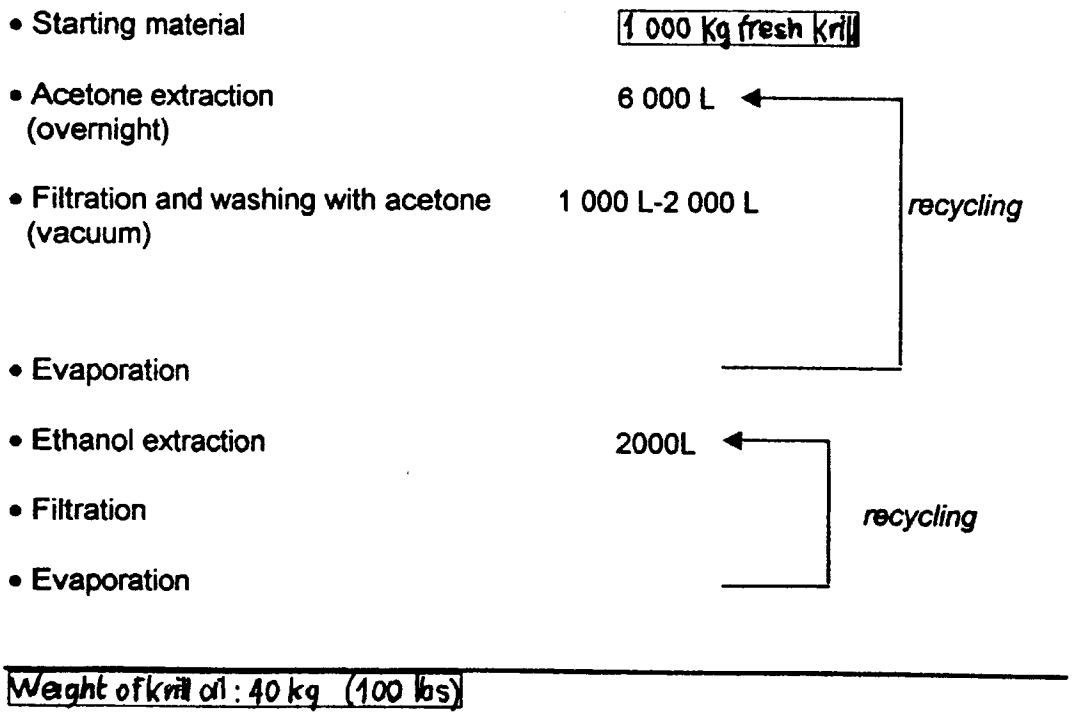
Figures 1 to 4 show chromatograms of fatty acid composition of *E. pacifica* lipids. On each of them, high proportions of 20:5 and 22:6 fatty acids (characteristic of marine oils) are noticeable and represented by two distinct peaks. The concentration of the sample on Figure 4 was lower than the others, so the peaks don't have the same amplitude. With retention times and amounts gave by the chromatograph, identification and compilation of the majority of the fatty acids have been done (see Table 10).

Figures 5 to 8 (TLC) show a higher proportion of neutral lipids as compared to phospholipids in marine oils.

The influence of incubation time on the efficiency of the acetone to extract lipids from *E. pacifica* is illustrated in Figure 9. Extraction is already completed at 2 h. With this time, we proceeded to determine the influence of the sample-acetone ratio (Figure 10). Results show that a ratio of 1:6 (w/v) produce the best yield. The second lipid extraction is carried out with ethanol. The incubation time in this solvent should be at least 30 min as indicated by the results of Figure 11. The volume of ethanol does not appear to be critical since the same yield was obtained with different volumes of ethanol.

One of the inventors, Mr Adrien Beaudoin, has tasted the different lipid fractions. No side effect was observed. The fraction I has the taste of the cod liver oil and the insoluble material tastes like salty shrimps.

DIAGRAM 1. KRILL LIPID EXTRACTION PROCESS



Weight of krill oil : 40 kg (100 lbs)

TABLE 1. EXTRACTION OF DRY KRILL LIPIDS (*E. pacifica*)

Exp. No.	Technique	Yield (%)	Total (%)
1-	acetone [a)]	8,00	
	ethanol [b)]	7,60	15,60
2-	"	19,70	
		6,90	26,60
3-	"	8,15	
		11,20	19,35
4-	"	6,80	
		13,60	20,40
			$\bar{x}$=20,49
			σ= 3,95
5-	Chlor : MeOH [c)]		15,50
6-	"		14,90
			$\bar{x}$=15,20
			σ= 0,30
7-	Combined acetone-ethanol [d)]		**14,30**

Determinations in triplicates (variation < 5 %).

[a)] :Extraction made with a sample-acetone ratio of 1:9 (w/v), no incubation.

[b)] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1 night at 4°C.

[c)] :Folch et al. 1957

[d)] :Extraction made with a sample-acetone-ethanol ratio of 1:5:5, no incubation.

TABLE 2. EXTRACTION OF FROZEN KRILL LIPIDS (*E. pacifica*)

Exp. No.	Technique	Yield (%)	Total (%)
1-	acetone [a]	2,26	
	ethanol [b]	2,14	4,40
2-	"	2,25	
		1,13	3,38
3-	"	2,71	
		1,80	4,50 [c]
4-	"	2,94	
		1,45	4,39 [c]
5-	"	2,44	
		1,43	3,87
6-	"	2,54	
		1,23	3,77
7-	"	2,58	
		1,46	4,04
8-	"	2,48	
		1,39	3,87
9-	"	2,46	
		1,72	4,18
			$\bar{x}$=4,04
			σ=0,34

Determinations in triplicates (variation < 5 %).

[a] :Extraction made with a sample-acetone ratio of 1:9 (w/v), incubated 1 night at 4°C.

[b] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1h at 4°C.

[c] :See Table 4 for total composition.

TABLE 3. EXTRACTION OF FROZEN KRILL LIPIDS (*M. norvegica*)

Exp. No.	Technique	Yield (%)	Total (%)
1-	acetone [a]	1,82	
	ethanol [b]	1,82	3,64
2-	"	1,15	
		2,35	3,50
3-	"	1,68	
		2,19	3,87
			$\bar{x}$=3,67
			σ=0,15

Determinations in triplicates (variation < 5 %).

[a] :Extraction made with a sample-acetone ratio of 1:9 (w/v), incubated 1 night at 4°C.

[b] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1 h at 4°C.

TABLE 4. FROZEN KRILL COMPOSITION (*E. pacifica*) on a fresh weight basis

Exp. No.	Lipids	Insoluble material	Water
3-	4,50	12,50	83,00
4-	4,39	11,50	84,11
	$\bar{x}$=4,44 σ=0,05	$\bar{x}$=12,00 σ= 0,50	$\bar{x}$=83,55 σ= 0,55

Determinations in triplicates (variation < 5 %).
Experience numbers refer to Table 2.

TABLE 5. INFLUENCE OF GRINDING ON EXTRACTION OF FROZEN KRILL LIPIDS (*M. norvegica*)

Exp. No.	Technique	Krill ground before 1st extraction	Yield (%)	Total (%)
1-	acetone [a]	yes	3,10	
	ethanol [b]		1,07	4,17
2-	"	no	2,14	
			1,39	3,53
3-	"	yes	3,32	
			1,14	4,46
4-	Chlor : MeOH [c]	yes		3,30
5-	"	yes		3,26

Determinations in triplicates (variation < 5 %).
[a] :Extraction made with a sample-acetone ratio of 1:6, incubated 2 h at 4°C
[b] :Extraction made with a sample-ethanol ratio of 1:2, incubated 30 min at 4°C.
[c] :Folch et al. 1957.

TABLE 6. EXTRACTION OF FROZEN *Calanus* LIPIDS (*Calanus* sp.)

Exp. No.	Technique	Yield (%)	Total (%)
1-	acetone [a]	6,18	
	ethanol [b]	2,04	8,22
2-	"	8,64	
		2,26	10.90
			$\bar{x}$=9,56
			σ=1,34

Determinations in triplicates (variation < 5 %).
[a] :Extraction made with a sample-acetone ratio of 1:9 (w/v), incubated 1 night at 4°C.
[b] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1 h at 4°C.

TABLE 7. EXTRACTION OF FRESH FISH.LIPIDS (Mackerel)

Exp. No.	Technique	Yield (%)	Total (%)
1-viscera	acetone [a]	6,11	
fish 1	ethanol [b]	0,59	6,70
2-tissues	"	3,78	
fish 1		0,91	4,69
3-viscera	"	10,46	
fish 2		0,57	11,03
4-tissues	"	6,65	
fish 2		1,41	8,06
5-viscera	"	8,39	
fish 3		0,66	9,05
6-tissues	"	5,27	
fish 3		0,97	6,24
7-viscera	"	8,47	
fish 4		0,69	9,16
8-tissues	"	8,40	
fish 4		1,02	9,42
9-viscera	Chlor:MeOH [c]		0,52
fish 1			
10-tissues	"		1,45
fish 1			

[a]:Extraction made with a sample-acetone ratio of 1:9 (w/v), incubation time:
-fish 1 viscera: 4h, fish 1 tissues: 23h
-fish 2 viscera: 23h45, fish 2 tissues: 45h30
-fish 3 viscera: 8 days 2h20, fish 3 tissues: 8 days 22h30
-fish 4 viscera: 17 days 23h, fish 4 tissues: 18 days 2h25

[b]:Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1h at 4°C.

[c]:Folch et al. 1957.

TABLE 8. EXTRACTION OF FRESH FISH LIPIDS (Trout)

Exp. No.	Technique	Yield (%)	Total (%)
1-viscera	acetone [a]	34,70	
	ethanol [b]	2,18	36,88
2-tissues	"	5,53	
		1,17	6,70
3-viscera	Chlor:MeOH [c]		39,81
4-tissues	"		14,93

Determinations in triplicates (variation < 5 %).
[a] :Extraction made with a sample-acetone ratio of 1:9 (w/v), incubated 1 night at 4°C.
[b] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1 h at 4°C.
[c] :Folch et al. 1957.

TABLE 9. EXTRACTION OF FRESH FISH LIPIDS (Herring)

Exp. No.	Technique	Yield (%)	Total (%)
1-tissues and viscera	acetone [a]	2,09	
	ethanol [b]	0,68	2.77
2-tissues and viscera	Chlor:MeOH [c]		5.95

Determination in triplicates (variation < 5 %).
[a] :Extraction made with a sample-acetone ratio of 1:9 (w/v), incubated 1 night at 4°.
[b] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1 h at 4°C.
[c] :Folch et al. 1957.

Table 10: Fatty acid composition *E. pacifica*

Solvent	Saturated	Unsaturated				Unidentified	
		Mono	Di	Poly	H-Poly		
chlo-meth	26,18	22,54	1,91	4,31	26,34	18,72	
acetone	21,4	22,18	1,75	4,67	24,52	25,49	
acetone	19,09	22,11	2,03	4,79	30,24	21,72	
ethanol	45,93	22,96	1,23	2,72	11,11	16,05	(500 µg/mL)
	45,96	22,98	1,24	2,48	11,18	16,15	(200 µg/mL)

Data expressed in percentage of total fatty acids (%).

TABLE 11. OPTIMAL CONDITIONS FOR LIPID EXTRACTION OF AQUATIC ANIMAL TISSUES (suggested procedure)

STEP	CONDITIONS
Grinding (if particles > 5mm)	4°C
Lipid extraction	sample-acetone ratio of 1:6 (w/v) 2h (including swirling 20 min) 4°C
Filtration	organic solvent resistant filter under reduced pressure
Washing	sample-acetone ratio of 1:2 (w/v) pure and cold acetone
Filtration	organic solvent resistant filter under reduced pressure
Evaporation	under reduced pressure
Oil-water separation	4°C
Lipid extraction	sample-ethanol ratio of 1:2 (w/v) pure ethanol 30 min 4°C
Filtration	organic solvent resistant filter under reduced pressure
Evaporation	under reduced pressure

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Although the present invention has been described herein above by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A method for extracting lipids from an aquatic animal tissue comprising the steps of:

a) suspending said animal aquatic tissue in an organic solvent;

b) extracting lipids by successive organic solvent treatment; and

c) collecting said lipids in a first fraction and an organic insoluble fraction.

2. The method of claim 1, wherein said organic solvent of a) is acetone.

3. The method of claim 1 or 2, wherein said organic solvent of b) is selected from at least one of acetone and alcohol.

4. The method of claim 1, 2 or 3, wherein said organic insoluble fraction comprises a dry residue fraction which is enriched in protein.

5. The method of claim 1, 2, 3 or 4, wherein said aquatic animal tissue is at least one tissue selected from the group consisting of krill tissue, *Calanus* tissue and fish tissue.

6. A lipid extract obtained by the method of claim 2, 3, 4 or 5.

7. A protein rich fraction obtained by the method of claim 4 or 5.

8. A lipid extract having the properties in accordance with the present invention.

CONFIDENTIEL

Injection Date : 98-03-24 20:09:39
Sample Name : 7
Acq. Operator : Chantal Beaudoin

Seq. Line : -
Vial : 1
Inj : 1
Inj Volume : Manually

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(modified after loading)

Méthode corrigée lors de l'installation de la nouvelle colonne 12 septembre 1997. Température du four 170 degré C et purge flow = 150 ml/min. Flux dans la colonne : 4,0 ml/min. Augmentation de la température a 175 degré C et le purge flow est descendu a 140 ml/min, le 13 mars 1998.

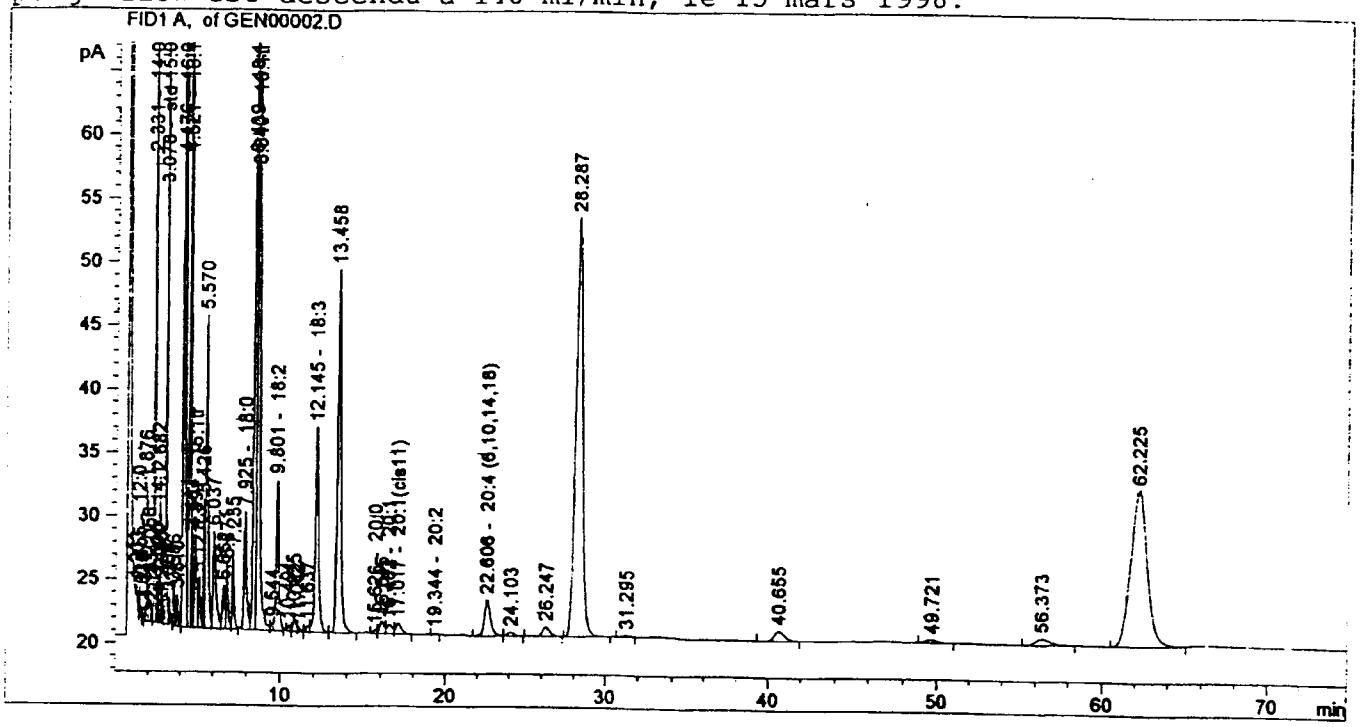

Figure 1: Gas-liquid chromatography of fatty acids from dry krill (chloroform-methanol).

CONFIDENTIEL

CONFIDENTIEL

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Seq. Line : -
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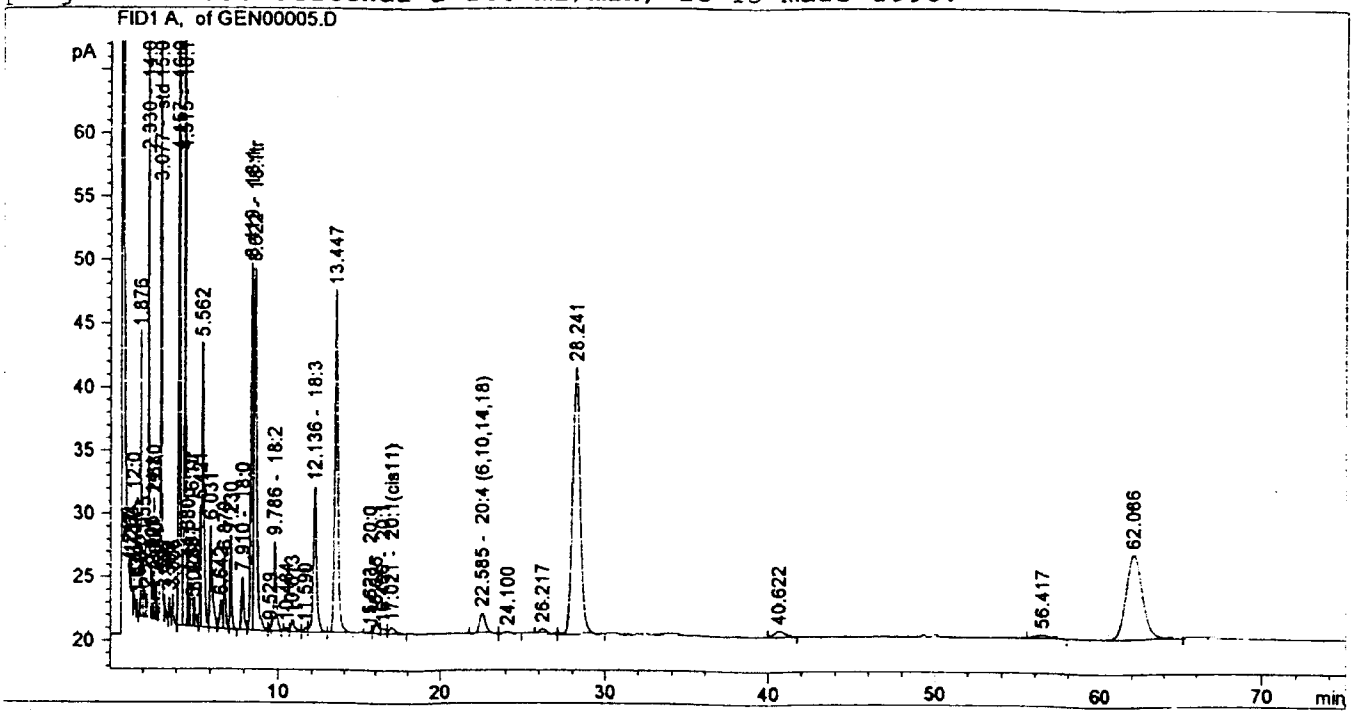

Figure 2: Gas-liquid chromatography of fatty acids from dry krill (acetone).

CONFIDENTIEL

CONFIDENTIEL

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Acq. Operator : Chantal Beaudoin

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(modified after loading)

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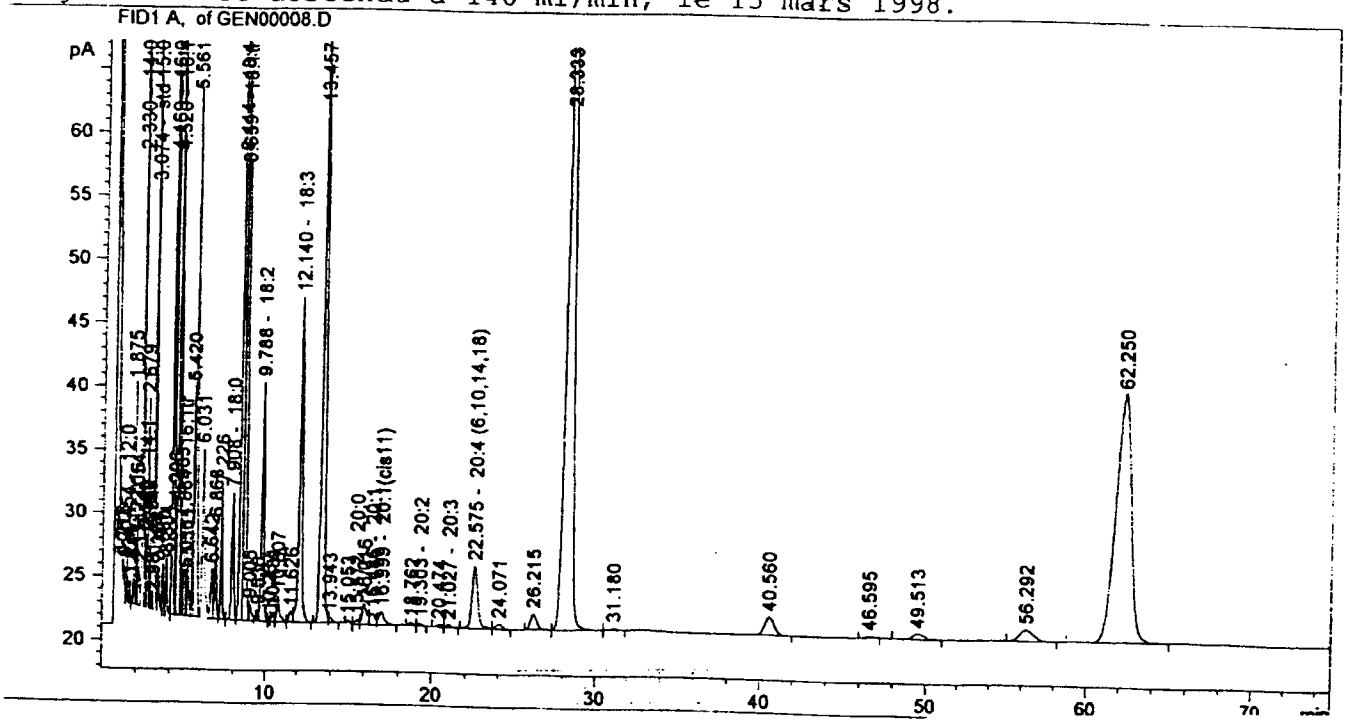

Figure 3: Gas-liquid chromatography of fatty acids from frozen krill (acetone).

CONFIDENTIEL

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Acq. Operator : Chantal Beaudoin

Seq. Line : -
Vial : 1
Inj : 1
Inj Volume : Manually

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Last changed : 98-04-02 17:28:39 by Chantal Beaudoin
(modified after loading)

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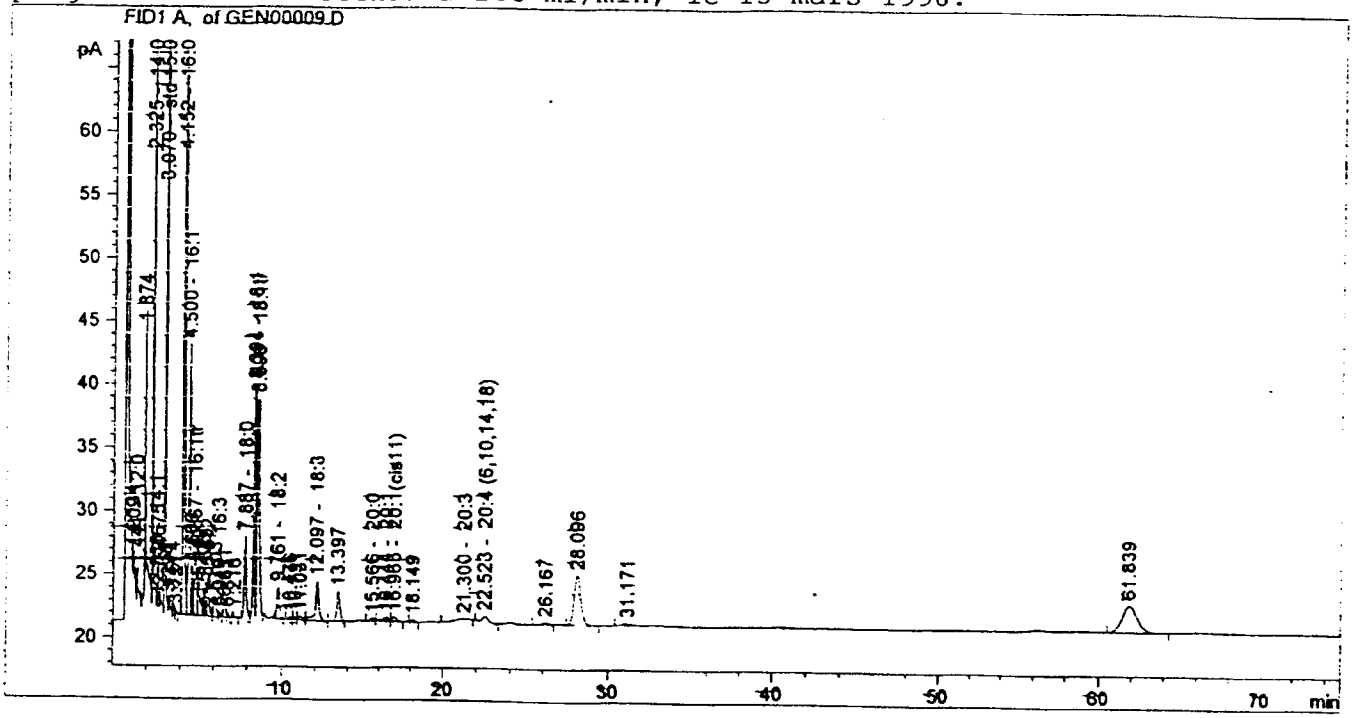


Figure 4: Gas-liquid chromatography of fatty acids from frozen krill (ethanol).

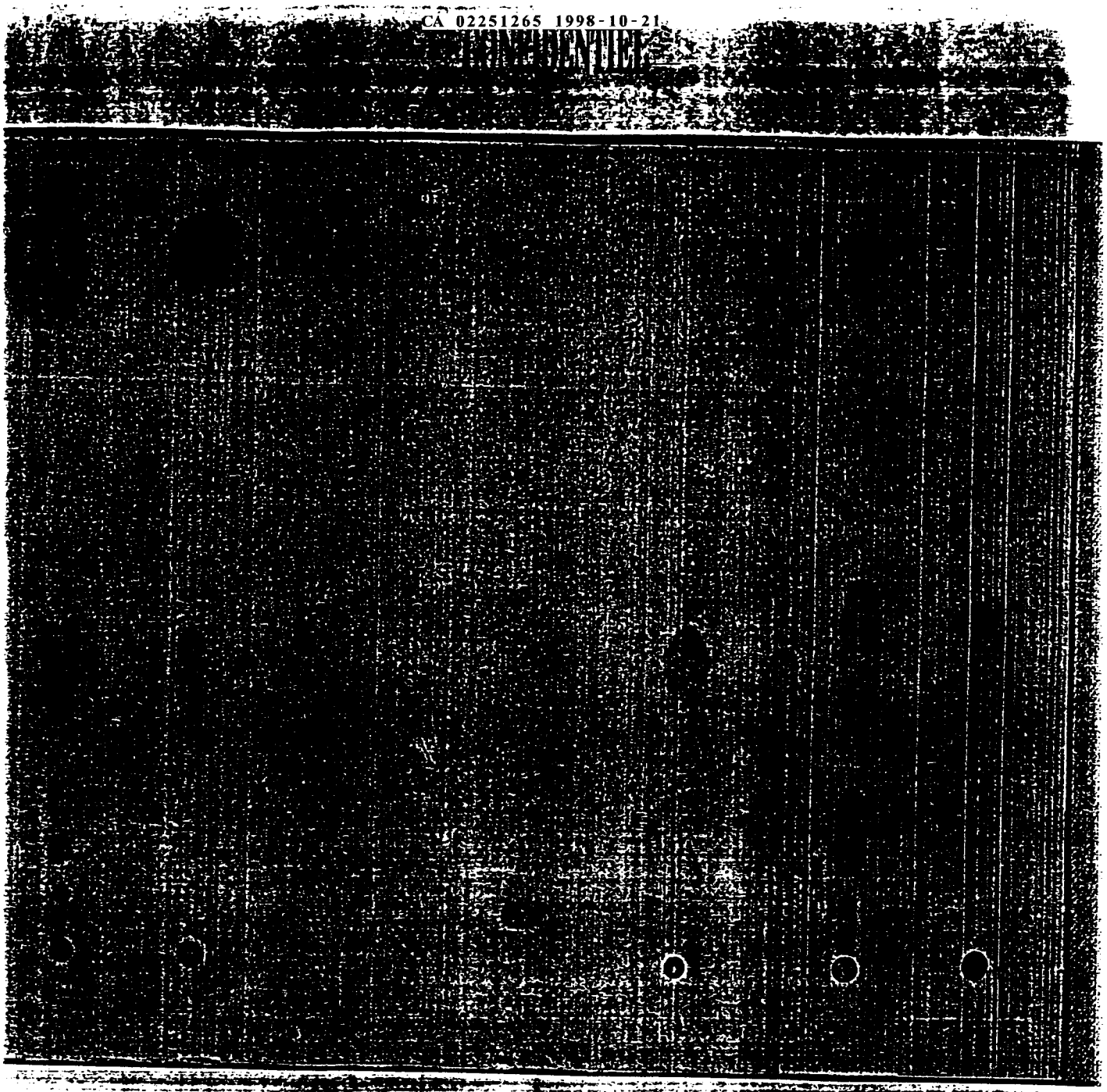


Figure 5: Thin-layer chromatography of neutral lipids of *Calanus* sp. (acetone), *Calanus* sp. (ethanol), sample of other interest, cholesterol 20mg/mL, egg (acetone), *M. norvegiça* (acetone) and *M. norvegica* (ethanol). Hexane-ethyl ether-acetic acid (90:10:1, v/v).

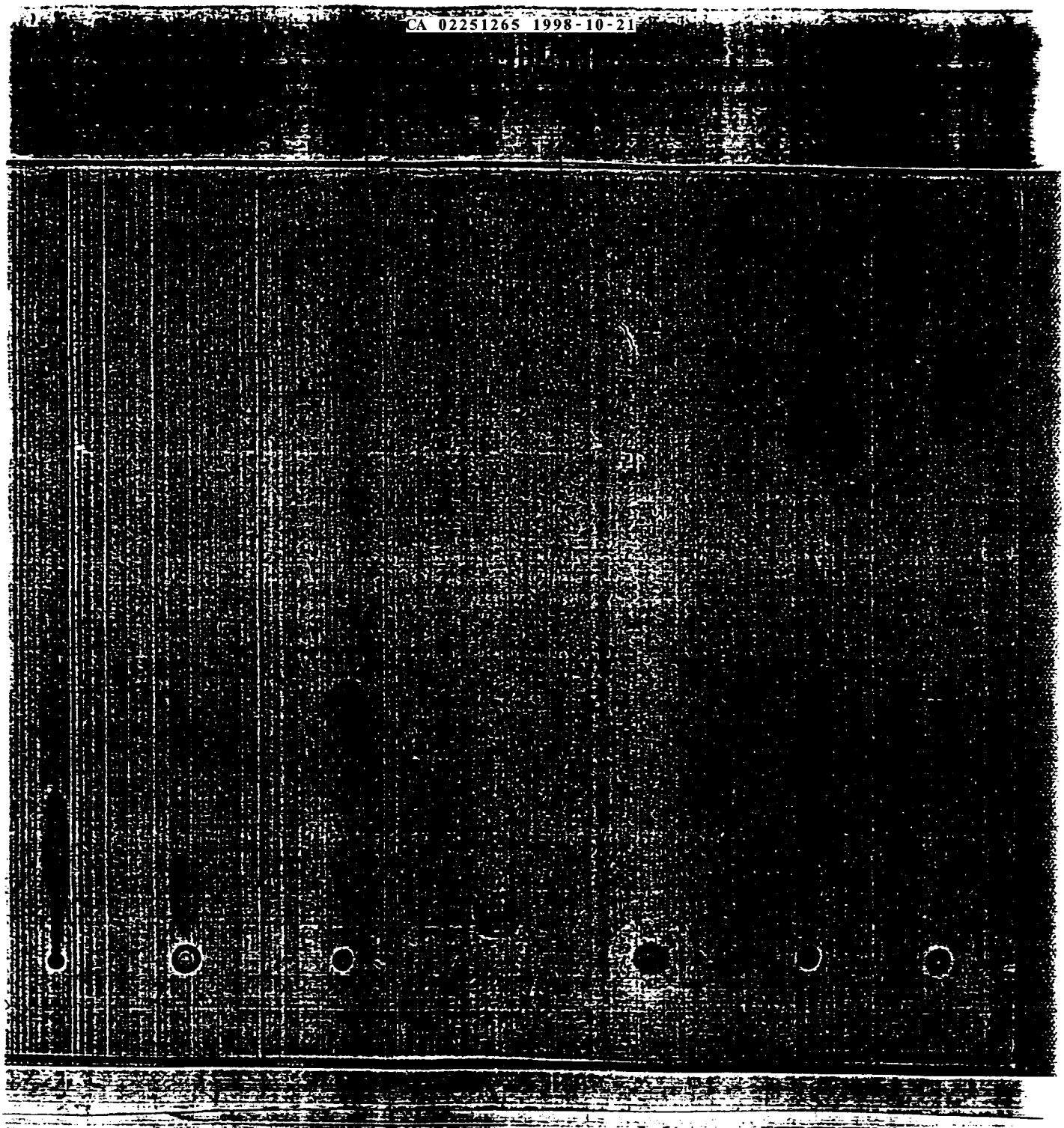


Figure 6: Thin-layer chromatography of neutral lipids of *E. pacifica* (acetone), *E. Pacifica* (ethanol), egg (acetone), cholesterol 20 mg/mL, sample of other interest, *Calanus* sp. (acetone) and *Calanus* sp. (ethanol). Hexane-ethyl ether-acetic acid (90:10:1, v/v).

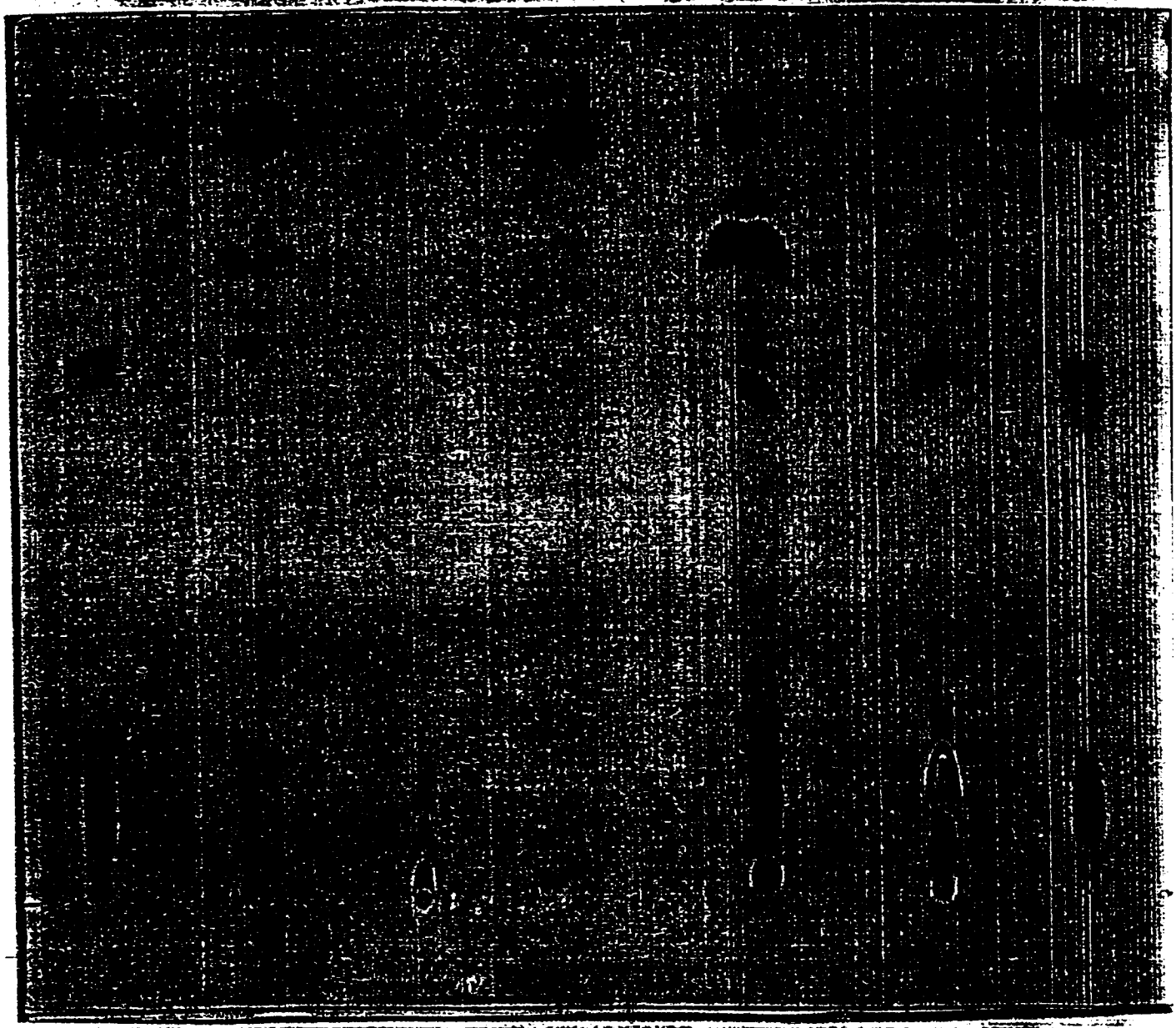


Figure 7: Thin-layer chromatography of phospholipids of *Calanus* sp. (acetone), *Calanus* sp. (ethanol), cholesterol 20 mg/mL, *M. norvegica* (acetone), *M. norvegica* (ethanol) and egg (acetone). Chloroform-methanol-water (80:25:2, v/v).

CONFIDENTIEL

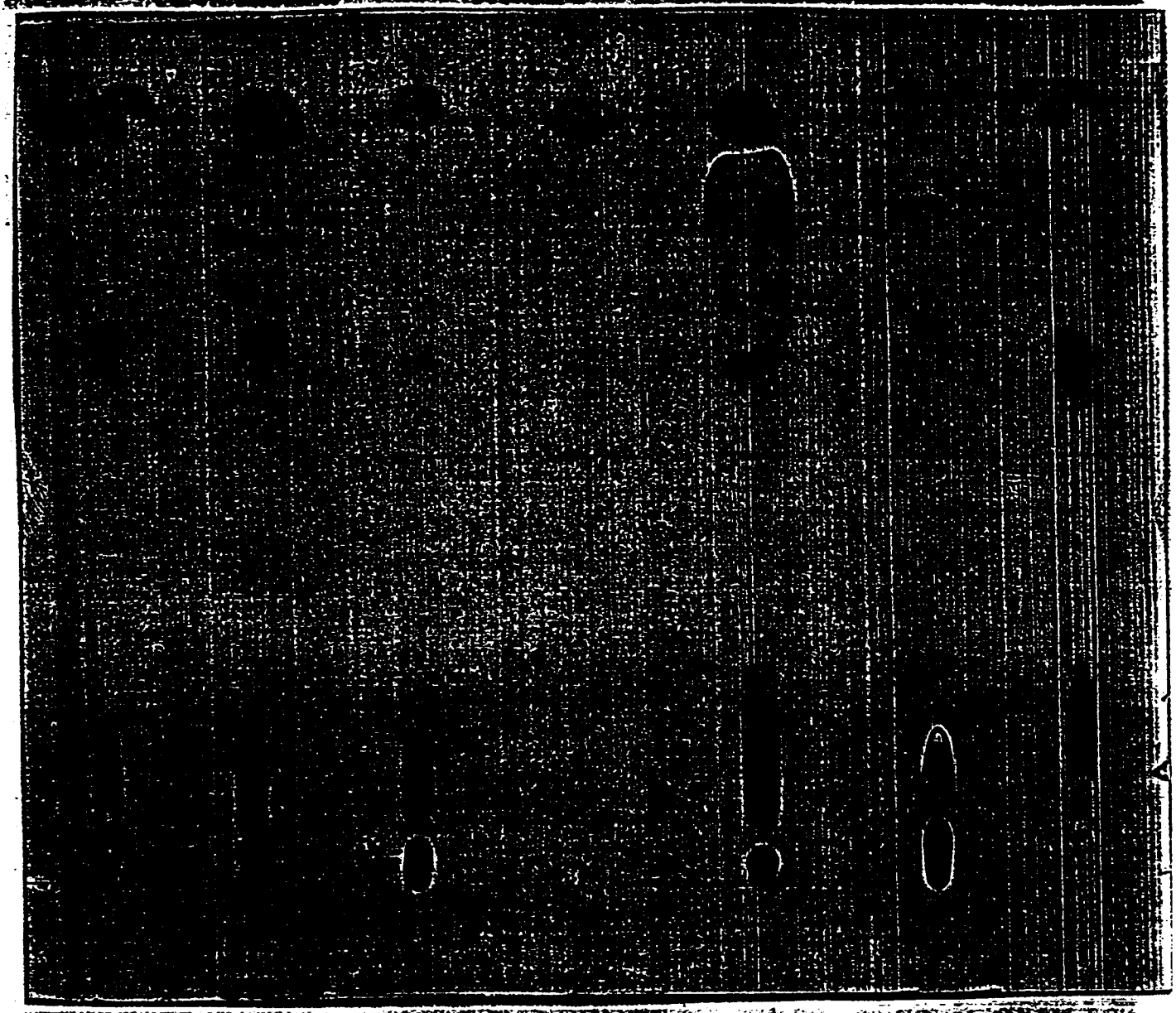


Figure 8: Thin-layer chromatography of phospholipids of *Calanus* sp. (acetone), *Calanus* sp. (ethanol), cholesterol 20 mg/mL, *E. pacifica* (acetone), *E. pacifica* (ethanol) and egg (acetone). Chloroform-metahnol-water (80:25:2), v/v).

FIGURE 9. INFLUENCE OF INCUBATION TIME IN ACETONE ON LIPID EXTRACTION (E. pacifica).

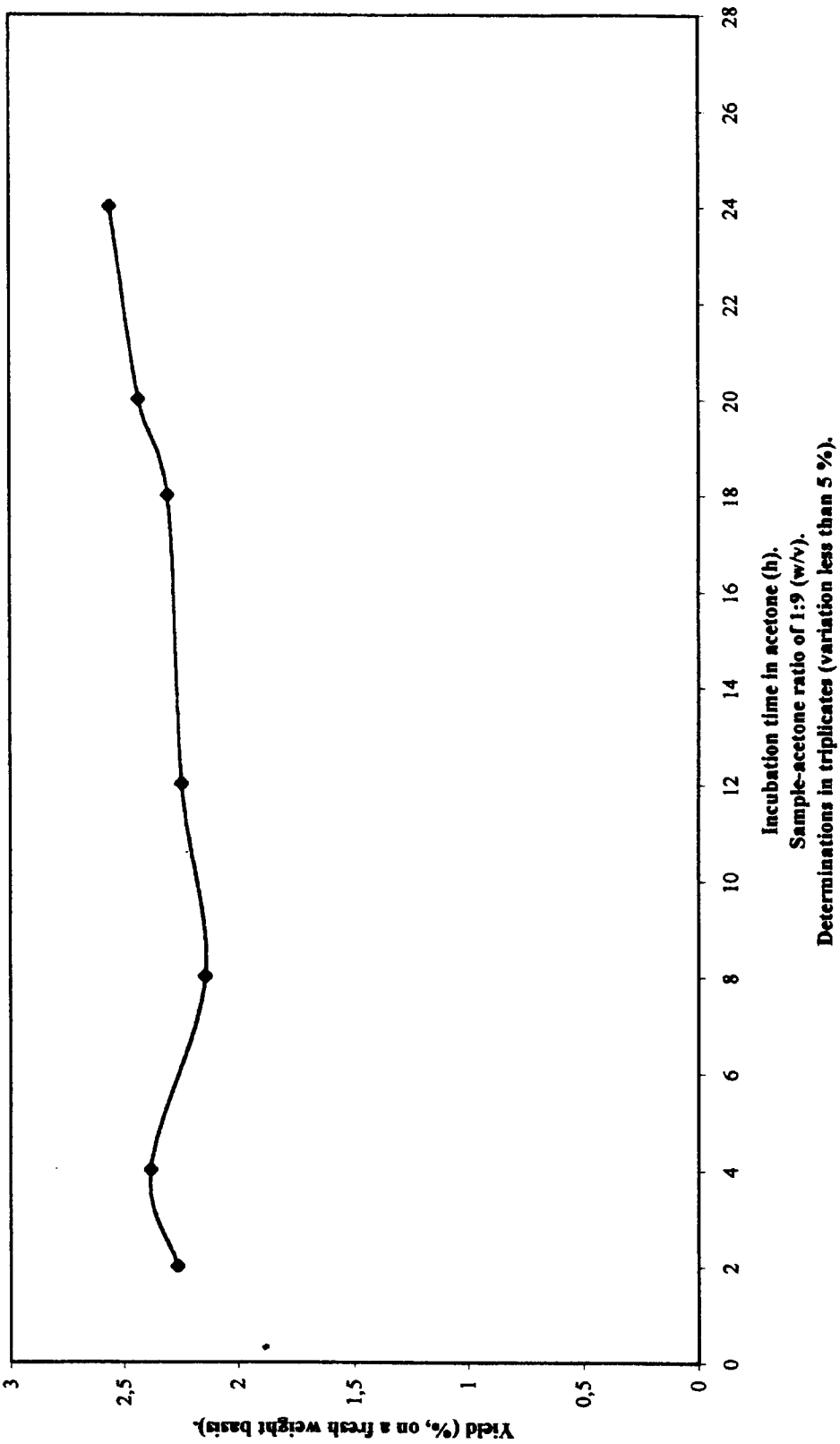
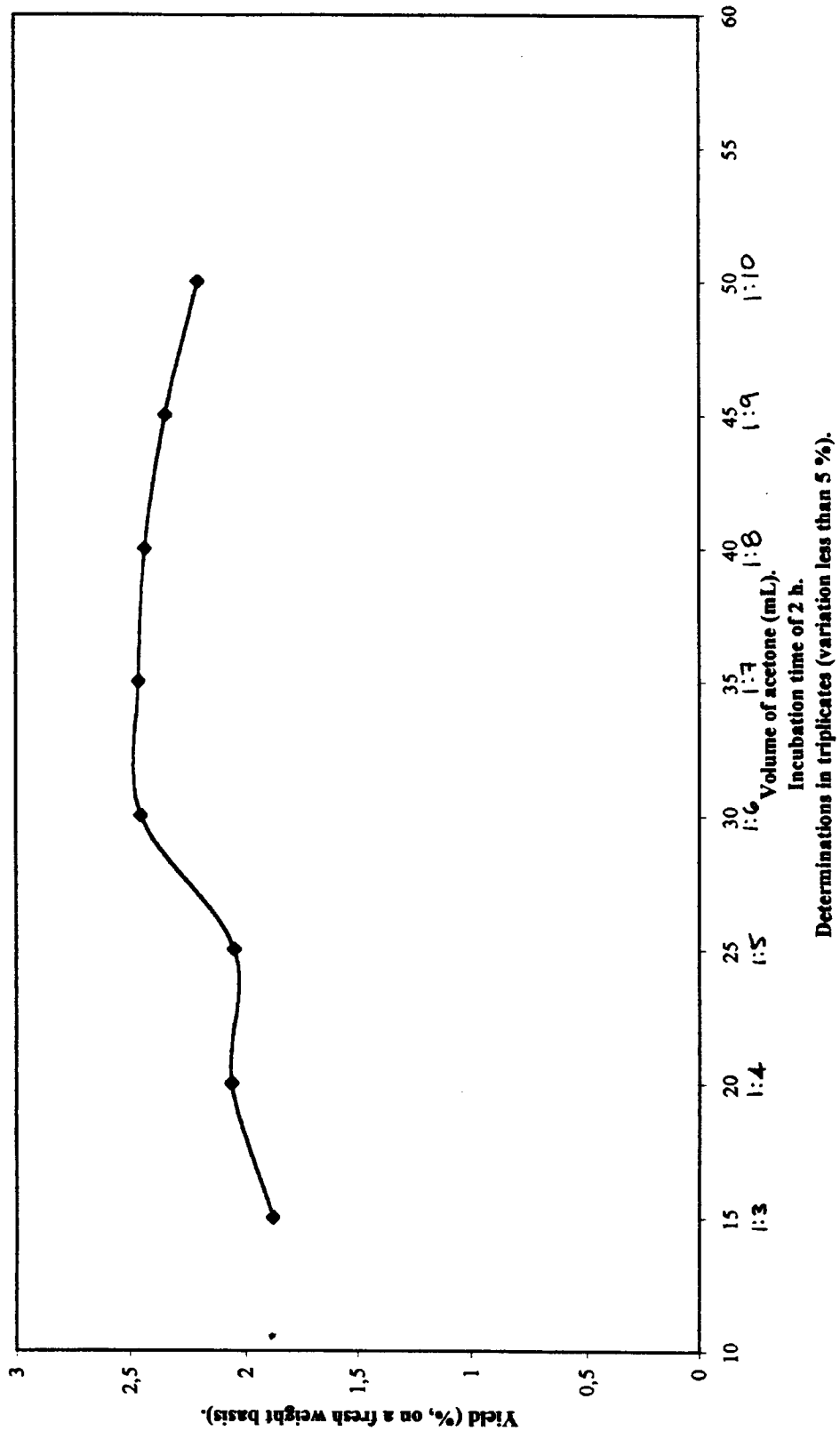


FIGURE 10. INFLUENCE OF THE VOLUME OF ACETONE ON LIPID EXTRACTION (E. pacifica).



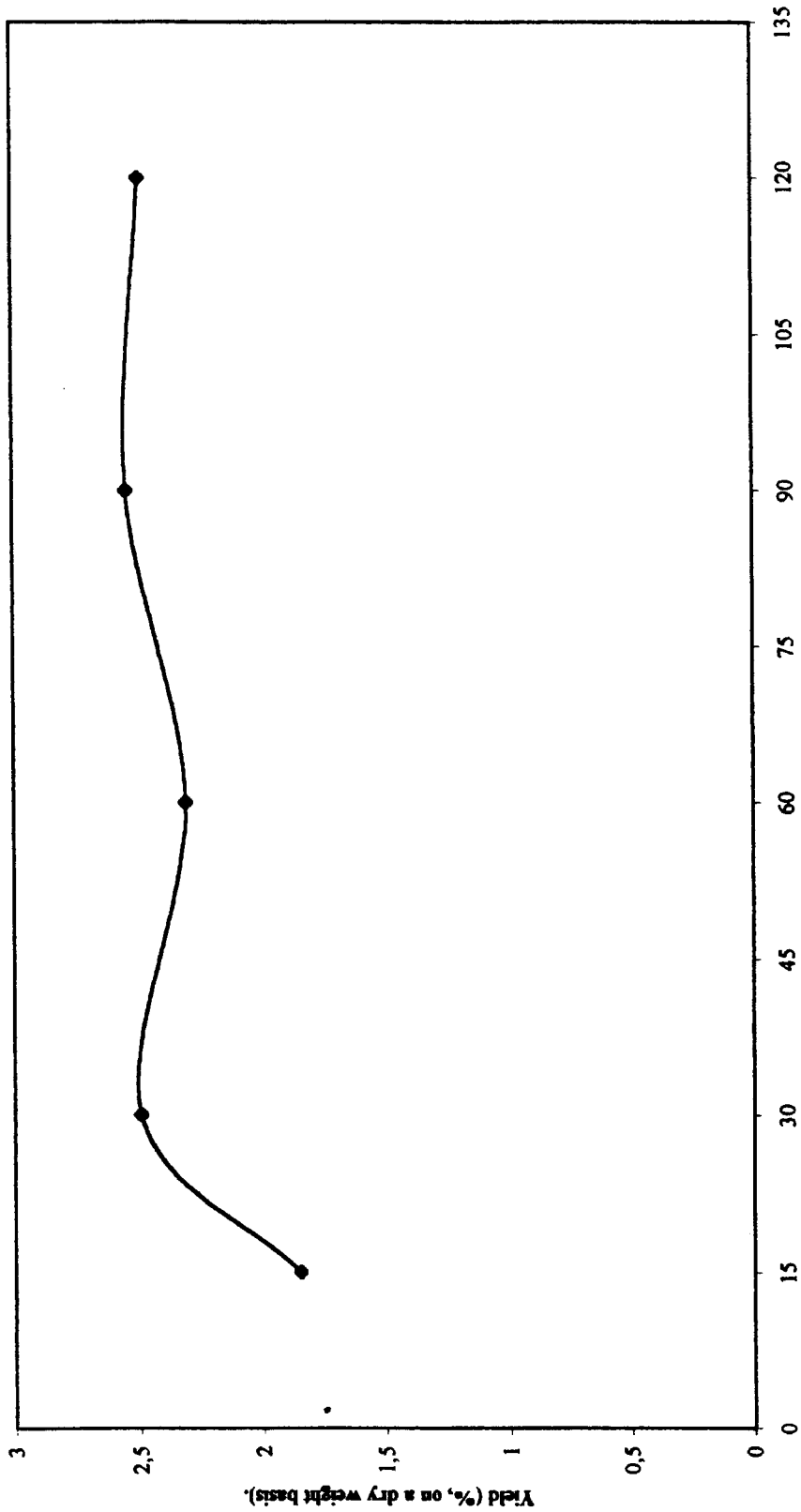
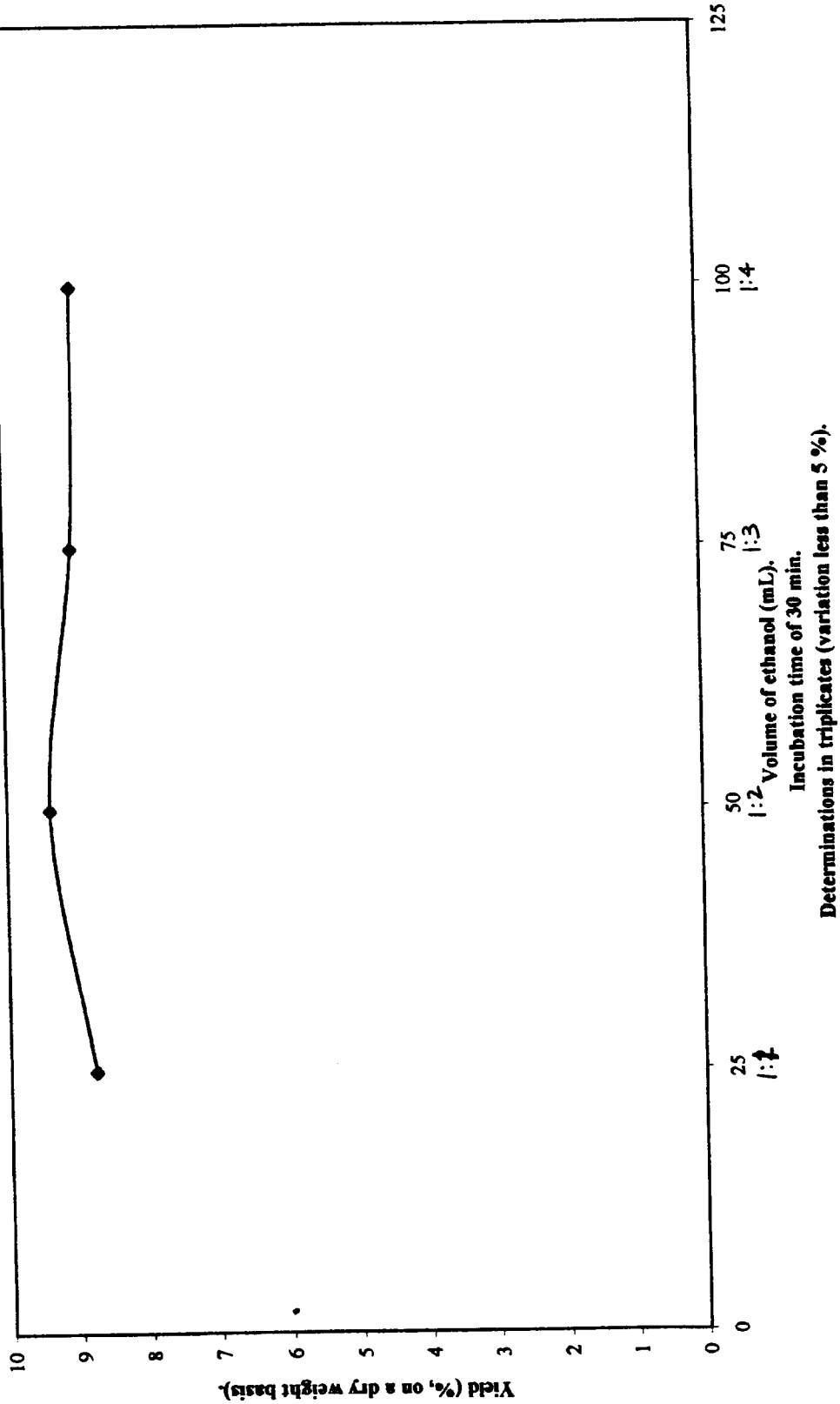


FIGURE 11. INFLUENCE OF INCUBATION TIME IN ETHANOL ON LIPID EXTRACTION (T. raschii).

FIGURE 12. INFLUENCE OF THE VOLUME OF ETHANOL ON LIPID EXTRACTION (E. pacifica).



⑲ 日本国特許庁（JP）　　⑪ 特許出願公開

⑫ 公開特許公報（A）　　昭60－153779

⑤Int.Cl.⁴	識別記号	庁内整理番号	㊸公開　昭和60年(1985) 8月13日
A 23 L 1/42		8412－4B	
A 61 K 9/48		6742－4C	
31/355	ADL	7330－4C	審査請求　未請求　発明の数 1　（全2頁）

㊹発明の名称　栄養補助食品

㉑特　願　昭59－10625

㉒出　願　昭59(1984) 1月24日

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明　細　書

1. 発明の名称

栄養補助食品

2. 特許請求の範囲

(1) ビタミンEおよび大豆レシチンをエイコサペンタエン酸含量の高い油に溶解してなる混合液状物をゼラチンのカプセル内に封入した栄養補助食品。

(2) エイコサペンタエン酸含量の高い油が、イワシ油、サバ油、イカ油、オキアミ油、ミンク鯨油等のごとき水産動物油である特許請求の範囲第(1)項記載の栄養補助食品。

3. 発明の詳細な説明

本発明はビタミンE、大豆レシチンおよびエイコサペンタエン酸含量の高い油を主成分とする新規な栄養補助食品に関するものである。

近年、①豊かな食生活がもたらす栄養バランスの偏り、②嗜好優先の食生活がもたらす偏食、過剰摂取、③運動、休息、栄養の健康保持バランスのくずれによる栄養損失、④高齢化社会に対応し得る補助栄養の必然性、等の要素を背景として栄養補助食品の需要が急激に増加しており、特に成人病は食生活の改善によって予防せんとする思想が強いため栄養補助食品が好まれて食されている。

本発明はこのような食生活上のニーズから導かれたものであり、①細胞の老化を防ぐ、②コレステロール値を下げて動脈硬化を防止する、③過酸化脂質の発生をおさえて細胞の活性化を促す、④血管を浄化して脳卒中や心筋梗塞を防止する等の効能を有するビタミンEと、①ビタミンEならびにエイコサペンタエン酸の吸収を促進する、②コレステロールを低下させて動脈硬化を防止する等の効能を有する大豆レシチンと、抗血小板凝集作用に基づく抗血栓、抗動脈硬化等の効能を有するエイコサペンタエン酸含量の高い油を組合せた新規な栄養補助食品を提供せんとするものである。

すなわち、本発明は、ビタミンEおよび大豆レシチンをエイコサペンタエン酸含量の高い油に溶解してなる混合液状物をゼラチンのカプセル内に封入した栄養補助食品である。

本発明において使用するビタミンEは、公知の製造法、例えば、植物油の不ケン化物を分子蒸留あるいはクロマトグラフイー等によって濃縮する方法で得られたものが適当であるが、その製造法は限定されるものではなく、また、その起源も限定されない。

小麦胚芽油、サフラワー油、米油、コーン油等の液状植物油中にはビタミンEが多く含まれているが、この含有量はせいぜい0.3％以下であるためこれをそのまま使用することは好ましくない。

本発明におけるビタミンEの配合量は、カプセル内に封

入する液状物全体中に占める割合が少なくとも1%必要であり、これ以下では生体内での生理活性作用が劣り、前記のごときビタミンEの効能が十分得られない。
また、ビタミンEと併用する大豆レシチンは、通常、大豆油の脱ガム工程で副生するガム質を脱水、乾燥して得られる大豆油を含んだ大豆リン脂質（所謂大豆レシチン）が適当であるが、アセトン、アルコール等により精製または濃縮されたレシチンを用いてもよく、また、ケファリン含量の少ないもしくはケファリン含量のない分別レシチンを使用することもできる。
この大豆レシチンが、カプセル内に封入する液状物全体中に占める割合は少なくとも1%必要であり、これ以下では前記のごとき大豆レシチンの効能が十分得られない。
さらに、ビタミンEと大豆レシチンを溶解するエイコサペンタエン酸（$C_{20:5}$）含量の高い油は、イワシ油、サバ油、イカ油、オキアミ油、ミンク鯨油等のごときエイコサペンタエン酸を8%以上含有する水産動物油の精製油、あるいはこれらの油から分別法等の手段によってエイコサペンタエン酸を濃縮して得られる油等が使用できる。
本発明におけるエイコサペンタエン酸含量の高い油の配合量は、カプセル内に封入する液状物全体中に占める割合が少なくとも10%必要であり、これ以下ではエイコサペンタエン酸の生体内での生理活性作用が劣り、前記のごときエイコサペンタエン酸の効能が十分得られない。
ビタミンEおよび大豆レシチンをエイコサペンタエン酸含量の高い油に溶解してなる混合液状物は、必要に応じ、これら各成分の有効濃度を維持できる範囲内において、小麦胚芽油、サフラワー油、米油、コーン油、大豆油、菜種油等の液状植物油で希釈することができる。
このようにして得られた混合液状物は、次いで、常法に従ってゼラチンのカプセル内に封入する。
この封入方法の一例としては、混合液状物をゼラチン、グリセリン、および水を溶融後射出成型したゼラチンカプセルに所定量注入し、その後、注入口を加熱密封して本発明の栄養補助食品を製造する。
ゼラチンカプセルの形状は球形、ラクビーボール形等任意である。
このようにして得られた本発明の栄養補助食品は、細胞の老化を防ぎ、コレステロール値を下げ、過酸化脂質の発生をおさえ、血管を浄化する等の作用を有するビタミンEと、ビタミンEおよびエイコサペンタエン酸の吸収を促進し、コレステロールを低下させる等の作用を有する大豆レシチンと、抗血小板凝集による抗血栓および抗動脈硬化等の作用を有するエイコサペンタエン酸を含有するものであるから、これら各生理活性成分の相互作用によって、血中コレステロールを下げ、高血圧を防ぎ、細胞を若返らせて活性化するほか、心筋梗塞、脳梗塞のごとき血栓性疾患等、循環器系成人病の治療および予防に効果を有する等、健康食品としての機能を発揮し得るものである。

次に本発明の実施例を示す。

実施例1.

ビタミンE 25重量部および大豆レシチン25重量部を精製イワシ油（エイコサペンタエン酸含量約16%）50重量部に混合し、約60℃に加温、撹拌して均一に溶解した。
一方、ゼラチン60重量部、グリセリン30重量部、水10重量部を均一に混合し、フイルム状にした後、容量約300mlのカプセル状に射出成型してゼラチン容器を製造した。
この容器に前記の混合液状物を注入し、しかる後、注入口を加熱密封して本発明の栄養補助食品を得た。

実施例2.

ビタミンE 30重量部および大豆レシチン30重量部を、市販のエイコサペンタエン酸濃縮油（日本油脂KK製、サンオメガ、エイコサペンタエン酸含量約25%）とサフラワー油を1：1の重量割合で混合したエイコサペンタエン酸含量の高い油40重量部に混合し、約60℃に加温、撹拌して均一に溶解した。
一方、ゼラチン60重量部、グリセリン30重量部、水10重量部を均一に混合し、フイルム状にした後、容量約300mlのカプセル状に射出成型してゼラチン容器を製造した。
この容器に前記の混合液状物を注入し、しかる後、注入口を加熱密封して本発明の栄養補助食品を得た。

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(54) Title of Invention Nutritional Supplement

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Specification

1. Title of Invention

Nutritional supplement

2. Scope of Claims

(1) A nutritional supplement being contained inside a gelatin capsule and comprising a nutritional liquid being formed by melting vitamin E, soybean lecithin in an oil having high eicosapentaenoic acid content.

(2) A nutritional supplement of claim 1 wherein the oil having high eicosapentaenoic acid content is a sea animal oil such as pilchard oil, mackerel oil, cuttlefish oil, krill oil, or mink oil.

3. Detailed Explanation of the Invention

The present invention relates to a new nutritional supplement having as its primary ingredients vitamin E, soybean lecithin, and an oil having high eicosapentaenoic acid content. In recent years, the demand for nutritional supplements has greatly increased due to factors such as (1) lack of nutritional balance brought about by a rich food culture, (2) pickiness and lack of food diversity caused by a selective food palette, (3) lack of nutrition brought about by a breakdown in balance of nutrition and health, exercise, and energy, and (4) the necessity for supplementary nutrition to correspond to an aging population. In particular, the desire to stem adult disease by the improvement of food life is strong, so people enjoy eating nutritional supplements.

The present invention arises out of the aforementioned nutritional needs and its object is to provide a new nutritional supplement from the combination of vitamin E, which (1) prevents the aging of cells, (2) lowers cholesterol and prevents the hardening of arteries, (3) prevents the occurrence of liquid fatty deposits and encourages cell life, (4) cleans and washes blood vessels and prevents brain or heart blood clots; soybean lecithin, which (1) encourages the absorption of vitamin E and eicosapentaenoic acid, (2) reduces cholesterol and prevents hardening of blood vessels and oil having high eicosapentaenoic acid content, which is effective anti-platelet aggregation, making it an anti-coagulant and acts to prevent the hardening of blood vessels.

In other words, the present invention is a nutritional supplement consisting of vitamin E and soybean lecithin melted in an oil having high eicosapentaenoic acid content in liquid form inserted into a gelatin capsule.

As for the vitamin E used in the present invention, items obtained by publicly known methods such as concentration by chromatography or molecular distillation of plant oils are appropriate, but the method of production thereof is not limited, and the source of energy is also not limited.

Vitamin E is included in great amounts in safflower oil, rice oil, corn oil, and other oil from water dwelling plants. However, the concentration

of the oil is at most 0.3%, so using any of these as-is is not desirable.

The amount of vitamin E included in the present invention must be at least 1% of the liquid included in the capsule. If less than this concentration is achieved the activity of the vitamin in the body is reduced, and the benefits of vitamin E cannot be achieved.

Also, soybean fatty substance containing soybean oil obtained by dehydrating and drying gum produced by the production of soybean gum is generally acceptable for the soybean lecithin used in conjunction with vitamin E, but it is also acceptable to use lecithin that has been sugared and concentrated via acetone or alcohol, and it is also possible to use separated lecithin that contains little of no keratin.

This soybean lecithin must be present in the liquid that is inserted into the capsule in a concentration of at least 1%. As noted before, if the concentration falls below this level the effects of the substance will not be sufficient. In addition, for oil with a high concentration of eicosapentaenoic acid (C20 : 5) that has vitamin E and soybean lecithin dissolved within it, an oil with a eicosapentaenoic acid content of 8% or higher may be used, such as that found in sea animal oils such as pilchard oil, mackerel oil, cuttlefish oil, krill oil, or mink oil, or an oil that was derived from one of the above and had was concentrated with respect to eicosapentaenoic acid content.

The concentration of oil having a high concentration of eicosapentaenoic acid in the present invention must be at least 10% of the volume of liquid in the capsule. If this concentration is not achieved then the effect of the oil in the body will be lacking and as noted before the effects will not be sufficient. A nutritional supplement formed by melting vitamin E and soybean lecithin in oil with a high concentration of eicosapentaenoic acid, within the scope wherein it is possible to maintain the relative levels of the various substances, may be diluted with safflower oil, rice oil, corn oil, soybean oil, or seaweed oil or other sea life oil. Mixed oil obtained in this way is inserted into a gelatin capsule under normal circumstances.

As one example of the method of sealing the substance in a capsule, the mixed liquid will be sealed in a gelatin capsule in a certain amount, the gelatin formed by melting gelatin, glycerin, and water. After that, the entry hole will be heated and sealed to create the nutritional substance of the present invention.

The form of the gelatin capsules may be spherical or in the form of a rugby ball.

Because the nutritional supplement of the present invention obtained in this way contains vitamin E which prevents the aging of cells, lowers cholesterol numbers, prevents excess fat deposits, and has other positive side effects, as well as contains soybean lecithin, which acts to lower cholesterol and promote the absorption of eicosapentaenoic acid, and contains eicosapentaenoic acid, which acts to prevent the hardening of blood vessels, the combined effects of the various ingredients act to reduce the cholesterol levels in the blood, prevent high blood pressure, reduce fat, and promote heart and brain function in brain and heart medical patients, and acts to treat and prevent adult circulatory diseases, and as such can be said to function as a nutritional supplement.

Below, the embodiments of the present invention are explained.

Embodiment 1.

25 parts vitamin E and 25 parts soybean lecithin are dissolved into 50 parts fish oil (containing 16% eicosapentaenoic acid), heated to around 60 degrees Celsius, and dried.

Then 60 parts gelatin, 30 parts glycerin, and 10 parts water are uniformly mixed, are made to take a film like form, are poured into a 300 milligram capsule and put into a gelatin container. The aforementioned compound is poured into this container, the entry point is heat sealed, and the nutritional supplement of the present invention is formed.

Embodiment 2

30 parts vitamin E by weight and 30 parts soybean lecithin are dissolved into a 1:1 mixture of safflower oil and market concentrated eicosapentaenoic acid oil (produced by Nippon Yushi Co. Ltd., Sun Omega, and containing 25% eicosapentaenoic acid), the mixture being 40 parts. The liquid is heated to around 60 degrees Celsius and dried.

Then 60 parts gelatin, 30 parts glycerin, and 10 parts water are uniformly mixed, are made to take a film like form, are poured into a 300 milligram capsule and put into a gelatin container. The aforementioned compound is poured into this container, the entry point is heat sealed, and the nutritional supplement of the present invention is formed.

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最終頁に続く

(54)【発明の名称】　痴呆症状改善薬

(57)【要約】
【目的】　痴呆症状を速やかに改善する、副作用のない痴呆症状改善薬を提供する。
【構成】　ドコサヘキサエン酸を有効成分として含有することを特徴とする痴呆症状改善薬。
【効果】　痴呆症による意欲の低下、せん妄、対人関係の悪化、徘徊、落ちつきのなさ等の精神症状、および／または痴呆症による計算能力の低下、判断力の低下、高次機能の低下等の知的機能の低下を改善する。

【特許請求の範囲】
【請求項１】　ドコサヘキサエン酸を有効成分として含有することを特徴とする痴呆症状改善薬。
【請求項２】　痴呆症状が痴呆症による精神症状である、請求項１に記載の痴呆症状改善薬。
【請求項３】　精神症状が、痴呆症による意欲の低下、せん妄、対人関係の悪化、落ちつきのなさ、および／または徘徊である、請求項２に記載の痴呆症状改善薬。
【請求項４】　痴呆症状が痴呆症による知的機能の低下である、請求項１に記載の痴呆症状改善薬。
【請求項５】　知的機能の低下が、痴呆症による計算能力の低下、判断力の低下、および／または高次機能の低下である、請求項１に記載の痴呆症状改善薬。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は痴呆症に伴う痴呆症状を改善する薬剤に関し、更に詳しくは、ドコサヘキサエン酸を有効成分として含有することを特徴とする痴呆症状改善薬に関するものである。
【０００２】
【従来技術】近年の人口の高齢化に伴い、痴呆症に対する薬剤の開発は医学的にも社会的にもますます重要な課題となっている。例えば、痴呆症患者の意欲の低下およびせん妄、あるいは対人関係でのトラブル等により、家族との関係が悪化して家庭介護が困難となることは最も大きな問題として指摘されている。従来種々の薬剤が痴呆症状改善薬として開発されてきたが、その効果は必ずしも満足しうるものではないばかりか、頭痛、めまい、自発性低下、感情障害、胃腸障害等の副作用を伴うことが多く、より優れた痴呆症状改善薬に対する期待は大きい。
【０００３】ドコサヘキサエン酸は脳や網膜の興奮性膜に多く含まれている不飽和脂肪酸で、アラキドン酸カスケードを阻害する作用を有していることが知られている。またこのほかに、幾つかの有用な生理作用を有することが知られており、例えば、脳機能改善組成物、学習能力増強剤、記憶力増強剤、痴呆予防剤、痴呆治療剤、または脳機能改善効果を有する機能性食品（特開平２－４９７２３号）、コリン作動性薬剤（特開平１－２７９８３０号）、血栓症治療剤（特開昭５７－３５５１２号）等の特許出願がなされている。これらの中で、特開平２－４９７２３号はドコサヘキサエン酸による学習能力や記憶力の増強及び血小板凝集の抑制作用を明らかにしているにすぎず、痴呆症状の改善については具体的開示は全くない。また、特開平１－２７９８３０号はドコサヘキサエン酸によりコリンエステラーゼ阻害剤であるフイゾスチグミンの脳への送達量が増加することに関するものである。
【０００４】
【発明が解決しようとする課題】本発明は、痴呆症状を速やかに改善する、副作用のない痴呆症状改善薬を提供することにある。
【０００５】
【課題を解決するための手段】本発明者らは、健康食品として広く知られているドコサヘキサエン酸を痴呆症患者に投与すると、その痴呆症状が速やかに改善されるという新たな知見に基づき、本発明を完成するに至った。
【０００６】すなわち、本発明は、ドコサヘキサエン酸を有効成分として含有することを特徴とする痴呆症状改善薬を提供する。
【０００７】本発明の痴呆症状改善薬は、多発梗塞性痴呆、脳血管性痴呆、脳機能障害による痴呆、ならびにアルツハイマー型痴呆等の痴呆症に随伴する精神症状（例えば、意欲の低下、せん妄、対人関係の悪化、落ちつきのなさ、徘徊等）あるいは知的機能の低下（例えば、計算能力の低下、判断力の低下、高次機能の低下等）などに適用される。
【０００８】本発明に用いるドコサヘキサエン酸とは、遊離酸をはじめ、その塩、エステル、グリセリド、リン脂質、コリン化合物、アスコルビン酸化合物、アミノ酸化合物等を意味するものである。このドコサヘキサエン酸を含む油としては、好ましくは総脂肪酸中のドコサヘキサエン酸（遊離酸として）の占める割合が１０％以上のものが良く、このようなものの例を上げるとイワシ、サバ、アジ、サケ、サンマなどの青背魚より抽出した魚油、マグロやカツオなどの大型海産魚の眼窩脂肪由来の魚油、微生物由来の油脂、オキアミ油、タラやイカ肝臓より抽出した海産物由来の油脂などが好ましい例として挙げられる。
【０００９】本発明の痴呆症状改善薬は治療のために経口的あるいは非経口的に投与することができる。経口投与剤とては散剤、顆粒剤、カプセル剤、錠剤などの固形製剤あるいはシロップ剤、エリキシル剤などの液状製剤とすることができる。また、非経口投与剤として注射剤とすることができる。これらの製剤は活性成分に薬理学的、製剤学的に認容される製造助剤を加えることにより常法に従って製造される。更に公知の技術により持続性製剤とすることも可能である。当該製造助剤を用いる場合は、本発明の痴呆症状改善薬中のドコサヘキサエン酸（遊離酸として）の配合量は通常は１０～１００重量％、好ましくは５０～１００重量％である。
【００１０】上記製造助剤としては、内服用製剤（経口剤）、注射用製材（注射剤）、粘膜投与剤（バッカル、トローチ、坐剤等）、外用剤（軟膏、貼付剤等）などの投与経路に応じた適当な製剤用成分から使用される。
【００１１】例えば、経口剤および粘膜投与剤にあっては、賦形剤（例：澱粉、乳糖、結晶セルロース、乳糖カルシウム、メタケイ酸アルミン酸マグネシウム、無水ケイ酸）、崩壊剤（例：カルボキシメチルセルロース、カルボキシメチルセルロースカルシウム）、滑沢剤（例：

ステアリン酸マグネシム、タルク）、コーテング剤（例：ヒドロキシエチルセルロース、白糖、ヒドロキシプロピルセルロース、ポリビニルピロリドン）、矯味剤などの製剤用成分が使用される。

【００１２】顆粒剤を製造するには湿式又は乾式造粒し、錠剤を製造するにはこれらの散剤及び顆粒剤をそのままあるいはステアリン酸マグネシウム、タルクなどの滑沢剤を加えて打錠すればよい。これらの顆粒又は錠剤はヒドロキシプロピルメチルセルロースフタレート、メタアクリル酸、メタアクリル酸メチルコポリマーなどの腸溶性基剤で被覆して腸溶性製剤、あるいはエチルセルロース、カルナウバロウ、硬化油などで被覆して持続性製剤とすることもできる。また、カプセル剤を製造するには散剤又は顆粒剤を硬カプセルに充填するか、活性成分をそのままあるいはグリセリン、ポリエチレングリコール、ゴマ油、オリーブ油などに溶解したのちゼラチン膜で被覆し軟カプセル剤とすることができる。

【００１３】経口投与用の液状製剤を製造するには活性成分と白糖、ソルビトール、グリセリンなどの甘味剤とを水に溶解して透明なシロップ剤、更に精油、エタノールなどを加えてエリキシル剤とするか、アラビアゴム、トラガント、ポリソルベート８０、カルボキシメチルセルロースナトリウムなどを加えて乳剤又は懸濁剤としてもよい。これらの液状製剤には所望により矯味剤、着色剤、保存剤などを加えてもよい。

【００１４】また注射剤にあっては、水性注射剤を構成し得る溶解剤ないし溶解補助剤（例：注射用蒸留水、生理食塩水、プロピレングリコール）、懸濁化剤（例：ポリソルベート８０などの界面活性剤）、ｐＨ調整剤（例：有機酸またはその金属塩）、安定剤などの製剤用成分が使用される。

【００１５】注射剤を製造するには活性成分を必要に応じ塩酸、水酸化ナトリウム、乳剤、乳酸ナトリウム、リン酸一水素ナトリウム、リン酸二水素ナトリウムなどのｐＨ調整剤、塩化ナトリウム、ブドウ糖などの等張化剤とともに注射用蒸留水に溶解し、無菌沪過してアンプルに充填するか、更にマンニトール、デキストリン、シクロデキストリン、ゼラチンなどを加えて真空下凍結乾燥し、用時溶解型の注射剤としてもよいし、活性成分にレシチン、ポリソルベート８０、ポリオキシエチレン硬化ヒマシ油などを加えて水中で乳化せしめ注射用乳剤とすることもできる。

【００１６】さらに外用剤にあっては、水性ないし油性の溶解剤ないし溶解補助剤（例：アルコール、脂肪酸エステル類）、粘着剤（例：カルボキシビニルポリマー、多糖類）、乳化剤（例：界面活性剤）などの製剤用成分が使用される。直腸投与剤を製造するには活性成分及びカカオ脂、脂肪酸のトリ、ジ及びモノグリセリド、ポリエチレングリコールなどの坐剤用基剤とを加湿して溶融し型に流しこんで冷却するか、活性成分をポリエチレングリコール、大豆油などに溶解したのちゼラチン膜で被覆すればよい。

【００１７】その他、上記構成を有する本発明の痴呆症状改善薬は、公知の製造法、例えば日本薬局方第１０版製剤総則記載の方法ないし適当な改良を加えた方法によっても製造することができる。

【００１８】とくに本発明の痴呆症状改善薬は、高純度の（例えば９０％以上）のドコサヘキサエン酸を軟カプセル剤の形態で投与するのが、投与が簡便な点で好ましい。

【００１９】本発明の痴呆症状改善薬の有効成分であるドコサヘキサエン酸の投与量は、患者の体重、症状等により異なるが、一般には一日当たり、１００～２０００ｍｇ／人程度であり、一日１回～数回に分けて投与する。

【００２０】以下、本発明を実施例により詳細に説明する。

【００２１】

【実施例】

試験例１．　精神症状の改善度評価試験

脳血管性痴呆患者１３名、アルツハイマー型痴呆患者（アルツハイマー病と老年痴呆）５名の対象者に対して、従来の薬剤に加えてＤＨＡ７０ｍｇ入りカプセルを１０錠～２０錠投与（以下、ＤＨＡ投与群と略）し、ＤＨＡの投与前と投与６ヶ月後の検査結果を比較検討した。併せて、従来の薬剤治療を継続した群（以下、投与不変群と略；２４名）を対照群として同様の検査を施行し、ＤＨＡ投与群との変化を比較した。結果を表１に示す。

【００２２】

【表１】

表１．　精神症状の改善度

	改善	やや改善	不変	悪化
脳血管性痴呆	9	1	2	1
アルツハイマー型痴呆	0	5	0	0

【００２３】なお、脳血管性痴呆における改善の内容は、主にせん妄が改善された症例が２例、主に意欲が改

善された症例が３例、主に対人関係が改善された症例が３例、主に徘徊が改善された症例が１例であった。また、アルツハイマー型痴呆においては、意欲、対人関係、落ちつきが改善された症例が、おのおの３例、１例、１例であった。投与不変群は、この間、症状の変化はみられず、全例不変と評価された。

【００２４】試験例２．　知的機能低下の改善度評価試験

試験例１と同じ対象者に対して、計算力、判断力及び高次機能の３項目を知的機能の簡易評価検査とし、コース立方体組合せテストを動作性知能の簡易評価検査とした。検査は、投与前と投与後６ヶ月後の計２回行い、結果は統計学的に処理をした。結果を表２に示す。

【００２５】

【表２】

表２．　知的機能の改善度

	DHA投与群		投与不変群	
知的機能	投与前（試験開始時）	6ヶ月後	試験開始時	6ヶ月後
計算力総点	6.2±3.3	6.9±3.0	3.4±2.9	3.1±3.3
判断力総点	4.6±3.0	5.5±3.3	4.1±2.9	2.6±2.4
高次機能	5.1±2.9	6.0±2.9	3.6±3.3	2.4±2.8

【００２６】なお、コース立方体およびＩＱ（動作性）試験においても、投与前後で改善が認められた。

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(54) [Title of the Invention] Medicine for Improvement of Dementia Symptoms

(57) [Abstract]
[Objective] To smoothly improve the symptoms of dementia and provide a medicine for said improvement without side effects.
[Structure] A medicine for improvement of dementia symptoms that has as a characteristic the inclusion of docosahexaenoic acid (DHA).
[Effect] The medicine improves the following ailments caused by dementia: loss of will, delirium, worsening of human relationships, loitering, manic psychological episodes and/or the reduction of powers of calculation, reduction of judgment, and reduction in the intellectual capacities and functioning of the higher functions.

[Scope of Claims]
[Claim 1] A medicine for improvement of dementia symptoms being characterized by including as an active ingredient DHA.
[Claim 2] A medicine for improvement of dementia symptoms of claim 1 that treats an adverse psychological state that is dementia.
[Claim 3] A medicine for improvement of dementia symptoms of claim 2 working to reduce will loss, delirium, worsening of human relationships, manic states, and/or loitering.
[Claim 4] A medicine for improvement of dementia symptoms of claim 1 working to reduce the loss of higher functions and of judgment brought about by dementia.
[Claim 5] A medicine for improvement of dementia symptoms of claim 1 working to reduce the loss of intellectual capacity, loss of facilities of calculation, loss of judgment, and/or loss of higher function due to dementia.

[Detailed Description of the Invention]
[0001]
[Industrial Field of Use] The present invention is in relation to a medicine for the betterment of mental symptoms that accompany dementia, and in particular relates to a medicine for the improvement of dementia symptoms that includes as an active ingredient DHA.
[0002]
[Related Art] With the aging of society in recent years, the development of medicine for the treatment of dementia has become more important both medically and socially. For example, a dementia patient may suffer worsening family relationships as a result of loss of will, delirium, or trouble in interpersonal relationships, and the looking after of the patient within the family becomes difficult. This has been pointed out as the most serious cause for concern. In past years many medicines have been developed for dementia, but the results haven't always been satisfactory. Furthermore, the traditional medicine can cause headache, dizziness, reduction in sex drive, emotional disturbances, and other side effects such as damage to the stomach. It is with this that there has been great expectation for the development of a new medicine for dementia.
[0003] DHA is present in abundance in the brain and the thick mucus membranes. DHA is known to stop the functioning of arachidonic acid. Also, in addition to this, it is known that DHA contains several useful biological functions. For example, the following patent applications have been made: substance for the increase of brain function, medicine for the improvement of academic performance, medicine for improvement of memory, dementia prevention substance, substance for the treatment of dementia, and functional food that improves brain function (Hei 2 – 49723), cholinergic agent (Hei 1 – 279830), agent for the treatment of thrombosis (Sho 57 – 35512), among others. Among these, patent application Hei 2 – 49723 shows that DHA can aid in the improvement of academic ability as well as increasing memory performance, and also acts to prevent the formation of platelet aggregation. However, this application said nothing more and did not hint at the specific application of DHA to dementia. Also, Application Hei 1 – 279830 is in relation to the increase of transmission volume to the brain of physostigmine, a cholinesterase antagonist, via the DHA.
[0004]
[Problem Solved by the Invention] The present invention provides a medicine to improve with the symptoms of dementia without providing side effects.
[0005]
[Method of Solving the Problem] The inventors of the present invention gave DHA, widely known for being a health food, to dementia patients, whereupon the symptoms of the dementia were immediately lessened, and based on that discovery gathered to file this application.
[0006] In other words, the present invention provides a medicine for the improvement of dementia symptoms that includes DHA.
[0007] The medicine for the improvement of dementia symptoms of the present invention is applied to psychological states accompanying dementia from multiple infarction, brain blood vessel function, brain damage, or Alzheimer's disease (such as loss of will, delirium, worsening of human relationships, mania, loitering, etc.) or the reduction in intellectual capabilities (for example a reduction in the powers of calculation, a reduction in judgment, or a reduction in higher order functions).
[0008] The DHA used in the present invention is an isolated acid, and refers to salt, ester, glyceride, phospholipids, choline compounds, ascorbic acid compounds, amino acid compounds. As for the oil that includes the DHA, an inclusion ratio of 10% or more DHA (as an isolated acid) within general fatty acids. As an example of such an oil, the fish oil extracted from blue backed fish such as Japanese pilchard, mackerel, horse mackerel, salmon, and Pacific saury, the fish oil from large ocean fish eye oil, such as that of the tuna or the shipjack tuna, oil coming from microorganisms, krill oil, and oil from industrial products extracted from the livers of Pacific cod and dolphins.
[0009] The medicine for the improvement of the symptoms of dementia of the present invention may be administered either orally or non-orally. For oral administration, powder, granule, capsule, lozenge, and other solid forms of administration are acceptable. Alternatively, the medicine may be administered as syrup, elixir, and other liquid forms. Also, for non-oral administration an injection can be given. By adding these forms of manufacturing to the approved medicine that is the active portion of the drug the medicine may be manufactured in the normal fashion. Furthermore, it is also possible to turn the medicine into extended release tablets via publicly known methods. When using those manufacturing helper substances the DHA levels within the medicine for the improvement of the symptoms of dementia of the present invention is between 10 and 100 % by weight, and preferably between 50 and 100 % by weight.
[0010] An appropriate manufacturing helper substance will be used in the above in accordance with the administration method, for example, internal use substances (oral medicine), injection use substances (injected medicine), adhesive administration substances (buccal, troche, and suppositories).
[0011] For example, in oral and adhesive administration excipients (example: starch, milk sugar, crystal cellulose, milk calcium, metakei acid aluminum acid magnesium, waterless silicic acid), collapse agents (example: carboxymethylcellulose, carboxymethyl cellulose calcium), lubricants (example: sterin acid magnesium, talc), coatings (example: hydroxyl methyl

cellulose, sugar, hydroxyl propyl cellulose), and taste making agents, and other production substances may be used.

[0012] In order to manufacture granules, wet or dry droplets are formed, and in order to produce pills, it is permissible to form the tablets with the powder and granules either left as they are or with additional stearic acid magnesium, talc, or other lubricant. These granules or tablets are coated with a stomach settling agent such as hydroxypropyl – methyl cellulose phthalate or methacrylic acid or methacrylic acid methyl copolymer, among others, and coating is made using stomach setting agent or ethyl cellulose, carnauba wax, hardened oil, or other substance. By doing so a durable pharmaceutical product may be produced. Also, in order to produce the medicine in capsule form, the powder or granules are filled into a hard capsule or the active ingredients are coated with a gelatin film either as is or after being melted into gelatin, polyethelyn glycol, sesame oil, olive oil, or other oil. In this way it is possible to generate a soft capsule.

[0013] In order to produce liquid medicine for oral administration, the active ingredient and a sweetener such as refined sugar, sorbitol, glycerol are dissolved in water, a clear syrup, essential oil, and ethanol are added making an elixir-like medicine, or alternatively gum arabic, tragacanth gum, polysorbate 80, carboxymethyl cellulose (CMC), or another such substance is added and an emulsion or a suspension is produced. This is also acceptable. Flavor agents, color changing agents, and/or preservatives may be added to the liquid solutions discussed herein, according to taste.

[0014] Also, stable production medicine components are used for injectable medicine, such as solution from water soluble injectable medicine and melted helper substances (example injection use distilled water, biological salt water, or propylene glycol), suspension substances (example: polysorbate 80 or other surfactant), pH regulation substances (example: organic acid or its metal salt).

[0015] In order to produce injection-use medication, the active ingredients are mixed with salts, sodium hydroxide, emulsion, emulsion natrium, dibasic sodium phosphate, sodium dihydrogen-phosphate, and other pH adjusting agents, sodium chloride, grape sugars, and other tonicity adjusting agents in injection use distilled water. The solution is sterilized and poured into an ampoule. Alternatively, mannitol, dextrin, cyclo-dextrin, gelatin, and other substances are added, fired into crystals under vacuum conditions, and placed into a form to be melted at the time of injection. To the active ingredients are added lecithin, polysolvent 80, polyoxyethylene hydrogenated castor oil, and other substances, melted into water and made into an injectable solution.

[0016] Additionally, water or oil soluble medicines or soluble helper substances (example: alcohol, fatty acid esters), adhesives (example: carboxy vinyl polymer multi-sugars), emulsifiers (example: surfactants), and other substances are used as ingredients in externally administrable medicine. In producing rectally administered medicine the active ingredients and cocoa butter, fatty acid salts, monoglycerides and other suppository use substances are humidified, melted, poured into a mold, hardened, and frozen. Alternatively, the active ingredient could be melted in polyethylene glycol, soybean oil, or other oil, and thereafter coated in a gelatin film.

[0017] Additionally, the medicine for the improvement of dementia symptoms of the present invention with the above listed characteristics may be produced using publicly known manufacturing methods, for example as stipulated in version 10 of the Pharmacy Act of Japan, noted in the manufacturing addendum, or a method that has appropriately modified the aforementioned method.

[0018] In particular, the medicine for the improvement of dementia of the present invention administration of a high purity concentration of DHA (for example, 90% or above) via a soft capsule is desirable because of the ease of administration.

[0019] The amount of DHA administered in the medicine to prevent the symptoms of dementia of the present invention will vary based on the body weight and health conditions of the patient, but in general, the dose will range from 100 to 2000 mg / person with between one and several administrations per day.

[0020] Below we explain the present invention in detail by following an embodiment of the present invention.

[0021]

[Embodiments]

Embodiment 1. Test to measure level of psychological improvement

The targets of this test were 13 cranial blood vessel related dementia patients and 5 Alzheimer's related dementia patients. In addition to traditional treatments, 10 – 20 capsules including 70 mg of DHA each were administered (hereinafter referred to as the "DHA Administration Group"), and the results of the test were compared before administration and 6 months after administration. Also, a group that continued traditional pharmaceutical treatments (hereinafter referred to as the "Unchanging Administration Group"; 24 individuals) were targeted for the same test and the variance from the DHA Administration Group was observed. The results appear in Table 1.

[0022]

[Table 1]

Table 1. Level of Improvement in Psychological State

	Recovered	Somewhat Recovered	No Change	Worsened
Cranial Blood Vessel Dementia	9	1	2	1
Alzheimer's Dementia	0	5	0	0

[0023] To further break down the content of the "improvements" seen in the cranial blood vessel related dementia patients, 2 cases of improvement in delirium were seen, 3 cases of greatly improved ambition were observed, 3 cases of improved loitering were observed. Also, among the Alzheimer's dementia patients we observed 1 case each of improved ambition, human relationships, and manic states, respectively, for a total of 3 observed improvements. The Unchanging Administration Group did not show any change in symptoms in this same period, and all cases were evaluated to have no change.

[0024] Embodiment 2. Test to measure improvement of loss of intellectual capacity. The calculation skills, judgment, and higher functions of the same test group as test 1 were evaluated. This test was a simple evaluation of intellectual abilities. Also, a course correction and pathfinding test was administered as a simple measure of motor control. The test was administered twice, once before administration of DHA and once 6 months after the administration of DHA. The results were statistically aggregated. The results are shown in Table 2.

[0025]

[Table 2]

Table 2. Level of Improvement of Intellectual Abilities

	DHA Admin Group		Unchanged Group	
Intellectual Ability	Preadmin (at test start time)	6 months post – admin	Pre-admin	6 months post - admin
Calculation Abilities Total	6.2 +- 3.3	6.9 +- 3.0	3.4 +- 2.4	3.1 +- 3.3
Judgment Total	4.6 +- 3.0	5.5 +- 3.3	4.1 +- 2.9	2.6 +- 2.4
Higher Function Total	5.1 +- 2.9	6.0 +- 2.9	3.6 +- 3.3	2.4 +- 2.8

[0026] Note that in both the motor and IQ tests, improvements were seen after the administration of DHA.

Continued from the front page

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First Named Inventor/Applicant Name:	Inge Bruheim
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44	Non Patent Literature	Declaration-Suzanne-Budge-8278351.pdf	3927519 0859756fa0a9d02754485cf66be15b452d57f85c	no	18
Warnings:					
Information:					
45	Non Patent Literature	Declaration-Suzanne-Budge-8383675.pdf	3927521 5c1e6f4a5b93ef8110d44fb8b212b8ca79d8374f	no	18
Warnings:					
Information:					
46	Non Patent Literature	Declaration-Thomas-Brenna-8278351.pdf	3695838 2581add7e497f969b6f6aa945fa7db91ef831477	no	244
Warnings:					
Information:					
47	Non Patent Literature	Declaration-Thomas-Brenna-8383675.pdf	3695838 2581add7e497f969b6f6aa945fa7db91ef831477	no	244
Warnings:					
Information:					
48	Non Patent Literature	Declaration-Thomas-Gundersen-8030348.pdf	8097462 801cce0cc9e8f1605c45969b59d9160562d45c74	no	67
Warnings:					
Information:					
49	Non Patent Literature	Declaration-Tina-Sampalis-8030348.pdf	5362024 88f2571deff892828a032272ae8893565e30228f	no	32
Warnings:					
Information:					
50	Non Patent Literature	Declaration-Van-Breemen-351.pdf	7328482 14fd532b4e75c4a9c8f248a19c1dc128661a411e	no	114
Warnings:					
Information:					
51	Non Patent Literature	Declaration-Van-Breemen-348.pdf	7582523 dc90897063f7959998aecc49dc82b0363e0cc256	no	114
Warnings:					
Information:					
52	Non Patent Literature	Declaration-Yeboah-8030348.pdf	8949960 8d8d99f6f57e09d61ba7cff1660b054b6c51d0b2	no	80
Warnings:					

Information:					
53	Non Patent Literature	Declaration-Yeboah-8278351.pdf	1065747 d57ffffa765ec1d35e9ec0247ea0b202308e1b0d	no	13
Warnings:					
Information:					
54	Non Patent Literature	Eichberg_1939_pp51-54.pdf	1479216 d5f765669acaa03a4bae6e29e5228fc35ade3d24	no	4
Warnings:					
Information:					
55	Non Patent Literature	Expert-Witness-Report-Theodore-Welch-337-TA-877.pdf	3552129 bc261e2e3e05c7b19d49ec0eef0fd1f83b3ba1f5	no	60
Warnings:					
Information:					
56	Non Patent Literature	Farkas_1998.pdf	475393 21cdd944cb15e1ef2529902da6e0bd59b1778975	no	4
Warnings:					
Information:					
57	Non Patent Literature	Final-Prospectus-May-11-2001.pdf	5062909 68f848dd6669646a0dc2e7a5d4c640945e6fffbb	no	75
Warnings:					
Information:					
58	Non Patent Literature	FisheriesAgency-Fujita_1985_pp273-307.pdf	18832464 5c37c9e743942c8e8b4217a3323baeba7c3164e8	no	22
Warnings:					
Information:					
59	Non Patent Literature	Folch_1957_pp497-509.pdf	1435175 b8f92cd7cc9fce68b370c3b8fa511f26be4d2b10	no	15
Warnings:					
Information:					
60	Non Patent Literature	Grant-Request-Ex-parte-Reexamination-351-patent.pdf	629763 f323ea1e0ef0ca39c053b49e9c3273df9e2461b5	no	14
Warnings:					
Information:					
			Total Files Size (in bytes):	196128755	

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bruheim et al.	Group No.:	1651
Serial No.:	14/020,162	Examiner:	D. K. Ware
Filed:	06 September 2013		
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

INFORMATION DISCLOSURE STATEMENT LETTER

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

Sir or Madam:

The citations listed in the attached IDS Form PTO-SB08 may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97. The Examiner is requested to make these citations of official record in this application.

Applicants wish to bring to the Examiner's attention that we are not providing copies of US Patents or published US patent applications as instructed under 37 CFR 1.98(a)(2).

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

The Commissioner is hereby authorized to charge any required fees or credit any overpayments to Attorney Deposit Account No.: 50-4302, referencing Attorney Docket No.: AKBM-14409/US-6/CON.

Respectfully submitted,

Dated: 14 January 2014

/J. Mitchell Jones/
J. Mitchell Jones
Registration No. 44,174
Casimir Jones S.C.
2275 Deming Way
Suite 310
Middleton, WI 53562
Phone: (608) 662-1277
Fax: (608) 662-1276

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON

CONFIRMATION NO. 4914

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

PUBLICATION NOTICE

Title:BIOEFFECTIVE KRILL OIL COMPOSITIONS

Publication No.US-2014-0005421-A1
Publication Date:01/02/2014

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON

CONFIRMATION NO. 4914

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

POA ACCEPTANCE LETTER

OC000000064717843

Date Mailed: 11/01/2013

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/29/2013.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/hchristian/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

■ Practitioners associated with Customer Number: 72960

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number

Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

■ The address associated with Customer Number: 72960

OR

☐

Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

Assignee Name and Address: Aker BioMarine AS
Fjordalleen 16
P.O. Box 1423 Vika
Oslo, Norway 0115

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	[signature]	Date	October 28, 2013
Name	HALLVARD MURI	Telephone	+4724130000
Title	CEO		

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Inge Bruheim et al.

Application No./Patent No.: 14/020,162 Filed/Issue Date: 06-Sep-2013

Titled: BIOEFFECTIVE KRILL OIL COMPOSITIONS

Aker BioMarine AS, a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

   The document was recorded in the United States Patent and Trademark Office at

   Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

   The document was recorded in the United States Patent and Trademark Office at

   Reel _____, Frame _____, or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☑ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/J. Mitchell Jones/	October 28, 2013
Signature	Date
J. Mitchell Jones	44,174
Printed or Typed Name	Title or Registration Number

ASSIGNMENT

WHEREAS, WE, Inge Bruheim, Snorre Tilseth and Daniele Mancinelli, hereinafter referred to as "ASSIGNORS," have invented certain new and useful improvements as described and set forth in the below-identified application for United States Letters Patent:

Title of Invention: **BIOEFFECTIVE KRILL OIL COMPOSITIONS**

Filing Date: 06-Sep-2013 Serial No.: 14/020,162

WHEREAS, Aker BioMarine AS, a Norway corporation having a place of business at Fjordallèen 16, P. O. Box 1423 Vika, Oslo, Norway 0115, hereinafter referred to as "ASSIGNEE," is desirous of acquiring the entire right, title and interest in said invention and application and in any Letters Patent which may be granted on the same;

NOW THEREFORE, TO ALL WHOM IT MAY CONCERN: Be it known that, for and in consideration of the sum of One Dollar ($1.00) lawful money paid to ASSIGNORS by ASSIGNEE, and other good and valuable consideration, receipt of which is hereby acknowledged, ASSIGNORS have sold, assigned and transferred, and by these presents do sell, assign and transfer unto said ASSIGNEE, and ASSIGNEE's successors and assigns, all right, title and interest in and to said invention, said application for United States Letters Patent, and any Letters Patent which may hereafter be granted on the same in the United States and all countries throughout the world, including any divisions, renewals, continuations in whole or in part, substitutions, conversions, reissues, or prolongations or extensions thereof, said interest to be held and enjoyed by said ASSIGNEE as fully and exclusively as it would have been held and enjoyed by said ASSIGNORS had this assignment and transfer not been made, to the full extent and term of any Letters Patent.

ASSIGNORS further agree that ASSIGNORS will, without charge to said ASSIGNEE, but at ASSIGNEE's expense, cooperate with ASSIGNEE in the prosecution of said application and/or applications; execute, verify, acknowledge, and deliver all such further papers, including applications for Letters Patent and for the reissue thereof, and instruments of assignment and transfer thereof; and perform such other acts as ASSIGNEE lawfully may request, to obtain or maintain Letters Patent for said invention and improvement in any and all countries, and to vest title thereto in said ASSIGNEE, or ASSIGNEE's successors and assigns.

IN TESTIMONY WHEREOF, ASSIGNORS have hereunto signed ASSIGNORS' names to this Assignment on the date indicated below.

Inge Bruheim 20/9-13
Inge Bruheim Date

Snorre Tilseth Date

Daniele Mancinelli Date

STATE OF)
) ss.
COUNTY OF)

On this 20 day of September, in the year of 2013, before me, the undersigned Notary Public or Witness, personally appeared the above-named ASSIGNORS, known to me (or proven to me on the basis of satisfactory evidence) to be the persons whose names are subscribed to the within instrument, and acknowledged that they executed the same.

Karen K. Kilday
NOTARY PUBLIC or WITNESS

SEAL

MY COMMISSION EXPIRES: _____

ASSIGNMENT

WHEREAS, WE, Inge Bruheim, Snorre Tilseth and Daniele Mancinelli, hereinafter referred to as "ASSIGNORS," have invented certain new and useful improvements as described and set forth in the below-identified application for United States Letters Patent:

Title of Invention: **BIOEFFECTIVE KRILL OIL COMPOSITIONS**

Filing Date: 06-Sep-2013 Serial No.: 14/020,162

WHEREAS, Aker BioMarine AS, a Norway corporation having a place of business at Fjordalléen 16, P. O. Box 1423 Vika, Oslo, Norway 0115, hereinafter referred to as "ASSIGNEE," is desirous of acquiring the entire right, title and interest in said invention and application and in any Letters Patent which may be granted on the same;

NOW THEREFORE, TO ALL WHOM IT MAY CONCERN: Be it known that, for and in consideration of the sum of One Dollar ($1.00) lawful money paid to ASSIGNORS by ASSIGNEE, and other good and valuable consideration, receipt of which is hereby acknowledged, ASSIGNORS have sold, assigned and transferred, and by these presents do sell, assign and transfer unto said ASSIGNEE, and ASSIGNEE's successors and assigns, all right, title and interest in and to said invention, said application for United States Letters Patent, and any Letters Patent which may hereafter be granted on the same in the United States and all countries throughout the world, including any divisions, renewals, continuations in whole or in part, substitutions, conversions, reissues, or prolongations or extensions thereof, said interest to be held and enjoyed by said ASSIGNEE as fully and exclusively as it would have been held and enjoyed by said ASSIGNORS had this assignment and transfer not been made, to the full extent and term of any Letters Patent.

ASSIGNORS further agree that ASSIGNORS will, without charge to said ASSIGNEE, but at ASSIGNEE's expense, cooperate with ASSIGNEE in the prosecution of said application and/or applications; execute, verify, acknowledge, and deliver all such further papers, including applications for Letters Patent and for the reissue thereof, and instruments of assignment and transfer thereof; and perform such other acts as ASSIGNEE lawfully may request, to obtain or maintain Letters Patent for said invention and improvement in any and all countries, and to vest title thereto in said ASSIGNEE, or ASSIGNEE's successors and assigns.

IN TESTIMONY WHEREOF, ASSIGNORS have hereunto signed ASSIGNORS' names to this Assignment on the date indicated below.

_____
Inge Bruheim Date

S. Tilseth 18. Sep. 2013
Snorre Tilseth Date

_____
Daniele Mancinelli Date

STATE OF)
) ss.
COUNTY OF)

On this 18 day of September, in the year of 2013, before me, the undersigned Notary Public or Witness, personally appeared the above-named ASSIGNORS, known to me (or proven to me on the basis of satisfactory evidence) to be the persons whose names are subscribed to the within instrument, and acknowledged that they executed the same.

Gerd K. Tilseth
NOTARY PUBLIC or WITNESS

SEAL

MY COMMISSION EXPIRES: _____

ASSIGNMENT

WHEREAS, WE, Inge Bruheim, Snorre Tilseth and Daniele Mancinelli, hereinafter referred to as "ASSIGNORS," have invented certain new and useful improvements as described and set forth in the below-identified application for United States Letters Patent:

Title of Invention: **BIOEFFECTIVE KRILL OIL COMPOSITIONS**

Filing Date: 06-Sep-2013 Serial No.: 14/020,162

WHEREAS, Aker BioMarine AS, a Norway corporation having a place of business at Fjordalléen 16, P. O. Box 1423 Vika, Oslo, Norway 0115, hereinafter referred to as "ASSIGNEE," is desirous of acquiring the entire right, title and interest in said invention and application and in any Letters Patent which may be granted on the same;

NOW THEREFORE, TO ALL WHOM IT MAY CONCERN: Be it known that, for and in consideration of the sum of One Dollar ($1.00) lawful money paid to ASSIGNORS by ASSIGNEE, and other good and valuable consideration, receipt of which is hereby acknowledged, ASSIGNORS have sold, assigned and transferred, and by these presents do sell, assign and transfer unto said ASSIGNEE, and ASSIGNEE's successors and assigns, all right, title and interest in and to said invention, said application for United States Letters Patent, and any Letters Patent which may hereafter be granted on the same in the United States and all countries throughout the world, including any divisions, renewals, continuations in whole or in part, substitutions, conversions, reissues, or prolongations or extensions thereof, said interest to be held and enjoyed by said ASSIGNEE as fully and exclusively as it would have been held and enjoyed by said ASSIGNORS had this assignment and transfer not been made, to the full extent and term of any Letters Patent.

ASSIGNORS further agree that ASSIGNORS will, without charge to said ASSIGNEE, but at ASSIGNEE's expense, cooperate with ASSIGNEE in the prosecution of said application and/or applications; execute, verify, acknowledge, and deliver all such further papers, including applications for Letters Patent and for the reissue thereof, and instruments of assignment and transfer thereof; and perform such other acts as ASSIGNEE lawfully may request, to obtain or maintain Letters Patent for said invention and improvement in any and all countries, and to vest title thereto in said ASSIGNEE, or ASSIGNEE's successors and assigns.

IN TESTIMONY WHEREOF, ASSIGNORS have hereunto signed ASSIGNORS' names to this Assignment on the date indicated below.

Inge Bruheim Date

Snorre Tilseth Date

Daniele Mancinelli 17/9/2013
Date

STATE OF)
) ss.
COUNTY OF)

On this 17 day of September, in the year of 2013, before me, the undersigned Notary Public or Witness, personally appeared the above-named ASSIGNORS, known to me (or proven to me on the basis of satisfactory evidence) to be the persons whose names are subscribed to the within instrument, and acknowledged that they executed the same.

Henrik Kildahl
~~NOTARY PUBLIC~~ or WITNESS

SEAL

MY COMMISSION EXPIRES: _____

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Inge Bruheim, et al.	Confirmation No.:	4914
Serial No.:	14/020,162	Group No.:	TBD
Filed:	06-Sep-2013	Examiner:	TBD
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

INVENTORS' OATH OR DECLARATION UNDER 37 C.F.R. 1.63 TRANSMITTAL LETTER

EFS WEB FILED
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir or Madam:

Applicant files herewith Form PTO/AIA/01 executed by the inventors for the above-referenced patent application. The oath or declaration fee in the amount of $140 was paid on September 6, 2013.

Should any additional fees be due, the Commissioner is hereby authorized to charge any required fees or credit any overpayments to Attorney Deposit Account No.: 50-4302, referencing Attorney Docket No.: AKBM-14409/US-6/CON.

Respectfully submitted,

Date: October 28, 2013

/J. Mitchell Jones/
J. Mitchell Jones
Registration No. 44,174
Casimir Jones S.C.
2275 Deming Way, Suite 310
Middleton, WI 53562
Tel.: 608-662-1277
Fax.: 608-662-1276

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/020,162 filed on 06-Sep-2013

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Inge Bruheim Date (Optional): 20/9-13

Signature: Inge Bruheim

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/020,162

filed on 06-Sep-2013

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Daniele Mancinelli Date (Optional): 17/9/2013

Signature: Daniele Mancinelli

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/020,162 filed on 06-Sep-2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Snorre Tilseth Date (Optional): 4. Sep-2013

Signature: S. Tilseth

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

Electronic Acknowledgement Receipt

EFS ID:	17239289
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Mallory Checkett
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON
Receipt Date:	29-OCT-2013
Filing Date:	06-SEP-2013
Time Stamp:	15:47:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	14409US6CONPowerofAttorneyforsubmission.pdf	303371 163d30681ea18b36b255e53b93f59b03f638f36d	no	6

Warnings:	
Information:	

2	Transmittal Letter	14409US6CONDeclarationTransmittal.pdf	78372 113678ca475f9eb88b524e0b3dcfe947946792a7	no	1
Warnings:					
Information:					
3	Oath or Declaration filed	14409US6CONDeclarationsEXEC.pdf	239051 4e654da35fdfd722f3330d99b1d1ce200d3bb407	no	3
Warnings:					
Information:					
			Total Files Size (in bytes):	620794	

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

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

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/020,162	09/06/2013	1653	1740	AKBM-14409/US-6/CON	11	2

CONFIRMATION NO. 4914

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

FILING RECEIPT

OC000000063953065

Date Mailed: 09/26/2013

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Inge Bruheim, Volda, NORWAY;
Snorre Tilseth, Bergen, NORWAY;
Daniele Mancinelli, Orsta, NORWAY;

Applicant(s)

AKER BIOMARINE AS, Oslo, NORWAY

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 12/057,775 03/28/2008
which claims benefit of 60/920,483 03/28/2007
and claims benefit of 60/975,058 09/25/2007
and claims benefit of 60/983,446 10/29/2007
and claims benefit of 61/024,072 01/28/2008

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 09/23/2013

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/020,162**

Projected Publication Date: 01/02/2014

Non-Publication Request: No

Early Publication Request: No

Title

BIOEFFECTIVE KRILL OIL COMPOSITIONS

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON

CONFIRMATION NO. 4914

72960
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

NOTICE

OC000000063953066

Date Mailed: 09/26/2013

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Inge Bruheim
  Snorre Tilseth
  Daniele Mancinelli

  Applicant may submit the inventor's oath or declaration at any time before the Notice of Allowance and Fee(s) Due, PTOL-85, is mailed.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/020,162

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1600

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

References cited in parent application to be filed in new continuation

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware
Attorney Docket Number	NATNUT-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2004/112767	WO		2004-12-29	Advanced Bionutrition Corp.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	NATNUT-14409/US-5/ORD

	1	European Search Report, EP Patent Application No. EP12187516, mailed June 10, 2013	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware
Attorney Docket Number	NATNUT-14409/US-5/ORD

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☒ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2013-08-01
Name/Print	J. Mitchell Jones	Registration Number	44174

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware, Deborah K.
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2002322233	AU		2003-02-17	Neptune Technologies & Bioressources, Inc.		☐
	2	04057853	JP		1992-02-25	CHLORINE ENG CORP LTD		☒

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware, Deborah K.
	Attorney Docket Number	AKBM-14409/US-5/ORD

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	CN Office Action mailed April 27, 2012, JP Patent Application No. 200880112125.6 (and English translation)	☒
	2	FRICKE, et al., Lipid, Sterol and Fatty Acid Composition of Antarctic Krill (Euphausia superba Dana), Lipids (1984) 19 (11): 821-827.	☐
	3	FRICKE, et al., 1-O-Alkylglycerolipids in Antarctic Krill (Euphausia Superba Dana), Comp. Biochem. Physiol. (1986) 85B(1): 131-134	☐
	4	GORDEEV, K.Y., et al. "Fatty Acid Composition of the Main Phospholipids of the Antarctic Krill, Euphausia superba," Chem. Nat. Cmpds. (1990) 26(2), pp. 143-147	☐
	5	GRANTHAM (1977) Southern Ocean Fisheries Survey Programme, FAO Rome, GLO/SO/77/3: 1-61.	☐
	6	RAVENTOS et al., Application and Posssibilities of Supercritical CO2 Extraction in Food Processing Industry: An Overview, Food Science and Technology International (2002) 8: 269-284	☐
	7	TANAKA, T., et al., Platelet-activating Factor (PAF)-like Phospholoipds Formed during Peroxidation of Phosphatidylcholines from Different Foodstuffs, Biosci. Biotech. Biochem. (1995) 59 (8), pp. 1389-93	☐
	8	WINTHER, et al., Elucidation of Phosphatidylcholine Composition in Krill Oil Extracted from Euphausia superba, Lipids (2011) 46: 25-36	☐

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Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2012-11-15
Name/Print	J. Mitchell Jones	Registration Number	44174

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Notice of References Cited	Application/Control No. 12/057,775	Applicant(s)/Patent Under Reexamination BRUHEIM ET AL.	
	Examiner DEBBIE K. WARE	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2003/0113432	06-2003	Yoshitomi et al.	426/643
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
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	J	US-			
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First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware
Attorney Docket Number	NATNUT-14409/US-5/ORD

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	1	JP-A-S52-114046	JP		1977-09-24	Kokai		☐
	2	JP-A-S51-125774	JP		1976-11-02	Nichiro Gyogyo et al.		☐

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	1	JP Office Action mailed February 23, 2012, JP Patent Application No. 2010-522444 (and English translation)	☐

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Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2012-03-21
Name/Print	J. Mitchell Jones	Registration Number	44174

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	1	2003-530448	JP		2003-10-14	Westfalia Separator Industry GmbH		☐

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	1	December 8, 2011 Office Action, KR Patent Application No. 10-2010-7006897 and its English translation	☐

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	2	8030348		2011-10-04	Sampalis, Fotni	
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	1	2004-534800	JP		2004-11-18	Kohyo		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware, Deborah K.
	Attorney Docket Number	NATNUT-14409/US-5/ORD

	2	07/080515	WO		2007-07-19	Aker Biomarine ASA		☐

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	1	SIKORSKI, E., "The Utilization of Krill For Food," Food Process Eng., 1:845-855 (1980)	☐
	2	BUDZINSKLI, E., et al., "Possibilities of processing and marketing of products made from Antarctic Krill", FAO Fish. Tech. Pap. (268) 46 pages (1985)	☐
	3	BUNEA R., et al.., "Evaluation of the Effects of Neptune Krill Oil on the Clinical Course of Hyperlipidemia," Alternative Medicine Review, Thorne Research Inc., Sandpoint, US, Vol. 9, No. 4, January 1, 2004	☐
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Examiner Name	Ware, Deborah K.
Attorney Docket Number	NATNUT-14409/US-5/ORD

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2012-01-24
Name/Print	J. Mitchell Jones	Registration Number	44174

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Notice of References Cited	Application/Control No. 12/057,775	Applicant(s)/Patent Under Reexamination BRUHEIM ET AL.	
	Examiner DEBBIE K. WARE	Art Unit 1651	Page 1 of 1

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2006/0193962	08-2006	Kamiya et al.	426/615
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Examiner Name	Susan Marie Hanley
Attorney Docket Number	NATNUT-14409/US-5/ORD

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	1	2000/25608	WO		2000-05-11	NIPPON SUISAN KAISHA, LTD.		☐
	2	2000/38708	WO		2000-07-06	PHAIRSON MEDICAL INC.		☐
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	Attorney Docket Number	NATNUT-14409/US-5/ORD

	4	2003/011873	WO		2003-02-13	NEPTUNE TECHNOLOGIES & BIORESSOURCES INC.		☐
	5	2005/004393	WO		2005-01-13	KONIN-KLIJKE PHILIPS ELECTRONICS N.V.		☐
	6	2005/037848	WO		2005-04-28	ENZYMOTEC LTD.		☐
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	15	2004/047554	WO		2004-06-10	PHARES PHARM RES NV		☐
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	17	1123368	EP		2008-04-09	UNIVERSITE DE SHERBROOKE		☐
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	21	1419768	EP		2009-01-07	NEPTUNE TECHNOLOGIES & BIORESSOURCES, INC.		☐
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	24	1392623	EP		2004-03-03	MARTEK BIOSCIENCES BOULDER CORPORATION		☐
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	Attorney Docket Number	NATNUT-14409/US-5/ORD

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	20	ENGLISH ABSTRACT; JP 2007-509131; See abstract from corresponding WO 2005/037848 filed herewith	☐
	21	ENGLISH ABSTRACT; JP 2007-518764; See abstract from corresponding WO 2005/070411 filed herewith	☐
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	23	ENGLISH ABSTRACT; JP 2006-502196; See abstract from corresponding WO 2004/028529 filed herewith	☐

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Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
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	1	20030044495		2003-03-06	KAGAN and BRAUN	

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	Art Unit	1636
	Examiner Name	
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	1	8701265	BR		1987-03-12	SATO		☐
	2	1098900	CA		1981-04-07	ROGOZHIN, et al		☐
	3	0609078	EP		1994-08-03	SCOTIA HOLDINGS PLC		☐
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	9	02049091	JP		1990-02-19	SUNTORY LTD		☐
	10	2215351	JP		1990-08-28	TAIYO FISHERY CO LTD.		☐
	11	2524217	JP		1996-08-14	TAIYO FISHERY CO LTD.		☐
	12	2963152	JP		1992-02-25	CHLORINE ENG CORP LTD		☐
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	14	3081692	JP		1994-07-19	CHLORINE ENG CORP LTD		☐
	15	3344887	JP		1997-07-08	IKEDA SHOKKEN KK		☐
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	Attorney Docket Number	NATNUT-14409/US-5/ORD

	20	4012665	JP		1992-01-17	MATSUSHITA ELECTRIC IND CO LTD		☐
	21	61281159	JP		1986-12-11	SHISEIDO CO LTD; NIPPON SUISAN KAISHA LTD.		☐
	22	2001-158736	JP	A	2001-06-12	SNOW BRAND MILK PROD CO LTD		☐
	23	2003-003192	JP	A	2003-01-08	UNITIKA LTD		☐
	24	2003-048831	JP	A	2003-02-21	SUNTORY LTD		☐
	25	2003-146883	JP	A	2003-05-21	SNOW BRAND MILK PROD CO LTD		☐
	26	2003-531857	JP	A	2003-10-28	HENDERSON		☐
	27	2004-525180	JP	A	2004-08-19	YEDA RESEARCH AND DEVELOPMENT CO. LTD.		☐
	28	2004-536059	JP	A	2004-12-02	MARTEK BIOSCIENCES BOULDER CORPORATION		☐
	29	2005-245379	JP	A	2005-09-15	NIPPON SUISAN KAISHA LTD		☐
	30	2006-069948	JP	A	2006-03-16	HIROSE YUKIHIRO		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	NATNUT-14409/US-5/ORD

	31	2006-083136	JP	A	2006-03-30	SUNTORY LTD		☐
	32	2006-290784	JP	A	2006-10-26	HIROSE YUKIHIRO		☐
	33	2006-316073	JP	A	2006-11-24	IBR ISRAELI BIOTECHNOLOGY RESEARCH LTD		☐
	34	2006-328014	JP	A	2006-12-07	HIROSE YUKIHIRO		☐
	35	2006-502196	JP	A	2006-01-19	SUNTORY LIMITED		☐
	36	2006-528233	JP	A	2006-12-14	BTG INTERNATIONAL LIMITED		☐
	37	2007-126455	JP	A	2007-05-24	FUJI CHEM IND CO LTD		☐
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	41	2007-518764	JP	A	2007-07-12	BRUZZESE		☐

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	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	NATNUT-14409/US-5/ORD

	42	220741	SU		1971-01-06	KRGUCHKOV		☐
	43	1986/06082	WO		1986-10-23	MAT-CON RADGIVENDE INGENIØRFIRMA A/S		☐
	44	1990/05765	WO		1990-05-31	MIKALSEN		☐
	45	1993/24142	WO		1993-12-09	PHAIRSON MEDICAL AB		☐
	46	1997/38585	WO		1997-10-23	THE UNIVERSITY OF BRITISH COLUMBIA		☐
	47	1997/39759	WO		1997-10-30	BRIGHAM AND WOMEN'S HOSPITAL		☐
	48	1998/34498	WO		1998-08-13	BIOZYME SYSTEMS INC.		☐
	49	1999/39589	WO		1999-08-12	BIOZYME SYSTEMS INC.		☐

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	ANDO and HATANO, 1988, "Isolation of apolipoproteins from carotenoid-carrying lipoprotein in the serum of chum salmon, Oncorhynchus keta", J. Lipid Research, 29: 1264-1271	☐

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	2	AOI et al., 2003, "Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice", Antioxidants & Redox Signaling, 5(1): 139-44	☐
	3	BRITTON, 1985, "General Carotenoid Methods", Methods in Enzymology, Vol 111, pp. 113-149	☐
	4	CALDER, 2006, "n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases", Am. J. Clin. Nutr., 83: 1505S	☐
	5	CHAREST et al., 2001, "Astaxanthin Extraction from Crawfish Shells by Supercritical CO2 with Ethanol as Cosolvent", J. Aquatic Food Product Technology, 10(3): 79-93	☐
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	7	CLARKE, 1980, "The Biochemical Composition of Krill, Euphausia superba dana,from South Georgia", J. Exp. Mar. Biol. Ecol., 43: 221-236	☐
	8	CZECZUGA, 1974, "Comparative Studies of Carotenoids in the Fauna of the Gullmar Fjord (Bohuslan, Sweden). II. Crustacea: Eupagurus bernhardus, Hyas coarctatus and Upogebia deltaura", Marine Biology, 28: 95-98	☐
	9	DE RITTER and PURCELL, 1981, "Carotenoid Analytical Methods", Carotenoids as Colorants and Vitamin A Precursors: Technological and Nutritional Applications, pp 815-882	☐
	10	DEUTCH, 1995, "Menstrual pain in Danish women correlated with low n-3 polyunsaturated fatty acid intake", Eur. J. Clin. Nutr., 49(7): 508-16	☐
	11	DIEZ et al., 2003, "The role of the novel adipocyte-derived hormone adiponectin in human disease", Eur. J. Endocrinol., 148(3): 293-300	☐
	12	ELLINGSEN et al., 1987, "Biochemistry of the autolytic processes in Antarctic krill post mortem. Autoproteolysis." Biochem. J. 246, 295-305	☐

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	13	EMODI, 1978, "Carotenoids: Properties and Applications", Food Technology, 32(5): 38	☐
	14	FELIX-VALENZUELA et al., 2001, "Supercritical CO2/Ethanol Extraction of Astaxanthin from Blue Crab (Callinectes Sapidus) Shell Waste", Journal of Food Process Engineering, 24: 101-112	☐
	15	FOX and SCHEER, 1941, "Comparative Studies of the Pigments of Some Pacific Coast Echinoderms", The Biological Bulletin, 441-455	☐
	16	FRICKE, et al., 1984, "Lipid, Sterol and Fatty Acid Composition of Antarctic Krill (Euphausia superba Dana)", Lipids, 19 (11): 821-827	☐
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	21	HARDARDOTTIR and KINSELLA, 1988, "Extraction of Lipid and Cholesterol from Fish Muscle with Supercritical Fluids" Journal of Food Science, 53(6): 1656-1658	☐
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	Art Unit	1636
	Examiner Name	
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	26	ITOH et al., 2007; "Increased adiponectin secretion by highly purified eicosapentaenoic acid in rodent models of obesity and human obese subjects", Arteriosclerosis, Thrombosis, and Vascular Biology; 27(9): 1918-1925	☐
	27	JOHNSON et al., 1978, "Simple Method for the Isolation of Astaxanthin from the Basidiomycetous Yeast Phaffia rhodozyma", Applied and Environmental Microbiology, 35(6): 1155-1159	☐
	28	KOLAKOWSKA, 1989, "Krill lipids after frozen storage of about one year in relation to storage time before freezing", Die Nahrung Food, 33(3): 241-244	☐
	29	KRIS-ETHERTON et al., 2002, "Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease", Circulation, 106:2747-2757	☐
	30	KRISTENSEN et al., 1989, "Dietary supplementation with n-3 polyunsaturated fatty acids and human platelet function: a review with particular emphasis on implications for cardiovascular disease", J. Intern. Med. Suppl. 731:141-50	☐
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	33	LAMBERTSON and BRAEKKAN, 1971, "Method of Analysis of Astaxanthin and its Occurrence in some Marine Products," J. Sci. Food. Agr., Vol 22(2): 99-101	☐
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	35	LOPEZ et al., 2004, "Selective extraction of astaxanthin from crustaceans by use of supercritical carbon dioxide", Talanta, 64: 726-731	☐
	36	MANDEVILLE, 1991, "Isolation and Identification of Carotenoid Pigments, Lipids and Flavor Active Components from Raw Commercial Shrimp Waste", Food Biotechnology, 5(2): 185-195	☐
	37	MEYERS and BLIGH, 1981, "Characterization of Astaxanthin Pigments from Heat-Processed Crawfish Waste", J. Agric. Food Chem., 29: 505-508	☐
	38	MEYERS, 1977, "Using Crustacean Meals and Carotenoid-Fortified Diets", Feedstuffs, Vol. 49(19)	☐
	39	MEYERS, 1994, "Developments in world aquaculture, feed formulations, and role of carotenoids", Pure & Appl. Chem, Vol. 66(5): 1069-1076	☐
	40	MILLS et al., 1989, "Dietary N-6 and N-3 fatty acids and salt-induced hypertension in the borderline hypertensive rat", Lipids, 24(1): 17-24	☐
	41	MOATES and VAN BENTEM, 1990, "Separating out the value", Food Science and Technology Today, 4(4): 213-214	☐
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	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	NATNUT-14409/US-5/ORD

	46	SAETHER et al., 1986, "Lipids of North Atlantic krill", J Lipid Res., 27(3):274-85.	☐
	47	SHAHIDI et al., 1998, "Carotenoid Pigments in Seafoods and Aquaculture" Critical Reviews in Food Science, 38(1): 1-67	☐
	48	SIDEHU et al., 1970, "Biochmical Composition and Nutritive Value of Krill (Euphausia superb dana)", J. Sci Food Agr., Vol 21, 293-296	☐
	49	SIMOPOULOS, 1991, "Omega-3 fatty acids in health and disease and in growth and development", Am. Clin. Nutr. 54:438-63	☐
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
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☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

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	Inge		Bruheim	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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	Snorre		Tilseth	

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Postal Code	5027	**Country** i	NO

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniele		Mancinelli	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Mailing Address of Inventor:

Address 1	Vikegeila 15		
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Title of the Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS		
Attorney Docket Number	AKBM-14409/US-6/CON	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	19	**Suggested Figure for Publication (if any)**	

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Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12057775	2008-03-28
Prior Application Status	Pending		Remove
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12057775	non provisional of	60920483	2007-03-28
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☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
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Application Number:	
Filing Date:	
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Filer:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
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Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1600**

Electronic Acknowledgement Receipt

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First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Vickie Hoeft
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Payment Type	Deposit Account
Payment was successfully received in RAM	$1600
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1		14409US6Application.pdf	272261 f3c56d7b2af3af918343d760ebcdc3230f29f757	yes	53

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	50
Claims	51	52
Abstract	53	53

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2	Drawings-only black and white line drawings	Drawings.pdf	6553768 452dd8982a6b7e75206db5fb4d4bbae6dcbcb379	no	19

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BIOEFFECTIVE KRILL OIL COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of pending U.S. Patent Application No. 12/057,775, filed March 28, 2008, which claims the benefit of expired U.S. Provisional Patent Application No. 60/920,483, filed March 28, 2007, expired U.S. Provisional Patent Application No. 60/975,058, filed September 25, 2007, expired U.S. Provisional Patent Application No. 60/983,446, filed October 29, 2007, and expired U.S. Provisional Patent Application No. 61/024,072, filed January 28, 2008, all of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

This invention relates to extracts from Antarctic krill that comprise bioactive fatty acids.

BACKGROUND OF THE INVENTION

In the Southern Ocean, off the coast of Antarctica, Antarctic krill (*Euphausia superba)* can be found in large quantities, ranging from 300-500 million metric tons of biomass. It feeds on phytoplankton during the short Antarctic summer. During winter, however, its food supply is limited to ice algae, bacteria, marine detritus as well as depleting body protein for energy.

In order to isolate the krill oil from the krill, solvent extraction methods have been used. See, e.g., WO 00/23546. Krill lipids have been extracted by placing the material in a ketone solvent (e.g. acetone) in order to extract the lipid soluble fraction. This method involves separating the liquid and solid contents and recovering a lipid rich fraction from the liquid fraction by evaporation. Further processing steps include extracting and recovering by evaporation the remaining soluble lipid fraction from the solid contents by using a solvent such as ethanol. See, e.g., WO 00/23546. The compositions produced by these methods are characterized by containing at least 75 μg/g astaxanthin, preferably 90 μg/g astaxanthin. Another krill lipid extract disclosed contained at least 250 μg/g canastaxanthin, preferably 270 μg/g canastaxanthin.

Krill oil compositions have been described as being effective for decreasing cholesterol, inhibiting platelet adhesion, inhibiting artery plaque formation, preventing hypertension, controlling arthritis symptoms, preventing skin cancer, enhancing transdermal transport, reducing

the symptoms of premenstrual symptoms or controlling blood glucose levels in a patient. See, e.g., WO 02/102394. In yet another application, a krill oil composition has been disclosed comprising a phospholipid and/or a flavonoid. The phospholipid content in the krill lipid extract could be as high as 60% w/w and the EPA/DHA content as high as 35% (w/w). See, e.g., WO 03/011873.

Furthermore, nutraceuticals, pharmaceuticals and cosmetics comprising the phospholipid extract were disclosed. Previously, it was also shown that supercritical fluid extraction using neat CO_2 could be used to prevent the extraction of phospholipids in order to extract the neutral lipid fraction from krill, which comprised of esterified and free astaxanthin. See, e.g., Yamaguchi et al., *J. Agric. Food Chem.* (1986), 34(5), 904-7. Supercritical fluid extraction with solvent modifier has previously been used to extract marine phospholipids from salmon roe, but has not been previously used to extract phospholipids from krill meal. See, e.g., Tanaka et al., J. Oleo Sci. (2004), 53(9), 417-424.

The methods described above rely on the processing of frozen krill that are transported from the Southern Ocean to the processing site. This transportation is both expensive and can result in degradation of the krill starting material. Data in the literature showing a rapid decomposition of the oil in krill explains why some krill oil currently offered as an omega-3 supplement in the marketplace contains very high amounts of partly decomposed phosphatidylcholine and also partly decomposed glycerides. Saether et al., Comp. Biochem Phys. B 83B(1): 51-55 (1986). The products offered also contain high levels of free fatty acids.

What is needed in the art are methods for processing krill that do not require transport of frozen krill material over long distances and the products produced by those methods.

SUMMARY OF THE INVENTION

In a first aspect of the invention is a composition characterized by comprising at least 65% (w/w) phospholipids.

In another aspect of the invention is a composition obtained from aquatic or marine sources, characterized by comprising 65% (w/w) phospholipids.

In yet another aspect of the invention is a composition obtained from krill, characterized by comprising at least 65% (w/w) phospholipids.

In another aspect of the invention is a composition obtained from krill, characterized by comprising at least 65% (w/w) phospholipids and at least 39% omega-3 fatty acids (w/w).

In yet another aspect of the invention is a composition obtained from krill, characterized by comprising at least 65% (w/w) phospholipids, at least 39% omega-3 fatty acids (w/w) and at least 580 mg/kg astaxanthin esters.

In another aspect of the invention is a composition obtained from krill, characterized by comprising at least 39% omega-3 fatty acids (w/w) and at least 580 mg/kg astaxanthin esters.

In yet another aspect of the invention is a composition obtained from krill, characterized by comprising at least 65% (w/w) phospholipids and at least 580mg/kg astaxanthin esters.

In yet another aspect, the present invention provides a krill oil effective for reducing insulin resistance, improving blood lipid profile, reducing inflammation or reducing oxidative stress.

In some embodiments, the present invention provides compositions comprising: from about 3% to 10% ether phospholipids on a w/w basis; from about 35% to 50% non-ether phospholipids on w/w basis, so that the total amount of ether phospholipids and non-ether phospholipids in the composition is from about 48% to 60% on a w/w basis; from about 20% to 45% triglycerides on a w/w basis; and from about 400 to about 2500 mg/kg astaxanthin. In some embodiments, the ether phospholipids are selected from the group consisting of alkylacylphosphatidylcholine, lyso-alkylacylphosphatidylcholine, alkylacylphosphatidylethanolamine, and combinations thereof. In some embodiments, the ether lipids are greater than 90% alkylacylphosphatidylcholine. In some embodiments, the non-ether phospholipids are selected from the group consisting of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and combinations thereof. In some embodiments, krill oil composition comprises a blend of lipid fractions obtained from krill. In some preferred embodiments, krill is *Euphausia superba*, although other krill species also find use in the present invention. Other krill species include, but are not limited to *E. pacifica, E. frigida, E. longirostris, E. triacantha, E. vallentini, Meganyctiphanes norvegica, Thysanoessa raschii* and *Thysanoessa inermis*. In some embodiments, the compositions comprise from about 25% to 30% omega-3 fatty acids as a percentage of total fatty acids and wherein from about 80% to 90% of said omega-3 fatty acids are attached to said phospholipids. In some embodiments, the present invention provides a capsule containing the foregoing compositions.

In further embodiments, the present inventions provide compositions comprising: from about 3% to 10% ether phospholipids on a w/w basis; and from about 400 to about 2500 mg/kg astaxanthin. In some embodiments, the compositions further comprise from about 35% to 50% non-ether phospholipids on w/w basis, so that the total amount of ether phospholipids and non-ether phospholipids in the composition is from about 38% to 60% on a w/w basis. In some embodiments, the compositions further comprise from about 20% to 45% triglycerides on a w/w basis. In some embodiments, the ether phospholipids are selected from the group consisting of alkylacylphosphatidylcholine, lyso-alkylacylphosphatidylcholine, alkylacylphosphatidylethanolamine, and combinations thereof. In some embodiments, the ether lipids are greater than 90% alkylacylphosphatidylcholine. In some embodiments, the non-ether phospholipids are selected from the group consisting of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and combinations thereof. In some embodiments, krill oil composition comprises a blend of lipid fractions obtained from krill. In some preferred embodiments, krill is *Euphausia superba*, although other krill species also find use in the present invention. Other krill species include, but are not limited to *E. pacifica, E. frigida, E. longirostris, E. triacantha, E. vallentini, Meganyctiphanes norvegica, Thysanoessa raschii* and *Thysanoessa inermis*. In some embodiments, the compositions comprise about 25% to 30% omega-3 fatty acids as a percentage of total fatty acids and wherein from about 80% to 90% of said omega-3 fatty acids are attached to said phospholipids. In some embodiments, the present invention provides a capsule containing the foregoing compositions.

In some embodiments, the present invention provides a composition comprising at least 65% (w/w) of phospholipids, said phospholipids characterized in containing at least 35% omega-3 fatty acid residues. In some preferred embodiments, the composition is derived from a marine or aquatic biomass. In some further preferred embodiments, the composition is derived from krill. In some embodiments, the composition comprises less than 2% free fatty acids. In some embodiments, composition comprises less than 10% triglycerides. In some preferred embodiments, the phospholipids comprise greater than 50% phosphatidylcholine. In some embodiments, the composition comprises at least 500 mg/kg astaxanthin esters. In some embodiments, the composition comprises at least 500 mg/kg astaxanthin esters and at least 36% (w/w) omega-3 fatty acids. In some embodiments, the composition comprises less than about

0.5g/100g total cholesterol. In some embodiments, the composition comprises less than about 0.45% arachidonic acid (w/w).

In some embodiments, the present invention provides a krill lipid extract comprising at least 500, 100, 1500, 2000, 2100, or 2200 mg/kg astaxanthin esters and at least 36% (w/w) omega-3 fatty acids. In further embodiments, the present invention provides a krill lipid extract comprising at least 100 mg/kg astaxanthin esters, at least 20% (w/w) omega-3 fatty acids, and less than about 0.45% arachidonic acid (w/w).

In some embodiments, the present invention provides methods comprising administering the foregoing compositions to a subject in an amount effective for reducing insulin resistance, reducing inflammation, improving blood lipid profile and reducing oxidative stress.

In some embodiments, the present invention provides a krill lipid extract comprising greater than about 80% triglycerides and greater than about 90, 100, 500, 1000, 1500, 200, 2100 or 2200 mg/kg astaxanthin esters. In some embodiments, the krill lipid extract is characterized in containing from about 5% to about 15% omega-3 fatty acid residues. In some embodiments, the krill lipid extract is characterized in containing less than about 5% phospholipids. In some embodiments, the krill lipid extract is characterized in comprising from about 5% to about 10% cholesterol.

In some embodiments, the present invention provides a krill meal composition comprising less than about 50g/kg total fat. In some embodiments, the krill meal composition comprises from about 5 to about 20 mg/kg astaxanthin esters. In some embodiments, the krill meal composition comprises greater than about 65% protein. In some embodiments, the krill meal composition of comprises greater than about 70% protein. In some further embodiments, the present invention provides an animal feed comprising the krill meal composition.

In some embodiments, the present invention provides methods of increasing flesh coloration in an aquatic species comprising feeding said aquatic species a composition comprising the krill meal described above. In some embodiments, the present invention provides methods of increasing growth and overall survival rate of aquatic species by feeding the krill meal described above.

In some embodiments, the present invention provides methods of producing krill oil comprising: a) providing krill meal; and b) extracting oil from said krill meal. In some embodiments, the krill meal is produced by heat-treating krill. In some embodiments, the krill

meal is stored prior to the extraction step. In some embodiments, the extracting step comprises extraction by supercritical fluid extraction. In some embodiments, the supercritical fluid extraction is a two step process comprising a first extraction step with carbon dioxide and a low concentration of a co-solvent (e.g., from about 1-10% co-solvent) and a second extraction step with carbon dioxide and a high concentration of a co-solvent (e.g., from about 10-30% co-solvent). In preferred embodiments, the co-solvent is a C_1-C_3 monohydric alcohol, preferably ethanol. In some embodiments, the present invention provides oil produced by the foregoing method.

In some embodiments, the present invention provides methods of production of krill oil comprising: a) providing fresh krill; b) treating said fresh krill to denature lipases and phospholipases in said fresh krill to provide a denatured krill product; and c) extracting oil from said denatured krill product. In some embodiments, the denaturation step comprises heating of said fresh krill. In some embodiments, the denaturation step comprises heating said fresh krill after grinding. In some embodiments, the methods further comprise storing said denatured krill product at room temperature or below between the denaturation step and the extraction step. In some embodiments, the enzyme denaturation step is achieved by application of heat. In some embodiments, the extraction step comprises use of supercritical carbon dioxide, with or without use of a polar modifier. In some embodiments, the extraction step comprises use of ethanol. In some embodiments, the extraction step is comprises ethanol extraction followed by acetone to precipitation of phospholipids. In some embodiments, the denatured krill product is a meal. In some embodiments, the present invention provides oil produced by the foregoing method.

In some embodiments, the present invention provides a composition comprising oil extracted from krill having a phosphatidylcholine content of greater then about 50% (w/w). In some embodiments, the oil has a phosphatidylcholine content of greater then about 70% (w/w). In some embodiments, the oil has a phosphatidylcholine content of greater then about 80% (w/w). In some embodiments, the composition comprises less than 2% free fatty acids. In some embodiments, the composition comprises less than 10% triglycerides. In some embodiments, the composition comprises at least 500 mg/kg astaxanthin esters. In some embodiments, the composition comprises less than about 0.45% arachidonic acid (w/w).

In some embodiments, the present invention provides composition comprising odorless krill oil. In some embodiments, the odorless krill oil comprises less than about 10 mg/kg (w/w)

trimethylamine. In some further embodiments, the present invention provides an odorless krill oil produced by the method comprising: extracting a neutral krill oil from a krill oil containing material by supercritical fluid extraction to provide a deodorized krill material, wherein said neutral krill oil contains odor causing compounds and extracting a polar krill oil from said deodorized krill material by supercritical fluid extraction with a polar entrainer to provide an essentially odorless krill oil.

In some embodiments, the present invention provides a composition comprising krill oil containing less than about 70 micrograms/kilogram (w/w) astaxanthin esters. In some embodiments, the compositions comprise less than about 50 micrograms/kilogram (w/w) astaxanthin esters. In some embodiments, the compositions comprise less than about 20 micrograms/kilogram (w/w) astaxanthin esters. In some embodiments, the compositions comprise less than about 5 micrograms/kilogram (w/w) astaxanthin esters.

In some embodiments, the present invention provides a krill oil produced by the process comprising: pumping fresh krill from a trawl onto a ship, heating the krill to provide a krill material, and extracting oil from the krill material.

In further embodiments, the present invention provides a blended krill oil composition comprising: from about 45% to 55% w/w phospholipids; from about 20% to 45% w/w triglycerides; and from about 400 to about 2500 mg/kg astaxanthin. In some embodiments, the blended krill oil product comprises a blend of lipid fractions obtained from *Euphausia superba*. In some embodiments, the composition comprises from about 25% to 30% omega-3 fatty acids as a percentage of total fatty acids and wherein from about 80% to 90% of said omega-3 fatty acids are attached to said phospholipids.

In still other embodiments, the present invention provides a *Euphausia superba* krill oil composition comprising: from about 30% to 60% w/w phospholipids; from about 20% to 50% triglycerides; from about 400 to about 2500 mg/kg astaxanthin; and from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

In still further embodiments, the present invention provides a dietary supplement comprising encapsulated *Euphausia superba* krill oil comprising from about 30% to 60% w/w phospholipids; from about 20% to 50% triglycerides; from about 400 to about 2500 mg/kg astaxanthin; and from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids

in said composition, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

In some embodiments, the present invention provides methods of making a *Euphausia superba* krill oil composition comprising: contacting *Euphausia superba* with a polar solvent to provide a polar extract comprising phospholipids; contacting *Euphausia superba* with a neutral solvent to provide a neutral extract comprising triglycerides and astaxanthin; combining said polar extract and said neutral extract to provide *Euphausia superba* krill oil comprising from about 30% to 60% w/w phospholipids; from about 20% to 50% triglycerides; from about 400 to about 2500 mg/kg astaxanthin; and from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids. In some embodiments, the methods further comprise the step of encapsulating the *Euphausia superba* krill oil. In some embodiments, the present invention provides a *Euphausia superba* krill oil produced by the methods described above.

In some embodiments, the present invention provides methods of producing a dietary supplement comprising; contacting *Euphausia superba* with a polar solvent to provide an polar extract comprising phospholipids; contacting *Euphausia* superba with a neutral solvent to provide a neutral extract comprising triglycerides and astaxanthin; combining said polar extract and said neutral extract to provide *Euphausia superba* krill oil comprising from about 30% to 60% w/w phospholipids; from about 20% to 50% triglycerides; from about 400 to about 2500 mg/kg astaxanthin; and from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids; and encapsulating said *Euphausia superba* krill oil.

In some embodiments, the present invention provides methods of reducing diet-induced hyperinsulinemia, insulin insensitivity, muscle mass hypertrophy, serum adiponectin reduction or hepatic steatosis comprising in a subject exposed to a high fat diet: administering to said subject exposed to a high fat diet an effective amount of a krill oil composition under conditions such that a condition selected from the group consisting of diet-induced hyperinsulinemia, insulin insensitivity, muscle mass hypertrophy, serum adiponectin reduction and hepatic steatosis is reduced. The present invention is not limited to any particular krill oil composition. In some embodiments, the krill oil composition is a *Euphausia superba* krill oil composition. The present invention is not limited to any particular formulation of krill oil. In some embodiments, the krill

oil composition is encapsulated. In some preferred embodiments, the effective amount of a krill oil composition is from 0.2 grams to 10 grams of said krill oil composition. In some embodiments, the krill oil composition comprises: from about 45% to 55% w/w phospholipids; from about 20% to 45% w/w triglycerides; and from about 400 to about 2500 mg/kg astaxanthin. In some embodiments, the krill oil composition comprises a blend of lipid fractions obtained from *Euphausia superba*. In some embodiments, the krill oil composition comprises from about 25% to 30% omega-3 fatty acids as a percentage of total fatty acids and wherein from about 80% to 90% of said omega-3 fatty acids are attached to said phospholipids. In some embodiments, the krill oil composition comprises from about 30% to 60% w/w phospholipids; from about 20% to 50% triglycerides; from about 400 to about 2500 mg/kg astaxanthin; and from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition, and wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

In some embodiments, the present invention provides methods of reducing diet-induced hyperinsulinemia, insulin insensitivity, muscle mass hypertrophy, serum adiponectin reduction or hepatic steatosis comprising in a subject consuming a high fat diet or a normal fat diet: administering to said subject consuming a high fat diet or a normal fat diet an effective amount of a krill oil composition under conditions such that a condition selected from the group consisting of diet-induced hyperinsulinemia, insulin insensitivity, muscle mass hypertrophy, serum adiponectin reduction and hepatic steatosis is reduced. The present invention is not limited to any particular krill oil composition. In some embodiments, the krill oil composition is a *Euphausia superba* krill oil composition. The present invention is not limited to any particular formulation of krill oil. In some embodiments, the krill oil composition is encapsulated. In some preferred embodiments, the effective amount of a krill oil composition is from 0.2 grams to 10 grams of said krill oil composition. In some embodiments, the krill oil composition comprises: from about 45% to 55% w/w phospholipids; from about 20% to 45% w/w triglycerides; and from about 400 to about 2500 mg/kg astaxanthin. In some embodiments, the krill oil composition comprises a blend of lipid fractions obtained from *Euphausia superba*. In some embodiments, the krill oil composition comprises from about 25% to 30% omega-3 fatty acids as a percentage of total fatty acids and wherein from about 80% to 90% of said omega-3 fatty acids are attached to said phospholipids. In some embodiments, the krill oil composition comprises from about 30% to 60% w/w phospholipids; from about 20% to 50% triglycerides; from about 400 to about

2500 mg/kg astaxanthin; and from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition, and wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

In some embodiments, the present invention provides methods of inducing diuresis in a subject comprising: administering to said subject an effective amount of a krill oil composition under conditions such that diuresis is induced. In some embodiments, the present invention provides methods of increasing muscle mass in a subject, comprising:
administering to said subject an effective amount of a krill oil composition under conditions such that muscle mass is increased. In some embodiments, the present invention provides methods of decreasing protein catabolism in a subject, comprising: administering to said subject an effective amount of a krill oil composition under conditions such that protein catabolism is decreased. In some embodiments, the present invention provides methods of decreasing lipid content in the heart of a subject, comprising: administering to said subject an effective amount of a krill oil composition under conditions such that lipid content in the heart of the subject is decreased. In some embodiments, the present invention provides methods of decreasing lipid content in the liver of a subject, comprising: administering to said subject an effective amount of a krill oil composition under conditions such that lipid content in the liver of the subject is decreased.

DESCRIPTION OF THE FIGURES

Figure 1. 31P NMR analysis of polar lipids in krill oil.

Figure 2. Blood lipid profiles in Zucker rats fed different forms of omega-3 fatty acids (TAG = FO, PL1 = NKO and PL2 = Superba).

Figure 3. Plasma glucose concentration in Zucker rats fed different forms of omega-3 fatty acids.

Figure 4. Plasma insulin concentration in Zucker rats fed different forms of omega-3 fatty acids.

Figure 5. Estimated HOMA-IR values in Zucker rats fed different forms of omega-3 fatty acids.

Figure 6. The effect of dietary omega-3 fatty acids on TNF production by peritoneal macrophages.

Figure 7. The effect of dietary omega-3 fatty acids on lipid accumulation in the liver.

Figure 8. The effect of dietary omega-3 fatty acids on lipid accumulation in the muscle.

Figure 9. The effect of dietary omega-3 fatty acids on lipid accumulation in the heart.

Figure 10. Relative concentrations of DHA in the brain in Zucker rats supplemented with omega-3 fatty acids.

Figure 11. Mean group body weights (g) in the collagen-induced male DBA/1 arthritic mice. B - PL2 is the krill oil group. * $p<0.05$, significantly different from Group A (Positive Control - Fish Oil) and Group C (Control).

Figure 12. Body weight for the various treatment groups.

Figure 13. Muscle weight for the various treatment groups.

Figure 14. Muscle to body weight ratio for the various treatment groups.

Figure 15. Serum adiopnectin levels (ng/ml) for the various treatment groups.

Figure 16. Serum insulin levels for the various treatment groups.

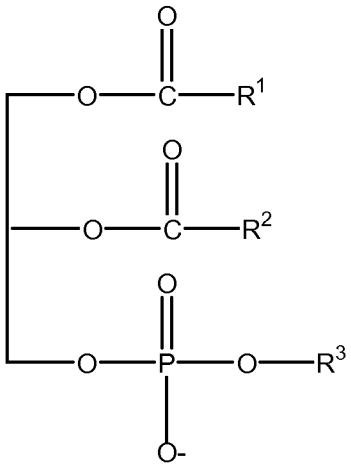
Figure 17. Blood glucose (mmol/l) levels in the various treatment groups.

Figure 18. HOMA-IR values for the various treatment groups.

Figure 19. Liver triglyceride levels (μmol/g) for the various treatment groups.

DEFINITIONS

As used herein, "phospholipid" refers to an organic compound having the following general structure:

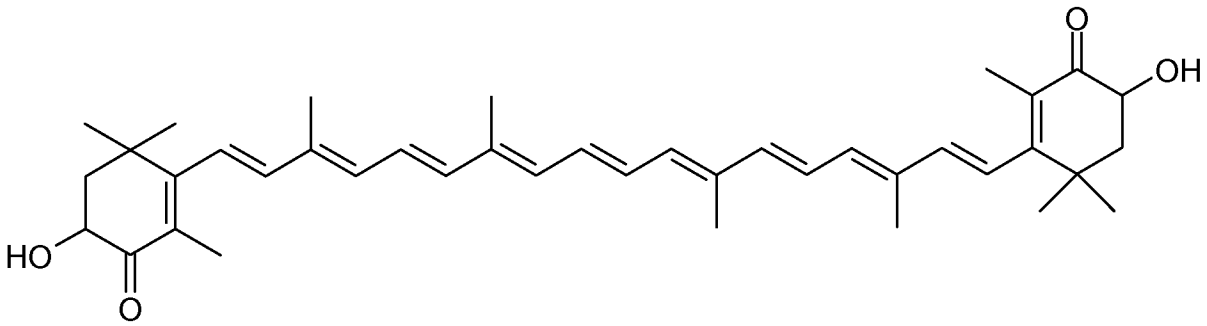


wherein R1 is a fatty acid residue, R2 is a fatty acid residue or –OH, and R3 is a –H or nitrogen containing compound choline ($HOCH_2CH_2N^+(CH_3)_3OH^-$), ethanolamine ($HOCH_2CH_2NH_2$), inositol or serine. R1 and R2 cannot simultaneously be OH. When R3 is an –OH, the compound is a diacylglycerophosphate, while when R3 is a nitrogen-containing compound, the compound is a phosphatide such as lecithin, cephalin, phosphatidyl serine or plasmalogen.

An "ether phospholipid" as used herein refers to a phospholipid having an ether bond at position 1 the glycerol backbone. Examples of ether phospholipids include, but are not limited to, alkylacylphosphatidylcholine (AAPC), lyso-alkylacylphosphatidylcholine (LAAPC), and alkylacylphosphatidylethanolamine (AAPE). A "non-ether phospholipid" is a phospholipid that does not have an ether bond at position 1 of the glycerol backbone.

As used herein, the term omega-3 fatty acid refers to polyunsaturated fatty acids that have the final double bond in the hydrocarbon chain between the third and fourth carbon atoms from the methyl end of the molecule. Non-limiting examples of omega-3 fatty acids include, 5,8,11,14,17-eicosapentaenoic acid (EPA), 4,7,10,13,16,19-docosahexanoic acid (DHA) and 7,10,13,16,19-docosapentanoic acid (DPA).

As used herein, astaxanthin refers to the following chemical structure:



As used herein, astaxanthin esters refer to the fatty acids esterified to OH group in the astaxanthin molecule.

As used herein, the term w/w (weight/weight) refers to the amount of a given substance in a composition on weight basis. For example, a composition comprising 50% w/w phospholipids means that the mass of the phospholipids is 50% of the total mass of the composition (i.e., 50 grams of phospholipids in 100 grams of the composition, such as an oil).

DETAILED DESCRIPTION OF THE INVENTION

This invention discloses novel krill oil compositions characterized by containing high levels of astaxanthin, phospholipids, included an enriched quantities of ether phospholipids, and omega-3 fatty acids. The krill oils compositions are extracted from krill meal using supercritical fluid extraction (SFE) with a co-solvent modifier. The krill meal has been processed on board a ship in Antarctica using live krill as starting material in order to ensure the highest possible quality of the krill meal. The krill oils are extracted from the krill meal in two stages, in step 1 the neutral fraction is extracted using neat supercritical CO_2 or in combination with 5% ethanol. The neutral fraction consisted mostly of triglycerides and cholesterol. In stage 2, the polar lipids (phospholipids) are extracted by adding at least 20% ethanol to the supercritical CO_2 extraction medium.

The present invention provides methods to avoid decomposition of glycerides and phospholipids in krill oil and compositions produced by those methods. The product obtained by these new methods is virtually free of enzymatically decomposed oil constituents. The solution to the problem is to incorporate a protein denaturation step on fresh krill prior to use of any extraction technology. Denaturation can be achieved by thermal stress or by other means. After denaturation, the oil can be extracted by an optional selection of nonpolar and polar solvents including use of supercritical carbon dioxide. Krill is adapted to a very efficient nutrient digestion at very low temperatures. Therefore the enzymes are sensitive to heat and the step of applying thermal denaturation of lipases and phospholipases does not imply use of very high temperatures. Surprisingly, it has been found that the use of mild denaturation conditions can greatly enhance the quality of krill oil.

Additionally, a major obstacle of several processes of extraction is the cost of removing water. This is particularly true for methods feasible for extraction of highly unsaturated lipids where freeze drying has been regarded as the method of choice to avoid oxidative breakdown of lipids. However, the lipids in krill are surprisingly stable against oxidative deterioration. Therefore, a process including moderate use of heat in the water removing process is feasible provided that the enzymes have been inactivated.

A. Krill Processing

The present invention provides methods for processing freshly caught krill at the site of capture and preferably on board a ship. After processing on board, the krill can be further subjected to extraction processes on board the ship or at a remote location away from the ship. The processing steps described herein also allow for the storage of krill material, preferably a krill meal for from about 1,2, 3, 4, 5, 6, 8, 9, 10, 11, or 12 months to about 24 to 36 months prior to processing.

In some preferred embodiments, freshly caught krill is first subjected to a protein denaturation step. The present invention is not limited to any particular method of protein denaturation. In some embodiments, the denaturation is accomplished by application of chemicals, heat, or combinations thereof. In some embodiments, freshly caught krill is wet pressed to obtain oil and meal. In some embodiments, the meal is then heated to a temperature of about 50°C to about 100°C for about 20 minutes to about an hour, preferably about 40 minutes to denature the proteins. In some embodiments, this material is then pressed to yield a press cake. When this method is used on krill, only a small amount of oil is released. Most of the oil is still present in the denatured meal. In some embodiments, antioxidants such as ethoxyquin or Vitamin E are added to the meal. However, as shown in the examples, the resulting meal is surprisingly stable. The stability can only partly be explained by addition of an antioxidant to the meal. This antioxidant can, after extraction of the oil from denatured meal, be removed by further processing steps. Alternatively the oil can be extracted rather shortly after production of the meal without any addition of antioxidant in the process. Further, storage conditions at a low to very low temperature can be applied if addition of antioxidant is not desired.

Krill oil extracted from denatured krill meal by supercritical fluid extraction even 19 months after the production of the meal contained virtually no decomposed phospholipids. This product turned out to be substantially different from samples of krill oil available in the market today. Previously described commercial krill processing procedures utilize krill that has been frozen immediately after catching followed by freeze drying and extraction at low temperatures. However, these processes only yield a suitable product if the time the krill is kept frozen is very short or the temperature is extremely low (-60°to -80°C). However, data provided herein clearly shows that if a step of denaturation of the proteins is added in front of an optional extraction method, an excellent krill oil can be produced even after a long time of storage. This

methodology also opens up for use of alternative methods to remove water prior to extraction, which in turn has a great impact on costs in full scale operation. If a long time of storage is desired, the denatured material should preferably be stored at low temperature preferably at -20°C.

In some embodiments, krill oil is extracted from the denatured krill meal. In some embodiments, the krill oil is extracted by contacting the krill meal with ethanol. In some embodiments, krill is then extracted with a ketone solvent such as acetone. In other embodiments, the krill oil is extracted by one or two step supercritical fluid extraction. In some embodiments, the supercritical fluid extraction uses carbon dioxide and neutral krill oil is produced. In some embodiments, the supercritical fluid extraction uses carbon dioxide with the addition of a polar entrainer, such as ethanol, to produce a polar krill oil. In some embodiments, the krill meal is first extracted with carbon dioxide followed by carbon dioxide with a polar entrainer, or vice versa. In some embodiments, the krill meal is first extracted with CO_2 supplemented with a low amount of a polar co-solvent (e.g., from about 1% to about 10%, preferably about 5%) such a C_1-C_3 monohydric alcohol, preferably ethanol, followed by extraction with CO_2 supplemented with a high amount of a polar co-solvent (from about 10% to about 30%, preferably about 23%) such as such a C_1-C_3 monohydric alcohol, preferably ethanol, or vice versa. Surprisingly, it has been found that use of a low amount of polar solvent in the CO_2 as an entrainer facilitates the extraction of neutral lipid components and astaxanthin in a single step. Use of the high of polar solvent as an entrainer in the other step facilitates extraction of ether phospholipids, as well as non-ether phospholipids.

The present invention is distinguished from previously described krill oil products, such as those described in U.S. Pat. No. 6,800,299 or WO 03/011873 and Neptune brand krill oil, by having substantially higher levels of non-ether phospholipids, ether phospholipids, and astaxanthin. The krill oils of the present invention also have unexpected and superior properties as compared to previously available krill oils. In particular, the krill oil of the present invention has been demonstrated to reduce blood LDL cholesterol levels, improve DHA transfer to the brain as well as reduce lipid accumulation in the liver and muscle while the previously described krill oil compositions do not have such a properties. Accordingly, in some embodiments, the present invention provides a krill oil composition, preferably a *Euphausia superba* krill oil composition, comprising from about 40% to about 60% w/w phospholipids, preferably from

about 45% to 55% w/w phospholipids and from about 300 mg/kg astaxanthin to about 2500 mg/kg astaxanthin, preferably from about 1000 to about 2200 mg/kg astaxanthin, more preferably from about 1500 to about 2200 mg/kg astaxanthin. In some preferred embodiments, the compositions comprise greater than about 1000, 1500, 1800, 1900, 2000, or 2100 mg/kg astaxanthin. In some preferred embodiments, the krill oil compositions of the present invention comprise from about 1%, 2%, 3% or 4% to about 8%, 10%, 12% or 15% w/w ether phospholipids or greater than about 4%, 5%, 6%, 7%, 8%, 9% or 10% ether phospholipids. In some embodiments the ether phospholipids are preferably alkylacylphosphatidylcholine, lyso-alkylacylphosphatidylcholine, alkylacylphosphatidyl-ethanolamine or combinations thereof. In some embodiments, the krill oil compositions comprise from about 1%, 2%, 3% or 4% to about 8%, 10%, 12% or 15% w/w ether phospholipids and from about 30%, 33%, 40%, 42%, 45%, 48%, 50%, 52%, 54%, 55% 56%, 58% to about 60% non-ether phospholipids so that the total amount of phospholipids (both ether and non-ether phospholipids) ranges from about 40% to about 60%. One of skill in the art will recognize that the range of 40% to 60% total phospholipids, as well as the other ranges of ether and non-ether phospholipids, can include other values not specifically listed within the range.

In further embodiments, the compositions comprise from about 20% to 45% w/w triglycerides; and from about 400 to about 2500 mg/kg astaxanthin. In some embodiments, the compositions comprise from about 20% to 35%, preferably from about 25% to 35%, omega-3 fatty acids as a percentage of total fatty acids in the composition, wherein from about 70% to 95%, or preferably from about 80% to 90% of the omega-3 fatty acids are attached to the phospholipids. In some embodiments, the present invention provides encapsulated *Euphausia superba* krill oil compositions. In some embodiments, the present invention provides a method of making a *Euphausia superba* krill oil composition comprising contacting *Euphausia superba* with a polar solvent to provide an polar extract comprising phospholipids, contacting *Euphausia superba* with a neutral solvent to provide a neutral extract comprising triglycerides and astaxanthin, and combining said polar extract and said neutral extract to provide the *Euphausia superba* krill oils described above. In some embodiments, fractions from polar and non-polar extractions are combined to provide a final product comprising the desired ether phospholipids, non-ether phospholipids, omega-3 moieties and astaxanthin. In other embodiments, the present invention provides methods of making a *Euphausia superba* (or other krill species) krill oil

comprising contacting a *Euphausia superba* preparation such as *Euphausia superba* krill meal under supercritical conditions with CO_2 containing a low amount of a polar solvent such as ethanol to extract neutral lipids and astaxanthin; contacting meal remaining from the first extraction step under supercritical conditions with CO_2 containing a high amount of a polar solvent such as ethanol to extract a polar lipid fraction containing ether and non-ether phospholipids; and then blending the neutral and polar lipid extracts to provide the compositions described above.

The krill oil extracted by the methods of the present invention contains few enzymatic breakdown products. Examples of the krill oil compositions of the present invention are provided in Tables 9-24. In some embodiments, the present invention provides a polar krill oil comprising at least 65% (w/w) of phospholipids, wherein the phospholipids are characterized in containing at least 35% omega-3 fatty acid residues. The present invention is not limited to the presence of any particular omega-3 fatty acid residues in the krill oil composition. In some preferred embodiments, the krill oil comprises EPA and DHA residues. In some embodiments, the krill oil compositions comprise less than about 5%, 4%, 3% or preferably 2% free fatty acids on a weight/weight (w/w) basis. In some embodiments, the krill oil compositions comprise less than about 25%, 20%, 15%, 10% or 5% triglycerides (w/w). In some embodiments, the krill oil compositions comprise greater than about 30%, 40%, 45%, 50%, 55%, 60%, or 65% phosphatidyl choline (w/w). In some embodiments, the krill oil compositions comprise greater than about 100, 200, 300, 400, or 500 mg/kg astaxanthin esters and up to about 700 mg/kg astaxanthin esters. In some embodiments, the present invention provides krill oil compositions comprising at least 500, 1000, 1500, 2000, 2100, or 2200 mg/kg astaxanthin esters and at least 36% (w/w) omega-3 fatty acids. In some embodiments, the krill oil compositions of the present invention comprise less than about 1.0g/100g, 0.5g/100g, 0.2g/100g or 0.1g/100g total cholesterol. In some embodiments, the krill oil compositions of the present invention comprise less than about 0.45

In some embodiments, the present invention provides a neutral krill oil extract comprising greater than about 70%, 75% 80%, 85% or 90% triglycerides. In some embodiments, the krill oil compositions comprise from about 50 to about 2500 mg/kg astaxanthin esters. In some embodiments, the krill oil compositions comprise from about 50, 100, 200, or 500 to about 750, 1000, 1500 or 2500 mg/kg astaxanthin esters. In some embodiments, the compositions comprise

from about 1% to about 30% omega-3 fatty acid residues, and preferably from about 5%-15% omega-3 fatty acid residues. In some embodiments, the krill oil compositions comprise less than about 20%, 15%, 10% or 5% phospholipids.

In some embodiments, the present invention provides krill oil containing less than about 70, 60, 50, 40, 30, 20, 10, 5 or 1 micrograms/kilogram (w/w) astaxanthin esters. In some embodiments, the krill oil is clear or only has a pale red color. In some embodiments, the low-astaxanthin krill oil is obtained by first extracting a krill material, such as krill oil, by supercritical fluid extraction with neat carbon dioxide. It is contemplated that this step removes astaxanthin from the krill material. In some embodiments, the krill material is then subjected to supercritical fluid extraction with carbon dioxide and a polar entrainer such as ethanol, preferably about 20% ethanol. The oil extracted during this step is characterized in containing low amounts of astaxanthin. In other embodiments, krill oil comprising astaxanthin is extracted by countercurrent supercritical fluid extraction with neat carbon dioxide to provide a low-astaxanthin krill oil.

In some embodiments, the present invention provides krill oil that is substantially odorless. By substantially odorless it is meant that the krill oil lacks an appreciable odor as determined by a test panel. In some embodiments, the substantially odorless krill oil comprises less than about 10, 5 or 1 milligrams/kilogram trimethylamine. In some preferred embodiments, the odorless krill oil is produced by first subjecting krill material to supercritical fluid extraction with neat carbon dioxide to remove odor causing compounds such as trimethylamine, followed by extraction with carbon dioxide with a polar entrainer such as ethanol.

In some embodiments, the present invention provides a delipidated krill meal produced after extraction of lipids from the krill meal. In some embodiments, the delipidated krill meal comprises krill protein. In some embodiments, the delipidated krill meal comprises less than about 200, 150, 120, 100, 75, 65, 60, 55, or 50 g/kg total fat. In some embodiments, the delipidated krill meal comprises from about 1 to about 100 mg/kg astaxanthin esters, and preferably from about 5 to about 20 mg/kg astaxanthin esters. In some embodiments, the delipidated krill meal comprises greater than about 60%, 65%, 70% or 75% krill protein. In some embodiments, the present invention provides animal feeds comprising the delipidated krill meal. In some embodiments, the animal feed is a fish feed or aquatic organism feed, such as shrimp feed, crab feed, or crawfish feed. In preferred embodiments, the krill meal is incorporated into

complete ration for the target organism. In preferred embodiments, the feed is provided in pelleted form. In many instances, compounds such as astaxanthin are removed during delipidation. The methods of the present invention provide a delipidated krill meal that retains significant amounts of astaxanthin. Accordingly, in some embodiments, the present invention provides methods of feeding aquatic organisms, comprising providing to the aquatic organism a feed comprising the delipidated krill meal described above. In other embodiments, the present invention provides methods of increasing flesh coloration in an aquatic species comprising feeding the aquatic species a comprising the delipidated krill meal described above.

B. Compositions Containing Krill Oil

In some embodiments, the compositions of this invention (such as those described in the preceding sections) are contained in acceptable excipients and/or carriers for oral consumption. The actual form of the carrier, and thus, the composition itself, is not critical. The carrier may be a liquid, gel, gelcap, capsule, powder, solid tablet (coated or non-coated), tea, or the like. The composition is preferably in the form of a tablet or capsule and most preferably in the form of a soft gel capsule. Suitable excipient and/or carriers include maltodextrin, calcium carbonate, dicalcium phosphate, tricalcium phosphate, microcrystalline cellulose, dextrose, rice flour, magnesium stearate, stearic acid, croscarmellose sodium, sodium starch glycolate, crospovidone, sucrose, vegetable gums, lactose, methylcellulose, povidone, carboxymethylcellulose, corn starch, and the like (including mixtures thereof). Preferred carriers include calcium carbonate, magnesium stearate, maltodextrin, and mixtures thereof. The various ingredients and the excipient and/or carrier are mixed and formed into the desired form using conventional techniques. The tablet or capsule of the present invention may be coated with an enteric coating that dissolves at a pH of about 6.0 to 7.0. A suitable enteric coating that dissolves in the small intestine but not in the stomach is cellulose acetate phthalate. Further details on techniques for formulation for and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

The dietary supplement may comprise one or more inert ingredients, especially if it is desirable to limit the number of calories added to the diet by the dietary supplement. For example, the dietary supplement of the present invention may also contain optional ingredients including, for example, herbs, vitamins, minerals, enhancers, colorants, sweeteners, flavorants,

inert ingredients, and the like. For example, the dietary supplement of the present invention may contain one or more of the following: ascorbates (ascorbic acid, mineral ascorbate salts, rose hips, acerola, and the like), dehydroepiandosterone (DHEA), Fo-Ti or Ho Shu Wu (herb common to traditional Asian treatments), Cat's Claw (ancient herbal ingredient), green tea (polyphenols), inositol, kelp, dulse, bioflavinoids, maltodextrin, nettles, niacin, niacinamide, rosemary, selenium, silica (silicon dioxide, silica gel, horsetail, shavegrass, and the like), spirulina, zinc, and the like. Such optional ingredients may be either naturally occurring or concentrated forms.

In some embodiments, the dietary supplements further comprise vitamins and minerals including, but not limited to, calcium phosphate or acetate, tribasic; potassium phosphate, dibasic; magnesium sulfate or oxide; salt (sodium chloride); potassium chloride or acetate; ascorbic acid; ferric orthophosphate; niacinamide; zinc sulfate or oxide; calcium pantothenate; copper gluconate; riboflavin; beta-carotene; pyridoxine hydrochloride; thiamin mononitrate; folic acid; biotin; chromium chloride or picolonate; potassium iodide; sodium selenate; sodium molybdate; phylloquinone; vitamin D3; cyanocobalamin; sodium selenite; copper sulfate; vitamin A; vitamin C; inositol; potassium iodide. Suitable dosages for vitamins and minerals may be obtained, for example, by consulting the U.S. RDA guidelines.

In further embodiments, the compositions comprise at least one food flavoring such as acetaldehyde (ethanal), acetoin (acetyl methylcarbinol), anethole (parapropenyl anisole), benzaldehyde (benzoic aldehyde), N butyric acid (butanoic acid), d or l carvone (carvol), cinnamaldehyde (cinnamic aldehyde), citral (2,6 dimethyloctadien 2,6 al 8, gera nial, neral), decanal (N decylaldehyde, capraldehyde, capric aldehyde, caprinaldehyde, aldehyde C 10), ethyl acetate, ethyl butyrate, 3 methyl 3 phenyl glycidic acid ethyl ester (ethyl methyl phenyl glycidate, strawberry aldehyde, C 16 aldehyde), ethyl vanillin, geraniol (3,7 dimethyl 2,6 and 3,6 octadien 1 ol), geranyl acetate (geraniol acetate), limonene (d , l , and dl), linalool (linalol, 3,7 dimethyl 1,6 octadien 3 ol), linalyl acetate (bergamol), methyl anthranilate (methyl 2 aminobenzoate), piperonal (3,4 methylenedioxy benzaldehyde, heliotropin), vanillin, alfalfa (Medicago sativa L.), allspice (Pimenta officinalis), ambrette seed (Hibiscus abelmoschus), angelic (Angelica archangelica), Angostura (Galipea officinalis), anise (Pimpinella anisum), star anise (Illicium verum), balm (Melissa officinalis), basil (Ocimum basilicum), bay (Laurus nobilis), calendula (Calendula officinalis), (Anthemis nobilis), capsicum (Capsicum frutescens), caraway (Carum carvi), cardamom (Elettaria cardamomum), cassia, (Cinnamomum cassia), cayenne pepper

(Capsicum frutescens), Celery seed (Apium graveolens), chervil (Anthriscus cerefolium), chives (Allium schoenoprasum), coriander (Coriandrum sativum), cumin (Cuminum cyminum), elder flowers (Sambucus canadensis), fennel (Foeniculum vulgare), fenugreek (Trigonella foenum graecum), ginger (Zingiber officinale), horehound (Marrubium vulgare), horseradish (Armoracia lapathifolia), hyssop (Hyssopus officinalis), lavender (Lavandula officinalis), mace (Myristica fragrans), marjoram (Majorana hortensis), mustard (Brassica nigra, Brassica juncea, Brassica hirta), nutmeg (Myristica fragrans), paprika (Capsicum annuum), black pepper (Piper nigrum), peppermint (Mentha piperita), poppy seed (Papayer somniferum), rosemary (Rosmarinus officinalis), saffron (Crocus sativus), sage (Salvia officinalis), savory (Satureia hortensis, Satureia montana), sesame (Sesamum indicum), spearmint (Mentha spicata), tarragon (Artemisia dracunculus), thyme (Thymus vulgaris, Thymus serpyllum), turmeric (Curcuma longa), vanilla (Vanilla planifolia), zedoary (Curcuma zedoaria), sucrose, glucose, saccharin, sorbitol, mannitol, aspartame. Other suitable flavoring are disclosed in such references as Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing, p. 1288-1300 (1990), and Furia and Pellanca, Fenaroli's Handbook of Flavor Ingredients, The Chemical Rubber Company, Cleveland, Ohio, (1971), known to those skilled in the art.

In other embodiments, the compositions comprise at least one synthetic or natural food coloring (e.g., annatto extract, astaxanthin, beet powder, ultramarine blue, canthaxanthin, caramel, carotenal, beta carotene, carmine, toasted cottonseed flour, ferrous gluconate, ferrous lactate, grape color extract, grape skin extract, iron oxide, fruit juice, vegetable juice, dried algae meal, tagetes meal, carrot oil, corn endosperm oil, paprika, paprika oleoresin, riboflavin, saffron, tumeric, tumeric and oleoresin).

In still further embodiments, the compositions comprise at least one phytonutrient (e.g., soy isoflavonoids, oligomeric proanthcyanidins, indol 3 carbinol, sulforaphone, fibrous ligands, plant phytosterols, ferulic acid, anthocyanocides, triterpenes, omega 3/6 fatty acids, conjugated fatty acids such as conjugated linoleic acid and conjugated linolenic acid, polyacetylene, quinones, terpenes, cathechins, gallates, and quercitin). Sources of plant phytonutrients include, but are not limited to, soy lecithin, soy isoflavones, brown rice germ, royal jelly, bee propolis, acerola berry juice powder, Japanese green tea, grape seed extract, grape skin extract, carrot juice, bilberry, flaxseed meal, bee pollen, ginkgo biloba, primrose (evening primrose oil), red clover,

burdock root, dandelion, parsley, rose hips, milk thistle, ginger, Siberian ginseng, rosemary, curcumin, garlic, lycopene, grapefruit seed extract, spinach, and broccoli.

In still other embodiments, the compositions comprise at least one vitamin (e.g., vitamin A, thiamin (B1), riboflavin (B2), pyridoxine (B6), cyanocobalamin (B12), biotin, ascorbic acid (vitamin C), retinoic acid (vitamin D), vitamin E, folic acid and other folates, vitamin K, niacin, and pantothenic acid). In some embodiments, the particles comprise at least one mineral (e.g., sodium, potassium, magnesium, calcium, phosphorus, chlorine, iron, zinc, manganese, flourine, copper, molybdenum, chromium, selenium, and iodine). In some particularly preferred embodiments, a dosage of a plurality of particles includes vitamins or minerals in the range of the recommended daily allowance (RDA) as specified by the United States Department of Agriculture. In still other embodiments, the particles comprise an amino acid supplement formula in which at least one amino acid is included (e.g., l-carnitine or tryptophan).

C. Uses of Krill Oil

Previously, it was disclosed that omega-3 fatty acids have anti-inflammatory properties. See, e.g., Calder. Am. J. Clin. Nutr. 83 (2006) 1505S. In addition, in it was disclosed that a phospholipid emulsion derived from a marine and/or synthetic origin comprising polyunsaturated fatty acids have anti-inflammatory and/or immuno-suppressive effects. See, e.g., 5,434,183. An embodiment of this invention is a krill oil composition effective for reducing inflammation i.e. reducing the levels of TNF-α, IL-1 beta, IL-6, IL-10, TGF beta and fibrinogen in the blood.

Type 2 diabetes is a metabolic disorder characterized by impaired glycemic control (high blood glucose levels). In type 2 diabetes, it is the tissue wide insulin resistance that contributes to the development of the disease. Strategies reducing insulin resistance or improving tissue sensitivity to insulin are recognized as beneficial in preventing type 2 diabetes. In healthy humans, a 3-week supplementation with fish oil (1.1 g EPA/d and 0.7 g DHA/d) decreased the insulin response to an oral glucose load by 40%. Omega-3 PUFA dietary enrichment resulted in lower glucose oxidation, higher fat oxidation, and increased glycogen storage; the glycemic response was unchanged, however, which indicates an improved sensitivity to insulin. In another embodiment of this invention is a krill oil composition effective for reducing the insulin resistance.

Krill oil has not been disclosed as being effective in treating one of the most important life style problems of modern societies, i.e., excess weight gain and obesity. Excess adipose tissue mass (overweight and obesity) is associated with low grade inflammation in adipose tissue and in the whole body reflecting the inflammatory mediators "spilling over" from fat tissue. Trayhurn et al., Br. J. Nutrition (2004), 92(3), 347-355. Inflammation appears to be an important link between obesity and metabolic syndrome/type-II diabetes as well as cardiovascular disease. Libby et al., J. Amer. Coll. Card. (2006), 48(9, Suppl. A), A33-A46. Thus, excess adipose tissue is an unhealthy condition. Weight reduction will improve the inflammatory condition, but persistent weight reduction is difficult to achieve. Omega-3 fatty acid supplementation may alleviate the inflammatory condition in adipose tissue and thus ideally complement the principal strategies of weight reduction i.e. low calorie diet and exercise. There are clinical studies in humans that demonstrate that omega-3 enhance the effect of very low calorie diet and exercise in reducing body fat mass. Kunesova et al., Physiological research / Academia Scientiarum Bohemoslovaca (2006), 55(1), 63-72. Although diet and exercise regime may fail to result in consistent decrease in weight in long term, the effect of omega-3 fatty acids alleviating the inflammatory condition in the adipose tissue may persist generating a condition that can be described as "healthy adipose tissue". Previously, it was shown that dietary omega-3 fatty acids can be used to reduce inflammation in adipose tissue without influencing level of obesity. Todoric et al., Diabetologia (2006), 49(9), 2109-2119. Reduction in adipose tissue inflammation was demonstrated by an increase in circulating levels of adiponectin. Adiponectin is an adipose tissue derived anti-inflammatory hormone. Results on the treatment of obese people with omega-3 fatty acids to alleviate circulating levels of inflammatory markers are inconclusive. Trebble et al., Br. J. Nutrition (2003), 90(2), 405-412. However, duration of these studies may not have been sufficient given the slow turnover of adipose tissue in humans. Itoh et al. found that 1.8 g/d of EPA increased adiponectin, a marker of adipose tissue derived inflammation, in a group of overweight subjects with metabolic syndrome. Itoh et al., Arteriosclerosis, Thrombosis, and Vascular Biology (2007), 27(9), 1918-1925.

An embodiment of the invention is the use of krill oil to increase serum adiponectin levels. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. Adiponectin is exclusively secreted from adipose tissue into the bloodstream and is very abundant in plasma relative to many hormones.

Levels of the hormone are inversely correlated with body mass index (BMI). The hormone plays a role in alleviating the metabolic dysregulation that may result in type 2 diabetes, obesity, atherosclerosis and non-alcoholic fatty liver disease (NAFLD). Díez et al., Eur. J. Endocrinol. 148 (3): 293-300; Ukkola et al., J. Mol. Med. 80 (11): 696-702.

Another embodiment of the invention is to use krill oil in an overweight and obese subjects for alleviating diet induced adipose tissue dysfunction and diet induced changes in the lipid metabolism.

In further embodiments, krill oil is effective in reducing risk factors of type 2 diabetes such as hyperinsulinemia and insulin resistance and cardiovascular disease risk factors in overweight subjects. In addition this invention discloses that krill oil is effective in preventing accumulation of fat in muscles and in the liver (liver steatosis).

It is well known in the art that the obese Zucker rat is a useful rat model to study metabolic Syndrome X and non-insulin dependent diabetes mellitus, including glucose tolerance, insulin resistance and hyperinsulinaemia. It has also been shown previously that astaxanthin is a powerful antioxidant, useful for prevention of oxidative stress in vivo and in Zucker rats using vitamin E. See, e.g., Aoi et al., (2003). Antioxidants & Redox Signaling. 5(1):139-44; Laight et al., Eur. J. Pharmacol. 377 (1999) 89.

In yet another embodiment of the invention is a krill oil composition effective of improving the blood lipid profile by increasing the HDL cholesterol levels, decreasing the LDL cholesterol and triglyceride levels. Hence the novel krill oil composition is effective for treating metabolic syndrome. Metabolic syndrome is defined as the coexistence of 3 or more components selected from the group: abdominal obesity, high serum triglyceride levels, low HDL levels, elevated blood pressure and high fasting plasma glucose levels.

In another embodiment of the invention, the krill oil compositions are found to be effective and safe for the treatment of metabolic syndrome in humans.

In still other embodiments, the krill oil compositions of the present invention find use in increasing or inducing diuresis. In some embodiments, the krill oil compositions of the present invention find use in decreasing protein catabolism and increasing the muscle mass of a subject.

In some embodiments, the kill oil composition of the present invention find use in the treatment of fatty heart disease and non-alcoholic fatty acid liver disease. Thus, the krill oil

compositions are useful for decreasing the lipid content of the heart and/or liver and/or muscle of a subject.

In yet another embodiment of the invention is a method to increase the transfer of DHA to the brain.

EXAMPLE 1

Antarctic krill (*Euphausia superba*) was captured and brought on board alive, before it was processed into krill meal, an oil (asta oil) and stickwater. The composition and properties of the krill meal was monitored during the processing and compared to a commercial competitor (Table 1 and 2). Furthermore, the amino acid composition of the krill meal and stickwater was determined (Table 3), showing that krill meal is a suitable feed source for to be used in aquaculture due to the presences of all the essential amino acids teleost fish require. During the krill meal processing a neutral oil (asta oil) is recovered, the chemical composition of the asta oil is shown in Tables 4 and 5.

Table 1. Composition of products from the processing line

	Round frozen krill	After decanter	After drier	Konstruktor Koshkin (Ukranian vessel)
Protein	13,5 g/100 g	20,9 g/100 g	58,5 g/100 g	60,2 g/100 g
Moisture	76,3 g/100 g	65,6 g/100 g	9,1 g/100 g	9,6 g/100 g
Lipid (Folch)	8,6 g/100 g	10 g/100 g	21,8 g/100 g	21,4 g/100 g
Free fatty acids	29,8 g/100 g	25,3 g/100 g	24,8 g/100 g	23,3 g/100 g
Total astaxanthin	53,3 mg/kg	81,3 mg/kg	145 mg/kg	126 mg/kg

Table 2. Lipid class composition in products from the processing line

Crude protein	Round frozen krill (g/100 g)	After decanter (g/100 g)	After drier (g/100 g)	Konstruktor Koshkin (Ukranian vessel) (g/100 g)
Wax ester/cholesterol ester	2,5	3,0	1,9	3,3
Triglycerides/pigments	30,2	33,7	29,3	32,2
Free fatty acids	15,1	2,5	9,0	5,9
Monoglycerides	3,9	Nd	1,3	Nd
PE	6,6	10,4	7,9	6,3

PS	1,2	1,6	1,4	2,7
PI	1,9	2,0	2,1	3,5
PC	28	35,9	32,0	32,1
Sphingomyeline/lyso PC	2,0	0,5	3,0	3,0

Nd= not detected

Table 3. Amino acids in krill meal and stick water

Amino acid	Total in meal (g/100 g protein)	Free in meal (g/100g protein)	Free in stickwater (g/100 g protein)
Aspartic acid	10,5	0,02	0,22
Glutamic acid	13,5	0,007	0,51
Hydroxiproline	<0,5	<0,001	<0,05
Serine	4,2	0,02	0,13
Glycine	4,4	0,18	3,28
Histidine	2,1	<0,01	<0,05
Arginine	6,7	0,56	4,86
Threonine	4,1	<0,01	0,22
Alanine	5,4	0,08	0,87
Proline	3,8	0,53	2,32
Tyrosine	4,0	0,01	0,2
Valine	5,0	0,02	0,13
Methionine	2,9	<0,01	0,12
Isoleucine	5,0	0,02	0,1
Leucine	7,8	0,14	0,19
Phenylalanine	4,4	0,01	0,1
Lysine	7,8	0,02	0,27
Cysteine/Cystine	1,4	<0,01	<0,05
Thryptophan	1,1	<0,02	<0,05
Creatinine		<0,01	<0,05
Asparagine		<0,01	0,05

Glutamine		<0,01	<0,05
3-aminopropanoic acid		0,5	8,99
Taurine		0,5	8,52
4-aminobutanoic acid		<0,01	<0,05
Citrulline		0,04	0,14
Carnosine		<0,01	<0,05
Anserine		<0,01	<0,05
Ornithine		0,02	1,04

3-aminopropanoic acid is also known as β-alanine

4-aminobutanoic acid is alos known as γ-aminobutyric acid or GABA

Table 4. Composition and quality parameters of asta oil.

Moisture	0,14 g/100 g
Insoluble impurities	0,02 g/100 g
Unsaponifable matter	1,5 g/100 g
Nitrogen	0,5 g/100 g
Free fatty acids	0,3 g/100 g
Peroxide value	<2 meq peroxide/kg oil
Ansidine value	<1
Phosphorous	23 mg/kg
Phopspholipids	575 mg/kg
Astaxanthin	1245 mg/kg

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Table 5. Fatty acid composition of the asta oil

Fatty Acid	**Asta oil**
File	
C4:0	0,00
C6:0	0,00
C8:0	0,00
C10:0	0,00
C12:0	0,00
C14:0	17,5
C14:1	0,00
C15:0	0,00
C16:0	19,3
C16:1	9,7
C18:0	1,2
C18:1	22,6
C18:2N6	1,4
C18:3N6	0,1

C18:3N3	0,7
C18:4N3	3,0
C20:0	0,1
C20:1	1,3
C20:2N6	<0,1
C20:3N6	0,1
C20:4N6	0,1
C20:3N3	<0,1
C20:4N3	0,2
C20:5N3 (EPA)	5,6
C22:0	0,1
C22:1	0,3
C22:2N6	0,0
C22:4N6	<0,1
C22:5N6	0,00
C22:5N3	0,2
C22:6N3 (DHA)	2,00
C24:1	0,03
Total	88,4
Saturated	38,0
Monounsaturated	33,9
Polyunsaturated	16,4
Total	88,4
Omega-3	11,9
Omega-6	1,6

EXAMPLE 2

The krill meal obtained in example 1 was then ethanol extracted according to the method disclosed in JP02215351. The results showed that around 22% fat from the meal could be extracted, somewhat lower than was extracted using Folch (25%). Table 6 shows the fatty acid composition of the krill meal and the krill oil extracted from the meal using ethanol. Table 7 shows the composition and properties of the krill meal and products before and after extraction, whereas table 8 shows the lipid composition.

Table 6. Fatty acid distribution in krill meal (g/100 g lipid) and the ethanol extracted krill oil.

Fatty Acid	Krill meal	EtOH KO
File		
C4:0	0,00	
C6:0	0,00	
C8:0	0,00	
C10:0	0,00	
C12:0	0,00	
C14:0	7,8	6,4
C14:1	0,00	
C15:0	0,00	
C16:0	15,8	14,7
C16:1	5,1	4,2
C18:0	0,9	0,7
C18:1	13,4	11,8
C18:2N6	1,1	1,2
C18:3N6	0,1	0,1
C18:3N3	0,4	0,4
C18:4N3	1,1	0,1
C20:0	0,1	0,1
C20:1	0,8	0,6
C20:2N6	<0,1	<0,1
C20:3N6	0,1	<0,1
C20:4N6	0,2	0,2
C20:3N3	<0,1	<0,1
C20:4N3	0,2	0,2
C20:5N3 (EPA)	10,5	10,4
C22:0	<0,1	<0,1
C22:1	0,5	0,4
C22:2N6	<0,1	<0,1
C22:4N6	<0,1	
C22:5N6	0,00	
C22:5N3	0,2	
C22:6N3 (DHA)	5,4	4,8
C24:1	0,03	
Saturated	24,6	21,9
Monounsaturated	19,9	17,0
Polyunsaturated	21,0	19,4
Total	65,5	58,2

Omega-3	18,2	17,0
Omega-6	1,3	

Table 7. Composition and properties of the krill meal and products after extraction

	Krill meal	Delipidated krill meal	EtOH extracted krill oil
Crude protein	586 g/kg	735 g/kg	
Fat (Folch)	250 g/kg	30 g/kg	
Moisture/ethanol	71 g/kg	134 g/kg	85 g/kg
Astaxanthin esters	144 mg/kg	10 mg/kg	117 mg/kg
Diesters	110 mg/kg	8,5 mg/kg	117 mg/kg
Monoesters	33 mg/kg	1,8 mg/kg	37 mg/kg
Biological digestable protein	854 g/kg protein	870 g/kg protein	
Flow number	4,8	1,9	
NH3	9 mg N/100 g	0	3 mg N/100 g
TMA	2 mg N/100 g	0	70 mg N/100 g
TMAO	125 mg N/100 g	0	456 mg N/100 g

Table 8. Lipid class distribution

	Krill meal	Delipidated krill meal	EtOH extracted KO
Cholesterol ester	3,5		
TG	32,7	37,4	31,1
FFA	7,8	14,1	16,0
Cholesterol	9,1	8,0	12,6
DG	1,1		3,3
MG	3,7		
Sphingolipid			2,8
PE	6,5	2,5	2,7
Cardiolipin		4,2	
PI	1,1	11,0	
PS	1,4		
PC	28,6	20,2	25,3
LPC	2,9	2,6	6,2
Total polar lipids	40,6	40,5	36,9

Total neutral lipids	54,2	59,5	63,1

EXAMPLE 3

The krill meal obtained in example 1 was then subjected to a supercritical fluid extraction method in two stages. During stage 1, 12.1% fat (neutral krill oil) was removed using neat CO_2 only at 300 bars, 60° C and for 30 minutes. In stage 2, the pressure was increased to 400 bar and 20% ethanol was added (v/v) for 90 minutes. This resulted in further extraction of 9% polar fat which hereafter is called polar krill oil. The total fatty acid composition of the polar krill oil, the neutral krill oil and a commercial product obtained from Neptune Biotech (Laval, Quebec, Canada) are listed in Table 9. In addition the fatty acid composition for the phospholipids (Table 10), the neutral lipids (Table 11), the free fatty acids, diglycerides (Table 12), triglycerides, lyso-phosphatidylcholine (LPC) (Table 13), phosphatidylcholine (PC), phosphatidylethanolamine (PE) (Table 14), phosphatidylinositol (PI) and phosphatidylserine (PS) (Table 15) are shown. Table 16 shows the level of astaxanthin and cholesterol for the different fractions.

Table 9. Total fatty acids compositions of the krill oil products (% (w/w))

	Total Fatty Acids		
Fatty Acid	**Neutral KO**	**Polar KO**	**NKO**
File			
C4:0	0,00	0,00	0,00
C6:0	0,00	0,00	0,00
C8:0	0,00	0,00	0,00
C10:0	0,00	0,00	0,00
C12:0	0,47	0,04	0,24
C14:0	22,08	3,28	12,48
C14:1	0,33	0,01	0,17
C15:0	0,58	0,36	0,52
C16:0	27,03	29,25	23,25
C16:1	0,07	0,01	8,44
C18:0	1,72	1,03	1,42
C18:1	30,29	13,57	18,92
C18:2N6	2,10	1,96	1,71
C18:3N6	0,30	0,21	0,00
C18:3N3	0,69	1,02	1,32
C18:4N3	0,05	1,81	3,50
C20:0	0,06	0,00	0,05
C20:1	1,87	0,80	1,16
C20:2N6	0,05	0,05	0,05

C20:3N6	0,22	0,73	0,04
C20:4N6	0,00	0,00	0,49
C20:3N3	0,09	0,09	0,06
C20:4N3	0,24	0,51	0,33
C20:5N3 (EPA)	7,33	29,88	16,27
C22:0	0,01	0,06	0,05
C22:1	0,64	1,78	0,82
C22:2N6	0,00	0,00	0,00
C22:4N6	0,00	0,00	0,07
C22:5N6	0,00	0,03	0,00
C22:5N3	0,21	0,67	0,36
C22:6N3 (DHA)	3,51	12,61	8,17
C24:0	0,05	0,00	0,01
C24:1	0,03	0,25	0,11
Total	100,00	100,00	100,00
Saturated	52,00	34,01	38,01
Monounsaturated	33,22	16,43	29,61
Polyunsaturated	14,77	49,56	32,37
Total	100,00	100,00	100,00
Omega-3	12,11	46,58	30,02
Omega-6	2,67	2,98	2,35

Table 10. Fatty acid composition of the phospholipid fraction (% (w/w)).

	Total Phospholipid		
Fatty Acid	**Neutral KO**	**Polar KO**	**Neptune KO**
File			
C4:0	0,00	0,00	0,00
C6:0	0,00	0,00	0,00
C8:0	0,00	0,00	0,00
C10:0	0,00	0,00	0,00
C12:0	0,00	0,00	0,00
C14:0	0,01	0,00	0,00
C14:1	0,42	0,01	0,01
C15:0	2,52	0,00	0,00
C16:0	4,73	35,78	32,81
C16:1	0,19	0,17	0,19
C18:0	6,31	1,18	1,55
C18:1	38,40	15,58	13,54
C18:2N6	4,18	2,16	1,90
C18:3N6	0,18	0,22	0,19

C18:3N3	1,02	1,05	1,48
C18:4N3	3,08	1,62	2,15
C20:0	0,27	0,00	0,07
C20:1	2,55	1,02	0,78
C20:2N6	0,19	0,06	0,06
C20:3N6	0,00	0,14	0,10
C20:4N6	0,57	0,62	0,64
C20:3N3	0,43	0,08	0,09
C20:4N3	0,17	0,45	0,42
C20:5N3 (EPA)	20,58	25,53	26,47
C22:0	0,14	0,06	0,00
C22:1	0,00	2,09	1,94
C22:2N6	0,25	0,71	0,85
C22:4N6	0,44	0,00	0,03
C22:5N6	0,11	0,00	0,00
C22:5N3	0,00	0,60	0,63
C22:6N3 (DHA)	10,93	10,30	13,34
C24:0	1,77	0,30	0,37
C24:1	0,59	0,28	0,38
Total	100,00	100,00	100,00
Saturated	15,74	37,32	34,81
Monounsaturated	42,14	19,15	16,84
Polyunsaturated	42,12	43,53	48,34
Total	100,00	100,00	100,00
Omega-3	36,22	39,62	44,56
Omega-6	5,91	3,90	3,78

Table 11. Fatty acid composition of the total neutral lipid fraction (% (w/w)).

	Total neutral lipid		
Fatty Acid	**Neutral KO**	**Polar KO**	**Neptune KO**
File			
C4:0	0,00	0,00	0,00
C6:0	0,00	0,00	0,00
C8:0	0,00	0,00	0,00
C10:0	0,00	0,00	0,00
C12:0	0,00	0,00	0,00
C14:0	20,35	11,31	18,44
C14:1	0,30	0,29	0,25
C15:0	0,53	1,53	0,62

C16:0	23,79	0,49	24,11
C16:1	12,42	5,22	11,86
C18:0	1,54	3,27	1,67
C18:1	26,81	33,09	23,82
C18:2N6	1,68	2,37	1,79
C18:3N6	0,20	0,23	0,25
C18:3N3	0,59	0,62	0,03
C18:4N3	0,03	1,27	0,05
C20:0	0,07	0,00	0,06
C20:1	1,63	1,41	1,39
C20:2N6	0,04	0,00	0,05
C20:3N6	0,18	0,94	0,01
C20:4N6	0,00	0,00	0,00
C20:3N3	0,09	0,00	0,01
C20:4N3	0,18	0,41	0,23
C20:5N3 (EPA)	5,88	19,26	9,68
C22:0	0,02	0,00	0,03
C22:1	0,56	0,60	0,53
C22:2N6	0,00	0,00	0,00
C22:4N6	0,00	0,00	0,04
C22:5N6	0,01	0,00	0,00
C22:5N3	0,17	0,27	0,22
C22:6N3 (DHA)	2,74	17,22	4,64
C24:0	0,15	0,00	0,17
C24:1	0,03	0,21	0,06
Total	100,00	100,00	100,00
Saturated	46,45	16,60	45,10
Monounsaturated	41,75	40,82	37,91
Polyunsaturated	11,80	42,59	16,99
Total	100,00	100,00	100,00
Omega-3	9,68	39,05	14,86
Omega-6	2,11	3,54	2,14

Table 12. Fatty acid composition of the diglyceride and free fatty acids (% (w/w)).

	Diglycerides			**Free fatty acids**		
Fatty Acid File	**Neutral KO**	**Polar KO**	**Neptune KO**	**Neutral KO**	**Polar KO**	**Neptune KO**
C4:0	0,00	0,00	0,00	0,00	0,00	0,00
C6:0	0,00	0,00	0,00	0,00	0,00	0,00
C8:0	0,00	0,00	0,00	0,00	0,00	0,00

C10:0	0,00	0,00	0,00	0,00	0,00	0,00
C12:0	0,00	0,00	0,00	0,00	0,00	0,00
C14:0	13,85	14,35	12,22	5,86	7,19	5,45
C14:1	0,18	0,00	0,17	0,05	0,00	0,08
C15:0	0,49	1,08	0,66	0,46	1,60	0,45
C16:0	23,68	35,24	25,81	28,30	29,37	21,12
C16:1	9,49	6,80	0,09	3,27	3,08	4,91
C18:0	1,56	3,63	1,89	1,13	2,43	0,99
C18:1	23,67	19,85	23,82	14,50	14,77	17,41
C18:2N6	1,79	0,21	1,90	1,69	0,97	1,86
C18:3N6	0,17	0,00	0,01	0,14	0,00	0,22
C18:3N3	0,69	0,00	1,19	0,85	0,00	1,34
C18:4N3	1,92	0,00	2,75	1,30	0,00	2,72
C20:0	0,00	0,00	0,00	0,00	0,00	0,00
C20:1	1,09	0,00	1,01	0,48	0,00	0,57
C20:2N6	0,00	0,00	0,00	0,00	0,00	0,00
C20:3N6	0,13	0,00	0,00	0,08	0,00	0,05
C20:4N6	0,45	0,00	0,64	0,78	0,00	1,43
C20:3N3	0,00	0,00	0,00	0,00	0,00	0,00
C20:4N3	0,35	0,00	0,43	0,39	0,00	0,43
C20:5N3 (EPA)	14,03	9,80	18,00	24,33	23,57	25,36
C22:0	0,18	0,00	0,10	0,00	0,00	0,05
C22:1	0,41	0,00	0,57	0,80	0,69	0,37
C22:2N6	0,28	0,00	0,50	0,46	0,00	0,54
C22:4N6	0,00	0,00	0,00	0,00	0,00	0,00
C22:5N6	0,00	0,00	0,00	0,00	0,00	0,00
C22:5N3	0,20	0,00	0,27	0,34	0,00	0,32
C22:6N3 (DHA)	4,74	9,04	7,53	14,31	16,33	13,95
C24:0	0,64	0,00	0,42	0,49	0,00	0,39
C24:1	0,00	0,00	0,00	0,00	0,00	0,00
Total	100,00	100,00	100,00	100,00	100,00	100,00
Saturated	40,40	54,30	41,10	36,24	40,59	28,45
Monounsaturated	34,84	26,64	25,66	19,09	18,54	23,34
Polyunsaturated	24,77	19,06	33,24	44,67	40,87	48,22
Total	100,00	100,00	100,00	100,00	100,00	100,00
Omega-3	21,95	18,85	30,18	41,51	39,90	44,13
Omega-6	2,82	0,21	3,05	3,15	0,97	4,09

Table 13. Fatty acid composition of the triglyceride and lyso-phophatidylcholine fractions (% (w/w)).

Fatty Acid	Triglycerides			Lyso PC		
	Neutral KO	Polar KO	Neptune KO	Neutral KO	Polar KO	Neptune KO
File						
C4:0	0,00	0,00	0,00	0,00	0,00	0,00
C6:0	0,00	0,00	0,00	0,00	0,00	0,00
C8:0	0,00	0,00	0,00	0,00	0,00	0,00
C10:0	0,00	0,00	0,00	0,00	0,00	0,00
C12:0	0,00	0,00	0,00	0,00	0,00	0,00
C14:0	23,06	26,65	25,13	19,38	4,27	2,87
C14:1	0,36	0,93	0,36	0,00	0,08	0,00
C15:0	0,56	2,64	0,78	0,00	0,52	0,45
C16:0	23,17	4,93	27,80	41,00	44,14	30,56
C16:1	13,68	11,58	0,04	0,00	1,84	2,24
C18:0	1,52	3,12	1,99	0,76	1,59	1,32
C18:1	27,83	34,39	27,92	6,65	14,24	11,29
C18:2N6	1,64	2,05	1,92	0,00	1,75	2,07
C18:3N6	0,20	0,00	0,30	0,00	0,00	0,06
C18:3N3	0,51	0,00	0,00	7,95	0,67	1,75
C18:4N3	1,99	0,00	4,83	0,00	1,11	2,46
C20:0	0,06	0,00	0,08	0,00	0,00	0,00
C20:1	1,67	0,00	1,76	0,00	0,52	0,00
C20:2N6	0,04	0,00	0,05	0,00	0,00	0,00
C20:3N6	0,05	0,00	0,01	0,00	0,00	0,54
C20:4N6	0,00	0,00	0,00	0,00	0,40	0,00
C20:3N3	0,05	0,00	0,07	0,00	0,00	0,00
C20:4N3	0,11	0,00	0,17	0,00	0,31	0,55
C20:5N3 (EPA)	2,10	7,97	4,44	0,00	18,59	28,48
C22:0	0,02	0,00	0,04	0,00	0,00	0,00
C22:1	0,37	0,00	0,42	0,00	1,46	0,91
C22:2N6	0,00	0,00	0,00	0,00	0,00	0,00
C22:4N6	0,01	0,00	0,01	0,00	0,00	0,00
C22:5N6	0,00	0,00	0,01	0,00	0,00	0,00
C22:5N3	0,10	0,00	0,16	0,00	0,41	0,62
C22:6N3 (DHA)	0,67	3,97	1,42	24,26	7,79	13,82
C24:0	0,26	1,78	0,26	0,00	0,32	0,00
C24:1	0,00	0,00	0,03	0,00	0,00	0,00
Total	100,00	100,00	100,00	100,00	100,00	100,00
Saturated	48,64	39,12	56,08	61,14	50,83	35,21
Monounsaturated	43,90	46,89	30,52	6,65	18,14	14,44
Polyunsaturated	7,45	13,99	13,41	32,20	31,02	50,35
Total	100,00	100,00	100,00	100,00	100,00	100,00

Omega-3	5,51	11,94	11,11	32,20	28,87	47,69
Omega-6	1,94	2,05	2,30	0,00	2,15	2,66

Table 14. Fatty acid composition of the phosphatidylcholine and the phosphatidylserine fractions (% (w/w)).

	PC			PS		
Fatty Acid File	**Neutral KO**	**Polar KO**	**Neptune KO**	**Neutral KO**	**Polar KO**	**Neptune KO**
C4:0	0,00	0,00	0,00	0,00	0,00	0,00
C6:0	0,00	0,00	0,00	0,00	0,00	0,00
C8:0	0,00	0,00	0,00	0,00	0,00	0,00
C10:0	0,00	0,00	0,00	0,00	0,00	0,00
C12:0	0,00	0,00	0,00	0,00	0,00	0,00
C14:0	0,75	3,29	2,77	7,60	9,52	2,31
C14:1	2,07	0,04	0,02	0,00	0,00	0,00
C15:0	1,34	0,00	0,00	3,83	0,00	0,00
C16:0	16,65	31,92	29,83	30,44	43,61	19,49
C16:1	0,96	0,01	0,17	9,96	3,47	2,79
C18:0	1,33	1,06	1,33	2,08	3,34	2,24
C18:1	34,34	13,55	11,16	0,00	7,37	11,87
C18:2N6	10,55	2,27	1,90	0,00	0,00	0,00
C18:3N6	1,44	0,25	0,20	0,00	0,00	0,00
C18:3N3	2,49	1,19	1,54	0,00	0,00	0,00
C18:4N3	2,38	1,92	2,41	0,00	0,00	0,00
C20:0	2,79	0,03	0,05	0,00	0,00	0,00
C20:1	2,42	0,82	0,74	0,00	0,00	0,00
C20:2N6	0,56	0,05	0,06	0,00	0,00	0,00
C20:3N6	0,67	0,13	0,09	0,00	0,00	0,00
C20:4N6	1,85	0,61	0,56	0,00	0,00	0,00
C20:3N3	3,94	0,07	0,06	0,00	0,00	0,33
C20:4N3	4,32	0,50	0,46	0,00	0,00	0,00
C20:5N3 (EPA)	1,08	29,85	30,09	25,84	15,81	16,35
C22:0	0,00	0,05	0,02	0,00	0,00	0,00
C22:1	2,77	0,00	1,87	0,00	0,00	0,00
C22:2N6	0,00	0,81	0,97	0,00	0,00	0,00
C22:4N6	0,00	0,01	0,02	0,00	0,00	0,00
C22:5N6	1,49	0,01	0,00	0,00	0,00	0,00
C22:5N3	1,48	0,67	0,68	0,00	0,00	0,00
C22:6N3 (DHA)	0,00	10,53	12,49	20,25	16,89	44,63
C24:0	2,34	0,10	0,18	0,00	0,00	0,00
C24:1	0,00	0,25	0,34	0,00	0,00	0,00
Total	100,00	100,00	100,00	100,00	100,00	100,00

Saturated	25,19	36,46	34,18	43,95	56,47	24,04
Monounsaturated	42,56	14,67	14,29	9,96	10,84	14,65
Polyunsaturated	32,25	48,87	51,53	46,09	32,69	61,31
Total	100,00	100,00	100,00	100,00	100,00	100,00
Omega-3	15,69	44,73	47,73	46,09	32,69	61,31
Omega-6	16,56	4,13	3,81	0,00	0,00	0,00

Table 15. Fatty acid composition of the phosphatidylinositol and phophatidylethanolamine fractions (% (w/w)).

	PI			**PE**		
Fatty Acid File	**Neutral KO**	**Polar KO**	**Neptune KO**	**Neutral KO**	**Polar KO**	**Neptune KO**
C4:0	0,00	0,00	0,00	0,00	0,00	0,00
C6:0	0,00	0,00	0,00	0,00	0,00	0,00
C8:0	0,00	0,00	0,00	0,00	0,00	0,00
C10:0	0,00	0,00	0,00	0,00	0,00	0,00
C12:0	0,00	0,00	0,00	0,00	0,00	0,00
C14:0	11,15	5,82	5,72	14,42	4,60	0,83
C14:1	3,03	0,66	0,00	0,00	0,00	0,10
C15:0	5,86	1,95	3,18	0,00	1,30	0,23
C16:0	37,02	30,66	31,39	35,91	31,21	18,38
C16:1	18,05	2,24	1,16	0,00	1,51	0,75
C18:0	6,72	2,83	5,56	12,72	16,70	1,84
C18:1	18,15	24,77	14,23	36,96	19,91	18,45
C18:2N6	0,00	2,67	0,00	0,00	2,62	0,85
C18:3N6	0,00	0,00	0,00	0,00	0,00	0,00
C18:3N3	0,00	0,00	0,00	0,00	0,00	0,33
C18:4N3	0,00	0,00	0,00	0,00	0,00	0,00
C20:0	0,00	0,00	0,00	0,00	0,00	0,00
C20:1	0,00	0,00	0,00	0,00	0,00	0,00
C20:2N6	0,00	0,00	0,00	0,00	0,00	0,00
C20:3N6	0,00	0,00	0,00	0,00	0,00	1,15
C20:4N6	0,00	0,00	0,00	0,00	0,00	0,00
C20:3N3	0,00	0,00	0,00	0,00	0,00	0,00
C20:4N3	0,00	0,00	0,00	0,00	0,00	0,00
C20:5N3 (EPA)	0,00	17,60	20,45	0,00	10,76	21,26
C22:0	0,00	0,00	0,00	0,00	0,00	0,00
C22:1	0,00	0,00	0,00	0,00	0,00	0,00

C22:2N6	0,00	0,00	0,00	0,00	0,00	0,00
C22:4N6	0,00	0,00	0,00	0,00	0,00	0,00
C22:5N6	0,00	0,00	0,00	0,00	0,00	0,00
C22:5N3	0,00	0,00	0,00	0,00	0,00	0,67
C22:6N3 (DHA)	0,00	10,79	18,32	0,00	11,39	35,16
C24:0	0,00	0,00	0,00	0,00	0,00	0,00
C24:1	0,00	0,00	0,00	0,00	0,00	0,00
Total	100,00	100,00	100,00	100,00	100,00	100,00
Saturated	60,76	41,26	45,84	63,04	53,81	21,28
Monounsaturated	39,24	27,67	15,39	36,96	21,42	19,30
Polyunsaturated	0,00	31,07	38,77	0,00	24,77	59,42
Total	100,00	100,00	100,00	100,00	100,00	100,00
Omega-3	0,00	28,40	38,77	0,00	22,15	57,43
Omega-6	0,00	2,67	0,00	0,00	2,62	1,99

Table 16. Compositional data for the novel krill oil composition obtained and NKO krill oil.

Compounds	**Neptune KO**	**Ethanol extracted KO**	**Polar KO**	**Neutral KO**
Astaxanthin esters	472 mg/kg	117 mg/kg	580 mg/kg	98 mg/kg
Astaxanthin free	11 mg/kg	< 1 mg/kg	<1 mg/kg	<1 mg/kg
Total cholesterol	1 g/100g	12 g/100g	< 0,5 g/100g	5,7 g/100g

EXAMPLE 4

Neutral lipids were extracted from krill meal (138 kg) using SFE with neat CO_2 (solvent ratio 25 kg/kg) at 500 bar and 75 °C. The neutral lipids were fractionated at 200 bar (75 °C) and at 60 bar (35 °C) at separator S1 and S2, respectively. The extract obtained in S1 (19,6 kg) were characterized and the results can be found in Tables 17A-C. The extract in table S2 (0,4 kg) were rich in water and were not further used. Next, the polar lipids were extracted using CO_2 at 500 bar, 20% ethanol and at a temperature of 75 °C. Using a solvent ratio of 32 (kg/kg) and collecting an extract of 18,2 kg using a separator at 60 bars and 35°C. The polar lipids were collected and analyzed (Tables 18A-C). Next, the polar lipids were mixed in a 50/50 ratio with the neutral

lipids collected from S1 before finally the ethanol was removed carefully by evaporation. The product obtained was red and transparent. If the ethanol is removed before the mixing if the fractions a transparent product is not obtained. The composition of the 50/50 red and transparent product can be found in Tables 19A-C.

Table 17A Fatty acid composition of the extract collected in S1

Fatty acid	**Unit**	**Amount**
14:0	g/100g	18,4
16:0	g/100g	22,2
18:0	g/100g	1,5
16:1 n-7	g/100g	10,9
18:1 (n-9) + (n-7) + (n-5)	g/100g	25,6
20:1 (n-9) + (n-7)	g/100g	1,8
22:1 (n-11) + (n-9) + (n-7)	g/100g	0,5
16:2 (n-4)	g/100g	1,3
16:4 (n-1)	g/100g	1,2
18:2 n-6	g/100g	1,3
18:3 n-3	g/100g	0,8
18:4 n-3	g/100g	2,9
20:5 n-3	g/100g	4,1
22:6 n-4	g/100g	1,7

Table 17B. Lipid class composition of the extract collected in S1

Lipid	**Unit**	**Amount**
Triacylglycerol	g/100g	84
Diacylglycerol	g/100g	0,7
Free fatty acids	g/100g	1,5
Cholesterol	g/100g	2,7
Cholesterol esters	g/100g	0,9

Table 17C. Miscellaneous analysis of the extract in S1.

Compound	Unit	Amount
Free astaxanthin	mg/kg	4,3
Astaxanthin esters	mg/kg	462
Trimethylamin	mg N/100 g	<1
Trimethylamineoxide	mg N/100 g	2

Table 18A Fatty acid composition of the extract collected after CO_2 and 20% ethanol in S1.

Fatty acid	Unit	Amount
14:0	g/100g	1,3
16:0	g/100g	13,8
18:0	g/100g	0,6
16:1 n-7	g/100g	0,9
18:1 (n-9) + (n-7) + (n-5)	g/100g	6,5
20:1 (n-9) + (n-7)	g/100g	0,6
22:1 (n-11) + (n-9) + (n-7)	g/100g	0,1
16:2 (n-4)	g/100g	<0,1
16:4 (n-1)	g/100g	<0,1
18:2 n-6	g/100g	0,8
18:3 n-3	g/100g	0,6
18:4 n-3	g/100g	1,0
20:5 n-3	g/100g	14,7
22:6 n-4	g/100g	6,5

Table 18B. Lipid class composition of the extract collected after CO_2 and 20% ethanol in S1.

Lipid	Unit	Amount
Triacylglycerol	g/100g	<0,5
Cholesterol	g/100g	<0,5
Phophatidylethanolamine	g/100g	1,6
Phosphatidylcholine	g/100g	67
Lyso-phophatidylcholine	g/100g	4,4

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Table 18C. Miscellaneous analysis of the extract in S1.

Compound	**Unit**	**Amount**
Trimethylamin	mg N/100 g	422
Trimethylamineoxide	mg N/100 g	239

Table 19A Fatty acid composition of the final blended product obtained in Example 4 in S1.

Fatty acid	**Unit**	**Amount**
14:0	g/100g	9,7
16:0	g/100g	18,5
18:0	g/100g	1,0
16:1 n-7	g/100g	5,8
18:1 (n-9) + (n-7) + (n-5)	g/100g	16,0
20:1 (n-9) + (n-7)	g/100g	1,2
22:1 (n-11) + (n-9) + (n-7)	g/100g	1,0
16:2 (n-4)	g/100g	0,3
16:4 (n-1)	g/100g	<0,1
18:2 n-6	g/100g	1,0
18:3 n-3	g/100g	0,8
18:4 n-3	g/100g	2,1
20:5 n-3	g/100g	10,7
22:6 n-4	g/100g	4,7

Table 19B. Lipid class composition of the final blended product obtained in Example 4.

Lipid	**Unit**	**Amount**
Triacylglycerol	g/100g	53
Diacylglycerol	g/100g	1,3
Free fatty acids	g/100g	0,5
Cholesterol	g/100g	0,6
Cholesterol esters	g/100g	<0,5
Phophatidylethanolamine	g/100g	<1

Phosphatidylcholine	g/100g	42
Lyso-phophatidylcholine	g/100g	5,9

Table 19C. Miscellaneous analysis of the final blended product obtained in example 4.

Compound	**Unit**	**Amount**
Free astaxanthin	mg/kg	1,1
Astaxanthin esters	mg/kg	151
Trimethylamin	mg N/100 g	109
Trimethylamineoxide	mg N/100 g	80

EXAMPLE 5

The asta oil obtained in example 1 was blended with the polar lipids obtained in example 4 in a ratio of 46:54 (v/v). Next the ethanol was removed by evaporation and a dark red and transparent product was obtained. The product was analyzed and the results can be found in Tables 20A-C. Furthermore, the product was encapsulated into soft gels successfully. During the encapsulation it was observed that any further increase in phospholipids and thereby viscosity will make it very difficult to encapsulate the final product.

Table 20A Fatty acid composition of the final blended product obtained in Example 5.

Fatty acid	**Unit**	**Amount**
14:0	g/100g	8,2
16:0	g/100g	17,7
18:0	g/100g	1,0
16:1 n-7	g/100g	4,9
18:1 (n-9) + (n-7) + (n-5)	g/100g	14,9
20:1 (n-9) + (n-7)	g/100g	1,1
22:1 (n-11) + (n-9) + (n-7)	g/100g	1,0
16:2 (n-4)	g/100g	0,4
16:4 (n-1)	g/100g	<0,1
18:2 n-6	g/100g	1,2

18:3 n-3	g/100g	0,8
18:4 n-3	g/100g	1,8
20:5 n-3	g/100g	10,6
22:6 n-4	g/100g	4,8

Table 20B. Lipid class composition of the final blended product obtained in Example 5.

Lipid	**Unit**	**Amount**
Triacylglycerol	g/100g	41
Diacylglycerol	g/100g	0,8
Free fatty acids	g/100g	1,2
Cholesterol	g/100g	0,4
Cholesterol esters	g/100g	0,3
Phophatidylethanolamine	g/100g	0,6
Phosphatidylcholine	g/100g	51
Lyso-phophatidylcholine	g/100g	<0,5
Total polar lipids	g/100g	52,4
Total neutral lipids	g/100g	43,6

Table 20C. Miscellaneous analysis of the final blended product obtained in Example 5

Compound	**Unit**	**Amount**
Free astaxanthin	mg/kg	12
Astaxanthin esters	mg/kg	1302
Trimethylamin	mg N/100 g	193
Trimethylamineoxide	mg N/100 g	1,7

EXAMPLE 6

Fresh krill was pumped from the harvesting trawl directly into an indirect steam cooker, and heated to 90C. Water and a small amount of oil were removed in a screw press before

ethoxyquin (antioxidant) was added and the denatured meal was dried under vacuum at a temperature not exceeding 80C. After 19 months storage in room temperature, a sample of the denatured meal was extracted in two steps with supercritical CO_2 in laboratory scale at a flow rate of 2ml/min at 100C and a pressure of 7500 psi. In the second step 20% ethanol was added to the CO_2. The two fractions collected were combined and analyzed by HPLC using ELS detection. The phosphatidylcholine was measured to 42.22% whereas the partly decomposed phosphatidylcholine was 1.68%. This data strongly contrasts the data obtained by analysis of a krill oil sample in the marketplace that showed a content of 9.05% of phosphatidylcholine and 4.60% of partly decomposed phosphatidylcholine.

EXAMPLE 7

Krill lipids were extracted from krill meal (a food grade powder) using supercritical fluid extraction with co-solvent. Initially, 300 bar pressure, 333°K and 5% ethanol (ethanol:CO_2, w/w) were utilized for 60 minutes in order to remove neutral lipids and astaxanthin from the krill meal. Next, the ethanol content was increased to 23% and the extraction was maintained for 3 hours and 40 minutes. The extract was then evaporated using a falling film evaporator and the resulting krill oil was finally filtered. The product obtained was then analyzed and the results can be found in Table 21.

Table 21. Analysis of the krill oil obtained using supercritical fluid extraction.

Parameter	**Value**
Ethanol	1.11% w/w
Water Content	2.98 % w/w
C20:5 n-3 (EPA)	19.9
C22:6 n-3 (DHA)	11.3
Total Omega 3	35.7
Total Omega 6	3.0
Total Phospholipids	50.55 wt%
Ratio Omega3-PL/Total Omega 3	77.6 % w/w
Ratio EPA- PL/Total EPA	84.4 %w/w
Ratio DHA-PL/Total DHA	74.7 %w/w
Triglycerides	25.9 g/100g
Astaxanthin	2091 mg/kg
Peroxide Value	<0.1

EXAMPLE 8

Krill oil was prepared according to the method described in example 7 extracting from the same krill meal. The oil was subjected to ^{31}P NMR analysis for the identification and quantification of the various forms of phospholipids. The analysis was performed according to the following methods: Samples (20 – 40 mg) were weighed into 1.5 ml centrifuge tubes. Next, NMR detergent (750 µl -10% Na cholate, 1% EDTA, pH 7.0 in H_2O+D_2O, 0.3 g L-1 PMG internal standard) was added. Next, the tube was placed in a oven at 60°C and periodically shaken/sonicated until completely dispersed. The solution was then transferred to a 5 ml NMR tube for analysis. Phosphorus NMR spectra were recorded on the two-channel Bruker Avance300 with the following instrument settings: spectrometer frequency 121.498MHz, sweep width 24,271 Hz, 64,000 data points, 30 degree excitation pulse, 576 transients were normally taken, each with an 8 second delay time and f.i.d. acquisition time of 1.35 sec. Spectra were processed with a standard exponential weighting function with 0.2 Hz line broadening before Fourier transformation.

Peaks were identified using known chemical shifts. Deacylation of samples with monomethylamine was also used on two samples for confirmation of peak identity and to achieve better peak resolution. Example spectra are presented in Figure 1. Peak area integration gave relative molar amounts of each lipid class. Weight percent values were calculated using molecular masses calculated from a krill sample fatty acid profile (average chain length = 18.6). Total PL levels were calculated from the PMG internal standard peak. The quantification of the phospholipids are shown in table 25 for both the raw material, the final product and for a commercially available krill oil (Neptune Krill Oil). The main polar ether lipids of the krill meal are alkylacylphosphatidylcholine (AAPC) at 7-9 % of total polar lipids, lyso-alkylacylphosphatidylcholine (LAAPC) at 1 % of total polar lipids (TPL) and alkylacylphosphatidyl-ethanolamine (AAPE) at < 1 % of TPL.

Table 22: Phospholipid profiles

	Type B krill powder	**NKO**	**Krill Oil obtained in Example 7**
PC	**66.0**	**68.6**	**75.3**
AAPC	**12.0**	**7.0**	**13.0**
PI			
1LPC	**1.2**	**1.3**	**0.4**
PS			
2LPC	**7.4**	**13.8**	**2.9**
LAAPC	**2.2**	**1.2**	**0.9**
PE	**6.0**	**3.4**	**3.4**
AAPE			**1.5**
SM			
GPC		**1.3**	
DHSM			
NAPE		**3.4**	
CL	**5.3**		**2.1**
LPE			**0.5**
LCL			
% PL in powder or lipid sample	**8.3**	**30.0**	**47.9**

Analysis has been carried out on the fatty acid and ether/alcohol profiles of the AAPC. The following results are presented in Table 23.

Table 23. Fatty acid profile of the alkylacylphosphatidylcholine.

AAPC fatty acid composition	AAPC alcohol composition alcohol	%
20:5(n-3) – 46.9%;	16:0	47.6
22:6(n-3) – 36.1%;	18:1	17.8
18:1(n-9) – 4.6%	16:1	14.1
22:5(n-3) – 2.6%	14:0	10
20:4(n-6) – 1.9%	18:0	8.6

21:5(n-3) – 1.5%	18:2	5.1
18:2(n-6) – 0.9%	17:0	4.4
16:1(n-9) – 0.8%	15:0-i	2.1
16:0 – 0.7%	15:0	1.7
phytanic – 0.6%	20:1	1.4
18:3(n-3) – 0.5%	15:0-a	1.3
18:4(n-3) – 0.4%	18:0-i	0.4
18:1(n-7) – 0.4%		
24:1 – 0.4%		
14:0 – 0.3%		

The rest of alcohols (i17:0, etc.), were less than 0.3% each. Only part of 20:1 was confirmed by GC-MS. Alcohol moieties composition of Krill AAPC was determined (identification was performed in the form of 1-alkyl-2,3-diTMS glycerols on GC-MS, % of total fatty alcohols were obtained by GC with FID). Ten other fatty acids were all below 0.3 % by mass.

EXAMPLE 9

The purpose of this experiment was to investigate the effect of different omega-3 fatty acid sources on metabolic parameters in the Zucker rat. The Zucker rat is a widely used model of obesity and insulin resistance. Obesity is due to a mutation in the leptin receptor which impairs the regulation of intake. Omega-3 sources compared in this study were fish oil (FO) and two types of krill oil. The krill oil were either from a commercial supplier (Neptune Krill oil) or prepared according to example 7 (Superba^{TM}). Four groups of rats (n = 6 per group) were fed *ad lib* either a control diet (CTRL) or a diet supplemented with a source of omega-3 fatty acids (FO, NKO, Superba). All diets supplied same amount of dietary fatty acids, oleic acid, linoleic acid and linolenic acid. Omega-3 diets (FO, NKO and Superba^{TM}) were additionally balanced for EPA and DHA content. The Zucker rats were 4 wk old at the start of the study with average initial weight of 250 g. At this stage the Zucker rats can be characterized as being pre-diabetic. Rats were fed the test diets for 4 wk after which they were sacrificed and blood and tissue samples were collected. Data presented in the following figures are means ± SE. This example shows that supplementation of the Zucker rat with krill oil prepared as in example 7 results in an improvement of metabolic parameters characteristic of the obesity induced type two diabetic condition. The effect induced by the novel krill oil is often more pronounced than the effect of

FO an in several cases greater than the effect induced by NKO. Specifically, the effects of the two types of krill oil differentiated with respect to the reduction of blood LDL cholesterol levels as well as lipid accumulation in the liver and muscle (Figure 2-9). Furthermore, the efficacy of transfer of DHA from the diet to the brain tissue was greatest with the krill oil prepared as in example 7 (Figure 10).

EXAMPLE 11

This example describes the effect of the supplementation of human diets with krill oil, fish oil (positive control), or a negative control oil (no omega-3 fatty acids) on blood urea nitrogen (BUN).

BUN measures the amount of nitrogen in the blood that comes from urea. BUN is used as a measure of renal function. Serum creatinine is, however, considered to be a more specific measure of renal function. In this study, krill oil decreased BUN by 11.8% while creatinine levels were unchanged. Thus, it is likely that the decrease in BUN is due to some other effect than improved renal function. BUN decreases if krill oil induced diuresis i.e. excretion of urine (diuretic effect).

BUN also decreases if body protein catabolism is reduced. Protein catabolism is a normal feature of body protein turnover. Many tissues express high protein turnover rates. For example the gastrointestinal system expresses high rates of protein turnover. In growing animals a reduction in GI protein catabolism improves weight gain. Mice supplemented with krill oil grew at a faster rate than mice supplemented with fish oil or control diet (Figure 11).

Table 24. The effect on blood urea nitrogen in humans for the different treatment groups.

	Control	Krill Oil	Menhaden oil	p
	n = 23	n = 24	n = 25	
BUN, mg/dL				
Baseline	11.5 (7.8, 13.8)	11.5 (9.5, 13.5)	11.5 (9.5, 14.0)	0.523
Δ from baseline, %	11.0 (-14.3, 26.1)	-11.8 (-20.0, 1.5)	9.1 (-9.1, 35.7)	0.014r

Creatinine, mg/dL				
Baseline	0.9 (0.7, 0.9)	0.9 (0.7, 0.9)	0.8 (0.8, 1.0)	0.952r (r)
Δ from baseline, %	0.0 (-9.6, 2.9)	0.0 (-2.0, 5.9)	0.0 (-5.9, 6.7)	0.416

EXAMPLE 12

The purpose of this experiment was to investigate the effect of dietary krill oil on metabolic parameters in high-fat fed mice and to compare the effect of dietary krill oil with that of fish oil containing the same amount of omega-3 fatty acids. Four groups of C57BL/6 mice (n = 10 per group) were fed 1) chow (N), 2) high fat diet comprising 21% butter fat and 0.15% cholesterol (HF), 3) high fat diet + krill oil (HFKO) or 4) high fat diet + fish oil (HFFO). Treatment 3 contained 2.25% (w/w) krill oil as prepared in example 5 (except that the astaxanthin content was 500 ppm) which were equivalent to 0.36% omega-3 fatty acids. Treatment 4 also contained 0.36% omega-3 fatty acids obtained from regular 18-12 fish oil. The diets were fed to the mice for 7 weeks with free access to drinking water. Data represented in this example means ± SE. Columns not sharing a common letter are significantly different ($P < 0.05$) by ANOVA followed by Tukey's multiple comparison test. N = normal chow diet (n = 10); HF = high-fat diet (n = 10); HFFO = high-fat diet supplemented with fish oil (n = 9); HFKO = high-fat diet supplemented with krill oil (n = 8). The data are presented in Figures 18-25.

This example shows that supplementation of high-fat fed mice with krill oil results in an amelioration of diet-induced hyperinsulinemia, insulin resistance, increase in muscle lipid content (measured as a change in muscle mass), serum adiponectin reduction and hepatic steatosis. These potentially beneficial atheroprotective effects were similar or greater than those achieved with a supplement containing a comparable level of omega-3 fatty acids (Figure 12-19).

CLAIMS

1. A method of production of krill oil from krill comprising:

a) denaturing the krill to provide a denatured krill product;

b) extracting the denatured krill product with supercritical fluid extraction to provide a polar krill oil comprising phospholipids;

c) formulating said polar krill oil for oral consumption.

2. The method of claim 1, wherein said supercritical fluid extraction comprises use of supercritical carbon dioxide.

3. The method of claim 1, wherein said polar krill oil comprises greater than about 40% phosphatidylcholine w/w.

4. The method of claim 1, wherein said polar krill oil comprises greater than about 45% phosphatidylcholine w/w.

5. The method of claim 1, wherein said polar krill oil comprises less than about 25% triglycerides w/w.

6. The method of claim 1, wherein said polar krill oil comprises at least 36% w/w omega-3 fatty acids.

7. The method of claim 1, wherein said krill oil comprises astaxanthin.

8. The method of claim 1, wherein said formulating further comprising formulating said polar krill oil for oral consumption by a human.

9. The method of claim 8, wherein said formulating further comprises formulating said polar krill oil in a capsule.

10. The method of claim 1, wherein said krill is *Euphausia superba*.

11. A method of production of krill oil from *Euphausia superba* krill comprising:

a) denaturing the *Euphausia superba* krill to provide a denatured krill product;

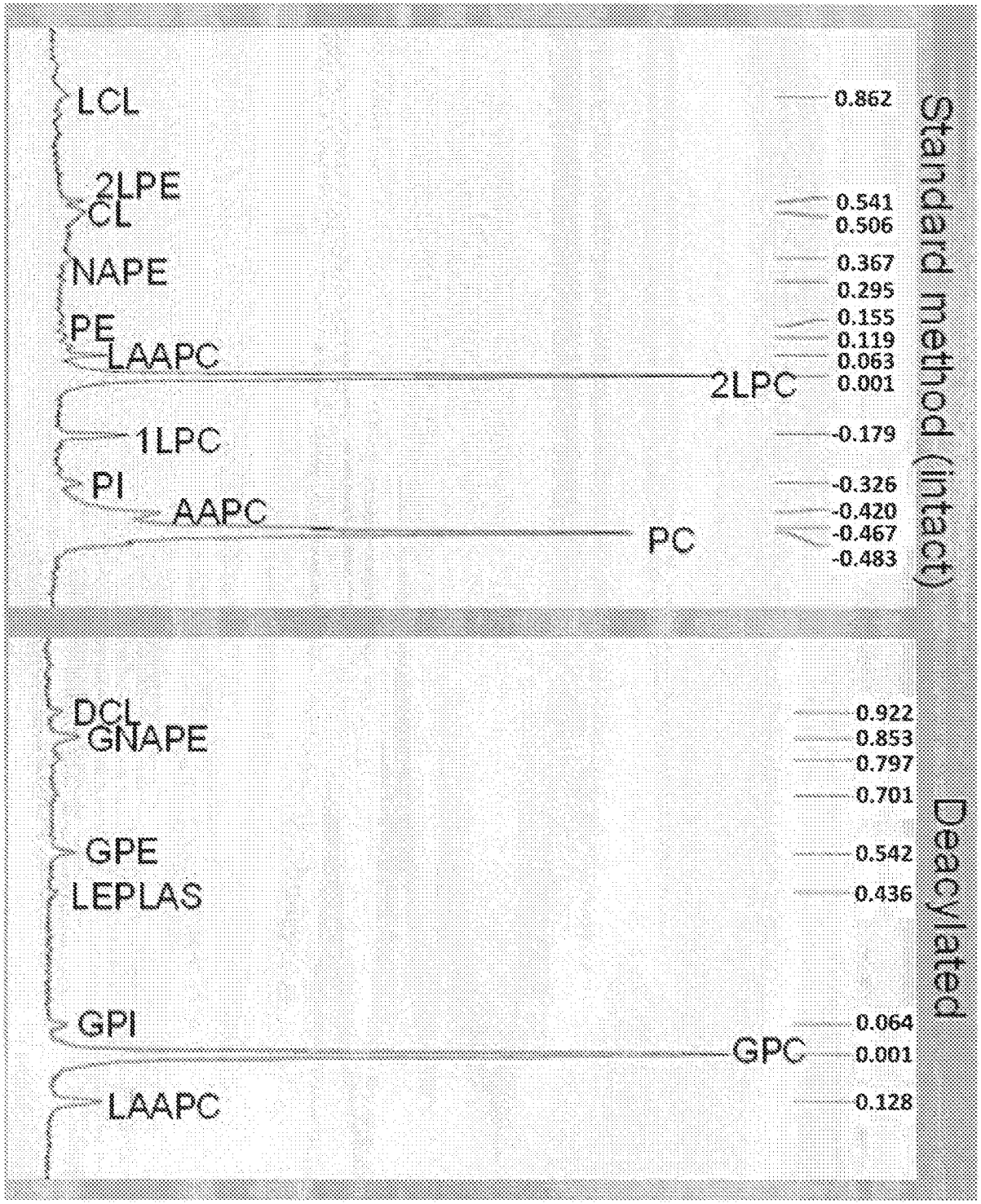
b) extracting the denatured krill product with supercritical fluid extraction to provide a polar krill oil comprising phospholipids;

c) formulating said polar krill oil in a capsule for oral consumption by a human.

Abstract

This invention discloses new krill oil compositions characterized by having high amounts of phospholipids, astaxanthin esters and/or omega-3 contents. The krill oils are obtained from krill meal using supercritical fluid extraction in a two stage process. Stage 1 removes the neutral lipid by extracting with neat supercritical CO_2 or CO_2 plus approximately 5% of a co-solvent. Stage 2 extracts the actual krill oils by using supercritical CO_2 in combination with approximately 20% ethanol. The krill oil materials obatined are compared with commercially available krill oil and found to be more bioeffective in a number of areas such as anti-inflammation, anti-oxidant effects, improving insulin resistances and improving blood lipid profile.

FIGURE 1


Standard method (intact)
LCL
2LPE
CL
NAPE
PE
LAAPC
2LPC
1LPC
PI
AAPC
PC
0.862
0.541
0.506
0.367
0.295
0.155
0.119
0.063
0.001
-0.179
-0.326
-0.420
-0.467
-0.483
Deacylated
DCL
GNAPE
GPE
LEPLAS
GPI
GPC
LAAPC
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0.853
0.797
0.701
0.542
0.436
0.064
0.001
0.128

FIGURE 2

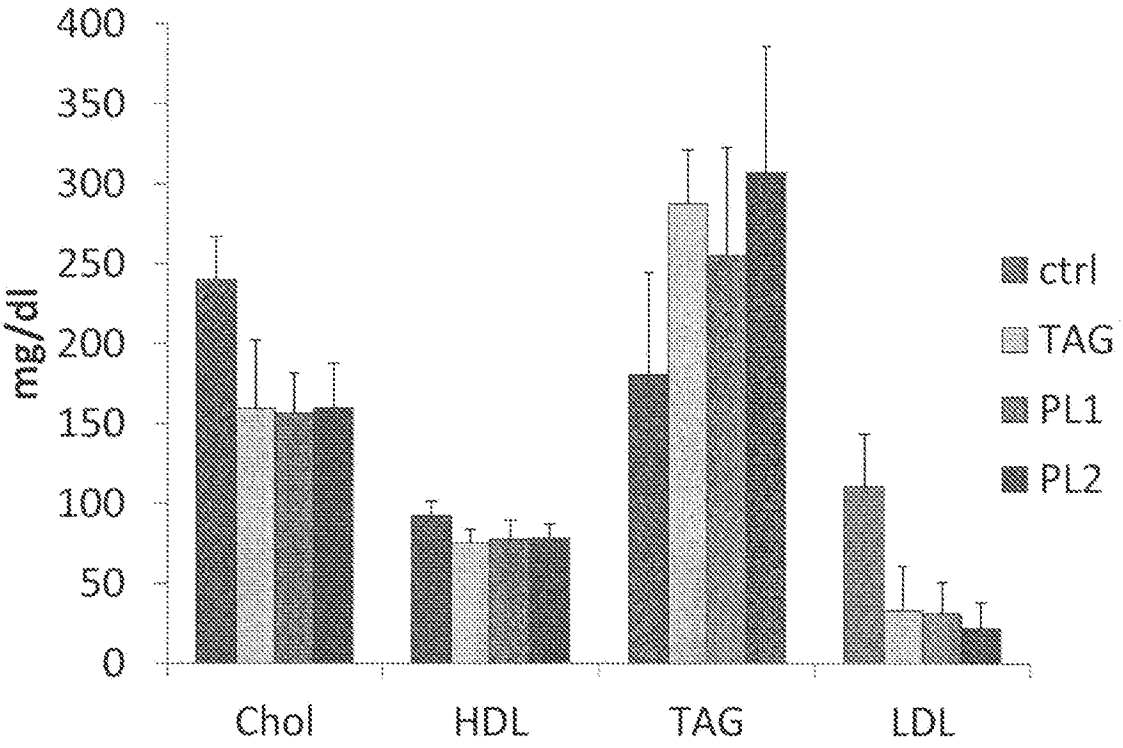

400
350
300
250
200
150
100
50
0
mg/dl
Chol
HDL
TAG
LDL
ctrl
TAG
PL1
PL2

FIGURE 3

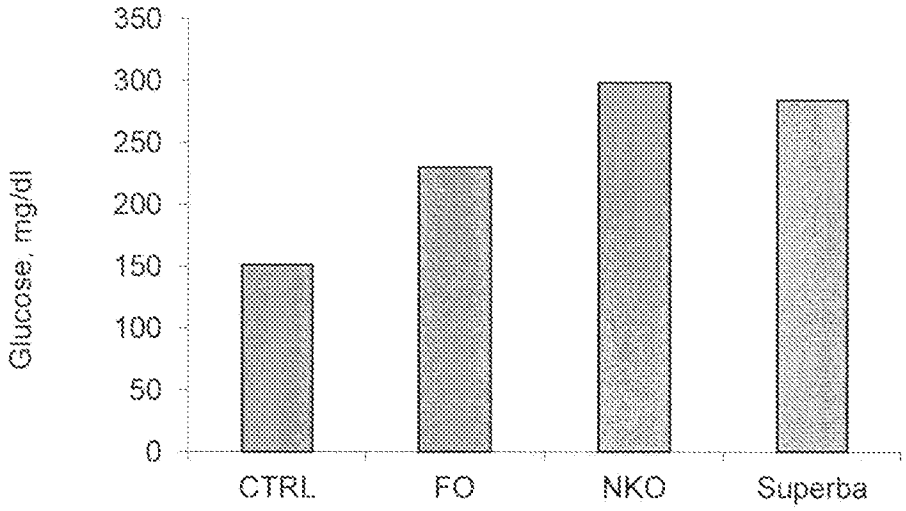


FIGURE 4

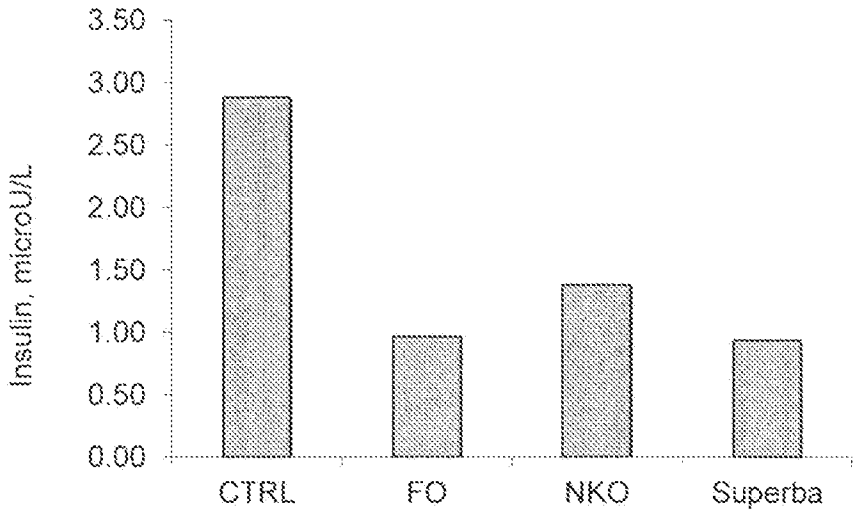

3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Insulin, microU/L
CTRL
FO
NKO
Superba

FIGURE 5

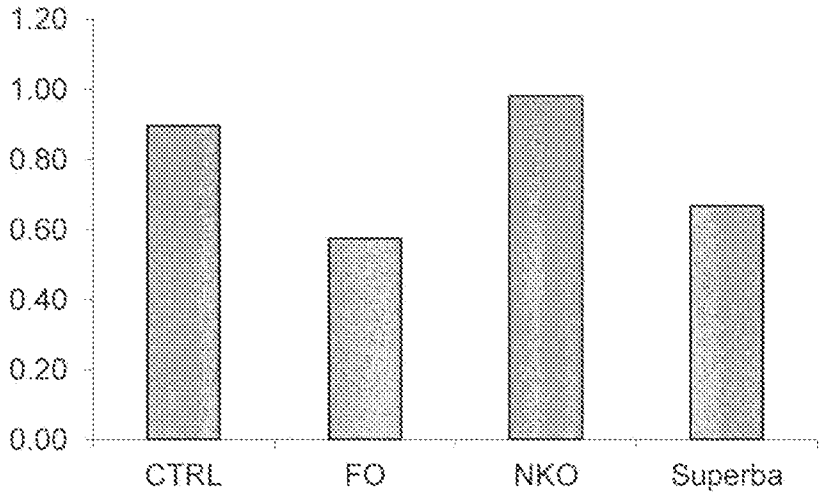


FIGURE 6

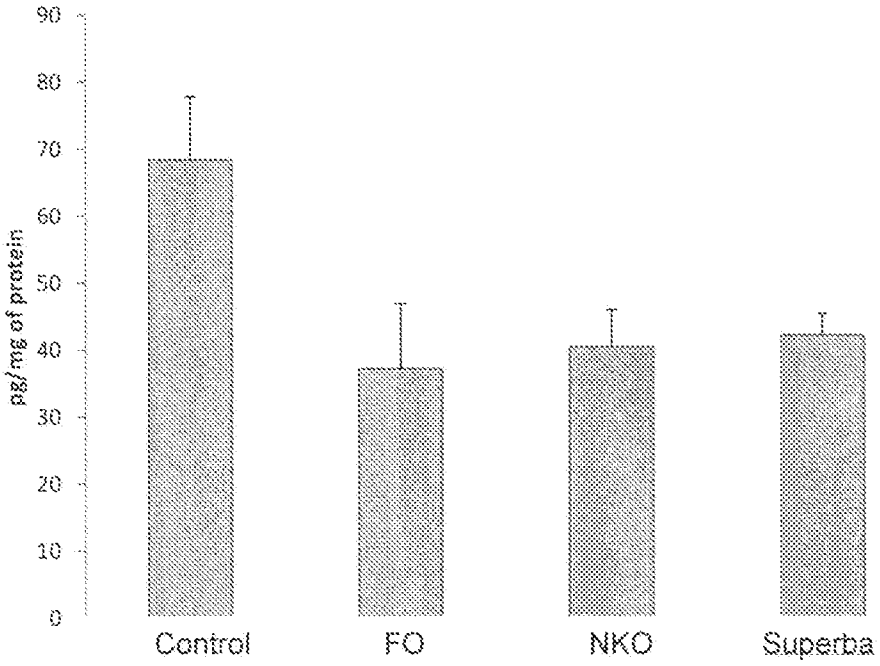

90
80
70
60
50
40
30
20
10
0
pg/mg of protein
Control
FO
NKO
Superba

FIGURE 7

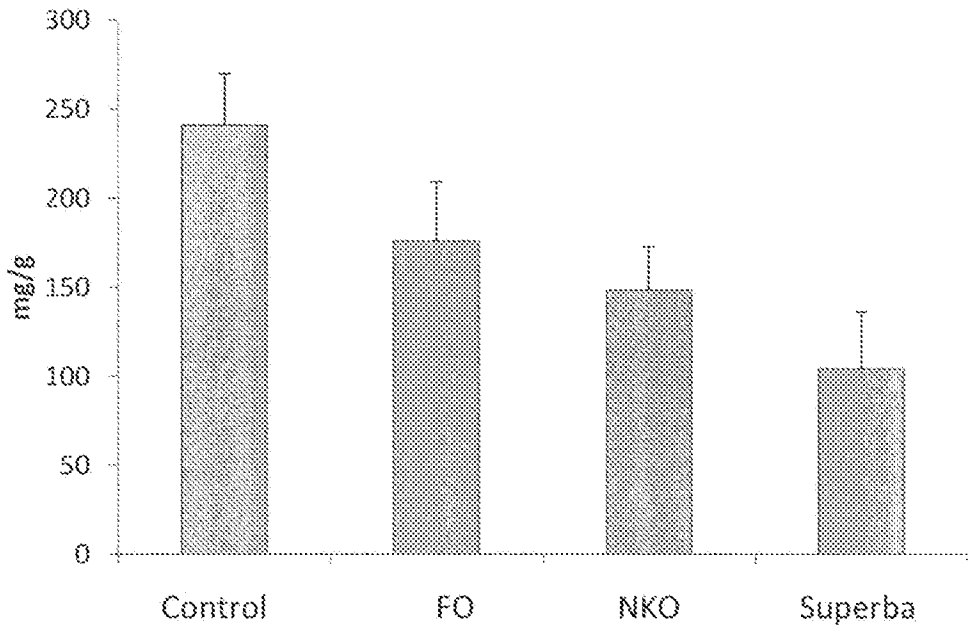


FIGURE 8

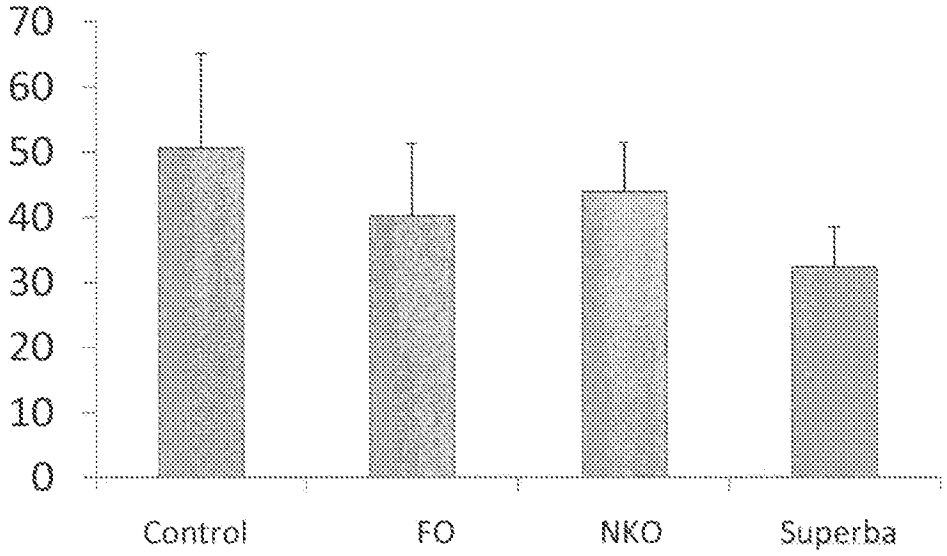


FIGURE 9

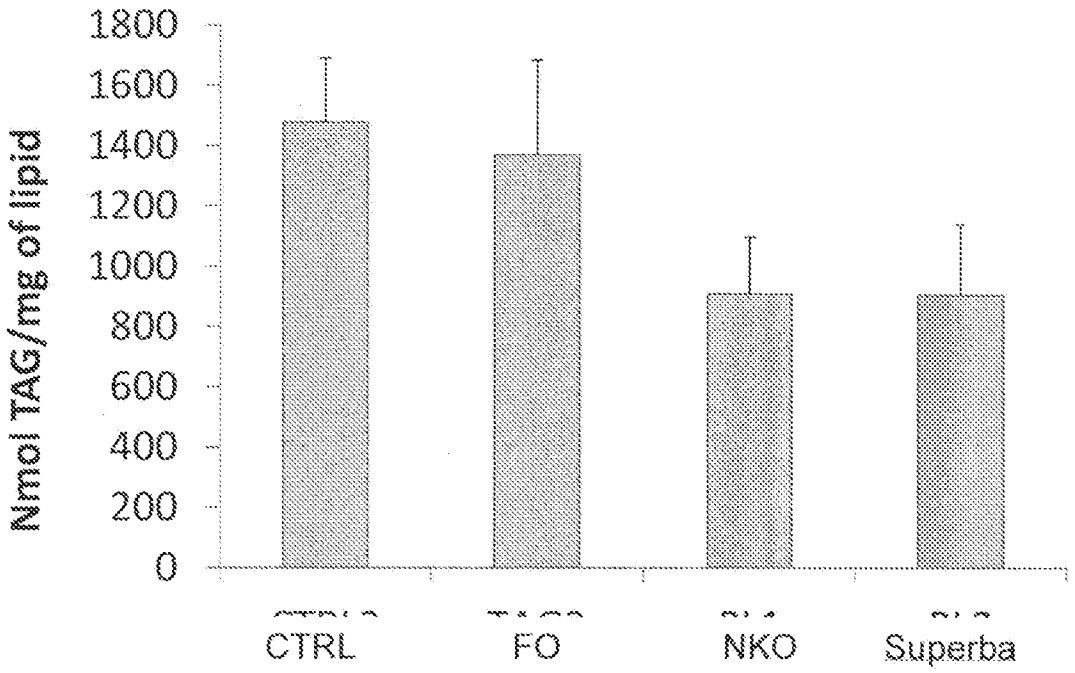

1800
1600
1400
1200
1000
800
600
400
200
0
Nmol TAG/mg of lipid
CTRL
FO
NKO
Superba

FIGURE 10

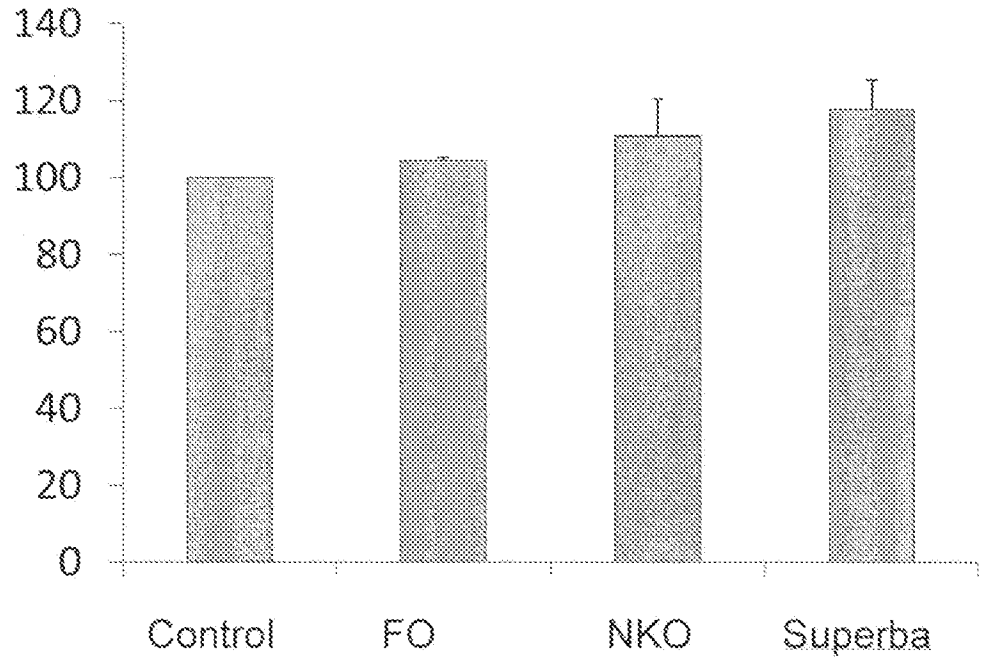
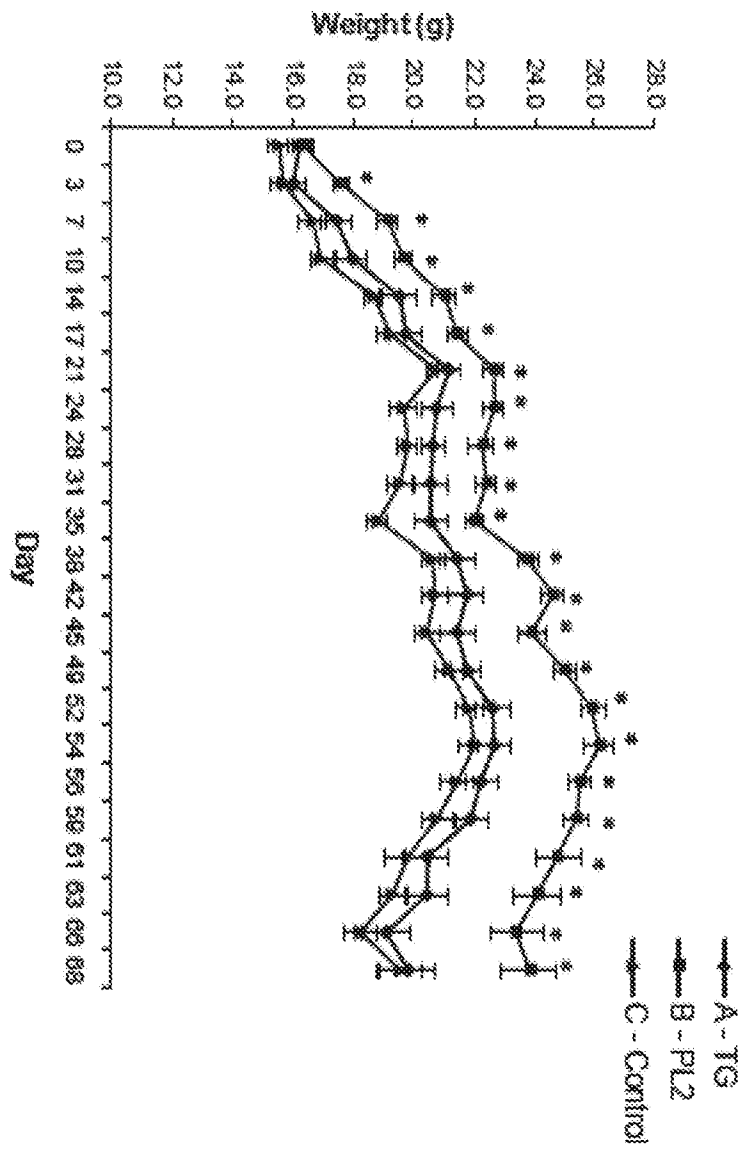


FIGURE 11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12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
Day
0 3 7 10 14 17 21 24 28 31 35 38 42 45 49 52 54 56 59 61 63 66 68
A - TG
B - PL2
C - Control

FIGURE 12

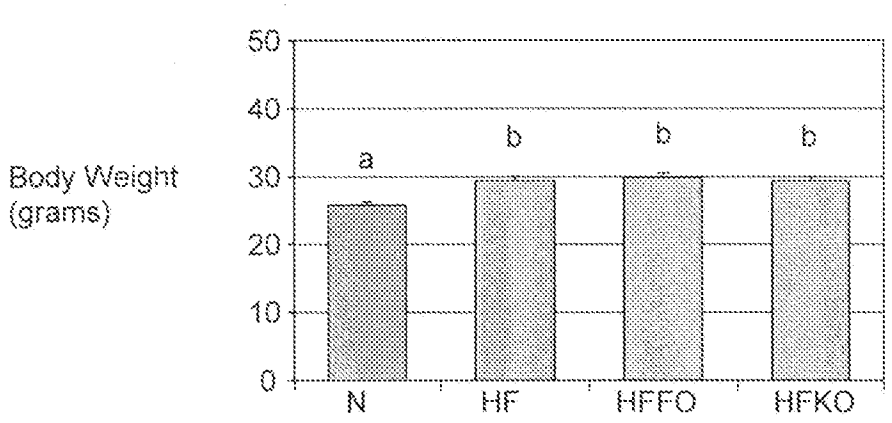


FIGURE 13

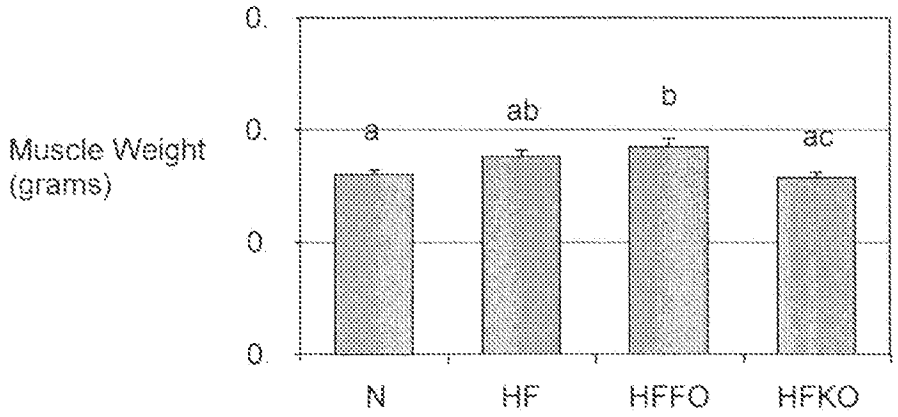


FIGURE 14

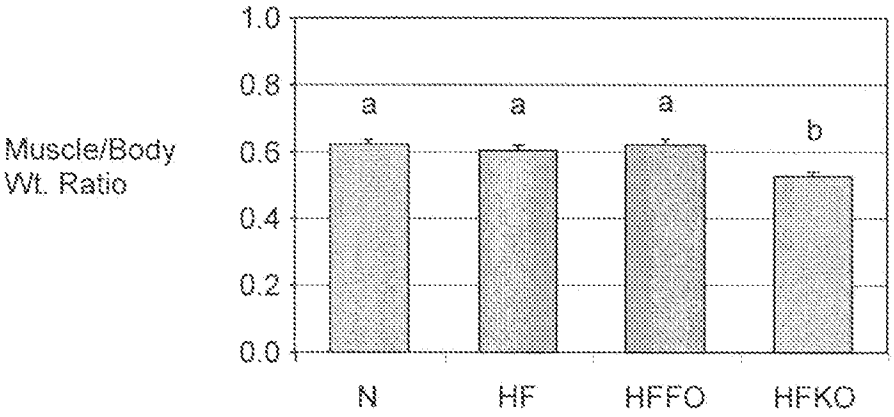


FIGURE 15

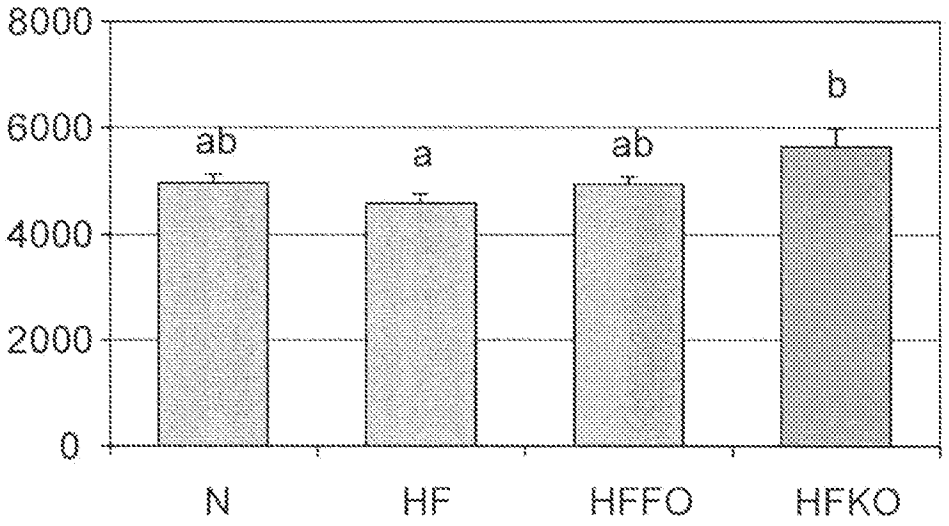


FIGURE 16

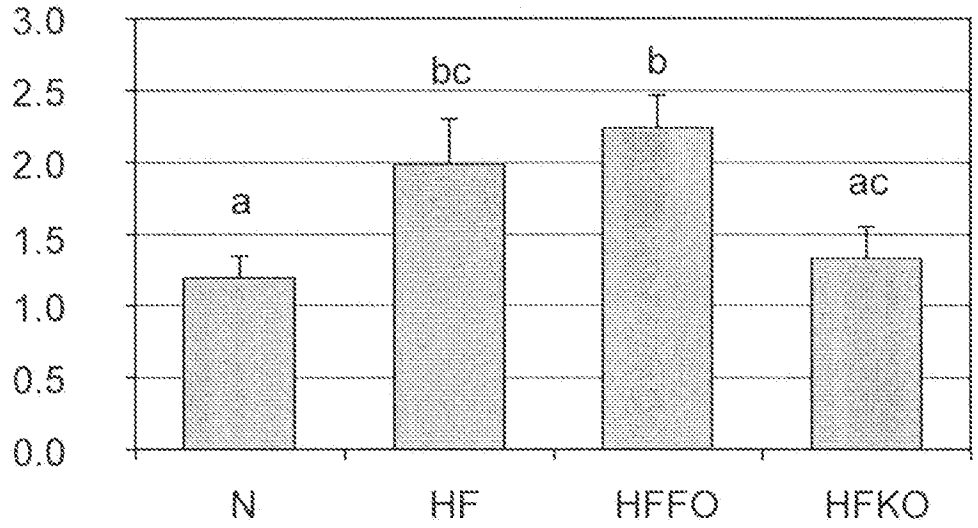


FIGURE 17

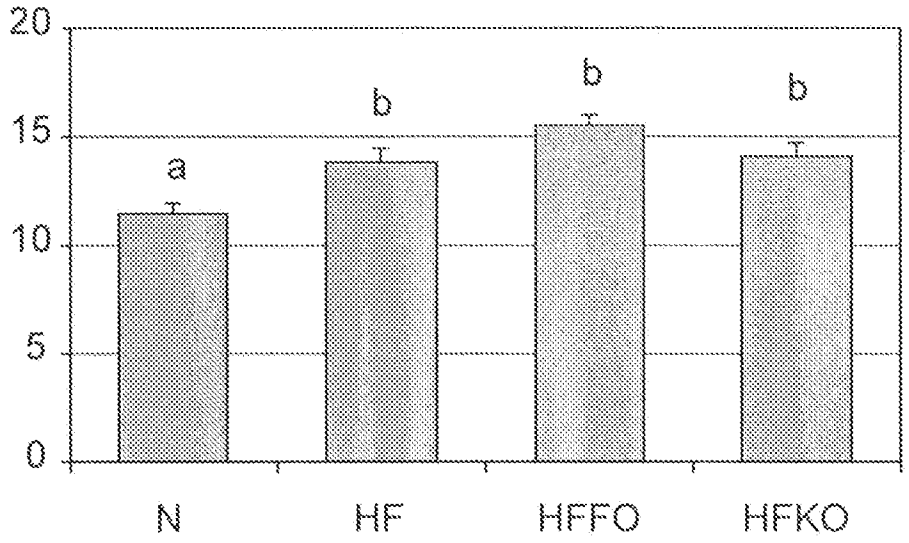


FIGURE 18

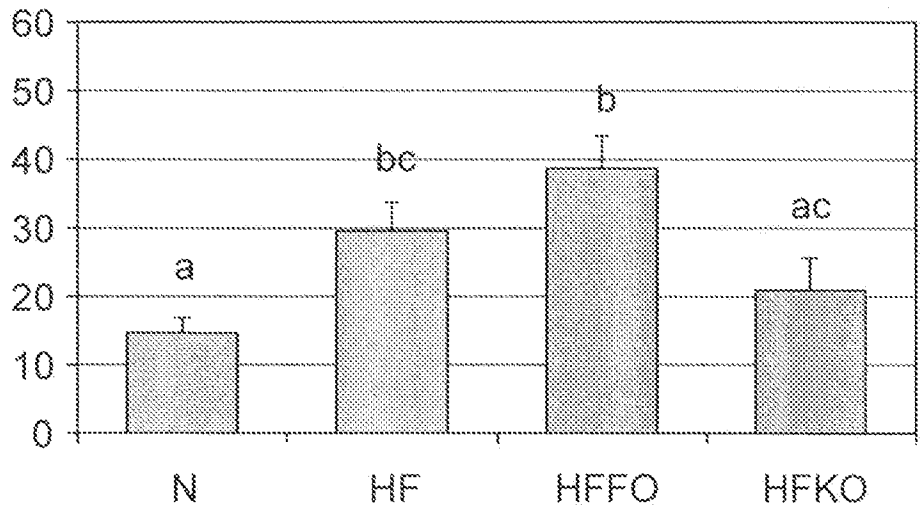
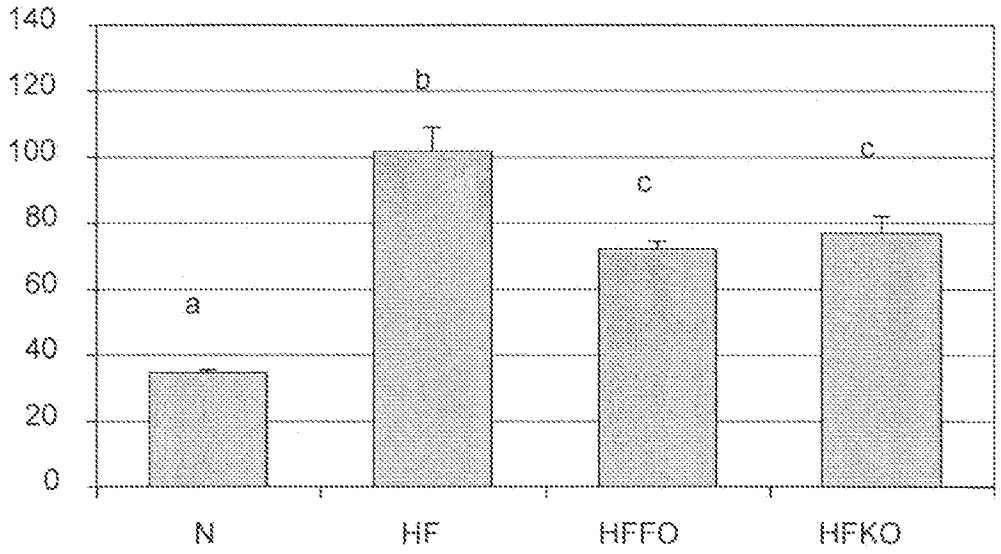


FIGURE 19



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Inge Bruheim, et al	Confirmation:	N/A
Serial No.:	N/A	Group No.:	N/A
Filed:	Herewith	Examiner:	N/A
Entitled:	**BIOEFFECTIVE KRILL OIL COMPOSITIONS**		

INFORMATION DISCLOSURE STATEMENT LETTER

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Sir or Madam:

The citations listed in the attached **IDS Form SB08A** may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97.

Applicants wish to bring to the Examiner's attention that we are not providing copies of US Patents as instructed under 37 CFR 1.98(a)(2). The Examiner is requested to make these citations of official record in this application.

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

Applicants wish to bring to the Examiner's attention that all of the references cited in this Information Disclosure Statement are from parent U.S. Patent Application No. 12/057,775 and all of the references in the file wrapper should be considered for this continuation application. Any and all foreign and non-patent literature documents can found in the file wrapper of U.S. Patent Application No. 12/057,775.

The Commissioner is hereby authorized to charge any required fees or credit any overpayments to Attorney Deposit Account No.: **50-4302**, referencing Attorney Docket No.: **NATNUT-14409/US-6/CON.**

Dated: September 6, 2013

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J. Mitchell Jones
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608.662.1277

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