UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,375,453 B2
APPLICATION NO. : 14/020162
DATED : June 28, 2016
INVENTOR(S) : Inge Bruheim et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Column 36, lines 23-24, should read:

percentage of total fatty acids in said polar krill oil.

Signed and Sealed this
Thirteenth Day of September, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

PTO/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,375,453

APPLICATION NO.: 14/020,162

ISSUE DATE : 28-Jun-2016

INVENTOR(S) : Inge Bruheim, Snorre Tilseth, Daniele Mancinelli

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 36, lines 23-24, should read:

percentage of total fatty acids in said polar krill oil.

MAILING ADDRESS OF SENDER (Please do not use customer number below):

Casimir Jones SC
2275 Deming Way, Suite 310
Middleton, WI 53562

Privacy Act Statement

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

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.:	9,375,453	Issued:	28-Jun-2016
Application No.:	14/020,162	Filing Date:	06-Sep-2013
Name of Patentee:	AKER BIOMARINE ANTARCTIC AS		
Title of Invention:	**METHODS FOR PRODUCING BIOEFFECTIVE KRILL OIL COMPOSITIONS**		

REQUEST FOR CERTIFICATE OF CORRECTION OF PATENT

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

Sir or Madam:

Pursuant to 35 U.S.C. §254 and 37 C.F.R. §1.322, applicants respectfully request that the Office issue a Certificate of Correction in the above-referenced patent. Applicants submit herewith Form PTO/SB/44 showing the correction that is requested.

1. Please correct the Letters Patent at Column 36, line 23, as follows:

   percentage of total fatty acids in said ~~composition~~ polar krill oil.

The correct text of the claim can be found in the Examiner's Amendment found in the Notice of Allowability mailed May 13, 2016.

Because the errors in the patent are a result of the Office's mistake, applicants believe that no fee is due. However, in the event a fee is due, the Commissioner is hereby authorized to charge the fee, or credit any overpayment, to Deposit Account 50-4302, referencing Attorney Docket No. **AKBM-14409/US-6/CON.**

Respectfully submitted,

CASIMIR JONES, S.C.

Dated: July 7, 2016

/J. Mitchell Jones/
J. Mitchell Jones
Reg. No. 44,174
2275 Deming Way, Suite 310
Middleton, WI 53562
608 662 1277

Electronic Acknowledgement Receipt

EFS ID:	26269814
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	METHODS FOR PRODUCING 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:	07-JUL-2016
Filing Date:	06-SEP-2013
Time Stamp:	15:06:37
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	Request for Certificate of Correction	14409US6CON_CoCFormSB44.pdf	164607 47ade4e7336515c4687460e10d790d8a500e40d0	no	2

Warnings:

Information:					
2	Request for Certificate of Correction	14409US6CON_Request_COC.pdf	79536 7fcfdee3b7a39022dda4bf2349e4dc2cc5675c5f	no	1
Warnings:					
Information:					
			Total Files Size (in bytes):	244143	

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 NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	06/28/2016	9375453	AKBM-14409/US-6/CON	4914

72960 7590 06/08/2016

Casimir Jones, S.C.
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MIDDLETON, WI 53562

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 41 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
Inge Bruheim, Volda, NORWAY;
Snorre Tilseth, Bergen, NORWAY;
Daniele Mancinelli, Orsta, NORWAY;

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14020162	BRUHEIM ET AL.
	Examiner	**Art Unit**
	DEBBIE K WARE	1651

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	12	17	28	33	50	49	34	65						
	2	13	18	29	34	51	50	35	66						
2	3	14	19	~~33~~	35	52	51								
3	4	15	20	37	36	53	52								
	5	16	21	38	37	54	53								
4	6	17	22	39	38	55	54								
	7	18	23	40	39	56	55								
5	8	19	24	41	40	57	56								
6	9	20	25	42	41	58	57								
	10	21	26	43	42	59	58								
	11	22	27	44	43	60	59								
7	12	23	28	45	44	61	60								
8	13	24	29	46	45	30	61								
9	14	25	30	47	46	31	62								
10	15	26	31	48	47	32	63								
11	16	27	32	49	48	~~36~~	64								

Change(s) applied
to document,
/A.E.M./
5/20/2016

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 61	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651 (Primary Examiner)	04/30/2016 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

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
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

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

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	4119619		1978-10-10	ROGOZHIN SERGEI VASILIEVICH et al.	
	2	5434183		1995-07-18	LARSSON-BACKSTROM	
Change(s) applied to document, /R.S./ 5/25/2016	3	6537787		2003-03-25	~~GILDAS~~ Breton	
	4	6800299		2004-10-05	BEAUDOIN & MARTIN	
	5	5266564		1993-11-30	MODELELL et al	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

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	20030044495		2003-03-06	KAGAN and BRAUN	

Receipt date: 09/06/2013

Receipt date: 09/0

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

72960 7590 05/26/2016

Casimir Jones, S.C.
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EXAMINER
WARE, DEBORAH K

ART UNIT	PAPER NUMBER
1651	

NOTIFICATION DATE	DELIVERY MODE
05/26/2016	ELECTRONIC

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

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

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

docketing@casimirjones.com
pto.correspondence@casimirjones.com

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Application No. : 14020162
Applicant : Bruheim
Filing Date : 09/06/2013
Date Mailed : 05/26/2016

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Anthony McPhail/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 14020162 **Drawings filed: 09/06/2013**

IDENTIFICATION OF DRAWING DEFICIENCIES

- [ ] There is a hole or the image thereof within the illustration. FIG(s)
- [ ] The illustration is penetrated or traversed by a solid or broken line that is not intended to be part of the drawing, such as a dark line caused by a flaw in the copying process. FIG(s)
- [ ] An ink stamp or the image thereof obscures part of the illustration. FIG(s)
- [ ] The drawing is marred by black smudges, obliterations, or fax/copier marks (for example, speckles or dots in a substantial portion of the drawing). FIG(s)
- [ ] Figure numbers are duplicated or missing. FIG(s)
- [ ] Drawing sheet or figure is missing. FIG(s)
- [ ] Numbers, letters, or reference characters in the drawing have been crossed out or are illegibly handwritten. FIG(s)
- [ ] The character of the lines, numbers, and letters is poor. FIG(s)
- [ ] The drawing's background shows that the original drawing was made on graph paper or other paper with a pattern or decoration. FIG(s)
- [ ] The FIG. number label is placed in a location that causes the drawing to be read upside down. FIG(s)
- [X] Data, a reference number, or part of the drawing is truncated or missing, or a lead line has no reference number. FIG(s) 9
- [ ] The drawing and/or the FIG. label contain(s) foreign language. FIG(s)
- [ ] This utility application contains a photograph of a view that is capable of being illustrated as a line drawing. FIG(s)
- [ ] A petition under 37 CFR 1.84(a)(2) to accept color drawings has been granted, but the brief description of the drawings in the specification does not contain (or has not been amended to contain) the paragraph required by 37 CFR 1.84(a)(2)(iii).
- [ ] This reissue application contains added and/or amended drawings that are not labeled as “New” or “Amended” or “Canceled” as required by 37 CFR 1.173(b)(3). FIG(s)
- [ ] This Design reissue application contains a drawing that is labeled as “Canceled” but is not surrounded by brackets, or a drawing that is surrounded by brackets but is not labeled as “Canceled.” See 37 CFR 1.173(b)(3). FIG(s)
- [ ] OTHER:
- [ ] COMMENTS:

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 14020162 Document Date: 05/26/2016

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Inge Bruheim et al.	Conf. No.:	4914
Serial No.:	14/020,162	Group No.:	1651
Filing Date:	06-Sep-2013	Examiner:	WARE
Entitled:	**METHODS FOR PRODUCING BIOEFFECTIVE KRILL OIL COMPOSITIONS**		

RESPONSE TO THE NOTICE TO FILE CORRECTED APPLICATION PAPERS MAILED MAY 26, 2016

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

In response to the Notice to File Corrected Application Papers mailed May 26, 2016, Applicant submits the following:

Amendments to the Drawings are on page 2 of this paper; and

Remarks are on page 3 of this paper.

AMENDMENTS TO THE DRAWINGS

Please replace Figures 9 (1 page) as originally filed in this application with Replacement Figure 9 (1 page) attached hereto.

REMARKS

In the Notice to File Corrected Application Papers mailed May 26, 2016, Applicants were notified that a part of Figure 9 filed September 6, 2013, is truncated or missing. Applicants submit herewith Replacement Figure 9. No new matter has been added.

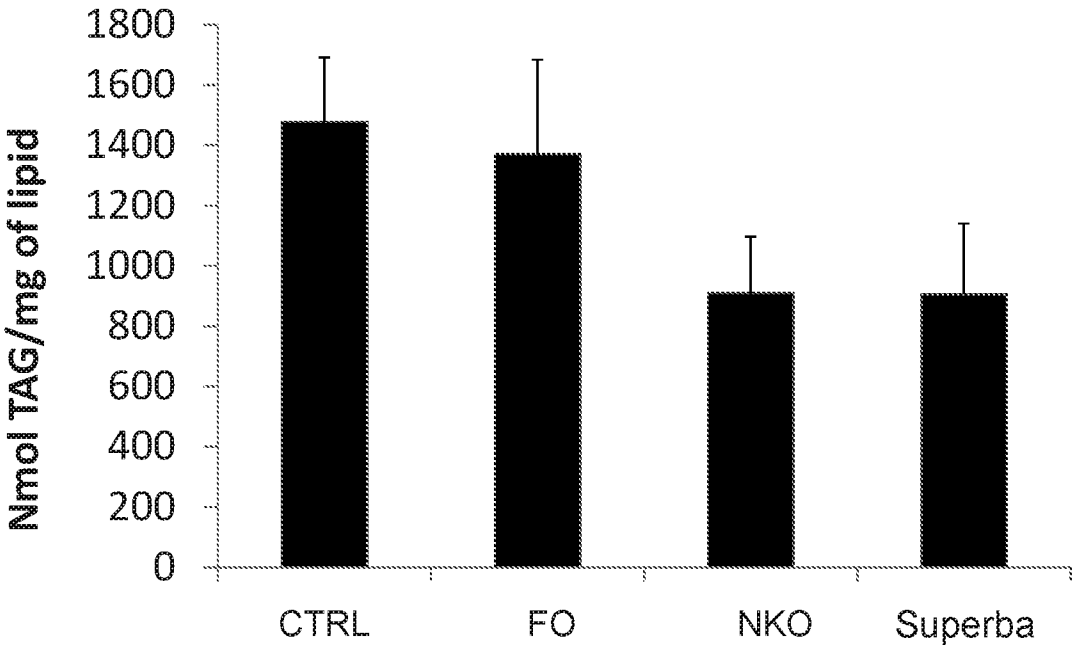
No fees are believed to be due in connection with this filing. Nevertheless, if the Director finds any additional fees to be due in connection with this, or any other filing, authorization is given to charge said fees to Deposit Account No. 50-4302, referencing attorney docket number AKBM-14409/US-6/CON.

Respectfully,

Dated: May 26, 2016

/ J. Mitchell Jones /
J. Mitchell Jones
Registration No. 44,174
CASIMIR JONES, S.C.
2275 Deming Way Suite 310
Middleton, WI 53562
608-662-1277

FIGURE 9


Nmol TAG/mg of lipid
1800
1600
1400
1200
1000
800
600
400
200
0
CTRL
FO
NKO
Superba

Electronic Acknowledgement Receipt

EFS ID:	25895779
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	METHODS FOR PRODUCING 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:	26-MAY-2016
Filing Date:	06-SEP-2013
Time Stamp:	14:53:49
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	14409US6CON_ResponseNoticeFileCorrectedAppPapers.pdf	92869 bc0bb71e0797a671401a5a911f43a1c94d97901d	no	3

Warnings:	
Information:	

2	Drawings-other than black and white line drawings	14409US6CON_ReplacementFigure9uspto.pdf	574821 adb115f5449bf97370717d150dfec2acd4af945c	no	1

Warnings:	
Information:	
Total Files Size (in bytes):	667690

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

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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	1651	5020	AKBM-14409/US-6/CON	11	2

CONFIRMATION NO. 4914

CORRECTED FILING RECEIPT

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

OC000000083077461

Date Mailed: 05/23/2016

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 ANTARCTIC AS, Stamsund, NORWAY

Power of Attorney: The patent practitioners associated with Customer Number 72960

Domestic Priority data as claimed by applicant

This application is a CON of 12/057,775 03/28/2008 PAT 9034388
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 Application via Priority Document Exchange: Yes

Permission to Access Search Results: No

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

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: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

METHODS FOR PRODUCING BIOEFFECTIVE KRILL OIL COMPOSITIONS

Preliminary Class

424

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).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

72960 7590 05/13/2016
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

TITLE OF INVENTION: BIOEFFECTIVE KRILL OIL COMPOSITIONS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/15/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
WARE, DEBORAH K	1651	424-520000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 Casimir Jones, S.C.
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: AKER BIOMARINE ANTARCTIC AS
(B) RESIDENCE: (CITY and STATE OR COUNTRY): STAMSUND, NORWAY

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 504302 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /J. Mitchell Jones/ Date May 17, 2016
Typed or printed name J. Mitchell Jones Registration No. 44,174

Electronic Patent Application Fee Transmittal

Application Number:	14020162
Filing Date:	06-Sep-2013
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Filer:	John Mitchell Jones/Mallory Checkett
Attorney Docket Number:	AKBM-14409/US-6/CON

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt

EFS ID:	25800912
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:	17-MAY-2016
Filing Date:	06-SEP-2013
Time Stamp:	15:11:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$960
RAM confirmation Number	1604
Deposit Account	504302
Authorized User	JONES, J. MITCHELL

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 CFR 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	14409US6CON_IssueFeePayment_uspto.pdf	1594798 6dcd845f633a84137fb6a7ad5db719ea7601af78	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30448 8d7f199ebb5a9133927e057a0db200f8edce3cde	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1625246		

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

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

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

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

UNITED STATES PATENT AND TRADEMARK OFFICE

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

72960 7590 05/13/2016

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

EXAMINER
WARE, DEBORAH K

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 05/13/2016

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

TITLE OF INVENTION: BIOEFFECTIVE KRILL OIL COMPOSITIONS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/15/2016

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

72960 7590 05/13/2016
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

TITLE OF INVENTION: BIOEFFECTIVE KRILL OIL COMPOSITIONS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/15/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
WARE, DEBORAH K	1651	424-520000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

72960 7590 05/13/2016

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

EXAMINER	
WARE, DEBORAH K	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 05/13/2016

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	14/020,162	BRUHEIM ET AL.	
	Examiner	Art Unit	
	DEBBIE K. WARE	1651	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Deborah K. Ware*. (3)_____.

(2) *J. Mitchell Jones, Esq.*. (4)_____.

Date of Interview: *22 March 2016*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *all pending claims as of 1/8/2016*.

Identification of prior art discussed: *Not discussed per se*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Applicants' Representative authorized changes by examiner amendment to place case into condition for allowance.

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Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

Notice of Allowability	Application No. 14/020,162	Applicant(s) BRUHEIM ET AL.	
	Examiner DEBBIE K. WARE	Art Unit 1651	AIA (First Inventor to File) Status No

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *3/30/2016*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1, 3, 4, 6, 8, 9 and 12-66*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

The present application has been examined under the pre-AIA first to invent provisions.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on 3/30/2016, 01/08/2016 and 12/17/2014, were received. The submissions are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with J. Mitchell Jones on March 22, 2016.

The application has been amended as follows:

In the abstract

at line 2, deleted "This invention discloses new" and inserted --New-- and deleted "characterized by" and inserted --are disclosed as-- .

In the title

before “BIOEFFECTIVE KRILL OIL COMPOSITIONS” inserted – METHODS FOR PRODUCING-- .

In the Claims

Claim 1, line 4, deleted "and"

, line 6, deleted “wherein” and inserted --and—

, line 7, deleted “comprising phospholipids is further characterized comprising” and inserted --comprises—

, line 8, after “said polar krill oil” inserted --,--

, line 9, deleted “in the”

, line 10, deleted "composition" and after "said polar krill oil" inserted --,--

, line 12, after “said polar krill oil” inserted --; and--

, line 13, inserted --c) formulating said polar krill oil with a carrier for oral consumption-- ;

Claim 6, line 1, deleted “w/w”

, line 2, after “omega-3 fatty acids” inserted --as a percentage of total fatty acids in said polar krill oil-- ;

Claim 9, line 2, after “said polar krill” inserted --oil in a capsule--;

Claim 15, line 2, deleted “composition” and inserted --polar krill oil--;

Claim 16, line 1, deleted “1” and inserted --15--;

Claim 24, line 1, before “krill oil” inserted --polar--;

Claim 25, line 1, before “krill oil” inserted --polar—

, line 2, deleted “composition” and inserted --polar krill oil--;

Claim 31, line 2, deleted “composition” and inserted --polar krill oil--;

Claim 33, line 1, deleted “25” and inserted --28--;

Claim 35, line 5, deleted “wherein” and inserted --and-- and deleted “comprising phospholipids is further"

, line 6, deleted “characterized in comprising” and inserted --comprises-- and deleted “;” and inserted --,--

, line 8, deleted “in the composition” and deleted “;” and inserted --,--;

Claim 38, line 1, deleted “w/w”

, line 2, after “omega-3 fatty acids” inserted --as a percentage of total fatty acids in said polar krill oil-- ;

Claim 42, line 2, deleted “composition” and inserted --polar krill oil--;

Claim 43, line 1, deleted “35” and inserted --42--;

Claim 50, line 1, before “krill oil” inserted --polar--;

Claim 51, line 1, before “krill oil” inserted --polar—

, line 2, deleted “composition” and inserted --polar krill oil--;

Claim 57, line 2, deleted “composition” and inserted --polar krill oil--;

Claim 63, line 1, deleted “comprising” and inserted --is a--;

Claim 66, line 1, deleted “comprising” and inserted --is a-- .

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBBIE K. WARE whose telephone number is (571)272-0924. The examiner can normally be reached on 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Renee Claytor can be reached on 571-272-8394. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Deborah K. Ware
Primary Examiner
Art Unit 1651

/DEBBIE K. WARE/
Primary Examiner, Art Unit 1651

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	14/020,162	BRUHEIM ET AL.	
	Examiner	Art Unit	
	DEBBIE K. WARE	1651	

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☐ Attachment

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. 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	Inge Bruheim
Art Unit	1651
Examiner Name	WARE, Deborah K.
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	4038722		1977-08-02	TERASE et al.	
	2	8586567		2013-11-19	SAMPALIS, F.	

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U.S.PATENT APPLICATION PUBLICATIONS Remove

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	20050003073		2005-01-06	PIVOVAROV et al.	
	2	20110256216		2011-10-20	LEFKOWITZ ANDREW R.	
	3	20110160161		2011-06-30	SAMPALIS, F.	

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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]

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	1	01/76385	WO		2001-10-18	Westfalia Separator Industry GmbH		
	2	S6323819	JP		1988-02-01	HARAK K. et al.		
	3	2012/139588	WO		2012-10-18	TRIPLENINE PHARMA AS		

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.	T5
	1	KOLAKOWSKI and GAJOWIECKI, "Optimization of autoproteolysis to obtain and edible product 'precipitate' from Antarctic krill," Seafood Science and Technology, pp. 331-336	
	2	"Neptune krill Oil's Unique Properties", INTERNET CITATION, 2011, URL:http://www.nowfoods.com/Products/ProductFAQs/081008/htm	
	3	GIGLIOTTI et al., "Extraction and characterisation of lipids from Antarctic krill (Euphausia superba)", Food Chemistry, 2011, vol. 125, no. 3, pages 1028-1036	
	4	ALI-NEHARI et al., "Characterization of purified phospholipids from krill () residues deoiled by supercritical carbon dioxide", Korean Journal of Chemical Engineering, 2012, vol. 29, no. 7	
	5	International Search Report and Written Opinion, International Patent Application No. PCT/IB2014/002130, mailed February 3, 2015	
	6	EP Opposition filed May 8, 2015 by Olympic Seafood AS, EP Patent No. 2144618	

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	7	ALLAHPICHAY et al., "Extraction of Growth Promoting Fractions from Non-muscle Krill Meal of Euphausia superba and its Effect on Fish Growth," Bulletin of the Japanese Society of Scientific Fisheries, 1984, 50(5): 821-826

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/Deborah Ware/	Date Considered	04/30/2016

*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.

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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)	2016-07-08
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.

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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.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Examiner Name	WARE
Attorney Docket Number	AKBM-14409/US-6/CON

	1	Third Party Submission, AU Patent Application No. 2014256345, filed October 12, 2015
	2	Third Party Submission, AU Patent Application No. 2014256345, filed December 22, 2015

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/Deborah Ware/	Date Considered	04/30/2016

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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.

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	14020162
Filing Date	2013-09-06
First Named Inventor	Inge Bruheim
Art Unit	1653
Examiner Name	NA
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	8697138		2014-04-15	Bruheim et al.	
	2	7488503		2009-02-10	Porzio et al	
	3	4749522		1988-06-07	Kamarei	
	4	4814111		1989-03-21	Kearns et al.	
	5	4133077		1979-01-09	Jasniewicz	

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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	20110130458		2011-06-02	Harald Breivik	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Inge Bruheim
Art Unit	1653
Examiner Name	NA
Attorney Docket Number	AKBM-14409/US-6/CON

	2	20080166420		2008-07-10	Scott F. Sones		
	3	20060078625		2006-04-13	Susie Rockway		
	4	20020076468		2002-06-20	Saxby		
	5	20030113432		2003-06-19	Yoshitomi		
	6	20100143571		2010-06-10	Breivik		
	7	20100160659		2010-06-24	Catchpole		
	8	20080166419		2008-07-10	Sones		

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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	40348	CL		1997-07-08	Tepual S.A.		☐
	2	89/01031	WO		1989-02-09	Pharmacia AB		☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Inge Bruheim
Art Unit	1653
Examiner Name	NA
Attorney Docket Number	AKBM-14409/US-6/CON

	3	89/10960	WO		1989-11-16	Pharmacia AB		☐
	4	97/38585	WO		1997-10-23	The University of British Columbia		☐
	5	98/34498	WO		1998-08-13	Biozyme Systems, Inc.		☐
	6	99/39589	WO		1999-08-12	Biozyme Systems Inc.		☐
	7	06/111633	WO		2006-10-26	SC DICOPHAR		☐
	8	07/123424	WO		2007-11-01	Catchpole		☐
	9	08/072563	WO		2008-06-19	Nippon Suisan Kaisha, Ltd.		☐

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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]
	1	EP Opposition filed February 13, 2014 by Olympic Seafood AS, EP Patent Application No. EP087189l0l6	☐
	2	BRZUSTOWICZ, Michael R., et al., "Controlling Membrane Cholesterol Content. A Role for Polyunsaturated (Docosahexaenoate) Phospholipids," Biochemistry (2002), 41, pp. 12509-12519	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14020162
	Filing Date	2013-09-06
	First Named Inventor	Inge Bruheim
	Art Unit	1653
	Examiner Name	NA
	Attorney Docket Number	AKBM-14409/US-6/CON

	3	JONG-HO LEE, "A Review: Antioxygenic and Peroxide-decomposing Activities of Antarctic Krill Lipids," J. Korean Soc. Food Mutr. 13(3) pp. 326-333 (1984)	☐
	4	KI WOONG CHO, et al., "Lipid and Fatty Acid Composition of the Antarctic Krill Euphausia superba," Ocean Research 21(2): 109-116 (1999)	☐
	5	HVATTUM, Erlend, et al., "Effect of soybean oil and fish oil on individual molecular species of Atlantic salmon...", Journal of Chromatography B, 748 (2000) 137-149	☐
	6	IGARASHI, Daisuke, et al., "Positional Distribution of DHA and EPA in Phosphatidylcholine and Phosphatidylethanolamine from Different Tissues of Squids," J. Oleo Sci. Vol. 50, No. 9 (2001)	☐
	7	TOCHIZAWA, Kaoru, et al., "Effects of Phospholipds Containing Docosahexaenoic Acid on Differentiation and Growth of HL-60 Human Promyelocytic Leukemia Cells," J. Jpn. Oil Chem. Soc. Vol. 46, No. 4 (1997)	☐
	8	ZEROUGA, Mustapha, et al., "Comparison of phosphatidylcholines containing one or two docosahexaenoic acyl chains on properties of phospholipid monolayers and bilayers," Biochimica et Biophysica Acta 1236 (1995) 266-272	☐
	9	EUNG-HO LEE, et al., "Studies on the Processing of Krill Sauce," J. Korean Soc. Food Nutr. 13(1) 97-106 (1984)	☐
	10	HYUN-KU KIM, et al., "Effects of Cooking and Drying Methods on the Polar Lipds Composition of Shrimp," Korean J. Food Sci. Technol. Vol. 21, No. 1, pp. 25-30 (1989)	☐
	11	SHON, Mi-Yae, et al., "Effects of Krill and Cadmium on Lipid Composition of Plasma in Cholesterol-Fed Rats," J. Korean Soc. Food Nutr. 23(1), 38-43 (1994)	☐
	12	Summons Materials downloaded from ESPACE on December 16, 2014 for EP Patent Application No. 08 718 910.6	☐

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14020162
	Filing Date	2013-09-06
	First Named Inventor	Inge Bruheim
	Art Unit	1653
	Examiner Name	NA
	Attorney Docket Number	AKBM-14409/US-6/CON

EXAMINER SIGNATURE			
Examiner Signature	/Deborah Ware/	Date Considered	04/30/2016

*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.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14020162
Filing Date	2013-09-06
First Named Inventor	Inge Bruheim
Art Unit	1653
Examiner Name	NA
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)	2014-12-16
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.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

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.

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14020162	BRUHEIM ET AL.
	Examiner	Art Unit
	DEBBIE K WARE	1651

CPC- SEARCHED

Symbol	Date	Examiner
A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 | A61K31/683 | A61K31/202 | A61K45/06 | A61K31/6615 | A61K8/553 | A61K2800/70 | A61K31/047 | A61K35/63 | A61K35/64 | A61K8/925 | A61K9/48	05/2015	dkw
A61K2300/00 | A61K31/122 | A61K31/23 | A61K31/683 | A61K31/685 | A61K31/202 | A61K31/232 | A61K31/355 | A61K31/663 | A61K35/74 | A61K9/4858 | A61K35/612 | A61K45/06 | A61K31/20 | A61K31/235 | A61K9/48	06/2015	dkw
A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 | A61K31/683 | A61K31/202 | A61K45/06 | A61K31/6615 | A61K8/553 | A61K2800/70 | A61K31/047 | A61K35/63 | A61K35/64 | A61K8/925 | A61K9/48	04/2016	dkw

CPC COMBINATION SETS - SEARCHED

Symbol	Date	Examiner

US CLASSIFICATION SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
CPC_WEST_INV_Searches: see search history print out	05/2015-06/2015	dkw
CPC_WEST_INV_Searches: see search history print out	04/2016	dkw

INTERFERENCE SEARCH

US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	CPC-West Interference Search: see search history print out	03/2016-04/2016	dkw

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14020162	BRUHEIM ET AL.
	Examiner	**Art Unit**
	DEBBIE K WARE	1651

CPC						
Symbol					**Type**	**Version**
A61K		35	/	612	F	2013-01-01
A61K		9	/	4858	I	2013-01-01
A61K		31	/	122	I	2013-01-01
A61K		31	/	23	I	2013-01-01
A61K		31	/	683	I	2013-01-01
A61K		31	/	685	I	2013-01-01
A61K		45	/	06	I	2013-01-01
C11B		3	/	006	I	2013-01-01
A61K		31	/	202	I	2013-01-01
A61K		9	/	48	I	2013-01-01
A61K		31	/	20	I	2013-01-01
A61K		31	/	235	I	2013-01-01
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CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
A61K		31	/	23	I	1	1	2013-01-01
A61K		2300	/	00	A	1	2	2013-01-01
A61K		31	/	683	I	2	1	2013-01-01
A61K		2300	/	00	A	2	2	2013-01-01
A61K		31	/	685	I	3	1	2013-01-01
A61K		2300	/	00	A	3	2	2013-01-01
A61K		31	/	122	I	4	1	2013-01-01
A61K		2300	/	00	A	4	2	2013-01-01

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 61	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651 (Primary Examiner)	04/30/2016 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14020162	BRUHEIM ET AL.
	Examiner	**Art Unit**
	DEBBIE K WARE	1651

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION												
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED							
		A	6	1	K	35 / 612								
CROSS REFERENCE(S)		A	6	1	K	9 / 48 (2006.01.01)								
		A	6	1	K	31 / 122 (2006.01.01)								
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)	A	6	1	K	31 / 683 (2006.01.01)								
		A	6	1	K	31 / 685 (2006.01.01)								
		C	1	1	B	3 / 00 (2006.01.01)								

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	61	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651	04/30/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14020162	BRUHEIM ET AL.
	Examiner	**Art Unit**
	DEBBIE K WARE	1651

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	12	17	28	33	50	49	34	65						
	2	13	18	29	34	51	50	35	66						
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10	15	26	31	48	47	32	63								
11	16	27	32	49	48	33	64								

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 61	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651 (Primary Examiner)	04/30/2016 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

WEST Search History for Application 14020162

Creation Date: 2016031917:32

Interference Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
krill.clm. and oil.clm. and phosphatidylcholine.clm.	PGPB, USPT, * No UPAD	n/a	OR	YES		06-01-2015
oil.clm. and superba.clm. and phospholipid.clm.	PGPB, USPT	13	OR	YES		03-19-2016
(oil.clm. and superba.clm. and phospholipid.clm.) and (denature or denaturing).clm.	PGPB, USPT	6	OR	YES		03-19-2016

Prior Art Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
9034388.pn.	USPT	n/a	OR	YES		06-01-2015
Inge.in. and Bruheim.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(Inge.in. and Bruheim.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(Inge.in. and Bruheim.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015

Snorre.in. and Tilseth.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(Snorre.in. and Tilseth.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(Snorre.in. and Tilseth.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
Daniele.in. and Mancinelli.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(Daniele.in. and Mancinelli.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(Daniele.in. and Mancinelli.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD,	n/a	OR	YES		06-01-2015

	FPRS					
krill and oil and phosphatidylcholine	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine) and astaxanthin	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine and astaxanthin) and triglycerides	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides) and omega	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides and omega) and fatty and acids	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides and omega and fatty and acids) and Euphausia and superba	PGPB, USPT, USOC, EPAB, JPAB,	n/a	OR	YES		06-01-2015

	DWPI, TDBD, FPRS					
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides and omega and fatty and acids and Euphausia and superba) and ((A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 | A61K31/683 | A61K31/202 | A61K45/06 | A61K31/6615 | A61K8/553 | A61K2800/70 | A61K31/047 | A61K35/63 | A61K35/64 | A61K8/925 | A61K9/4858 | A61K31/7036 | A61K2800/522 | A61K9/0019 | A61K31/07 | A61K31/133 | A61K31/198 | A61K31/232 | A61K31/352 | A61K31/353 | A61K31/575 | A61K31/661 | A61K36/05 | A61K36/535 | A61K36/537 | A61K36/55 | A61K38/1767 | A61K9/107 | A61K9/2009 | A61K9/2054 | A61K9/2866 | A61K9/4808 | A61K31/216 | A61K31/366 | A61K31/375 | A61K31/40 | A61K31/405 | A61K31/44 | A61K31/47 | A61K31/505 | A61K31/612 | A61K31/05 | A61K31/14 | A61K31/194 | A61K31/20 | A61K31/355 | A61K31/407 | A61K31/66 | A61K35/60 | A61K41/0028 | A61K47/46 | A61K8/4986 | A61K9/0095 | A61K9/08 | A61K9/1075 | A61K9/127 | A61K9/1271 | A23V2002/00 | A23V2250/187 | A23V2250/1846 | A23V2250/1868 | A23V2250/185 | A23V2250/1848 | A23V2250/1852 | A23V2250/70 | A23V2250/1882 | A23V2200/324 | A23V2200/326 | A23V2200/02 | A23V2200/322 | A23V2250/026 | A23V2250/1866 | A23V2250/211 | A23V2250/2116 | A23V2250/2136 | A23V2250/5432 | A23V2250/702 | A23V2250/708 | A23L1/3006 | A23L1/33 | A23L1/3008 | A23L1/302 | A23L1/305 | A23L1/30 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/0026 | A23L1/3002 | A23L1/0041 | A23L1/2753 | A23L1/303 | A23L1/304 | A23L1/29 | A23L1/326 | A23D9/013 | A23D9/007 | A23D9/02 | A23D7/011 | A23D9/00 | C11B1/06 | C11B1/10 | C11B3/006 | C11B13/00 | C11B1/02 | C11B1/16 | C11B1/025 | C11B1/104 | A61Q19/00 | A61Q1/06 | A61Q17/04 | A61Q19/007 | A61Q1/10 | A61Q1/12 | A23K1/164 | A23K1/103 | A23K1/1606 | A23K1/188 | A23K1/1755 | Y02W30/74 | A23J7/00 | A23J1/04 | A23J3/34 | C07F9/10 | C07F9/103 | C07F9/117 | C07F9/106 | C07D407/14 | C07K14/43509 | C07K19/00 | A23G3/40 | A23G3/364 | A23G3/368 | A23G3/54).CPC.)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015

9028877.pn.	USPT	n/a	OR	YES		06-01-2015
9034388.pn.	USPT	n/a	OR	YES		06-01-2015
8697138.pn.	USPT	n/a	OR	YES		06-01-2015
8557297.pn.	USPT	n/a	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides and omega and fatty and acids and Euphausia and superba and ((A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 | A61K31/683 | A61K31/202 | A61K45/06 | A61K31/6615 | A61K8/553 | A61K2800/70 | A61K31/047 | A61K35/63 | A61K35/64 | A61K8/925 | A61K9/4858 | A61K31/7036 | A61K2800/522 | A61K9/0019 | A61K31/07 | A61K31/133 | A61K31/198 | A61K31/232 | A61K31/352 | A61K31/353 | A61K31/575 | A61K31/661 | A61K36/05 | A61K36/535 | A61K36/537 | A61K36/55 | A61K38/1767 | A61K9/107 | A61K9/2009 | A61K9/2054 | A61K9/2866 | A61K9/4808 | A61K31/216 | A61K31/366 | A61K31/375 | A61K31/40 | A61K31/405 | A61K31/44 | A61K31/47 | A61K31/505 | A61K31/612 | A61K31/05 | A61K31/14 | A61K31/194 | A61K31/20 | A61K31/355 | A61K31/407 | A61K31/66 | A61K35/60 | A61K41/0028 | A61K47/46 | A61K8/4986 | A61K9/0095 | A61K9/08 | A61K9/1075 | A61K9/127 | A61K9/1271 | A23V2002/00 | A23V2250/187 | A23V2250/1846 | A23V2250/1868 | A23V2250/185 | A23V2250/1848 | A23V2250/1852 | A23V2250/70 | A23V2250/1882 | A23V2200/324 | A23V2200/326 | A23V2200/02 | A23V2200/322 | A23V2250/026 | A23V2250/1866 | A23V2250/211 | A23V2250/2116 | A23V2250/2136 | A23V2250/5432 | A23V2250/702 | A23V2250/708 | A23L1/3006 | A23L1/33 | A23L1/3008 | A23L1/302 | A23L1/305 | A23L1/30 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/0026 | A23L1/3002 | A23L1/0041 | A23L1/2753 | A23L1/303 | A23L1/304 | A23L1/29 | A23L1/326 | A23D9/013 | A23D9/007 | A23D9/02 | A23D7/011 | A23D9/00 | C11B1/06 | C11B1/10 | C11B3/006 | C11B13/00 | C11B1/02 | C11B1/16 | C11B1/025 | C11B1/104 | A61Q19/00 | A61Q1/06 | A61Q17/04 | A61Q19/007 | A61Q1/10 | A61Q1/12 | A23K1/164 | A23K1/103 | A23K1/1606 | A23K1/188 | A23K1/1755 | Y02W30/74 | A23J7/00 | A23J1/04 | A23J3/34 | C07F9/10 | C07F9/103 | C07F9/117 | C07F9/106 | C07D407/14 | C07K14/43509 |	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015

C07K19/00 | A23G3/40 | A23G3/364 | A23G3/368 | A23G3/54).CPC.)) and (capsule or encapsulated)						
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides and omega and fatty and acids and Euphausia and superba and ((A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 | A61K31/683 | A61K31/202 | A61K45/06 | A61K31/6615 | A61K8/553 | A61K2800/70 | A61K31/047 | A61K35/63 | A61K35/64 | A61K8/925 | A61K9/4858 | A61K31/7036 | A61K2800/522 | A61K9/0019 | A61K31/07 | A61K31/133 | A61K31/198 | A61K31/232 | A61K31/352 | A61K31/353 | A61K31/575 | A61K31/661 | A61K36/05 | A61K36/535 | A61K36/537 | A61K36/55 | A61K38/1767 | A61K9/107 | A61K9/2009 | A61K9/2054 | A61K9/2866 | A61K9/4808 | A61K31/216 | A61K31/366 | A61K31/375 | A61K31/40 | A61K31/405 | A61K31/44 | A61K31/47 | A61K31/505 | A61K31/612 | A61K31/05 | A61K31/14 | A61K31/194 | A61K31/20 | A61K31/355 | A61K31/407 | A61K31/66 | A61K35/60 | A61K41/0028 | A61K47/46 | A61K8/4986 | A61K9/0095 | A61K9/08 | A61K9/1075 | A61K9/127 | A61K9/1271 | A23V2002/00 | A23V2250/187 | A23V2250/1846 | A23V2250/1868 | A23V2250/185 | A23V2250/1848 | A23V2250/1852 | A23V2250/70 | A23V2250/1882 | A23V2200/324 | A23V2200/326 | A23V2200/02 | A23V2200/322 | A23V2250/026 | A23V2250/1866 | A23V2250/211 | A23V2250/2116 | A23V2250/2136 | A23V2250/5432 | A23V2250/702 | A23V2250/708 | A23L1/3006 | A23L1/33 | A23L1/3008 | A23L1/302 | A23L1/305 | A23L1/30 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/0026 | A23L1/3002 | A23L1/0041 | A23L1/2753 | A23L1/303 | A23L1/304 | A23L1/29 | A23L1/326 | A23D9/013 | A23D9/007 | A23D9/02 | A23D7/011 | A23D9/00 | C11B1/06 | C11B1/10 | C11B3/006 | C11B13/00 | C11B1/02 | C11B1/16 | C11B1/025 | C11B1/104 | A61Q19/00 | A61Q1/06 | A61Q17/04 | A61Q19/007 | A61Q1/10 | A61Q1/12 | A23K1/164 | A23K1/103 | A23K1/1606 | A23K1/188 | A23K1/1755 | Y02W30/74 | A23J7/00 | A23J1/04 | A23J3/34 | C07F9/10 | C07F9/103 | C07F9/117 | C07F9/106 | C07D407/14 | C07K14/43509 | C07K19/00 | A23G3/40 | A23G3/364 | A23G3/368 | A23G3/54).CPC.) and (capsule or encapsulated)) and Euphausia and superba	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-01-2015

20040241249	PGPB	n/a	OR	YES		06-01-2015
(20040241249) and "omega"	PGPB	n/a	OR	YES		06-01-2015
(20040241249 and "omega") and "astaxanthin"	PGPB	n/a	OR	YES		06-01-2015
(20040241249 and "omega" and "astaxanthin") and "triglyceride"	PGPB	n/a	OR	YES		06-01-2015
(20040241249 and "omega" and "astaxanthin" and "triglyceride") and superba	PGPB	n/a	OR	YES		06-01-2015
(20040241249 and "omega" and "astaxanthin" and "triglyceride" and superba) and capsule	PGPB	n/a	OR	YES		06-01-2015
20040241249	PGPB	n/a	OR	YES		06-01-2015
(20040241249) and supercritical	PGPB	n/a	OR	YES		06-01-2015
(20040241249) and supercritical	PGPB	n/a	OR	YES		06-01-2015
9034388.pn.	USPT	n/a	OR	YES		06-29-2015
9034388.pn.	USPT	n/a	OR	YES		06-29-2015
9028877.pn.	USPT	n/a	OR	YES		06-29-2015
krill.clm. and denaturing.clm. and supercritical.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
krill and denaturing and supercritical	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
(krill and denaturing and supercritical) and supercritical.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
Inge.in. and Bruheim.in.		n/a	OR	YES		06-29-2015

	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS					
(Inge.in. and Bruheim.in.) and krill.clm. and denaturing.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
Snorre.in. and Tilseth.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
(Snorre.in. and Tilseth.in.) and supercritical.clm. and denaturing.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
Daniele.in. and Mancinelli.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
(Daniele.in. and Mancinelli.in.) and krill.clm. and denaturing.clm. and supercritical.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD,	n/a	OR	YES		06-29-2015

	FPRS					
(krill and denaturing and supercritical) and ((A61K2300/00 | A61K31/122 | A61K31/23 | A61K31/683 | A61K31/685 | A61K31/202 | A61K31/232 | A61K31/355 | A61K31/663 | A61K35/74 | A61K9/4858 | A61K35/612 | A61K45/06 | A61K31/20 | A61K31/235 | A61K9/48 | A23L1/3006 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/33 | A23L1/0041 | A23L1/2753 | A23L1/302 | A23L1/303 | C11B3/006 | C11B1/025 | C11B1/10 | C11B1/104 | A23D9/007 | A23D9/013 | A23D9/02 | A23J1/04 | A23J3/34 | C07K14/43509 | C07K19/00 | A23K1/164).CPC.)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	n/a	OR	YES		06-29-2015
Euphausia and superba and oil and phospholipid and astaxanthin	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	138	OR	YES		03-19-2016
(Euphausia and superba and oil and phospholipid and astaxanthin) and non-ether and triglycerides	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	28	OR	YES		03-19-2016
(Euphausia and superba and oil and phospholipid and astaxanthin and non-ether and triglycerides) and ether	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	28	OR	YES		03-19-2016
(Euphausia and superba and oil and phospholipid and astaxanthin and non-ether and triglycerides) and ((A61K2300/00 | A61K31/122 | A61K31/685 | A61K31/683 | A61K31/23 | A61K35/612 | A61K31/202 | A61K9/4858 | A61K31/20 | A61K31/235 | A61K45/06 | A61K9/48 | A61K31/133 | A61K31/198 | A61K31/575 | A61K35/60 | A61K38/1767 | A61K9/2009 | A61K9/2054 | A61K9/2866 | A61K31/661 | A61K31/194 |	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	28	OR	YES		03-19-2016

C11B3/006 | C11B1/06 | C11B1/10 | A23V2002/00 | A23V2250/1868 | A23V2250/187 | A23V2200/322 | A23V2250/1866 | A23V2250/1882 | A23V2250/5432 | A23L1/3006 | A23L1/305 | A23L1/33 | A23L1/0026 | A23L1/3008 | A23L1/30 | A23L1/29 | A23L1/302 | A23L1/326 | A23D9/013 | A23D7/011 | A23D9/00 | A23K1/103 | A23K1/1606 | A23K1/164 | A23K1/188 | C07F9/103 | A23J7/00 | A23G3/364 | A23G3/368 | A23G3/40 | A23G3/54).CPC.)						
Inge.in. and Bruheim.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	112	OR	YES		03-19-2016
(Inge.in. and Bruheim.in.) and oil.clm. and (krill or superba).clm. and phospholipid.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	20	OR	YES		03-19-2016
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Snorre.in. and Tilseth.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	104	OR	YES		03-19-2016
(Snorre.in. and Tilseth.in.) and oil.clm. and (krill or superba).clm. and phospholipid.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI,	18	OR	YES		03-19-2016

	TDBD, FPRS					
(Snorre.in. and Tilseth.in. and oil.clm. and (krill or superba).clm. and phospholipid.clm.) and ether.clm. and non-ether.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	6	OR	YES		03-19-2016
Daniele.in. and Mancinelli.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	67	OR	YES		03-19-2016
(Daniele.in. and Mancinelli.in.) and oil.clm. and (krill or superba).clm. and phospholipid.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	15	OR	YES		03-19-2016
(Daniele.in. and Mancinelli.in. and oil.clm. and (krill or superba).clm. and phospholipid.clm.) and ether.clm. and non-ether.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	6	OR	YES		03-19-2016

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NEWS	26	DEC 24	Additional Experimental Spectra Now Available in CAS REGISTRY

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                  Goes Live
 NEWS 28  JAN 26  Revision of DWPI Fragmentation Codes for 2015
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                  Information and the 2015 MeSH Thesaurus
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                  Patent Index
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                  Information
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COPYRIGHT (C) 2015 IFI CLAIMS(R) Patent Services (IFI)

=> s L1
L2          40 L1

=> s L2 and phospholipid
L3          30 L2 AND PHOSPHOLIPID

=> dup rem L3
PROCESSING COMPLETED FOR L3
L4           30 DUP REM L3 (0 DUPLICATES REMOVED)

=> d L4 1-30

L4   ANSWER 1 OF 30  USPAT2 on STN
AN     2008:312554  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Tilseth, Snorre, Bergen, NORWAY
       Banni, Sebastiano, Cagliari, ITALY
       Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ANTARTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9034388          B2  20150519
AI     US 2008-57775           20080328 (12)
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2386
INCL   INCLM: 424/520.000
NCL    NCLM:  424/520.000
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CPC    CPCI   A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685
              [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0035-56 [I]; A23D0009-00 [I]; A61K0031-66 [I]; A61K0031-661
              [I]; A61K0031-685 [I]; A61P0003-02 [I]

L4   ANSWER 2 OF 30  USPAT2 on STN
AN     2015:4195  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
PA     Aker Biomarine Antarctic AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9028877          B2  20150512
AI     US 2014-14490176        20140918 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2412
INCL   INCLM: 424/520.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]
       IPCI-2 A61K0045-06 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0009-48
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0031-202 [I]
       IPCR   A61K0035-56 [I]

L4   ANSWER 3 OF 30  USPATFULL on STN
AN     2015:186362  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
PI     US 20150164963      A1  20150618
AI     US 2015-14620784    A1  20150212 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
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       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1937
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48
              [I]; A61K0031-235 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683
              [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122
              [I], A61K2300-00
IPC    IPCI   A61K0035-612 [I]; A61K0031-235 [I]; A61K0009-48 [I]; A61K0031-122
              [I]; A61K0031-20 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 4 OF 30  USPATFULL on STN
AN     2015:178202  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
PI     US 20150157669      A1  20150611
AI     US 2015-14620779    A1  20150212 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1930
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-685 [I]; A61K0031-23
              [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00;
              A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-612 [I]; A61K0031-685 [I]; A61K0031-23 [I]; A61K0031-122
              [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 5 OF 30  USPATFULL on STN
AN     2015:55132  USPATFULL
TI     METHOD FOR MAKING KRILL MEAL
IN     Tilseth, Snorre, Bergen, NORWAY
       H.o slashed.stmark, .O slashed.istein, Loddefjord, NORWAY
USPA   Aker BioMarine AS, Oslo, NORWAY
PI     US 20150050403      A1  20150219
AI     US 2014-14490204    A1  20140918 (14)
RLI    Continuation of Ser. No. US 2008-201325, filed on 29 Aug 2008, PENDING
PRAI   US 2007-60968765        20070829 (60)
DT     Utility
FS     APPLICATION
LN.CNT 2192
INCL   INCLM: 426/417.000
       INCLS: 554/008.000
NCL    NCLM:  426/417.000
       NCLS:  554/008.000
CPC    CPCI   C11B0001-10 [I]; A23L0001-33 [I]; A23V2002-00
IPC    IPCI   C11B0001-10 [I]; A23L0001-33 [I]
       IPCR   C11B0001-10 [I]; A23L0001-33 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 6 OF 30  USPATFULL on STN
AN     2015:33475  USPATFULL
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TI Method for Processing Crustaceans to Produce Low Fluoride/Low Trimethyl Amine Products Thereof
IN Bruheim, Inge, Volda, NORWAY
Griinari, Mikko, Espoo, FINLAND
Ervik, Jon Reidar, Aalesund, NORWAY
Remoy, Stig Rune, Fosnavag, NORWAY
Remoy, Even, Fosnavaag, NORWAY
Cameron, John, Fosnavaag, NORWAY
USPA OLYMPIC SEAFOOD AS, Fosnavaag, NORWAY
PI US 20150030751 A1 20150129
AI US 2014-14370324 A1 20121221 (14)
WO 2012-IB3004 20121221
20140702 PCT 371 date
RLI Continuation-in-part of Ser. No. US 2012-13342664, filed on 3 Jan 2012, Pat. No. US 8557297
DT Utility
FS APPLICATION
LN.CNT 2061
INCL INCLM: 426/608.000
NCL NCLM: 426/608.000
CPC CPCI A23L0001-33 [I]
IPC IPCI A23L0001-33 [I]
IPCR A23L0001-33 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 7 OF 30 USPATFULL on STN
AN 2015:33442 USPATFULL
TI OXIDIXABLE FATTY ACID COMPOSITION DELIVERY FORM
IN Saebo, Asgeir, Eidsnes, NORWAY
PA AKER BIOMARINE ANTARCTIC AS, Oslo, NORWAY (non-U.S. corporation)
PI US 20150030718 A1 20150129
AI US 2014-14384286 A1 20130311 (14)
WO 2013-IB865 20130311
20140910 PCT 371 date
PRAI US 2012-61609628 20120312 (61)
DT Utility
FS APPLICATION
LN.CNT 925
INCL INCLM: 426/002.000
INCLS: 426/576.000; 426/072.000; 426/073.000
NCL NCLM: 426/002.000
NCLS: 426/072.000; 426/073.000; 426/576.000
CPC CPCI A23G0003-40 [I]; A23G0003-368 [I]; A23V2002-00, A23V2250-1866, A23V2250-1868, A23V2250-187, A23V2250-1882, A23V2250-5432
IPC IPCI A23G0003-40 [I]; A23G0003-36 [I]
IPCR A23G0003-40 [I]; A23G0003-36 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 8 OF 30 USPATFULL on STN
AN 2015:4199 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
Tilseth, Snorre, Bergen, NORWAY
Mancinelli, Daniele, Orsta, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20150004227 A1 20150101
AI US 2014-14490221 A1 20140918 (14)
RLI Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)

DT Utility
FS APPLICATION
LN.CNT 1955
INCL INCLM: 424/456.000
INCLS: 424/522.000; 424/451.000
NCL NCLM: 424/456.000
NCLS: 424/451.000; 424/522.000
CPC CPCI A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-56 [I]
IPCR A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 9 OF 30 USPATFULL on STN
AN 2015:4195 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
Tilseth, Snorre, Bergen, NORWAY
Mancinelli, Daniele, Orsta, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20150004223 A1 20150101
US 9028877 B2 20150512
AI US 2014-14490176 A1 20140918 (14)
RLI Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
DT Utility
FS APPLICATION
LN.CNT 1983
INCL INCLM: 424/451.000
INCLS: 424/522.000
NCL NCLM: 424/520.000
CPC CPCI A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-56 [I]
IPCI-2 A61K0045-06 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0009-48 [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I]; A61K0031-202 [I]
IPCR A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 10 OF 30 USPAT2 on STN
AN 2011:251469 USPAT2
TI Solvent-free process for obtaining phospholipids and neutral enriched krill oils
IN Katevas, Dimitri Sclabos, Santiage, CHILE
Toro Guerra, Raul R., Santiage, CHILE
Chiong Lay, Mario M., Santiage, CHILE
PA Tharos. Ltd., Santiago, CHILE (non-U.S. corporation)
Lonza, Ltd., Basel, SWITZERLAND (non-U.S. corporation)
PI US 8772516 B2 20140708
AI US 2011-13096644 20110428 (13)
RLI Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009,

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       PENDING
DT     Utility
FS     GRANTED
LN.CNT 1996
INCL   INCLM: 554/023.000
       INCLS: 554/008.000; 554/078.000
NCL    NCLM:  554/023.000
       NCLS:  554/008.000; 554/078.000
CPC    CPCI   C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
       CPCI-2 C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
IPC    IPCI   C11B0001-00 [I]; C07F0009-10 [I]
       IPCI-2 C11B0001-00 [I]
       IPCR   C11B0001-00 [I]
EXF    554/8; 554/23; 554/78

L4   ANSWER 11 OF 30  USPAT2 on STN
AN     2011:117391  USPAT2
TI     Methods of using krill oil to treat risk factors for cardiovascular,
       metabolic, and inflammatory disorders
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Cohn, Jeffery, Sydney, AUSTRALIA
       Griinari, Mikko, Espoo, FINLAND
       Mancinelli, Daniele, Orsta, NORWAY
       Hoem, Nils, Oslo, NORWAY
       Vik, Hogne, Eiksmarka, NORWAY
       Banni, Sebastiano, Calgliari, ITALY
PA     Aker Biomarine AS, Oslo, NORWAY (non-U.S. corporation)
PI     US 8697138          B2  20140415
AI     US 2010-790575          20100528 (12)
RLI    Continuation-in-part of Ser. No. US 2008-57775, filed on 28 Mar 2008,
       PENDING
PRAI   US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
       US 2009-61181743        20090528 (61)
       US 2007-60920483        20070328 (60)
DT     Utility
FS     GRANTED
LN.CNT 2694
INCL   INCLM: 424/538.000
       INCLS: 424/283.100
NCL    NCLM:  424/538.000; 424/522.000
       NCLS:  424/283.100; 426/002.000
CPC    CPCI   A61K0035-612 [I]
       CPCI-2 A61K0035-612 [I]
IPC    IPCI   A61K0035-56 [I]; A61P0009-10 [I]; A61P0003-04 [I]; A61P0003-00
              [I]
       IPCI-2 A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
       IPCR   A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
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L4 ANSWER 12 OF 30 USPATFULL on STN
AN 2014:407114 USPATFULL
TI METHODS OF USING KRILL OIL TO TREAT RISK FACTORS FOR CARDIOVASCULAR, METABOLIC, AND INFLAMMATORY DISORDERS
IN BRUHEIM, Inge, Volda, NORWAY
TILSETH, Snorre, Bergen, NORWAY
COHN, Jeffery, Sydney, AUSTRALIA
GRIINARI, Mikko, Espoo, FINLAND
BANNI, Sebastiano, Calgliari, ITALY
MANCINELLI, Daniele, Orsta, NORWAY
HOEM, Nils, Oslo, NORWAY
VIK, Hogne, Eiksmarka, NORWAY
PA AKER BIOMARINE AS, Oslo, NORWAY (non-U.S. corporation)
PI US 20140363517 A1 20141211
AI US 2014-14244532 A1 20140403 (14)
RLI Division of Ser. No. US 2010-790575, filed on 28 May 2010, Pat. No. US 8697138 Continuation-in-part of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
US 2009-61181743 20090528 (61)
US 2007-60920483 20070328 (60)
DT Utility
FS APPLICATION
LN.CNT 2476
INCL INCLM: 424/522.000
NCL NCLM: 424/522.000
CPC CPCI A61K0035-612 [I]
IPC IPCI A61K0035-56 [I]
IPCR A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 13 OF 30 USPATFULL on STN
AN 2014:399230 USPATFULL
TI SOLVENT-FREE PROCESS FOR OBTAINING PHOSPHOLIPIDS AND NEUTRAL ENRICHED KRILL OILS
IN SCLABOS KATEVAS, Dimitri, La Reina, CHILE
TORO GUERRA, Raul R., La Reina, CHILE
CHIONG LAY, Mario M., La Reina, CHILE
USPA Tharos, Ltd., La Reina, CHILE
Lonza, Ltd., Basel, SWITZERLAND
PI US 20140356447 A1 20141204
AI US 2014-14310134 A1 20140620 (14)
RLI Continuation of Ser. No. US 2011-13096644, filed on 28 Apr 2011, Pat. No. US 8772516 Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009, PENDING
DT Utility
FS APPLICATION
LN.CNT 1991
INCL INCLM: 424/522.000
INCLS: 554/008.000; 426/608.000; 426/643.000
NCL NCLM: 424/522.000
NCLS: 426/608.000; 426/643.000; 554/008.000
CPC CPCI C11B0001-16 [I]; A61K0035-64 [I]; A61K0008-925 [I]; A61Q0017-04 [I]; A61Q0019-007 [I]; A61Q0001-12 [I]; A23D0009-007 [I]; A23L0001-33 [I]; A23D0009-013 [I]; A61K2800-70; A23V2002-00
IPC IPCI C11B0001-16 [I]; A61K0008-92 [I]; A61Q0017-04 [I]; A23D0009-013 [I]; A61Q0001-12 [I]; A23D0009-007 [I]; A23L0001-33 [I]; A61K0035-64 [I]; A61Q0019-00 [I]
IPCR C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23L0001-33

[I]; A61K0008-92 [I]; A61K0035-64 [I]; A61Q0001-12 [I];
A61Q0017-04 [I]; A61Q0019-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 14 OF 30 USPATFULL on STN
AN 2014:307870 USPATFULL
TI OMEGA-3 PHOSPHOLIPID SUPPLEMENTS FOR IMPROVED BRAIN MATURITY
IN Berge, Kjetil, Oslo, NORWAY
Burri, Lena, Oslo, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20140274968 A1 20140918
AI US 2014-14204592 A1 20140311 (14)
PRAI US 2013-61783574 20130314 (61)
DT Utility
FS APPLICATION
LN.CNT 898
INCL INCLM: 514/120.000
NCL NCLM: 514/120.000
CPC CPCI A61K0031-661 [I]; A61K0031-23 [I]; A61K0031-194 [I]; A23V2002-00,
A23V2200-322, A23V2250-1868, A23V2250-187
IPC IPCI A61K0031-661 [I]; A61K0031-194 [I]; A61K0031-23 [I]
IPCR A61K0031-661 [I]; A61K0031-194 [I]; A61K0031-23 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 15 OF 30 USPATFULL on STN
AN 2014:201306 USPATFULL
TI EICOSAPENTAENOIC ACID (EPA) FORMULATIONS
IN WAIBEL, Brian J., Kennett Square, PA, UNITED STATES
Schonemann, Hans, Newburyport, MA, UNITED STATES
Krukonis, Val, Lexington, MA, UNITED STATES
Kagan, Michael, Jerusalem, ISRAEL
USPA Qualitas Health, Ltd., UNITED STATES
PI US 20140179781 A1 20140626
AI US 2013-13797802 A1 20130312 (13)
PRAI US 2012-61745740 20121224 (61)
DT Utility
FS APPLICATION
LN.CNT 4171
INCL INCLM: 514/560.000
INCLS: 426/601.000; 426/607.000
NCL NCLM: 514/560.000
NCLS: 426/601.000; 426/607.000
CPC CPCI A61K0031-202 [I]; A23L0001-3008 [I]; A23V2002-00, A23V2250-1846,
A23V2250-185, A23V2250-187, A23V2250-2136
IPC IPCI A61K0031-202 [I]; A23L0001-30 [I]
IPCR A61K0031-202 [I]; A23L0001-30 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 16 OF 30 USPATFULL on STN
AN 2014:119010 USPATFULL
TI New Method For Making Krill Meal
IN Tilseth, Snorre, Bergen, NORWAY
H.o slashed.stmark, .O slashed.istein, Loddefjord, NORWAY
PA Aker BioMarine AS, Oslo, NORWAY (non-U.S. corporation)
PI US 20140107072 A1 20140417
AI US 2013-14136848 A1 20131220 (14)
RLI Division of Ser. No. US 2008-201325, filed on 29 Aug 2008, PENDING
PRAI US 2007-60968765 20070829 (60)
DT Utility
FS APPLICATION
LN.CNT 2214
INCL INCLM: 514/078.000

NCL NCLM: 514/078.000
CPC CPCI A61K0031-685 [I]; A23L0001-33 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-133 [I]; A61K0031-575 [I]; A61K0031-198 [I]
IPC IPCI A61K0031-685 [I]; A61K0031-122 [I]; A61K0031-198 [I]; A61K0031-133 [I]; A61K0031-575 [I]; A23L0001-33 [I]; A61K0031-202 [I]
IPCR A61K0031-685 [I]; A23L0001-33 [I]; A61K0031-122 [I]; A61K0031-133 [I]; A61K0031-198 [I]; A61K0031-202 [I]; A61K0031-575 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 17 OF 30 USPATFULL on STN
AN 2014:89396 USPATFULL
TI OMEGA-3 PHOSPHOLIPID SUPPLEMENTS FOR FEMALES
IN Berge, Kjetil, Oslo, NORWAY
Hoem, Nils, Oslo, NORWAY
USPA Aker Biomarine AS, Oslo, NORWAY
PI US 20140080791 A1 20140320
AI US 2013-14028738 A1 20130917 (14)
PRAI US 2012-61703009 20120919 (61)
DT Utility
FS APPLICATION
LN.CNT 832
INCL INCLM: 514/120.000
NCL NCLM: 514/120.000
CPC CPCI A61K0031-661 [I]; A61K0035-60 [I], A61K2300-00; A61K0035-612 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00; A61K0031-202 [I], A61K2300-00
IPC IPCI A61K0031-661 [I]
IPCR A61K0031-661 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 18 OF 30 USPATFULL on STN
AN 2014:11777 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
Tilseth, Snorre, Bergen, NORWAY
Mancinelli, Daniele, Orsta, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20140010888 A1 20140109
AI US 2013-14020155 A1 20130906 (14)
RLI Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
DT Utility
FS APPLICATION
LN.CNT 1898
INCL INCLM: 424/522.000
NCL NCLM: 424/522.000
CPC CPCI A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-56 [I]; A61K0031-202 [I]; A61K0031-122 [I]
IPCR A61K0035-56 [I]; A61K0031-122 [I]; A61K0031-202 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 19 OF 30 USPATFULL on STN
AN 2014:5400 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
Tilseth, Snorre, Bergen, NORWAY

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       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20140005421      A1  20140102
AI     US 2013-14020162    A1  20130906 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1908
INCL   INCLM: 554/008.000
NCL    NCLM:  554/008.000
CPC    CPCI   C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
IPC    IPCI   C11B0003-00 [I]
       IPCR   C11B0003-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 20 OF 30  USPAT2 on STN
AN     2012:168278  USPAT2
TI     Method for processing crustaceans and products thereof
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Ervik, Jon Reidar, Aalesund, NORWAY
       Remoy, Stig Rune, Fosnavag, NORWAY
PA     Olympic Seafood, AS, Fosnavaag, GERMANY, FEDERAL REPUBLIC OF (non-U.S.
       corporation)
PI     US 8557297          B2  20131015
AI     US 2012-13342664        20120103 (13)
RLI    Continuation of Ser. No. US 1900-63488, PENDING A 371 of International
       Ser. No. WO 2009-NO322, filed on 14 Sep 2009
DT     Utility
FS     GRANTED
LN.CNT 1435
INCL   INCLM: 424/538.000
       INCLS: 435/068.100; 435/325.000; 435/381.000; 500/300.000; 500/359.000;
              426/665.000; 426/417.000
NCL    NCLM:  424/538.000; 530/300.000
       NCLS:  426/417.000; 426/665.000; 435/068.100; 435/325.000; 435/381.000;
              530/300.000; 530/359.000; 554/008.000; 554/021.000; 554/084.000
CPC    CPCI   C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02
              [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I];
              A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I];
              A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I];
              C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104
              [I]
       CPCI-2 C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02
              [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I];
              A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I];
              A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I];
              C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104
              [I]
IPC    IPCI   C11B0001-10 [I]; C07F0009-02 [I]; C07K0014-00 [I]; C07K0002-00
              [I]
       IPCI-2 A61K0035-64 [I]
       IPCR   A61K0035-64 [I]

L4   ANSWER 21 OF 30  USPAT2 on STN
AN     2010:256169  USPAT2
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TI     Phospholipid and protein tablets
IN     Tilseth, Snorre, Bergen, NORWAY
       Hoem, Nils, Oslo, NORWAY
PA     Aker Biomarine ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 8372812          B2  20130212
AI     US 2010-711822          20100224 (12)
PRAI   US 2009-61155758        20090226 (61)
DT     Utility
FS     GRANTED
LN.CNT 3399
INCL   INCLM: 514/021.920
       INCLS: 514/762.000; 424/464.000; 424/476.000; 424/477.000
NCL    NCLM:  514/021.920; 514/005.500
       NCLS:  424/464.000; 424/476.000; 424/477.000; 514/762.000; 514/691.000
CPC    CPCI   A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
              A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
              A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
              A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
              [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
       CPCI-2 A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
              A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
              A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
              A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
              [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
IPC    IPCI   A61K0038-02 [I]
       IPCI-2 A61K0038-17 [I]; A61K0031-01 [I]; A61K0009-20 [I]; A61K0009-38
              [I]; A61K0009-42 [I]
       IPCR   A61K0038-17 [I]; A61K0009-20 [I]; A61K0009-38 [I]; A61K0009-42
              [I]; A61K0031-01 [I]

L4   ANSWER 22 OF 30  USPATFULL on STN
AN     2013:254433  USPATFULL
TI     REDUCED FLUORIDE CRUSTACEAN OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Ervik, Jon Reidar, Aalesund, NORWAY
       Remoy, Stig Rune, Fosnavaag, NORWAY
PA     Olympic Seafood AS, Fosnavaag, NORWAY (non-U.S. corporation)
PI     US 20130225794      A1  20130829
AI     US 2013-13856642    A1  20130404 (13)
RLI    Division of Ser. No. US 2012-13342664, filed on 3 Jan 2012, PENDING
       Continuation of Ser. No. US 2011-13063488, filed on 24 May 2011, PENDING
       A 371 of International Ser. No. WO 2009-NO322, filed on 14 Sep 2009
DT     Utility
FS     APPLICATION
LN.CNT 1430
INCL   INCLM: 530/359.000
       INCLS: 554/078.000; 530/350.000
NCL    NCLM:  530/359.000
       NCLS:  530/350.000; 554/078.000
CPC    CPCI   C11B0003-006 [I]; C07K0019-00 [I]; C07K0014-43509 [I]
IPC    IPCI   C11B0003-00 [I]; C07K0014-435 [I]; C07K0019-00 [I]
       IPCR   C11B0003-00 [I]; C07K0014-435 [I]; C07K0019-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 23 OF 30  USPATFULL on STN
AN     2013:186762  USPATFULL
TI     PHOSPHOLIPID AND PROTEIN TABLETS
IN     Tilseth, Snorre, Bergen, NORWAY
       Hoem, Nils, Oslo, NORWAY
PA     AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 20130165393      A1  20130627
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AI US 2013-13748013 A1 20130123 (13)
RLI Continuation of Ser. No. US 2010-711822, filed on 24 Feb 2010, Pat. No. US 8372812
PRAI US 2009-61155758 20090226 (61)
DT Utility
FS APPLICATION
LN.CNT 3145
INCL INCLM: 514/021.920
INCLS: 264/113.000
NCL NCLM: 514/021.920
NCLS: 264/113.000
CPC CPCI A61K0031-122 [I]; A61K0038-1767 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612 [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
IPC IPCI A61K0031-122 [I]; A61K0038-17 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 24 OF 30 USPATFULL on STN
AN 2012:168278 USPATFULL
TI Method For Processing Crustaceans And Products Thereof
IN Bruheim, Inge, Volda, NORWAY
Griinari, Mikko, Espoo, FINLAND
Ervik, Jon Reidar, Aalesund, NORWAY
Remoy, Stig Rune, Fosnavag, NORWAY
PA Emerald Fisheries (non-U.S. corporation)
PI US 20120149867 A1 20120614
US 8557297 B2 20131015
AI US 2012-13342664 A1 20120103 (13)
RLI Continuation of Ser. No. US 2011-13063488, filed on 24 May 2011, PENDING
A 371 of International Ser. No. WO 2009-NO322, filed on 14 Sep 2009
DT Utility
FS APPLICATION
LN.CNT 1449
INCL INCLM: 530/300.000
INCLS: 554/008.000; 554/021.000; 554/084.000; 530/359.000
NCL NCLM: 424/538.000; 530/300.000
NCLS: 426/417.000; 426/665.000; 435/068.100; 435/325.000; 435/381.000; 530/300.000; 530/359.000; 554/008.000; 554/021.000; 554/084.000
CPC CPCI C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02 [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I]; A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I]; A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I]; C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104 [I]
CPCI-2 C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02 [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I]; A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I]; A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I]; C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104 [I]
IPC IPCI C11B0001-10 [I]; C07F0009-02 [I]; C07K0014-00 [I]; C07K0002-00 [I]
IPCI-2 A61K0035-64 [I]
IPCR A61K0035-64 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 25 OF 30 USPATFULL on STN
AN 2011:251469 USPATFULL
TI SOLVENT-FREE PROCESS FOR OBTAINING PHOSPHOLIPIDS AND NEUTRAL ENRICHED KRILL OILS
IN Sclabos Katevas, Dimitri, Santiage, CHILE
Toro Guerra, Raul R., Santiage, CHILE

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       Chiong Lay, Mario M., Santiage, CHILE
PA     THAROS LTD., Santiago, CHILE (non-U.S. corporation)
       LONZA LTD., Basel, SWITZERLAND (non-U.S. corporation)
PI     US 20110224450      A1  20110915
       US 8772516          B2  20140708
AI     US 2011-13096644    A1  20110428 (13)
RLI    Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009,
       PENDING
DT     Utility
FS     APPLICATION
LN.CNT 2021
INCL   INCLM: 554/023.000
       INCLS: 554/008.000; 554/078.000
NCL    NCLM:  554/023.000
       NCLS:  554/008.000; 554/078.000
CPC    CPCI   C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
       CPCI-2 C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
IPC    IPCI   C11B0001-00 [I]; C07F0009-10 [I]
       IPCI-2 C11B0001-00 [I]
       IPCR   C11B0001-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 26 OF 30  USPATFULL on STN
AN     2011:117391  USPATFULL
TI     METHODS OF USING KRILL OIL TO TREAT RISK FACTORS FOR CARDIOVASCULAR,
       METABOLIC, AND INFLAMMATORY DISORDERS
IN     BRUHEIM, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Cohn, Jeffery, Sydney, AUSTRALIA
       Griinari, Mikko, Espoo, FINLAND
       Mancinelli, Daniele, Orsta, NORWAY
       Hoem, Nils, Oslo, NORWAY
       Vik, Hogne, Eiksmarka, NORWAY
       Banni, Sebastiano, Calgliari, ITALY
PA     Aker BioMarine A.S.A., Oslo, NORWAY (non-U.S. corporation)
PI     US 20110104297      A1  20110505
       US 8697138          B2  20140415
AI     US 2010-790575      A1  20100528 (12)
RLI    Continuation-in-part of Ser. No. US 2008-57775, filed on 28 Mar 2008,
       PENDING
PRAI   US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
       US 2009-61181743        20090528 (61)
       US 2007-60920483        20070328 (60)
DT     Utility
FS     APPLICATION
LN.CNT 2547
INCL   INCLM: 424/522.000
       INCLS: 426/002.000
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NCL    NCLM:  424/538.000; 424/522.000
       NCLS:  424/283.100; 426/002.000
CPC    CPCI   A61K0035-612 [I]
       CPCI-2 A61K0035-612 [I]
IPC    IPCI   A61K0035-56 [I]; A61P0009-10 [I]; A61P0003-04 [I]; A61P0003-00
              [I]
       IPCI-2 A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
       IPCR   A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 27 OF 30  USPATFULL on STN
AN     2010:256169  USPATFULL
TI     PHOSPHOLIPID AND PROTEIN TABLETS
IN     Tilseth, Snorre, Bergen, NORWAY
       Hoem, Nils, Oslo, NORWAY
PA     AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 20100227792      A1  20100909
       US 8372812          B2  20130212
AI     US 2010-711822      A1  20100224 (12)
PRAI   US 2009-61155758        20090226 (61)
DT     Utility
FS     APPLICATION
LN.CNT 3112
INCL   INCLM: 514 2
NCL    NCLM:  514/021.920; 514/005.500
       NCLS:  424/464.000; 424/476.000; 424/477.000; 514/762.000; 514/691.000
CPC    CPCI   A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
              A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
              A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
              A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
              [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
       CPCI-2 A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
              A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
              A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
              A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
              [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
IPC    IPCI   A61K0038-02 [I]
       IPCI-2 A61K0038-17 [I]; A61K0031-01 [I]; A61K0009-20 [I]; A61K0009-38
              [I]; A61K0009-42 [I]
       IPCR   A61K0038-17 [I]; A61K0009-20 [I]; A61K0009-38 [I]; A61K0009-42
              [I]; A61K0031-01 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 28 OF 30  USPATFULL on STN
AN     2010:255355  USPATFULL
TI     LOW VISCOSITY PHOSPHOLIPID COMPOSITIONS
IN     Tilseth, Snorre, Bergen, NORWAY
PA     AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 20100226977      A1  20100909
AI     US 2010-711553      A1  20100224 (12)
RLI    Continuation-in-part of Ser. No. US 2008-201325, filed on 29 Aug 2008,
       PENDING
PRAI   US 2009-61155767        20090226 (61)
       US 2007-60968765        20070829 (60)
DT     Utility
FS     APPLICATION
LN.CNT 2394
INCL   INCLM: 424/456.000
       INCLS: 426/601.000; 426/417.000; 514/078.000
NCL    NCLM:  424/456.000
       NCLS:  426/417.000; 426/601.000; 514/078.000
CPC    CPCI   A23D0009-013 [I]; A23D0007-011 [I]; A23J0007-00 [I]; A23K0001-103
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[I]; A23K0001-1606 [I]; A23K0001-164 [I]; A23K0001-188 [I];
A23L0001-30 [I]; A23L0001-3006 [I]; A23L0001-3008 [I];
A23L0001-305 [I]; A23L0001-326 [I]; A61K0035-612 [I];
C07F0009-103 [I]; C11B0001-06 [I]
IPC IPCI A61K0031-685 [I]; A23D0009-00 [I]; A23D0009-02 [I]; A61K0009-48
[I]; A61P0009-00 [I]; A61P0019-00 [I]; A61P0029-00 [I]
IPCR A61K0031-685 [I]; A23D0009-00 [I]; A23D0009-02 [I]; A61K0009-48
[I]; A61P0009-00 [I]; A61P0019-00 [I]; A61P0029-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 29 OF 30 USPATFULL on STN
AN 2009:67318 USPATFULL
TI METHOD FOR MAKING KRILL MEAL
IN Tilseth, Snorre, Bergen, NORWAY
Hostmark, Oistein, Loddefjord, NORWAY
PA Aker BioMarine ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 20090061067 A1 20090305
AI US 2008-201325 A1 20080829 (12)
PRAI US 2007-60968765 20070829 (60)
DT Utility
FS APPLICATION
LN.CNT 2307
INCL INCLM: 426/602.000
INCLS: 426/417.000; 210/149.000; 426/480.000; 426/609.000; 426/648.000;
426/608.000; 366/145.000; 366/147.000
NCL NCLM: 426/602.000
NCLS: 210/149.000; 366/145.000; 366/147.000; 426/417.000; 426/480.000;
426/608.000; 426/609.000; 426/648.000
CPC CPCI A61K0031-685 [I]; A23D0009-013 [I]; A23K0001-103 [I];
A23K0001-1606 [I]; A23K0001-164 [I]; A23K0001-188 [I];
A23L0001-3006 [I]; A23L0001-305 [I]; A23L0001-33 [I];
A61K0031-122 [I]; A61K0031-133 [I]; A61K0031-198 [I];
A61K0031-202 [I]; A61K0031-575 [I]; A61K0035-612 [I];
C07F0009-103 [I]; C11B0001-06 [I]
IPC IPCI A23D0007-005 [I]; A23D0007-02 [I]; A23D0007-04 [I]; A23L0001-29
[I]; B01F0015-06 [I]; A23L0001-33 [I]; A23L0001-326 [I];
B01D0021-30 [I]
IPCR A23D0007-005 [I]; A23D0007-02 [I]; A23D0007-04 [I]; A23L0001-29
[I]; A23L0001-326 [I]; A23L0001-33 [I]; B01D0021-30 [I];
B01F0015-06 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 30 OF 30 USPATFULL on STN
AN 2008:312554 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
Griinari, Mikko, Espoo, FINLAND
Tilseth, Snorre, Bergen, NORWAY
Banni, Sebastiano, Cagliari, ITALY
Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
Mancinelli, Daniele, Orsta, NORWAY
PA AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 20080274203 A1 20081106
US 9034388 B2 20150519
AI US 2008-57775 A1 20080328 (12)
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
DT Utility
FS APPLICATION
LN.CNT 2199

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INCL   INCLM: 424/522.000
       INCLS: 514/121.000; 514/078.000; 514/114.000; 426/601.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685
              [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0035-56 [I]; A23D0009-00 [I]; A61K0031-66 [I]; A61K0031-661
              [I]; A61K0031-685 [I]; A61P0003-02 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> s L4 and phosphatidylcholine
L5          29 L4 AND PHOSPHATIDYLCHOLINE

=> d L4 1-30

L4   ANSWER 1 OF 30  USPAT2 on STN
AN     2008:312554  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Tilseth, Snorre, Bergen, NORWAY
       Banni, Sebastiano, Cagliari, ITALY
       Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ANTARTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9034388          B2  20150519
AI     US 2008-57775           20080328 (12)
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2386
INCL   INCLM: 424/520.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685
              [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
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              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0035-56 [I]; A23D0009-00 [I]; A61K0031-66 [I]; A61K0031-661
              [I]; A61K0031-685 [I]; A61P0003-02 [I]

L4   ANSWER 2 OF 30  USPAT2 on STN
AN     2015:4195  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
PA     Aker Biomarine Antarctic AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9028877          B2  20150512
AI     US 2014-14490176        20140918 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2412
INCL   INCLM: 424/520.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]
       IPCI-2 A61K0045-06 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0009-48
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0031-202 [I]
       IPCR   A61K0035-56 [I]

L4   ANSWER 3 OF 30  USPATFULL on STN
AN     2015:186362  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
PI     US 20150164963      A1  20150618
AI     US 2015-14620784    A1  20150212 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1937
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48
              [I]; A61K0031-235 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683
              [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122
              [I], A61K2300-00
IPC    IPCI   A61K0035-612 [I]; A61K0031-235 [I]; A61K0009-48 [I]; A61K0031-122
              [I]; A61K0031-20 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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L4   ANSWER 4 OF 30 USPATFULL on STN
AN   2015:178202 USPATFULL
TI   BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN   Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
PI   US 20150157669   A1 20150611
AI   US 2015-14620779   A1 20150212 (14)
RLI   Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483   20070328 (60)
       US 2007-60975058   20070925 (60)
       US 2007-60983446   20071029 (60)
       US 2008-61024072   20080128 (61)
DT   Utility
FS   APPLICATION
LN.CNT 1930
CPC   CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-685 [I]; A61K0031-23
              [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00;
              A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC   IPCI   A61K0035-612 [I]; A61K0031-685 [I]; A61K0031-23 [I]; A61K0031-122
              [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 5 OF 30 USPATFULL on STN
AN   2015:55132 USPATFULL
TI   METHOD FOR MAKING KRILL MEAL
IN   Tilseth, Snorre, Bergen, NORWAY
       H.o slashed.stmark, .O slashed.istein, Loddefjord, NORWAY
USPA   Aker BioMarine AS, Oslo, NORWAY
PI   US 20150050403   A1 20150219
AI   US 2014-14490204   A1 20140918 (14)
RLI   Continuation of Ser. No. US 2008-201325, filed on 29 Aug 2008, PENDING
PRAI   US 2007-60968765   20070829 (60)
DT   Utility
FS   APPLICATION
LN.CNT 2192
INCL   INCLM: 426/417.000
       INCLS: 554/008.000
NCL   NCLM: 426/417.000
       NCLS: 554/008.000
CPC   CPCI   C11B0001-10 [I]; A23L0001-33 [I]; A23V2002-00
IPC   IPCI   C11B0001-10 [I]; A23L0001-33 [I]
       IPCR   C11B0001-10 [I]; A23L0001-33 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 6 OF 30 USPATFULL on STN
AN   2015:33475 USPATFULL
TI   Method for Processing Crustaceans to Produce Low Fluoride/Low Trimethyl
       Amine Products Thereof
IN   Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Ervik, Jon Reidar, Aalesund, NORWAY
       Remoy, Stig Rune, Fosnavag, NORWAY
       Remoy, Even, Fosnavaag, NORWAY
       Cameron, John, Fosnavaag, NORWAY
USPA   OLYMPIC SEAFOOD AS, Fosnavaag, NORWAY
PI   US 20150030751   A1 20150129
AI   US 2014-14370324   A1 20121221 (14)
       WO 2012-IB3004   20121221
                               20140702 PCT 371 date

RLI Continuation-in-part of Ser. No. US 2012-13342664, filed on 3 Jan 2012, Pat. No. US 8557297
DT Utility
FS APPLICATION
LN.CNT 2061
INCL INCLM: 426/608.000
NCL NCLM: 426/608.000
CPC CPCI A23L0001-33 [I]
IPC IPCI A23L0001-33 [I]
IPCR A23L0001-33 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 7 OF 30 USPATFULL on STN
AN 2015:33442 USPATFULL
TI OXIDIXABLE FATTY ACID COMPOSITION DELIVERY FORM
IN Saebo, Asgeir, Eidsnes, NORWAY
PA AKER BIOMARINE ANTARCTIC AS, Oslo, NORWAY (non-U.S. corporation)
PI US 20150030718 A1 20150129
AI US 2014-14384286 A1 20130311 (14)
WO 2013-IB865 20130311
20140910 PCT 371 date
PRAI US 2012-61609628 20120312 (61)
DT Utility
FS APPLICATION
LN.CNT 925
INCL INCLM: 426/002.000
INCLS: 426/576.000; 426/072.000; 426/073.000
NCL NCLM: 426/002.000
NCLS: 426/072.000; 426/073.000; 426/576.000
CPC CPCI A23G0003-40 [I]; A23G0003-368 [I]; A23V2002-00, A23V2250-1866, A23V2250-1868, A23V2250-187, A23V2250-1882, A23V2250-5432
IPC IPCI A23G0003-40 [I]; A23G0003-36 [I]
IPCR A23G0003-40 [I]; A23G0003-36 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 8 OF 30 USPATFULL on STN
AN 2015:4199 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
Tilseth, Snorre, Bergen, NORWAY
Mancinelli, Daniele, Orsta, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20150004227 A1 20150101
AI US 2014-14490221 A1 20140918 (14)
RLI Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
DT Utility
FS APPLICATION
LN.CNT 1955
INCL INCLM: 424/456.000
INCLS: 424/522.000; 424/451.000
NCL NCLM: 424/456.000
NCLS: 424/451.000; 424/522.000
CPC CPCI A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-56 [I]
IPCR A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 9 OF 30 USPATFULL on STN
AN 2015:4195 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
    Tilseth, Snorre, Bergen, NORWAY
    Mancinelli, Daniele, Orsta, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20150004223 A1 20150101
    US 9028877 B2 20150512
AI US 2014-14490176 A1 20140918 (14)
RLI Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60920483 20070328 (60)
    US 2007-60975058 20070925 (60)
    US 2007-60983446 20071029 (60)
    US 2008-61024072 20080128 (61)
DT Utility
FS APPLICATION
LN.CNT 1983
INCL INCLM: 424/451.000
    INCLS: 424/522.000
NCL NCLM: 424/520.000
CPC CPCI A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
    CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-56 [I]
    IPCI-2 A61K0045-06 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0009-48 [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I]; A61K0031-202 [I]
    IPCR A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 10 OF 30 USPAT2 on STN
AN 2011:251469 USPAT2
TI Solvent-free process for obtaining phospholipids and neutral enriched krill oils
IN Katevas, Dimitri Sclabos, Santiage, CHILE
    Toro Guerra, Raul R., Santiage, CHILE
    Chiong Lay, Mario M., Santiage, CHILE
PA Tharos. Ltd., Santiago, CHILE (non-U.S. corporation)
    Lonza, Ltd., Basel, SWITZERLAND (non-U.S. corporation)
PI US 8772516 B2 20140708
AI US 2011-13096644 20110428 (13)
RLI Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009, PENDING
DT Utility
FS GRANTED
LN.CNT 1996
INCL INCLM: 554/023.000
    INCLS: 554/008.000; 554/078.000
NCL NCLM: 554/023.000
    NCLS: 554/008.000; 554/078.000
CPC CPCI C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553 [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I]; A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10; A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007

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              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
       CPCI-2 C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
IPC    IPCI   C11B0001-00 [I]; C07F0009-10 [I]
       IPCI-2 C11B0001-00 [I]
       IPCR   C11B0001-00 [I]
EXF    554/8; 554/23; 554/78

L4   ANSWER 11 OF 30  USPAT2 on STN
AN     2011:117391  USPAT2
TI     Methods of using krill oil to treat risk factors for cardiovascular,
       metabolic, and inflammatory disorders
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Cohn, Jeffery, Sydney, AUSTRALIA
       Griinari, Mikko, Espoo, FINLAND
       Mancinelli, Daniele, Orsta, NORWAY
       Hoem, Nils, Oslo, NORWAY
       Vik, Hogne, Eiksmarka, NORWAY
       Banni, Sebastiano, Calgliari, ITALY
PA     Aker Biomarine AS, Oslo, NORWAY (non-U.S. corporation)
PI     US 8697138          B2  20140415
AI     US 2010-790575          20100528 (12)
RLI    Continuation-in-part of Ser. No. US 2008-57775, filed on 28 Mar 2008,
       PENDING
PRAI   US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
       US 2009-61181743        20090528 (61)
       US 2007-60920483        20070328 (60)
DT     Utility
FS     GRANTED
LN.CNT 2694
INCL   INCLM: 424/538.000
       INCLS: 424/283.100
NCL    NCLM:  424/538.000; 424/522.000
       NCLS:  424/283.100; 426/002.000
CPC    CPCI   A61K0035-612 [I]
       CPCI-2 A61K0035-612 [I]
IPC    IPCI   A61K0035-56 [I]; A61P0009-10 [I]; A61P0003-04 [I]; A61P0003-00
              [I]
       IPCI-2 A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
       IPCR   A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]

L4   ANSWER 12 OF 30  USPATFULL on STN
AN     2014:407114  USPATFULL
TI     METHODS OF USING KRILL OIL TO TREAT RISK FACTORS FOR CARDIOVASCULAR,
       METABOLIC, AND INFLAMMATORY DISORDERS
IN     BRUHEIM, Inge, Volda, NORWAY
       TILSETH, Snorre, Bergen, NORWAY
       COHN, Jeffery, Sydney, AUSTRALIA
       GRIINARI, Mikko, Espoo, FINLAND
       BANNI, Sebastiano, Calgliari, ITALY
       MANCINELLI, Daniele, Orsta, NORWAY
       HOEM, Nils, Oslo, NORWAY
       VIK, Hogne, Eiksmarka, NORWAY
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PA AKER BIOMARINE AS, Oslo, NORWAY (non-U.S. corporation)
PI US 20140363517 A1 20141211
AI US 2014-14244532 A1 20140403 (14)
RLI Division of Ser. No. US 2010-790575, filed on 28 May 2010, Pat. No. US 8697138 Continuation-in-part of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
US 2009-61181743 20090528 (61)
US 2007-60920483 20070328 (60)
DT Utility
FS APPLICATION
LN.CNT 2476
INCL INCLM: 424/522.000
NCL NCLM: 424/522.000
CPC CPCI A61K0035-612 [I]
IPC IPCI A61K0035-56 [I]
IPCR A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 13 OF 30 USPATFULL on STN
AN 2014:399230 USPATFULL
TI SOLVENT-FREE PROCESS FOR OBTAINING PHOSPHOLIPIDS AND NEUTRAL ENRICHED KRILL OILS
IN SCLABOS KATEVAS, Dimitri, La Reina, CHILE
TORO GUERRA, Raul R., La Reina, CHILE
CHIONG LAY, Mario M., La Reina, CHILE
USPA Tharos, Ltd., La Reina, CHILE
Lonza, Ltd., Basel, SWITZERLAND
PI US 20140356447 A1 20141204
AI US 2014-14310134 A1 20140620 (14)
RLI Continuation of Ser. No. US 2011-13096644, filed on 28 Apr 2011, Pat. No. US 8772516 Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009, PENDING
DT Utility
FS APPLICATION
LN.CNT 1991
INCL INCLM: 424/522.000
INCLS: 554/008.000; 426/608.000; 426/643.000
NCL NCLM: 424/522.000
NCLS: 426/608.000; 426/643.000; 554/008.000
CPC CPCI C11B0001-16 [I]; A61K0035-64 [I]; A61K0008-925 [I]; A61Q0017-04 [I]; A61Q0019-007 [I]; A61Q0001-12 [I]; A23D0009-007 [I]; A23L0001-33 [I]; A23D0009-013 [I]; A61K2800-70; A23V2002-00
IPC IPCI C11B0001-16 [I]; A61K0008-92 [I]; A61Q0017-04 [I]; A23D0009-013 [I]; A61Q0001-12 [I]; A23D0009-007 [I]; A23L0001-33 [I]; A61K0035-64 [I]; A61Q0019-00 [I]
IPCR C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23L0001-33 [I]; A61K0008-92 [I]; A61K0035-64 [I]; A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 14 OF 30 USPATFULL on STN
AN 2014:307870 USPATFULL
TI OMEGA-3 PHOSPHOLIPID SUPPLEMENTS FOR IMPROVED BRAIN MATURITY
IN Berge, Kjetil, Oslo, NORWAY
Burri, Lena, Oslo, NORWAY
USPA AKER BIOMARINE AS, Oslo, NORWAY
PI US 20140274968 A1 20140918
AI US 2014-14204592 A1 20140311 (14)
PRAI US 2013-61783574 20130314 (61)

DT Utility
FS APPLICATION
LN.CNT 898
INCL INCLM: 514/120.000
NCL NCLM: 514/120.000
CPC CPCI A61K0031-661 [I]; A61K0031-23 [I]; A61K0031-194 [I]; A23V2002-00, A23V2200-322, A23V2250-1868, A23V2250-187
IPC IPCI A61K0031-661 [I]; A61K0031-194 [I]; A61K0031-23 [I]
IPCR A61K0031-661 [I]; A61K0031-194 [I]; A61K0031-23 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 15 OF 30 USPATFULL on STN
AN 2014:201306 USPATFULL
TI EICOSAPENTAENOIC ACID (EPA) FORMULATIONS
IN WAIBEL, Brian J., Kennett Square, PA, UNITED STATES
Schonemann, Hans, Newburyport, MA, UNITED STATES
Krukonis, Val, Lexington, MA, UNITED STATES
Kagan, Michael, Jerusalem, ISRAEL
USPA Qualitas Health, Ltd., UNITED STATES
PI US 20140179781 A1 20140626
AI US 2013-13797802 A1 20130312 (13)
PRAI US 2012-61745740 20121224 (61)
DT Utility
FS APPLICATION
LN.CNT 4171
INCL INCLM: 514/560.000
INCLS: 426/601.000; 426/607.000
NCL NCLM: 514/560.000
NCLS: 426/601.000; 426/607.000
CPC CPCI A61K0031-202 [I]; A23L0001-3008 [I]; A23V2002-00, A23V2250-1846, A23V2250-185, A23V2250-187, A23V2250-2136
IPC IPCI A61K0031-202 [I]; A23L0001-30 [I]
IPCR A61K0031-202 [I]; A23L0001-30 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 16 OF 30 USPATFULL on STN
AN 2014:119010 USPATFULL
TI New Method For Making Krill Meal
IN Tilseth, Snorre, Bergen, NORWAY
H.o slashed.stmark, .O slashed.istein, Loddefjord, NORWAY
PA Aker BioMarine AS, Oslo, NORWAY (non-U.S. corporation)
PI US 20140107072 A1 20140417
AI US 2013-14136848 A1 20131220 (14)
RLI Division of Ser. No. US 2008-201325, filed on 29 Aug 2008, PENDING
PRAI US 2007-60968765 20070829 (60)
DT Utility
FS APPLICATION
LN.CNT 2214
INCL INCLM: 514/078.000
NCL NCLM: 514/078.000
CPC CPCI A61K0031-685 [I]; A23L0001-33 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-133 [I]; A61K0031-575 [I]; A61K0031-198 [I]
IPC IPCI A61K0031-685 [I]; A61K0031-122 [I]; A61K0031-198 [I]; A61K0031-133 [I]; A61K0031-575 [I]; A23L0001-33 [I]; A61K0031-202 [I]
IPCR A61K0031-685 [I]; A23L0001-33 [I]; A61K0031-122 [I]; A61K0031-133 [I]; A61K0031-198 [I]; A61K0031-202 [I]; A61K0031-575 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 17 OF 30 USPATFULL on STN
AN 2014:89396 USPATFULL
TI OMEGA-3 PHOSPHOLIPID SUPPLEMENTS FOR FEMALES

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IN     Berge, Kjetil, Oslo, NORWAY
       Hoem, Nils, Oslo, NORWAY
USPA   Aker Biomarine AS, Oslo, NORWAY
PI     US 20140080791      A1  20140320
AI     US 2013-14028738    A1  20130917 (14)
PRAI   US 2012-61703009        20120919 (61)
DT     Utility
FS     APPLICATION
LN.CNT 832
INCL   INCLM: 514/120.000
NCL    NCLM:  514/120.000
CPC    CPCI   A61K0031-661 [I]; A61K0035-60 [I], A61K2300-00; A61K0035-612 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00; A61K0031-202 [I], A61K2300-00
IPC    IPCI   A61K0031-661 [I]
       IPCR   A61K0031-661 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 18 OF 30  USPATFULL on STN
AN     2014:11777  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20140010888      A1  20140109
AI     US 2013-14020155    A1  20130906 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1898
INCL   INCLM: 424/522.000
NCL    NCLM:  424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23
              [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685
              [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-202 [I]; A61K0031-122 [I]
       IPCR   A61K0035-56 [I]; A61K0031-122 [I]; A61K0031-202 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 19 OF 30  USPATFULL on STN
AN     2014:5400  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20140005421      A1  20140102
AI     US 2013-14020162    A1  20130906 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1908
INCL   INCLM: 554/008.000
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NCL NCLM: 554/008.000
CPC CPCI C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI C11B0003-00 [I]
IPCR C11B0003-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 20 OF 30 USPAT2 on STN
AN 2012:168278 USPAT2
TI Method for processing crustaceans and products thereof
IN Bruheim, Inge, Volda, NORWAY
Griinari, Mikko, Espoo, FINLAND
Ervik, Jon Reidar, Aalesund, NORWAY
Remoy, Stig Rune, Fosnavag, NORWAY
PA Olympic Seafood, AS, Fosnavaag, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
PI US 8557297 B2 20131015
AI US 2012-13342664 20120103 (13)
RLI Continuation of Ser. No. US 1900-63488, PENDING A 371 of International Ser. No. WO 2009-NO322, filed on 14 Sep 2009
DT Utility
FS GRANTED
LN.CNT 1435
INCL INCLM: 424/538.000
INCLS: 435/068.100; 435/325.000; 435/381.000; 500/300.000; 500/359.000; 426/665.000; 426/417.000
NCL NCLM: 424/538.000; 530/300.000
NCLS: 426/417.000; 426/665.000; 435/068.100; 435/325.000; 435/381.000; 530/300.000; 530/359.000; 554/008.000; 554/021.000; 554/084.000
CPC CPCI C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02 [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I]; A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I]; A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I]; C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104 [I]
CPCI-2 C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02 [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I]; A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I]; A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I]; C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104 [I]
IPC IPCI C11B0001-10 [I]; C07F0009-02 [I]; C07K0014-00 [I]; C07K0002-00 [I]
IPCI-2 A61K0035-64 [I]
IPCR A61K0035-64 [I]

L4 ANSWER 21 OF 30 USPAT2 on STN
AN 2010:256169 USPAT2
TI Phospholipid and protein tablets
IN Tilseth, Snorre, Bergen, NORWAY
Hoem, Nils, Oslo, NORWAY
PA Aker Biomarine ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 8372812 B2 20130212
AI US 2010-711822 20100224 (12)
PRAI US 2009-61155758 20090226 (61)
DT Utility
FS GRANTED
LN.CNT 3399
INCL INCLM: 514/021.920
INCLS: 514/762.000; 424/464.000; 424/476.000; 424/477.000
NCL NCLM: 514/021.920; 514/005.500

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       NCLS:  424/464.000; 424/476.000; 424/477.000; 514/762.000; 514/691.000
CPC    CPCI   A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
              A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
              A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
              A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
              [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
       CPCI-2 A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
              A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
              A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
              A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
              [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
IPC    IPCI   A61K0038-02 [I]
       IPCI-2 A61K0038-17 [I]; A61K0031-01 [I]; A61K0009-20 [I]; A61K0009-38
              [I]; A61K0009-42 [I]
       IPCR   A61K0038-17 [I]; A61K0009-20 [I]; A61K0009-38 [I]; A61K0009-42
              [I]; A61K0031-01 [I]

L4   ANSWER 22 OF 30  USPATFULL on STN
AN     2013:254433  USPATFULL
TI     REDUCED FLUORIDE CRUSTACEAN OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Ervik, Jon Reidar, Aalesund, NORWAY
       Remoy, Stig Rune, Fosnavaag, NORWAY
PA     Olympic Seafood AS, Fosnavaag, NORWAY (non-U.S. corporation)
PI     US 20130225794      A1  20130829
AI     US 2013-13856642    A1  20130404 (13)
RLI    Division of Ser. No. US 2012-13342664, filed on 3 Jan 2012, PENDING
       Continuation of Ser. No. US 2011-13063488, filed on 24 May 2011, PENDING
       A 371 of International Ser. No. WO 2009-NO322, filed on 14 Sep 2009
DT     Utility
FS     APPLICATION
LN.CNT 1430
INCL   INCLM: 530/359.000
       INCLS: 554/078.000; 530/350.000
NCL    NCLM:  530/359.000
       NCLS:  530/350.000; 554/078.000
CPC    CPCI   C11B0003-006 [I]; C07K0019-00 [I]; C07K0014-43509 [I]
IPC    IPCI   C11B0003-00 [I]; C07K0014-435 [I]; C07K0019-00 [I]
       IPCR   C11B0003-00 [I]; C07K0014-435 [I]; C07K0019-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 23 OF 30  USPATFULL on STN
AN     2013:186762  USPATFULL
TI     PHOSPHOLIPID AND PROTEIN TABLETS
IN     Tilseth, Snorre, Bergen, NORWAY
       Hoem, Nils, Oslo, NORWAY
PA     AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 20130165393      A1  20130627
AI     US 2013-13748013    A1  20130123 (13)
RLI    Continuation of Ser. No. US 2010-711822, filed on 24 Feb 2010, Pat. No.
       US 8372812
PRAI   US 2009-61155758        20090226 (61)
DT     Utility
FS     APPLICATION
LN.CNT 3145
INCL   INCLM: 514/021.920
       INCLS: 264/113.000
NCL    NCLM:  514/021.920
       NCLS:  264/113.000
CPC    CPCI   A61K0031-122 [I]; A61K0038-1767 [I]; A61K0031-122 [I],
              A61K2300-00 [I]; A61K0035-612 [I], A61K2300-00 [I]; A61K0031-685
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              [I], A61K2300-00 [I]
IPC    IPCI   A61K0031-122 [I]; A61K0038-17 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 24 OF 30  USPATFULL on STN
AN     2012:168278  USPATFULL
TI     Method For Processing Crustaceans And Products Thereof
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Ervik, Jon Reidar, Aalesund, NORWAY
       Remoy, Stig Rune, Fosnavag, NORWAY
PA     Emerald Fisheries (non-U.S. corporation)
PI     US 20120149867      A1  20120614
       US 8557297          B2  20131015
AI     US 2012-13342664    A1  20120103 (13)
RLI    Continuation of Ser. No. US 2011-13063488, filed on 24 May 2011, PENDING
       A 371 of International Ser. No. WO 2009-NO322, filed on 14 Sep 2009
DT     Utility
FS     APPLICATION
LN.CNT 1449
INCL   INCLM: 530/300.000
       INCLS: 554/008.000; 554/021.000; 554/084.000; 530/359.000
NCL    NCLM:  424/538.000; 530/300.000
       NCLS:  426/417.000; 426/665.000; 435/068.100; 435/325.000; 435/381.000;
              530/300.000; 530/359.000; 554/008.000; 554/021.000; 554/084.000
CPC    CPCI   C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02
              [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I];
              A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I];
              A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I];
              C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104
              [I]
       CPCI-2 C11B0003-006 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23D0009-02
              [I]; A23J0001-04 [I]; A23J0003-34 [I]; A23L0001-0152 [I];
              A23L0001-0153 [I]; A23L0001-3006 [I]; A23L0001-3053 [I];
              A23L0001-3252 [I]; A23L0001-33 [I]; C07K0014-43509 [I];
              C07K0019-00 [I]; C11B0001-025 [I]; C11B0001-10 [I]; C11B0001-104
              [I]
IPC    IPCI   C11B0001-10 [I]; C07F0009-02 [I]; C07K0014-00 [I]; C07K0002-00
              [I]
       IPCI-2 A61K0035-64 [I]
       IPCR   A61K0035-64 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 25 OF 30  USPATFULL on STN
AN     2011:251469  USPATFULL
TI     SOLVENT-FREE PROCESS FOR OBTAINING PHOSPHOLIPIDS AND NEUTRAL ENRICHED
       KRILL OILS
IN     Sclabos Katevas, Dimitri, Santiage, CHILE
       Toro Guerra, Raul R., Santiage, CHILE
       Chiong Lay, Mario M., Santiage, CHILE
PA     THAROS LTD., Santiago, CHILE (non-U.S. corporation)
       LONZA LTD., Basel, SWITZERLAND (non-U.S. corporation)
PI     US 20110224450      A1  20110915
       US 8772516          B2  20140708
AI     US 2011-13096644    A1  20110428 (13)
RLI    Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009,
       PENDING
DT     Utility
FS     APPLICATION
LN.CNT 2021
INCL   INCLM: 554/023.000
       INCLS: 554/008.000; 554/078.000
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NCL NCLM: 554/023.000
NCLS: 554/008.000; 554/078.000
CPC CPCI C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553 [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I]; A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10; A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007 [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I]; Y02W0030-74
CPCI-2 C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I]; A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553 [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I]; A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10; A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007 [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I]; Y02W0030-74
IPC IPCI C11B0001-00 [I]; C07F0009-10 [I]
IPCI-2 C11B0001-00 [I]
IPCR C11B0001-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 26 OF 30 USPATFULL on STN
AN 2011:117391 USPATFULL
TI METHODS OF USING KRILL OIL TO TREAT RISK FACTORS FOR CARDIOVASCULAR, METABOLIC, AND INFLAMMATORY DISORDERS
IN BRUHEIM, Inge, Volda, NORWAY
Tilseth, Snorre, Bergen, NORWAY
Cohn, Jeffery, Sydney, AUSTRALIA
Griinari, Mikko, Espoo, FINLAND
Mancinelli, Daniele, Orsta, NORWAY
Hoem, Nils, Oslo, NORWAY
Vik, Hogne, Eiksmarka, NORWAY
Banni, Sebastiano, Calgliari, ITALY
PA Aker BioMarine A.S.A., Oslo, NORWAY (non-U.S. corporation)
PI US 20110104297 A1 20110505
US 8697138 B2 20140415
AI US 2010-790575 A1 20100528 (12)
RLI Continuation-in-part of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
US 2009-61181743 20090528 (61)
US 2007-60920483 20070328 (60)
DT Utility
FS APPLICATION
LN.CNT 2547
INCL INCLM: 424/522.000
INCLS: 426/002.000
NCL NCLM: 424/538.000; 424/522.000
NCLS: 424/283.100; 426/002.000
CPC CPCI A61K0035-612 [I]
CPCI-2 A61K0035-612 [I]
IPC IPCI A61K0035-56 [I]; A61P0009-10 [I]; A61P0003-04 [I]; A61P0003-00 [I]
IPCI-2 A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
IPCR A61K0035-64 [I]; A61K0045-00 [I]; A61K0047-44 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 27 OF 30 USPATFULL on STN
AN 2010:256169 USPATFULL
TI PHOSPHOLIPID AND PROTEIN TABLETS

IN Tilseth, Snorre, Bergen, NORWAY
    Hoem, Nils, Oslo, NORWAY
PA AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 20100227792 A1 20100909
    US 8372812 B2 20130212
AI US 2010-711822 A1 20100224 (12)
PRAI US 2009-61155758 20090226 (61)
DT Utility
FS APPLICATION
LN.CNT 3112
INCL INCLM: 514 2
NCL NCLM: 514/021.920; 514/005.500
    NCLS: 424/464.000; 424/476.000; 424/477.000; 514/762.000; 514/691.000
CPC CPCI A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
           A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
           A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
           A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
           [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
    CPCI-2 A23L0001-0026 [I]; A23L0001-3006 [I]; A23L0001-305 [I];
           A23L0001-33 [I]; A61K0009-2009 [I]; A61K0009-2054 [I];
           A61K0009-2866 [I]; A61K0031-122 [I]; A61K0031-685 [I];
           A61K0035-612 [I]; A61K0031-122 [I], A61K2300-00 [I]; A61K0035-612
           [I], A61K2300-00 [I]; A61K0031-685 [I], A61K2300-00 [I]
IPC IPCI A61K0038-02 [I]
    IPCI-2 A61K0038-17 [I]; A61K0031-01 [I]; A61K0009-20 [I]; A61K0009-38
           [I]; A61K0009-42 [I]
    IPCR A61K0038-17 [I]; A61K0009-20 [I]; A61K0009-38 [I]; A61K0009-42
           [I]; A61K0031-01 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 28 OF 30 USPATFULL on STN
AN 2010:255355 USPATFULL
TI LOW VISCOSITY PHOSPHOLIPID COMPOSITIONS
IN Tilseth, Snorre, Bergen, NORWAY
PA AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 20100226977 A1 20100909
AI US 2010-711553 A1 20100224 (12)
RLI Continuation-in-part of Ser. No. US 2008-201325, filed on 29 Aug 2008,
    PENDING
PRAI US 2009-61155767 20090226 (61)
    US 2007-60968765 20070829 (60)
DT Utility
FS APPLICATION
LN.CNT 2394
INCL INCLM: 424/456.000
    INCLS: 426/601.000; 426/417.000; 514/078.000
NCL NCLM: 424/456.000
    NCLS: 426/417.000; 426/601.000; 514/078.000
CPC CPCI A23D0009-013 [I]; A23D0007-011 [I]; A23J0007-00 [I]; A23K0001-103
           [I]; A23K0001-1606 [I]; A23K0001-164 [I]; A23K0001-188 [I];
           A23L0001-30 [I]; A23L0001-3006 [I]; A23L0001-3008 [I];
           A23L0001-305 [I]; A23L0001-326 [I]; A61K0035-612 [I];
           C07F0009-103 [I]; C11B0001-06 [I]
IPC IPCI A61K0031-685 [I]; A23D0009-00 [I]; A23D0009-02 [I]; A61K0009-48
           [I]; A61P0009-00 [I]; A61P0019-00 [I]; A61P0029-00 [I]
    IPCR A61K0031-685 [I]; A23D0009-00 [I]; A23D0009-02 [I]; A61K0009-48
           [I]; A61P0009-00 [I]; A61P0019-00 [I]; A61P0029-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 29 OF 30 USPATFULL on STN
AN 2009:67318 USPATFULL
TI METHOD FOR MAKING KRILL MEAL

IN Tilseth, Snorre, Bergen, NORWAY
    Hostmark, Oistein, Loddefjord, NORWAY
PA Aker BioMarine ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 20090061067 A1 20090305
AI US 2008-201325 A1 20080829 (12)
PRAI US 2007-60968765 20070829 (60)
DT Utility
FS APPLICATION
LN.CNT 2307
INCL INCLM: 426/602.000
    INCLS: 426/417.000; 210/149.000; 426/480.000; 426/609.000; 426/648.000; 426/608.000; 366/145.000; 366/147.000
NCL NCLM: 426/602.000
    NCLS: 210/149.000; 366/145.000; 366/147.000; 426/417.000; 426/480.000; 426/608.000; 426/609.000; 426/648.000
CPC CPCI A61K0031-685 [I]; A23D0009-013 [I]; A23K0001-103 [I]; A23K0001-1606 [I]; A23K0001-164 [I]; A23K0001-188 [I]; A23L0001-3006 [I]; A23L0001-305 [I]; A23L0001-33 [I]; A61K0031-122 [I]; A61K0031-133 [I]; A61K0031-198 [I]; A61K0031-202 [I]; A61K0031-575 [I]; A61K0035-612 [I]; C07F0009-103 [I]; C11B0001-06 [I]
IPC IPCI A23D0007-005 [I]; A23D0007-02 [I]; A23D0007-04 [I]; A23L0001-29 [I]; B01F0015-06 [I]; A23L0001-33 [I]; A23L0001-326 [I]; B01D0021-30 [I]
    IPCR A23D0007-005 [I]; A23D0007-02 [I]; A23D0007-04 [I]; A23L0001-29 [I]; A23L0001-326 [I]; A23L0001-33 [I]; B01D0021-30 [I]; B01F0015-06 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 30 OF 30 USPATFULL on STN
AN 2008:312554 USPATFULL
TI BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN Bruheim, Inge, Volda, NORWAY
    Griinari, Mikko, Espoo, FINLAND
    Tilseth, Snorre, Bergen, NORWAY
    Banni, Sebastiano, Cagliari, ITALY
    Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
    Mancinelli, Daniele, Orsta, NORWAY
PA AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI US 20080274203 A1 20081106
    US 9034388 B2 20150519
AI US 2008-57775 A1 20080328 (12)
PRAI US 2007-60920483 20070328 (60)
    US 2007-60975058 20070925 (60)
    US 2007-60983446 20071029 (60)
    US 2008-61024072 20080128 (61)
DT Utility
FS APPLICATION
LN.CNT 2199
INCL INCLM: 424/522.000
    INCLS: 514/121.000; 514/078.000; 514/114.000; 426/601.000
NCL NCLM: 424/520.000
CPC CPCI A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
    CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00

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IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0035-56 [I]; A23D0009-00 [I]; A61K0031-66 [I]; A61K0031-661
              [I]; A61K0031-685 [I]; A61P0003-02 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


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     (FILE 'HOME' ENTERED AT 10:17:58 ON 29 JUN 2015)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BIOSIS,
     BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CROPB,
     CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGU, EMBAL, EMBASE,
     ESBIOBASE, FOMAD, FROSTI, FSTA, GENBANK, IFIALL, ...' ENTERED AT 10:18:10
     ON 29 JUN 2015
                SEA DENATUR? AND KRILL AND SUPERCRITICAL AND EXTRACTION AND OIL
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               0*  FILE ADISNEWS
               0*  FILE BIOTECHABS
               0*  FILE BIOTECHDS
               0*  FILE BIOTECHNO
               0*  FILE CEABA-VTB
               0*  FILE CIN
               0*  FILE FOMAD
               0*  FILE FROSTI
               4   FILE IFIALL
               0*  FILE KOSMET
               0*  FILE NTIS
               0*  FILE PASCAL
              27   FILE USPATFULL
               9   FILE USPAT2
L1              QUE DENATUR? AND KRILL AND SUPERCRITICAL AND EXTRACTION AND OIL
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     FILE 'USPAT2, USPATFULL, IFIALL' ENTERED AT 10:19:11 ON 29 JUN 2015
L2           40 S L1
L3           30 S L2 AND PHOSPHOLIPID
L4           30 DUP REM L3 (0 DUPLICATES REMOVED)
L5           29 S L4 AND PHOSPHATIDYLCHOLINE

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      95.91      97.86

STN INTERNATIONAL LOGOFF AT 10:22:21 ON 29 JUN 2015



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                  Patent Index
 NEWS  4  APR 15  USPATFULL/USPAT2 Now Include Corporate Patent Applicant
                  Information
 NEWS  5  MAY 22  Country Coverage in Derwent World Patent Index Extended to
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 NEWS  6  MAY 28  Partner with CAS to help shape the future of CAS products!
 NEWS  7  JUL 2   Major Update to GBFULL Improves Quality of Full Text
 NEWS  8  JUL 7   100 Millionth Small Molecule Added to CAS REGISTRY
 NEWS  9  SEP 15  New Version of Emtree Introduces over 800 New Terms to
                  Embase on Classic STN and New STN
 NEWS 10  NOV 25  Change to PI field in CAplus records
 NEWS 11  DEC 17  Latest Release of New STN Enhances Search Functionality,
                  Workflow, and Efficiency
 NEWS 12  JAN 11  PatentPak Now available to STN Express 8.6 and STN on the
                  Web customers
 NEWS 13  JAN 11  CAplus Family of Files Updated with New Data to Support
                  PatentPak in STN
 NEWS 14  JAN 14  The Derwent World Patents Index (DWPI): Latest Manual Code
                  Revision is now live
 NEWS 15  FEB 9   Emtree in Classic STN Updated for 2016 with Additional
                  Thesaurus Fields and Expanded Terminology
 NEWS 16  MAR 23  CHEMLIST Content Expanded with the Addition of Information
                  from Vermont
 NEWS 17  APR 26  Data Quality Improved in CNFULL and FRFULL

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              AND CURRENT DISCOVER FILE IS DATED 21 MAR 2016.

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=> index bioscience
FILE 'WPIDS' ACCESS NOT AUTHORIZED
FILE 'WPINDEX' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
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FULL ESTIMATED COST                                    0.54       0.54

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHABS,
       BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CROPB, CROPU, DDFB,
       DDFU, DGENE, DISSABS, DRUGB, DRUGU, EMBAL, EMBASE, ESBIOBASE, FOMAD,
       FROSTI, FSTA, GENBANK, IFIALL, ...' ENTERED AT 20:18:30 ON 29 APR 2016

46 FILES IN THE FILE LIST IN STNINDEX

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=> s Euphausia and superba and denatur?(p)lipases and krill(p)oil
          0*  FILE ADISNEWS
          0*  FILE BIOTECHABS
          0*  FILE BIOTECHDS
          0*  FILE BIOTECHNO
          0*  FILE CEABA-VTB
          0*  FILE CIN
          0*  FILE FOMAD
          0*  FILE FROSTI
          3   FILE IFIALL
          0*  FILE KOSMET
          0*  FILE NTIS
  33 FILES SEARCHED...
          0*  FILE PASCAL
  41 FILES SEARCHED...
          7   FILE USPATFULL
          6   FILE USPAT2

   3 FILES HAVE ONE OR MORE ANSWERS,   46 FILES SEARCHED IN STNINDEX

L1   QUE EUPHAUSIA AND SUPERBA AND DENATUR?(P) LIPASES AND KRILL(P) OIL

=> file ifiall uspatfull uspat2
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                                                     ENTRY    SESSION
FULL ESTIMATED COST                                   1.94       2.48

FILE 'IFIALL' ENTERED AT 20:19:38 ON 29 APR 2016
COPYRIGHT (C) 2016 IFI CLAIMS(R) Patent Services (IFI)

FILE 'USPATFULL' ENTERED AT 20:19:38 ON 29 APR 2016
CA INDEXING COPYRIGHT (C) 2016 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPAT2' ENTERED AT 20:19:38 ON 29 APR 2016
CA INDEXING COPYRIGHT (C) 2016 AMERICAN CHEMICAL SOCIETY (ACS)

=> s L1
L2          16 L1

=> dup rem L2
PROCESSING COMPLETED FOR L2
L3           13 DUP REM L2 (3 DUPLICATES REMOVED)

=> d L3 1-13

L3   ANSWER 1 OF 13  USPAT2 on STN
AN     2014:11777  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
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       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9320765          B2  20160426
AI     US 2013-14020155        20130906 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, Pat. No.
       US 9034388
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2500
NCL    NCLM:  424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23
              [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685
              [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0009-4858 [I];
              A61K0031-122 [I]; A61K0031-20 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-235 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00;
              A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00;
              A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-202 [I]; A61K0031-122 [I]
       IPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; C11B0003-00
              [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; A61K0031-202 [I]; A61K0031-20 [I]; A61K0031-235
              [I]
       IPCR   A61K0035-56 [I]; A61K0031-122 [I]; A61K0031-202 [I]

L3   ANSWER 2 OF 13  IFIALL  COPYRIGHT 2016 IFI on STN DUPLICATE 1
AN    14054223  IFIALL
TI    BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN    Bruheim Inge (NO); Tilseth Snorre (NO); Mancinelli Daniele (NO)
PA    Unassigned or assigned to individual (68000)
PPA   Aker Biomarine As; Aker Biomarine Antarctic As (Probable)
PI    US 20150004223  A1  20150101
AI    US 2014-490176      20140918  (14)
RLI   US 2008-57775       20080328 CONTINUATION           PENDING
PRAI  US 2007-920483P      20070328 (Provisional)
      US 2007-975058P      20070925 (Provisional)
      US 2007-983446P      20071029 (Provisional)
      US 2008-24072P       20080128 (Provisional)
FI    US 20150004223      20150101
DT    Utility; Patent Application - First Publication
FS    CHEMICAL
      APPLICATION
ED    Entered STN: 5 Jan 2015
      Last Updated on STN: 16 Feb 2015
CLMN  30

L3   ANSWER 3 OF 13  IFIALL  COPYRIGHT 2016 IFI on STN DUPLICATE 2
AN    06651654  IFIALL
TI    BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN    Bruheim Inge (NO); Tilseth Snorre (NO); Mancinelli Daniele (NO)
PA    Aker Biomarine Antarctic A S NO
PI    US 9028877      B2  20150512
      US 20150004223  A1  20150101
AI    US 2014-490176      20140918  (14)
RLI   US 2008-57775       20080328 CONTINUATION           9034388
PRAI  US 2007-920483P      20070328 (Provisional)
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      US 2007-975058P      20070925 (Provisional)
      US 2007-983446P      20071029 (Provisional)
      US 2008-24072P       20080128 (Provisional)
FI    US 9028877          20150512
      US 9034388
DT    Utility; Reassigned; Granted Patent - Utility, with Pre-Grant Publication
FS    CHEMICAL
      GRANTED
ED    Entered STN: 13 May 2015
      Last Updated on STN: 18 Jun 2015
CLMN  19

L3   ANSWER 4 OF 13  USPATFULL on STN
AN     2015:186362  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
PI     US 20150164963      A1  20150618
       US 9072752          B2  20150707
AI     US 2015-14620784    A1  20150212 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1937
NCL    NCLM:  001/001.000; 424/538.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48
              [I]; A61K0031-235 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683
              [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122
              [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48
              [I]; A61K0031-235 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683
              [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122
              [I], A61K2300-00
IPC    IPCI   A61K0035-612 [I]; A61K0031-235 [I]; A61K0009-48 [I]; A61K0031-122
              [I]; A61K0031-20 [I]
       IPCI-2 A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48
              [I]; A61K0031-235 [I]
       IPCR   A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-20
              [I]; A61K0031-235 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 5 OF 13  USPATFULL on STN
AN     2015:178202  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY
PI     US 20150157669      A1  20150611
       US 9119864          B2  20150901
AI     US 2015-14620779    A1  20150212 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
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DT     Utility
FS     APPLICATION
LN.CNT 1930
NCL    NCLM:  001/001.000; 424/451.000
       NCLS:  424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-685 [I]; A61K0031-23
              [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00;
              A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0009-4858 [I];
              A61K0031-122 [I]; A61K0031-20 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-235 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00;
              A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00;
              A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-612 [I]; A61K0031-685 [I]; A61K0031-23 [I]; A61K0031-122
              [I]
       IPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-23
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I];
              C11B0003-00 [I]; A61K0031-202 [I]; A61K0031-20 [I]; A61K0031-235
              [I]
       IPCR   A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-20
              [I]; A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-235 [I];
              A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-00
              [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 6 OF 13  USPATFULL on STN
AN     2015:4199  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20150004227      A1  20150101
       US 9078905          B2  20150714
AI     US 2014-14490221    A1  20140918 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1955
INCL   INCLM: 424/456.000
       INCLS: 424/522.000; 424/451.000
NCL    NCLM:  001/001.000; 424/456.000
       NCLS:  424/451.000; 424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685
              [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0035-612
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I];
              C11B0003-00 [I]; A61K0031-202 [I]
       IPCR   A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0035-612 [I];
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              A61K0045-06 [I]; C11B0003-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 7 OF 13  USPAT2 on STN
AN     2015:178202  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9119864          B2  20150901
AI     US 2015-14620779        20150212 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2400
NCL    NCLM:  001/001.000; 424/451.000
       NCLS:  424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-685 [I]; A61K0031-23
              [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00;
              A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0009-4858 [I];
              A61K0031-122 [I]; A61K0031-20 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-235 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00;
              A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00;
              A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-612 [I]; A61K0031-685 [I]; A61K0031-23 [I]; A61K0031-122
              [I]
       IPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-23
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I];
              C11B0003-00 [I]; A61K0031-202 [I]; A61K0031-20 [I]; A61K0031-235
              [I]
       IPCR   A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-20
              [I]; A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-235 [I];
              A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-00
              [I]

L3   ANSWER 8 OF 13  USPAT2 on STN
AN     2015:4199  USPAT2
TI     Bioeffective krill oil compositions
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI     US 9078905          B2  20150714
AI     US 2014-14490221        20140918 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2423
NCL    NCLM:  001/001.000; 424/456.000
       NCLS:  424/451.000; 424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
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A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-56 [I]
IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0035-612 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I]; C11B0003-00 [I]; A61K0031-202 [I]
IPCR A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0035-612 [I]; A61K0045-06 [I]; C11B0003-00 [I]

L3 ANSWER 9 OF 13 USPAT2 on STN
AN 2015:186362 USPAT2
TI Bioeffective krill oil compositions
IN Bruheim, Inge, Volde, NORWAY
Tilseth, Snorre, Bergen, NORWAY
Mancinelli, Daniele, Orsta, NORWAY
PA AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI US 9072752 B2 20150707
AI US 2015-14620784 20150212 (14)
RLI Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)
US 2007-60983446 20071029 (60)
US 2008-61024072 20080128 (61)
DT Utility
FS GRANTED
LN.CNT 2411
NCL NCLM: 001/001.000; 424/538.000
CPC CPCI A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48 [I]; A61K0031-235 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
CPCI-2 A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48 [I]; A61K0031-235 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC IPCI A61K0035-612 [I]; A61K0031-235 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-20 [I]
IPCI-2 A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0009-48 [I]; A61K0031-235 [I]
IPCR A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-20 [I]; A61K0031-235 [I]

L3 ANSWER 10 OF 13 USPAT2 on STN
AN 2008:312554 USPAT2
TI Bioeffective krill oil compositions
IN Bruheim, Inge, Volda, NORWAY
Griinari, Mikko, Espoo, FINLAND
Tilseth, Snorre, Bergen, NORWAY
Banni, Sebastiano, Cagliari, ITALY
Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
Mancinelli, Daniele, Orsta, NORWAY
PA AKER BIOMARINE ANTARTIC AS, Stamsund, NORWAY (non-U.S. corporation)
PI US 9034388 B2 20150519
AI US 2008-57775 20080328 (12)
PRAI US 2007-60920483 20070328 (60)
US 2007-60975058 20070925 (60)

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       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     GRANTED
LN.CNT 2386
INCL   INCLM: 424/520.000
NCL    NCLM:  424/520.000; 424/522.000
       NCLS:  426/601.000; 514/078.000; 514/114.000; 514/121.000
CPC    CPCI   A61K0035-612 [I]; A61K0009-48 [I]; A61K0009-4858 [I];
              A61K0031-122 [I]; A61K0031-20 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-235 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00;
              A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00;
              A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0009-48 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06 [I];
              C11B0003-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 11 OF 13  USPATFULL on STN
AN     2014:11777  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20140010888      A1  20140109
       US 9320765          B2  20160426
AI     US 2013-14020155    A1  20130906 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1898
INCL   INCLM: 424/522.000
NCL    NCLM:  424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-122 [I]; A61K0031-202 [I]; A61K0031-23
              [I], A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685
              [I], A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0009-4858 [I];
              A61K0031-122 [I]; A61K0031-20 [I]; A61K0031-202 [I]; A61K0031-23
              [I]; A61K0031-235 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00;
              A61K0031-683 [I], A61K2300-00; A61K0031-685 [I], A61K2300-00;
              A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-202 [I]; A61K0031-122 [I]
       IPCI-2 A61K0035-612 [I]; A61K0009-48 [I]; A61K0031-122 [I]; C11B0003-00
              [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I];
              A61K0045-06 [I]; A61K0031-202 [I]; A61K0031-20 [I]; A61K0031-235
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       IPCR   A61K0035-56 [I]; A61K0031-122 [I]; A61K0031-202 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 12 OF 13  USPATFULL on STN
AN     2014:5400  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20140005421      A1  20140102
AI     US 2013-14020162    A1  20130906 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1908
INCL   INCLM: 554/008.000
NCL    NCLM:  554/008.000
CPC    CPCI   C11B0003-006 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
IPC    IPCI   C11B0003-00 [I]
       IPCR   C11B0003-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 13 OF 13  IFIALL  COPYRIGHT 2016 IFI on STN DUPLICATE 3
AN    11934106  IFIALL
TI    BIOEFFECTIVE KRILL OIL COMPOSITIONS; Having high amounts of
      phospholipids, astaxanthin esters and/or omega-3 contents;
      antiinflammation, antioxidant effects, improving insulin resistance and
      blood lipid profile
IN    Banni Sebastiano (IT); Bruheim Inge (NO); Cohn Jeffrey Stuart (AU);
      Griinari Mikko (FI); Mancinelli Daniele (NO); Tilseth Snorre (NO)
PA    Aker BioMarine ASA NO (79725)
PI    US 20080274203  A1  20081106
AI    US 2008-57775       20080328  (12)
PRAI  US 2007-920483P      20070328 (Provisional)
      US 2007-975058P      20070925 (Provisional)
      US 2007-983446P      20071029 (Provisional)
FI    US 20080274203      20081106
DT    Utility; Patent Application - First Publication
FS    CHEMICAL
      APPLICATION
ED    Entered STN: 7 Nov 2008
      Last Updated on STN: Jan 2011
CLMN  90


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     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BIOSIS,
     BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CROPB,
     CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGU, EMBAL, EMBASE,
     ESBIOBASE, FOMAD, FROSTI, FSTA, GENBANK, IFIALL, ...' ENTERED AT 20:18:30
     ON 29 APR 2016
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               0*  FILE BIOTECHDS
               0*  FILE BIOTECHNO
               0*  FILE CEABA-VTB
               0*  FILE CIN
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               0*  FILE FROSTI
               3   FILE IFIALL
               0*  FILE KOSMET
               0*  FILE NTIS
               0*  FILE PASCAL
               7   FILE USPATFULL
               6   FILE USPAT2
L1              QUE EUPHAUSIA AND SUPERBA AND DENATUR?(P) LIPASES AND KRILL(P)
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L3            13 DUP REM L2 (3 DUPLICATES REMOVED)

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LOGOFF? (Y)/N/HOLD:y
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                     ENTRY    SESSION
FULL ESTIMATED COST                                  41.66      44.14

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PTO/SB/08a (03-15)
Approved for use through 07/31/2016. 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	Inge Bruheim
Art Unit	1651
Examiner Name	WARE
Attorney Docket Number	AKBM-14409/US-6/CON

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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.	T[5]

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

	1	Third Party Submission, AU Patent Application No. 2014256345, filed October 12, 2015
	2	Third Party Submission, AU Patent Application No. 2014256345, filed December 22, 2015

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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.

[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	Inge Bruheim
	Art Unit	1651
	Examiner Name	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)	2016-03-30
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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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.

Electronic Patent Application Fee Transmittal

Application Number:	14020162
Filing Date:	06-Sep-2013
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

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	25342432
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:	30-MAR-2016
Filing Date:	06-SEP-2013
Time Stamp:	15:00:38
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	1532
Deposit Account	504302
Authorized User	JONES, J. MITCHELL

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 CFR 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	14409US6CON_IDS_Letter_3-30-2016.pdf	82009 77e80005eeae93e3f23056be9dba266f3d1751f3	no	1
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	14409US6CON_IDS_3-30-2016.pdf	1035191 55f18c5c4b37cef4813ce59b5f74c5e709f6f919	no	4
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
3	Other Reference-Patent/App/Search documents	20160106_NotificationofFurtherMaterialFiledunderSection27_12-22-2015.pdf	7720718 f7df0e502ea57a1e2f8fe9de14933cd1b998d9b7	no	47
Warnings:					
Information:					
4	Other Reference-Patent/App/Search documents	20151016__USPTO_APO_NotificationofMa.pdf	25328409 45c0d6874a23a1bde817b1bbbe5f5357b0ba7f39	no	148
Warnings:					
Information:					
5	Other Reference-Patent/App/Search documents	20151016_USPTO_APO_NotificationofMa.pdf	18152653 d661e514d828fc1faf438fab1757d859b5dd09d5	no	108
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	30598 8dac326a6e8eb6e5399ec4a6f86a2623bdff1edd	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	52349578		

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:	Inge Bruheim, et al	Confirmation:	4914
Serial No.:	14/020,162	Group No.:	1651
Filed:	06-Sep-2013	Examiner:	Ware
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: March 30, 2016

/J. Mitchell Jones/
J. Mitchell Jones
Registration No. 44,174
CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, WI 53562
608.662.1277

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Inge Bruheim et al.	Art Unit:	1651
Serial No.:	14/020,162	Examiner:	Ware
Filed:	06-Sep-2013	Conf. No.:	4914
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

STATEMENT OF THE SUBSTANCE OF THE INTERVIEW OF MARCH 16, 2016

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

Examiner Ware:

Applicant's attorney confirms receipt of the Interview Summary mailed March 24, 2016. A telephonic interview was conducted on March 16, 2016 between Examiner Deborah K. Ware and Applicant's representative, J. Mitchell Jones. Applicant wishes to thank the Examiner for the interview and agrees with the Examiner's summary of the topics discussed.

Dated: March 28, 2016

/J. Mitchell Jones/

J. Mitchell Jones
Reg. No. 44,174
CASIMIR JONES, SC
2275 Deming Way, Suite 310
Middleton, Wisconsin 53562
Tel: 608-662-1277

Electronic Acknowledgement Receipt

EFS ID:	25314938
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:	28-MAR-2016
Filing Date:	06-SEP-2013
Time Stamp:	16:37:30
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	Applicant summary of interview with examiner	14409US6CON_InterviewSummary.pdf	85127 5d6a5e6ab3b274ddad1a7596bfcc7c7c2ad7060e	no	1

Warnings:	
Information:	

Total Files Size (in bytes):	85127

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 NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

72960 7590 03/24/2016

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

EXAMINER
WARE, DEBORAH K

ART UNIT	PAPER NUMBER
1651	

NOTIFICATION DATE	DELIVERY MODE
03/24/2016	ELECTRONIC

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

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

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

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	14/020,162	BRUHEIM ET AL.	
	Examiner	Art Unit	
	DEBBIE K. WARE	1651	

All participants (applicant, applicant's representative, PTO personnel):

(1) *DEBBIE K. WARE*. (3)_____.

(2) *J. MITCHELL JONES*. (4)_____.

Date of Interview: *16 March 2016*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *all pending claims as of 1/8/2016*.

Identification of prior art discussed: *art of record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Applicants discussed the amendments most recently filed and how the cited prior art does not teach or motivate one of ordinary skill in the art to increase ether phospholipid content of kirll oil. Examiner confirmed that Applicants have overcome the outstanding obviousness double patenting issues because the terminal disclaimer has been approved. Furthermore, Examiner Ware will reconsider the claims on the merits as presented in the amendment of 1/8/2016. No agreement was reached at this time.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/DEBBIE K. WARE/ Primary Examiner, Art Unit 1651	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bruheim et al.	Art Unit: 1651
Serial No.:	14/020,162	Examiner: Ware
Filed:	09/06/2013	Confirmation: 4914
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

RESPONSE TO OFFICE ACTION MAILED JULY 8, 2015

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

Examiner Ware:

This communication is responsive to the Office Action mailed July 8, 2015. A three-month extension of time is submitted with this Response. The Commissioner is hereby authorized to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No. **AKBM-14409/US-6/CON**. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIM AMENDMENTS:

1. (Currently amended) A method of production of polar krill oil from *Euphausia superba* ~~krill~~ comprising:

a) treating ~~denaturing~~ the *Euphausia superba* ~~krill~~ to denature lipases and phospholipases to provide a denatured krill product; and

b) ~~extracting~~ contacting the denatured krill product with ~~supercritical fluid extraction~~ a polar solvent to extract ~~provide~~ a polar krill oil comprising phospholipids, wherein said polar krill oil comprising phospholipids is further characterized in comprising greater than about 3% ether phospholipids w/w of said polar krill oil; from about 27% to 50% non-ether phospholipids w/w of said polar krill oil so that the amount of total phospholipids in the composition is from about 30% to 60% w/w of said polar krill oil; from about 20% to 50% triglycerides w/w of said polar krill oil, and astaxanthin esters in amount of greater than about 100 mg/kg of said polar krill oil;

~~c) formulating said polar krill oil for oral consumption~~.

2. Cancelled.

3. (Currently amended) The method of claim 1, wherein said polar krill oil comprises greater than about 40% phosphatidylcholine w/w of said polar krill oil.

4. (Currently amended) The method of claim 1, wherein said polar krill oil comprises greater than about 45% phosphatidylcholine w/w of said polar krill oil.

5. Cancelled.

6. (Original) The method of claim 1, wherein said polar krill oil comprises at least 36% w/w omega-3 fatty acids.

7. Cancelled.

8. (Currently amended) The method of claim 1, wherein said method further comprises formulating ~~further comprising formulating~~ said polar krill oil for oral consumption by a human.

9. (Currently amended) The method of claim 8, wherein said formulating ~~further~~ comprises encapsulating ~~formulating~~ said polar krill ~~oil in a capsule~~.

10-11. Cancelled.

12. (New) The method of claim 1, wherein said polar krill oil contains greater than about 200 mg/kg astaxanthin esters.

13. (New) The method of claim 1, wherein said polar krill oil contains greater than about 300 mg/kg astaxanthin esters.

14. (New) The method of claim 1, wherein said polar krill oil contains greater than about 400 mg/kg astaxanthin esters.

15. (New) The method of claim 1, wherein said polar krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition.

16. (New) The method of claim 1, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

17. (New) The method of claim 9, wherein said polar krill oil is encapsulated in a soft gel capsule.

18. (New) The method of claim 1, further comprising combining said polar krill oil with a plant phytonutrient.

19. (New) The method of claim 1, wherein said polar krill oil comprises greater than about 4% w/w ether phospholipids.

20. (New) The method of claim 19, wherein said polar krill oil comprises greater than about 200 mg/kg astaxanthin esters.

21. (New) The method of claim 19, wherein said polar krill oil comprises greater than about 300 mg/kg astaxanthin esters.

22. (New) The method of claim 20, wherein said polar krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said polar krill oil.

23. (New) The method of claim 22, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

24. (New) The method of claim 19, wherein said krill oil comprises greater than about 400 mg/kg astaxanthin esters.

25. (New) The method of claim 24, wherein said krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition.

26. (New) The method of claim 25, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

27. (New) The method of claim 21, further comprising combining said polar krill oil with a plant phytonutrient.

28. (New) The method of claim 1, wherein said polar krill oil comprises greater than about 5% w/w ether phospholipids.

29. (New) The method of claim 28, wherein said polar krill oil comprises greater than about 200 mg/kg astaxanthin esters.

30. (New) The method of claim 28, wherein said polar krill oil comprises greater than about 300 mg/kg astaxanthin esters.

31. (New) The method of claim 29, wherein said polar krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition.

32. (New) The method of claim 31, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

33. (New) The method of claim 25, wherein said polar krill oil comprises greater than about 400 mg/kg astaxanthin esters.

34. (New) The method of claim 29, further comprising combining said polar krill oil with a plant phytonutrient.

35. (New) A method of production of polar krill oil from *Euphausia superba* comprising:

a) treating the *Euphausia superba* to denature lipases and phospholipases to provide a denatured krill product;

b) contacting the denatured krill product with a polar solvent to extract a polar krill oil comprising phospholipids, wherein said polar krill oil comprising phospholipids is further characterized in comprising greater than about 3% ether phospholipids w/w of said polar krill oil; from about 27% to 50% non-ether phospholipids w/w of said polar krill oil so that the amount of total phospholipids in the composition is from about 30% to 60% w/w of said polar krill oil; from about 20% to 50% triglycerides w/w of said polar krill oil, and astaxanthin esters in amount of greater than about 100 mg/kg of said polar krill oil; and

c) encapsulating said polar krill oil in a soft gel capsule.

36. (New) The method of claim 35, wherein said polar krill oil comprises greater than about 40% phosphatidylcholine w/w of said polar krill oil.

37. (New) The method of claim 35, wherein said polar krill oil comprises greater than about 45% phosphatidylcholine w/w of said polar krill oil.

38. (New) The method of claim 35, wherein said polar krill oil comprises at least 36% w/w omega-3 fatty acids.

39. (New) The method of claim 35, wherein said polar krill oil contains greater than about 200 mg/kg astaxanthin esters.

40. (New) The method of claim 35, wherein said polar krill oil contains greater than about 300 mg/kg astaxanthin esters.

41. (New) The method of claim 35, wherein said polar krill oil contains greater than about 400 mg/kg astaxanthin esters.

42. (New) The method of claim 35, wherein said polar krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition.

43. (New) The method of claim 35, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

44. (New) The method of claim 35, further comprising combining said polar krill oil with a plant phytonutrient.

45. (New) The method of claim 35, wherein said polar krill oil comprises greater than about 4% w/w ether phospholipids.

46. (New) The method of claim 45, wherein said polar krill oil comprises greater than about 200 mg/kg astaxanthin esters.

47. (New) The method of claim 45, wherein said polar krill oil comprises greater than about 300 mg/kg astaxanthin esters.

48. (New) The method of claim 45, wherein said polar krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said polar krill oil.

49. (New) The method of claim 48, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

50. (New) The method of claim 45, wherein said krill oil comprises greater than about 400 mg/kg astaxanthin esters.

51. (New) The method of claim 50, wherein said krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition.

52. (New) The method of claim 51, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

53. (New) The method of claim 45, further comprising combining said polar krill oil with a plant phytonutrient.

54. (New) The method of claim 35, wherein said polar krill oil comprises greater than about 5% w/w ether phospholipids.

55. (New) The method of claim 54, wherein said polar krill oil comprises greater than about 200 mg/kg astaxanthin esters.

56. (New) The method of claim 54, wherein said polar krill oil comprises greater than about 300 mg/kg astaxanthin esters.

57. (New) The method of claim 55, wherein said polar krill oil comprises from about 20% to 35% omega-3 fatty acids as a percentage of total fatty acids in said composition.

58. (New) The method of claim 57, wherein from about 70% to 95% of said omega-3 fatty acids are attached to said phospholipids.

59. (New) The method of claim 54, wherein said polar krill oil comprises greater than about 400 mg/kg astaxanthin esters.

60. (New) The method of claim 54, further comprising combining said polar krill oil with a plant phytonutrient.

61. (New) The method of claim 1, wherein the treating step occurs on a ship.

62. (New) The method of claim 1, wherein said *Euphausia superba* is freshly caught.

63. (New) The method of claim 1, wherein said treating comprising heat treatment.

64. (New) The method of claim 35, wherein the treating step occurs on a ship.

65. (New) The method of claim 35, wherein said *Euphausia superba* is freshly caught.

66. (New) The method of claim 35, wherein said treating comprising heat treatment.

REMARKS

Claims 1, 3, 4, 6, 8, 9 and 12-66 are pending and under examination following entry of this response. Claims 1, 3, 4, 8 and 9 have been amended. Claims 2, 5, 7 and 10-11 have been cancelled. New claims 12-66 have been entered. Support for the amendments may be found in the specification at pages 2-4, 15-16 and 18 among other places. No new matter has been added. All amendments and cancellation of claims are made without acquiescing to any of the Examiner's arguments or rejections, and solely for the purpose of expediting the patent application process and without waiving the right to prosecute the cancelled claims (or similar claims) in the future.

Terminal disclaimers over US9034388, US9028877, US9119864 (USSN14/620,779) and USSN 14/490,204 are being submitted herewith. If the Examiner believes additional terminal disclaimers are needed she is invited to contact Applicant's representative.

The pending rejections are addressed in order below.

Indefiniteness. The claims have been amended to correct the issues noted by the Examiner and to further clarify the use of w/w.

Anticipation. The claims are rejected as allegedly being anticipated by JP Abstract 04-057853 and as obvious over JP4057853, US4814111, US 2006/0078625 and US8057825. It is initially noted that the claims have been amended to contain additional limitations as compared to those allowed in related patent US9028877, to which a terminal disclaimer is filed herewith. Thus, it is urged that the claims are in condition for allowance.

With respect to the anticipation rejection over JP Abstract 04-057853, Applicant has amended the claims to clarify that the extraction process with a polar solvent yields a krill oil with a specified phospholipid content and particularly a high ether phospholipid content. The Japanese abstract discloses a protease treated and mechanically ground composition:

> Krill shells are treated with a protease to decompose the protein in the shells and the treatment product is filtered. The residue of filtration is dried to give treated shells having a water content of 6-8% and a mean particle size of 200 μm or lower. The treated shells are put into an extraction vessel 5.

The purpose of the process is to extract a coloring pigment from krill shells: "To prepare a reddish orange coloring matter having a high safety in a high concn. by extracting, with CO2 in a supercritical state, krill shells of which the protein has been decomposed by a protease."

Applicants respectfully submit that the alleged prior art process, which uses only krill shells, is substantially different from the claimed process which uses a polar solvent to extract a specified phospholipid-rich oil. Extraction from krill shells, even if they have residual material associated with the shells, would not yield a high phospholipid krill oil as claimed. The shells of krill are primarily composed of chitin which is a carbohydrate, not a protein. Therefore, the residual material would be primarily chitin. Furthermore, the extracted material would not contain ether phospholipids, phospholipids, or triglycerides in the specified amounts.

Obviousness. The claims are rejected as obvious over JP4057853, US4814111, US 2006/0078625 and US8057825. First, the claims have been amended to specify that the extracted oil contains at least 3% ether phospholipids. None of the cited references, alone or in combination, teach or suggest this limitation. In fact, as shown in the papers submitted herewith, a person of skill in the art would not have been motivated to extract oil from krill with an increased amount of ether phospholipids. It was known in the art at the time of the invention that ether phospholipids are precursors for compounds with known, pro-inflammatory activity that mimic Platelet Activating factor (PAF). In this regard, the Examiner's attention is respectfully directed to Marathe et al., J. Biol. Chem. (1999) 274(10):28395-28404, which is attached for the Examiner's convenience. This paper, as well as many others in the field, indicates that ether phospholipids are precursors for compounds with a much higher PAF-like activity (800 fold higher) than non-ether phospholipids. Tanaka et al., Biosci. Biotech. Biochem. 59(8):1389-93 (1995) demonstrates that the deleterious PAF-like analogs described in Marathe et al. (1999) 274(10):28395-28404 are readily formed from ether phospholipids found in krill. In fact, as described in the Tanaka abstract:

> The activity of oxidized krill PC, which was equivalent of 89.8 ± 8.8 pmol 16:0 PAF/umol of starting PC, was about five times those of oxidized PCs from salmon roe and sea urchin eggs and about 50 times that of oxidized hen yolk PC.

Based on this information, and the known platelet aggregation and inflammatory activity of PAF, one of skill in the art would not be motivated to increase the ether phospholipid content of prior art krill oil and would in fact have been motivated to avoid increasing the ether phospholipid content of krill oil. Certainly, one of skill in the art would not have expected that increasing the ether phospholipid content of krill oil would lead to increased health benefits. For this reason, Applicant respectfully submits that a person of skill in the art would not have sought to increase the ether phospholipid content of prior art krill oil and would have been motivated to do just the opposite, i.e., to decrease the ether phospholipid content of krill oil. Thus, the presently claimed compositions with increased ether phospholipid content are not obvious.

Applicant respectfully requests that this rejection be withdrawn and the claims passed to allowance.

CONCLUSION

If a telephone interview would aid in the prosecution of this application, the Examiner is encouraged to call the undersigned collect at (608) 662-1277.

Dated: January 8, 2016

/J. Mitchell Jones/
John Mitchell Jones
Registration No. 44,174
Casimir Jones, S.C.
2275 Deming Way, Suite 310
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PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): AKBM-14409/US-6/CON

Application Number: 14/020,162

Filed: 06-Sep-2013

For: BIOEFFECTIVE KRILL OIL COMPOSITIONS

Art Unit: 1651

Examiner: WARE, DEBORAH

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☑ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 1,400
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 504302.

☑ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☑ attorney or agent of record. Registration number 44174.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/J. Mitchell Jones/ — Signature

January 8, 2016 — Date

J. Mitchell Jones — Typed or printed name

608-662-1277 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☑ * Total of 1 forms are submitted.

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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
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PTO/SB/08a (03-15)
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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	Inge Bruheim
Art Unit	1651
Examiner Name	WARE, Deborah K.
Attorney Docket Number	AKBM-14409/US-6/CON

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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	4038722		1977-08-02	TERASE et al.	
	2	8586567		2013-11-19	SAMPALIS, F.	

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	1	20050003073		2005-01-06	PIVOVAROV et al.	
	2	20110256216		2011-10-20	LEFKOWITZ ANDREW R.	
	3	20110160161		2011-06-30	SAMPALIS, F.	

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	Art Unit	1651
	Examiner Name	WARE, Deborah K.
	Attorney Docket Number	AKBM-14409/US-6/CON

	1	01/76385	WO		2001-10-18	Westfalia Separator Industry GmbH		
	2	S6323819	JP		1988-02-01	HARAK K. et al.		
	3	2012/139588	WO		2012-10-18	TRIPLENINE PHARMA AS		

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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.	T[5]
	1	KOLAKOWSKI and GAJOWIECKI, "Optimization of autoproteolysis to obtain and edible product 'precipitate' from Antarctic krill," Seafood Science and Technology, pp. 331-336	
	2	"Neptune krill Oil's Unique Properties", INTERNET CITATION, 2011, URL:http://www.nowfoods.com/Products/ProductFAQs/081008/htm	
	3	GIGLIOTTI et al., "Extraction and characterisation of lipids from Antarctic krill (Euphausia superba)", Food Chemistry, 2011, vol. 125, no. 3, pages 1028-1036	
	4	ALI-NEHARI et al., "Characterization of purified phospholipids from krill () residues deoiled by supercritical carbon dioxide", Korean Journal of Chemical Engineering, 2012, vol. 29, no. 7	
	5	International Search Report and Written Opinion, International Patent Application No. PCT/IB2014/002130, mailed February 3, 2015	
	6	EP Opposition filed May 8, 2015 by Olympic Seafood AS, EP Patent No. 2144618	

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

	7	ALLAHPICHAY et al., "Extraction of Growth Promoting Fractions from Non-muscle Krill Meal of Euphausia superba and its Effect on Fish Growth," Bulletin of the Japanese Society of Scientific Fisheries, 1984, 50(5): 821-826

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[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.

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	First Named Inventor	Inge Bruheim
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	Examiner Name	WARE, Deborah K.
	Attorney Docket Number	AKBM-14409/US-6/CON

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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).

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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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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)	2016-07-08
Name/Print	J. Mitchell Jones	Registration Number	44174

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/271,209	23 February 2001 (23.02.2001)	US

(71) Applicant *(for all designated States except US)*: **WESTFALIA SEPARATOR INDUSTRY GMBH** [DE/DE]; Werner-Habig-Strasse 1, D-59302 Oelde (DE).

(72) Inventors; and

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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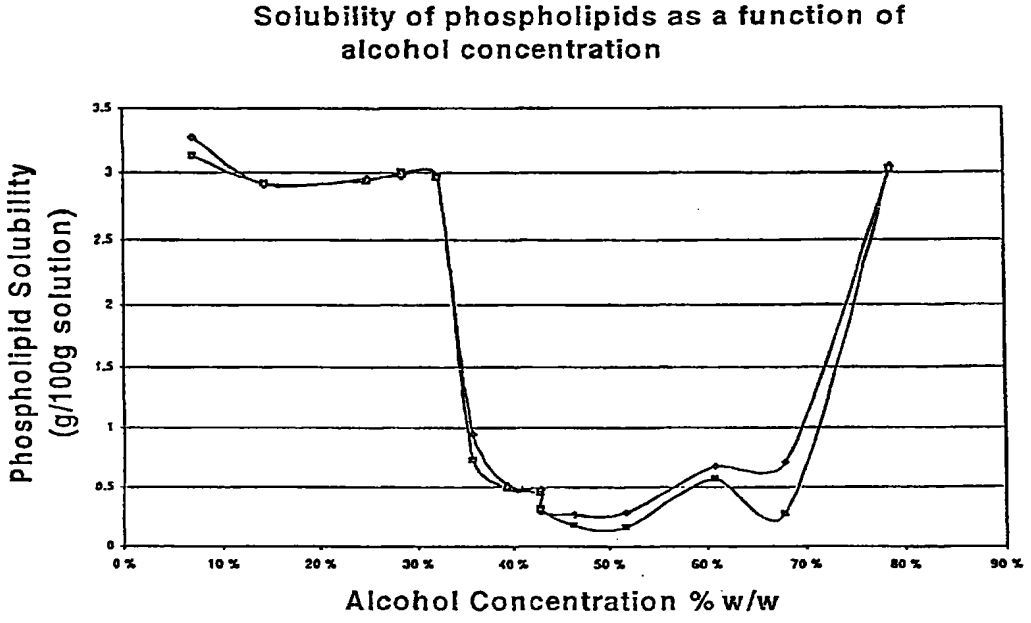
Published:
— *with international search report*

[Continued on next page]

(54) Title: METHOD FOR THE FRACTIONATION OF OIL AND POLAR LIPID-CONTAINING NATIVE RAW MATERIALS USING ALCOHOL AND CENTRIFUGATION



(57) Abstract: A process for the production of polar lipid-rich materials and preferably phospholipids. Preferably the polar lipid-rich materials are separated and recovered from native raw materials by extraction with water-soluble organic solvent and use of density separation to separate the resulting mixture.

WO 01/76385 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD FOR THE FRACTIONATION OF OIL AND POLAR LIPID-CONTAINING NATIVE RAW MATERIALS USING ALCOHOL AND CENTRIFUGATION

FIELD OF THE INVENTION

The present invention relates to a process for the separation and recovery of polar lipid-rich fractions from mixtures such as native raw materials. Other fractions in the raw materials can also be recovered.

BACKGROUND OF THE INVENTION

Examples of polar lipids include phospholipids (e.g. phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, phosphatidylglycerol, diphosphatidylglycerols), cephalins, sphingolipids (sphingomyelins and glycosphingolipids), and glycoglycerolipids. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, amino alcohols, and carbohydrates. They are generally considered to be structural lipids, playing important roles in the structure of the membranes of plants, microbes and animals. Because of their chemical structure, polar lipids exhibit a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents. The term polar lipid within the present description is not limited to natural polar lipids but also includes chemically modified polar lipids. Although the term oil has various meanings, as used herein, it will refer to the triacylglycerol fraction.

One of the important characteristics of polar lipids, and especially phospholipids, is that they commonly contain polyunsaturated fatty acids (PUFAs: fatty acids with 2 or more unsaturated bonds). In many plant, microbial and animal systems, they are especially enriched in the highly unsaturated fatty acids (HUFAs: fatty acids with 4 or more unsaturated bonds) of the omega-3 and omega-6 series. Although these highly unsaturated fatty acids are considered unstable in triacylglycerol form, they exhibit enhanced stability when incorporated in phospholipids.

The primary sources of commercial PUFA-rich phospholipids are soybeans and canola seeds. These biomaterials do not contain any appreciable amounts of HUFAs unless they have been genetically modified. The phospholipids (commonly called lecithins) are routinely recovered from these oilseeds as a by-product of the vegetable oil extraction process. For example, in the production of soybean or canola oil, the beans

(seeds) are first heat-treated and then crushed, ground, and/or flaked, followed by extraction with a non-polar solvent such as hexane. Hexane removes the triacylglycerol-rich fraction from the seeds together with a varying amount of polar lipids (lecithins). The extracted oil is then de-gummed (lecithin removal) either physically or chemically as a part of the normal oil refining process and the precipitated lecithins recovered. One disadvantage of this process is the use of the non-polar solvents such as hexane presents toxicity and flammability problems that must be dealt with.

The crude lecithin extracted in the "de-gumming" process can contain up to about 33% oil (triacylglycerols). One preferred method for separating this oil from the crude lecithin is by extraction with acetone. The oil (triacylglycerols) is soluble in acetone and the lecithin is not. The acetone solution is separated from the precipitate (lecithin) by centrifugation and the precipitate dried under first a fluidized bed drier and then a vacuum drying oven to recover the residual acetone as the product is dried. Drying temperatures of 50-70°C are commonly used. The resulting dried lecithins contain approximately 2-4% by weight of oil (triacylglycerols). Process temperatures above 70°C can lead to thermal decomposition of the phospholipids. However, even at temperatures below 70°C the presence of acetone leads to the formation of products that can impair the organoleptic quality of the phospholipids. These by-products can impart musty odors to the product and also a pungent aftertaste.

To avoid use of non-polar solvents such as hexane and avoid the negative side effects of an acetone-based process, numerous processes have also been proposed involving the use of supercritical fluids, especially supercritical CO_2. For example, U.S. Patent No. 4,367,178 discloses the use of supercritical CO_2 to partially purify crude soy lecithin preparation by removing the oil from the preparation. German Patent Nos. DE-A 30 11 185 and DE-A 32 29 041 disclose methods for de-oiling crude lecithin with supercritical CO_2 and ethane respectively. Other supercritical processes have been proposed which include adding small amounts of hydrocarbons such as propane to the supercritical CO_2 to act as entraining agents. However, supercritical fluid extraction systems are very capital expensive and cannot be operated continuously. Further, extraction times are long and the biomaterials must be dried before extraction, and this increases the difficulties of stabilizing the resulting dry product with antioxidants. All of these factors make the supercritical process one of the most expensive options for extracting and recovering polar-lipid material or mixtures of these materials. As a result,

alternative processes using extraction with liquid hydrocarbons at lower pressures have been described. For example U.S. Patent No. 2,548,434 describes a method for de-oiling oilseed materials and recovering crude lecithin using a liquid hydrocarbon at lower pressures (35-45 bars) but elevated temperatures (79° to 93°C). U.S. Patent No. 5,597,602 describes a similar process that operates at even lower pressures and temperatures. However, even with these improvements supercritical fluid extraction remains very expensive and is not currently used to produce phospholipids for food use on a large commercial scale.

The primary commercial source of HUFA-rich polar lipids is egg yolk. Two primary methods are used for the recovery of egg phospholipids on an industrial scale. Both require the drying of the egg yolk before extraction. In the first process the dried egg yolk powder is extracted first with acetone to remove the triacylglycerols. This is then followed by an extraction with pure alcohol to recover the phospholipids. In the second process, pure alcohol is used to extract an oil/lecithin fraction from the dried egg yolk. The oil/lecithin phase is then extracted with acetone to remove the triacylglycerols, leaving behind a lecithin fraction. Both of these methods require the use of acetone, which has the disadvantages discussed above.

Canadian Patent No. 1,335,054 describes a process for extracting fresh liquid egg yolk into protein, oil and lecithin fractions by the use of ethanol, elevated temperatures, filtration and low temperature crystallization with further filtration. The purity of the lecithin product is not disclosed. However one skilled in the art would expect that the lecithin fraction produced by this process would not be very pure. There would still be very significant amounts of oil associated with the lecithin because the chilling process would primarily remove the triglycerides containing saturated fatty acids. Those containing some unsaturated fatty acids would remain more soluble at low temperatures. Additionally, the filtration and the chilling/filtration processes employed in this method would be labor intensive and difficult to turn into a continuous process. In light of the current state of the art, there remains a need for an improved extraction technology for food-grade polar lipid products that is less expensive to operate and which protects the overall quality of the HUFAs in the polar lipid products.

SUMMARY OF THE INVENTION

In accordance with the present invention, an improved process is provided for recovering polar lipids from native biomaterials, which does not involve the disadvantages of the prior art. One embodiment of the invention resides in a process for recovering polar lipids and/or polar lipid-containing mixtures from biomaterials using both high and low water-soluble organic solvent concentrations and centrifugation.

In accordance with an embodiment of the present invention, a process for fractionation of an oil-, polar lipid-, and protein-containing mixture is provided. The process includes the steps of adding a high concentration of water-soluble organic solvent to the mixture, separating protein from the mixture by subjecting the mixture to density separation, e.g., using gravity or centrifugal force, to form a protein-rich fraction and a polar lipid/oil-rich fraction, reducing of the concentration of water-soluble organic solvent in the polar lipid/oil-rich fraction, and subjecting this fraction to density separation, e.g., using gravity or centrifugal force, to form a polar lipid-rich fraction and an oil-rich fraction.

In accordance with another embodiment of the present invention, a process for recovering polar lipid from a polar lipid-containing mixture employing the use of a water-soluble organic solvent, wherein the relatively high solubility of polar lipid in a water-soluble organic solvent, in which the water-soluble organic solvent comprises greater than 68 percent by weight of the aqueous solution, followed by process steps which utilize water-soluble organic solvent concentrations of from about 5 to about 35% by weight, are employed to assist in the recovery.

In accordance with another embodiment of the present invention, a process for fractionation of an oil-, polar lipid-, and protein-containing mixture is provided. The process includes the steps of adding a high concentration water-soluble organic solvent to the oil-, polar lipid-, and protein-containing mixture, and separating protein from the mixture to form a protein-rich fraction and a polar lipid/oil-rich fraction. As used herein, the term "high concentration water-soluble organic solvent" will mean greater than 68 percent organic solvent, preferably greater than 80 percent organic solvent, more preferably greater than 90 percent, more preferably from about 80 percent to about 95 percent organic solvent.

Preferably, the process steps are conducted under oxygen-reduced atmospheres that can include use of inert or non-reactive gases (e.g. nitrogen, carbon dioxide, argon,

etc), use of solvent vapors, use of a partial or full vacuum, or any combination of the above.

BRIEF DESCRIPTION OF THE FIGURES

The present invention may be more readily understood by reference to the following figures, wherein

FIG. 1 is a graphical representation of the solubility of phospholipids, a form of polar lipids, as a function of alcohol concentration.

FIG. 2 is a graphical representation of a phospholipid extraction process (as an example of a polar lipid extraction process) based on a high concentration of alcohol.

DETAILED DESCRIPTION OF THE INVENTION

Because of their bipolar nature, polar lipids (including phospholipids) are of significant commercial interest as wetting and emulsifying agents. These properties may also help make HUFAs in the phospholipids more bioavailable, in addition to enhancing their stability. These properties make phospholipids ideal forms of ingredients for use in nutritional supplements, food, infant formula and pharmaceutical applications.

We have unexpectedly found that polar lipids are soluble not only in high water-soluble organic solvent concentrations (e.g., at water-soluble organic solvent concentrations greater than about 68% w/w) but also in low water-soluble organic solvent concentrations (less than about 35% water-soluble organic solvent w/w) (FIG. 1). As used herein, water-soluble organic solvent concentration means the weight percentage of water-soluble organic solvent in an aqueous solution. The aqueous solution includes added water and water present in the materials. For the purpose of this invention, phospholipids are described as "soluble" if they do not settle or separate from the continuous phase (sometimes also called supernatant or light phase) when subjected to centrifugation by equipment described in this invention. In the water-soluble organic solvent concentration range from about 35% w/w to about 68% w/w water-soluble organic solvent, polar lipids exhibit significantly lower solubility. The present invention exploits this property of polar lipids (enhanced solubility/dispersibility at low water-soluble organic solvent concentrations), which can then be exploited in several ways (along with the high solubility of phospholipids in high water-soluble organic solvent

concentrations) to develop processes for inexpensively extracting and recovering polar lipids, and especially phospholipids, from native biomaterials.

Native biomaterials that are rich in HUFA-containing polar lipids include fish, crustaceans, microbes, eggs, brain tissue, milk, meat and plant material including oilseeds. As used herein, the terms fish, crustaceans, microbes, eggs, brain tissue, milk, meat and plant material including oilseeds will include genetically modified versions thereof. The content of phospholipids in these materials is generally low, usually ranging from 0.1% to about 4% by wet weight. As a result large amounts of raw materials need to be processed to recover these phospholipids. Because of the high costs of prior extraction techniques, phospholipids and especially HUFA-enriched phospholipids were very expensive and therefore restricted to use in the infant formula, pharmaceutical and cosmetic industries. One of the advantages of the present invention is that it provides for the extraction of polar lipids, and in particular phospholipids, in a cost-effective manner.

A polar lipid recovery process utilizing high concentrations of water-soluble organic solvent in a polar lipid/oil concentration step followed by the use of low water-soluble organic solvent concentrations in a step recovering the polar lipids from the oil phase is outlined in FIG. 2. Liquid egg yolk is used as the polar-lipid rich biomaterial in this example. It is understood, however, that other polar lipid-containing biomaterials (e.g. fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds) could also be processed in a similar manner with minor modification to the process.

In the first step of the process 12, the material is dried, if necessary. For a more efficient recovery of the protein, the material is optionally subjected to size reduction 14. A water-soluble organic solvent (e.g., alcohol) is added 14. The concentration of water-soluble organic solvent in the water-soluble organic solvent/water solution is at least about 68% w/w, preferably at least about 80% w/w, preferably at least about 90% w/w, more preferably from about 80 to about 95% w/w, more preferably from about 85 to about 95% w/w, and more preferably from about 90 to about 95% w/w. The more moisture that is present in the material, the greater the amount and/or the higher the concentration of the water-soluble organic solvent that will be needed to achieve the desired concentration when mixed with the material. In other words, if the material is relatively dry, less water-soluble organic solvent and/or lower concentration water-soluble organic solvent can be employed. On the other hand, if the material is relatively

wet, more water-soluble organic solvent and/or higher concentration water-soluble organic solvent must be employed.

The denatured protein 20 is then separated by density separation 18. Since proteins are not soluble in high concentrations of water-soluble organic solvent, they precipitate (while the polar lipids and oil dissolves in the high concentration water-soluble organic solvent) and the precipitated proteins 20 are separated from the polar lipid/oil-enriched fraction 22 by density separation 18, e.g., using gravity or centrifugal force. Using egg yolk as an example, this results in two fractions being recovered: (1) a fraction with approximately 60-95% oil (as % dry weight) and about 5-40% dry weight as polar lipids; and (2) a protein fraction, preferably with more than 90% of the protein of the egg yolk.

If it is desired to separate the polar lipid from the oil, the oil/polar lipid fraction 22 is mixed 26 with water 24 to a final concentration of water-soluble organic solvent in water of from about 5 to about 35% w/w, preferably from about 20 to about 35% w/w, more preferably from about 25 to about 30% w/w. A less desirable alternative would be to dry the oil/polar lipid fraction 22 and then add a water-soluble organic solvent, and water as necessary, to achieve the desired concentration of water-soluble organic solvent. The polar lipid is then separated from the oil by means of density separation 28. A polar lipid-enriched fraction 30 and an oil-enriched fraction 32 are formed. Further processing can be performed on the polar lipid-enriched fraction 30 and/or oil-enriched fraction 32 as desired or necessary. For example, counter-current washing/centrifugation or cross-current washing/separation of the oil and polar lipid products can be employed to improve the purity of the products and economics of the overall process.

In an alternative embodiment, the drying step can be eliminated. For example, instead of drying a material such as eggs, wet eggs can be used. The process is similar to that described above, however, the drying step is eliminated. As a result, a larger amount and/or higher concentration of water-soluble organic solvent is employed to precipitate the protein.

Because of the simplicity of the equipment required in the process, the entire process can very easily be conducted under a reduced-oxygen atmosphere (e.g., nitrogen, a preferred embodiment of the process), further protecting any HUFAs in the polar lipids from oxidation. For example, a gas tight decanter can be used to separate protein from the mixture. A suitable decanter is model CA 226-28 Gas Tight available from Westfalia

Separator Industry GmbH of Oelde Germany, which is capable of continuous separation of protein from suspensions with high protein solids content in a centrifugal field. A gas tight separator useful for separating polar lipids from oil is model SC 6-06-576 Gas Tight available from Westfalia Separator Industry GmbH of Oelde Germany, which is capable of continuous separation of polar lipids from oil in a centrifugal field.

The concentration of water-soluble organic solvent in the protein removal step is preferably greater than about 68% w/w, more preferably greater than about 70% w/w, more preferably greater than about 80% w/w, more preferably greater than about 90% w/w. In principle, it is believed that the higher the water-soluble organic solvent concentration, the stronger the protein contraction, but the more nonpolar the aqueous/water-soluble organic solvent phase, more polar lipids may be dissolved in the oil phase. The appropriate concentration and temperature must therefore be found, for example, by conducting a few preliminary experiments (centrifuge tests), for each raw material.

The present invention, in various embodiments, includes components, methods, processes, systems and/or apparatus substantially as depicted and described herein, including various embodiments, subcombinations, and subsets thereof. Those of skill in the art will understand how to make and use the present invention after understanding the present disclosure. The present invention, in various embodiments, includes providing devices and processes in the absence of items not depicted and/or described herein or in various embodiments hereof, including in the absence of such items as may have been used in previous devices or processes, *e.g.*, for improving performance, achieving ease and/or reducing cost of implementation.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or

equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

We claim:

1. A process for fractionation of an oil-, polar lipid-, and protein-containing mixture, comprising the steps:

(a) adding a water-soluble organic solvent to said mixture and separating protein from said mixture to form a protein-rich fraction and a polar lipid/oil-rich fraction;

(b) reducing the concentration of water-soluble organic solvent in said polar lipid/oil-rich fraction; and

(c) subjecting the water/water-soluble organic solvent and polar lipid/oil-rich fraction to density separation to form a polar lipid-rich fraction and an oil-rich fraction.

2. The process of Claim 1, wherein the separation of protein of step (a) comprises the steps:

(a) adding water-soluble organic solvent to said oil-, polar lipid-, and protein-containing mixture to obtain a water-soluble organic solvent concentration of a least about 68% w/w; and

(b) separating by density separation the resulting mixture into a protein-rich fraction and a polar lipid/oil-rich fraction

3. The process of Claim 1, wherein said oil-, polar lipid-, and protein-containing mixture is derived from eggs.

4. The process of Claim 1, wherein water-soluble organic solvent is recovered from the protein-rich fraction and the polar lipid/oil-rich fraction after the density separation.

5. The process of Claim 1, wherein said polar lipid/oil-rich fraction formed in step (a) comprises from about 5% to about 40% by weight polar lipid and from about 60% to about 95% by weight oil.

6. The process of Claim 1, wherein said protein-rich fraction formed in step (a) comprises from about 80% to about 95% by weight protein on a dry basis.

7. The process of Claim 1, wherein said oil-, polar lipid-, and protein-containing mixture further comprises cholesterol and a substantial amount of said cholesterol reports to said oil-rich fraction pursuant to the separation of step (c).

8. The process of Claim 1, wherein said water-soluble organic solvent in step (a) is present in a water-soluble organic solvent/water mixture in which said water-soluble

organic solvent comprises from about 80% to about 95% by weight of said water-soluble organic solvent/water mixture.

9. The process of Claim 1, wherein said water-soluble organic solvent in step (c) is present in a water-soluble organic solvent/water mixture in which said water-soluble organic solvent comprises from about 5% to about 35% by weight of said water-soluble organic solvent/water mixture.

10. The process of Claim 1, wherein said water-soluble organic solvent is recovered by countercurrent washing, evaporation or drying.

11. The process of Claim 1, wherein said polar lipid-rich fraction is dried to recover water-soluble organic solvent, washed with an water-soluble organic solvent/water mixture comprising greater than about 80% by weight water-soluble organic solvent in order to precipitate residual protein and further dried to recover the water-soluble organic solvent.

12. The process of Claim 11, wherein the addition of said water-soluble organic solvent results in the precipitation of at least some of said protein, which is recovered by density separation.

13. The process as claimed in Claims 1-12, wherein said water-soluble organic solvent comprises a polar solvent.

14. The process as claimed in Claims 1-13, wherein said water-soluble organic solvent comprises an alcohol.

15. The process as claimed in Claims 1-14, wherein said water-soluble organic solvent comprises a C_1-C_8 alcohol.

16. The process as claimed in Claims 1-15, wherein said water-soluble organic solvent comprises isopropanol, ethanol or mixtures thereof.

17. The process as claimed in Claims 1-16, wherein the pH during processing is from pH 4 to about pH 10.

18. The process as claimed in Claims 1-17, wherein said mixture is obtained from at least one of eggs, fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds.

19. The process as claimed in Claims 1-18, wherein at least 60% of the polar lipids originally present in the mixture are recovered in a polar lipid-rich fraction.

20. The process as claimed in Claims 1-19, wherein at least 80% of the polar lipids originally present in the mixture are recovered in a polar lipid-rich fraction.

21. A process for recovering polar lipid from a polar lipid-containing mixture employing the use of a water-soluble organic solvent, wherein the relatively high solubility of polar lipid in an aqueous solution of the water-soluble organic solvent, in which the water-soluble organic solvent comprises more than 68 percent by weight of the aqueous solution, followed by employing the use of a water-soluble organic solvent, wherein the relatively high solubility of polar lipid in an aqueous solution of the water-soluble organic solvent, in which the water-soluble organic solvent comprises less than 35 percent by weight of the aqueous solution, are employed to assist in said recovery.

22. The process as claimed in Claim 21, wherein said mixture is obtained from at least one of eggs, fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds.

23. The process of any of Claims 1-22, wherein said polar lipid comprises a phospholipid.

24. The process of any of Claims 1-23, wherein at least a portion of said process is performed in an oxygen-reduced atmosphere.

25. The process as claimed in Claims 1-24, wherein said mixture is obtained from at least one of eggs, fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds.

26. The process as claimed in Claim 1, wherein said steps of adding and subjecting are repeated at least once.

27. A process for fractionation of an undried oil-, polar lipid-, and protein-containing mixture, comprising the steps:

(a) adding water-soluble organic solvent to said oil-, polar lipid-, and protein-containing mixture to obtain a water-soluble organic solvent concentration of a least about 68% w/w; and

(b) separating by density separation the resulting mixture into a protein-rich fraction and a polar lipid/oil-rich fraction.

28. An oil-containing, polar lipid-containing or protein-containing product produced by any of the processes of Claims 1-27.

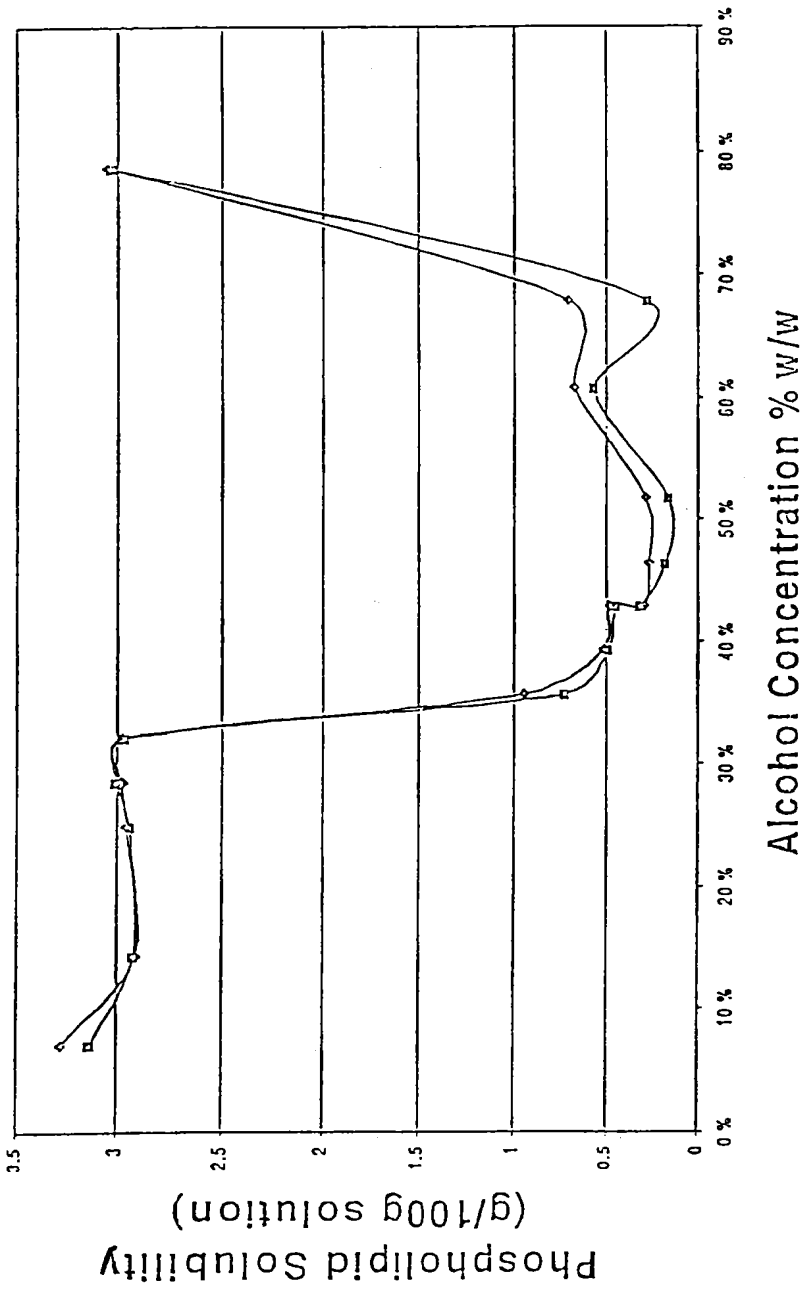


Figure 1. Solubility of phospholipids as a function of alcohol concentration

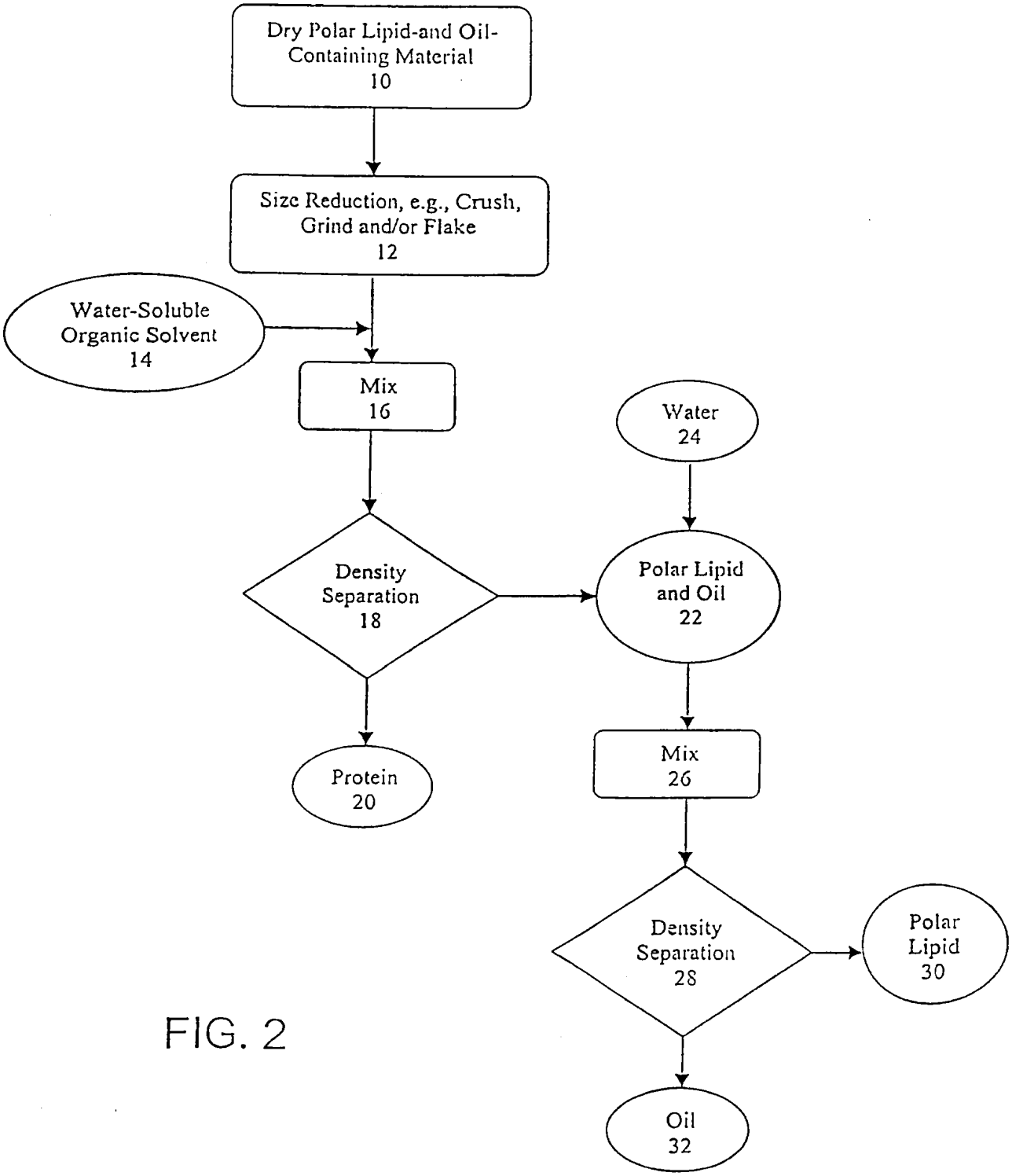


FIG. 2

INTERNATIONAL SEARCH REPORT

Interna. al Application No
PCT/IB 01/00963

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23J1/08 C11B1/00 C11B7/00 A23D9/013 A23J7/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23J C11B A23D

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 883 273 A (MCCOMBS CHARLES ALLAN ET AL) 16 March 1999 (1999-03-16) column 6, line 53 -column 7, line 43 column 8, line 45-55 claims 1-24; examples 1,8	1-4, 7-10, 13-25, 27,28
A		5,6,11, 12,26

X	WO 97 27274 A (ABBOTT LAB) 31 July 1997 (1997-07-31) page 11, paragraph 4 -page 12, paragraph 1 page 14, paragraph 4 -page 15, paragraph 2 claim 1; examples 1,9	1-4, 7-10, 13-25, 27,28
Y		5,6
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2001	01/10/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rooney, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna. il Application No
PCT/IB 01/00963

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 917 068 A (BARNICKI SCOTT DONALD ET AL) 29 June 1999 (1999-06-29) column 5, line 32-42; claim 1; example 7	1-4,7, 10, 13-16, 18-20, 23,25,28
Y	CA 1 335 054 A (CANADIAN EGG MARKETING AGENCY) 4 April 1995 (1995-04-04) cited in the application See whole document	5,6
A	US 4 157 404 A (FUKINBARA ITARU ET AL) 5 June 1979 (1979-06-05) column 2, line 39 -column 3, line 8	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat · ıl Application No
PCT/IB 01/00963

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US 4157404 A	05-06-1979	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

(19) Japan Patent Office (JP)
(12) Laid-open Patent Application Gazette (A)
(11) Laid-open Application No. S63-23819
(43) Date of Laid-open Publication: Feb. 1, 1988
(51) Int. Cl. 4
A 61 K 35/60
ID Code
ACB
Internal Control No.
8615-4C
Examination: Not Requested Yet No. of Claims: 1 (Total4 pages)
(54) Title of Invention: Platelet Aggregation Inhibitor
(21) Application No.: S61-167540
(22) Application Date: July 16, 1986
(72) Inventor: Shoichi MURATA, Sugamata Heights #504, 3-1-1 Imaizumi, Utsunomiya City, Tochigi Prefecture
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(74) Agent: Mitsuyuki ARIGA, Patent Agent, and two others

Abstract:
PURPOSE: The titled inhibitor capable of being orally administered, having low side effects and improved stability, containing an extract of krill with an organic solvent as an active ingredient.
CONSTITUTION: An inhibitor of blood platelet aggregation containing an extract of krill with an organic solvent as an active ingredient. The extract is obtained by adding krill to an organic solvent such as chloroform, etc., stirring it preferably at 15W60°C for 2W24hr and filtering. The extract can be directly used as it is, or concentrated or dried and used and further a phospholipid fraction can be collected from the extract. To collect the phospholipid fraction, the extract is concentrated, dripped little by little in to a large amount of cold acetone and the phospholipid fraction is precipitated. A dose is 1W20g/day calculated as phospholipid, the inhibitor is preferably administered orally and the dose can be increased depending upon symptoms.

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　昭63－23819

⑤Int.Cl.⁴	識別記号	庁内整理番号	㊸公開　昭和63年(1988)2月1日
A 61 K 35/60	ACB	8615－4C	

審査請求　未請求　発明の数 1　（全4頁）

㊹発明の名称　血小板凝集抑制剤

㉑特　願　昭61－167540
㉒出　願　昭61(1986)7月16日

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明　細　書

1. 発明の名称

血小板凝集抑制剤

2. 特許請求の範囲

1. オキアミの有機溶剤抽出物を有効成分として含有する血小板凝集抑制剤。

2. オキアミの有機溶剤抽出物がリン脂質画分である特許請求の範囲第1項記載の血小板凝集抑制剤。

3. 発明の詳細な説明

〔産業上の利用分野〕

本発明は血小板凝集抑制剤に関し、更に詳細にはオキアミを有機溶剤で抽出することにより得られる副作用が少なく安定性の高い血小板凝集抑制剤に関する。

〔従来の技術〕

血管内に血栓が形成されると血流が著しく阻害され、組織に重大な障害をもたらすことが知られている。この血栓形成による疾患、すなわち、血栓症の病態生理において、血栓形成因子として血管壁の性状、血流速度及び血液成分の三つの因子が挙げられ、これらは互いに影響しあい重要な役割を果していることが知られている。このうち、血液成分の1つである血小板の役割は非常に重要であり、血小板の凝集亢進により血栓症の誘発の可能性が実験的に、また疫学的に証明されている。さらに最近、動脈硬化症の発症メカニズムの解明に従い、動脈硬化症の発症第一段階が損傷血管内皮細胞への血小板凝集であることが

明らかとなつてきた。そこで近年血栓症の治療・予防のため、抗血小板凝集剤であるアスピリン、インドメサシン、フエニルブタゾン、クロフイブレート、デキストランサルフエート、パパベリン、ヘパリン等の薬剤、あるいはエイコサペンタエン酸等の高度不飽和脂肪酸の、経口的もしくは静脈注射による投与がおこなわれている。

〔発明が解決しようとする問題点〕

しかしながら、上記薬剤はいずれも副作用、薬効、安定性のいずれかの面において十分に満足し得るものでなく、これらに代る、副作用がなく、薬効、安定性の面でも優れた血小板凝集抑制剤の開発が望まれていた。また、日常的に投与可能とするために、当該血小板凝集抑制剤は経口投与できるものであることが望まれていた。

〔問題を解決するための手段〕

このような実情に鑑み、本発明者らは上記要望を満足する血小板凝集抑制剤を得べく鋭意研究をおこなつた結果、オキアミの有機溶剤抽出物は優れた血小板凝集抑制作用を有すること及びこのものは副作用が少なく、安定性も優れ、しかも経口投与できることを見出し、本発明を完成した。

したがつて本発明はオキアミの有機溶剤抽出物を有効成分として含有する血小板凝集抑制剤を提供するものである。

本発明で使用するオキアミとしては、例えば、スーパーバ(superba)、クリスタロロフイアス(crystallo rophias)、フリジダ(frigida)、トリアカンサ(triacantha)、ベランテイニ(Vellantini)、ロージロストリス(lougirostris)、ルーセンス(lucens)、シミリス(similis)、スピニフエラ(spinifera)、レクルバ(recurva)等のオイフアウシア(Euphausia)属、マカルーラ(macarura)、ビシナ(Vicina)、グレグリア(gregaria)等のシサノエシサ(Thysanoessa)属等のいずれでも使用可能であつて、特別な種類に限定されない。

なお、原料であるこのオキアミは全世界の海洋に分布し、特に南極周辺に多く、生息量は数億トン～２０億トンといわれており、現在の全世界漁獲量に匹敵する５０００～７０００万トン／年の漁獲も可能と考えられているもので、原料供給の面でも安定供給が可能である。

このオキアミの有機溶剤による抽出は常法によりおこなわれる。例えばオキアミ若しくはその粉砕物をクロロホルム、ベンゼン、ブタノール、エーテル等の非極性溶剤又はクロロホルム・メタノール、エーテル・エタノール、ブタノール・水等の非極性溶剤と極性溶剤の混液等の有機溶剤中に投入し、好ましくは１５～６０℃で２～２４時間撹拌し、沪過することによりおこなわれる。有機溶剤のうち、特に好ましいものとしては、クロロホルム、クロロホルム・メタノール及びブタノール・水があげられる。

このようにして得られたオキアミの有機溶

剤抽出物は、これをそのまま、あるいは濃縮若しくは乾燥して血小板凝集抑制剤として用いることもできるが、更に該抽出物からリン脂質画分を取り出し、用いることもできる。リン脂質画分を取り出す方法としては、例えば上記抽出物を濃縮した後、大量の冷アセトン中に小量ずつ滴下してリン脂質画分を沈澱させる方法及び薄層クロマトグラフィー等により分離・採取する方法が例示される。

本発明の血小板凝集抑制剤は、叙上の如くして得られたオキアミの有機溶剤抽出物又はこれから得られたリン脂質画分を必要に応じて公知の医薬用担体と配合することにより製造される。なお、オキアミの有機溶剤抽出物を用いる場合、抽出物中の有機溶剤は水と置換させ、除去することが好ましい。

斯くして得られた本発明の血小板凝集抑制剤は、成人男子に対し、抽出物もしくはリン脂質画分中のリン脂質量として1～20g/日程度経口投与することが好ましいが、症状の程度によつては更に投与量を増やすことも可能である。

なお、本発明の血小板凝集抑制剤の有効成分である抽出物及びリン脂質画分は、これらに含まれるリン脂質のラットに対するLD$_{50}$（経口）が25g/kgであることからわかるように極めて安全性の高いものである。

〔作用〕

本発明のオキアミ有機溶剤抽出物の血小板凝集抑制作用の機作は詳細には解明されていないがオキアミ有機溶剤抽出物に含有されるエイコサペンタエン酸等の高度不飽和脂肪酸を多量に有するリン脂質に由来するものと考えられる。

〔発明の効果〕

叙上の如く、本発明のオキアミ有機溶剤抽出物及び、特にこれから導かれるリン脂質画分は経口投与にて優れた血小板凝集抑制作用を示し、しかも毒性・副作用もないので極めて優れた血小板凝集抑制剤である。

〔実施例〕

以下に実施例をあげて本発明を更に具体的に説明する。

実施例1.

凍結乾燥されたオキアミ〔オイファウシア・スパーバ（Euphausia superba）〕を細かく砕き、この粉砕物500gにブタノール・水（65：35）混液4000mlを加え、50℃で20時間、撹拌させながら脂質を抽出し、沪別することによりブタノール・水抽出液を得た。

沪別したブタノール・水抽出液を減圧濃縮し、ブタノールを除去後、さらに濃縮してブタノール・水抽出液40g（水含量28g）を得た。

実施例2.

(1) 凍結乾燥されたオキアミ〔オイファウシア・スパーバ（Euphausia Superba）〕を細かく砕き、この粉砕物500gに1000mlのクロロホルムを加え、40℃で20時間、撹拌させな

がら脂質を抽出し、戸別することによりクロロホルム抽出液を得た。

(B) 戸別したクロロホルム抽出液を減圧濃縮し、乾固した後、水-エタノール(1:1)溶液を50mℓ添加し、乾固物質を溶解させ、再度減圧濃縮しエタノールを除去し、オキアミクロロホルム抽出物37g(水含量25g)を得た。

実施例3.

実施例2.(I)と同様にして調製したクロロホルム抽出液を約10mℓとなるまで減圧濃縮した後、2000mℓのアセトンの中へ撹拌させながら滴下し、リン脂質画分を沈澱させた。沈澱物を減圧戸過し採取した後、水-エタノール1:1の混合溶液30mℓに溶解し、減圧濃縮によりエタノールを除去した。オキアミのクロロホルム抽出物のリン脂質画分20g(水含量15g)が得られた。

実施例4.

実施例1.実施例2.及び実施例3.で得たオキアミ抽出物及びそのリン脂質画分について、それらの血小板凝集抑制作用を試験した。すなわち、1群10匹のウイスター系雄ラット(体重200g)にゾンデを用いオキアミ抽出物及びリン脂質画分をそれぞれ0.5mℓずつ2週間毎日胃内投与した。実験最終日にネンタール麻酔下腹部大動脈より3.8%のクエン酸ナトリウム液を1/10量加え採血した。採血した血液は500g、22℃で6分間遠心して上清をPRP(Platelet Rich plasma)とした。

残りの血液を1000g、22℃で15分間遠心して上清を乏血小板血漿(Platelet Poor plasma, PPP)とした。血小板凝集能はエルマ社製アグリテックTE-500を用いて測定し、最大凝集率及び最大凝集時間で示した。なお、血小板凝集能は血小板数がPRP200mℓ中5~7×10⁷個となるようにPPPで稀釈し、凝集惹起物質としてADP20μmol生理食塩水溶液20mℓを添加した。また、対照群としては、水のみ投与したラットを用いた。この結果を第1表に示す。

第1表

投与群	最大凝集率(%)	最大凝集時間(分)
オキアミエタノール-水抽出物	59.8	3.16
オキアミクロロホルム抽出物	60.3	3.14
オキアミリン脂質画分	52.8	2.89
水	68.5	3.48

以上の結果からオキアミ有機溶媒抽出物特にそのリン脂質画分は血小板凝集抑制作用を有することが明らかとなつた。

製剤例

組成:

①オキアミリン脂質*	100mg
②乳糖	100mg
③ケイ酸アルミニウム	50mg
④ステアリン酸マグネシウム	5mg

*完全に抽出溶剤を除去した粉末物(実施例2.)

製法:

①~④を混合し、打錠する。

以上

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[Continued on next page]

(54) Title: A PROCESS FOR THE ISOLATION OF A PHOSPHOLIPID

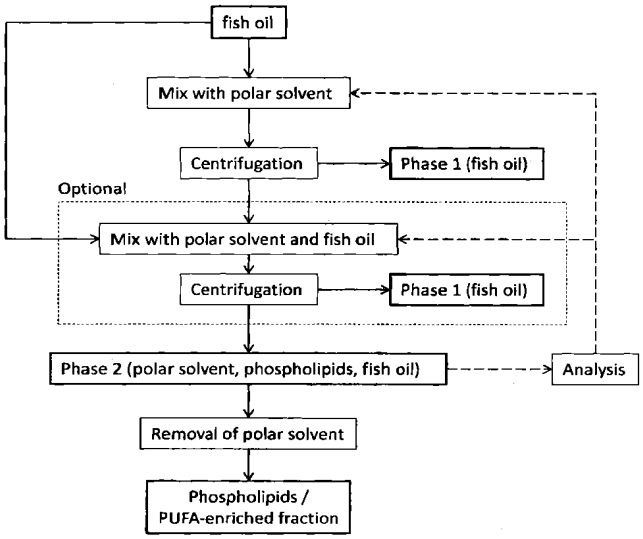

Fig. 1

(57) Abstract: The present invention relates to processes for the isolation of a phospholipid and for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of -providing a fish oil containing lipids and phospholipids; -mixing the fish oil with a polar solvent; -centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction; -isolating a phospholipid from the polar fraction or isolating a PUFA-enriched fraction from the polar fraction. The fish oil may be provided by -extracting a fish material with an extractant solvent; -removing the extractant solvent to provide the fish oil; -optionally subjecting the fish oil to a solid-liquid separation. The isolated phospholipids and PUFA's may be used as additives for functional foods, as a dietary supplement and for pharmaceutical application.

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A process for the isolation of a phospholipid

Field of the invention

This invention relates to processes for the isolation of phospholipids and for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from marine products. Marine phospholipids, in particular those comprising long chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are useful as additives for functional foods, as a dietary supplement and for pharmaceutical application. Marine phospholipids may provide beneficial effects to the health of both humans and animals.

Prior art

In recent years phospholipids comprising polyunsaturated fatty acids have been found to play important roles in physiology. Phospholipids have therefore attracted much attention as candidate materials for functional foods and in pharmaceutical applications.

Phospholipids are found in many sources of biological material, such as plant material or matter derived from animals. Marine animals comprise a particular promising source of phospholipids due to the specific composition of these phospholipids, in particular the amount of PUFA's, such as omega-3 fatty acids, e.g. eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), in marine phospholipids is large.

Phospholipids typically comprise a central glycerol moiety with two fatty acid chains and a phosphate group that may be further derivatised. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, amino alcohols, and carbohydrates. Phospholipids may also be referred to as polar lipids, and in the context of this application the terms "phospholipid" and "polar lipid" may be used interchangeably. Phospholipids are generally considered to be structural lipids, playing important roles in e.g. the structure of the membranes of plants, microbes and animals. Examples of phospholipids are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, phosphatidylglycerol, diphosphatidylglycerols. Because of their chemical structure, phos-

pholipids have a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents.

One important characteristic of marine phospholipids is that they commonly contain PUFA's with two or more unsaturated bonds, in particular with four or more unsaturated bonds. The lipid moieties of phospholipids are commonly of the omega-3 type, which often exhibit enhanced stability, e.g. oxidative stability, when incorporated into phospholipids.

Several methods exist in the prior art to extract and isolate phospholipids from raw materials. Such methods typically involve solvent extraction coupled with additional unit operations. Several examples of prior art processes are provided below.

WO2001/76385 discloses a process for the production of polar lipid-rich materials, e.g. phospholipids, from biomaterials that are rich in polar lipids with highly unsaturated fatty acids, i.e. fatty acids with four or more unsaturated bonds. Appropriate biomaterials for the process of WO2001/76385 include fish, crustaceans, microbes, eggs, brain tissue, milk, meat and plant material including oilseeds. Egg yolks are considered the primary commercial source of polar lipids rich in highly unsaturated fatty acids.

The process of WO2001/76385 comprises extracting polar lipids from the biomaterial using a water-soluble organic solvent (e.g. an alcohol) at a concentration of water soluble organic solvent of at least 68% in water. Denatured protein, which is not soluble in high concentrations of water-soluble organic solvent, is then separated by density separation, such as using gravity or centrifugal force, as a precipitate. The polar lipid/oil enriched liquid fraction may then be mixed with water to a final concentration of water-soluble organic solvent in water of from 5 to 35% to precipitate polar lipid, and polar lipid is then separated from the oil by means of density separation. An exemplary unit operation for density separation in WO2001/76385 is a decanter centrifuge.

US 6,372,460 discloses a method to provide a DHA phospholipid material, in particular from algae and other single celled organisms that contain a significant amount of DHA. In an example dried biomass (an alga) is extracted with hexane to provide a DHA-rich hexane fraction, which is centrifuged to remove fine particles. DHA-phospholipids are then precipitated chemically and the DHA-phospholipids subsequently collected by centrifuga-

tion.

JP2006-311853 discloses a method for producing a phospholipid composition from fish and shellfish. It is a particular concern of JP2006-311853 to provide a phospholipid composition free of heavy metals, such as cadmium. In the process of JP2006-311853 the starting material, e.g. fish waste is boiled with water. The boiled material is then separated into a solid and a liquid phase using centrifugal separation and/or filtration. The solid phase is then subjected to an organic solvent extraction process. The organic solvent may be methanol, ethanol, propanol, butanol, acetone, chloroform, methylene chloride, hexane or aqueous acetone. The organic solvent is then removed from the extract, now free of heavy metals, which is subjected to chromatographic purification.

JP2008-255182 describes a process for producing a phospholipid composition from an edible source, such as an edible portion and internal organs of fish and shellfishes. In the process of JP2008-255182 the starting material is initially heated with micro-waves to inactivate enzymes that may otherwise hydrolyse the phospholipids of interest. The heat-treated material is then extracted with a solvent, such as ethanol, hexane or acetone with ethanol being preferred.

JP2008-044907 provides the manufacture of phospholipid from solvent extraction of fish with the aim of improving the quality of the obtained phospholipid. The fish material is extracted with a non-polar solvent, e.g. hexane, heptane, isooctane, or benzene, a polar solvent, for example, methanol, ethanol, isopropanol, diethylether, ethyl acetate, acetone or a mixture of a non-polar solvent and a polar solvent, in particular a mixture of hexane and ethanol. The solvent is then removed from the extract, and the obtained fraction is then purified using adsorption filtration on diatomaceous earth.

WO2000/23456 discloses a method for extraction of lipid fractions from marine and aquatic animals, e.g. krill or fish. The method comprises suspending marine and aquatic material in a ketone such as acetone to extract lipids. The extraction may be carried out by successive acetone and alcohol treatments, e.g. using isopropanol or t-butanol, and the extraction should be performed at a temperature of about 5°C or less. The solubilised lipid fractions may then be separated from the solid material by techniques

such as filtration, centrifugation or sedimentation, with filtration being preferred. It appears from WO2000/23456 that the method disclosed therein may provide a fraction enriched in phospholipids. The method of WO2000/23456 is used specifically for extraction of phospholipids derived from natural marine or aquatic sources in WO2003/011873.

WO 2006/106325 discloses processes for the production of phospholipid compositions, e.g. marine phospholipids. One process of WO 2006/106325 comprises extracting a fish meal with an organic solvent to produce a lipid-containing liquid, and subjecting the liquid to microfiltration. The organic solvent may be a solvent in which phospholipids and triglycerides are soluble, such as hexane, isohexane, cyclohexane or heptane. According to WO 2006/106325 phospholipids aggregate into large molecular weight micellar structures in the non-polar alkane solvent, whereas all neutral lipids are dissolved in molecular disperse solution. The phospholipid micelles are considered too big to diffuse across microfiltration membranes having pore sizes of 0.1 to 0.5 µm, and phospholipids can therefore be isolated in this process.

In another process of WO 2006/106325 the alkane solvent extract may be subjected to solvent stripping and the extract or residue may be contacted with a second solvent in which neutral lipids are more soluble than polar lipids whereby to precipitate a phospholipid composition. The second solvent may be supercritical carbon dioxide, propane, carbon dioxide/propane mixtures, ethanol/water mixtures or ketones with acetone being preferred.

Several processes are known for separating phospholipids from oils of plant origin. However, the content of phospholipids in plant oil is typically different from that of fish oil. Thus, for example a plant oil may contain from 0.5 to 3 % phospholipids whereas the content in fish oil will normally be below 0.5 %, e.g. close to 0 %. Furthermore, the lipid composition of a fish oil will also be different from the lipid composition of a plant oil. For example, plant oils such as olive oil, rape seed oil and linseed oil do not contain omega-3 acids containing more than 18 carbon atoms, whereas phospholipids containing fatty acids with more than 18 carbon atoms, e.g. EPA (20 carbon atoms) and DHA (22 carbon atoms) are found in fish; these PUFA's are of particular interest. Moreover, in the processing of a plant oil the aim is typically the complete separation of oil from phospholipids without regard to keeping the phospholipids intact. Thus, plant phospholipids, "lecithins", are commonly

hydrolysed using e.g. acid or enzymes, in order to make them hydrophilic to ease their removal from plant oils.

US 4584141 discloses a modified conventional degumming process for removing impurities from triglyceride oils. Exemplary oils are plant oils, e.g. sunflower oil and soybean oil, although the process is also suggested for use with safflower oil, cottonseed oil, grapeseed oil, corn oil, rapeseed oil, rice bran oil, tallow and fish oil. In the process of US 4584141 the oil is mixed with hydrolysed phosphatide and water before separating the oil into an oil portion and a sludge portion and separating the sludge portion into an aqueous phase and an oil phase. US 4584141 thus requires addition of hydrolysed phospholipid, and it is therefore not suitable for isolating phospholipids as a product.

US 6172247 relates to methods for refining vegetable oils and by-products thereof. The process for refining vegetable oil uses organic acid, for example to produce a refined vegetable oil with improved odour, flavour, and storage stability, and a reduced content of e.g. free fatty acids and phosphatides. The process involves admixing a dilute aqueous organic acid solution with a heated stream of crude vegetable oil to give an acid-oil blend and separating a hydrated impurities phase and a purified vegetable oil phase. The hydrated impurities phase is a phosphatide concentrate and comprises hydrolysed lecithin. US 6172247 further discloses a "Lecithin Deodorizing" process comprising adding hydrogen peroxide to the hydrolysed lecithin fraction. US 6172247 require as a minimum addition of organic acid or hydrogen peroxide to provide the advantages of the processes, and it is not disclosed how intact phospholipids may be isolated, and further US 6172247 is limited to plant oils.

US2006/110521 relates to non-hydrogenated or partially hydrogenated non-animal oils, and US2006/110521 discloses processes for their preparation. The oil is prepared in the steps of preparation, cracking and dehulling, conditioning, milling, flaking or pressing, extracting, degumming, refining, bleaching and deodorising. Oil extraction may be performed using a solvent, such as n-hexane or isohexane, and degumming to remove the hydratable phosphatides is performed by adding water and heating. The process of US2006/110521 is however considered ill-suited for treating fish since these contain significant quantities of EPA and DHA.

US2005/129739 suggests that phospholipids can be recovered from fish, microalgae, or fungi through a physical or chemical degumming process. However, the degumming process is not disclosed, and further the only processes for oil extraction discussed in US2005/129739 are for extraction from plant material.

EP 0269277 discloses a process for degumming triglyceride oils for removing phospholipids or gums from the oils. The object of EP 0269277 is to produce an oil product with a reduced phosphorus content in the oil, and this is achieved by dispersing in the oil an organic acid or acid anhydride, at a temperature not greater than about 40°C, subsequently dispersing water in the oil, while maintaining this temperature, and then separating a sludge containing the gums from the oil. In the treatment according to EP 0269277 the phospholipids in the oil will be hydrolysed and hydrated by the process, and therefore the process is not suited for extracting intact phospholipids.

In light of the above there is a need for a robust and scaleable process capable of processing large amounts of raw material to obtain a phospholipid product. In particular, there is a need for an efficient process to isolate phospholipids and to provide a PUFA-enriched product from raw material derived from fish. The present invention addresses these points.

Disclosure of the invention

The present invention relates to a process for the isolation of a phospholipid from a fish oil. The process comprises the steps of:

-providing a fish oil containing lipids and phospholipids;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a phospholipid from the polar fraction.

In another aspect the invention relates to a process for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of:

-providing a fish oil containing PUFA's;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;
-isolating a PUFA-enriched fraction from the polar fraction.

In certain embodiments of the processes, the step of providing the fish oil comprises:
-extracting a fish material with an extractant solvent;
-removing the extractant solvent to provide the fish oil;
-optionally subjecting the fish oil to a solid-liquid separation.

Any fish oil is appropriate for the processes as long as the fish oil contains both lipids and phospholipids and/or PUFA's, and the fish oil may be obtained from any species of fish. In this context, the term "fish" covers both vertebrate and invertebrate species of marine animals, such as fish, molluscs, e.g. octopuses, squid and cuttlefish, or crustaceans, e.g. krill, shrimps, crabs, lobsters, mantis shrimp, woodlice, sandhoppers. Fish of particular relevance comprise sand eel (*Hyperoplus* sp., *Gymnammodytes* sp. or *Ammodytes* sp., e.g. *Hyperoplus lanceolatus*), sprat (*Sprattus sprattus*), herring (*Clupea* sp., e.g. *Clupea harengus*), anchovy (*Engraulis* sp., e.g. *Engraulis ringens)*, boarfish (*Capros aper)*, Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*Micromesistius poutassou*), and Jack Mackerel (*Trachurus murphyi*). Certain embodiments of the invention employ a fish material. The term "fish material" is to be understood broadly and may comprise any material derived from a fish as defined in the invention. The fish material may especially be any material derived from fish meal production. The fish material may also be derived from fish which has not been subjected to heat treatment; for example the fish material may be fish waste or the like from the production of fish for human consumption.

Any type of phospholipid from fish is relevant for the present process, and the term phospholipid within the present description is not limited to natural polar lipids but also includes chemically modified polar lipids. Phospholipids containing PUFA's are of particular interest in the present invention. The process of the invention is especially suitable for the isolation of an intact phospholipid. In particular the phospholipid is not hydrolysed in the process, and in certain embodiments of the invention no additive, which may hydrolyse a phospholipid is added in the process. Relevant compounds that may hydrolyse a phospholipid comprise acids, e.g. phosphoric acid, organic acids,

e.g. citric acid, acid anhydrides, hydrogen peroxide, and enzymes, e.g. lipases and phospholipases. The intact phospholipid comprising both fatty acid chains and the phosphate group attached to the central glycerol moiety will stabilise PUFA's, in particular EPA and DHA, from degradation, such as oxidative degradation. Furthermore, in other embodiments no surfactant is added in the process.

The fish oil may be obtained using any available process although the fish oil may advantageously be obtained according to the invention. When the phospholipids are obtained according to the invention the contents of contaminants, such as heavy metals, e.g. lead, cadmium, pesticides and pesticide break-down products, e.g. toxaphen, chlordan, DDD, DDE, DDT, endosulfan, endrin, heptachlor, hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), other harmful compounds, e.g. dioxins, polychlorinated biphenyls (PCBs), persistent organic pollutants (POPs) will be reduced. Thus, when a fish material is processed according to the invention the isolated phospholipids will contain unwanted contaminants in amounts acceptable for use in food products for humans or animals.

Any polar solvent can be used in the invention. Importantly, the polar solvent should be able to extract phospholipids from the fish oil. The polar solvent is selected such that it is immiscible with the fish oil, so that addition of the polar solvent to the fish oil will create a two-phase system. A preferred polar solvent is water.

Phospholipids may be found in a micellar form with the polar "head" facing the centre of the micelle or facing the solvent depending on the polarity of the solvent. In particular, the phospholipids may have a "critical micelle concentration" or CMC, so that when the phospholipids are present above this concentration in a solvent they will form micelles with the type of micelles depending on the polarity of the solvent. For example, when present in a polar solvent above the CMC the phospholipids will form micelles with the polar moiety facing the polar solvent. Below the CMC the phospholipids may be found in a generally dissolved form in either of a polar or an apolar solvent. The present inventors have now surprisingly found that when a fish oil containing phospholipids and/or PUFA's is mixed with a polar solvent it is possible to preferentially extract the phospholipids and/or PUFA's to the polar solvent in a micellar form by carefully considering the ratio of polar solvent to

fish oil and the nature of the polar solvent. The amount of polar solvent should be sufficient for the phospholipids to form micelles, and it will depend on the amount of phospholipids and free fatty acids. This allows that the phospholipids, and thereby also PUFA's, are extracted and isolated from the fish oil; in particular, the simple nature of the extraction, i.e. mixing a fish oil and a polar solvent, allows the process to be used in industrial scale. Furthermore, the invention allows that a fish oil fraction may be enriched in PUFA's, e.g. EPA and DHA, since these are common among the fatty acids chains of phospholipids in fish oil. The processes of the invention may further comprise analysing the polar fraction or the concentrated polar fraction for the presence of an excess of polar solvent, e.g. excess relative to the formation of phospholipid micelles. The analysis may be used to control, e.g. adjust, the amount of polar solvent used in the upstream polar solvent extraction. This is especially useful when the process is performed under continuous operation. The ratio of polar solvent to fish oil will generally be about 5:95 to about 25:75, although it is also possible to use an excess of polar solvent to fish oil. Using an excess of polar solvent evidently requires larger volumes of solvent and therefore using the ratio of about 5:95 to about 25:75 is especially advantageous in an industrial process since smaller scale equipment, e.g. centrifuges, can be employed. The reduced process volumes and the smaller scale equipment allow faster processing of the fish oil as less polar solvent has to be separated from the fish oil. Furthermore, by careful choice of the ratio of polar solvent to fish oil it is possible to minimise the amount of fish lipids trapped in the phospholipid micelles and thereby increase the purity of the phospholipids in the polar fraction.

Certain embodiments of the invention comprise a second extraction with the polar solvent. Thus, the process may further comprise the steps of:

-mixing the polar fraction with the polar solvent and fish oil;

-separating the mixture of the polar fraction, the polar solvent and the fish oil into a concentrated polar fraction and a lipid fraction. The separation is preferably a centrifugation. The concentrated polar fraction may also be analysed for the presence of an excess of polar solvent as described above. In general, the same considerations as for the first extraction with the polar solvent apply. However, in this second extraction fish oil, e.g. fish oil which has not been treated according to the invention or fish oil which has been extracted

from fish material with an extractant solvent according to the invention, is added, e.g. simultaneously, with the polar solvent to the polar fraction. The ratio of polar solvent to the polar fraction and the fish oil will generally be up to about 5% polar solvent, e.g. about 1% to about 4%, preferably about 2%; about 25% to about 75%, e.g. about 40% to about 60%, preferably about 50% fish oil and polar fraction to balance. This second extraction allows that a higher concentration of phospholipids can be obtained in the concentrated polar fraction compared to the polar fraction from the first polar solvent extraction. In particular, the polar fraction from the first polar solvent extraction will be enriched in phospholipids and the higher concentration of phospholipids is advantageous in sequestering further phospholipids from the additional, untreated fish oil added in the second polar solvent extraction. Thus, the second polar solvent extraction will provide a synergistic concentrating effect on phospholipids and PUFA's in the combined treated and untreated fish oil to provide an even higher concentration of phospholipids and PUFA's in the products obtained after removal of the polar solvent. For example, aqueous extraction of a fish oil provided from an ethanol-extracted fish material may yield a phospholipid product from the polar fraction with a phospholipid content of 15% and a content of EPA+DHA of about 25-30%. The second aqueous extraction may yield a phospholipid product from the concentrated polar fraction with a phospholipid content of 40% and a correspondingly increased content of EPA+DHA.

Several steps of the processes of the invention may comprise a centrifugation. In the context of the invention the term "centrifugation" and derived forms include any type of centrifugation, in particular using centrifuges suited for industrial scale of operation, e.g. disk stack centrifuges, decanter centrifuges, solid bowl centrifuges etc.

The transfer of the phospholipids and PUFA's from the fish oil to the polar solvent may take place instantaneously when the polar solvent is mixed with the fish oil, or the mixing step may have any duration as desired.

In certain embodiments it may be necessary to physically mix the polar solvent with the fish oil. For example, the mixing may be performed in a vessel equipped with a stirring blade, an impeller, a Rushton turbine, a propeller or the like, or the mixing vessel may otherwise be fitted to agitate the mixture of the fish oil with the polar solvent. In particular, when the mixture

of the fish oil with the polar solvent is physically mixed this generally involves subjecting the mixture to shear stress.

The mixing may take place at any temperature at which the polar solvent is liquid, e.g. the temperature may be decreased below ambient temperature, the mixing may take place at ambient temperature or the temperature may be increased during mixing. A high temperature will generally allow that the phospholipids are extracted at a higher rate than when the extraction is performed at a lower temperature. The temperature may thus be increased to any value below the boiling point of the polar solvent. In other embodiments, the mixing may take place at a decreased or at ambient temperature. In yet further embodiments, the temperature may be increased or decreased from the initial mixing temperature so that the temperature is changed during the mixing.

Following extraction of the phospholipids and PUFA's from the fish oil in the mixing step the mixture of the fish oil with the polar solvent is centrifuged to separate the two phases, i.e. the polar fraction comprising the phospholipids from the lipid fraction comprising other lipids from the fish oil. The centrifugal separation may be performed at an increased temperature. Any industrial centrifuge may be employed, e.g. a disk stack centrifuge, a decanter centrifuge, a solid bowl centrifuge. The separation of the two phases may advantageously be performed in a disk stack centrifuge. The centrifugal separation will provide a polar fraction with phospholipids and also a fish oil product depleted in phospholipids; another aspect of the invention relates to the phospholipid-depleted fish oil product obtainable in the process of the invention. In further embodiments of the processes the polar fraction is subjected to a second centrifugal separation, e.g. in a disk stack centrifuge, to concentrate the phospholipids and PUFA's further.

The polar solvent fraction, or phase, from the centrifugal separation comprises the phospholipids and PUFA's, and in the process of the invention the phospholipids are isolated from the polar solvent fraction. Likewise, a PUFA-enriched fraction may be isolated from the polar fraction. The isolation may comprise any appropriate method, such as evaporation of the polar solvent, distillation, e.g. vacuum distillation, of the polar solvent, or the phospholipids and/or PUFA's may be isolated adsorptively, e.g. using a chromatographic membrane or matrix or an adsorptive material such as diatoma-

ceous earth, or the phospholipids may be isolated using nano- or ultrafiltration. In the context of the invention "vacuum distillation" generally refers to a unit operation where heat is applied to the polar fraction with the simultaneous lowering of the pressure above the polar fraction in order to drive out the polar solvent from the polar face with the phospholipids. The term may also be used in the context of removal of an extractant solvent. Furthermore, the heat applied may be moderate, e.g. to a maximum of about 40°C to avoid heat modification of phospholipids. The phospholipids may be further dried, e.g. by subjecting the phospholipids to additional heat treatment, optionally at a decreased pressure. Removal of polar solvent and drying of the phospholipids may be performed in the same operation.

In another aspect the invention relates to the phospholipids obtainable in the process of the invention. In yet another aspect the invention relates to the PUFA's obtainable in the process of the invention.

In a specific embodiment of the process of the invention the fish oil is provided by extracting lipids and phospholipids, i.e. "fish oil", from a fish material as described above. Appropriate fish materials are fish meal, optionally in the form of pellets, presscake, e.g. from fish meal production, unprocessed fish, whole fish, specific parts of fish, such as skin, bone, meat, organs, e.g. fish liver, or fish waste etc.; in particular, the "fish material" may be a material derived from fish at any stage in the production of fish meal or the fish material may be derived from fish at any stage in the production of fish for human consumption. The fish material is extracted with an extractant solvent. Any solvent capable of extracting lipids including phospholipids is contemplated for use in the invention. The extractant solvent may be polar or apolar. Relevant apolar solvents comprise hydrocarbon solvents. The extractant solvent may also be supercritical carbon dioxide. Apolar solvents, such as hexane, e.g. isohexane, are preferred as extractant solvent in some embodiments. Other embodiments employ ethanol or ethanol-water-mixtures as extractant solvent.

The extraction will generally involve contacting a fish material with the extractant solvent. In a specific embodiment the fish material is a fish meal, e.g. in the form of pellets, although the fish meal may also be extracted without prior pelletisation. In another embodiment, the fish material is a presscake from fish meal production, and in yet another embodiment

whole fish or parts of fish are extracted with the extractant solvent. The fish material, e.g. fish meal, or fish meal pellets, is mixed with the extractant solvent, and the extraction with the extractant solvent may be performed under application of shear stress to the mixture of the fish material and the extractant solvent, for example using a stirring blade, an impeller, a Rushton turbine, a propeller or the like. The duration of the extraction step may be selected freely, e.g. the extraction may take place instantaneously, or the extraction may have a duration up to e.g. 24 hours. The extraction may advantageously be performed as a continuous process.

The extraction with the extractant solvent may be performed at ambient temperature or lower, or the temperature may be increased during the extraction, e.g. to any temperature up to the boiling point of the extractant solvent. In general, an increased temperature will result in a faster extraction of the phospholipids and PUFA's and lipids from the fish material. Ambient temperature or lower may be employed when it is of interest to ensure that the phospholipids and PUFA's are not modified by exposure to high temperature.

After the extraction with the extractant solvent it may be desirable to remove the extracted fish material from the extract. The extracted fish material will generally comprise particulate material of a relatively large size, e.g. from sub-millimetre up to the size of the pellets, if applicable. Any solid-liquid unit operation capable of separating such particulate from the extractant solvent may be applied to remove the extracted fish material from the extract. For example, the extracted fish material may be removed from the extract using sieving, filtration or centrifugation. In a further aspect the invention relates to the extracted fish material obtainable in the process.

The extractant solvent is removed from the extract following the extraction. Any appropriate method may be used to remove the extractant solvent, such as distillation, e.g. vacuum distillation, or evaporation. The extractant solvent removed from the extract may be recycled in the process to be added to and contacted with a further portion of fish material or fish material pellets. This allows for an efficient continuous processing of fish material to isolate phospholipids.

The fish oil resulting from the removal of the extractant solvent may be subjected to a solid-liquid separation prior to processing to isolate phos-

pholipids as described above. Any solid-liquid unit operation may be employed, although filtration is preferred. In a further aspect the invention relates to a protein product obtainable by filtration of the extract.

The embodiments of the process of the invention disclosed above may advantageously be performed under continuous operation. An advantage of continuous operation is hygiene since all process steps may be carried out in closed systems to prevent contamination from air or operators. Furthermore, the stability of the product, e.g. phospholipids and PUFA's, is improved since storage in tanks and the like is minimised in a continuous process. Continuous operation is particularly advantageous since it allows efficient processing of large quantities of material, e.g. in the order of hundreds of tonnes. Efficient processing of such quantities of material is particularly relevant for isolating a product from a starting material where the product is present in low amounts, such as isolating phospholipids from fish material. Furthermore, when the process steps allow continuous operation simple integration of the process steps in a process train of industrial scale is possible.

Thus, in yet a further aspect the invention relates to an integrated continuous process for producing a product from a fish material, such as a fish meal or fish meal pellets. The product may be a phospholipid product or a PUFA-product. The term "integrated" is to be understood broadly, but it especially refers to a situation where a process stream, such as a waste stream, e.g. a stream of solvent, e.g. extractant solvent or polar solvent, removed from a process step is recycled in an earlier, or upstream, process step. For example, in this process the fish material is extracted with an extractant solvent as described above, before removal of the extractant solvent likewise as described above. The removed extractant solvent may be recycled in the process, although further extractant solvent may also be added to retain the mass balance of extractant solvent in the process. In specific embodiments solid-liquid separation unit operations are included in the process following the extraction and following the removal of the extractant solvent. The fish oil is then treated to isolate phospholipids as described above. Thus, the fish oil is mixed with the polar solvent in a vessel appropriate for continuous processing before leading the process stream to a centrifuge likewise suited for continuous operation. The stream of polar solvent containing phospholipids is then led to the removal of polar solvent optionally combined with

a drying step, e.g. by treating at increased temperature and decreased pressure. This operation may also be performed continuously, and the polar solvent may be recycled and added to fish oil provided from the prior extraction step. In certain embodiments the mixing and extraction steps are performed at increased temperatures. However, in a specific embodiment, e.g. where the fish material is fish which has not been subjected to heat treatment, all process steps are performed without subjecting the fish material to excessive temperatures, e.g. temperatures above 40°C, at any stage of the process. An integrated process may further comprise analysing the polar fraction and/or the optional concentrated polar fraction for the presence of an excess of polar solvent and controlling the amount polar solvent added to the fish oil or the mixture of polar fraction and fish oil based on the result of the analysis. Thus, the analysis may provide information to a feedback loop allowing adjustment of the amount(s) of polar solvent added in the respective polar solvent extractions to the optimal ratio of polar solvent to fish oil or mixture of polar fraction and fish oil.

It is within the knowledge of the skilled person to design the integrated process for continuous operation in order to isolate phospholipids from fish material when considering the amount of fish material to be processed and the amount of phospholipids contained in the fish material. For example, the skilled person can select reactor vessels, and their required size and capacity, appropriate for continuous operation and calculate the necessary residence times in the vessels and the corresponding material flow rates in the vessels. All steps for which an increased temperature is relevant as outlined above, are preferably performed at increased temperature. This will advantageously minimise the risk of microbial contamination, and further lead to a faster overall process.

Brief description of the figures

In the following the invention will be explained in greater detail with the aid of examples of embodiments and with reference to the schematic drawings, in which

Fig. 1 shows a process diagram of an embodiment of the invention;

Fig. 2 shows a process diagram of an embodiment of the invention;

Fig. 3 shows a process diagram of an embodiment of the invention.

Detailed description of the invention

The present invention relates to a process for isolation of a phospholipid from a fish oil comprising the steps of:

-providing a fish oil containing lipids and phospholipids;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a phospholipid from the polar fraction.

In another aspect invention relates to a process for producing a poly-unsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of:

-providing a fish oil containing PUFA's;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a PUFA-enriched fraction from the polar fraction. In the context of the present invention a PUFA is a fatty acid containing more than 18 carbon atoms and two or more unsaturated bonds. Preferred PUFA's are EPA and DHA.

A process diagram of the invention is illustrated in Fig. 1.Fig. 1 shows the process with the optional second polar solvent extraction indicated, and furthermore, Fig. 1 illustrates how the result of the analysis for excess polar solvent may be used to control the upstream polar solvent extraction(s).

The fish oil may be provided by:

-extracting a fish material with an extractant solvent;

-removing the extractant solvent to provide the fish oil;

-optionally subjecting the fish oil to a solid-liquid separation.

Specific embodiments of the processes are illustrated in Fig. 2 and Fig. 3. Fig. 2 and Fig. 3 indicate the optional second polar solvent extractions. The processes in Fig. 2 and Fig. 3 may both provide a phospholipid product or a PUFA-product, and both may be integrated to be performed as integrated continuous processes where e.g. solvent streams are recycled to be used in

upstream extraction steps. Further, both processes may comprise analysis steps, as described above, to provide information for use regarding addition of polar solvent in the respective extractions.

The fish oil is mixed with a polar solvent. The "polar solvent" is immiscible with the fish oil, but the polarity of the solvent allows that phospholipids and PUFA's are extracted from the fish oil due to the formation of phospholipid micelles in the polar solvent. Any solvent with this capability is contemplated for use in the process of the invention. In particular, polar solvents typically have a high dielectric constant, such as above 15. A preferred polar solvent is water, e.g. deionised water. The ratio of polar solvent to fish oil will generally be from about 5:95 to about 25:75. The amount of polar solvent to fish oil will typically dependent on the exact nature of the polar solvent. For example, when water is selected as the polar solvent the ratio of water to fish oil may be from about 10:90 to about 20:80. The optimal amount of polar solvent may be determined by analysis of the polar fraction and the result of the analysis may be used to adjust the amount of polar solvent to be mixed with the fish oil. In particular when the process is performed continuously the result of the analysis may be employed in a feed-back loop to optimise the process when it is running. Specific embodiments of the invention thus comprise the step of analysing the polar fraction, or optionally the concentrated polar fraction, for the presence of an excess of polar solvent. The result of the analysis may be used to adjust, in particular during continuous operation, the amount of polar solvent mixed with the fish oil. Thus, for example when a relatively dense polar solvent, such as water, is used the amount of polar solvent to be mixed with the fish oil or the mixture of the polar fraction and the fish oil may be determined by subjecting a sample from the polar fraction to lab scale centrifugation and checking the test tube for the presence of free polar solvent in the bottom of the tube. The presence of free polar solvent will indicate that an excess amount of polar solvent was present during the step of mixing the fish oil with water. The amount of polar solvent to be added in the continuous process may be adjusted to the minimum excess required which is optimal for the separation.

When the processes of the invention comprise a second polar solvent extraction of the polar fraction as outlined above, the concentrated polar fraction may also be analysed for excess of polar solvent as explained above. The

duration of the mixing step should be sufficient to provide a polar fraction, e.g. an aqueous fraction, enriched in phospholipids and PUFA's and a lipid fraction depleted in phospholipids. The mixing may be for any predetermined period of time and the mixing is not limited regarding the temperature. However, the duration of the mixing should be sufficient to separate the phospholipids from the fish oil.

The mixing temperature may be selected to optimise extraction of phospholipids and PUFA's, and in certain embodiments it is generally increased from ambient temperature to a temperature below the boiling point of the polar solvent. For example, when the polar solvent is water the temperature may be from about 50°C to about 95°C or higher, such as about 60°C, about 70°C, about 80°C or about 90°C. An increased temperature may provide a faster extraction of the phospholipids and PUFA's from the fish oil. In another embodiment the mixing temperature is maintained in a range from below ambient, e.g. about 5°C, to moderately increased, e.g. to about 40°C, such as about 10°C, about 20°C or about 30°C. Certain species of phospholipids and especially PUFA's, may be modified by high temperatures, and in this temperature range it can be ensured that the phospholipids and PUFA's are not modified, e.g. damaged by the high temperature. In particular it may be of interest to keep the temperature as low as possible. In some embodiments all process steps are performed at a low temperature, and in others some steps may be performed at low temperature whereas others are performed at increased temperature. In general, brief exposure of a fish material or a mixture or an extract etc. in a step of the process of the invention to high temperature will not be detrimental to the phospholipids. In particular, a process stream or the phospholipid product may be subjected to pasteurisation without modifying the phospholipids. Thus, any step of the inventive process may also comprise a pasteurisation step. Pasteurisation is well known to the skilled person.

The mixing time will typically be up to about 1 hour, such as about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes or about 60 minutes. In a specific embodiment water is used as the polar solvent, which is mixed with the fish oil at a ratio of 15:85 for about 20 minutes at about 80°C, preferably in a continuous process. This ratio of water to fish oil may also be used in embodiments using other mixing tem-

peratures. Likewise, this ratio is also relevant for other polar solvents.

The mixture, i.e. the two-phase system, with the polar fraction and the lipid fraction is centrifuged to separate the polar fraction from the lipid fraction, optionally at an increased temperature, e.g. at a temperature of about 40°C to about 75°C, e.g. at about 70°C. In particular, an increased temperature may be used when the preceding mixing step is performed at an increased temperature, and further when subsequent removal of the polar solvent by vacuum distillation is intended, centrifugation at an increased temperature is preferred. Likewise, when the mixing temperature is kept low, as defined above, to ensure that phospholipids are not modified due to heating, it may be of interest to maintain the temperature in this range in the centrifugation step. In general, the polar solvent may be present as drops or droplets in the fish oil. Further, the phospholipids in micellar form in the polar solvent may function as surfactants to create an "oil-in-polar-solvent emulsion", e.g. an oil-in-water emulsion. Any centrifugation operation capable of separating two liquid phases, e.g. in the form of drops or droplets of one phase in the other, may be employed, but it is preferred that a disk stack centrifuge is used. A particularly preferred embodiment employs two consecutive disk stack centrifuges to centrifuge the mixture of the fish oil and the polar solvent, or optionally the mixture of the polar fraction, the polar solvent and the fish oil. In this embodiment the first centrifuge serves to separate water and phospholipids, i.e. the polar fraction or concentrated polar fraction, from the lipid fraction. The subsequent, e.g. serially connected, disk stack centrifuge concentrates the phospholipids in the polar fraction or concentrated polar fraction from the upstream disk stack centrifuge. In a specific set-up the first centrifuge has a distance between the disks of 0.6 mm, and the second centrifuge has a distance between the disks of 0.8 mm.

The polar solvent is subsequently removed from the mixture of the polar fraction e.g. by vacuum distillation. For example, when the polar solvent is water it may be removed by increasing the temperature to be in the range of about 60°C to about 85°C, e.g. about 80°C or about 85°C while reducing the pressure so that the water boils, e.g. while reducing the pressure to about -0,7 bar to about -0,9 bar. The water may thus be removed from the phospholipid fraction, which is further dried, in about 1 hour to about

3 hours. It is also possible to employ a different combination of temperature and pressure, but when the process employs increased temperatures, the temperature and pressure are typically selected such that the water is boiling. Likewise, in embodiments where excessive temperatures are avoided to prevent modification of phospholipids it may be desirable to maintain a moderate temperature when removing the polar solvent. These considerations also apply when other polar solvents are employed. The temperature may advantageously be increased using indirect steam when relevant.

In another embodiment of the process of the invention, fish material is extracted with an extractant solvent to provide fish oil for isolation of phospholipids. In a preferred embodiment the fish material is fish meal, which may be pelletised prior to extraction, e.g. at a temperature of about 50°C, for example with addition of steam to optimise pelletisation. In yet another embodiment, the fish material is a presscake from the production of fish meal. In very broad terms the "presscake" refers to the material obtained after initially heating fish or fish material to coagulate protein, rupture fat depots and liberate oil and physico-chemically bound water, followed by pressing (or optionally centrifugation) to, at least partially, remove liquids from the mass. The presscake may be extracted directly or the presscake may be subjected to disruption or comminution or the like prior to extraction. When presscake is treated according to the process of the invention the fish oil extracted with the extractant solvent comprises a higher content of phospholipids since the neutral oils have been removed during the pressing. This further allows that smaller amounts, e.g. relative to the amount of fish material, of extractant solvent are employed. Presscake is therefore a preferred fish material in the present invention. In a further embodiment, whole fish or parts of fish are extracted with the extractant solvent, specifically the whole fish or parts of fish may be extracted without any prior heat treatment. When the fish material has not been subjected to prior heat treatment, whole fish may be extracted directly, or the whole fish may be subjected to comminution or disruption prior to extraction. The extraction may take place in any appropriate vessel. In particular, the extraction vessel may be provided with a device to apply shear stress to the mixture of the fish material and the extractant solvent, e.g. the vessel or extractor may be equipped with stirrer blades or the like.

In the context of the present invention, the term "extractant solvent" refers to any solvent that may extract a lipid fraction, e.g. fish oil or phospholipids and PUFA's, from a fish material. Typical extractant solvents comprise apolar solvents, such as alkanes, e.g. pentane, hexane, heptane, octane etc., and aromatic hydrocarbons, e.g. benzene, toluene, and the like. An apolar solvent may also be referred to as a "non-polar solvent". Hydrocarbon solvents comprising heteroatoms may also be employed as extractant solvent, as long as the hydrocarbon solvent may extract a lipid fraction comprising phospholipids from a fish material. The extractant solvent is preferably liquid at ambient temperature and pressure. A preferred extractant solvent is hexane, in particular isohexane. It is noted that in the context of the present invention supercritical carbon dioxide is also contemplated for use as an extractant solvent. Other relevant extractant solvents are alcohols, such as methanol, ethanol, e.g. 96% ethanol in water, propanol, isopropanol or butanol, optionally mixed with water, ketones, such as acetone, ethers or esters etc. It is also possible to employ mixtures of two or more extractant solvents. In a specific embodiment the extractant solvent is ethanol or a mixture of ethanol and water, e.g. with a concentration of ethanol in water from 10% up to 30%, or with a concentration of ethanol in water above 70%, for example the concentration of ethanol may be about 80% or about 85%. In a preferred embodiment the extractant solvent is 96% ethanol. When 96% ethanol is employed to extract presscake the ratio of ethanol to presscake is typically from about 1:2 to about 1:5, preferably about 1:3. The extraction time may be about 2 hours, at the temperature about 65°C.

The extraction may be performed at ambient or lower temperature, or it may be performed at an increased temperature. For example, in one embodiment the extraction may be performed at a temperature in the range of about 40°C to about 70°C, such as about 40°C, about 50°C, about 60°C, or about 70°C. In another embodiment the extraction with the extractant solvent is performed at a low temperature of about 5°C to about 40°C, e.g. about 10°C, at about 20°C or about 30°C. When the extraction is performed at low temperature other process steps may also be performed at low temperature. Extraction at increased temperature can increase the extraction efficiency, and in particular the temperature may be controlled to increase the efficiency of extraction of phospholipids, which may be extracted selec-

tively, e.g. extraction at about 50°C to about 60°C when the extractant solvent is isohexane will provide optimal extraction of phospholipids using this solvent. The extraction temperature is preferably below the boiling point of the extractant solvent. The same considerations for employing a low temperature in the step of mixing fish oil with the polar solvent generally apply also for extraction with the extractant solvent and any subsequent steps.

The duration of the extraction is not limited and may be selected to provide sufficient extraction of lipids, especially phospholipids, from the fish material. For example, the duration may be from about 0.5 hours to about 10 hours or more, e.g. about 1 hours, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours or about 10 hours. Extraction with isohexane may be performed with a duration of e.g. about 2 hours.

Extraction of the fish material with the extractant solvent will result in a liquid fraction comprising the lipids, including also polar lipids, such as phospholipids and PUFA's, from the fish material. The liquid phase comprising the lipids and the extractant solvent may be referred to as an "extract". This extract may be subjected to a solid-liquid separation to remove solid debris, e.g. extracted fish material, from the liquid phase with the phospholipids. This liquid phase may also be referred to as a "crude oil". Any appropriate solid-liquid separation operation may be employed, for example, sieving, filtration, centrifugation.

The extractant solvent can be removed from the crude oil or the extract using any appropriate method. In particular, the extractant solvent may be removed from the crude oil or the extract using increased temperature and decreased pressure (referred to in the context of the invention as "vacuum distillation"). For example, isohexane may be removed by increasing the temperature to about 70°C to about 90°C, e.g. about 85°C under a reduced pressure (e.g. under "vacuum") of about 5 mbar to about 50 mbar. Under these conditions isohexane may be removed in about 10 minutes to about 20 minutes. Removal of the extractant solvent from the extract or crude oil will provide a fish oil comprising both polar and non-polar lipids from the fish material. The fish oil is preferably free of extractant solvent, e.g. the fish oil contains less than 10 ppm extractant solvent, such as less than 5 ppm or less than 2 ppm extractant solvent. The extractant solvent is preferably recycled

in the process by adding to fish material to be processed according to the invention.

The fish oil may be subjected to a solid-liquid separation, such as filtration to remove residual protein and other impurities. For example, the fish oil may be subjected to a first filtration to remove crude material followed by a finer filtration step to remove fines.

In an embodiment of the invention the processing of fish material to fish oil will result in phospholipids with reduced contents of unwanted contaminants. For example, the phospholipids will comply with standards of the European Union regarding concentrations of contaminants.

In a specific embodiment, an integrated process is set up as a continuous process, in which about 10 tonnes/hour of fish material is extracted with about 15 tonnes/hour of isohexane as explained above. Removal of the isohexane yields about 1.5 tonnes/hour of fish oil from which phospholipids are isolated according to the invention. Thus, the process is evidently scaleable to a large industrial scale.

The invention will now be explained in the following non-limiting examples. As will be evident to the skilled person variations are possible without deviating from the invention.

Comparative example

A batch of fish oil was prepared from sprat according to a prior art technique. The composition of the fish oil thus prepared is summarised in Table 1.

Table 1 Fatty acid composition of fish oil prepared according to the prior art.

Fatty acid	**Danish sprat**
	%
C14:0	6.4
C15:0	0.8
C16:0	18.9
C16:1	5.7
C18:0	3.1
C18:1	18.5

Fatty acid	Danish sprat
C18:2	2.2
C18:3	1.7
C18:4n3	<0.01
C20:1	6.8
C20:4n6	0.5
C20:5n3 (EPA)	8.9
C22:1	6.9
C22:5n3 (DPA)	0.9
C22:6n3 (DHA)	13.2

Example 1

A batch of 500 tonnes of fish meal was treated in a continuous plant according to the invention. The raw material fish meal was extracted with isohexane as an extractant solvent following initial pelletisation. After evaporation of the isohexane the fish oil was extracted with water as a polar solvent before centrifugation in a disk stack centrifuge. Isohexane removed from the fish oil was recycled in the process. The phospholipids were finally isolated from the polar fraction by drying to remove the water. The parameter values employed in the process are summarised in Table 2 below.

Table 2 Process parameters for phospholipid preparation

Unit operation	Reaction conditions	Product
Pelletisation	50°C	
Extraction with isohexane	2 hours 52°C	
Sieving to remove dry matter		
Isohexane removal (evaporation)	10 mbar 85°C	60 tonnes of fish oil with phospholipids
Filtering and polishing		
Mixing with water at a water:fish oil ratio of	50°C	

Unit operation	Reaction conditions	Product
15:85		
Extraction under agitation	20 minutes 60°C	
Centrifugation in a disk stack centrifuge		Polar fraction with phospholipids; Lipid fraction of phospholipid depleted fish oil
Phospholipid isolation (water removal to 1% moisture)	2 hours 5 mbar 85°C	10 tonnes of product containing 40% phospholipids and 60% fish oil with 26% EPA+DHA

The dry matter occurring after the solid-liquid separation steps represented protein products of the invention, and the lipid fraction from the centrifugation represented a phospholipid depleted fish oil product of the invention. The polar fraction with phospholipids and the product obtained from this fraction after water removal represented different embodiments of the phospholipid product obtainable in the process of the invention. The composition of the fish oil provided by the extraction is compared to the composition of the final product in Table 3 and Table 4 below.

Table 3 Fatty acid composition of fish oil prepared according to an embodiment of the invention

Fatty acid	Extracted fish oil	Final product
	%	%
C14:0	5.5	4.2
C15:0	0.5	0.5
C16:0	16.8	18.8
C16:1	10.2	6.6
C18:0	3.1	4.9
C18:1	9.7	10.9
C18:2n6	2.1	2.0
C18:3n6	0.5	0.2
C18:3n3	1.1	0.9

Fatty acid	Extracted fish oil	Final product
C18:4n3	2.9	1.8
C20:1	3.4	1.5
C20:4n6	0.7	1.0
C20:5n3 (EPA)	12.3	13.5
C22:1	0.2	1.4
C22:5n3 (DPA)	0.8	1.3
C22:6n3 (DHA)	15.4	19.3
C24:1	0.8	0.1

Table 4 Phospholipid composition of fish oil prepared according to an embodiment of the invention

Phospholipids	Extracted fish oil	Final product
Phosphatidylcholine	6.3	16.1
Lyso-phosphatidylcholine	1.2	5.4
Phosphatidylinositol	0.7	1.8
Spingomyelin	1.6	3.5
Phosphathidylethanolamin	1.8	4.5
Lyso-phosphathidylethanolamin	0.5	1.4
Acylphosphatidylethanolamine	2.1	6.3
Phosphatic acid	0.3	0.9
Lyso-phosphatic acid	0.1	0.2
Total phospholipids	16.6	44.3

It is evident from Table 3 and Table 4 that the process of the invention provided a product enriched in phospholipids, and that the process of the invention further provided a product enriched in PUFA's compared to the process of the prior art.

Example 2

Fish were heated up to 85°C and pressed to provide a presscake, which was subjected to continuous ethanol (96% ethanol in water) extraction for two hours at 65°C. The extracted presscake was subjected to solid-liquid separation to separate a crude oil containing ethanol from the extracted presscake. Ethanol was evaporated at 85°C under vacuum to provide an

ethanol-free fish oil, which was filtered to remove debris from the fish oil. The fish oil was then extracted with water as a polar solvent at 80°C for 20 minutes followed by treatment in a disk stack centrifuge at 70°C. The polar fraction from the centrifugation was mixed with fish oil and water at a ratio of 48% polar fraction to 50% fish oil and 2% water, and the mixture was extracted at 80°C for 20 minutes. The extracted mixture was then centrifuged in a disk stack centrifuge at 70°C before removal of the water by drying at 85°C under vacuum. This yielded a product enriched in phospholipids and PUFA's. The composition of the fish oil provided by the ethanol extraction is compared to the composition of the final product in Table 5 and Table 6 below.

Table 5 Fatty acid composition of fish oil prepared according to an embodiment of the invention

Fatty acid	**Ethanol extracted fish oil**	**Final product**
	%	%
C14:0	1.9	1.6
C15:0	0.2	0.5
C16:0	22.7	18.8
C16:1	3.2	4.5
C18:0	4.8	4.9
C18:1	12.6	10.9
C18:2n6	0.6	1.5
C18:3	0.4	0.2
C18:4n3	0.6	1.8
C20:1	1.6	1.5
C20:4n6	0.8	1.0
C20:5n3 (EPA)	9.7	10.5
C22:1	1.8	1.4
C22:6n3 (DHA)	19.7	24.3

Table 6 Phospholipid composition of fish oil prepared according to an embodiment of the invention

Phospholipids	Ethanol extracted fish oil	Final product
Phosphatidylcholine	9.5	24.2
Lyso-phosphatidylcholine	1.3	3.3
Phosphatidylinositol	0.9	2.3
Spingomyelin	0.9	2.3
Phosphathidylethanolamin	1.4	3.6
Lyso-phosphathidylethanolamin	0.3	0.8
Acylphosphatidylethanolamine	0.8	2.1
Phosphatic acid	0.1	0.3
Lyso-phosphatic acid	0.1	0.3
Total phospholipids	15.6	>40

It is evident from Table 5 and Table 6 that the process of the invention provided a product enriched in phospholipids, and that the process of the invention further provided a product enriched in PUFA's compared to the process of the prior art.

PATENT CLAIMS

1. A process for the isolation of a phospholipid from a fish oil comprising the steps of:

-providing a fish oil containing lipids and phospholipids;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a phospholipid from the polar fraction.

2. A process for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of:

-providing a fish oil containing PUFA's;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a PUFA-enriched fraction from the polar fraction.

3. The process according any one of claims 1 to 2, wherein the step of providing the fish oil comprises:

-extracting a fish material with an extractant solvent;

-removing the extractant solvent to provide the fish oil;

-optionally subjecting the fish oil to a solid-liquid separation.

4. The process according to any one of claims 1 to 3, wherein the ratio of polar solvent to fish oil is about 5:95 to about 25:75.

5. The process according to any one of claims 1 to 4 further comprising the steps of:

-mixing the polar fraction with the polar solvent and fish oil;

-separating the mixture of the polar fraction, the polar solvent and the fish oil into a concentrated polar fraction and a lipid fraction.

6. The process according to claim 5, wherein the step of separating comprises centrifuging the mixture of the polar fraction, the polar solvent and the fish oil to separate a concentrated polar fraction from a lipid fraction.

7. The process according to claim 5 or 6, wherein mixture comprises up to about 5% polar solvent; about 25% to about 75% fish oil and polar fraction to balance.

8. The process according to any one of claims 1 to 7, wherein the polar solvent is water.

9. The process according to any one of claims 1 to 8, wherein no additive, which may hydrolyse a phospholipid, is added in the process, such as those selected from the group consisting of acids, e.g. phosphoric acid, organic acids, e.g. citric acid, acid anhydrides, hydrogen peroxide, and enzymes, e.g. lipases and phospholipases.

10. The process according to any one of claims 1 and 3 to 9, wherein intact phospholipids are isolated.

11. The process according to any one of claims 2 to 9, wherein the PUFA's are eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).

12. The process according to any one of claims 1 to 11 further comprising analysing the polar fraction or the concentrated polar fraction for the presence of an excess of polar solvent.

13. The process according to any one of claims 1 to 12 further comprising the step of centrifuging the polar fraction or the concentrated polar fraction to concentrate the phospholipids and/or the PUFA's.

14. The process according to any one of claims 1 to 13, wherein the fish material is derived from fish meal production, such as a fish meal or a presscake.

15. The process according to any one of claims 1 to 14, wherein the fish material is derived from sand eel (*Hyperoplus* sp., *Gymnammodytes* sp. or *Ammodytes* sp., e.g. *Hyperoplus lanceolatus*), sprat (*Sprattus sprattus*), herring (*Clupea* sp., e.g. *Clupea harengus*), anchovy (*Engraulis* sp., e.g. *Engraulis ringens)*, boarfish (*Capros aper)*, Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*Micromesistius poutassou*), or Jack Mackerel (*Trachurus murphyi*).

16. The process according to any one of claims 1 to 14, wherein the fish material is derived from krill, shrimps, crabs, lobsters, mantis shrimp, woodlice, sandhoppers.

17. The process according to any one of claims 1 to 16, wherein the fish material is derived from fish which has not been subjected to heat treatment.

18. The process according to any one of claims 1 to 17, wherein the step of mixing the fish oil or the mixture of the polar fraction and the fish oil with the polar solvent is performed at an increased temperature.

19. The process according to any one of claims 1 to 18, wherein the step of mixing the fish oil or the mixture of the polar fraction and the fish oil

with the polar solvent is performed at a temperature of about 5°C to about 40°C.

20. The process according to any one of claims 1 to 19, wherein the isolation of the phospholipid from the polar fraction or the concentrated polar fraction comprises vacuum distillation of the polar fraction to remove the polar solvent.

21. The process according to any one of claims 1 to 20, wherein the centrifugation is performed in a disk stack centrifuge.

22. The process according to any one of claims 3 to 21, wherein the extractant solvent is an apolar solvent, e.g. hexane.

23. The process according to any one of claims 3 to 21, wherein the extractant solvent is ethanol or a mixture of ethanol and water.

24. The process according to any one of claims 3 to 21, wherein the extractant solvent is 96% ethanol, and the ratio of ethanol to fish material is from about 1:2 to about 1:5, preferably about 1:3.

25. The process according to claim 23, wherein the temperature is 65°C.

26. The process according to any one of claims 3 to 23, wherein the extraction with the extractant solvent is performed at an increased temperature.

27. The process according to any one of claims 3 to 23, wherein the extraction with the extractant solvent is performed at a temperature of about 5°C to about 40°C.

28. The process according to any one of claims 1 to 27, wherein the process is performed under continuous operation.

29. An integrated continuous process for producing a phospholipid product or a PUFA-product from a fish material comprising treating a fish material according to the process of any one of claims 3 to 28, wherein a process stream is recycled in an earlier process step.

30. An integrated continuous process according to claim 29 further comprising analysing the polar fraction or the concentrated polar fraction for the presence of an excess of polar solvent and controlling the amount polar solvent added to the fish oil or the mixture of polar fraction and fish oil based on the result of the analysis.

31. A phospholipid product obtainable in the process according to any one of claims 1 to 30.

32. A PUFA-product obtainable in the process according to any one of claims 2 to 30.

33. A phospholipid-depleted and/or PUFA-depleted fish oil product obtainable in the process according to any one of claims 1 to 30.

34. A protein product obtainable from the extractant solvent-extracted fish material of the process according to any one of claims 1 to 30.

35. A protein product obtainable from the polar solvent-extracted fish material of the process according to any one of claims 1 to 30.

36. An extracted fish material obtainable in the process according to any one of claims 1 to 30.

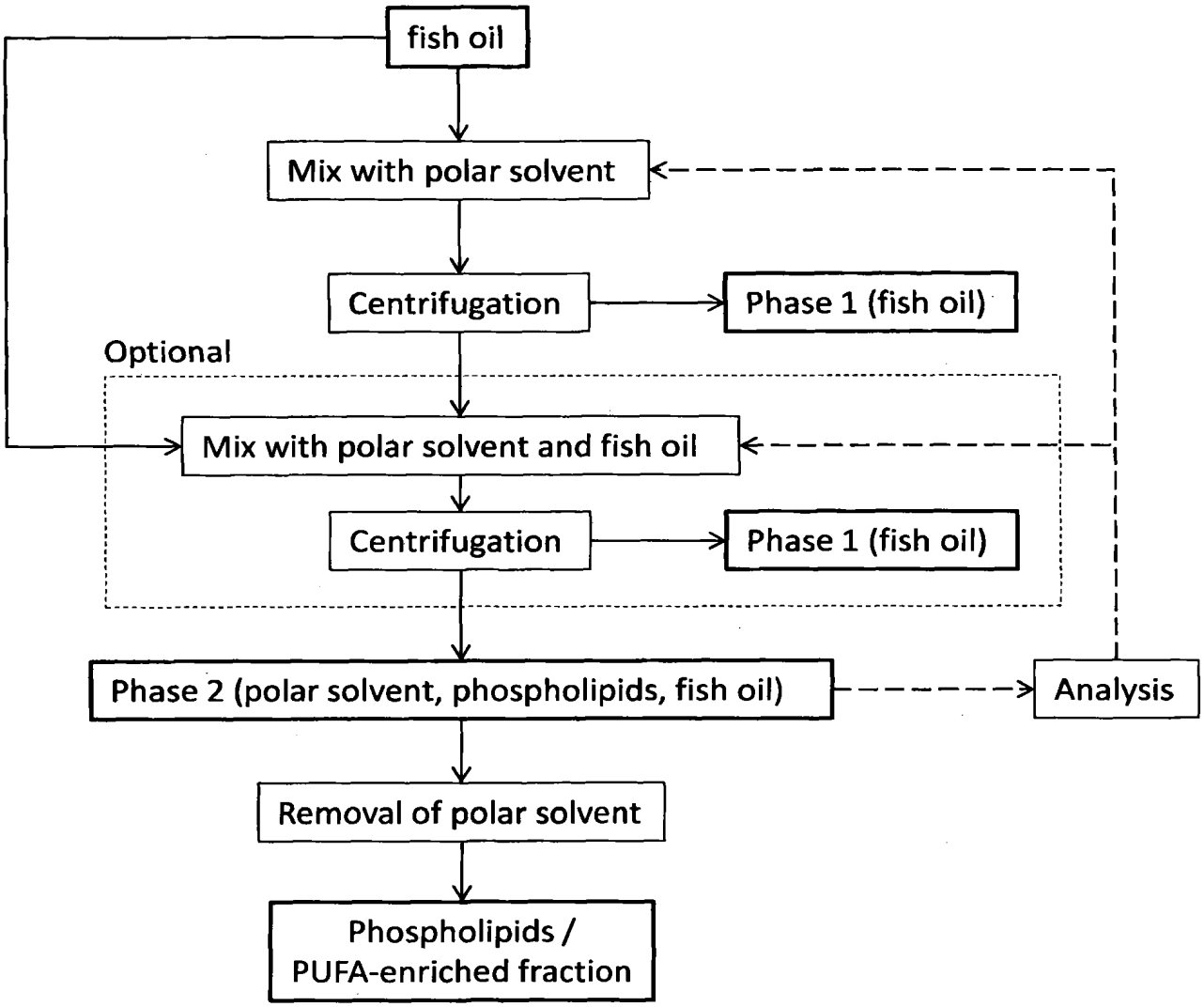

Fig. 1

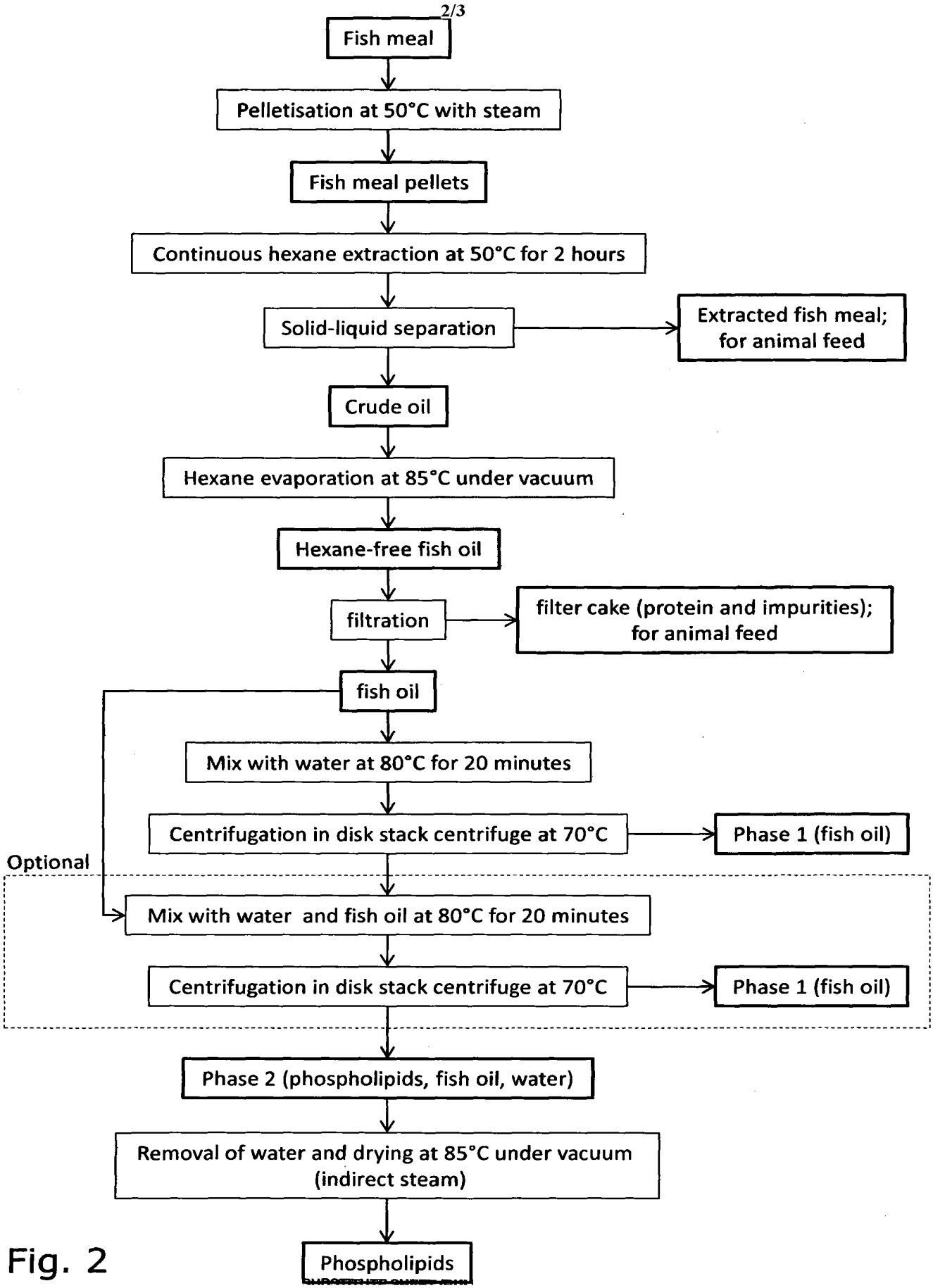

Fig. 2

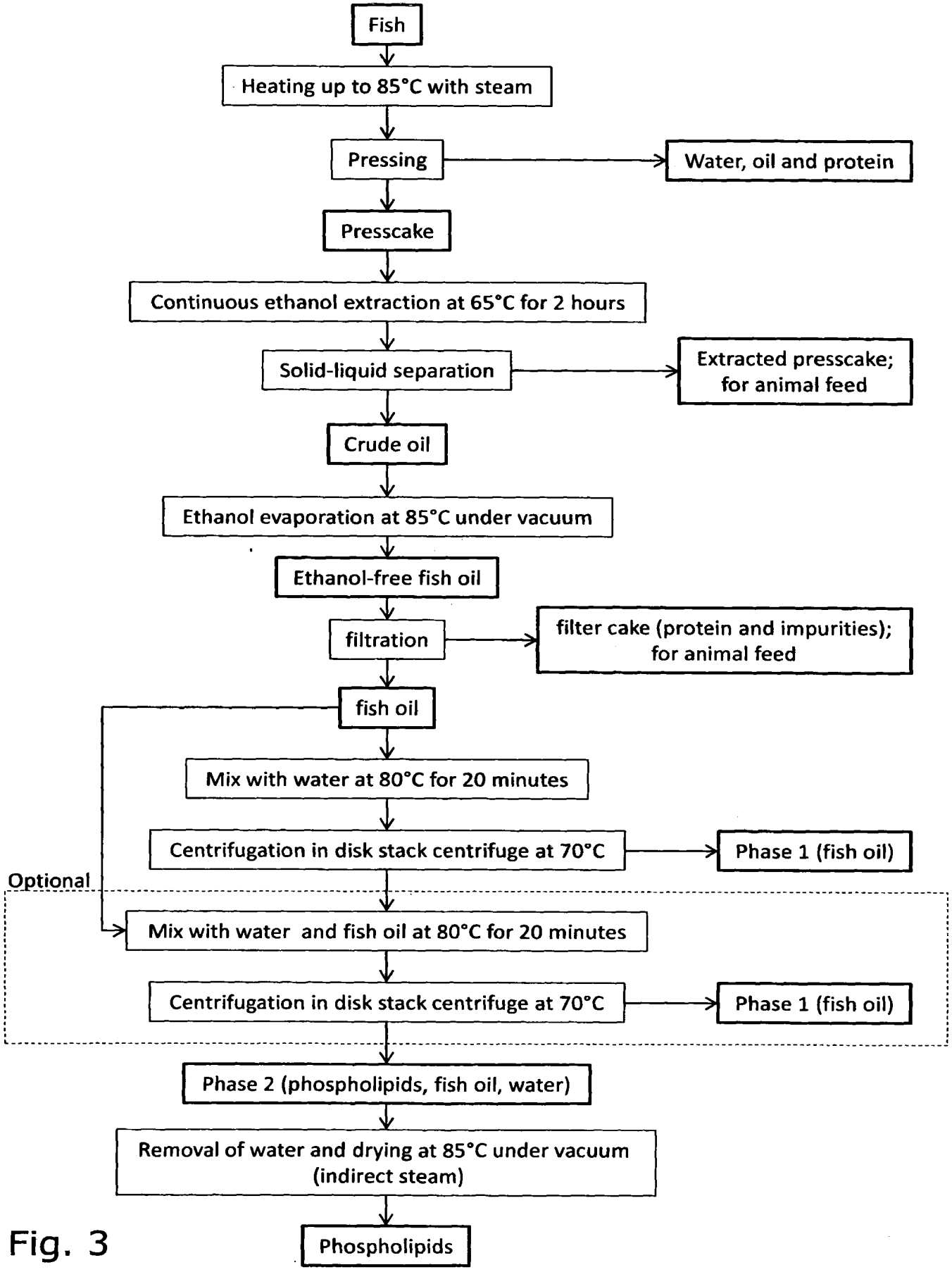

Fig. 3

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[Continued on next page]

(54) Title: A PROCESS FOR THE ISOLATION OF A PHOSPHOLIPID

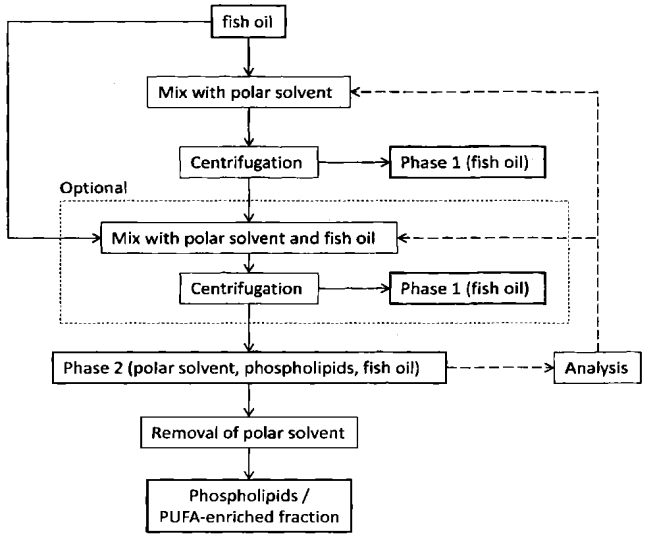

Fig. 1

(57) Abstract: The present invention relates to processes for the isolation of a phospholipid and for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of -providing a fish oil containing lipids and phospholipids; -mixing the fish oil with a polar solvent; -centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction; -isolating a phospholipid from the polar fraction or isolating a PUFA-enriched fraction from the polar fraction. The fish oil may be provided by -extracting a fish material with an extractant solvent; -removing the extractant solvent to provide the fish oil; -optionally subjecting the fish oil to a solid-liquid separation. The isolated phospholipids and PUFA's may be used as additives for functional foods, as a dietary supplement and for pharmaceutical application.

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 462 054 A (STATOILHYDRO ASA [NO]) 27 January 2010 (2010-01-27) page 13, line 27 - page 14, line 25; example 1	1-36
X	US 2005/129739 A1 (KOHN GERHARD [DE] ET AL) 16 June 2005 (2005-06-16) cited in the application paragraph [0037] - paragraph [0041]	1-36
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Saettel, Damien

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INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050124

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Form PCT/ISA/210 (continuation of second sheet) (April 2005)

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Information on patent family members

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2012/050124

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AR 054213 A1	13-06-2007
		AT 469957 T	15-06-2010
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JMJ

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

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

(PCT Rule 44.1)

To:
Jones, J. Mitchell
CASIMIR JONES, S.C.
2275 Deming Way, Suite 310
Middleton, WI 53562
ETATS-UNIS D'AMERIQUE

Art. 19 Amend. 4.3.15

FEB 13 2015
CASIMIR JONES, S.C.

DOCKETED
FEB 16 2015
CASIMIR JONES S.C.

Applicant's or agent's file reference AKBM33382WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/IB2014/002130	International filing date *(day/month/year)* 13 June 2014 (13-06-2014)
Applicant AKER BIOMARINE AS	Date of mailing *(day/month/year)* 3 February 2015 (03-02-2015)

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

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   How? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

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

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

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with any applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

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

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

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

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

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	KOUROUSSENKO, Svetlana Tel: +31 (0)70 340-2885

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
AKBM33382WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IB2014/002130	13 June 2014 (13-06-2014)	14 June 2013 (14-06-2013)
Applicant		
AKER BIOMARINE AS		

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

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

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

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

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

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/002130

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11B1/10 A23G3/40
ADD.

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)
C11B A23G

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)
EPO-Internal, BIOSIS, COMPENDEX, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/76385 A1 (WESTFALIA SEPARATOR IND GMBH [DE]; HRUSCHKA STEFFEN M [DE]; KIRCHNER S) 18 October 2001 (2001-10-18) page 6, line 14 - page 8, line 14; claims 1,9,14-18; figure 2 -----	1-52
X	JP S63 23819 A (KAO CORP) 1 February 1988 (1988-02-01) the whole document & HARA K ET AL: "Medicine for preventing platelet aggregation - comprises organic solvent extract of euphausia, as active ingredient", WPI/THOMSON, 16 July 1986 (1986-07-16), XP002430959, the whole document ----- -/--	37-55

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier 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
28 January 2015	03/02/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Saettel, Damien

3

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/256216 A1 (LEFKOWITZ ANDREW R [US]) 20 October 2011 (2011-10-20) paragraphs [0050], [0051]; claims 19-22	56-58
A	"Neptune krill Oil's Unique Properties", INTERNET CITATION, 30 September 2011 (2011-09-30), pages 1-3, XP002660404, Retrieved from the Internet: URL:http://www.nowfoods.com/Products/ProductFAQs/081008/htm [retrieved on 2011-09-30] the whole document	1-58
A	WO 2012/139588 A2 (TRIPLENINE PHARMA AS [DK]; SOERENSEN HANS OTTO [DK]; JENSEN NILS CHRIS) 18 October 2012 (2012-10-18) claims 1,8-10,13,16; figures; example 2	1-58
A	GIGLIOTTI J C ET AL: "Extraction and characterisation of lipids from Antarctic krill (Euphausia superba)", FOOD CHEMISTRY, ELSEVIER LTD, NL, vol. 125, no. 3, 1 April 2011 (2011-04-01), pages 1028-1036, XP027477867, ISSN: 0308-8146, DOI: 10.1016/J.FOODCHEM.2010.10.013 [retrieved on 2010-11-04] the whole document	1-58
A	ABDELKADER ALI-NEHARI ET AL: "Characterization of purified phospholipids from krill () residues deoiled by supercritical carbon dioxide", KOREAN JOURNAL OF CHEMICAL ENGINEERING, SPRINGER US, BOSTON, vol. 29, no. 7, 2 February 2012 (2012-02-02), pages 918-924, XP035079782, ISSN: 1975-7220, DOI: 10.1007/S11814-011-0273-4 the whole document	1-58

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2014/002130

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AT 447332 T	15-11-2009
		AU 783066 B2	22-09-2005
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		CN 103635564 A	12-03-2014
		EP 2697345 A2	19-02-2014
		PE 04962014 A1	07-05-2014
		US 2014031569 A1	30-01-2014
		WO 2012139588 A2	18-10-2012

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2014/002130	13.06.2014	14.06.2013

International Patent Classification (IPC) or both national classification and IPC
INV. C11B1/10 A23G3/40

Applicant
AKER BIOMARINE AS

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

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

2. **FURTHER ACTION**

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

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

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

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Telephone No. +31 70 340-0

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

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

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

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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

1. Statement

Novelty (N)	Yes:	Claims	4, 6, 7, 10-30, 33-36, 39-41, 43, 44, 46-48, 51, 54
	No:	Claims	1-3, 5, 8, 9, 31, 32, 37, 38, 42, 45, 49, 50, 52, 53, 55-58
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-58
Industrial applicability (IA)	Yes:	Claims	1-58
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 WO 01/76385 A1 (WESTFALIA SEPARATOR IND GMBH [DE]; HRUSCHKA STEFFEN M [DE]; KIRCHNER S) 18 October 2001 (2001-10-18)

D2 JP S63 23819 A (KAO CORP) 1 February 1988 (1988-02-01); & HARA K ET AL: "Medicine for preventing platelet aggregation - comprises organic solvent extract of euphausia, as active ingredient", WPI/THOMSON, 16 July 1986 (1986-07-16), XP002430959,

D3 US 2011/256216 A1 (LEFKOWITZ ANDREW R [US]) 20 October 2011 (2011-10-20)

2 Claim 37

Claim 37 is defined by some technical features of the phospholipids compounds in a composition. These features are not always described in the prior art.

However, these features are believed to be inherent to any krill phospholipids, which always have these molar percentages of phosphatidylcholine and these weight percentages of omega-3 fatty acids.

Depending on the krill extraction method, the obtained phospholipids will be more or less concentrated in the final composition, but their inherent features, those claimed in claim 37, will always be present.

3 Novelty

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-3, 5, 8, 9, 31, 32, 37, 38, 42, 45, 49, 50, 52, 53 and 55-58 is not new in the sense of Article 33(2) PCT.

3.1 Novelty over D1

The document D1 discloses (see p. 6, l. 14 - p. 8, l. 14; fig. 2; cl. 1, 9, 14-18) a process for extracting phospholipids from a biological source (such as fish, crustaceans..), comprising:

- contacting said source with a concentrated protic solvent (alcohol such as isopropanol or ethanol at a concentration of 90-95% most preferably),
- separating the phospholipid solution from proteins,
- adding water to a concentration of the alcohol of 25-30% most preferably, thus a reduction of 60-70%,
- separation of the phospholipids,
- washing the polar lipids.

The document D1 therefore removes novelty from the subject-matter of claims 1-3, 5, 8, 9, 31 and 32.

3.2 Novelty over D2

The document D2 discloses (see the whole document) a phospholipid composition obtained by extracting a krill product with a mixture of butanol and water and the use of this composition for oral administration to reduce platelet aggregation.

Taking into account the remark made under paragraph 2, and given the fact that the phospholipids of D2 are obtained by an extraction of krill using water and alcohol without additives which could hydrolyse phospholipids (such as acids, peroxides and enzymes), as in the process of the Application, the document D2 therefore removes novelty from the subject-matter of claims 37, 38, 42, 45, 49, 50, 52, 53 and 55.

3.3 Novelty over D3

The document D3 discloses (see par. 50, 51; cl. 19-22) a gummi candy product and the process for its obtention, comprising blending krill phospholipids into a gel matrix and forming the final gummi product.

Taking into account the remark made under paragraph 2, the document D3 therefore removes the novelty of claims 56-58.

4 Inventive step

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 4, 6, 7, 10-30, 33-36, 39-41, 43, 44, 46-48, 51 and 54 does not involve an inventive step in the sense of Article 33(3) PCT.

The features of these claims are either known from the prior art, or can be obtained by the skilled person in a simple routine work, without any inventive input, with the knowledge of the field, depending on the features of the final product he wishes to obtain. Claims 4, 6, 7, 10-30, 33-36, 39-41, 43, 44, 46-48, 51 and 54 are therefore not considered as being inventive.

BNSDOCID: <XS_____2006100103CF_I_>

EUROPEAN PATENT OFFICE

Patent Abstracts of Japan

PUBLICATION NUMBER : 63023819
PUBLICATION DATE : 01-02-88

APPLICATION DATE : 16-07-86
APPLICATION NUMBER : 61167540

APPLICANT : KAO CORP;

INVENTOR : HARA KENJI;

INT.CL. : A61K 35/60

TITLE : INHIBITOR OF BLOOD PLATELET AGGREGATION

ABSTRACT : PURPOSE: The titled inhibitor capable of being orally administered, having low side effects and improved stability, containing an extract of krill with an organic solvent as an active ingredient.

CONSTITUTION: An inhibitor of blood platelet aggregation containing an extract of krill with an organic solvent as an active ingredient. The extract is obtained by adding krill to an organic solvent such as chloroform, etc., stirring it preferably at 15~60°C for 2~24hr and filtering. The extract can be directly used as it is, or concentrated or dried and used and further a phospholipid fraction can be collected from the extract. To collect the phospholipid fraction, the extract is concentrated, dripped little by little in to a large amount of cold acetone and the phospholipid fraction is precipitated. A dose is 1~20g/day calculated as phospholipid, the inhibitor is preferably administered orally and the dose can be increased depending upon symptoms.

XP-002660404

Neptune Krill Oil's Unique Properties

Read Neptune's Response to ConsumerLab.com®'s Report

Neptune Krill Oil (NKO™) is known as being a fish oil alternative that similarly supplies the biologically essential Omega-3 fatty acids known as EPA and DHA. However, unlike ordinary fish oil, NKO™ provides these fatty acids in a natural food complex that includes phospholipids and astaxanthin, which give NKO™ some unique and interesting biological properties.

Basic information about the type of crustacean (Euphausia superba) that NKO™ is derived from and how it is sustainably harvested appear elsewhere on this website; click on this link to access it: http://www.nowfoods.com/Products/ProductFAQs/M099553.htm?cat=FAQ

Let's go beyond that to review some other qualities of NKO™ based on its special mixture of nutritional components:

EPA and DHA Omega-3 Fatty Acids

DHA and EPA are the two most active polyunsaturated fatty acids in the human body, contributing to all health benefits associated with Omega-3 fatty acids. Since the health benefits of these nutrients have been very well documented, that won't be repeated here. A link to one such article is available by clicking on this link: http://www.nowfoods.com/M006938.htm?cat=Sports%20Nutrition

Krill oil, like fish oil, is a rich source of these essential fatty acids (EFAs). However, in krill oil these fatty acids are bound to phospholipids, enhancing their bioavailability. Unlike the common triglyceride form of omega-3 fatty acids in fish oil, the phospholipid form found in krill oil can easily form micelles; which allow them to mix with water (unlike other oils) and absorb into the body without the usual need for bile salts, enhancing ease of digestion and bioavailability.

IN 1,000 mg (one gram) of NKO™ the typical Omega-3 Fatty Acid level is 230 - 300 mg. For the main two essential Omega-3 fatty acids, the typical Eicosapentaenoic Acid (EPA) content is 140 - 160 mg and for Docosahexaenoic Acid (DHA) content it is 80 - 90 mg.

NKO™, like fish oils, naturally contains smaller amounts of other fatty acids. Its typical Omega-6 Fatty Acid content is 10 - 20 mg per gram; and its Omega-9 Fatty Acid content is typically 60 - 80 mg per gram.

Phospholipids

Phospholipids have received justifiable scientific interest because of their ability to support healthy body structures and functions. Phospholipids are a group of phosphorus-containing fats that are found in virtually every cell in the body.

Phospholipids are a class of fat-like molecules that promote cell membrane fluidity, facilitate cell-to-cell signaling, and assist in the processing of nutrients by the cell. Membranes are the fatty barriers that surround every cell, and many structures within the cell. As we age, our cell membranes tend to get stiffer and this makes them more resistant to the movement of molecules within the membrane, as well as to the transfer of molecules into and out of the cell. This decrease in molecular movement can result in reduced communication between cells, ultimately reducing healthy cellular function.

Lecithin is the most common dietary source of phospholipids, which are found in many animal tissues and organs as an important constituent of biological membranes. It is also widespread in the plant world and abundant in legumes, cereals, and seed embryos; as well as in egg yolks and some vegetable oils. Phospholipids also play a role in memory and cognitive function, nerve health, cardiovascular health, and liver function.

While the best known phospholipid source is lecithin, commonly found in egg yolks and commercially distilled from highly purified soybean oil (http://www.nowfoods.com/Search/nf_search_results.htm?search=lecithin), more recent products like sunflower lecithin (http://www.nowfoods.com/Search/nf_search_results.htm?search=sunflower+lecithin) and krill oil (http://www.nowfoods.com/Search/nf_search_results.htm?search=sunflower+lecithin) are getting more recognition as alternative sources.

Natural sources of phospholipids typically provide a complex mixture of phosphatides that consist primarily of phosphatidylcholine (phosphatidyl choline, or PC), plus smaller amounts of phosphatidylethanolamine (phosphatidyl ethanolmine, or PE), phosphatidylinositol

(phosphatidyl inositol, or PI), and minor amounts of other components including phosphatidylserine (phosphatidyl serine, or PS).

NKO™ is a natural, soy-free, egg-free source of phospholipids. About 40% of NKO™ by weight consists of phospholipids (typically 390-420 mg/gm). Phospholipids from animal sources contain a higher percentage of longer-chain fatty acids with a higher degree of unsaturation than plant source phospholipids. That, combined with the high levels of EPA and DHA essential omega-3 fatty acids in krill oil, make it more closely resemble human brain phospholipids. In fact, one study showed that phospholipid-bound fatty acids were absorbed twice as well by the infant brain of primates as the triglyceride form. http://www.ncbi.nlm.nih.gov/pubmed/11861929

Phosphatidylcholine

Phosphatidylcholine (phosphatidyl choline, or PC) typically represents about 80% of the phospholipid content of NKO™. Phosphatidylcholine is one of the primary membrane phospholipids; which help form the outer layer of cell and other biological membranes and is the layer that is exposed to water and water-soluble substances. (The inner layer of these membranes is typically averse to water and faces the inside of cells, where there are commonly more fatty acid chains.) Phosphatidylcholine is also the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially HDL (the so-called "good cholesterol"). Research has shown that PC is beneficial for mood, memory and cognitive function, and neurological health. http://lipidlibrary.aocs.org/Lipids/pc/index.htm

Phosphatidylethanolamine

Phosphatidylethanolamine (phosphatidyl ethanolamine or PE) is the next most abundant phospholipid in NKO™; typically present at levels of 2-5% of NKO's phospholipid total, by weight. PE is frequently the main lipid component of microbial membranes, can amount to 20% of liver phospholipids, and up to 45% of brain phospholipids. Higher proportions are found in the mitochondria than in other cellular organelles. PE is a key building block of membrane bilayers; where phospholipids are stacked two molecules thick with the water soluble ends facing away from the middle and toward both the inside and outside of the cell to facilitate contact with the mostly watery environment on each side of the membrane. This makes the animal or human cell bilayered membrane a virtual "sandwich" with a layer of cholesterol placed between the two stacked phospholipids for rigidity. That middle layer also acts as a barrier to substances entering or leaving the cell without help from other molecules coating the membrane surface, such as carbohydrates and proteins, which serve as receptor sites for messenger molecules. Receptors facilitate interaction with the cell membrane, allowing molecular cell-to-cell communication signals to pass from the outside to the inside of the cell. The water soluble phospholipid ends on both surfaces of the cell membrane allow only partial penetration of water-borne substances through that cell membrane because the water resistant phospholipid ends and the inner layer of cholesterol form a barrier unless specific receptors aid that passage. http://lipidlibrary.aocs.org/lipids/pe/index.htm

Phosphatidylinositol

Phosphatidylinositol (phosphatidyl inositol, or PI) is another phospholipid in NKO™; typically present at levels of 1-3% of NKO's phospholipid total, by weight. Phosphatidylinositol is especially abundant in brain tissue, where it can amount to 10% of the phospholipids, but it is present in all tissues and cell types. Phosphatidylinositol is known to be the anchor that links a variety of proteins to the plasma membrane. (More precisely, to the external leaflet of the plasma membrane via a glycosyl bridge). http://lipidlibrary.aocs.org/Lipids/pi/index.htm

Phosphatidylserine

Phosphatidylserine (phosphatidyl serine or PS) is another naturally occurring phospholipid in NKO™; typically present at levels of 1-2% of NKO's phospholipid total, by weight. PS is critical for the normal functioning of all cells and is the phospholipid most concentrated in the brain and nervous system, where it is important for the conduction of nerve impulses. PS is essential for the accumulation, storage, and release of neurotransmitters; thus supporting brain and cognitive functions, including mood and memory. The greatest concentration in humans is in myelin found in brain tissue, and it is also relatively abundant in the plasma membrane of cells and in cells' endoplasmic reticulum (cellular organelles that serve as facilities for protein synthesis and storage, steroid synthesis and storage, and ion storage).
http://lipidlibrary.aocs.org/Lipids/ps/index.htm

Sphingomyelin

Sphingomyelin is another naturally occurring phospholipid in NKO™, typically present at levels of 1-2% of NKO's phospholipid total, by weight. Only found in animals and humans, it is the sphingolipid analogue of phosphatidylcholine, and contains phosphorylcholine in a complex molecular structure. Sphingolipids are named that because of their once enigmatic nature. In sphingolipids, fatty acids are linked via amide bonds to a long-chain base ("sphingoid").

Sphingomyelin is in all animal cell membranes, where it is by far the most abundant sphingolipid. While it can comprise up to 50% of the lipids in some tissues, it is usually lower in concentration than phosphatidylcholine. Sphingomyelin makes up about 10% of the lipids in the brain. It is the single most abundant lipid in the red blood cells (erythrocytes) of most ruminant animals, where it actually replaces phosphatidylcholine. As with phosphatidylcholine, sphingomyelin usually is in greatest concentration in the plasma membrane of cells. In the membrane of the human eye lens, sphingomyelin can comprise over 50% of the total phospholipid content.

There is a lot of variability in the fatty acid content of sphingomyelin. About 60% of the fatty acids of the sphingomyelin of the grey matter of the human brain consist of stearic acid (18:0); proving that this sometimes maligned fatty acid that is present in virtually every natural plant and animal fat is essential to human life in general, and our brain structures and functions in particular. Sphingomyelin is also present in

testes and sperm; in this case comprised of very long chain fatty acids.

One important function of sphingomyelin is to serve as a chemically distinct substitute for phosphatidylcholine in forming a building block of membranes; it does this by forming a stable, chemically resistant outer "leaflet" of the plasma membrane's lipid bilayer. Sphingomyelin and cholesterol metabolism are closely integrated, and it has been suggested that sphingomyelin may control the distribution of cholesterol in cells; these two substances are most abundant in the same types of membranes. Sphingomyelin has a role in supporting intracellular messengers and cell membrane components. It also has been shown to support a healthy HDL/LDL cholesterol ratio.
http://lipidlibrary.aocs.org/Lipids/sph/index.htm

Astaxanthin

Astaxanthin is a naturally occurring carotenoid that, because of its unique structure, provides a wide range of antioxidant benefits. Astaxanthin can help to protect cell membranes against free radical attack and potentiates the action of other antioxidants like Vitamins E and C. Scientific studies have demonstrated that Astaxanthin can help to support a healthy inflammatory response, enhance immune function, and provide neurological support.

Astaxanthin belongs to the same family of fat-soluble carotenoid molecules as the yellow/orange colored Beta-Carotene. It differs from Beta-Carotene in that its molecular structure contains two additional oxygen groups in each ring structure. This gives it a deep red color and classifies it as a xanthophyll, with up to 10 times stronger free radical scavenging activity. Another difference is that, unlike Beta-Carotene, Astaxanthin cannot be converted to Vitamin A in the human body.

The characteristic pink or red color of salmon and trout is reflective of the presence of accumulated Astaxanthin that they get from their diet by consuming krill, krill-consuming smaller fish, or similar sources. NKO™ typically contains about 1.0 - 1.5 mg of astaxanthin per 1,000 mg (per gram).

NKO™

NKO™ (Neptune Krill Oil) is a patented and trademarked dietary ingredient. For additional information about Neptune Krill Oil, please visit this website: http://neptunekrilloil.com/

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Extraction and characterisation of lipids from Antarctic krill (*Euphausia superba*)

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ABSTRACT

There is significant commercial interest in oil extraction from krill because it is rich in omega-3 polyunsaturated fatty acids (*n*-3 PUFA) such as eicosapentaenoic (EPA, 20:5*n*3) and docosahexaenoic (DHA, 22:6*n*3) acids. The objectives were to determine oil extraction efficiency using different solvent systems and the composition of extracted oil and spent krill following extraction. Extraction efficiency was the highest ($P < 0.05$) for one-step extraction using freeze-dried krill with 1:12 or 1:30 krill:solvent ratio (w:v) compared to Folch, Soxhlet, and conventional two-step extraction. Extracted oils contained predominantly phospholipids (20–33%), polar non-phospholipids (64–77%), and minor triglycerides (1–3%). Triglycerides contained much less ($P < 0.05$) total *n*-3 (4.0%), DHA (1.1%), and EPA (2.3%), but more ($P < 0.05$) saturated FA (38.7%) than phospholipids (total *n*-3-47.4%, DHA-18.0%, EPA-28.2%, saturated FA-23.5%). Antioxidant capacity of krill oil extracted by one-step extraction (9.4–14.2 μmol Trolox Equivalents/ml oil) was generally similar to antioxidant capacity of krill oil extracted by ethanol (22.9), but greater ($P < 0.05$) than antioxidant capacity of krill oil extracted by acetone (1.2) and Folch method (1.5). The spent krill following oil extraction contained protein (72.9–75.8%, dry basis). Based on the extraction efficiency and composition of the extracted oil, the one-step extraction using 1:12 krill:solvent ratio is recommended.

1. Introduction

Antarctic krill (*Euphausia superba*) are small, shrimp-like crustaceans. Commercial capture is simple because krill form high-density surface swarms. Despite their small size, krill likely has the largest biomass of any multi-cellular animal species on earth (Nicol, James, & Pitcher, 1987). Although it is difficult to accurately determine the sustainable biomass for krill harvest, this significant resource may be comparable to the biomass of all other aquatic species currently harvested. The total annual capture from all fisheries has been approximately 130 million tons (MT) since 2000 (FAO, 2007). By comparison, krill biomass has been estimated at 400–1550 MT with a sustainable harvest at 70–200 MT (Suzuki & Shibata, 1990). However, newer estimates suggest that the krill biomass may be lower (Priddle, Boyd, Whitehouse, Murphy, & Croxall, 1998; Smetacek & Nicol, 2005). Nicol and Foster (2003) estimated the annual krill capture to be 0.1 MT, making krill an underutilized species. However, due to the role that krill play in marine ecology, an internationally monitored and governed ecosystem approach is a necessity for a long-term sustainability of this fishery (Everson, 2000; Hureau, 1985; Laws, 1985).

Grantham (1977) reported that krill contains 77.9–83.1% moisture, 0.4–3.6% lipids, 11.9–15.4% protein, and ~2% chitin and glucides. Saether, Ellingsen, and Mohr (1986) determined that due to seasonality lipid content ranges widely from 12–50% (dry basis). Lipid content and its composition in krill also depend on species, age, and the time between capture and freezing (Kolakowska, 1991). Kolakowska, Kolakowski, and Szczygielski (1994) reported that the *n*-3 PUFA, EPA and DHA are particularly abundant, which was attributed to krill consuming single-cell marine micro-algae. However, shellfish are often perceived as high in cholesterol; and therefore, reduce its acceptance as food by consumers. Cholesterol level in krill is higher than fish, but lower than shrimp (Tou, Jaczynski, & Chen, 2007). Also, it is important to emphasise that two-thirds of the sterols in shellfish are non-cholesterol sterols, which interfere with absorption of dietary cholesterol (Feeley, Criner, & Watt, 1972; Vahouny, Connor, Roy, Lin, & Gallo, 1981).

Despite its potential as a high quality lipid and protein source (Bridges, Gigliotti, Altman, Jaczynski, & Tou, 2010; Chen, Tou, & Jaczynski, 2009; Gigliotti, Jaczynski, & Tou, 2008; Gigliotti, Smith, Jaczynski, & Tou, 2010; Tou et al., 2007), the use of krill as human food has been limited (Suzuki & Shibata, 1990). Krill is mainly used by reduction fisheries for manufacture of fish feeds due to its high astaxanthin content. In addition, encapsulated krill oil is used as a dietary supplement with various potential health benefits including protection against cardiovascular disease (CVD) (Bunea, El

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Farrah, & Deutsch, 2004; Sampalis, 2007; Sampalis et al., 2003). Bunea et al. (2004) attributed some of these benefits to the *n*-3 PUFA in krill being mainly associated with phospholipids (PL); unlike in fish where the *n*-3 PUFA are associated with triglycerides (TG). The oxidative stability of krill oil has been attributed to its antioxidants content, in particular astaxanthin (Suzuki & Shibata, 1990). Krill oil may be valuable in the development of nutraceutical food products (Kassis, Beamer, Matak, Tou, and Jaczynski, 2010; Kassis, Drake, Beamer, Matak, & Jaczynski, 2010; Kassis, Gigliotti, Beamer, Tou, and Jaczynski, submitted for publication).

A major hindrance to commercial processing of krill and development of new krill-based food products may be due to high activity of krill lipases and proteases (Anheller, Hellgren, Karlstam, & Vincent, 1989). These enzymes are released immediately upon the demise of krill, resulting in autolysis, which leads to a rapid spoilage. The enzymes combined with its small size makes krill processing for human food a significant challenge. Another concern is high fluoride content in the exoskeleton. However, centrifugation removes fluoride (Christians & Leinemann, 1983; Karl et al., 1986).

Krill oil is currently extracted by two-step solvent extraction using acetone and ethanol in the first and second step, respectively (Beaudoin & Martin, 2004; Sampalis, 2007). However, this extraction requires two separate extraction steps and takes a relatively long time. In addition, the two-step extraction does not mention water removal from krill prior to oil extraction. Water interferes with solvent extraction and water removal prior to oil extraction results in greatly improved extraction efficiency and less water in the extracted oil (Dunford, Temelli, & LeBlanc, 1997; Nilsson, 1996). Another process to extract krill oil takes advantage of superctitical-CO_2 entrained with up to 20% ethanol (Bruheim et al., 2008). However, this process requires thermal inactivation of lipases at over 50 °C prior to oil extraction. Although heat likely inactivates lipases resulting in reduced hydrolysis of ester bonds and consequently fewer free FA, it simultaneously denatures heat-labile krill muscle proteins (Carvajal, Lanier, & Macdonald, 2005).

Due to structural changes, the recovery of denatured proteins would be difficult and even if krill proteins were recovered, the proteins would exhibit reduced functionalities (i.e., gel-forming ability, extractability, water-holding-capacity, etc.). Bruheim et al.'s (2008) process is similar to the two-step solvent extraction (Beaudoin & Martin, 2004; Sampalis, 2007), but does not require water removal prior to processing. However, freeze-drying of krill prior to oil extraction with superctitical-CO_2 has been shown to increase extraction efficiency approximately three times (Yamaguchi et al., 1986). The protein remaining in the residual spent krill following oil extraction can be recovered with techniques such as isoelectric solubilisation/precipitation and if protein functionalities are retained, this protein could be used in human food products contributing to the fuller use of this tremendous resource (Chen & Jaczynski, 2007a,b; Chen et al., 2009; Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2010; Jaczynski, 2010; Torres, Chen, Rodrigo-Garcia, & Jaczynski, 2007).

It is hypothesised that one-step extraction with acetone:ethanol mixture for 2 h from whole krill will result in high extraction efficiency. The objectives were to determine oil extraction efficiency from whole krill using different solvent systems and characterise the composition of extracted lipids and residual spent krill following oil extraction.

2. Materials and methods

2.1. Sample preparation and oil extraction

Whole frozen Antarctic krill (*Euphausia superba*) was obtained from Krill Canada (Langley, BC, Canada). The krill blocks were transported overnight to the West Virginia University food science laboratory in heavily insulated industrial strength boxes filled with dry ice. Upon arrival the boxes were immediately stored at −80 °C until use. Whole frozen krill was freeze-dried without thawing (VirTis Genesis 35SQ Super XL freeze-dryer, Virtis, Gardiner, NY, USA), vacuum-packed and stored at −80 °C until processed. A flow diagram of oil extraction from krill is shown is Fig. 1.

In the one-step extraction, oil was extracted from freeze-dried krill using 1:1 acetone:ethanol (v:v) solvent mixture (ACS grade acetone, Fisher Scientific, Fairlawn, NJ, USA; ACS grade 95% ethanol, Pharmco, Brookfield, CT, USA). The following krill:solvent ratios were tested 1:6, 1:9, 1:12, and 1:30 (w:v). The weight of the initial freeze-dried krill was recorded in order to determine extraction efficiency (see below). The 1:30 ratio was used in the present study to mimic the 1:6 ratio of whole fresh krill as described by Beaudoin and Martin (2004) as well as Sampalis (2007). Fresh whole krill is currently used in commercial oil extraction (Beaudoin & Martin, 2004; Bruheim et al., 2008; Sampalis, 2007). However, in the present study freeze-dried krill was used (Dunford et al., 1997; Yamaguchi et al., 1986). Therefore, 1:30 ratio (freeze-dried krill) used in the present study approximately corresponded to 1:6 ratio (fresh whole krill) during commercial krill oil extraction based on the lipid content in relation to the solvent volume. Freeze-dried krill was dispersed in the solvent mixture (acetone:ethanol) by homogenisation for 30 s using a laboratory blender (model 51BL31, Waring Commercial, Torrington, CT, USA). Oil extraction was conducted for 2 h at 4 °C using a continuous shaker (model Excella E25R, New Brunswick Scientific, Edison, NJ, USA) followed by centrifugation at 10,000*g* and 4 °C for 20 min (model Sorvall RC-5B Refrigerated Superspeed, Kendro Laboratory Products, Newtown, CT, USA). The supernatant (i.e., extracted krill oil) was decanted and air dried at atmospheric pressure. The sediment (residual spent krill including protein, shell, etc.) was also dried under air at atmospheric pressure and analysed for crude protein (Kjeldahl), total fat (Soxhlet), and ash content (see below).

In the two-step extraction oil was extracted from freeze-dried krill using two separate extractions. The weight of the initial freeze-dried krill was recorded in order to determine extraction efficiency (see below). The freeze-dried krill was first mixed with acetone at a 1:6 ratio (krill:acetone, w:v), centrifuged, and then the sediment was mixed with ethanol at a 1:6 ratio (krill:ethanol, w:v), followed by final centrifugation. Therefore, two separate extracts were obtained. Acetone extract was obtained in step 1 and ethanol extract in step 2. In step 1, freeze-dried krill was dispersed in acetone by homogenisation for 30 s using the laboratory blender. Oil extraction in step 1 was conducted for 2 h at 4 °C using the continuous shaker followed by centrifugation at 10,000*g* and 4 °C for 20 min. The supernatant (i.e. acetone extract) was decanted and air dried at atmospheric pressure; while the sediment was subjected to step 2 of the extraction. Step 2 was conducted in the same manner as step 1 except ethanol was used instead of acetone. Therefore, total extraction time (i.e., step 1 and 2) was 4 h. Following step 2, final centrifugation at 10,000*g* and 4 °C for 20 min was applied. The supernatant (i.e., ethanol extract) was decanted and air dried at atmospheric pressure. The sediment (residual spent krill including protein, shell, etc.) was analysed for crude protein (Kjeldahl), total fat (Soxhlet), and ash content (see below).

Dry krill oils (i.e., krill oil from one-step extraction, acetone extract from two-step extraction, and ethanol extract from two-step extraction) were clarified in 2:1 chloroform:methanol (v:v) mixture (ACS grade chloroform, Fisher Scientific, Fairlawn, NJ, USA; HPLC grade methanol, Fisher Scientific, Fairlawn, NJ, USA) with 20 ml of 10% NaCl in water added to a separation funnel (Folch, Lees, & Sloane, 1957). This clarification removed any residual water in the krill oil samples. Sufficient volume of the chloroform:methanol mixture was added until there was no visible separate layers. Following clar-

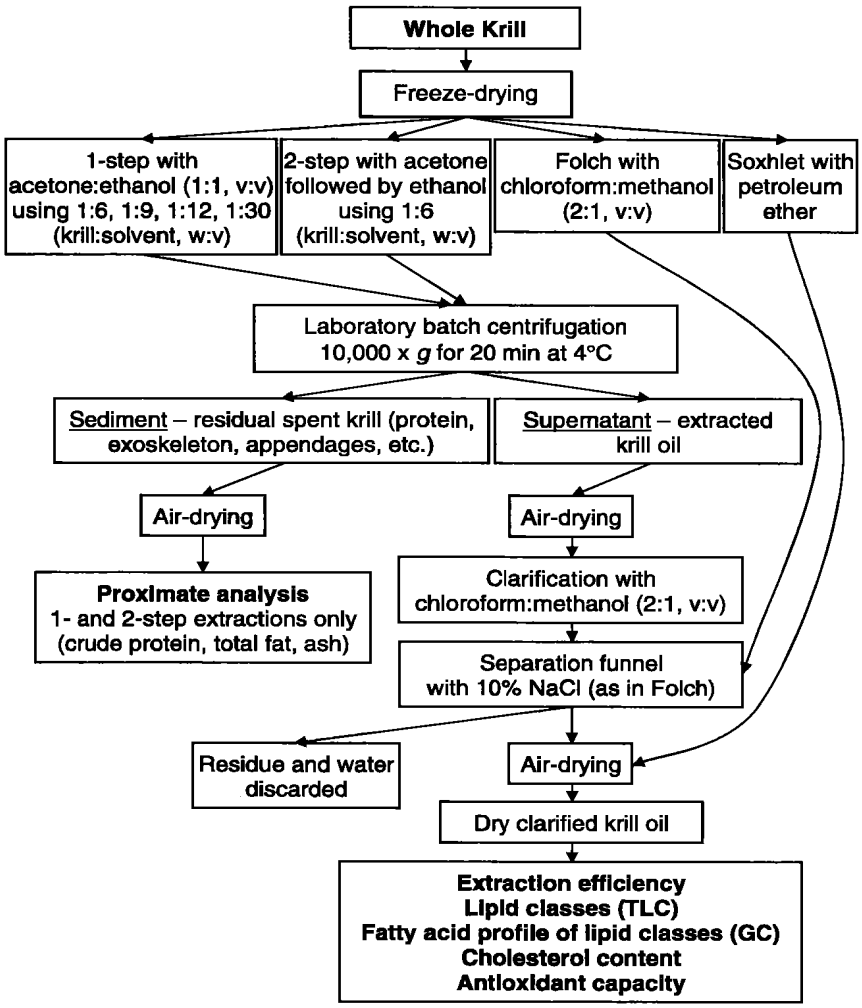

Fig. 1. A flow diagram for oil extraction from krill and subsequent analyses of the extracted oil and residual spent krill.

ification, the krill oil samples were air re-dried at atmospheric pressure. Dry clarified krill oils were weighed and compared to the initial weight of the freeze-dried krill subjected to extraction in order to determine extraction efficiency (see below).

For comparison, oil was also extracted from freeze-dried krill using the Folch method (Folch et al., 1957) and Soxhlet extraction (AOAC, 1995). In Folch method, 3 g of freeze-dried krill were dispersed in 60 ml of the 2:1 chloroform:methanol mixture by homogenisation for 30 s using the laboratory blender, followed by filtration through Whatmann #40 paper (Whatman International, Maidstone, UK) in the separation funnel. A 20 ml aliquot of 10% NaCl in water was added to the separation funnel and the mixture was manually shaken and allowed to stand until the phases had completely separated. The bottom organic solvent phase was removed and air dried at atmospheric pressure. The drying resulted in solvent evaporation yielding krill oil. The weight of krill oil was recorded in order to determine extraction efficiency. In Soxhlet extraction, 3 g of freeze-dried krill were dispersed in petroleum ether (ACS grade petroleum ether, VWR International, Bridgeport, NJ, USA) (AOAC, 1995). The extraction was carried out in a standard Soxhlet apparatus for 18 h (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006, 2007), followed by air drying at atmospheric pressure. The drying resulted in evaporation of petroleum ether yielding krill oil. The weight of krill oil was recorded in order to determine extraction efficiency.

In preliminary experiments, the above solvent systems were applied to extract oil from fresh krill and freeze-dried krill. The extraction efficiency was significantly lower ($P < 0.05$) for all solvent systems using fresh krill when compared to freeze-dried krill. These preliminary experiments confirmed earlier reports by Yamaguchi et al. (1986) and Dunford et al. (1997). Therefore, only freeze-dried krill was used in the present study. All oil extractions were performed in triplicate ($n = 3$) using the same batch of freeze-dried krill.

2.2. Determination of extraction efficiency

The weight of the initial freeze-dried krill prior to the extraction with different solvent systems was recorded. Following extraction, the weight of extracted oil was also recorded. The weight of acetone extract and ethanol extract were combined in order to determine an overall extraction efficiency of the two-step extraction. The extraction efficiency was determined according to the following equation:

$$\text{Extraction efficiency} = \frac{\text{weight of extracted krill oil(g)}}{\text{weight of freeze-dried krill subjected to extraction(g)}} \times 100 \quad (1)$$

Extraction efficiency is expressed as g of extracted oil per 100 g of freeze-dried krill subjected to the extraction.

2.3. Determination of lipid classes in extracted krill oil

Thin layer chromatography (TLC) was applied to resolve lipid classes of oils extracted by one-step, two-step, Folch, or Soxhlet. For krill oil extracted by the two-step extraction, TLC and subsequent densitometry analysis were applied to acetone and ethanol extracts. A 10 ml aliquot of extracted krill oil was dissolved in 1:1 chloroform:methanol (v:v) and loaded onto TLC plates (Whatman K6F silica plates with 60 A pore sizes, P.J. Cobert Associates, St. Louis, MO). The TLC plates were developed using a 80:20:1.5 hexane:ether:acetic acid solution (v:v:v) as a mobile phase. Once developed, plates were air dried for 5 min.

Plate images were captured using a digital camera interfaced with a PC and spot densitometer (Fluorchem 8000, Alpha Innotech Corp., San Leandro, CA) using transluminating white light (Alpha Innotech Corp., San Leandro, CA). The images were analysed using the Fluorchem software (version 1.0, Alpha Innotech Corp., San Leandro, CA). Phospholipids (PL) and triglycerides (TG) were identified using R_F values obtained from triolein (Sigma–Aldrich, St. Louis, MO) and soybean lecithin (Fisher Scientific, Fairlawn, NJ) standards. Once identified, the bands corresponding to PL, TG, and polar non-PL class were scraped from the TLC plates and suspended in 1:1 chloroform:methanol (v:v) for determination of fatty acid profile (FAP) by gas chromatography (GC). The densitometry data are reported as mean values (±standard deviation) of at least three replicates and the mean values are expressed as percent of lipid class in total krill oil.

2.4. Fatty acid profile (FAP) of extracted krill oil

The PL and TG were separately scraped from the TLC plates. The FAP of the PL and TG scrapes was determined (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2007, 2008a, 2008b; Folch et al., 1957; Taskaya, Chen, Beamer, Tou, & Jaczynski, 2009). FA were transmethylated by the addition of 4 ml of 4 g/100 ml H_2SO_4 in anhydrous methanol and heated in a water bath set at 90 °C for 60 min. The mixture was saponified by transferring through a Na_2SO_4-filled glass Pasteur pipette and subsequently dried under N_2 in a water bath set at 60 °C for 60 min. The FA methyl esters (FAME) were re-suspended in filtered iso-octane (HPLC grade iso-octane, Fisher Scientific, Fairlawn, NJ, USA). The FAME were analysed by a gas chromatograph (GC) (model CP-3800, Varian Analytical Instruments, Walnut Creek, CA, USA) using flame ionisation detector fitted with a WCOT-fused silica capillary column (50 m length, 0.25 mm inside diameter; Varian Analytical Instruments, Walnut Creek, CA, USA). Injection and detection temperatures were maintained at 220 °C and column temperature was 190 °C. The stationary phase was CP-Silica 88 (Varian Analytical Instruments, Walnut Creek, CA, USA). Nitrogen was the carrier gas and a split ratio of 10 to 1 was used. The FA were identified by comparing their retention times with those of known standards and references (Ackman, 1980). Peak area and the amount of each FA were computed by an integrator using the Star GC workstation (version 6, Varian Analytical Instruments, Walnut Creek, CA, USA). The data are reported as mean values (±standard deviation) of at least three replicates and the mean values are expressed as percent of a fatty acid in total fatty acids.

2.5. Determination of cholesterol content in extracted krill oil

Due to the prevalence of polar non-PL, the TLC and densitometry analysis did not allow determination of cholesterol content. For krill oil extracted with the two-step extraction, cholesterol content was separately determined for acetone and ethanol extracts. Cholesterol content of oil extracted from krill by one-step, two-step, Folch, and Soxhlet procedure was determined using a colorimetric assay (EMD Chemicals Inc., Darmstadt, Germany). This cholesterol assay relies on the oxidation of cholesterol by added cholesterol oxidase and yielding H_2O_2. The resulting H_2O_2 interacts with a cholesterol probe to produce resorufin that is detected spectrophotometrically.

Krill oil samples were prepared in a cholesterol reaction buffer. In a 96 well microplate, 50 μl of the buffered krill oil samples were mixed with 50 μl of reaction mix (containing cholesterol oxidase and cholesterol probe). Samples were covered and incubated at 37 °C for 1 h. Absorbance was read at 570 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA). In order to determine cholesterol content in krill oil, standard curve was constructed using cholesterol standards mixed with 50 μl of the reaction mix. The cholesterol content is reported as mean values (± standard deviation) of at least three replicates and the mean values are expressed as grams of cholesterol per 100 grams of krill oil.

2.6. Antioxidant capacity of extracted krill oil

It has been reported that krill oil is rich in antioxidants, in particular astaxanthin (Kolakowska, 1991; Kolakowska et al., 1994; Suzuki & Shibata, 1990; Tou et al., 2007). These antioxidants were likely included in the polar non-PL class. Therefore, total antioxidant capacity for the oil extracted from krill by the different extraction methods was determined according to a colorimetric antioxidant assay (Cayman Chemical Company, Ann Arbor, MI). For krill oil extracted with the two-step extraction, total antioxidant capacity was separately determined for acetone and ethanol extracts. This antioxidant assay relies on the ability of endogenous antioxidants in krill oil to inhibit the metmyoglobin-mediated oxidation of ABTS (2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^+$. ABTS and metmyoglobin were added to krill oil samples as reagents and the amount of $ABTS^+$ was determined spectrophotometrically. The capacity of the endogenous antioxidants in krill oil to prevent ABTS oxidation was compared with that of a water-soluble tocopherol analogue, trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

In a 96 well microplate, 10 μl of krill oil samples were mixed with 10 μl of metmyoglobin and 150 μl of ABTS. To initiate the reaction, 40 μl of H_2O_2 was added to each well. Samples were covered and incubated at room temperature on a shaker for 5 min. Absorbance was read at 750 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA). In order to determine trolox

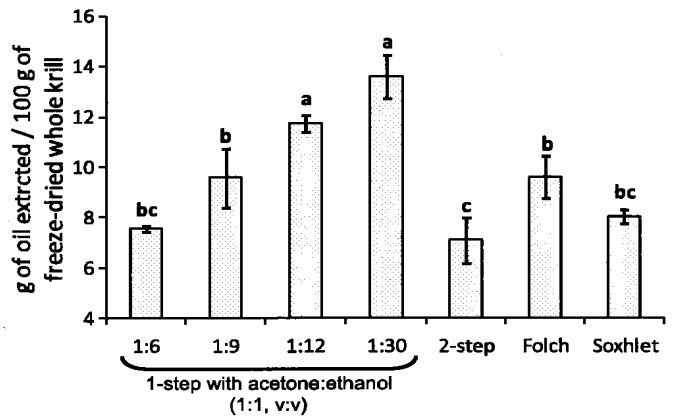

Fig. 2. Extraction efficiency* of different solvent systems for extraction of oil from freeze-dried whole krill. *Different letters on the top of data bars indicate significant differences (Tukey's test, $P < 0.05$) between mean values (±SD, $n = 3$).

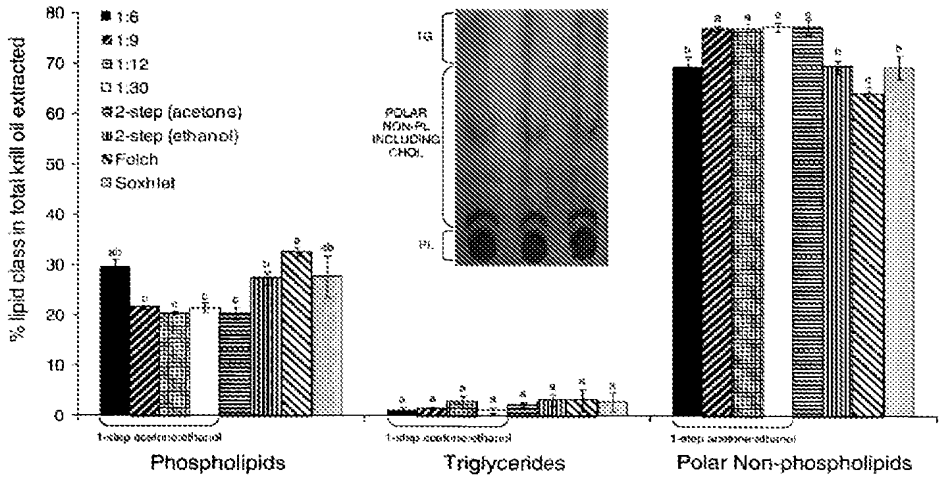

Fig. 3. Densitometry analysis of thin layer chromatography (TLC) plates. For two-step extraction, TLC plates with acetone and ethanol extracts were analysed separately. Insert: An example TLC plate showing lipid classes of krill oil extracted with one-step extraction using 1:12 krill:solvent ratio (w:v) (triplicate). TG – triglycerides, PL – phospholipids, CHOL – cholesterol. Different letters on the top of data bars indicate significant differences (Tukey's test, $P < 0.05$) between mean values (±SD, $n = 3$) within a lipid class.

equivalent (TE) for krill oil, standard curve was constructed using trolox standards mixed with 10 μl of metmyoglobin and 150 μl of ABTS. The antioxidant capacity is reported as mean values (±standard deviation) of at least three replicates and the mean values are expressed as μmols of TE per ml of krill oil.

2.7. Proximate analysis of the residual spent krill following oil extraction

To determine proximate composition (i.e., crude protein, total fat, and ash content) on dry basis, the residual spent krill was analysed for moisture content. A sample (2 g) was placed on an aluminium dish (Fisher Scientific, Fairlawn, NJ, USA), spread evenly across the dish and oven dried (105 °C for 24 h) (AOAC, 1995). The crude protein, ash content, and total fat were determined in the residual spent krill following oil extraction with one- and two-step extraction only (i.e., sediment in Fig. 1). Crude protein was determined by Kjeldahl assay (AOAC, 1995). Ash content was determined by incinerating samples in a muffle furnace at 550 °C for 24 h (AOAC, 1995). Residual fat content was determined according to the Soxhlet extraction method (AOAC, 1995). The proximate data are reported as mean values (±standard deviation) of at least three replicates and the mean values are expressed as grams per 100 g of the residual spent krill following oil extraction (dry weight basis).

2.8. Statistical analysis

The oil extraction experiments were performed in triplicate ($n = 3$). For each triplicate, at least three measurements were performed. One-way independent measures analyses of variance (ANOVA) were used to determine individual differences between treatments except for the differences of FA content between TG and PL where Student's t-test was used. Post-hoc analysis was conducted using Tukey's test with a significance level of ($P < 0.05$). ANOVA statistical comparisons were conducted using KYPlot (KyensLab, Tokyo, Japan).

3. Results and discussion

3.1. Extraction efficiency

Extraction of krill oil using the two-step procedure resulted in similar ($P > 0.05$) efficiency as the one-step procedure using a krill:solvent ratio of 1:6 (Fig. 2). Extraction efficiency for the one-step procedure increased ($P < 0.05$) as the krill:solvent ratio increased, with 1:12 and 1:30 ratios having the greatest ($P < 0.05$) efficiencies. There was no ($P > 0.05$) significant difference in extraction efficiency between the one-step procedure using 1:12 and 1:30 ratio. The krill:solvent ratio is a critical parameter for extraction efficiency using the one-step procedure. Likely, if the krill:solvent ratio were increased in the two-step extraction, the extraction efficiency would similarly increase. However, one-step extraction is simpler than the two-step procedure; and therefore, one-step extraction with acetone:ethanol (1:1, v:v) using 1:12 krill:solvent ratio is recommended.

3.2. Lipid classes in extracted krill oil

Major lipid classes of the extracted krill oils were separated using TLC (Fig. 3 insert), quantified by densitometry and presented as % of lipid class in total krill oil extracted (Fig. 3). The extraction procedure did not ($P > 0.05$) affect TG content in the extracted oils, which ranged 1.0–3.2% (Fig. 3). However, extraction procedure had an effect ($P < 0.05$) on PL content, with Folch, Soxhlet, and 1-step (1:6 krill:solvent ratio) extractions having the highest ($P < 0.05$) content of PL. The PL content of all extracted oils ranged from 20.4–32.7% (Fig. 3). A high PL content in krill oil has been described previously, with total PL accounting for approximately 40% of krill oil (Bottino, 1975). The high PL content makes krill oil unique as compared to other dietary lipids. The TG content in fish oils is approximately 60% (Tou et al., 2007).

The major lipid classes in all of the oils extracted from krill were the polar non-PL classes (>60%). These classes consist of cholesterol, mono- and di-glycerides, and the red pigment primarily astaxanthin. The association of the red pigment with the polar non-PL classes made quantifying each class individually with TLC difficult. Thus, these classes were combined and accounted for as the polar non-PL class. The one-step extraction with krill:solvent ratios greater than 1:6 and the acetone fraction from the two-step extraction had the highest ($P < 0.05$) polar non-PL class content. The differences in the polar non-PL class content should be of commercial interest because an extraction procedure that yields the oil with lower cholesterol content, but higher antioxidant capacity would provide the healthiest oil. Therefore, cholesterol content and total antioxidant capacity were determined (see Section 3.4 and 3.5, respectively).

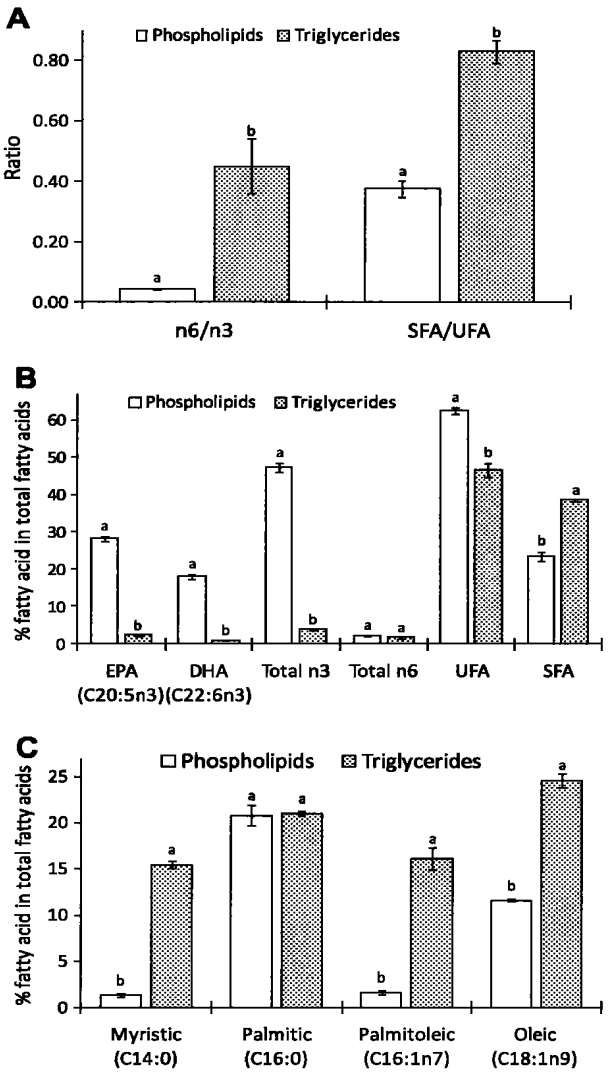

Fig. 4. Major FA* associated with phospholipids (PL) and triglycerides (TG) in oil extracted from krill by one-step extraction using 1:12 krill:solvent ratio (w:v). The PL, TG, and polar non-PL class were resolved with TLC (Fig. 3). The polar non-PL showed no detectable FA. This class likely contained antioxidants including astaxanthin. *n*3 – ω-3 FA, *n*6 – ω-6 FA, UFA – total unsaturated FA, SFA – total saturated FA, EPA – eicosapentaenoic acid (20:5n3), DHA – docosahexaenoic acid (22:6*n*3). *Different letters on the top of data bars indicate significant differences (Student's *t*-test, $P < 0.05$) between mean values (±SD, $n = 3$) within the FA.

3.3. Fatty acid profile (FAP) of extracted krill oil

The PL was the major lipid class containing FA (PL – 20–33% vs. TG – 1–3%; Fig. 3). Only PL and TG had detectable levels of FA. Preliminary experiments were conducted to determine if the different extraction methods had an effect ($P < 0.05$) on the FAP. The FAP was not ($P > 0.05$) different regardless of the extraction method (data not shown). Therefore, extraction methods tested in the present study did not ($P > 0.05$) affect FAP of the extracted oils. In addition, Fig. 2 indicates that one-step extraction resulted in the highest ($P < 0.05$) extraction efficiency for 1:12 and 1:30 krill:solvent ratio (w:v); while the extraction efficiency at 1:12 and 1:30 was similar ($P > 0.05$). Therefore, only FAP for PL and TG extracted with one-step extraction using 1:12 krill:solvent ratio (w:v) is reported (Fig. 4).

Following TLC analysis, TG and PL spots were scraped from the TLC plates and methylated for FAP by GC (Fig. 4). The ratios of *n*6/*n*3 and saturated FA/unsaturated FA were lower ($P < 0.05$) in PL than TG (Fig. 4A). Oil with a lower *n*-6/*n*-3 and saturated FA/unsaturated FA is considered healthier. EPA and DHA were predominant ($P < 0.05$) in PL, contributing to much higher ($P < 0.05$) content of total *n*-3 FA and unsaturated FA in PL than in TG (Fig. 4B). EPA and DHA are the bioactive *n*-3 PUFA associated with health benefits such as reduced risk of CVD (cardiovascular disease). The TG had a higher ($P < 0.05$) content of myristic, palmitoleic, and oleic acid than PL (Fig. 4C).

The PL and TG require different digestive enzymes and therefore, there may be differences in bioavailability and tissue accretion of *n*-3 PUFA. In turn, this may result in different physiological and health effects (Amate, Gil, & Ramirez, 2001, 2002; Matthews et al., 2002). Therefore, having EPA and DHA esterified as PL in krill oil may have significant implications for human health. The preferential esterification of EPA and DHA into PL is intriguing and has been noted previously (Saether et al., 1986). However, available data comparing the benefits of consuming ω-3 PUFA as krill oil compared to other sources is scarce. Therefore, further studies are needed to compare and understand the nutritional value and health effects of krill oil versus other sources of *n*-3 PUFA.

3.4. Cholesterol content in extracted krill oil

The one-step extraction procedures yielding oil with the highest ($P < 0.05$) content of polar non-PL (one-step extractions in Fig. 3) did not ($P < 0.05$) result in the highest cholesterol content (Fig. 5A). The one-step procedures with krill:solvent ratios $\geq 1:9$ generally had the lowest cholesterol content. Cholesterol content of the two-step, Folch, and Soxhlet procedure was similar to the content of the polar non-PL (Fig. 3), suggesting that the cholesterol content contributed more to the content of polar non-PL in these procedures than the one-step procedures.

In general, cholesterol content of krill is lower than shrimp, and slightly higher than fish (Tou et al., 2007). Krill oil resulted in 18% reduction in total serum cholesterol compared to 6% with fish oil in hyperlipidemic patients (Bunea et al., 2004). The hypolipidemic effects of seafood oils are not fully understood, but are significantly influenced by *n*-3 PUFA. Since krill oil is rich in *n*-3 PUFA bound in PL and is low in cholesterol, the hypolipidemic effects of krill oil could be additive.

3.5. Antioxidant capacity of extracted krill oil

The red colour of krill oil is due to the carotenoid astaxanthin, a potent antioxidant. Frozen krill contains 3–4 mg of carotenoids/100 g and astaxanthin is >80% of the total carotenoids (Yamaguchi et al., 1983). By measuring the total antioxidant capacity of the krill oil extracted in the present study, the antioxidative effect of astaxanthin was accounted for. *In vitro* studies have shown that astaxanthin decreases membrane oxidative injury to a greater degree than α-tocopherol, an antioxidant commonly used in food products (Kurashige, Okimasu, Inoue, & Utsumi, 1990).

Oils extracted with the one-step procedure using 1:9 and 1:30 krill:solvent ratios as well as the ethanol extract of the two-step procedure had the greatest ($P < 0.05$) antioxidant capacity (Fig. 5B). The antioxidant capacities of oils extracted with one-step procedures was similar to their respective polar non-PL content (Fig. 3), suggesting that astaxanthin was the primary component of their polar non-PL. Despite having the highest ($P < 0.05$) content of polar non-PL (Fig.3), the acetone extract of the two-step procedure had the lowest ($P < 0.05$) antioxidant capacity. Therefore, the polar non-PL of the acetone extract may be predominately cholesterol. The Folch extraction also yielded oil with the lowest ($P < 0.05$) antioxidant capacity.

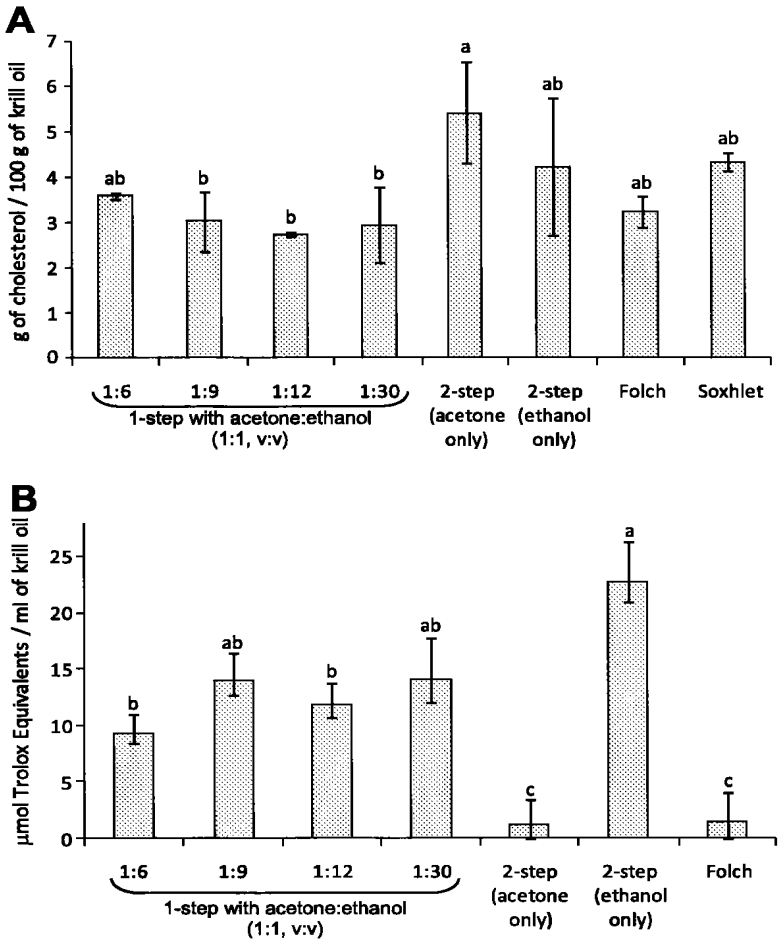

Fig. 5. Cholesterol content (A) and antioxidant capacity (B) of oil extracted from freeze-dried whole krill using different solvent systems. *Different letters on the top of data bars indicate significant differences (Tukey's test, $P < 0.05$) between mean values (±SD, $n = 3$).

Table 1
Crude protein*, total fat*, and ash content* of the residual spent krill following oil extraction with one- and two-step extraction.

	Extraction method				
	1-Step extraction with acetone:ethanol (1:1, v:v) at different krill:solvent ratio (w:v)				2-Step extraction
	1:6	1:9	1:12	1:30	
Crude protein (g/100 g, dry basis)	72.9 ± 1.0 a	73.8 ± 0.8 a	74.8 ± 0.8 a	75.8 ± 1.0 a	75.3 ± 4.0 a
Ash content (g/100 g, dry basis)	17.7 ± 0.7 a	17.3 ± 0.5 a	17.9 ± 0.5 a	16.1 ± 0.6 b	15.6 ± 0.4 b
Total fat (g/100 g, dry basis)	6.4 ± 1.0 a	6.5 ± 1.2 a	4.4 ± 1.0 b	3.7 ± 0.7 b	3.4 ± 1.1 b

* Different letters indicate significant differences (Tukey's test, $P < 0.05$) between mean values (±SD, $n = 3$) within the same row.

3.6. Proximate composition of krill following oil extraction

Following oil extraction, the residual spent krill was removed from the solvent/oil mixture by centrifugation. The spent krill was collected in the sediment (Fig. 1). Table 1 shows that the sediment was primarily composed of protein (~74 g/100 g, dry weight). The amount of protein in the residual spent krill was not ($P > 0.05$) affected by oil extraction procedure. The lipid content decreased ($P < 0.05$) as the krill:solvent ratio increased from 1:9 to 1:30 in the one-step extraction. The two-step extraction and one-step extraction using 1:12 and 1:30 krill:solvent ratios resulted in the lowest ($P < 0.05$) lipid content in the residual spent krill. The ash content in the residual spent krill was comparable to the ash content in whole krill prior to extraction (ash 17.4 g/100 g, dry basis, Chen et al., 2009). Therefore, the fluoride (F) content was likely high and would need to be reduced if the protein in the residual spent krill were to be recovered and used for human/animal consumption.

The proximate data clearly shows that there is high protein content in the residual spent krill; though ash (and likely F) is also high. The protein could be recovered with the isoelectric solubilisation/precipitation (ISP). It has been shown that the proteins recovered from whole krill and other aquatic resources using ISP yield good quality heat-set gels (Chen & Jaczynski, 2007a; Taskaya, Chen, Beamer, and Jaczynski, 2009; Taskaya, Chen, & Jaczynski, 2009). Application of ISP to whole krill also significantly reduces ash content (and likely F) in the recovered proteins (Chen, Tou, & Jaczynski, 2007; Chen et al., 2009). Therefore, a fuller utilisation

of krill as a human food would be possible by extracting oil as shown in the present study, followed by protein recovery by ISP for subsequent inclusion in human food products.

4. Conclusions

Based on the present study, subjecting freeze-dried krill to one-step extraction (acetone:ethanol, 1:1, v:v) using 1:12 krill:solvent ratio (w:v) resulted in the highest oil extraction efficiency. Phospholipids (PL) were the main lipid class in the extracted oil containing omega-3 fatty acids (*n*-3 FA). The PL contained considerably more total *n*-3 FA, unsaturated FA, eicosapentaenoic (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n3) acids, but less saturated FA and similar content of total *n*-6 FA compared to triglycerides (TG). The oil extracted from freeze-dried krill with one-step procedure using 1:12 krill:solvent ratio also had low cholesterol content and high antioxidant capacity compared to other treatments. The protein left over in the residual spent krill following oil extraction could potentially be recovered with isoelectric solubilisation/precipitation (ISP). The ISP also results in reduction of ash, and thus, fluoride content in the ISP-recovered krill protein.

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Characterization of purified phospholipids from krill (*Euphausia superba*) residues deoiled by supercritical carbon dioxide

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Abstract—Purification of phospholipids (PL) from the Antarctic krill (*Euphausia superba*) using a two-step extraction process has been investigated. Using supercritical carbon dioxide (SC-CO_2) extraction with optimal extractions conditions of 45 °C, 25 MPa, and CO_2 flow rate of 22 g/min, most of the neutral lipids were extracted. PC, PE and PI were then extracted in a second step conducted with modified existing method using ethanol, hexane and acetone as solvents. The major PL of krill residues was quantified by high performance liquid chromatography (HPLC-ELSD). The fatty acid compositions of total PL, PC, PE and PI were analyzed by gas chromatography (GC). A significant amount of polyunsaturated fatty acids (PUFA) was present in both total and PLs fractions. The purified PLs were characterized by their acid value, peroxide value, and the oxidative stability. The purity of PL ranged between 93 and 97% and was evaluated by spectrophotometry.

Key words: Krill, Phospholipids Purification, Supercritical Carbon Dioxide, Oxidative Stability

INTRODUCTION

As with fish, the Antarctic krill is a rich source of the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, unlike fish oil the EPA and DHA of krill oil are in the form of phospholipids (PL), giving it new properties and making it potentially more potent [1]. PL, which is a natural and integral part of cell function and is more readily absorbed increasing bioavailability, is a general term that includes all lipids containing phosphorus. Usually, the analysis of PL is based either on the determination of their total fatty acids by gas chromatography or the determination of the PL classes (phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylserine, sphingomyelin, lysophosphatidylcholine etc.) with high performance liquid chromatography (HPLC) [2].

Commercially, PL comes from soybeans, egg yolk, or brain tissue [3]. Until now, the soybean is the most frequent and studied source of lecithin. However, lecithin from soybean is rich in mainly saturated fatty acids with some lower unsaturated fatty acids. It does not contain some important polyunsaturated fatty acids (PUFA) including EPA and DHA. Egg yolk has also been used widely as a source of lecithin.

Several methods were compared for recovery and purification of mixtures of lipids and, more specifically, for PL; however, for more difficult isolation, the results and recoveries vary, depending on the type of phase used and the nature of the sample matrix and composition [4]. Some methods that were originally used for PL separation from meat [5] gave low recoveries when applied to other matrixes. In recent years, supercritical fluid extraction technology (SFE), which is used as an alternative for lipid extraction to organic solvent extraction, has received much attention, because it allows a reduction in extraction time, requires little sample manipulation, and involves a much lower solvent consumption, leading to extracts of increased purity [6,7]. Some works refer to the application of SC-CO_2 extraction of marine materials to obtain PUFA. Yamaguchi et al. [8] reported on the extraction of lipids from Antarctic krill. According to their results, only non-polar components such as cholesterol, carotenoid, triacylglycerols and their derivatives were extracted. PL did not appear in the extracted fractions. SC-CO_2 does not provide a means to dissolve PL, but it can be recovered by the addition of a polar entrainer to SC-CO_2 [9]. The choice of a suitable co-solvent must be based on some considerations such as thermodynamics and food safety [10]. Some researchers have already studied the role of ethanol as a co-solvent. Prosise [11] reported that ethanol was an excellent solvent for isolating PL for food use. But all other neutral lipids with PL are also extracted by ethanol. Therefore, further steps are needed to purify the PL.

Our objective was to optimize and improve existing methods to isolate an enriched PL fraction from dry krill and characterize the purified PL. First, SFE conditions were optimized to extract the majority of neutral lipids. Then, in a second processing step, the residual extracted krill, containing PLs, were extracted with ethanol solvent.

MATERIALS AND METHODS

1. Materials and Chemicals

The krill (*Euphausia superba*) were collected from Dongwon F & B Co., S. Korea. The krill blocks were stored at −80 °C for no longer than 1 year before being used experimentally. The carbon dioxide (99.99% pure) was supplied by KOSEM, Korea. Linoleic acid and trolox were purchased from Sigma-Aldrich, Germany. All other chemicals used in different analysis were of analytical or HPLC

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grade.

2. Sample Preparation

The krill samples (mean body length, 5.15 cm; mean body weight, 0.65 g) were freeze-dried for about 72 h. Then the dried samples were crushed and sieved (700 μm) by mesh. The dried samples, called raw materials, were used for oil extraction by SC-CO_2 and for PL isolation by organic solvent.

3. SC-CO_2 Extraction

Fifty grams of freeze dried krill sample was loaded into 200 mL stainless steel extraction vessel containing cotton at the bottom of the SFE unit for extracting oil from krill. Before plugging with a cap, another layer of cotton was used at the top of the sample. CO_2 was pumped into the vessel by high pressure pump up to the desired pressure, which was regulated by a back pressure regulator. The vessel temperature was maintained by a heater. Flow rates and accumulated gas volume passing through the apparatus were measured with a gas flow meter. The effects of temperature and pressure on lipid extraction from krill were studied at 35-45 ℃ and 15-25 MPa at a constant extraction time of 2.5 h. The flow rates of CO_2 were kept constant at 22 g/min for all extraction conditions. The extraction yield was determined and the krill residues remaining in the vessel were stored at −80 ℃ until further analysis.

4. Purification of PL

The multiple-step procedure for extracting PL from krill is outlined in Fig. 1. The sample (45 g) was extracted according to the procedure described by Palacios and Wang [12], with modification. For the initial extraction, 200 mL of ethanol (95%) was added to 45 g of krill extracted residues (at 45 ℃ and 25 MPa) in a 300-mL centrifuge bottle and stirred by a magnetic stirrer about 12 h. The mixture was then centrifuged at 1,900 rpm for 10 min, and the supernatant containing ethanol, some polar lipids, and some neutral lipids was transferred to a separator funnel with double volume of hexane. After one hour (1 h), the hexane phase was removed, and the ethanol phase was mixed with an additional double volume of hexane and left for 2 h for phase separation. The ethanol was removed from the ethanol extract by rotary vacuum evaporator at 40 ℃, the remaining lipid material was dissolved in 10 mL of hexane and transferred to a 150-mL centrifuge bottle where 60 mL (about fifth volume) of chilled acetone (4 ℃) was added and carefully stirred to precipitate the PL. The centrifuge bottle was then placed in an ice-water bath for 15 min and centrifuged at 1,500 rpm. The precipitate was the purified PL. To investigate the effect of the presence of neutral lipids on the ethanol extraction yield, raw krill samples were used in the same purification method. Three replicates of the samples were carried out.

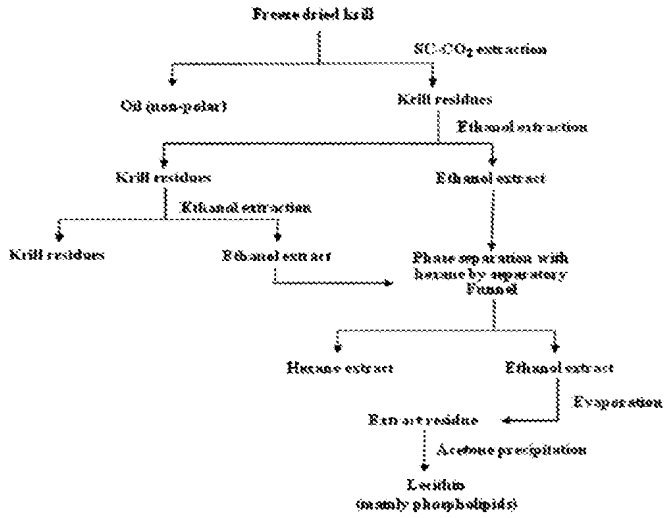

Fig. 1. Summary of the purification steps.

5. Quantification of Purity of Isolated PL

The PL content of lecithin was measured according to Stewart [13] by a colorimetric method based on the formation of a complex between PL and ammonium ferrothiocyanate. Briefly, 0.35 mg of dry lecithin was dissolved in 2 mL of chloroform. Then, 1 mL of a solution prepared from ferric chloride (27 g/L) and ammonium ferrothiocyante (30 g/L) was added. After vortexing, the mixture was centrifuged at 1,000 rpm for 15 min. The lower phase was collected and the absorbance was recorded at 488 nm. A calibration curve was made by standard PC and the result was expressed as gram equivalents of PC per gram of lecithin.

6. Major PL Quantification by HPLC-ELSD

PL composition was determined using a Jasco (LC-Net II/ADC) HPLC system coupled with an evaporative light scattering detector (ELSD), model 400 (Jasco, Japan), model 126 solvent delivery modules. Appropriate dilutions of sample were injected (20 μl injection loop) onto a diol normal-phase silica column (250 mm×4.6 mm i.d., with integral guard column; Advanced Separations Technologies, Waters). The analysis was carried out according to the method of Letter [14]. Extracted lecithin was dissolved in chloroform and injected (20 μL) into injector. The mobile phase was isopropyl alcohol, hexane and water. The drift tube temperature of ELSD was set at 60 ℃. The pressure of nitrogen gas as a nebulizer was 50 PSI. The quantification of PL was performed based on the peak area of standard PL, PC, PE and PI. The millennium software was used to analyze the data obtained by HPLC.

7. PLs Characterization

Hexane-insoluble matter, humidity and acid value of the purified PL were determined using the AOCS [15] official methods (Ja 3-87, Ja 2b-87, Ja 6-55). Analyses were performed in triplicate.

7-1. Free Fatty Acids

Free fatty acids of purified PL from krill were analyzed as described by Bernardez et al. [16]. Briefly, 50 mg (approximately) of sample was placed into Pyrex tubes with the addition of 3 ml of cyclohexane, and then 1 ml of cupric acetate-pyridine reagent was added. Tubes were vortexed for 30 s. After centrifugation at 2,000 rpm for 10 min, the upper layer was read at 710 nm. The FFA content was measured on a calibration curve constructed from oleic acid standards. Copper reagent was prepared according to Lowry and Tinsley [17]. A 5% (w/v) aqueous solution of cupric acetate was prepared and filtered. Then the pH of cupric acetate solution was adjusted to 6.1 by using pyridine.

7-2. Peroxide Value

Peroxide value was determined according to AOCS [15] method Cd 8-53 by modified amount. 1.0 g of krill purified PL was dissolved in 6 mL of acetic acid-chloroform (3 : 2) solution. Then 0.1 mL of saturated KI solution was added to the mixtures and the solution was allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution. The solu-

tion was titrated with 0.1 N of sodium thiosulfate until the yellow iodine color had almost disappeared. Then 0.4 mL of starch indicator solution was added and again titrated until the blue color disappeared. A blank determination was conducted with the same procedure. Peroxide value was expressed as milliequivalents peroxide/1,000 g sample.

7-3. Thin Layer Chromatography (TLC) of the Purified PL Fractions

The PL was separated by TLC for the determination of fatty acid compositions of PLs fractions. TLC was carried out using silica gel 60 plates (pre-coated with a 0.20-mm layer of silica gel 60) were obtained from ALUGRAM (Germany). The sample was applied (0.3 mg) on the plate and developed with a solvent system of dichloromethane : methanol : glacial acetic acid (60 : 30 : 10) (18). Spots were visualized by exposure to iodine vapor or by charring with 50% sulfuric acid at 130 °C for 30 min. Standard mixtures of PL were run in parallel with the sample for identification of spots. Spots were then scraped off in a screw cap tube separately and extracted from the silica using the solvent system of chloroform : ethanol : water (2 : 2 : 1, v/v/v). The chloroform phase was collected by phase separation and evaporated by vacuum rotary evaporator. The purity of the remaining residues of each polar lipid was again checked by TLC. PC, PE and PI from the spots were extracted as described above. This purified PC, PE and PI were used for fatty acid compositions.

7-4. Analysis of Fatty Acids Composition by Gas Chromatography

Fatty acids analysis of the oils extracted using SC-CO_2, lecithin, and purified PC, PE and PI was performed by GC. Methyl esters of fatty acids from total lipid extracts were prepared according to AOCS [19]. The gas chromatographic apparatus for the analysis of fatty acid composition was an HP 5790II equipped with a flame ionization detector (FID) and a capillary column DB-wax. Nitrogen was used as a carrier gas (1.0 $mLmin^{-1}$) of fatty acid methyl esters. The oven temperature was programmed, starting at a constant temperature of 130 °C for 3 min, and then increased to 240 °C at a rate of 4 °Cmin^{-1} and hold at 240 °C for 10 min. Injector and detector temperatures were 250 °C. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, USA).

7-5. Oxidative Stability

The oxidative stability was measured by the oxidation of emulsion of purified PL in water (deionized and degassed water) at 37 °C. Four emulsions of PL in water (w/w) (linoleic acid 5%, PL 5%, water 90%; PL 5%, water 95%; torolox 1%, PL 4%, water 95%; linoleic acid 10%, water 90% (control)) were prepared. The mixtures were properly homogenized by a homogenizer. Oxidative stabilities were checked by thiocaynate and thiobarbituric acid method (TBA). Linoleic acid and standard trolox were used to compare oxidative stability of purified PL.

7-5-1. Thiocyanate Method

This method was conducted according to Mitsuda et al. [20]. The peroxide formed by lipid peroxidation reacts with ferrous chloride and forms ferric ions. Ferric ions then unite with ammonium thiocyanate and produce ferric thiocyanate. Briefly, 0.1 mL of emulsion solution was added to 4.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500 nm (UVIKON 933, Kontron Instruments). Every 24 h interval during incubation, the absorbance was recorded.

7-5-2. TBA Method

The TBA method measures free radicals present after peroxide oxidation. The TBA value was measured according to Ottolenghi [21]. Briefly, 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid were added to 1 ml of emulsion solution. The mixture was placed in a boiling water bath (100 °C) for 10 min. After cooling, the mixture was centrifuged at 3,000 rpm for 20 min. Absorbance of supernatant was measured at 532 nm by a uv/visible spectrophotometer (UVIKON 933, Kontron Instruments).

8. Statistical Analysis

All experiments were performed in triplicate and each set of results was averaged. The standard deviations were used as the basis for the error bars shown in the figures. The least significant difference at the 95% confident ($P<0.05$) level was calculated by Duncan test using Statistical Analysis System (SAS Ver. 9.1, SAS Institute, USA).

RESULTS AND DISCUSSION

1. SC-CO_2 Extraction

Fig. 2 shows the extraction curves of krill oil by SC-CO_2 at different temperatures (35, 40 and 45 °C) and pressure (15, 20 and 25 MPa) as well as the complete yield data obtained in each experimental run are shown in Fig. 3. Extraction yields varied from 4-6% to 10-11.5%. The highest yield (11.5%) was at 45 °C and 25 MPa. The amount of extracted oil increased with the increasing of CO_2 mass, depending on the pressure and temperature. In this work, the amount of extracted krill oil per solvent (CO_2) mass used was increased over the entire extraction period, until almost all the oil was extracted. On the other hand, the oil extraction yield increased either with pressure at constant temperature or with temperature at constant pressure. By the increase in pressure, the density of the SC-CO_2 was increased and hence the solvating power. Morita and Kajimoto [22] reported that the effect of pressure can be attributed to the increase in solvent power and by the strengthening of inter-

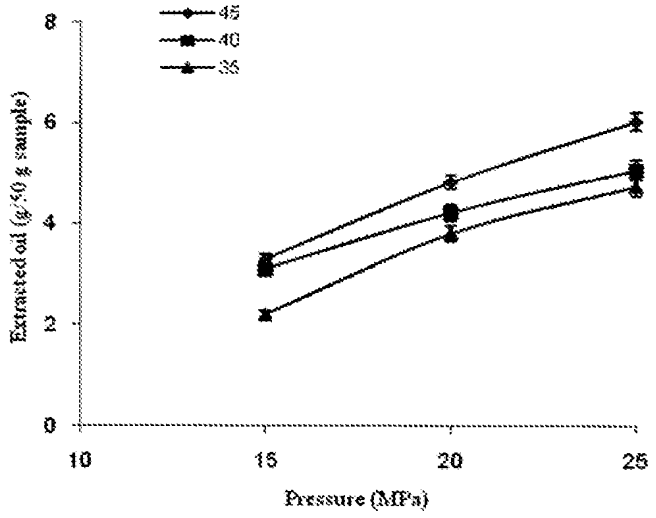

Fig. 2. SC-CO_2 extraction of oil from krill at different temperatures and pressure.

molecular physical interactions. Similar results were found by De Azevedo et al. [23]. The oil yield increased with the increase in temperature. By the increase of temperature, the density of SC-CO_2 was decreased, but it increased the oil component vapor pressure and that enhanced the extraction yield. Macias et al. [24] has also mentioned that the yield depends on a complex balance between the decrease in the SC-CO_2 density and the increase in vapor pressure.

2. Fatty Acids Composition

The fatty acid composition of the lipids of different species of Antarctic krill has already been published [25]. In this study, the fatty acid profile of the oil extracted by SC-CO_2 under different conditions and at different times was slightly similar. Whereas, the most abundant fatty acid was palmitic acid (16 : 0) representing 23.3% of fatty acids in krill oil (Table 1) and the main fatty acids were 18 : 1, 16 : 1, 14 : 0, 20 : 5 n-3, 22 : 6 n-3. The comparison between the fatty acid composition of the total lipids in freeze dried krill and in krill meal [8], as reported in Table 2, shows a significant differences in the 14 : 0, 16 : 1 and in 20 : 3.

3. Organic Solvent Extraction for Comparison and Solvent Selection

To investigate the effect of neutral lipids on the efficiency of the extraction by ethanol, forty-five grams of raw krill was treated using the same purification method, and the purified PL composition was compared with that obtained from SC-CO_2 extracted residues. Also, with the aim of comparing the extraction efficiency of organic solvents in order to select the solvent with highest yield of PL content and safety for human body, different organic solvents were used for the extraction of PL from both raw materials and SC-CO_2 extracted residues. The results of the comparison are reported in Table 2. The highest yield was from SC-CO_2 extracted residues, but the most effective solvent was ethanol with 42.7%.

Table 1. Fatty acids composition from SC-CO_2 extracted krill oil

Fatty acids[a] (%)	SC-CO_2 krill oil	Krill meal [8]
14 : 0	11.2±0.12	19.1
16 : 0	23.3±0.14	18.8
18 : 0	0.87±0.32	1.3
16 : 1	12.31±0.23	16.3
17 : 1	1.02±0.42	1.70
18 : 1	20.11±0.33	22.4
18 : 2 n-6	2.21±0.15	3.56
18 : 3	0.8±0.26	0.26
18 : 4	2.1±0.13	3.21
20 : 1	1.4±0.24	1.86
20 : 3	0.17±0.31	0.09
20 : 4 n-6	0.17±0.43	0.55
20 : 5 n-3	8.24±0.25	6.62
22 : 5 n-3	0.28±0.17	0.18
22 : 6 n-3	3.18±0.25	2.71
24 : 1	0.16±0.22	0.42

[a]The results showed mean value±standard deviation

Table 2. Organic solvent extraction from raw krill and SC-CO_2 extracted residues for comparison and solvent selection

Solvent	Total PL purified[a] (%)	
	Raw krill	SC-CO_2 treated krill
Petroleum ether	26.5±0.52	29±0.41
Acetone	18.6±0.35	20±0.24
2-Propanol	33.5±0.43	36±0.35
Ethanol	37.4±0.51	42.7±0.16

[a]The results showed mean value±standard deviation

Table 3. Major PLs composition

PLs distribution[a] (%)	
PC	80.4±0.64
PE	14.9±0.46
PI	0.7±0.72

[a]The results showed mean value±standard deviation

4. Major PL Composition

Major PL compositions are shown in Table 3. The main PLs of krill were PC and PE. PC and PE content of krill was 80.4% and 14.9% of total PL, respectively. PI was also present but with very low percentage (0.7%) of total PL. Kusumoto et al. [26] reported that PL from krill (*Euphausia pacifica*) contained (36.2-53.8%) of PC and (3.4-17.5%) of PE. Gordeev et al. [27] and Fricke et al. [18] also reported that PC content of PL from krill (*Euphausia superba*) was 3 to 5 times higher than PE content, which was similar to this study. The PL content of krill from different catches may vary considerably. The variations are probably due to both differences in nutritional status and maturity of the roe of krill, as well as to different isolation and quantification process used.

5. PLs Characterization

The isolated PL was almost pure (97%). It was found that the deoiled krill residues contained 8.06% of total PL. Analysis of the purified krill PL shows a composition in agreement with the range reported in the literature for krill PL [18]. PL content variation in krill may due to different factors, such as sample age, fishing area, season, extraction process and time, extraction efficiency by different solvents etc. The results from krill PL characterization are summarized in Table 4. Hexane-insoluble matter of PL was low (<1%) indicating almost the purity of PL.

5-1. Free Fatty Acids

FFAs are responsible for the acidity of oil. Changes of FFA content are mainly related to hydrolytic reactions in the lipid. FFA content of the purified PL was 2.34 g/100 g of PL. The amount of FFA and peroxide value agreed with the results of Ackman et al. [28] and Sargent and Falk-Petersen [29], who reported values in the range of 2-8% of the dry weight. But contrast the result (0.6%) reported by Saether et al. [30], who mentioned the special precautions taken

Table 4. Characterization of the purified PLs

Paramater	Value
Hexane-insoluble matter[a] (%)	0.91±0.73
Moisture[a] (%)	2.13±0.65
Acid value[a] (mg KOH/g)	23.07±0.24
Peroxide value[a] (milliequivalent/1,000 g)	4.66±0.28
Free fatty acids[a] (g/100 g)	2.34±0.34

[a]The results showed mean value±standard deviation

in their study to avoid postmortem lipolysis. Also, higher temperature and storage time caused a significant increment of the FFA in the hake byproducts oil [31]. Previous studies reported that the oxidation rate of Antarctic krill (*Euphausia superba*) lipid is very slow and no peroxides are accumulated even after a long-term storage [32].

5-2. Acid Value and Peroxide Value

The acid value and peroxide values of purified PL from SC-CO_2 extracted krill residues are given in Table 4. Acid value was used to determine the acidity of PL. On the other hand, peroxide value is used as a measurement of rancidity of lipids which occurs by auto oxidation. Generally, peroxide and acid values of commercially available soy lecithin (mainly PL) are found up to 12 milliequivalent/1,000 g and 30 mg KOH/g of lecithin, respectively. The PL isolated from krill contained free fatty acids which increased its acid value.

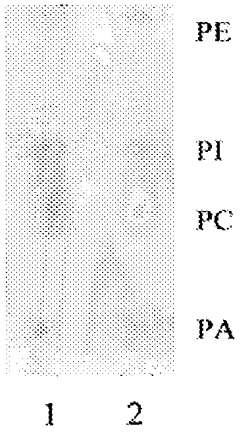

Fig. 3. Thin-layer chromatograms of PL purified from SC-CO_2 extracted residues (1) and from raw krill (2): PA, PC, PI, and PE.

Table 5. Fatty acid composition of the PLs fraction (% (w/w))

F.A. as methyl esters[a]	Total PL	PC	PE	PI
14 : 0	1.12	2.78	4.32	10.15
15 : 0	1.50	1.21	1.55	5.63
16 : 0	4.69	18.05	25.66	36.02
16 : 1	0.21	0.70	1.16	19.33
18 : 0	10.28	6.20	1.33	8.72
18 : 1	29.16	33.09	16.77	20.15
18 : 2 n-6	3.50	11.54	2.17	ND
20 : 4 n-6	18.07	24.35	9.50	ND
20 : 5 n-3	20.57	2.08	26.30	ND
22 : 6 n-3	10.90	ND	10.79	ND
Total (%)	100.0	100.0	100.0	100.0
Saturated	11.59	28.24	33.31	60.52
Unsaturated	82.41	71.76	66.69	39.48

[a]Results were average of two determinants. Standard error of the fatty acid constituents was on the order of about ±2%. For total PL, fatty acids showed which was present more than 1% of total fatty acids
ND: not detected

5-3. Fatty Acid Composition of PL

To analyze fatty acid compositions of major PL, the PL were separated by preparative TLC (Fig. 3). The fatty acid compositions of isolated PL, PC, PE and PI are shown in Table 5. The predominant fatty acid in total PL and in PC subfraction was oleic acid (18 : 1) (29.16% and 33.9%, respectively). Arachidonic acid (20 : 4 n-6) was abundant in PC subfraction (24.35%) and in total PLs in different percentages (18.07%). Palmitic acid (16 : 0) was predominant in PI subfraction (36.02%), but also was highly present in all subfractions. Stearic acid (18 : 0) varied from 1.33% to 10.28% in all subfractions. PC can be an important source of essential PUFA; the present study showed that PC contained almost 40% of PUFA. Several works have confirmed that in marine crustaceans PE is a highly unsaturated PL [33]. Because of the high levels of PUFA that contain, particularly EPA and DHA, PE has been considered as a reserve for the adaptation of membrane fluidity to changes in temperature [34]. In agreement with these previous studies, these results showed that PE contained 26.30% of EPA and 10.79% of DHA.

The amount of the unsaturated fatty acids in total PL was very

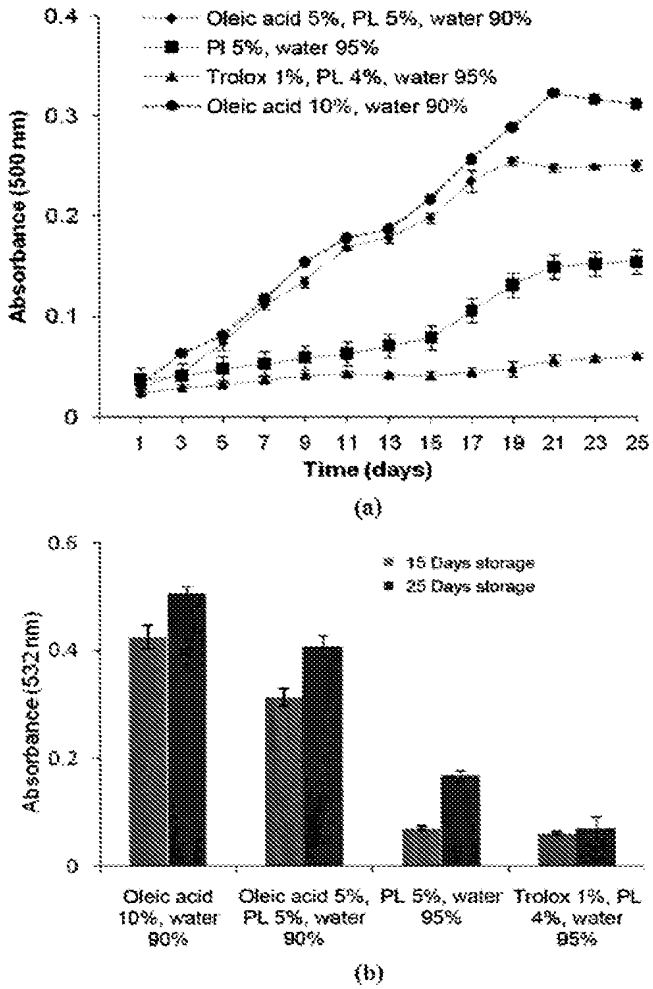

Fig. 4. Oxidative stability of the purified krill PL. (a) Thiocyanate method, (b) Thiobarbituric acid method.

high (82.41%), while the percentage of the saturated fatty acids was found 11.59%. The unsaturated/saturated ratio varied from 0.6 to 7.11 for the total PL and for all subfractions. The PUFA were the most abundant of the unsaturated fatty acids of total PL, PC, and of the PE subfraction. The results obtained are in agreement with those of other authors [27].

5-4. Oxidative Stability

Instead of determining the absolute state of oxidation of incubated sample, the oxidation trend was evaluated. The oxidative stabilities of PL are shown in Fig. 4(a) and (b). It has been reported that the oxidation rate of Antarctic krill (*Euphausia superba*) lipid is very slow [32]. In this study, PL with linoleic acid showed increase in absorbance values from first day. The increase in absorbance value was an indicator of auto oxidation by formation of peroxides during incubation. PL showed low absorbance value, indicating low level of lipid peroxidation until 17 days (Fig. 4(a)). And it showed increased oxidation after 19 days. On the other hand, PL with trolox showed higher oxidative stability than that of the other emulsions. Trolox, which is an antioxidant, inhibited the peroxide formation by lipid peroxidation in a certain period. The control showed increase in absorbance values from day 1 and reached on day 19 and dropped on day 23. In addition, the PL showed slightly high absorbance compared to PL with linoleic acid. It might be due to the presence of peroxide from the oxidation of some neutral lipid (impurities) existing in the emulsion of PL. In TBA method, the absorbance measured on day 15 was slightly similar between PL and PL with trolox emulsion. However, it was high on PL with linoleic acid emulsion, indicating low oxidative stability (Fig. 4(b)). On the other hand, a significant increase in absorbance was found on day 25 for PL emulsion. It has also been shown that the major constituents of the PUFA of PL in krill are EPA and DHA, which were the most susceptible to oxidation; in soybean, linoleate, and in egg yolk, arachidonate. Therefore, it can be supposed that the oxidative deterioration of PL proceeds most rapidly in krill, less rapidly in egg yolk and least rapidly in soybean [35]. However, PL showed high oxidative stability, which can be explained by the presence of the natural antioxidant, astaxanthin in PL, since Krill is a major source of astaxanthin, which has strong antioxidant activity [36]. Gogolewski et al. [37] also reported that long chain polyunsaturated fatty acid esterified with polar lipids had synergistic effect with antioxidant. Previous studies reported the high oxidative stabilities of PL from animal and plant sources by applying different methods [12,38].

CONCLUSION

The suitable conditions for the purification of PL from krill were examined. With a two-step extraction process, it is possible to isolate a PL-rich extract from krill. By SFE with CO_2 most of the neutral lipids were extracted from the Antarctic krill. The functional lipids in the spent solids from this first stage SFE were enriched with the removal of the neutral lipids. PC, PE and PI were then extracted in a second step using ethanol, hexane and acetone as solvents. Under optimum conditions, the final yield estimated was about 42.7% (w/w) of krill lipid. EPA formed the most abundant molecular species in PE, but not in PC and PI. While, arachidonic acid (20 : 4 n-6) was mainly present in PC and PE but was absent in PI. Also, the purified PL showed a potent antioxidant activity. Therefore, further work could be done on the isolation and characterization of the individual compounds in the extracts, which are responsible for antioxidant activity. These results show a great potential for utilization of SFE to obtain a functional, value-added ingredient from marine product, with great economic significance.

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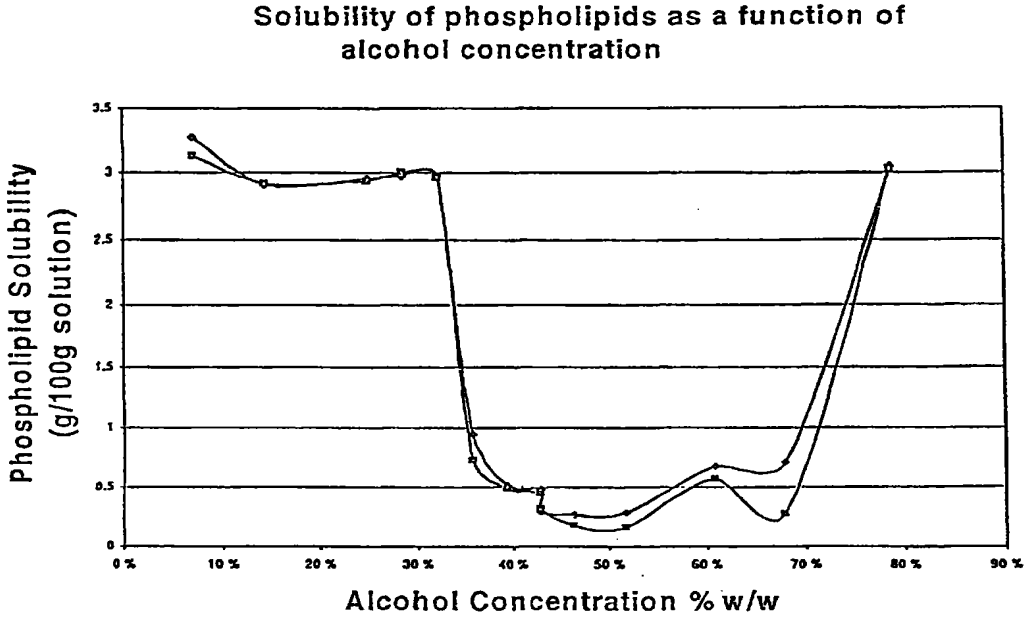
(54) Title: METHOD FOR THE FRACTIONATION OF OIL AND POLAR LIPID-CONTAINING NATIVE RAW MATERIALS USING ALCOHOL AND CENTRIFUGATION



(57) Abstract: A process for the production of polar lipid-rich materials and preferably phospholipids. Preferably the polar lipid-rich materials are separated and recovered from native raw materials by extraction with water-soluble organic solvent and use of density separation to separate the resulting mixture.

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METHOD FOR THE FRACTIONATION OF OIL AND POLAR LIPID-CONTAINING NATIVE RAW MATERIALS USING ALCOHOL AND CENTRIFUGATION

FIELD OF THE INVENTION

The present invention relates to a process for the separation and recovery of polar lipid-rich fractions from mixtures such as native raw materials. Other fractions in the raw materials can also be recovered.

BACKGROUND OF THE INVENTION

Examples of polar lipids include phospholipids (e.g. phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, phosphatidylglycerol, diphosphatidylglycerols), cephalins, sphingolipids (sphingomyelins and glycosphingolipids), and glycoglycerolipids. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, amino alcohols, and carbohydrates. They are generally considered to be structural lipids, playing important roles in the structure of the membranes of plants, microbes and animals. Because of their chemical structure, polar lipids exhibit a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents. The term polar lipid within the present description is not limited to natural polar lipids but also includes chemically modified polar lipids. Although the term oil has various meanings, as used herein, it will refer to the triacylglycerol fraction.

One of the important characteristics of polar lipids, and especially phospholipids, is that they commonly contain polyunsaturated fatty acids (PUFAs: fatty acids with 2 or more unsaturated bonds). In many plant, microbial and animal systems, they are especially enriched in the highly unsaturated fatty acids (HUFAs: fatty acids with 4 or more unsaturated bonds) of the omega-3 and omega-6 series. Although these highly unsaturated fatty acids are considered unstable in triacylglycerol form, they exhibit enhanced stability when incorporated in phospholipids.

The primary sources of commercial PUFA-rich phospholipids are soybeans and canola seeds. These biomaterials do not contain any appreciable amounts of HUFAs unless they have been genetically modified. The phospholipids (commonly called lecithins) are routinely recovered from these oilseeds as a by-product of the vegetable oil extraction process. For example, in the production of soybean or canola oil, the beans

(seeds) are first heat-treated and then crushed, ground, and/or flaked, followed by extraction with a non-polar solvent such as hexane. Hexane removes the triacylglycerol-rich fraction from the seeds together with a varying amount of polar lipids (lecithins). The extracted oil is then de-gummed (lecithin removal) either physically or chemically as a part of the normal oil refining process and the precipitated lecithins recovered. One disadvantage of this process is the use of the non-polar solvents such as hexane presents toxicity and flammability problems that must be dealt with.

The crude lecithin extracted in the "de-gumming" process can contain up to about 33% oil (triacylglycerols). One preferred method for separating this oil from the crude lecithin is by extraction with acetone. The oil (triacylglycerols) is soluble in acetone and the lecithin is not. The acetone solution is separated from the precipitate (lecithin) by centrifugation and the precipitate dried under first a fluidized bed drier and then a vacuum drying oven to recover the residual acetone as the product is dried. Drying temperatures of 50-70°C are commonly used. The resulting dried lecithins contain approximately 2-4% by weight of oil (triacylglycerols). Process temperatures above 70°C can lead to thermal decomposition of the phospholipids. However, even at temperatures below 70°C the presence of acetone leads to the formation of products that can impair the organoleptic quality of the phospholipids. These by-products can impart musty odors to the product and also a pungent aftertaste.

To avoid use of non-polar solvents such as hexane and avoid the negative side effects of an acetone-based process, numerous processes have also been proposed involving the use of supercritical fluids, especially supercritical CO_2. For example, U.S. Patent No. 4,367,178 discloses the use of supercritical CO_2 to partially purify crude soy lecithin preparation by removing the oil from the preparation. German Patent Nos. DE-A 30 11 185 and DE-A 32 29 041 disclose methods for de-oiling crude lecithin with supercritical CO_2 and ethane respectively. Other supercritical processes have been proposed which include adding small amounts of hydrocarbons such as propane to the supercritical CO_2 to act as entraining agents. However, supercritical fluid extraction systems are very capital expensive and cannot be operated continuously. Further, extraction times are long and the biomaterials must be dried before extraction, and this increases the difficulties of stabilizing the resulting dry product with antioxidants. All of these factors make the supercritical process one of the most expensive options for extracting and recovering polar-lipid material or mixtures of these materials. As a result,

alternative processes using extraction with liquid hydrocarbons at lower pressures have been described. For example U.S. Patent No. 2,548,434 describes a method for de-oiling oilseed materials and recovering crude lecithin using a liquid hydrocarbon at lower pressures (35-45 bars) but elevated temperatures (79° to 93°C). U.S. Patent No. 5,597,602 describes a similar process that operates at even lower pressures and temperatures. However, even with these improvements supercritical fluid extraction remains very expensive and is not currently used to produce phospholipids for food use on a large commercial scale.

The primary commercial source of HUFA-rich polar lipids is egg yolk. Two primary methods are used for the recovery of egg phospholipids on an industrial scale. Both require the drying of the egg yolk before extraction. In the first process the dried egg yolk powder is extracted first with acetone to remove the triacylglycerols. This is then followed by an extraction with pure alcohol to recover the phospholipids. In the second process, pure alcohol is used to extract an oil/lecithin fraction from the dried egg yolk. The oil/lecithin phase is then extracted with acetone to remove the triacylglycerols, leaving behind a lecithin fraction. Both of these methods require the use of acetone, which has the disadvantages discussed above.

Canadian Patent No. 1,335,054 describes a process for extracting fresh liquid egg yolk into protein, oil and lecithin fractions by the use of ethanol, elevated temperatures, filtration and low temperature crystallization with further filtration. The purity of the lecithin product is not disclosed. However one skilled in the art would expect that the lecithin fraction produced by this process would not be very pure. There would still be very significant amounts of oil associated with the lecithin because the chilling process would primarily remove the triglycerides containing saturated fatty acids. Those containing some unsaturated fatty acids would remain more soluble at low temperatures. Additionally, the filtration and the chilling/filtration processes employed in this method would be labor intensive and difficult to turn into a continuous process. In light of the current state of the art, there remains a need for an improved extraction technology for food-grade polar lipid products that is less expensive to operate and which protects the overall quality of the HUFAs in the polar lipid products.

SUMMARY OF THE INVENTION

In accordance with the present invention, an improved process is provided for recovering polar lipids from native biomaterials, which does not involve the disadvantages of the prior art. One embodiment of the invention resides in a process for recovering polar lipids and/or polar lipid-containing mixtures from biomaterials using both high and low water-soluble organic solvent concentrations and centrifugation.

In accordance with an embodiment of the present invention, a process for fractionation of an oil-, polar lipid-, and protein-containing mixture is provided. The process includes the steps of adding a high concentration of water-soluble organic solvent to the mixture, separating protein from the mixture by subjecting the mixture to density separation, e.g., using gravity or centrifugal force, to form a protein-rich fraction and a polar lipid/oil-rich fraction, reducing of the concentration of water-soluble organic solvent in the polar lipid/oil-rich fraction, and subjecting this fraction to density separation, e.g., using gravity or centrifugal force, to form a polar lipid-rich fraction and an oil-rich fraction.

In accordance with another embodiment of the present invention, a process for recovering polar lipid from a polar lipid-containing mixture employing the use of a water-soluble organic solvent, wherein the relatively high solubility of polar lipid in a water-soluble organic solvent, in which the water-soluble organic solvent comprises greater than 68 percent by weight of the aqueous solution, followed by process steps which utilize water-soluble organic solvent concentrations of from about 5 to about 35% by weight, are employed to assist in the recovery.

In accordance with another embodiment of the present invention, a process for fractionation of an oil-, polar lipid-, and protein-containing mixture is provided. The process includes the steps of adding a high concentration water-soluble organic solvent to the oil-, polar lipid-, and protein-containing mixture, and separating protein from the mixture to form a protein-rich fraction and a polar lipid/oil-rich fraction. As used herein, the term "high concentration water-soluble organic solvent" will mean greater than 68 percent organic solvent, preferably greater than 80 percent organic solvent, more preferably greater than 90 percent, more preferably from about 80 percent to about 95 percent organic solvent.

Preferably, the process steps are conducted under oxygen-reduced atmospheres that can include use of inert or non-reactive gases (e.g. nitrogen, carbon dioxide, argon,

etc), use of solvent vapors, use of a partial or full vacuum, or any combination of the above.

BRIEF DESCRIPTION OF THE FIGURES

The present invention may be more readily understood by reference to the following figures, wherein

FIG. 1 is a graphical representation of the solubility of phospholipids, a form of polar lipids, as a function of alcohol concentration.

FIG. 2 is a graphical representation of a phospholipid extraction process (as an example of a polar lipid extraction process) based on a high concentration of alcohol.

DETAILED DESCRIPTION OF THE INVENTION

Because of their bipolar nature, polar lipids (including phospholipids) are of significant commercial interest as wetting and emulsifying agents. These properties may also help make HUFAs in the phospholipids more bioavailable, in addition to enhancing their stability. These properties make phospholipids ideal forms of ingredients for use in nutritional supplements, food, infant formula and pharmaceutical applications.

We have unexpectedly found that polar lipids are soluble not only in high water-soluble organic solvent concentrations (e.g., at water-soluble organic solvent concentrations greater than about 68% w/w) but also in low water-soluble organic solvent concentrations (less than about 35% water-soluble organic solvent w/w) (FIG. 1). As used herein, water-soluble organic solvent concentration means the weight percentage of water-soluble organic solvent in an aqueous solution. The aqueous solution includes added water and water present in the materials. For the purpose of this invention, phospholipids are described as "soluble" if they do not settle or separate from the continuous phase (sometimes also called supernatant or light phase) when subjected to centrifugation by equipment described in this invention. In the water-soluble organic solvent concentration range from about 35% w/w to about 68% w/w water-soluble organic solvent, polar lipids exhibit significantly lower solubility. The present invention exploits this property of polar lipids (enhanced solubility/dispersibility at low water-soluble organic solvent concentrations), which can then be exploited in several ways (along with the high solubility of phospholipids in high water-soluble organic solvent

concentrations) to develop processes for inexpensively extracting and recovering polar lipids, and especially phospholipids, from native biomaterials.

Native biomaterials that are rich in HUFA-containing polar lipids include fish, crustaceans, microbes, eggs, brain tissue, milk, meat and plant material including oilseeds. As used herein, the terms fish, crustaceans, microbes, eggs, brain tissue, milk, meat and plant material including oilseeds will include genetically modified versions thereof. The content of phospholipids in these materials is generally low, usually ranging from 0.1% to about 4% by wet weight. As a result large amounts of raw materials need to be processed to recover these phospholipids. Because of the high costs of prior extraction techniques, phospholipids and especially HUFA-enriched phospholipids were very expensive and therefore restricted to use in the infant formula, pharmaceutical and cosmetic industries. One of the advantages of the present invention is that it provides for the extraction of polar lipids, and in particular phospholipids, in a cost-effective manner.

A polar lipid recovery process utilizing high concentrations of water-soluble organic solvent in a polar lipid/oil concentration step followed by the use of low water-soluble organic solvent concentrations in a step recovering the polar lipids from the oil phase is outlined in FIG. 2. Liquid egg yolk is used as the polar-lipid rich biomaterial in this example. It is understood, however, that other polar lipid-containing biomaterials (e.g. fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds) could also be processed in a similar manner with minor modification to the process.

In the first step of the process 12, the material is dried, if necessary. For a more efficient recovery of the protein, the material is optionally subjected to size reduction 14. A water-soluble organic solvent (e.g., alcohol) is added 14. The concentration of water-soluble organic solvent in the water-soluble organic solvent/water solution is at least about 68% w/w, preferably at least about 80% w/w, preferably at least about 90% w/w, more preferably from about 80 to about 95% w/w, more preferably from about 85 to about 95% w/w, and more preferably from about 90 to about 95% w/w. The more moisture that is present in the material, the greater the amount and/or the higher the concentration of the water-soluble organic solvent that will be needed to achieve the desired concentration when mixed with the material. In other words, if the material is relatively dry, less water-soluble organic solvent and/or lower concentration water-soluble organic solvent can be employed. On the other hand, if the material is relatively

wet, more water-soluble organic solvent and/or higher concentration water-soluble organic solvent must be employed.

The denatured protein 20 is then separated by density separation 18. Since proteins are not soluble in high concentrations of water-soluble organic solvent, they precipitate (while the polar lipids and oil dissolves in the high concentration water-soluble organic solvent) and the precipitated proteins 20 are separated from the polar lipid/oil-enriched fraction 22 by density separation 18, e.g., using gravity or centrifugal force. Using egg yolk as an example, this results in two fractions being recovered: (1) a fraction with approximately 60-95% oil (as % dry weight) and about 5-40% dry weight as polar lipids; and (2) a protein fraction, preferably with more than 90% of the protein of the egg yolk.

If it is desired to separate the polar lipid from the oil, the oil/polar lipid fraction 22 is mixed 26 with water 24 to a final concentration of water-soluble organic solvent in water of from about 5 to about 35% w/w, preferably from about 20 to about 35% w/w, more preferably from about 25 to about 30% w/w. A less desirable alternative would be to dry the oil/polar lipid fraction 22 and then add a water-soluble organic solvent, and water as necessary, to achieve the desired concentration of water-soluble organic solvent. The polar lipid is then separated from the oil by means of density separation 28. A polar lipid-enriched fraction 30 and an oil-enriched fraction 32 are formed. Further processing can be performed on the polar lipid-enriched fraction 30 and/or oil-enriched fraction 32 as desired or necessary. For example, counter-current washing/centrifugation or cross-current washing/separation of the oil and polar lipid products can be employed to improve the purity of the products and economics of the overall process.

In an alternative embodiment, the drying step can be eliminated. For example, instead of drying a material such as eggs, wet eggs can be used. The process is similar to that described above, however, the drying step is eliminated. As a result, a larger amount and/or higher concentration of water-soluble organic solvent is employed to precipitate the protein.

Because of the simplicity of the equipment required in the process, the entire process can very easily be conducted under a reduced-oxygen atmosphere (e.g., nitrogen, a preferred embodiment of the process), further protecting any HUFAs in the polar lipids from oxidation. For example, a gas tight decanter can be used to separate protein from the mixture. A suitable decanter is model CA 226-28 Gas Tight available from Westfalia

Separator Industry GmbH of Oelde Germany, which is capable of continuous separation of protein from suspensions with high protein solids content in a centrifugal field. A gas tight separator useful for separating polar lipids from oil is model SC 6-06-576 Gas Tight available from Westfalia Separator Industry GmbH of Oelde Germany, which is capable of continuous separation of polar lipids from oil in a centrifugal field.

The concentration of water-soluble organic solvent in the protein removal step is preferably greater than about 68% w/w, more preferably greater than about 70% w/w, more preferably greater than about 80% w/w, more preferably greater than about 90% w/w. In principle, it is believed that the higher the water-soluble organic solvent concentration, the stronger the protein contraction, but the more nonpolar the aqueous/water-soluble organic solvent phase, more polar lipids may be dissolved in the oil phase. The appropriate concentration and temperature must therefore be found, for example, by conducting a few preliminary experiments (centrifuge tests), for each raw material.

The present invention, in various embodiments, includes components, methods, processes, systems and/or apparatus substantially as depicted and described herein, including various embodiments, subcombinations, and subsets thereof. Those of skill in the art will understand how to make and use the present invention after understanding the present disclosure. The present invention, in various embodiments, includes providing devices and processes in the absence of items not depicted and/or described herein or in various embodiments hereof, including in the absence of such items as may have been used in previous devices or processes, *e.g.*, for improving performance, achieving ease and/or reducing cost of implementation.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or

equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

We claim:

1. A process for fractionation of an oil-, polar lipid-, and protein-containing mixture, comprising the steps:

(a) adding a water-soluble organic solvent to said mixture and separating protein from said mixture to form a protein-rich fraction and a polar lipid/oil-rich fraction;

(b) reducing the concentration of water-soluble organic solvent in said polar lipid/oil-rich fraction; and

(c) subjecting the water/water-soluble organic solvent and polar lipid/oil-rich fraction to density separation to form a polar lipid-rich fraction and an oil-rich fraction.

2. The process of Claim 1, wherein the separation of protein of step (a) comprises the steps:

(a) adding water-soluble organic solvent to said oil-, polar lipid-, and protein-containing mixture to obtain a water-soluble organic solvent concentration of a least about 68% w/w; and

(b) separating by density separation the resulting mixture into a protein-rich fraction and a polar lipid/oil-rich fraction

3. The process of Claim 1, wherein said oil-, polar lipid-, and protein-containing mixture is derived from eggs.

4. The process of Claim 1, wherein water-soluble organic solvent is recovered from the protein-rich fraction and the polar lipid/oil-rich fraction after the density separation.

5. The process of Claim 1, wherein said polar lipid/oil-rich fraction formed in step (a) comprises from about 5% to about 40% by weight polar lipid and from about 60% to about 95% by weight oil.

6. The process of Claim 1, wherein said protein-rich fraction formed in step (a) comprises from about 80% to about 95% by weight protein on a dry basis.

7. The process of Claim 1, wherein said oil-, polar lipid-, and protein-containing mixture further comprises cholesterol and a substantial amount of said cholesterol reports to said oil-rich fraction pursuant to the separation of step (c).

8. The process of Claim 1, wherein said water-soluble organic solvent in step (a) is present in a water-soluble organic solvent/water mixture in which said water-soluble

organic solvent comprises from about 80% to about 95% by weight of said water-soluble organic solvent/water mixture.

9. The process of Claim 1, wherein said water-soluble organic solvent in step (c) is present in a water-soluble organic solvent/water mixture in which said water-soluble organic solvent comprises from about 5% to about 35% by weight of said water-soluble organic solvent/water mixture.

10. The process of Claim 1, wherein said water-soluble organic solvent is recovered by countercurrent washing, evaporation or drying.

11. The process of Claim 1, wherein said polar lipid-rich fraction is dried to recover water-soluble organic solvent, washed with an water-soluble organic solvent/water mixture comprising greater than about 80% by weight water-soluble organic solvent in order to precipitate residual protein and further dried to recover the water-soluble organic solvent.

12. The process of Claim 11, wherein the addition of said water-soluble organic solvent results in the precipitation of at least some of said protein, which is recovered by density separation.

13. The process as claimed in Claims 1-12, wherein said water-soluble organic solvent comprises a polar solvent.

14. The process as claimed in Claims 1-13, wherein said water-soluble organic solvent comprises an alcohol.

15. The process as claimed in Claims 1-14, wherein said water-soluble organic solvent comprises a C_1-C_8 alcohol.

16. The process as claimed in Claims 1-15, wherein said water-soluble organic solvent comprises isopropanol, ethanol or mixtures thereof.

17. The process as claimed in Claims 1-16, wherein the pH during processing is from pH 4 to about pH 10.

18. The process as claimed in Claims 1-17, wherein said mixture is obtained from at least one of eggs, fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds.

19. The process as claimed in Claims 1-18, wherein at least 60% of the polar lipids originally present in the mixture are recovered in a polar lipid-rich fraction.

20. The process as claimed in Claims 1-19, wherein at least 80% of the polar lipids originally present in the mixture are recovered in a polar lipid-rich fraction.

21. A process for recovering polar lipid from a polar lipid-containing mixture employing the use of a water-soluble organic solvent, wherein the relatively high solubility of polar lipid in an aqueous solution of the water-soluble organic solvent, in which the water-soluble organic solvent comprises more than 68 percent by weight of the aqueous solution, followed by employing the use of a water-soluble organic solvent, wherein the relatively high solubility of polar lipid in an aqueous solution of the water-soluble organic solvent, in which the water-soluble organic solvent comprises less than 35 percent by weight of the aqueous solution, are employed to assist in said recovery.

22. The process as claimed in Claim 21, wherein said mixture is obtained from at least one of eggs, fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds.

23. The process of any of Claims 1-22, wherein said polar lipid comprises a phospholipid.

24. The process of any of Claims 1-23, wherein at least a portion of said process is performed in an oxygen-reduced atmosphere.

25. The process as claimed in Claims 1-24, wherein said mixture is obtained from at least one of eggs, fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds.

26. The process as claimed in Claim 1, wherein said steps of adding and subjecting are repeated at least once.

27. A process for fractionation of an undried oil-, polar lipid-, and protein-containing mixture, comprising the steps:

(a) adding water-soluble organic solvent to said oil-, polar lipid-, and protein-containing mixture to obtain a water-soluble organic solvent concentration of a least about 68% w/w; and

(b) separating by density separation the resulting mixture into a protein-rich fraction and a polar lipid/oil-rich fraction.

28. An oil-containing, polar lipid-containing or protein-containing product produced by any of the processes of Claims 1-27.

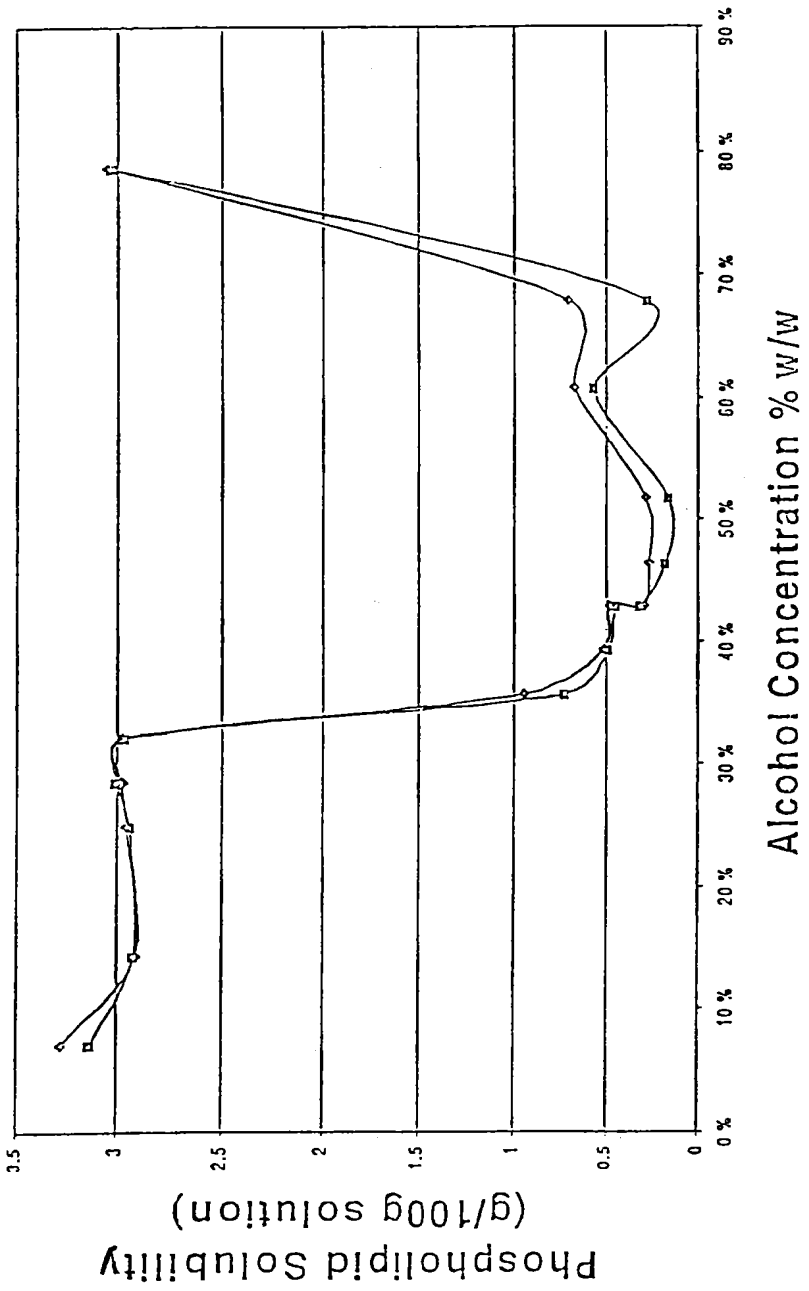

Figure 1. Solubility of phospholipids as a function of alcohol concentration

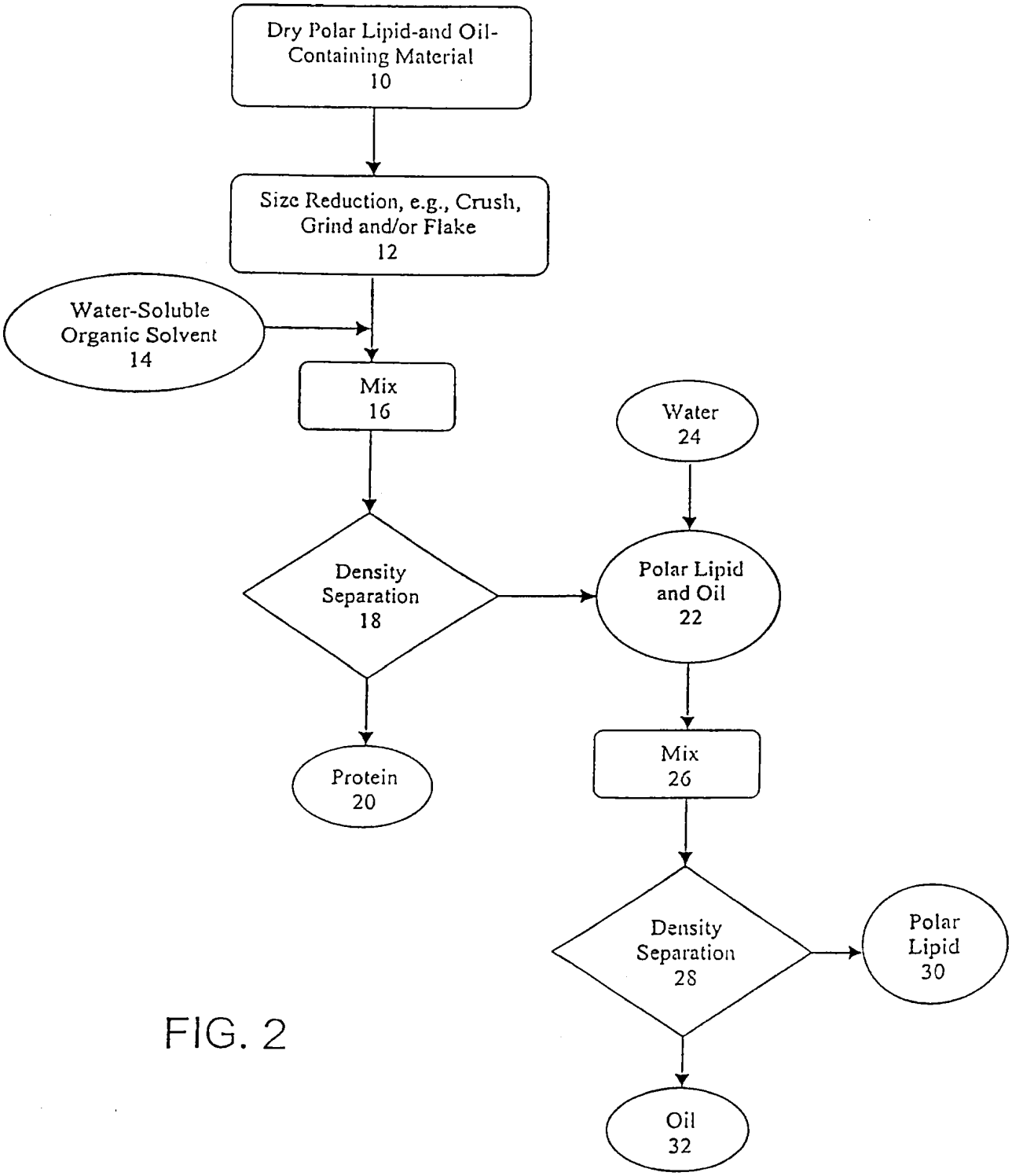

FIG. 2

INTERNATIONAL SEARCH REPORT

Interna. al Application No
PCT/IB 01/00963

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23J1/08 C11B1/00 C11B7/00 A23D9/013 A23J7/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23J C11B A23D

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 883 273 A (MCCOMBS CHARLES ALLAN ET AL) 16 March 1999 (1999-03-16) column 6, line 53 -column 7, line 43 column 8, line 45-55 claims 1-24; examples 1,8	1-4, 7-10, 13-25, 27,28
A		5,6,11, 12,26

X	WO 97 27274 A (ABBOTT LAB) 31 July 1997 (1997-07-31) page 11, paragraph 4 -page 12, paragraph 1 page 14, paragraph 4 -page 15, paragraph 2 claim 1; examples 1,9	1-4, 7-10, 13-25, 27,28
Y		5,6
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2001	01/10/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rooney, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna... Application No
PCT/IB 01/00963

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 917 068 A (BARNICKI SCOTT DONALD ET AL) 29 June 1999 (1999-06-29) column 5, line 32-42; claim 1; example 7	1-4,7, 10, 13-16, 18-20, 23,25,28
Y	CA 1 335 054 A (CANADIAN EGG MARKETING AGENCY) 4 April 1995 (1995-04-04) cited in the application See whole document	5,6
A	US 4 157 404 A (FUKINBARA ITARU ET AL) 5 June 1979 (1979-06-05) column 2, line 39 -column 3, line 8	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat · ıl Application No
PCT/IB 01/00963

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5883273 A	16-03-1999	WO 9727275 A1	31-07-1997
WO 9727274 A	31-07-1997	US 6063946 A	16-05-2000
		AU 1846597 A	20-08-1997
		WO 9727274 A1	31-07-1997
US 5917068 A	29-06-1999	CN 1209160 A	24-02-1999
		EP 0870006 A1	14-10-1998
		JP 2000502740 T	07-03-2000
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		WO 9103946 A1	04-04-1991
US 4157404 A	05-06-1979	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　昭63－23819

⑤1Int.Cl.⁴	識別記号	庁内整理番号	㊸公開　昭和63年(1988)2月1日
A 61 K 35/60	ACB	8615－4C	

審査請求　未請求　発明の数 1　（全4頁）

⑤4発明の名称　血小板凝集抑制剤

㉑特　願　昭61－167540

㉒出　願　昭61(1986)7月16日

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明　細　書

1. 発明の名称

血小板凝集抑制剤

2. 特許請求の範囲

1. オキアミの有機溶剤抽出物を有効成分として含有する血小板凝集抑制剤。

2. オキアミの有機溶剤抽出物がリン脂質画分である特許請求の範囲第1項記載の血小板凝集抑制剤。

3. 発明の詳細な説明

〔産業上の利用分野〕

本発明は血小板凝集抑制剤に関し、更に詳細にはオキアミを有機溶剤で抽出することにより得られる副作用が少なく安定性の高い血小板凝集抑制剤に関する。

〔従来の技術〕

血管内に血栓が形成されると血流が著しく阻害され、組織に重大な障害をもたらすことが知られている。この血栓形成による疾患、すなわち、血栓症の病態生理において、血栓形成因子として血管壁の性状、血流速度及び血液成分の三つの因子が挙げられ、これらは互いに影響しあい重要な役割を果していることが知られている。このうち、血液成分の1つである血小板の役割は非常に重要であり、血小板の凝集亢進により血栓症の誘発の可能性が実験的に、また疫学的に証明されている。さらに最近、動脈硬化症の発症メカニズムの解明に従い、動脈硬化症の発症第一段階が損傷血管内皮細胞への血小板凝集であることが

明らかとなつてきた。そこで近年血栓症の治療・予防のため、抗血小板凝集剤であるアスピリン、インドメサシン、フエニルブタゾン、クロフイブレート、デキストランサルフエート、パパベリン、ヘパリン等の薬剤、あるいはエイコサペンタエン酸等の高度不飽和脂肪酸の、経口的もしくは静脈注射による投与がおこなわれている。

〔発明が解決しようとする問題点〕

しかしながら、上記薬剤はいずれも副作用、薬効、安定性のいずれかの面において十分に満足し得るものでなく、これらに代る、副作用がなく、薬効、安定性の面でも優れた血小板凝集抑制剤の開発が望まれていた。また、日常的に投与可能とするために、当該血小板凝集抑制剤は経口投与できるものであることが望まれていた。

〔問題を解決するための手段〕

このような実情に鑑み、本発明者らは上記要望を満足する血小板凝集抑制剤を得べく鋭意研究をおこなつた結果、オキアミの有機溶剤抽出物は優れた血小板凝集抑制作用を有すること及びこのものは副作用が少なく、安定性も優れ、しかも経口投与できることを見出し、本発明を完成した。

したがつて本発明はオキアミの有機溶剤抽出物を有効成分として含有する血小板凝集抑制剤を提供するものである。

本発明で使用するオキアミとしては、例えば、スパーバ(superba)、クリスタロロフイアス(crystallo rophias)、フリジダ(frigida)、トリアカンサ(triacantha)、ベランテイニ(Vellantini)、ロージロストリス(lougirostris)、ルーセンス(lucens)、シミリス(similis)、スピニフエラ(spinifera)、レクルバ(recurva)等のオイフアウシア(Euphausia)属、マカルーラ(macarura)、ビシナ(Vicina)、グレグリア(gregaria)等のシサノエシサ(Thysanoessa)属等のいずれでも使用可能であつて、特別な種類に限定されない。

なお、原料であるこのオキアミは全世界の海洋に分布し、特に南極周辺に多く、生息量は数億トン～20億トンといわれており、現在の全世界漁獲量に匹敵する5000～7000万トン／年の漁獲も可能と考えられているもので、原料供給の面でも安定供給が可能である。

このオキアミの有機溶剤による抽出は常法によりおこなわれる。例えばオキアミ若しくはその粉砕物をクロロホルム、ベンゼン、ブタノール、エーテル等の非極性溶剤又はクロロホルム-メタノール、エーテル-エタノール、ブタノール-水等の非極性溶剤と極性溶剤の混液等の有機溶剤中に投入し、好ましくは15～60℃で2～24時間撹拌し、沪過することによりおこなわれる。有機溶剤のうち、特に好ましいものとしては、クロロホルム、クロロホルム-メタノール及びブタノール-水が挙げられる。

このようにして得られたオキアミの有機溶

剤抽出物は、これをそのまま、あるいは濃縮若しくは乾燥して血小板凝集抑制剤として用いることもできるが、更に該抽出物からリン脂質画分を取り出し、用いることもできる。リン脂質画分を取り出す方法としては、例えば上記抽出物を濃縮した後、大量の冷アセトン中に小量ずつ滴下してリン脂質画分を沈殿させる方法及び薄層クロマトグラフィー等により分離・採取する方法が例示される。

本発明の血小板凝集抑制剤は、叙上の如くして得られたオキアミの有機溶剤抽出物又はこれから得られたリン脂質画分を必要に応じて公知の医薬用担体と配合することにより製造される。なお、オキアミの有機溶剤抽出物を用いる場合、抽出物中の有機溶剤は水と置換させ、除去することが好ましい。

斯くして得られた本発明の血小板凝集抑制剤は、成人男子に対し、抽出物もしくはリン脂質画分中のリン脂質量として1～20g/日程度経口投与することが好ましいが、症状の程度によつては更に投与量を増やすことも可能である。

なお、本発明の血小板凝集抑制剤の有効成分である抽出物及びリン脂質画分は、これらに含まれるリン脂質のラットに対するLD$_{50}$（経口）が25g/Kgであることからわかるように極めて安全性の高いものである。

〔作用〕

本発明のオキアミ有機溶剤抽出物の血小板凝集抑制作用の機作は詳細には解明されていないがオキアミ有機溶剤抽出物に含有されるエイコサペンタエン酸等の高度不飽和脂肪酸を多量に有するリン脂質に由来するものと考えられる。

〔発明の効果〕

叙上の如く、本発明のオキアミ有機溶剤抽出物及び、特にこれから導かれるリン脂質画分は経口投与にて優れた血小板凝集抑制作用を示し、しかも毒性・副作用もないので極めて優れた血小板凝集抑制剤である。

〔実施例〕

以下に実施例をあげて本発明を更に具体的に説明する。

実施例1.

凍結乾燥されたオキアミ〔オイファウシア・スパーバ（Euphausia superba）〕を細かく砕き、この粉砕物500gにブタノール-水（65：35）混液4000mlを加え、50℃で20時間、撹拌させながら脂質を抽出し、戸別することによりブタノール-水抽出液を得た。

戸別したブタノール-水抽出液を減圧濃縮し、ブタノールを除去後、さらに濃縮してブタノール-水抽出液40g（水含量28g）を得た。

実施例2.

(1) 凍結乾燥されたオキアミ〔オイファウシア・スパーバ（Euphausia Superba）〕を細かく砕き、この粉砕物500gに1000mlのクロロホルムを加え、40℃で20時間、撹拌させな

がら脂質を抽出し、戸別することによりクロロホルム抽出液を得た。

(II) 戸別したクロロホルム抽出液を減圧濃縮し、乾固した後、水-エタノール(1:1)溶液を50mℓ添加し、乾固物質を溶解させ、再度減圧濃縮しエタノールを除去し、オキアミクロロホルム抽出物37g(水含量25g)を得た。

実施例3.

実施例2.(I)と同様にして調製したクロロホルム抽出液を約10mℓとなるまで減圧濃縮した後、2000mℓのアセトンの中へ撹拌させながら滴下し、リン脂質画分を沈澱させた。沈澱物を減圧戸過し採取した後、水-エタノール1:1の混合溶液30mℓに溶解し、減圧濃縮によりエタノールを除去した。オキアミのクロロホルム抽出物のリン脂質画分20g(水含量15g)が得られた。

実施例4.

実施例1.実施例2.及び実施例3.で得たオキアミ抽出物及びそのリン脂質画分について、それらの血小板凝集抑制作用を試験した。すなわち、1群10匹のウイスター系雄ラット(体重200g)にゾンデを用いオキアミ抽出物及びリン脂質画分をそれぞれ0.5mℓずつ2週間毎日胃内投与した。実験最終日にネンブタール麻酔下腹部大動脈より3.8%のクエン酸ナトリウム液を1/10量加え採血した。採血した血液は500g、22℃で6分間遠心して上清をPRP(Platelet Rich plasma)とした。

残りの血液を10000g、22℃で15分間遠心して上清を乏血小板血漿(Platelet Poor plasma, PPP)とした。血小板凝集能はエルマ社製アグリテックTE-500を用いて測定し、最大凝集率及び最大凝集時間で示した。なお、血小板凝集能は血小板数がPRP200μℓ中5~7×10⁷個となるようにPPPで稀釈し、凝集惹起物質としてADP20μmol生理食塩水溶液20μℓを添加した。また、対照群としては、水のみ投与したラットを用いた。この結果を第1表に示す。

第1表

投与群	最大凝集率(%)	最大凝集時間(分)
オキアミブタノール-水抽出物	59.8	3.16
オキアミクロロホルム抽出物	60.3	3.14
オキアミリン脂質画分	52.8	2.89
水	68.5	3.48

以上の結果からオキアミ有機溶媒抽出物特にそのリン脂質画分は血小板凝集抑制作用を有することが明らかとなつた。

製剤例

組成:

①オキアミリン脂質*	100mg
②乳糖	100mg
③ケイ酸アルミニウム	50mg
④ステアリン酸マグネシウム	5mg

*完全に抽出溶剤を除去した粉末物(実施例2.)

製法:

①~④を混合し、打錠する。

以上

US 20110256216A1

(19) **United States**

(12) **Patent Application Publication**

Lefkowitz

(10) **Pub. No.: US 2011/0256216 A1**

(43) **Pub. Date: Oct. 20, 2011**

(54) **PROBIOTIC CONFECTION AND LIPID COMPOSITIONS**

(76) Inventor: **Andrew R. Lefkowitz**, Mayfield Heights, OH (US)

(21) Appl. No.: **13/086,675**

(22) Filed: **Apr. 14, 2011**

Related U.S. Application Data

(60) Provisional application No. 61/323,914, filed on Apr. 14, 2010, provisional application No. 61/390,355, filed on Oct. 6, 2010.

Publication Classification

(51) **Int. Cl.**

A61K 9/48	(2006.01)
A61K 9/00	(2006.01)
A61P 1/00	(2006.01)
A61K 35/74	(2006.01)

(52) **U.S. Cl.** **424/451**; 424/93.46; 424/439

(57) **ABSTRACT**

The present application relates to probiotic confection-based compositions comprising lactic acid-producing bacteria and oil-based compositions comprising the same.

PROBIOTIC CONFECTION AND LIPID COMPOSITIONS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 61/323,914, filed on Apr. 14, 2010 and U.S. Ser. No. 61/390,355, filed on Oct. 6, 2010, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present application relates to probiotic confection and lipid compositions comprising lactic acid-producing bacteria.

BACKGROUND OF THE INVENTION

[0003] The gastrointestinal microflora plays a number of vital roles in maintaining gastrointestinal tract function and overall physiological health. The growth and metabolism of the many individual bacterial species inhabiting the gastrointestinal tract depend primarily upon the substrates available to them, most of which are derived from the diet. These findings have led to attempts to modify the composition and metabolic activities of the bacterial community through diet, primarily with probiotics, which are live microbial food supplements.

[0004] Probiotic organisms are non-pathogenic, non-toxigenic, retain viability during storage, and typically survive passage through the stomach and small intestine. Since probiotics do not generally permanently colonize the host, they need to be ingested regularly for health promoting properties to persist.

SUMMARY OF THE INVENTION

[0005] The invention is based on the discovery that lactic acid-producing bacteria, particularly *Bacillus* species, remain viable and retain their beneficial probiotic properties in/on confection-based compositions as well as lipid or oil-based compositions. Accordingly, the invention describes probiotic confection-based compositions and seafood/fish oil soft-gels or capsules. Specifically, the invention provides an isolated *Bacillus coagulans* in such compositions. The compositions are suitable for human or animal consumption.

[0006] The invention provides probiotic confection-based compositions comprising a confection and an isolated *Bacillus coagulans* bacterium. The *Bacillus coagulans* bacterium is coated on the exterior surface of the confection. Alternatively, the *Bacillus coagulans* bacterium is inside the confection itself. For example, the bacterium is incorporated throughout the confection. Optionally, the composition further comprises a granulated or powder sugar coating or dusting on the exterior surface of the confection. For example, a sugar-sanded jelly confection is characterized by a flexible candy base structure and a sugar sanding layer or coating that comprises *B. coagulans* spores or vegetative cells in an admixture with a granulated or powdered sugar or other sweetener.

[0007] Suitable confections include hard sweets, fudge, toffee, liquorice, chocolate, jelly candy, marshmallow, and marzipan. Preferably, the jelly candy is a gelatin-based gummi candy. Exemplary gummi candies include gummi bears, gummi worms, gummi frogs, gummi hamburgers, gummi cherries, gummi soda bottles, gummi sharks, gummi army men, gummi hippopotami, gummi lobsters, gummi watermelons, gummi octopuses, gummi apples, gummi peaches, and gummi oranges. Preferably, the probiotic confection-based composition is a gummi bear with isolated *Bacillus coagulans* coated on the external surface. The terms "gummi" and "gummy" are used interchangeably herein.

[0008] In one aspect, the isolated *Bacillus coagulans* comprise between about 0.01% to about 50% by weight of the confection-based composition. Optionally, the isolated *Bacillus coagulans* comprise between about 0.01% and about 10% by weight of the confection-based composition. Preferably, the isolated *Bacillus coagulans* comprise between about 0.01% and about 5% by weight of the confection-based composition, e.g., about 1%, about 2%, about 3%, about 4%, or about 5% by weight of the confection-based composition.

[0009] The invention also provides bacterial species including *Bacillus coagulans*, e.g., *Bacillus coagulans* hammer, preferably *Bacillus coagulans* hammer strain Accession No. ATCC 31284, or one or more strains derived from *Bacillus coagulans* hammer strain Accession No. ATCC 31284 (e.g., ATCC Numbers: GBI-20, ATCC Designation Number PTA-6085; GBI-30 or BC^{30}, ATCC Designation Number PTA-6086; and GBI-40, ATCC Designation Number PTA-6087; see U.S. Pat. No. 6,849,256 to Farmer).

[0010] Optionally, the isolated *Bacillus coagulans* is in the form of a spore. Alternatively, the isolated *Bacillus coagulans* is in the form of a vegetative cell. In another aspect, the isolated *Bacillus coagulans* is in the form of a mixture of vegetative cells and spores. The *Bacillus coagulans* is predominantly in spore form, e.g., about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100% spores. Alternatively, the *Bacillus coagulans* is predominantly in vegetative form, e.g., about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100% vegetative cells.

[0011] The invention provides compositions comprising a dry mix for confection-based compositions comprising sugar and an isolated *Bacillus coagulans* bacterium. The dry mix is between 1% and 50% *Bacillus coagulans* bacterium, e.g., about 5%, about 10%, about 15%, about 20%, about 25%, about 35%, about 45%, or about 50% *Bacillus coagulans* bacterium. Preferably, the dry mix is about 15% *Bacillus coagulans* bacterium. For example, about 100 pounds of dry mix contains about 15 pounds of *Bacillus coagulans* bacterium and about 85 pounds of sugar.

[0012] The dry mix is between about 1% and about 50% by weight of the confection-based composition, e.g., about 1% to about 20%, about 5% to about 15%; about 6%, about 7%, about 8%, about 9%, or about 10% by weight of the confection-based composition. For example, a 3 gram confection-based composition contains about 7% dry mix by weight of the confection-based composition. A 3.8 to 4 gram confection-based composition contains about 8-9% dry mix by weight of the confection-based composition.

[0013] The invention also provides methods of making a probiotic confection-based composition. First, a confection (e.g., a gummi bear) is provided and heated to about 100° C. to make the confection "sticky". Subsequently, isolated *Bacillus coagulans* bacterium and sugar are applied to an external surface of the confection, thereby making a probiotic confection-based composition. Preferably, the confection is a gummi bear. The isolated *Bacillus coagulans* comprise between 1% and 10% by weight of the confection-based composition. In one aspect, the isolated *Bacillus coagulans* is *Bacillus coagulans* hammer strain Accession No. ATCC

31284. The isolated *Bacillus coagulans* is selected from the group consisting of GBI-30 strain (ATCC Designation Number PTA-6086), GBI-20 strain (ATCC Designation Number PTA-6085), and GBI-40 strain (ATCC Designation Number PTA-6087). Optionally, the isolated *Bacillus coagulans* is in the form of a spore. Alternatively, the isolated *Bacillus coagulans* is in the form of a vegetative cell. In another aspect, the isolated *Bacillus coagulans* is in the form of a mixture of vegetative cells and spores.

[0014] *Bacillus coagulans* bacteria are included in the confection-based compositions of this invention. Bacterial species include *Bacillus coagulans*, e.g., *Bacillus coagulans* hammer, preferably *Bacillus coagulans* hammer strain Accession No. ATCC 31284, or one or more strains derived from *Bacillus coagulans* hammer strain Accession No. ATCC 31284 (e.g., ATCC Numbers: GBI-20, ATCC Designation Number PTA-6085; GBI-30 or BC^{30}, ATCC Designation Number PTA-6086; and GBI-40, ATCC Designation Number PTA-6087; see U.S. Pat. No. 6,849,256 to Farmer).

[0015] The invention also provides a probiotic seafood/fish oil-based composition comprising fish oil and an isolated *Bacillus coagulans* bacterium. For example, the fish oil, e.g., salmon, cod (e.g., cod liver) contains omega-3 fatty acids. Alternatively, the oil is omega-3 fatty acid krill oil. The oil comprises eicosapentaenoic acid or docosahexaenoic acid. The composition is encapsulated in as soft-shelled capsule or a soft gelatin capsule. Alternatively, the composition is a gelatin-based gummi candy. In one aspect, the isolated *Bacillus coagulans* comprise between 0.01% and 10% by weight of the composition, e.g., about 1% to about 10%; about 2% to about 9%; or about 5% to about 8% by weight of the composition.

[0016] In some cases, the isolated *Bacillus coagulans* is in the form of a mixture of vegetative cells and spores. Preferably, the isolated *Bacillus coagulans* is in the form of a spore. More preferably, the bacterium is present as at least 90% spores, e.g., 95%, 98%, or 99% spores. The isolated *Bacillus coagulans* is selected from the group consisting of GBI-30 strain (ATCC Designation Number PTA-6086), GBI-20 strain (ATCC Designation Number PTA-6085), and GBI-40 strain (ATCC Designation Number PTA-6087).

[0017] The *Bacillus coagulans* Hammer strains of the invention are non-pathogenic and generally regarded as safe for use in human nutrition (i.e., GRAS classification) by the U.S. Federal Drug Administration (FDA) and the U.S. Department of Agriculture (USDA), and by those skilled in the art. Furthermore, the *Bacillus coagulans* Hammer strains of the invention germinate at or below human body temperature, rendering them useful as probiotics. Many *Bacillus coagulans* strains outside the Hammer group have mostly industrial applications, little or no nutritional benefit, and environmental contaminants that have not been evaluated for safety. Moreover, many other non-Hammer strains of *Bacillus coagulans* grow optimally at temperatures that exceed human body temperature and, thus, do not germinate efficiently in the human body. Such strains are less or not suitable as probiotics for human consumption.

[0018] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, Genbank/NCBI accession numbers, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

DETAILED DESCRIPTION OF THE INVENTION

[0019] Probiotic organisms are non-pathogenic, non-toxigenic, retain viability during storage, and survive passage through the stomach and small intestine. Non-pathogenic lactic acid-producing bacteria (i.e., "lactic acid bacteria"), such as the exemplary *Bacillus coagulans*, remain viable and retain their beneficial probiotic properties in confection-based compositions, such as those prepared in boiling water. Specifically, the probiotic organisms described herein, e.g., *Bacillus coagulans* strain GBI-30 or BC^{30}, ATCC Designation Number PTA-6086, survive the harsh manufacturing processes of the confection-based compositions described below.

Probiotic Lactic Acid-Producing Bacteria

[0020] A probiotic lactic acid-producing bacteria suitable for use in the methods and compositions of the invention produces acid and is non-pathogenic. Purified and/or isolated *Bacillus coagulans* is particularly useful as a probiotic in the compositions described herein. By "purified" or "substantially purified" is meant a *Bacillus coagulans* bacterium that is substantially free of contaminating microorganisms or other macromolecules, e.g., polysaccharides, nucleic acids, or proteins.

[0021] The confection-based compositions include a lactic acid-producing bacteria, such as a spore-forming *Bacillus* species, such as *B. coagulans*. Preferably, the spore-forming *Bacillus* species of the invention is *B. coagulans* Hammer. There are many suitable bacteria identified as described herein, although the invention is not limited to currently known bacterial species insofar as the purposes and objectives of the bacteria is described. The property of acid production is important to the effectiveness of the probiotic lactic acid-producing bacteria of this invention.

[0022] Exemplary methods and compositions are described herein using *Bacillus coagulans* as a probiotic. Purified and/or isolated *Bacillus coagulans* is particularly useful as a probiotic in confection-based compositions. Probiotic *B. coagulans* is non-pathogenic and is generally regarded as safe (i.e., GRAS classification) by the U.S. Federal Drug Administration (FDA) and the U.S. Department of Agriculture (USDA), and by those skilled in the art.

[0023] *Bacillus coagulans* is a non-pathogenic gram positive spore-forming bacteria that produces L(+) lactic acid (dextrorotatory) in fermentation conditions. It has been isolated from natural sources, such as heat-treated soil samples inoculated into nutrient medium (Bergey's Manual off Systemic Bacteriology, Vol. 2, Sneath, P. H. A., et al., eds., Williams & Wilkins, Baltimore, Md., 1986). Purified *B. coagulans* strains have served as a source of enzymes including endonucleases (e.g., U.S. Pat. No. 5,200,336); amylase (U.S. Pat. No. 4,980,180); lactase (U.S. Pat. No. 4,323,651); and cyclo-malto-dextrin glucano-transferase (U.S. Pat. No.

5,102,800). *B. coagulans* has been used to produce lactic acid (U.S. Pat. No. 5,079,164). A strain of *B. coagulans* (referred to as *L. sporogenes*; Sakaguti & Nakayama (ATCC 31284)) has been combined with other lactic acid producing bacteria and *B. natto* to produce a fermented food product from steamed soybeans (U.S. Pat. No. 4,110,477).

[0024] Bacterial species include *Bacillus coagulans*, e.g., *Bacillus coagulans* hammer, preferably *Bacillus coagulans* hammer strain Accession No. ATCC 31284, or one or more strains derived from *Bacillus coagulans* hammer strain Accession No. ATCC 31284 (e.g., ATCC Numbers: GBI-20, ATCC Designation Number PTA-6085; GBI-30 (BC^{30}), ATCC Designation Number PTA-6086; and GBI-40, ATCC Designation Number PTA-6087; see U.S. Pat. No. 6,849,256 to Farmer).

[0025] *Bacillus coagulans* was previously mis-characterized as a *Lactobacillus* and labeled as *Lactobacillus sporogenes* (Nakamura et al. 1988. *Int. J. Syst. Bacteria* 38: 63-73). However, initial classification was incorrect because *Bacillus coagulans* produces spores and excretes L(+)-lactic acid through metabolism. Both of these characteristics provide key features to the utility of *Bacillus coagulans*. These developmental and metabolic aspects required that the bacterium be classified as a lactic acid *Bacillus*. In addition, it is not generally appreciated that classic *Lactobacillus* species are unsuitable for colonization of the gut due to their instability in the harsh (i.e., acidic) pH environment of the bile, particularly human bile. By contrast, *Bacillus coagulans* is able to survive and colonize the gastrointestinal tract in the bile environment and even grown in this low pH range.

Probiotic Activity of *Bacillus coagulans*

[0026] It is well-documented clinically that many species of bacterial, mycotic and yeast pathogens possess the ability to cause a variety of gastrointestinal disorders including, but not limited to: disruption of normal gastrointestinal biochemical function, necrosis of gastrointestinal tissues, and disruption of the bioabsorption of nutrients, and like conditions. The probiotic microorganism-containing compositions described herein inhibit these pathogens. Thus, the compositions are useful in the prophylactic or therapeutic treatment of conditions associated with infection by these aforementioned pathogens. The probiotic confection-based compositions of the invention are also used in the methods described herein for boosting the immune system.

[0027] In one aspect, a *Bacillus coagulans* strain is included in the composition in the form of vegetative cells. In another aspect, the *Bacillus coagulans* strain is included in the composition in the form of spores. The invention also provides for including the *Bacillus coagulans* strain in the composition in the form of a powder, a dried cell mass, a stabilized paste, or a stabilized gel.

[0028] Because *Bacillus* spores are heat and pressure-resistant and can be stored as a dry powder, they are particularly useful for formulation into and manufacture of products such as the various confection-based compositions described herein. A *Bacillus* species is well suited for the present invention, particularly species having the ability to form spores which are relatively resistant to heat and other conditions, making them ideal for storage (shelf-life) in product formulations, e.g., confection-based compositions. Due to the shelf-stable properties of the *Bacillus coagulans* strains described herein, e.g., *Bacillus coagulans* strain GBI-30 or BC^{30}, ATCC Designation Number PTA-6086, the product formulations of the invention are not confined to a refrigerator and may be stored at room temperature.

[0029] The *Bacillus coagulans* of the invention survives storage (shelf-life) from about 12 days to about 2 years; from about 1 month to about 18 months; from about 3 months to about 1 year; or from about 6 months to about 9 months.

[0030] The probiotic organisms described herein, e.g., *Bacillus coagulans* strain GBI-30 or BC^{30}, ATCC Designation Number PTA-6086, promote digestive and oral health and support the immune system. The ability of *Bacillus coagulans* to inhibit various bacterial pathogens was quantitatively ascertained by use of an in vitro assay. This assay is part of a standardized bacterial pathogen screen (developed by the U.S. Food and Drug Administration(FDA)) and is commercially available on solid support disks (DIFCO® BACTROL® Antibiotic Disks). To perform the assay, potato-dextrose plates (DIFCO®) were initially prepared using standard procedures. The plates were then individually inoculated with the bacteria (approximately 1.5×10^6 CFU) to be tested so as to form a confluent bacterial bed.

[0031] Inhibition of microorganisms (e.g. gastrointestinal pathogens) by *Bacillus coagulans* was subsequently ascertained by placing approximately 1.8×10^6 CFU of *Bacillus coagulans* in 10 μl of broth or buffer, directly in the center of the potato-dextrose plate with one test locus being approximately 8 mm in diameter per plate. A minimum of three test loci were used for each assay. The negative control consisted of a 10 μl volume of a sterile saline solution, whereas the positive control consisted of a 1 μl volume of glutaraldehyde. The plates were then incubated for approximately about 18 hr at 30° C., at which time the zones of inhibition were measured. As designated herein, "excellent inhibition" means the zone was 10 mm or greater in diameter; and "good inhibition" means the zone was greater than 2 mm in diameter but less than 10 mm in diameter.

[0032] As expected, no "inhibition" was seen with the negative, saline control, and excellent "inhibition" (approximately 16.2 mm diameter; average of three tests) was seen with the positive, glutaraldehyde control. For the enteric microorganisms tested, the following inhibition by *Bacillus coagulans* was found: (i) *Clostridium* species—excellent inhibition; (ii) *Escherichia coli*—excellent inhibition; (iii) *Clostridium* species—excellent inhibition, where the zone of inhibition was consistently greater than 15 mm in diameter. Similarly, excellent inhibition was also seen for the opportunistic pathogens *Pseudornonas aeruginosa*, and *Staphylococcus aureus*. Pathogenic enteric bacteria which were inhibited by *Bacillus coagulans* activity include, but are not limited to: *Staphylococcus aureus; Staphylococcus epidermidis; Streptococcus pyogenes; Pseudomonas aeruginosa; Escherichia coli* (enterohemorragic species); numerous *Clostridium* species (e.g., *Clostridium perfingens, Clostridium botulinum, Clostridium tributrycum, Clostridium sporogenes*, and the like); *Gardnereia vaginails; Proponbacterium aenes; Aeromonas hydrophia; Aspergillus* species; *Proteus* species; and *Klebsiella* species.

Micro-Encapsulation

[0033] In one aspect, the lactic-acid producing bacteria are incorporated into a microcapsule coating prior to addition to the confection-based composition, using any micro-encapsulation process well-known in the art. The isolated *Bacillus coagulans* are packaged, or encapsulated, within another material in order to protect the bacteria from the surrounding

environment. The capsules of the invention range in size from one-thousandth of a millimeter to seven millimeters. The internal ingredients of the microcapsule are released from their shells in various ways, including mechanical rupture of the capsule wall, dissolution of the wall, melting of the wall and diffusion through the wall. Thus, micro-encapsulation provides additional protection to the isolated *Bacillus* bacterium during heat processing of the confection-based compositions of the invention. Physical methods of micro-encapsulation include pan coating, air-suspension coating, centrifugal extrusion, vibrational nozzle, and spray-drying. Chemical methods of micro-encapsulation include interfacial polymerization, in-situ polymerization, and matrix polymerization.

[0034] Alternatively, the lactic-acid producing bacteria is added to the confection-based composition without micro-encapsulation.

Probiotic Confection-Based Compositions

[0035] The invention is directed to the surprising discovery that lactic acid-producing bacteria, particularly *Bacillus* species, remain viable and retain their beneficial probiotic properties in confection-based compositions. The confection compositions are suitable for human or animal consumption. In one aspect, the confection-based compositions are administered to children under 18 years of age, e.g., under 15 years of age, under 10 years of age, or under 5 years of age. Alternatively, the confection-based compositions are administered to children and adults of all ages.

[0036] Confectionery includes food items that are rich in sugar or artificial sweeteners, any one or type of which is called a "confection". The words "candy" or "sweets" are also used for the term "confectionery". Candy is made by dissolving sugar in water or milk to form a syrup, which is boiled until it reaches the desired concentration or starts to caramelize. The type of candy depends on the ingredients and how long the mixture is boiled, while the final texture of candy depends on the sugar concentration. As the syrup is heated, it boils, water evaporates, the sugar concentration increases, and the boiling point rises. Thus, boiling temperature corresponds to a particular sugar concentration. In general, higher temperatures and greater sugar concentrations result in hard, brittle candies, while lower temperatures result in softer candies. Candy names come from the process used to test the syrup before thermometers became affordable: a small spoonful of syrup was dropped into cold water, and the characteristics of the resulting lump were evaluated to determine the concentration of the syrup. Long strings of hardened sugar indicate "thread" stage, while a smooth lump indicates "ball" stages, with the corresponding hardness described. The "crack" stages are indicated by a ball of candy so brittle that the rapid cooling from the water literally causes it to crack. Candy comes in an endless variety of textures from soft and chewy to hard and brittle.

[0037] There are a variety of categories and types of confectionery. Hard sweets are based on sugars cooked to the hard-crack stage, including suckers, lollipops, jawbreakers (or gobstoppers), lemon drops, peppermint drops and disks, candy canes, rock candy, etc. Hard sweets also include candies often mixed with nuts, such as brittle. Others contain flavorings including coffee, such as Kopiko. Fudge is a confection of milk and sugar boiled to the soft-ball stage. Toffee (or Taffy or Tuffy) is based on sugars cooked to the soft-ball stage and then pulled to create an elastic texture. Tablet is a crumbly milk-based soft and hard candy, based on sugars cooked to the soft-ball stage, and comes in several forms, such as wafers and heart shapes. Liquorice, which contains extract of the liquorice root, is chewier and more resilient than gum/gelatin candies, but still designed for swallowing. Other types of confection include chocolates, marshmallow, marzipan, and divinity. Jelly candies include those based on sugar and starch, pectin, gum, or gelatin, e.g., jelly beans, gumdrops, jujubes, cola bottles, and gummies.

[0038] Suitable gummi confections include bears, rings, worms, frogs, snakes, hamburgers, cherries, sharks, penguins, hippos, lobsters, octopuses, apples, peaches, oranges, and spiders. Suitable gummi bear sizes range from the standard candy size (or smaller), to gummi bears that weigh several kilograms. Gummi confections come in a variety of flavors, including raspberry, orange, strawberry, pineapple, and lemon.

[0039] Traditional gummi confection (e.g., gummi bears) is made from sugar, glucose syrup, starch, flavoring, food coloring, citric acid, and gelatin. Suitable gelling agents and hydrocolloids can be selected by one of ordinary skill in the art. Examples include gums, carrageenan, gelatin, pectin, high methoxy pectin, alginates, and agar. One of ordinary skill in the art can select a suitable gelling agent or hydrocolloid depending on the desired final texture of the starch molded piece. There are some gummi confections made with pectin or starch instead of gelatin, making them suitable for vegetarians. An exemplary organic gummi confection is made with most all natural ingredients, such as organic tapioca syrup, organic evaporated cane juice, gelatin, organic grape juice concentrate, citric acid, lactic acid, ascorbic acid, colors added (black, carrot juice concentrate, turmeric, annatto), natural flavors, organic sunflower oil, and carnauba wax.

[0040] Large sour gummi bears are larger and flatter than traditional gummi bears, have a softer texture, and include fumaric acid or other acid ingredients to produce a sour flavor. Sour "gummies" are produced by forming a sweet, flavored, and chewy core and subsequently dusting the exterior with a food acid, such as citric acid. The gelling ingredient in the core of these products is ordinarily gelatin or pectin. The acidic exterior is applied by use of a wetting agent or food adhesive. Some manufacturers produce sour bears with a different texture, based on starch instead of gelatin. Typically, starch produces a shorter (cleaner bite, less chewy) texture than gelatin.

[0041] Confection-based compositions, such as those described herein, are made from a variety of ingredients known to those skilled in the art. The confection-based compositions are prepared by combining confection ingredients and a liquid, e.g., water or milk. In one aspect, the composition is prepared by combining confection ingredients and a liquid, and heating the resulting combination. Optionally, the combination is heated (heat-processed) using applied heat, a flame, or a microwave. The confection-based composition is boiled in hot water, e.g., stovetop boiling, addition of boiling water to a container, or microwaving the confection-based composition along with water. In one aspect, boiling water (about 100° C.) is added to a combination of confection ingredients and *Bacillus coagulans* bacteria.

[0042] Mass production of gummi confection (e.g., gummi bears) includes mixing the gummi confection ingredients and pouring the resulting mixture into many starched-lined (e.g., corn starch-lined) trays/molds. The corn starch prevents the

gummy bears from sticking to the mold and lets them release easily once they are set. First, the desired character molds are created and, if necessary, duplicated with a machine. Optionally, starch powder is applied to the character molds. Gummi confection ingredients, such as sugar, glucose syrup, gelatin, and water are mixed together and heated. In one aspect, the ingredients are mixed with colors and flavors that give the bears their signature look and taste. The molten gelatin mixture is poured into the molds and allowed to cool and set prior to packaging or consumption. Preferably, the gummi confection is subsequently heated and placed in a large drum tumbler to apply a composition of isolated *Bacillus coagulans* and a sweetener (e.g., a sugar).

[0043] More specifically, as described in WO/2009/102575, production of gummi confection includes the following. A colloid batch and a puree batch are formed and combined with corn syrup and sugar to form a base slurry. The colloid batch comprises a solution of the gelling agent in water at a level of from 5 to 15% by weight of the gelling agent, more preferably from 7 to 12% of the gelling agent based on the total weight of the colloid batch. The colloid batch is held at a temperature of 170 to 190° F. The puree batch preferably comprises water, fruit puree and/or high fructose corn syrup or other sweeteners, thin boiling starch, and sodium citrate. It is held at a temperature of from 65 to 75° F. Preferably, the fruit puree has a Brix of from 10 to 45, more preferably from 25 to 40. Optionally, the puree batch includes a plurality of fruit purees. The fruit puree comprises a typical fruit puree, a fruit juice, or a fruit powder. The puree batch comprises from 30 to 40% by weight water, from 0 to 40% by weight fruit puree, from 0 to 40% by weight high fructose corn syrup, from 25 to 35% by weight thin boiling starch, and from 0.0 to 2.0% by weight sodium citrate. In a mixing kettle from 25 to 40% by weight of additional corn syrup is combined with from 15 to 40% by weight of fine granulated sugar, from 10 to 15% by weight of the colloid batch and from 20 to 30% by weight of the puree batch to form the base slurry. Preferably, the corn syrup is approximately 42 DE corn syrup, however, as would be understood by one of ordinary skill in the art other DE corn syrups could be used. The base slurry components are completely mixed and held at 130 to 150° F. in a holding tank.

[0044] The base slurry is then cooked to bring the Brix to from 70 to 85 Brix, more preferably to a Brix of from 75 to 80. In one embodiment the base slurry is passed through a coil cooker and heated to a temperature of from 250 to 325° F. to cook it. Other cooking methods could be used as will be understood by one of ordinary skill in the art. The cooked base slurry is preferably subjected to vacuum to further increase the Brix into the desired range. The cooked base slurry is held at approximately 200° F. until used. An acidulant solution is preferably added along with color and flavor to the cooked base slurry just prior to deposition in the starch molds. In one aspect, the acidulant solution comprises ascorbic acid present in an amount of from 15 to 20% by weight, citric acid present in an amount of from 10 to 20% by weight, and malic acid present in an amount of from 5 to 10% by weight with the remainder comprising water. As would be understood by one of ordinary skill in the art, other edible acids could be used in place of or in addition to those listed. In one aspect, 95 to 97% by weight of cooked base slurry is combined with from 2 to 3% by weight of the acidulant solution and the remainder comprises flavors and colors. Optionally, the acidulant solution is used to bring the pH of the base slurry to from 2.6 to 3.2. One of ordinary skill in the art would have no difficulty selecting suitable colors and flavors. The combined mixture is then deposited into starch molds, e.g., using a Mogul starch molding machine. Such starch molding machines are well known by those of ordinary skill in the art. In one aspect, from 0.3 to 3 grams of the base slurry is deposited into each mold cavity. The starch trays with deposited base slurry are transferred to a drying room where there are held for 12 to 48 hours. Optionally, the trays are first held at a temperature of from 130 to 150° F. for from 10 to 15 hours, and then cooled to 70 to 80° F. and held at that temperature for from 6 to 12 hours. The gelled starch molded food pieces are then removed from the trays, the starch is recycled.

[0045] Preferably, the confections of the invention further comprise a sweetener (e.g., a granulated or powder sugar) coating on the exterior surface of the confection. The sweeteners can comprise one or more monosaccharides or disaccharides. Examples include sugar, sucrose, invert sugar, dextrose, lactose, honey, malt syrup, malt syrup solids, maltose, fructose, granular fructose, maple syrup, rice syrup, rice syrup solids, sorghum syrup, refiners syrup, corn syrup, corn syrup solids, high fructose corn syrup, molasses, or combinations thereof.Sandin g sugar comprises cane sugar, beet sugar, date sugar, sucanat, granulated fructose or an artificial sweetener (e.g., Sweet-n-Low®, NutraSweet®, or Equal®) and *B. coagulans* in spore form, freeze-dried vegetative cell form, or a combination thereof. Other artificial sweeteners include acesulfame K, aspartame, sucralose, d-tagatose, and combinations thereof.

[0046] The probiotic organisms described herein, e.g., *Bacillus coagulans* strain GBI-30 or BC^{30}, ATCC Designation Number PTA-6086, uniquely survive the harsh manufacturing and cooking processes of the confection-based compositions. The confection-based compositions are processed for packaging by separating the confection-based compositions from starch (e.g., corn starch). The confection-based compositions are heated to about 100° C. to make them "sticky". Subsequently, the confection-based compositions are placed in a drum tumbler, wherein the probiotic/sugar coating is applied. *Bacillus coagulans* is blended with sugar prior to application to the surface of the confection-based composition. The dry mix for confection-based compositions comprises sugar and an isolated *Bacillus coagulans* bacterium. The dry mix is between 1% and 50% *Bacillus coagulans* bacterium, e.g., about 5%, about 10%, about 15%, about 20%, about 25%, about 35%, about 45%, or about 50% *Bacillus coagulans* bacterium. Preferably, the dry mix is about 15% *Bacillus coagulans* bacterium and 85% sugar. For example, about 100 pounds of dry mix contains about 15 pounds of *Bacillus coagulans* bacterium and about 85 pounds of sugar.

[0047] The dry mix is between about 1% and about 50% by weight of the confection-based composition, e.g., about 1% to about 20%, about 5% to about 15%; about 6%, about 7%, about 8%, about 9%, or about 10% by weight of the confection-based composition. For example, a 3 gram confection-based composition contains about 7% dry mix by weight of the confection-based composition. A 3.8 to 4 gram confection-based composition contains about 8-9% dry mix by weight of the confection-based composition.

[0048] Alternatively, the isolated *Bacillus coagulans* bacterium is added directly to the confection ingredients prior to heating, molding, and subsequent cooling of the confection.

In this manner, the probiotic is introduced into the confection itself, instead of on the surface of the confection-based composition.

[0049] As the recommended dietary allowances (RDA or recommended daily intake; RDI) is about 1×10^9 bacterium (according to EU guidelines), preferably, the confection-based composition comprises at least about 1×10^9 viable bacteria. In another aspect, the confection-based composition comprises at least about 1×10^6 to 1×10^7; at least about 1×10^7 to 1×10^8; or at least about 1×10^8 to 1×10^9 viable bacteria.

Probiotic Seafood Oil-Based Compositions

[0050] The invention is also directed to the surprising discovery that lactic acid-producing bacteria, particularly *Bacillus* species, remain viable and retain their beneficial probiotic properties in seafood/fish oil-based compositions. By "seafood" is meant any fish or shellfish from the sea used for food. Specifically, the probiotic organisms described herein, e.g., *Bacillus coagulans* strain GBI-30 or BC^{30}, ATCC Designation Number PTA-6086, survive in the fish oil-based compositions described below. The seafood/fish oil-based compositions are packaged in soft-shelled capsules or soft gelatin capsules or in the form of sugar/gelatin (gummi) confections, e.g., both the *Bacillus coagulans* spores or bacterium and the fish oil are encapsulated together. For example, the fish oil and bacterial spores are incorporated into confection-based compositions, such as the gummi confections described herein. Preferably, the bacterium is present as at least 90% spores, e.g., 95%, 98%, or 99% spores. The fish oil-based compositions are suitable for human or animal consumption. In one aspect, the fish oil-based compositions are administered to children under 18 years of age, e.g., under 15 years of age, under 10 years of age, or under 5 years of age. Alternatively, the fish oil-based compositions are administered to children and adults of all ages.

[0051] Fish oil contains two omega-3 fatty acids: eicosapentaenoic acid (EPA; all-cis-5,8,11,14,17-eicosapentaenoic acid) and docosahexaenoic acid (DHA; all-cis-4,7,10,13,16,19-docosahexaenoic acid). Omega-3 fatty acids (n-3 fatty acids or ω-3 fatty acids) are a family of unsaturated fatty acids that have a carbon-carbon double bond at the n-3 position, e.g., at the third carbon bond from the terminal methyl end (n) of the fatty acid. Although fish are a dietary source of omega-3 fatty acids, fish do not produce the fatty acids themselves. Instead, omega-3 fatty acids, such as EPA and DHA are synthesized by microalgae and plankton that live in seawater. Fish accumulate omega-3 fatty acids by either consuming the microalgae that produce the fatty acids, or by eating smaller prey fish that have consumed the omega-3 fatty acids found in microalgae. Thus, fatty predatory fish like mackerel, lake trout, flounder, albacore tuna and salmon possess high levels of omega-3 fatty acids. Krill oil is also a source of omega-3 fatty acids.

[0052] The process by which oil is extracted from fish begins with cooking the fish product through a process of steam heating, wherein the fish will reach a top temperature of almost 100° C. This important step not only sterilizes the fish, but also causes the proteins to coagulate and the alteration of cell membranes to aid in the extraction of the oil from the dry material. In some cases, the raw fish is hashed (cut into pieces) prior to steam cooking. After cooking, the mass of fish is pressed or centrifuged to separate the fat-free dry solids (mass of fish) from the liquid (oil & water). This process also creates a fish presscake, which is used by many facilities for the production of fish meal commonly used in animal feed. The liquid collected from mass of fish contains not only water, but also fish oil, salts, proteins, and even undesired waste particles and toxins. The liquid (oil & water) is further filtered to separate the oil and water. At this point, the unrefined fish oil (also referred to as crude fish oil) has not undergone any portion of the refining process.

[0053] When fish oil is extracted from fish, so too are the free fatty acids and toxins that are present in the fish. In some cases, fish oils are refined and processed to remove impurities from the fish oil and to enhance the fatty acid potency. Ultra-refined fish oil has been through sophisticated and intensive filtering and refining processes (e.g., winterization) to produce pure and concentrated oil that is as far as possible, free from contaminants. During winterization, the oil is chilled to allow filtration of the saturated fats and particles that form at colder temperatures. Mercury and other metals are subsequently removed before the oil is converted to ethyl esters, subjected to trans-esterification, and molecular or vacuum distillation to remove other fats and undesirable elements and to concentrate the oil. Optionally, fish oil is combined with preservatives and other ingredients suitable for mammalian consumption. For example, in some cases, acid clay is added to remove the pungent smell from the fish oil.

[0054] The omega-3 fatty acids derived from the tissues of oily fish, e.g., salmon, herring, anchovies, sardines, tuna, pollock, cod, catfish, flounder, grouper, halibut, mahi mahi, orange roughy, red snapper, shark, swordfish, tilefish, and king mackerel have many health benefits. For example, the omega-3 fatty acids found in fish oil reduce inflammation, slow the spread of cancerous tissue, regulate cholesterol levels, improve cardiovascular health, boost the immune system, and protect the brain from a variety of disorders, such as clinical depression, anxiety, Alzheimer's disease, and Parkinson's disease.

[0055] The fish oil-based and confection-based compositions are formulated in many configurations, because the bacterium is present as a vegetative cell or as a spore, or both, depending on the species and form of the probiotic organism. The cells/spores are formulated in a variety of compositions suited for use in a fish oil-based or confection-based composition. In one aspect, the bacterium is present as a mixture of spores and vegetative cells. In another aspect, the bacterium is present as at least 90% spores, e.g., 95%, 98%, or 99% spores. Optionally, prior to addition to the fish oil-based or confection-based compositions of the invention, the *Bacillus coagulans* cells are cultured in liquid in the absence of or with limited quantities of a food source to induce sporulation. In another aspect, heat gun spray drying kills about 50%, about 75%, about 90%, about 95%, or about 99% of vegetative cells prior to addition to the fish oil-based or confection-based compositions of the invention.

[0056] In one aspect, *Bacillus coagulans* bacteria in the form of a spray-dried powder is included in or on the surface of the confection-based composition described herein. Preferably, the isolated *Bacillus coagulans* is in the form of a spore. The isolated *Bacillus coagulans* are at least 85%, at least 90%, at least 95%, or at least 99% pure spores. Alternatively, the isolated *Bacillus coagulans* is in the form of a vegetative cell. In one aspect, the isolated *Bacillus coagulans* are at least 85%, at least 90%, or at least 95% pure vegetative cells. In another aspect, the isolated *Bacillus coagulans* is in the form of a mixture of vegetative cells and spores. The *Bacillus coagulans* mixture is 90% spores, 10% vegetative

cells; 75% spores, 25% vegetative cells; 60% spores, 40% vegetative cells; 50% spores, 50% vegetative cells; 60% vegetative cells, 40% spores; 75% vegetative cells; 25% spores; or 90% vegetative cells, 10% spores.

[0057] The *Bacillus* and/or *Bacillus coagulans* is applied using any of a variety of known methods including, for example, applying a powder, spray-drying the probiotic onto the confection-based composition, or soaking the composition in a solution containing the probiotic. Alternatively, the *Bacillus* bacterium is mixed with the confection ingredients (e.g., gummi ingredients) prior to boiling in water.

[0058] Any of a variety of methods for placing the bacterial composition into a fish oil-based or confection-based composition can be used. In one aspect, a "spray-dry" method is used, in which the compositions are exposed in a low humidity chamber to an atomized mix containing a liquid composition, where the chamber is subsequently exposed to approximately 80-110° F. to dry the liquid, thereby impregnating the material of fish oil-based or confection-based composition with the components.

[0059] A typical concentration is from approximately 1×10^7 to 1×10^{12} CFU; 1×10^8 to 1×10^{11} CFU; or 1×10^9 to 1×10^{10} CFU of viable bacterium or spores/g of fish oil or confection matrix or sanding sugar. Sanding sugar comprises cane sugar, beet sugar, date sugar, sucanat, granulated fructose or an artificial sweetener (e.g., Sweet-n-Low®, NutraSweet®, or Equal®) and *B. coagulans* in spore form, freeze-dried vegetative cell form, or a combination thereof. Following drying, the fish oil-based composition or confection is ready for immediate use or for storage in a sterile package, e.g., a 3-ounce package (e.g., a bag or a bottle), a 6-ounce package, a 9-ounce package, a 12-ounce package, a 15-ounce package, an 18-ounce package, or a 24-ounce package.

[0060] The active ingredients (i.e., live bacteria or extracellular components), comprise between about 0.01% to about 10%; 0.01% to about 1%; or about 0.05% to about 0.1% by weight of the probiotic fish oil-based or confection-based composition. Optionally, the isolated *Bacillus coagulans* comprise about 1 mg to about 10 g; about 10 mg to about 1 g; or about 25 mg to about 75 mg by weight of the probiotic composition. Most preferably, the amount of *Bacillus coagulans* bacteria is about 5×10^7 colony forming units (CFU) of bacteria per gram of food matrix.

[0061] In one aspect, the amount of bacteria is about 10^4 to 10^{14} colony forming units (CFU) of bacteria per gram of probiotic composition (i.e., vegetative cells and/or bacterial spores), preferably 10^5 to 10^{13} CFU/g of fish oil or confection matrix. Alternatively, the concentrations are 10^8 to 10^{13} CFU/g; 10^9 to 10^{12} CFU/g; or 10^{10} to 10^{11} CFU/g of fish oil or confection matrix. In one aspect, the amount of bacteria is about 1×10^6 CFU per gram of fish oil or confection matrix. The actual amount in a fish oil-based or confection-based composition will vary depending upon the amounts of composition to be dispersed into the fish oil or confection composition and upon routes of dispersal.

[0062] In one aspect, the invention provides for storing the fish oil-based or confection-based composition in a sterile package at room temperature prior to consumption. Alternatively, the composition is consumed immediately.

[0063] By way of example, and not of limitation, *Bacillus coagulans* spores are incorporated into any type of dry or lyophilized product which is dissolved or mixed with hot water, so long as the temperature of the *Bacillus coagulans* spore-containing mixture is raised to the required heat-shock temperature (i.e., 80° C. for 5 minutes) necessary for germination of the spores. The *Bacillus coagulans* spores may either be incorporated into the dry or lyophilized product by the manufacturer of the product or by the consumer during preparation. The fish oil-based or confection-based composition is subsequently boiled in hot water, e.g., stovetop boiling, addition of boiling water to a container, or microwaving the fish oil-based or confection-based composition along with water.

[0064] The *Bacillus coagulans* spores survive storage (shelf-life), i.e., retain viability or the ability to germinate at physiological conditions (e.g., ingestion), from about 12 days to about 2 years; from about 1 month to about 18 months; from about 3 months to about 1 year; or from about 6 months to about 9 months.

Example 1

Preparation of *Bacillus coagulans* Cultures

[0065] *Bacillus coagulans* Hammer bacteria (ATCC Accession No. 31284) was inoculated and grown to a cell density of about 10^8 to 10^9 cells/ml in nutrient broth containing 5 g Peptone, 3 g Meat extract, 10-30 mg $MnSO_4$, and 1,000 ml distilled water, adjusted to pH 7.0, using a standard airlift fermentation vessel at 30° C. The range of $MnSO_4$ acceptable for sporulation is 1 mg/l to 1 g/l. The vegetative cells can actively reproduce up to 45° C., and the spores are stable up to 90° C. After fermentation, the *B. coagulans* bacterial cells or spores are collected using standard methods (e.g., filtration, centrifugation) and the collected cells and spores can be lyophilized, spray-dried, air-dried or frozen. The supernatant from the cell culture is collected and used as an extracellular agent secreted by *B. coagulans*.

[0066] A typical yield from the above culture is in the range of about 10^9 to 10^{10} viable spores and more typically about 100 to 150 billion cells/spores per gram before drying. Spores maintain at least 90% viability after drying when stored at room temperature for up to ten years, and thus the effective shelf life of a composition containing *B. coagulans* Hammer spores at room temperature is about 10 years.

Example 2

Preparation of *Bacillus coagulans* Spores

[0067] A culture of dried *B. coagulans* spores was prepared as follows. Ten million spores were inoculated into a one liter culture containing 24 g potato dextrose broth, 10 g of enzymic-digest of poultry and fish tissue, 5 g of FOS and 10 g $MnSO_4$. The culture was maintained for 72 hours under a high oxygen environment at 37° C. to produce culture having about 150 billion cells per gram of culture. Thereafter, the culture was filtered to remove culture medium liquid, and the bacterial pellet was resuspended in water and freeze-dried. The freeze-dried powder is then ground to a fine powder using standard good manufacturing practice (GMP).

Example 3

Bacillus coagulans Spores Survive in the Gastric Environment

[0068] This study was performed in order to determine the survivability rate of *Bacillus coagulans* spores as they pass through the stomach. Samples of *Bacillus coagulans* spores

were subjected to a simulated gastric environment for varying lengths of time in order to attain their survivability rate. First, a homogeneous sample of raw material *Bacillus coagulans* of at least 12 grams was prepared. Saline solution at pH 1 was prepared using 3N HCl (150 mls each into six 250 ml media bottles) and sterilized. Additional saline solutions with pH 2 and 3 were prepared similarly, resulting in 6 sterile 250 ml bottles, each containing 150 ml pH adjusted saline. Six sterile 250 ml media bottles each containing 150 ml normal saline solution were prepared and sterilized. Phosphate buffer (~400 ml) was prepared at pH 7.2. Test tubes (24) were prepared and sterilized, each containing 9 ml of phosphate buffer pH 7.2. Test tubes (120) were prepared, each containing 9 ml of normal saline. GYE (glucose-yeast extract) agar medium was prepared and sterilized and cooled to 45° C. in a water bath. Samples (24) of raw material were weighed, each ~500 milligrams (theoretically equivalent to 10 billion spores). The samples were added to media bottles at 37° C. and incubated half for 20 minutes the other half for 120 minutes. After 20 and 120 minutes incubation, respectively, the samples were mixed to uniformity and pipet 1 ml into 9 ml of sterile phosphate buffer pH 7.2. After all 12 samples from each time point were placed into test tubes containing sterile phosphate buffer, serial dilutions were made until 6 tubes had been used for each sample. The final dilution for the final two test tubes were 3×10^7 and 3×10^8, which gave a count of roughly 300 and 30 CFU, respectively. The final 2 test tubes from each sample were placed into 70° C. water bath for 30 minutes. After 30 minutes, they were cooled immediately to 45° C. Three sterile petri plates per tube were set out. 1.0 ml from the heat-treated tube was added into each petri plate, then 15 ml of sterile molten GYE Agar medium (at 45° C.) was poured into each of the petri plates and mixed thoroughly. When solidified, the plates were incubated in an inverted position for 48 hours at 40° C. The individual colonies were counted. Results were expressed as CFU per gram as shown in Table 1 below. $1.0E+10=1\times10^{10}$.

TABLE 1

Sample	20 Minutes Incubation Spore Count, CFU/gram	120 Minutes Incubation Spore Count, CFU/gram
Normal Saline-A	1.90E+10	1.88E+10
Normal Saline-B	2.12E+10	2.00E+10
Normal Saline-C	1.64E+10	2.06E+10
Average	1.89E+10	1.98E+10
Saline pH 1.0-D	2.08E+09	5.98E+07
Saline pH 1.0-E	1.47E+09	0.00E+00
Saline pH 1.0-F	3.59E+09	0.00E+00
Average	2.38E+09	1.99E+07
Saline pH 2.0-G	3.63E+09	3.46E+09
Saline pH 2.0-H	4.47E+09	2.48E+09
Saline pH 2.0-I	3.58E+09	2.82E+09
Average	3.89E+09	2.92E+09
Saline pH 3.0-J	1.65E+10	1.13E+10
Saline pH 3.0-K	1.35E+10	1.11E+10
Saline pH 3.0-L	1.80E+10	1.39E+10
Average	1.60E+10	1.21E+10

Other Embodiments

[0069] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[0070] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

[0071] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

What is claimed is:

1. A probiotic confection-based composition comprising a confection and an isolated *Bacillus coagulans* spore.

2. The probiotic confection-based composition of claim **1**, wherein said *Bacillus coagulans* spore is coated on the exterior surface of said confection.

3. The probiotic confection-based composition of claim **2**, wherein said composition further comprises a granulated or powder sugar coating on the exterior of said confection.

4. The probiotic confection-based composition of claim **1**, wherein said *Bacillus coagulans* spore is inside of said confection.

5. The probiotic confection-based composition of claim **1**, wherein said confection is selected from the group consisting of hard sweets, fudge, toffee, liquorice, chocolate, jelly candy, marshmallow, and marzipan.

6. The probiotic confection-based composition of claim **5**, wherein said jelly candy is a gelatin-based gummi candy.

7. The probiotic confection-based composition of claim **6**, wherein said gummi candy is in the shape of a bear, a worm, a frog, a hamburger, a cherry, a soda bottle, a shark, an army man, a hippopotamus, a lobster, a watermelon, an octopus, an apple, a peach, or an orange.

8. The probiotic confection-based composition of claim **1**, wherein said isolated *Bacillus coagulans* comprise between 0.01% and 10% by weight of said composition.

9. The probiotic confection-based composition of claim **1**, wherein said isolated *Bacillus coagulans* is *Bacillus coagulans* hammer strain Accession No. ATCC 31284.

10. The probiotic confection-based composition of claim **1**, wherein said isolated *Bacillus coagulans* is selected from the group consisting of GBI-30 strain (ATCC Designation Number PTA-6086), GBI-20 strain (ATCC Designation Number PTA-6085), and GBI-40 strain (ATCC Designation Number PTA-6087).

11. A composition comprising a dry mix for confection-based compositions comprising sugar and an isolated *Bacillus coagulans* spore.

12. The composition of claim **11**, wherein said *Bacillus coagulans* spore comprises 15% of said dry mix.

13. A method of making a probiotic confection-based composition comprising:

providing a confection;

heating said confection;

applying an isolated *Bacillus coagulans* spore to an external surface of said confection;

thereby making a probiotic confection-based composition.

14. The method of claim **13**, further comprising applying a sugar to an external surface of said confection.

15. The method of claim **13**, wherein said confection is a gummi bear

16. The method of claim **13**, wherein said isolated *Bacillus coagulans* is *Bacillus coagulans* hammer strain Accession No. ATCC 31284.

17. The method of claim **13**, wherein said isolated *Bacillus coagulans* is selected from the group consisting of GBI-30 strain (ATCC Designation Number PTA-6086), GBI-20 strain (ATCC Designation Number PTA-6085), and GBI-40 strain (ATCC Designation Number PTA-6087).

18. The method of claim **13**, wherein said isolated *Bacillus coagulans* comprise between 1% and 10% by weight of said confection-based composition.

19. A probiotic oil-based composition comprising seafood oil and an isolated *Bacillus coagulans* spore.

20. The probiotic oil-based composition of claim **19**, wherein said probiotic oil-based composition is selected from the group consisting of salmon oil, cod liver oil, and krill oil.

21. The probiotic oil-based composition of claim **19**, wherein said composition is encapsulated in as soft-shelled capsule or a soft gelatin capsule.

22. The probiotic oil-based composition of claim **19**, wherein said composition is a gelatin-based gummi candy.

23. The probiotic oil-based composition of claim **19**, wherein said seafood oil is eicosapentaenoic acid or docosahexaenoic acid.

24. The probiotic oil-based composition of claim **19**, wherein said isolated *Bacillus coagulans* comprise between 0.01% and 10% by weight of said composition.

25. The probiotic oil-based composition of claim **19**, wherein said isolated *Bacillus coagulans* is selected from the group consisting of GBI-30 strain (ATCC Designation Number PTA-6086), GBI-20 strain (ATCC Designation Number PTA-6085), and GBI-40 strain (ATCC Designation Number PTA-6087).

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[Continued on next page]

(54) Title: A PROCESS FOR THE ISOLATION OF A PHOSPHOLIPID

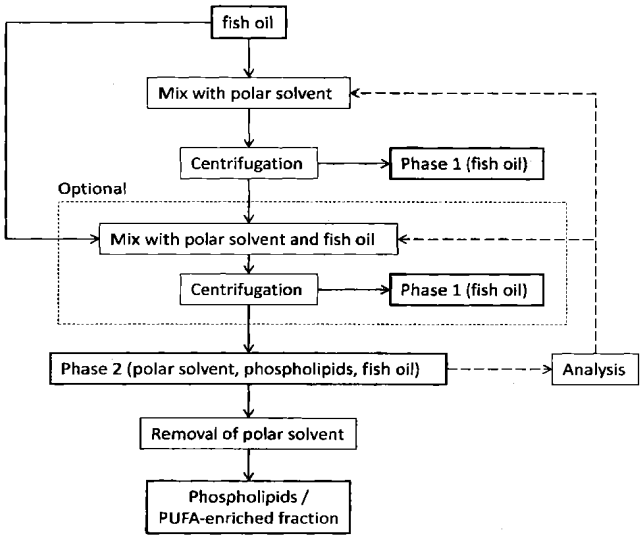

Fig. 1

(57) Abstract: The present invention relates to processes for the isolation of a phospholipid and for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of -providing a fish oil containing lipids and phospholipids; -mixing the fish oil with a polar solvent; -centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction; -isolating a phospholipid from the polar fraction or isolating a PUFA-enriched fraction from the polar fraction. The fish oil may be provided by -extracting a fish material with an extractant solvent; -removing the extractant solvent to provide the fish oil; -optionally subjecting the fish oil to a solid-liquid separation. The isolated phospholipids and PUFA's may be used as additives for functional foods, as a dietary supplement and for pharmaceutical application.

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A process for the isolation of a phospholipid

Field of the invention

This invention relates to processes for the isolation of phospholipids and for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from marine products. Marine phospholipids, in particular those comprising long chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are useful as additives for functional foods, as a dietary supplement and for pharmaceutical application. Marine phospholipids may provide beneficial effects to the health of both humans and animals.

Prior art

In recent years phospholipids comprising polyunsaturated fatty acids have been found to play important roles in physiology. Phospholipids have therefore attracted much attention as candidate materials for functional foods and in pharmaceutical applications.

Phospholipids are found in many sources of biological material, such as plant material or matter derived from animals. Marine animals comprise a particular promising source of phospholipids due to the specific composition of these phospholipids, in particular the amount of PUFA's, such as omega-3 fatty acids, e.g. eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), in marine phospholipids is large.

Phospholipids typically comprise a central glycerol moiety with two fatty acid chains and a phosphate group that may be further derivatised. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, amino alcohols, and carbohydrates. Phospholipids may also be referred to as polar lipids, and in the context of this application the terms "phospholipid" and "polar lipid" may be used interchangeably. Phospholipids are generally considered to be structural lipids, playing important roles in e.g. the structure of the membranes of plants, microbes and animals. Examples of phospholipids are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, phosphatidylglycerol, diphosphatidylglycerols. Because of their chemical structure, phos-

pholipids have a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents.

One important characteristic of marine phospholipids is that they commonly contain PUFA's with two or more unsaturated bonds, in particular with four or more unsaturated bonds. The lipid moieties of phospholipids are commonly of the omega-3 type, which often exhibit enhanced stability, e.g. oxidative stability, when incorporated into phospholipids.

Several methods exist in the prior art to extract and isolate phospholipids from raw materials. Such methods typically involve solvent extraction coupled with additional unit operations. Several examples of prior art processes are provided below.

WO2001/76385 discloses a process for the production of polar lipid-rich materials, e.g. phospholipids, from biomaterials that are rich in polar lipids with highly unsaturated fatty acids, i.e. fatty acids with four or more unsaturated bonds. Appropriate biomaterials for the process of WO2001/76385 include fish, crustaceans, microbes, eggs, brain tissue, milk, meat and plant material including oilseeds. Egg yolks are considered the primary commercial source of polar lipids rich in highly unsaturated fatty acids.

The process of WO2001/76385 comprises extracting polar lipids from the biomaterial using a water-soluble organic solvent (e.g. an alcohol) at a concentration of water soluble organic solvent of at least 68% in water. Denatured protein, which is not soluble in high concentrations of water-soluble organic solvent, is then separated by density separation, such as using gravity or centrifugal force, as a precipitate. The polar lipid/oil enriched liquid fraction may then be mixed with water to a final concentration of water-soluble organic solvent in water of from 5 to 35% to precipitate polar lipid, and polar lipid is then separated from the oil by means of density separation. An exemplary unit operation for density separation in WO2001/76385 is a decanter centrifuge.

US 6,372,460 discloses a method to provide a DHA phospholipid material, in particular from algae and other single celled organisms that contain a significant amount of DHA. In an example dried biomass (an alga) is extracted with hexane to provide a DHA-rich hexane fraction, which is centrifuged to remove fine particles. DHA-phospholipids are then precipitated chemically and the DHA-phospholipids subsequently collected by centrifuga-

tion.

JP2006-311853 discloses a method for producing a phospholipid composition from fish and shellfish. It is a particular concern of JP2006-311853 to provide a phospholipid composition free of heavy metals, such as cadmium. In the process of JP2006-311853 the starting material, e.g. fish waste is boiled with water. The boiled material is then separated into a solid and a liquid phase using centrifugal separation and/or filtration. The solid phase is then subjected to an organic solvent extraction process. The organic solvent may be methanol, ethanol, propanol, butanol, acetone, chloroform, methylene chloride, hexane or aqueous acetone. The organic solvent is then removed from the extract, now free of heavy metals, which is subjected to chromatographic purification.

JP2008-255182 describes a process for producing a phospholipid composition from an edible source, such as an edible portion and internal organs of fish and shellfishes. In the process of JP2008-255182 the starting material is initially heated with micro-waves to inactivate enzymes that may otherwise hydrolyse the phospholipids of interest. The heat-treated material is then extracted with a solvent, such as ethanol, hexane or acetone with ethanol being preferred.

JP2008-044907 provides the manufacture of phospholipid from solvent extraction of fish with the aim of improving the quality of the obtained phospholipid. The fish material is extracted with a non-polar solvent, e.g. hexane, heptane, isooctane, or benzene, a polar solvent, for example, methanol, ethanol, isopropanol, diethylether, ethyl acetate, acetone or a mixture of a non-polar solvent and a polar solvent, in particular a mixture of hexane and ethanol. The solvent is then removed from the extract, and the obtained fraction is then purified using adsorption filtration on diatomaceous earth.

WO2000/23456 discloses a method for extraction of lipid fractions from marine and aquatic animals, e.g. krill or fish. The method comprises suspending marine and aquatic material in a ketone such as acetone to extract lipids. The extraction may be carried out by successive acetone and alcohol treatments, e.g. using isopropanol or t-butanol, and the extraction should be performed at a temperature of about 5°C or less. The solubilised lipid fractions may then be separated from the solid material by techniques

such as filtration, centrifugation or sedimentation, with filtration being preferred. It appears from WO2000/23456 that the method disclosed therein may provide a fraction enriched in phospholipids. The method of WO2000/23456 is used specifically for extraction of phospholipids derived from natural marine or aquatic sources in WO2003/011873.

WO 2006/106325 discloses processes for the production of phospholipid compositions, e.g. marine phospholipids. One process of WO 2006/106325 comprises extracting a fish meal with an organic solvent to produce a lipid-containing liquid, and subjecting the liquid to microfiltration. The organic solvent may be a solvent in which phospholipids and triglycerides are soluble, such as hexane, isohexane, cyclohexane or heptane. According to WO 2006/106325 phospholipids aggregate into large molecular weight micellar structures in the non-polar alkane solvent, whereas all neutral lipids are dissolved in molecular disperse solution. The phospholipid micelles are considered too big to diffuse across microfiltration membranes having pore sizes of 0.1 to 0.5 µm, and phospholipids can therefore be isolated in this process.

In another process of WO 2006/106325 the alkane solvent extract may be subjected to solvent stripping and the extract or residue may be contacted with a second solvent in which neutral lipids are more soluble than polar lipids whereby to precipitate a phospholipid composition. The second solvent may be supercritical carbon dioxide, propane, carbon dioxide/propane mixtures, ethanol/water mixtures or ketones with acetone being preferred.

Several processes are known for separating phospholipids from oils of plant origin. However, the content of phospholipids in plant oil is typically different from that of fish oil. Thus, for example a plant oil may contain from 0.5 to 3 % phospholipids whereas the content in fish oil will normally be below 0.5 %, e.g. close to 0 %. Furthermore, the lipid composition of a fish oil will also be different from the lipid composition of a plant oil. For example, plant oils such as olive oil, rape seed oil and linseed oil do not contain omega-3 acids containing more than 18 carbon atoms, whereas phospholipids containing fatty acids with more than 18 carbon atoms, e.g. EPA (20 carbon atoms) and DHA (22 carbon atoms) are found in fish; these PUFA's are of particular interest. Moreover, in the processing of a plant oil the aim is typically the complete separation of oil from phospholipids without regard to keeping the phospholipids intact. Thus, plant phospholipids, "lecithins", are commonly

hydrolysed using e.g. acid or enzymes, in order to make them hydrophilic to ease their removal from plant oils.

US 4584141 discloses a modified conventional degumming process for removing impurities from triglyceride oils. Exemplary oils are plant oils, e.g. sunflower oil and soybean oil, although the process is also suggested for use with safflower oil, cottonseed oil, grapeseed oil, corn oil, rapeseed oil, rice bran oil, tallow and fish oil. In the process of US 4584141 the oil is mixed with hydrolysed phosphatide and water before separating the oil into an oil portion and a sludge portion and separating the sludge portion into an aqueous phase and an oil phase. US 4584141 thus requires addition of hydrolysed phospholipid, and it is therefore not suitable for isolating phospholipids as a product.

US 6172247 relates to methods for refining vegetable oils and by-products thereof. The process for refining vegetable oil uses organic acid, for example to produce a refined vegetable oil with improved odour, flavour, and storage stability, and a reduced content of e.g. free fatty acids and phosphatides. The process involves admixing a dilute aqueous organic acid solution with a heated stream of crude vegetable oil to give an acid-oil blend and separating a hydrated impurities phase and a purified vegetable oil phase. The hydrated impurities phase is a phosphatide concentrate and comprises hydrolysed lecithin. US 6172247 further discloses a "Lecithin Deodorizing" process comprising adding hydrogen peroxide to the hydrolysed lecithin fraction. US 6172247 require as a minimum addition of organic acid or hydrogen peroxide to provide the advantages of the processes, and it is not disclosed how intact phospholipids may be isolated, and further US 6172247 is limited to plant oils.

US2006/110521 relates to non-hydrogenated or partially hydrogenated non-animal oils, and US2006/110521 discloses processes for their preparation. The oil is prepared in the steps of preparation, cracking and dehulling, conditioning, milling, flaking or pressing, extracting, degumming, refining, bleaching and deodorising. Oil extraction may be performed using a solvent, such as n-hexane or isohexane, and degumming to remove the hydratable phosphatides is performed by adding water and heating. The process of US2006/110521 is however considered ill-suited for treating fish since these contain significant quantities of EPA and DHA.

US2005/129739 suggests that phospholipids can be recovered from fish, microalgae, or fungi through a physical or chemical degumming process. However, the degumming process is not disclosed, and further the only processes for oil extraction discussed in US2005/129739 are for extraction from plant material.

EP 0269277 discloses a process for degumming triglyceride oils for removing phospholipids or gums from the oils. The object of EP 0269277 is to produce an oil product with a reduced phosphorus content in the oil, and this is achieved by dispersing in the oil an organic acid or acid anhydride, at a temperature not greater than about 40°C, subsequently dispersing water in the oil, while maintaining this temperature, and then separating a sludge containing the gums from the oil. In the treatment according to EP 0269277 the phospholipids in the oil will be hydrolysed and hydrated by the process, and therefore the process is not suited for extracting intact phospholipids.

In light of the above there is a need for a robust and scaleable process capable of processing large amounts of raw material to obtain a phospholipid product. In particular, there is a need for an efficient process to isolate phospholipids and to provide a PUFA-enriched product from raw material derived from fish. The present invention addresses these points.

Disclosure of the invention

The present invention relates to a process for the isolation of a phospholipid from a fish oil. The process comprises the steps of:

-providing a fish oil containing lipids and phospholipids;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a phospholipid from the polar fraction.

In another aspect the invention relates to a process for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of:

-providing a fish oil containing PUFA's;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;
-isolating a PUFA-enriched fraction from the polar fraction.

In certain embodiments of the processes, the step of providing the fish oil comprises:
-extracting a fish material with an extractant solvent;
-removing the extractant solvent to provide the fish oil;
-optionally subjecting the fish oil to a solid-liquid separation.

Any fish oil is appropriate for the processes as long as the fish oil contains both lipids and phospholipids and/or PUFA's, and the fish oil may be obtained from any species of fish. In this context, the term "fish" covers both vertebrate and invertebrate species of marine animals, such as fish, molluscs, e.g. octopuses, squid and cuttlefish, or crustaceans, e.g. krill, shrimps, crabs, lobsters, mantis shrimp, woodlice, sandhoppers. Fish of particular relevance comprise sand eel (*Hyperoplus* sp., *Gymnammodytes* sp. or *Ammodytes* sp., e.g. *Hyperoplus lanceolatus*), sprat (*Sprattus sprattus*), herring (*Clupea* sp., e.g. *Clupea harengus*), anchovy (*Engraulis* sp., e.g. *Engraulis ringens)*, boarfish (*Capros aper)*, Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*Micromesistius poutassou*), and Jack Mackerel (*Trachurus murphyi*). Certain embodiments of the invention employ a fish material. The term "fish material" is to be understood broadly and may comprise any material derived from a fish as defined in the invention. The fish material may especially be any material derived from fish meal production. The fish material may also be derived from fish which has not been subjected to heat treatment; for example the fish material may be fish waste or the like from the production of fish for human consumption.

Any type of phospholipid from fish is relevant for the present process, and the term phospholipid within the present description is not limited to natural polar lipids but also includes chemically modified polar lipids. Phospholipids containing PUFA's are of particular interest in the present invention. The process of the invention is especially suitable for the isolation of an intact phospholipid. In particular the phospholipid is not hydrolysed in the process, and in certain embodiments of the invention no additive, which may hydrolyse a phospholipid is added in the process. Relevant compounds that may hydrolyse a phospholipid comprise acids, e.g. phosphoric acid, organic acids,

e.g. citric acid, acid anhydrides, hydrogen peroxide, and enzymes, e.g. lipases and phospholipases. The intact phospholipid comprising both fatty acid chains and the phosphate group attached to the central glycerol moiety will stabilise PUFA's, in particular EPA and DHA, from degradation, such as oxidative degradation. Furthermore, in other embodiments no surfactant is added in the process.

The fish oil may be obtained using any available process although the fish oil may advantageously be obtained according to the invention. When the phospholipids are obtained according to the invention the contents of contaminants, such as heavy metals, e.g. lead, cadmium, pesticides and pesticide break-down products, e.g. toxaphen, chlordan, DDD, DDE, DDT, endosulfan, endrin, heptachlor, hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), other harmful compounds, e.g. dioxins, polychlorinated biphenyls (PCBs), persistent organic pollutants (POPs) will be reduced. Thus, when a fish material is processed according to the invention the isolated phospholipids will contain unwanted contaminants in amounts acceptable for use in food products for humans or animals.

Any polar solvent can be used in the invention. Importantly, the polar solvent should be able to extract phospholipids from the fish oil. The polar solvent is selected such that it is immiscible with the fish oil, so that addition of the polar solvent to the fish oil will create a two-phase system. A preferred polar solvent is water.

Phospholipids may be found in a micellar form with the polar "head" facing the centre of the micelle or facing the solvent depending on the polarity of the solvent. In particular, the phospholipids may have a "critical micelle concentration" or CMC, so that when the phospholipids are present above this concentration in a solvent they will form micelles with the type of micelles depending on the polarity of the solvent. For example, when present in a polar solvent above the CMC the phospholipids will form micelles with the polar moiety facing the polar solvent. Below the CMC the phospholipids may be found in a generally dissolved form in either of a polar or an apolar solvent. The present inventors have now surprisingly found that when a fish oil containing phospholipids and/or PUFA's is mixed with a polar solvent it is possible to preferentially extract the phospholipids and/or PUFA's to the polar solvent in a micellar form by carefully considering the ratio of polar solvent to

fish oil and the nature of the polar solvent. The amount of polar solvent should be sufficient for the phospholipids to form micelles, and it will depend on the amount of phospholipids and free fatty acids. This allows that the phospholipids, and thereby also PUFA's, are extracted and isolated from the fish oil; in particular, the simple nature of the extraction, i.e. mixing a fish oil and a polar solvent, allows the process to be used in industrial scale. Furthermore, the invention allows that a fish oil fraction may be enriched in PUFA's, e.g. EPA and DHA, since these are common among the fatty acids chains of phospholipids in fish oil. The processes of the invention may further comprise analysing the polar fraction or the concentrated polar fraction for the presence of an excess of polar solvent, e.g. excess relative to the formation of phospholipid micelles. The analysis may be used to control, e.g. adjust, the amount of polar solvent used in the upstream polar solvent extraction. This is especially useful when the process is performed under continuous operation. The ratio of polar solvent to fish oil will generally be about 5:95 to about 25:75, although it is also possible to use an excess of polar solvent to fish oil. Using an excess of polar solvent evidently requires larger volumes of solvent and therefore using the ratio of about 5:95 to about 25:75 is especially advantageous in an industrial process since smaller scale equipment, e.g. centrifuges, can be employed. The reduced process volumes and the smaller scale equipment allow faster processing of the fish oil as less polar solvent has to be separated from the fish oil. Furthermore, by careful choice of the ratio of polar solvent to fish oil it is possible to minimise the amount of fish lipids trapped in the phospholipid micelles and thereby increase the purity of the phospholipids in the polar fraction.

Certain embodiments of the invention comprise a second extraction with the polar solvent. Thus, the process may further comprise the steps of:

-mixing the polar fraction with the polar solvent and fish oil;

-separating the mixture of the polar fraction, the polar solvent and the fish oil into a concentrated polar fraction and a lipid fraction. The separation is preferably a centrifugation. The concentrated polar fraction may also be analysed for the presence of an excess of polar solvent as described above. In general, the same considerations as for the first extraction with the polar solvent apply. However, in this second extraction fish oil, e.g. fish oil which has not been treated according to the invention or fish oil which has been extracted

from fish material with an extractant solvent according to the invention, is added, e.g. simultaneously, with the polar solvent to the polar fraction. The ratio of polar solvent to the polar fraction and the fish oil will generally be up to about 5% polar solvent, e.g. about 1% to about 4%, preferably about 2%; about 25% to about 75%, e.g. about 40% to about 60%, preferably about 50% fish oil and polar fraction to balance. This second extraction allows that a higher concentration of phospholipids can be obtained in the concentrated polar fraction compared to the polar fraction from the first polar solvent extraction. In particular, the polar fraction from the first polar solvent extraction will be enriched in phospholipids and the higher concentration of phospholipids is advantageous in sequestering further phospholipids from the additional, untreated fish oil added in the second polar solvent extraction. Thus, the second polar solvent extraction will provide a synergistic concentrating effect on phospholipids and PUFA's in the combined treated and untreated fish oil to provide an even higher concentration of phospholipids and PUFA's in the products obtained after removal of the polar solvent. For example, aqueous extraction of a fish oil provided from an ethanol-extracted fish material may yield a phospholipid product from the polar fraction with a phospholipid content of 15% and a content of EPA+DHA of about 25-30%. The second aqueous extraction may yield a phospholipid product from the concentrated polar fraction with a phospholipid content of 40% and a correspondingly increased content of EPA+DHA.

Several steps of the processes of the invention may comprise a centrifugation. In the context of the invention the term "centrifugation" and derived forms include any type of centrifugation, in particular using centrifuges suited for industrial scale of operation, e.g. disk stack centrifuges, decanter centrifuges, solid bowl centrifuges etc.

The transfer of the phospholipids and PUFA's from the fish oil to the polar solvent may take place instantaneously when the polar solvent is mixed with the fish oil, or the mixing step may have any duration as desired.

In certain embodiments it may be necessary to physically mix the polar solvent with the fish oil. For example, the mixing may be performed in a vessel equipped with a stirring blade, an impeller, a Rushton turbine, a propeller or the like, or the mixing vessel may otherwise be fitted to agitate the mixture of the fish oil with the polar solvent. In particular, when the mixture

of the fish oil with the polar solvent is physically mixed this generally involves subjecting the mixture to shear stress.

The mixing may take place at any temperature at which the polar solvent is liquid, e.g. the temperature may be decreased below ambient temperature, the mixing may take place at ambient temperature or the temperature may be increased during mixing. A high temperature will generally allow that the phospholipids are extracted at a higher rate than when the extraction is performed at a lower temperature. The temperature may thus be increased to any value below the boiling point of the polar solvent. In other embodiments, the mixing may take place at a decreased or at ambient temperature. In yet further embodiments, the temperature may be increased or decreased from the initial mixing temperature so that the temperature is changed during the mixing.

Following extraction of the phospholipids and PUFA's from the fish oil in the mixing step the mixture of the fish oil with the polar solvent is centrifuged to separate the two phases, i.e. the polar fraction comprising the phospholipids from the lipid fraction comprising other lipids from the fish oil. The centrifugal separation may be performed at an increased temperature. Any industrial centrifuge may be employed, e.g. a disk stack centrifuge, a decanter centrifuge, a solid bowl centrifuge. The separation of the two phases may advantageously be performed in a disk stack centrifuge. The centrifugal separation will provide a polar fraction with phospholipids and also a fish oil product depleted in phospholipids; another aspect of the invention relates to the phospholipid-depleted fish oil product obtainable in the process of the invention. In further embodiments of the processes the polar fraction is subjected to a second centrifugal separation, e.g. in a disk stack centrifuge, to concentrate the phospholipids and PUFA's further.

The polar solvent fraction, or phase, from the centrifugal separation comprises the phospholipids and PUFA's, and in the process of the invention the phospholipids are isolated from the polar solvent fraction. Likewise, a PUFA-enriched fraction may be isolated from the polar fraction. The isolation may comprise any appropriate method, such as evaporation of the polar solvent, distillation, e.g. vacuum distillation, of the polar solvent, or the phospholipids and/or PUFA's may be isolated adsorptively, e.g. using a chromatographic membrane or matrix or an adsorptive material such as diatoma-

ceous earth, or the phospholipids may be isolated using nano- or ultrafiltration. In the context of the invention "vacuum distillation" generally refers to a unit operation where heat is applied to the polar fraction with the simultaneous lowering of the pressure above the polar fraction in order to drive out the polar solvent from the polar face with the phospholipids. The term may also be used in the context of removal of an extractant solvent. Furthermore, the heat applied may be moderate, e.g. to a maximum of about 40°C to avoid heat modification of phospholipids. The phospholipids may be further dried, e.g. by subjecting the phospholipids to additional heat treatment, optionally at a decreased pressure. Removal of polar solvent and drying of the phospholipids may be performed in the same operation.

In another aspect the invention relates to the phospholipids obtainable in the process of the invention. In yet another aspect the invention relates to the PUFA's obtainable in the process of the invention.

In a specific embodiment of the process of the invention the fish oil is provided by extracting lipids and phospholipids, i.e. "fish oil", from a fish material as described above. Appropriate fish materials are fish meal, optionally in the form of pellets, presscake, e.g. from fish meal production, unprocessed fish, whole fish, specific parts of fish, such as skin, bone, meat, organs, e.g. fish liver, or fish waste etc.; in particular, the "fish material" may be a material derived from fish at any stage in the production of fish meal or the fish material may be derived from fish at any stage in the production of fish for human consumption. The fish material is extracted with an extractant solvent. Any solvent capable of extracting lipids including phospholipids is contemplated for use in the invention. The extractant solvent may be polar or apolar. Relevant apolar solvents comprise hydrocarbon solvents. The extractant solvent may also be supercritical carbon dioxide. Apolar solvents, such as hexane, e.g. isohexane, are preferred as extractant solvent in some embodiments. Other embodiments employ ethanol or ethanol-water-mixtures as extractant solvent.

The extraction will generally involve contacting a fish material with the extractant solvent. In a specific embodiment the fish material is a fish meal, e.g. in the form of pellets, although the fish meal may also be extracted without prior pelletisation. In another embodiment, the fish material is a presscake from fish meal production, and in yet another embodiment

whole fish or parts of fish are extracted with the extractant solvent. The fish material, e.g. fish meal, or fish meal pellets, is mixed with the extractant solvent, and the extraction with the extractant solvent may be performed under application of shear stress to the mixture of the fish material and the extractant solvent, for example using a stirring blade, an impeller, a Rushton turbine, a propeller or the like. The duration of the extraction step may be selected freely, e.g. the extraction may take place instantaneously, or the extraction may have a duration up to e.g. 24 hours. The extraction may advantageously be performed as a continuous process.

The extraction with the extractant solvent may be performed at ambient temperature or lower, or the temperature may be increased during the extraction, e.g. to any temperature up to the boiling point of the extractant solvent. In general, an increased temperature will result in a faster extraction of the phospholipids and PUFA's and lipids from the fish material. Ambient temperature or lower may be employed when it is of interest to ensure that the phospholipids and PUFA's are not modified by exposure to high temperature.

After the extraction with the extractant solvent it may be desirable to remove the extracted fish material from the extract. The extracted fish material will generally comprise particulate material of a relatively large size, e.g. from sub-millimetre up to the size of the pellets, if applicable. Any solid-liquid unit operation capable of separating such particulate from the extractant solvent may be applied to remove the extracted fish material from the extract. For example, the extracted fish material may be removed from the extract using sieving, filtration or centrifugation. In a further aspect the invention relates to the extracted fish material obtainable in the process.

The extractant solvent is removed from the extract following the extraction. Any appropriate method may be used to remove the extractant solvent, such as distillation, e.g. vacuum distillation, or evaporation. The extractant solvent removed from the extract may be recycled in the process to be added to and contacted with a further portion of fish material or fish material pellets. This allows for an efficient continuous processing of fish material to isolate phospholipids.

The fish oil resulting from the removal of the extractant solvent may be subjected to a solid-liquid separation prior to processing to isolate phos-

pholipids as described above. Any solid-liquid unit operation may be employed, although filtration is preferred. In a further aspect the invention relates to a protein product obtainable by filtration of the extract.

The embodiments of the process of the invention disclosed above may advantageously be performed under continuous operation. An advantage of continuous operation is hygiene since all process steps may be carried out in closed systems to prevent contamination from air or operators. Furthermore, the stability of the product, e.g. phospholipids and PUFA's, is improved since storage in tanks and the like is minimised in a continuous process. Continuous operation is particularly advantageous since it allows efficient processing of large quantities of material, e.g. in the order of hundreds of tonnes. Efficient processing of such quantities of material is particularly relevant for isolating a product from a starting material where the product is present in low amounts, such as isolating phospholipids from fish material. Furthermore, when the process steps allow continuous operation simple integration of the process steps in a process train of industrial scale is possible.

Thus, in yet a further aspect the invention relates to an integrated continuous process for producing a product from a fish material, such as a fish meal or fish meal pellets. The product may be a phospholipid product or a PUFA-product. The term "integrated" is to be understood broadly, but it especially refers to a situation where a process stream, such as a waste stream, e.g. a stream of solvent, e.g. extractant solvent or polar solvent, removed from a process step is recycled in an earlier, or upstream, process step. For example, in this process the fish material is extracted with an extractant solvent as described above, before removal of the extractant solvent likewise as described above. The removed extractant solvent may be recycled in the process, although further extractant solvent may also be added to retain the mass balance of extractant solvent in the process. In specific embodiments solid-liquid separation unit operations are included in the process following the extraction and following the removal of the extractant solvent. The fish oil is then treated to isolate phospholipids as described above. Thus, the fish oil is mixed with the polar solvent in a vessel appropriate for continuous processing before leading the process stream to a centrifuge likewise suited for continuous operation. The stream of polar solvent containing phospholipids is then led to the removal of polar solvent optionally combined with

a drying step, e.g. by treating at increased temperature and decreased pressure. This operation may also be performed continuously, and the polar solvent may be recycled and added to fish oil provided from the prior extraction step. In certain embodiments the mixing and extraction steps are performed at increased temperatures. However, in a specific embodiment, e.g. where the fish material is fish which has not been subjected to heat treatment, all process steps are performed without subjecting the fish material to excessive temperatures, e.g. temperatures above 40°C, at any stage of the process. An integrated process may further comprise analysing the polar fraction and/or the optional concentrated polar fraction for the presence of an excess of polar solvent and controlling the amount polar solvent added to the fish oil or the mixture of polar fraction and fish oil based on the result of the analysis. Thus, the analysis may provide information to a feedback loop allowing adjustment of the amount(s) of polar solvent added in the respective polar solvent extractions to the optimal ratio of polar solvent to fish oil or mixture of polar fraction and fish oil.

It is within the knowledge of the skilled person to design the integrated process for continuous operation in order to isolate phospholipids from fish material when considering the amount of fish material to be processed and the amount of phospholipids contained in the fish material. For example, the skilled person can select reactor vessels, and their required size and capacity, appropriate for continuous operation and calculate the necessary residence times in the vessels and the corresponding material flow rates in the vessels. All steps for which an increased temperature is relevant as outlined above, are preferably performed at increased temperature. This will advantageously minimise the risk of microbial contamination, and further lead to a faster overall process.

Brief description of the figures

In the following the invention will be explained in greater detail with the aid of examples of embodiments and with reference to the schematic drawings, in which

Fig. 1 shows a process diagram of an embodiment of the invention;

Fig. 2 shows a process diagram of an embodiment of the invention;

Fig. 3 shows a process diagram of an embodiment of the invention.

Detailed description of the invention

The present invention relates to a process for isolation of a phospholipid from a fish oil comprising the steps of:

-providing a fish oil containing lipids and phospholipids;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a phospholipid from the polar fraction.

In another aspect invention relates to a process for producing a poly-unsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of:

-providing a fish oil containing PUFA's;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a PUFA-enriched fraction from the polar fraction. In the context of the present invention a PUFA is a fatty acid containing more than 18 carbon atoms and two or more unsaturated bonds. Preferred PUFA's are EPA and DHA.

A process diagram of the invention is illustrated in Fig. 1.Fig. 1 shows the process with the optional second polar solvent extraction indicated, and furthermore, Fig. 1 illustrates how the result of the analysis for excess polar solvent may be used to control the upstream polar solvent extraction(s).

The fish oil may be provided by:

-extracting a fish material with an extractant solvent;

-removing the extractant solvent to provide the fish oil;

-optionally subjecting the fish oil to a solid-liquid separation.

Specific embodiments of the processes are illustrated in Fig. 2 and Fig. 3. Fig. 2 and Fig. 3 indicate the optional second polar solvent extractions. The processes in Fig. 2 and Fig. 3 may both provide a phospholipid product or a PUFA-product, and both may be integrated to be performed as integrated continuous processes where e.g. solvent streams are recycled to be used in

upstream extraction steps. Further, both processes may comprise analysis steps, as described above, to provide information for use regarding addition of polar solvent in the respective extractions.

The fish oil is mixed with a polar solvent. The "polar solvent" is immiscible with the fish oil, but the polarity of the solvent allows that phospholipids and PUFA's are extracted from the fish oil due to the formation of phospholipid micelles in the polar solvent. Any solvent with this capability is contemplated for use in the process of the invention. In particular, polar solvents typically have a high dielectric constant, such as above 15. A preferred polar solvent is water, e.g. deionised water. The ratio of polar solvent to fish oil will generally be from about 5:95 to about 25:75. The amount of polar solvent to fish oil will typically dependent on the exact nature of the polar solvent. For example, when water is selected as the polar solvent the ratio of water to fish oil may be from about 10:90 to about 20:80. The optimal amount of polar solvent may be determined by analysis of the polar fraction and the result of the analysis may be used to adjust the amount of polar solvent to be mixed with the fish oil. In particular when the process is performed continuously the result of the analysis may be employed in a feed-back loop to optimise the process when it is running. Specific embodiments of the invention thus comprise the step of analysing the polar fraction, or optionally the concentrated polar fraction, for the presence of an excess of polar solvent. The result of the analysis may be used to adjust, in particular during continuous operation, the amount of polar solvent mixed with the fish oil. Thus, for example when a relatively dense polar solvent, such as water, is used the amount of polar solvent to be mixed with the fish oil or the mixture of the polar fraction and the fish oil may be determined by subjecting a sample from the polar fraction to lab scale centrifugation and checking the test tube for the presence of free polar solvent in the bottom of the tube. The presence of free polar solvent will indicate that an excess amount of polar solvent was present during the step of mixing the fish oil with water. The amount of polar solvent to be added in the continuous process may be adjusted to the minimum excess required which is optimal for the separation.

When the processes of the invention comprise a second polar solvent extraction of the polar fraction as outlined above, the concentrated polar fraction may also be analysed for excess of polar solvent as explained above. The

duration of the mixing step should be sufficient to provide a polar fraction, e.g. an aqueous fraction, enriched in phospholipids and PUFA's and a lipid fraction depleted in phospholipids. The mixing may be for any predetermined period of time and the mixing is not limited regarding the temperature. However, the duration of the mixing should be sufficient to separate the phospholipids from the fish oil.

The mixing temperature may be selected to optimise extraction of phospholipids and PUFA's, and in certain embodiments it is generally increased from ambient temperature to a temperature below the boiling point of the polar solvent. For example, when the polar solvent is water the temperature may be from about 50°C to about 95°C or higher, such as about 60°C, about 70°C, about 80°C or about 90°C. An increased temperature may provide a faster extraction of the phospholipids and PUFA's from the fish oil. In another embodiment the mixing temperature is maintained in a range from below ambient, e.g. about 5°C, to moderately increased, e.g. to about 40°C, such as about 10°C, about 20°C or about 30°C. Certain species of phospholipids and especially PUFA's, may be modified by high temperatures, and in this temperature range it can be ensured that the phospholipids and PUFA's are not modified, e.g. damaged by the high temperature. In particular it may be of interest to keep the temperature as low as possible. In some embodiments all process steps are performed at a low temperature, and in others some steps may be performed at low temperature whereas others are performed at increased temperature. In general, brief exposure of a fish material or a mixture or an extract etc. in a step of the process of the invention to high temperature will not be detrimental to the phospholipids. In particular, a process stream or the phospholipid product may be subjected to pasteurisation without modifying the phospholipids. Thus, any step of the inventive process may also comprise a pasteurisation step. Pasteurisation is well known to the skilled person.

The mixing time will typically be up to about 1 hour, such as about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes or about 60 minutes. In a specific embodiment water is used as the polar solvent, which is mixed with the fish oil at a ratio of 15:85 for about 20 minutes at about 80°C, preferably in a continuous process. This ratio of water to fish oil may also be used in embodiments using other mixing tem-

peratures. Likewise, this ratio is also relevant for other polar solvents.

The mixture, i.e. the two-phase system, with the polar fraction and the lipid fraction is centrifuged to separate the polar fraction from the lipid fraction, optionally at an increased temperature, e.g. at a temperature of about 40°C to about 75°C, e.g. at about 70°C. In particular, an increased temperature may be used when the preceding mixing step is performed at an increased temperature, and further when subsequent removal of the polar solvent by vacuum distillation is intended, centrifugation at an increased temperature is preferred. Likewise, when the mixing temperature is kept low, as defined above, to ensure that phospholipids are not modified due to heating, it may be of interest to maintain the temperature in this range in the centrifugation step. In general, the polar solvent may be present as drops or droplets in the fish oil. Further, the phospholipids in micellar form in the polar solvent may function as surfactants to create an "oil-in-polar-solvent emulsion", e.g. an oil-in-water emulsion. Any centrifugation operation capable of separating two liquid phases, e.g. in the form of drops or droplets of one phase in the other, may be employed, but it is preferred that a disk stack centrifuge is used. A particularly preferred embodiment employs two consecutive disk stack centrifuges to centrifuge the mixture of the fish oil and the polar solvent, or optionally the mixture of the polar fraction, the polar solvent and the fish oil. In this embodiment the first centrifuge serves to separate water and phospholipids, i.e. the polar fraction or concentrated polar fraction, from the lipid fraction. The subsequent, e.g. serially connected, disk stack centrifuge concentrates the phospholipids in the polar fraction or concentrated polar fraction from the upstream disk stack centrifuge. In a specific set-up the first centrifuge has a distance between the disks of 0.6 mm, and the second centrifuge has a distance between the disks of 0.8 mm.

The polar solvent is subsequently removed from the mixture of the polar fraction e.g. by vacuum distillation. For example, when the polar solvent is water it may be removed by increasing the temperature to be in the range of about 60°C to about 85°C, e.g. about 80°C or about 85°C while reducing the pressure so that the water boils, e.g. while reducing the pressure to about -0,7 bar to about -0,9 bar. The water may thus be removed from the phospholipid fraction, which is further dried, in about 1 hour to about

3 hours. It is also possible to employ a different combination of temperature and pressure, but when the process employs increased temperatures, the temperature and pressure are typically selected such that the water is boiling. Likewise, in embodiments where excessive temperatures are avoided to prevent modification of phospholipids it may be desirable to maintain a moderate temperature when removing the polar solvent. These considerations also apply when other polar solvents are employed. The temperature may advantageously be increased using indirect steam when relevant.

In another embodiment of the process of the invention, fish material is extracted with an extractant solvent to provide fish oil for isolation of phospholipids. In a preferred embodiment the fish material is fish meal, which may be pelletised prior to extraction, e.g. at a temperature of about 50°C, for example with addition of steam to optimise pelletisation. In yet another embodiment, the fish material is a presscake from the production of fish meal. In very broad terms the "presscake" refers to the material obtained after initially heating fish or fish material to coagulate protein, rupture fat depots and liberate oil and physico-chemically bound water, followed by pressing (or optionally centrifugation) to, at least partially, remove liquids from the mass. The presscake may be extracted directly or the presscake may be subjected to disruption or comminution or the like prior to extraction. When presscake is treated according to the process of the invention the fish oil extracted with the extractant solvent comprises a higher content of phospholipids since the neutral oils have been removed during the pressing. This further allows that smaller amounts, e.g. relative to the amount of fish material, of extractant solvent are employed. Presscake is therefore a preferred fish material in the present invention. In a further embodiment, whole fish or parts of fish are extracted with the extractant solvent, specifically the whole fish or parts of fish may be extracted without any prior heat treatment. When the fish material has not been subjected to prior heat treatment, whole fish may be extracted directly, or the whole fish may be subjected to comminution or disruption prior to extraction. The extraction may take place in any appropriate vessel. In particular, the extraction vessel may be provided with a device to apply shear stress to the mixture of the fish material and the extractant solvent, e.g. the vessel or extractor may be equipped with stirrer blades or the like.

In the context of the present invention, the term "extractant solvent" refers to any solvent that may extract a lipid fraction, e.g. fish oil or phospholipids and PUFA's, from a fish material. Typical extractant solvents comprise apolar solvents, such as alkanes, e.g. pentane, hexane, heptane, octane etc., and aromatic hydrocarbons, e.g. benzene, toluene, and the like. An apolar solvent may also be referred to as a "non-polar solvent". Hydrocarbon solvents comprising heteroatoms may also be employed as extractant solvent, as long as the hydrocarbon solvent may extract a lipid fraction comprising phospholipids from a fish material. The extractant solvent is preferably liquid at ambient temperature and pressure. A preferred extractant solvent is hexane, in particular isohexane. It is noted that in the context of the present invention supercritical carbon dioxide is also contemplated for use as an extractant solvent. Other relevant extractant solvents are alcohols, such as methanol, ethanol, e.g. 96% ethanol in water, propanol, isopropanol or butanol, optionally mixed with water, ketones, such as acetone, ethers or esters etc. It is also possible to employ mixtures of two or more extractant solvents. In a specific embodiment the extractant solvent is ethanol or a mixture of ethanol and water, e.g. with a concentration of ethanol in water from 10% up to 30%, or with a concentration of ethanol in water above 70%, for example the concentration of ethanol may be about 80% or about 85%. In a preferred embodiment the extractant solvent is 96% ethanol. When 96% ethanol is employed to extract presscake the ratio of ethanol to presscake is typically from about 1:2 to about 1:5, preferably about 1:3. The extraction time may be about 2 hours, at the temperature about 65°C.

The extraction may be performed at ambient or lower temperature, or it may be performed at an increased temperature. For example, in one embodiment the extraction may be performed at a temperature in the range of about 40°C to about 70°C, such as about 40°C, about 50°C, about 60°C, or about 70°C. In another embodiment the extraction with the extractant solvent is performed at a low temperature of about 5°C to about 40°C, e.g. about 10°C, at about 20°C or about 30°C. When the extraction is performed at low temperature other process steps may also be performed at low temperature. Extraction at increased temperature can increase the extraction efficiency, and in particular the temperature may be controlled to increase the efficiency of extraction of phospholipids, which may be extracted selec-

tively, e.g. extraction at about 50°C to about 60°C when the extractant solvent is isohexane will provide optimal extraction of phospholipids using this solvent. The extraction temperature is preferably below the boiling point of the extractant solvent. The same considerations for employing a low temperature in the step of mixing fish oil with the polar solvent generally apply also for extraction with the extractant solvent and any subsequent steps.

The duration of the extraction is not limited and may be selected to provide sufficient extraction of lipids, especially phospholipids, from the fish material. For example, the duration may be from about 0.5 hours to about 10 hours or more, e.g. about 1 hours, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours or about 10 hours. Extraction with isohexane may be performed with a duration of e.g. about 2 hours.

Extraction of the fish material with the extractant solvent will result in a liquid fraction comprising the lipids, including also polar lipids, such as phospholipids and PUFA's, from the fish material. The liquid phase comprising the lipids and the extractant solvent may be referred to as an "extract". This extract may be subjected to a solid-liquid separation to remove solid debris, e.g. extracted fish material, from the liquid phase with the phospholipids. This liquid phase may also be referred to as a "crude oil". Any appropriate solid-liquid separation operation may be employed, for example, sieving, filtration, centrifugation.

The extractant solvent can be removed from the crude oil or the extract using any appropriate method. In particular, the extractant solvent may be removed from the crude oil or the extract using increased temperature and decreased pressure (referred to in the context of the invention as "vacuum distillation"). For example, isohexane may be removed by increasing the temperature to about 70°C to about 90°C, e.g. about 85°C under a reduced pressure (e.g. under "vacuum") of about 5 mbar to about 50 mbar. Under these conditions isohexane may be removed in about 10 minutes to about 20 minutes. Removal of the extractant solvent from the extract or crude oil will provide a fish oil comprising both polar and non-polar lipids from the fish material. The fish oil is preferably free of extractant solvent, e.g. the fish oil contains less than 10 ppm extractant solvent, such as less than 5 ppm or less than 2 ppm extractant solvent. The extractant solvent is preferably recycled

in the process by adding to fish material to be processed according to the invention.

The fish oil may be subjected to a solid-liquid separation, such as filtration to remove residual protein and other impurities. For example, the fish oil may be subjected to a first filtration to remove crude material followed by a finer filtration step to remove fines.

In an embodiment of the invention the processing of fish material to fish oil will result in phospholipids with reduced contents of unwanted contaminants. For example, the phospholipids will comply with standards of the European Union regarding concentrations of contaminants.

In a specific embodiment, an integrated process is set up as a continuous process, in which about 10 tonnes/hour of fish material is extracted with about 15 tonnes/hour of isohexane as explained above. Removal of the isohexane yields about 1.5 tonnes/hour of fish oil from which phospholipids are isolated according to the invention. Thus, the process is evidently scaleable to a large industrial scale.

The invention will now be explained in the following non-limiting examples. As will be evident to the skilled person variations are possible without deviating from the invention.

Comparative example

A batch of fish oil was prepared from sprat according to a prior art technique. The composition of the fish oil thus prepared is summarised in Table 1.

Table 1 Fatty acid composition of fish oil prepared according to the prior art.

Fatty acid	Danish sprat
	%
C14:0	6.4
C15:0	0.8
C16:0	18.9
C16:1	5.7
C18:0	3.1
C18:1	18.5

Fatty acid	Danish sprat
C18:2	2.2
C18:3	1.7
C18:4n3	<0.01
C20:1	6.8
C20:4n6	0.5
C20:5n3 (EPA)	8.9
C22:1	6.9
C22:5n3 (DPA)	0.9
C22:6n3 (DHA)	13.2

Example 1

A batch of 500 tonnes of fish meal was treated in a continuous plant according to the invention. The raw material fish meal was extracted with isohexane as an extractant solvent following initial pelletisation. After evaporation of the isohexane the fish oil was extracted with water as a polar solvent before centrifugation in a disk stack centrifuge. Isohexane removed from the fish oil was recycled in the process. The phospholipids were finally isolated from the polar fraction by drying to remove the water. The parameter values employed in the process are summarised in Table 2 below.

Table 2 Process parameters for phospholipid preparation

Unit operation	Reaction conditions	Product
Pelletisation	50°C	
Extraction with isohexane	2 hours 52°C	
Sieving to remove dry matter		
Isohexane removal (evaporation)	10 mbar 85°C	60 tonnes of fish oil with phospholipids
Filtering and polishing		
Mixing with water at a water:fish oil ratio of	50°C	

Unit operation	Reaction conditions	Product
15:85		
Extraction under agitation	20 minutes 60°C	
Centrifugation in a disk stack centrifuge		Polar fraction with phospholipids; Lipid fraction of phospholipid depleted fish oil
Phospholipid isolation (water removal to 1% moisture)	2 hours 5 mbar 85°C	10 tonnes of product containing 40% phospholipids and 60% fish oil with 26% EPA+DHA

The dry matter occurring after the solid-liquid separation steps represented protein products of the invention, and the lipid fraction from the centrifugation represented a phospholipid depleted fish oil product of the invention. The polar fraction with phospholipids and the product obtained from this fraction after water removal represented different embodiments of the phospholipid product obtainable in the process of the invention. The composition of the fish oil provided by the extraction is compared to the composition of the final product in Table 3 and Table 4 below.

Table 3 Fatty acid composition of fish oil prepared according to an embodiment of the invention

Fatty acid	Extracted fish oil	Final product
	%	%
C14:0	5.5	4.2
C15:0	0.5	0.5
C16:0	16.8	18.8
C16:1	10.2	6.6
C18:0	3.1	4.9
C18:1	9.7	10.9
C18:2n6	2.1	2.0
C18:3n6	0.5	0.2
C18:3n3	1.1	0.9

Fatty acid	Extracted fish oil	Final product
C18:4n3	2.9	1.8
C20:1	3.4	1.5
C20:4n6	0.7	1.0
C20:5n3 (EPA)	12.3	13.5
C22:1	0.2	1.4
C22:5n3 (DPA)	0.8	1.3
C22:6n3 (DHA)	15.4	19.3
C24:1	0.8	0.1

Table 4 Phospholipid composition of fish oil prepared according to an embodiment of the invention

Phospholipids	Extracted fish oil	Final product
Phosphatidylcholine	6.3	16.1
Lyso-phosphatidylcholine	1.2	5.4
Phosphatidylinositol	0.7	1.8
Spingomyelin	1.6	3.5
Phosphathidylethanolamin	1.8	4.5
Lyso-phosphathidylethanolamin	0.5	1.4
Acylphosphatidylethanolamine	2.1	6.3
Phosphatic acid	0.3	0.9
Lyso-phosphatic acid	0.1	0.2
Total phospholipids	16.6	44.3

It is evident from Table 3 and Table 4 that the process of the invention provided a product enriched in phospholipids, and that the process of the invention further provided a product enriched in PUFA's compared to the process of the prior art.

Example 2

Fish were heated up to 85°C and pressed to provide a presscake, which was subjected to continuous ethanol (96% ethanol in water) extraction for two hours at 65°C. The extracted presscake was subjected to solid-liquid separation to separate a crude oil containing ethanol from the extracted presscake. Ethanol was evaporated at 85°C under vacuum to provide an

ethanol-free fish oil, which was filtered to remove debris from the fish oil. The fish oil was then extracted with water as a polar solvent at 80°C for 20 minutes followed by treatment in a disk stack centrifuge at 70°C. The polar fraction from the centrifugation was mixed with fish oil and water at a ratio of 48% polar fraction to 50% fish oil and 2% water, and the mixture was extracted at 80°C for 20 minutes. The extracted mixture was then centrifuged in a disk stack centrifuge at 70°C before removal of the water by drying at 85°C under vacuum. This yielded a product enriched in phospholipids and PUFA's. The composition of the fish oil provided by the ethanol extraction is compared to the composition of the final product in Table 5 and Table 6 below.

Table 5 Fatty acid composition of fish oil prepared according to an embodiment of the invention

Fatty acid	**Ethanol extracted fish oil**	**Final product**
	%	%
C14:0	1.9	1.6
C15:0	0.2	0.5
C16:0	22.7	18.8
C16:1	3.2	4.5
C18:0	4.8	4.9
C18:1	12.6	10.9
C18:2n6	0.6	1.5
C18:3	0.4	0.2
C18:4n3	0.6	1.8
C20:1	1.6	1.5
C20:4n6	0.8	1.0
C20:5n3 (EPA)	9.7	10.5
C22:1	1.8	1.4
C22:6n3 (DHA)	19.7	24.3

Table 6 Phospholipid composition of fish oil prepared according to an embodiment of the invention

Phospholipids	Ethanol extracted fish oil	Final product
Phosphatidylcholine	9.5	24.2
Lyso-phosphatidylcholine	1.3	3.3
Phosphatidylinositol	0.9	2.3
Spingomyelin	0.9	2.3
Phosphathidylethanolamin	1.4	3.6
Lyso-phosphathidylethanolamin	0.3	0.8
Acylphosphatidylethanolamine	0.8	2.1
Phosphatic acid	0.1	0.3
Lyso-phosphatic acid	0.1	0.3
Total phospholipids	15.6	>40

It is evident from Table 5 and Table 6 that the process of the invention provided a product enriched in phospholipids, and that the process of the invention further provided a product enriched in PUFA's compared to the process of the prior art.

PATENT CLAIMS

1. A process for the isolation of a phospholipid from a fish oil comprising the steps of:

-providing a fish oil containing lipids and phospholipids;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a phospholipid from the polar fraction.

2. A process for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of:

-providing a fish oil containing PUFA's;

-mixing the fish oil with a polar solvent;

-centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction;

-isolating a PUFA-enriched fraction from the polar fraction.

3. The process according any one of claims 1 to 2, wherein the step of providing the fish oil comprises:

-extracting a fish material with an extractant solvent;

-removing the extractant solvent to provide the fish oil;

-optionally subjecting the fish oil to a solid-liquid separation.

4. The process according to any one of claims 1 to 3, wherein the ratio of polar solvent to fish oil is about 5:95 to about 25:75.

5. The process according to any one of claims 1 to 4 further comprising the steps of:

-mixing the polar fraction with the polar solvent and fish oil;

-separating the mixture of the polar fraction, the polar solvent and the fish oil into a concentrated polar fraction and a lipid fraction.

6. The process according to claim 5, wherein the step of separating comprises centrifuging the mixture of the polar fraction, the polar solvent and the fish oil to separate a concentrated polar fraction from a lipid fraction.

7. The process according to claim 5 or 6, wherein mixture comprises up to about 5% polar solvent; about 25% to about 75% fish oil and polar fraction to balance.

8. The process according to any one of claims 1 to 7, wherein the polar solvent is water.

9. The process according to any one of claims 1 to 8, wherein no additive, which may hydrolyse a phospholipid, is added in the process, such as those selected from the group consisting of acids, e.g. phosphoric acid, organic acids, e.g. citric acid, acid anhydrides, hydrogen peroxide, and enzymes, e.g. lipases and phospholipases.

10. The process according to any one of claims 1 and 3 to 9, wherein intact phospholipids are isolated.

11. The process according to any one of claims 2 to 9, wherein the PUFA's are eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).

12. The process according to any one of claims 1 to 11 further comprising analysing the polar fraction or the concentrated polar fraction for the presence of an excess of polar solvent.

13. The process according to any one of claims 1 to 12 further comprising the step of centrifuging the polar fraction or the concentrated polar fraction to concentrate the phospholipids and/or the PUFA's.

14. The process according to any one of claims 1 to 13, wherein the fish material is derived from fish meal production, such as a fish meal or a presscake.

15. The process according to any one of claims 1 to 14, wherein the fish material is derived from sand eel (*Hyperoplus* sp., *Gymnammodytes* sp. or *Ammodytes* sp., e.g. *Hyperoplus lanceolatus*), sprat (*Sprattus sprattus*), herring (*Clupea* sp., e.g. *Clupea harengus*), anchovy (*Engraulis* sp., e.g. *Engraulis ringens)*, boarfish (*Capros aper)*, Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*Micromesistius poutassou*), or Jack Mackerel (*Trachurus murphyi*).

16. The process according to any one of claims 1 to 14, wherein the fish material is derived from krill, shrimps, crabs, lobsters, mantis shrimp, woodlice, sandhoppers.

17. The process according to any one of claims 1 to 16, wherein the fish material is derived from fish which has not been subjected to heat treatment.

18. The process according to any one of claims 1 to 17, wherein the step of mixing the fish oil or the mixture of the polar fraction and the fish oil with the polar solvent is performed at an increased temperature.

19. The process according to any one of claims 1 to 18, wherein the step of mixing the fish oil or the mixture of the polar fraction and the fish oil

with the polar solvent is performed at a temperature of about 5°C to about 40°C.

20. The process according to any one of claims 1 to 19, wherein the isolation of the phospholipid from the polar fraction or the concentrated polar fraction comprises vacuum distillation of the polar fraction to remove the polar solvent.

21. The process according to any one of claims 1 to 20, wherein the centrifugation is performed in a disk stack centrifuge.

22. The process according to any one of claims 3 to 21, wherein the extractant solvent is an apolar solvent, e.g. hexane.

23. The process according to any one of claims 3 to 21, wherein the extractant solvent is ethanol or a mixture of ethanol and water.

24. The process according to any one of claims 3 to 21, wherein the extractant solvent is 96% ethanol, and the ratio of ethanol to fish material is from about 1:2 to about 1:5, preferably about 1:3.

25. The process according to claim 23, wherein the temperature is 65°C.

26. The process according to any one of claims 3 to 23, wherein the extraction with the extractant solvent is performed at an increased temperature.

27. The process according to any one of claims 3 to 23, wherein the extraction with the extractant solvent is performed at a temperature of about 5°C to about 40°C.

28. The process according to any one of claims 1 to 27, wherein the process is performed under continuous operation.

29. An integrated continuous process for producing a phospholipid product or a PUFA-product from a fish material comprising treating a fish material according to the process of any one of claims 3 to 28, wherein a process stream is recycled in an earlier process step.

30. An integrated continuous process according to claim 29 further comprising analysing the polar fraction or the concentrated polar fraction for the presence of an excess of polar solvent and controlling the amount polar solvent added to the fish oil or the mixture of polar fraction and fish oil based on the result of the analysis.

31. A phospholipid product obtainable in the process according to any one of claims 1 to 30.

32. A PUFA-product obtainable in the process according to any one of claims 2 to 30.

33. A phospholipid-depleted and/or PUFA-depleted fish oil product obtainable in the process according to any one of claims 1 to 30.

34. A protein product obtainable from the extractant solvent-extracted fish material of the process according to any one of claims 1 to 30.

35. A protein product obtainable from the polar solvent-extracted fish material of the process according to any one of claims 1 to 30.

36. An extracted fish material obtainable in the process according to any one of claims 1 to 30.

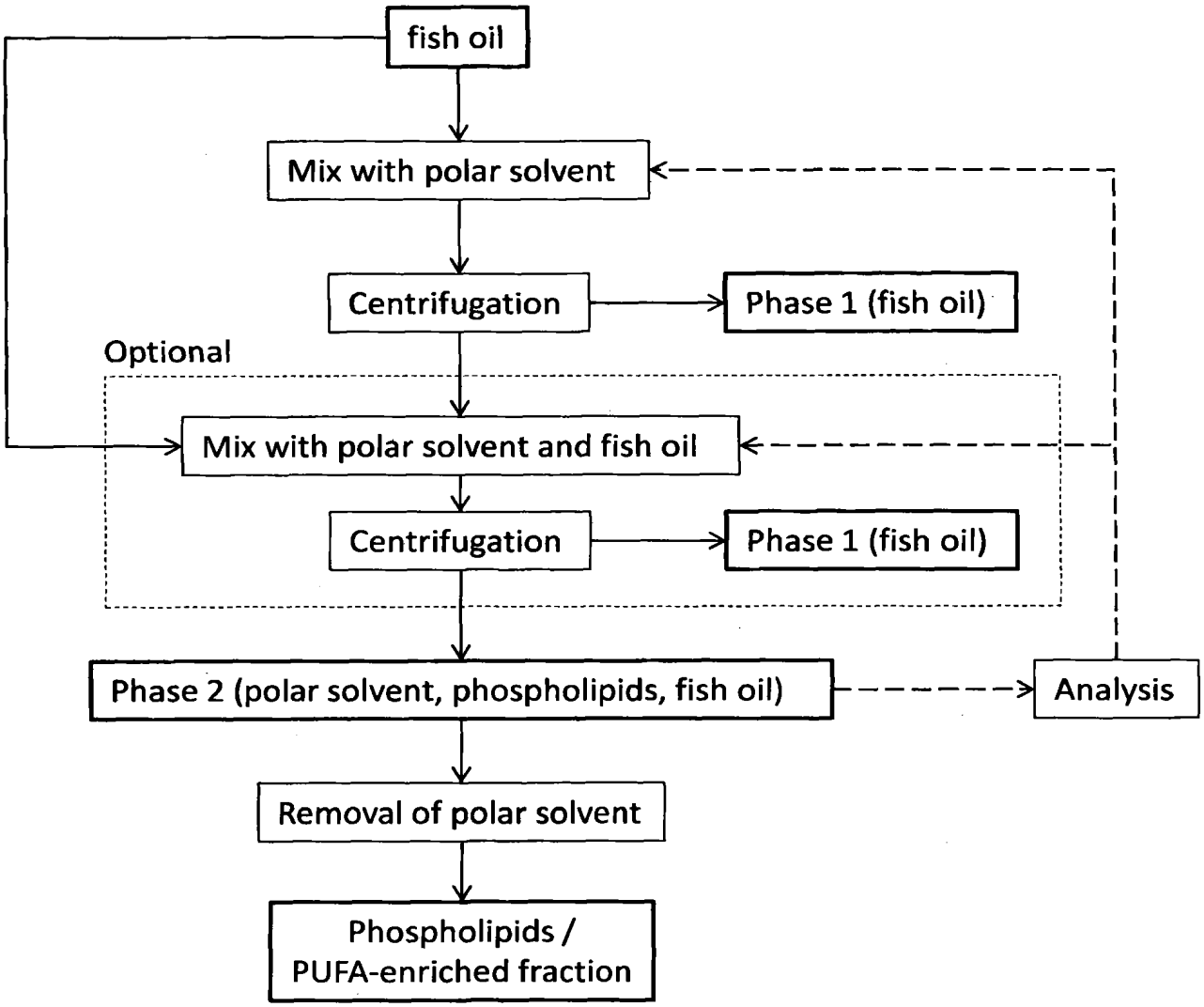

Fig. 1

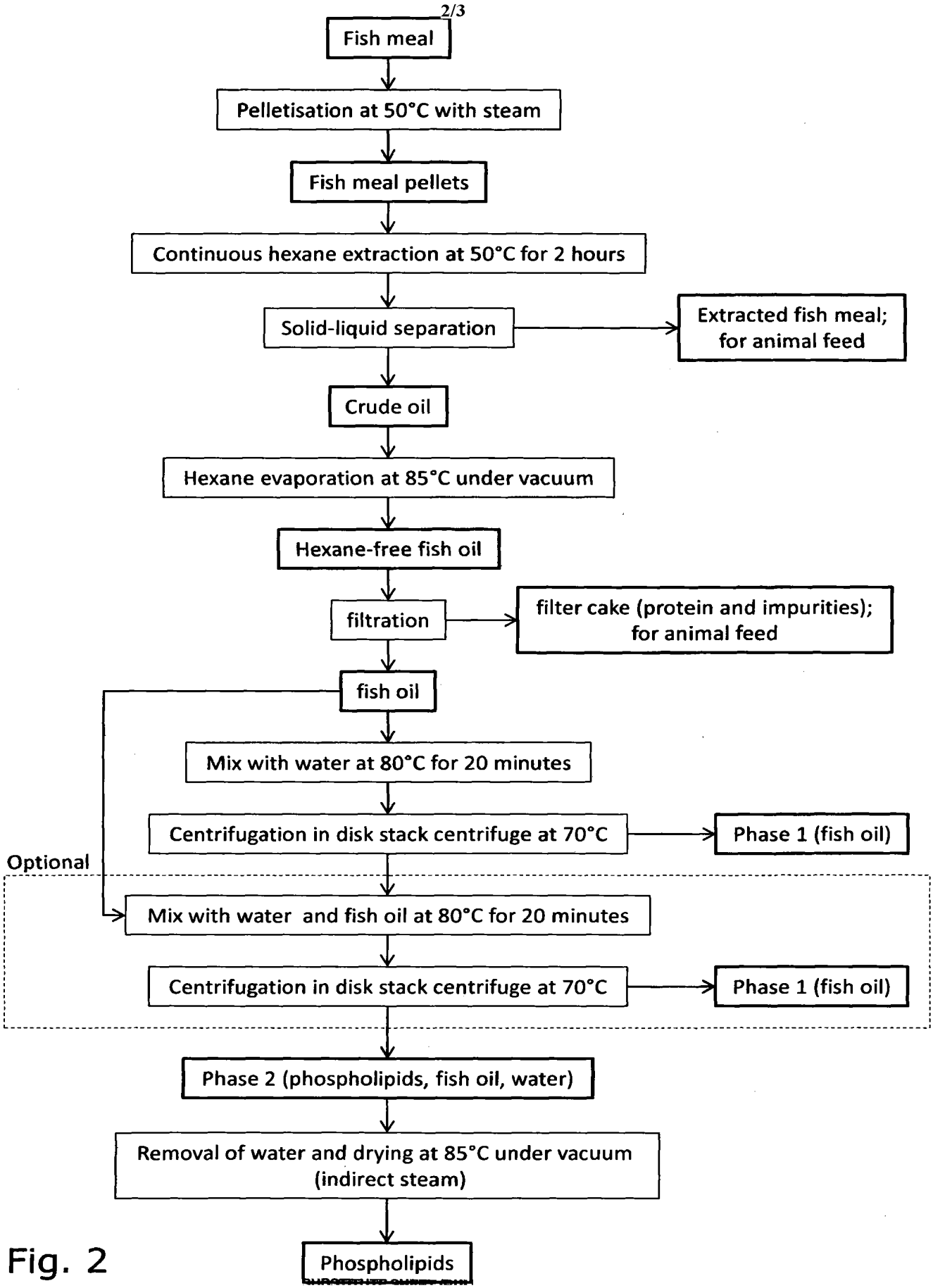

Fig. 2

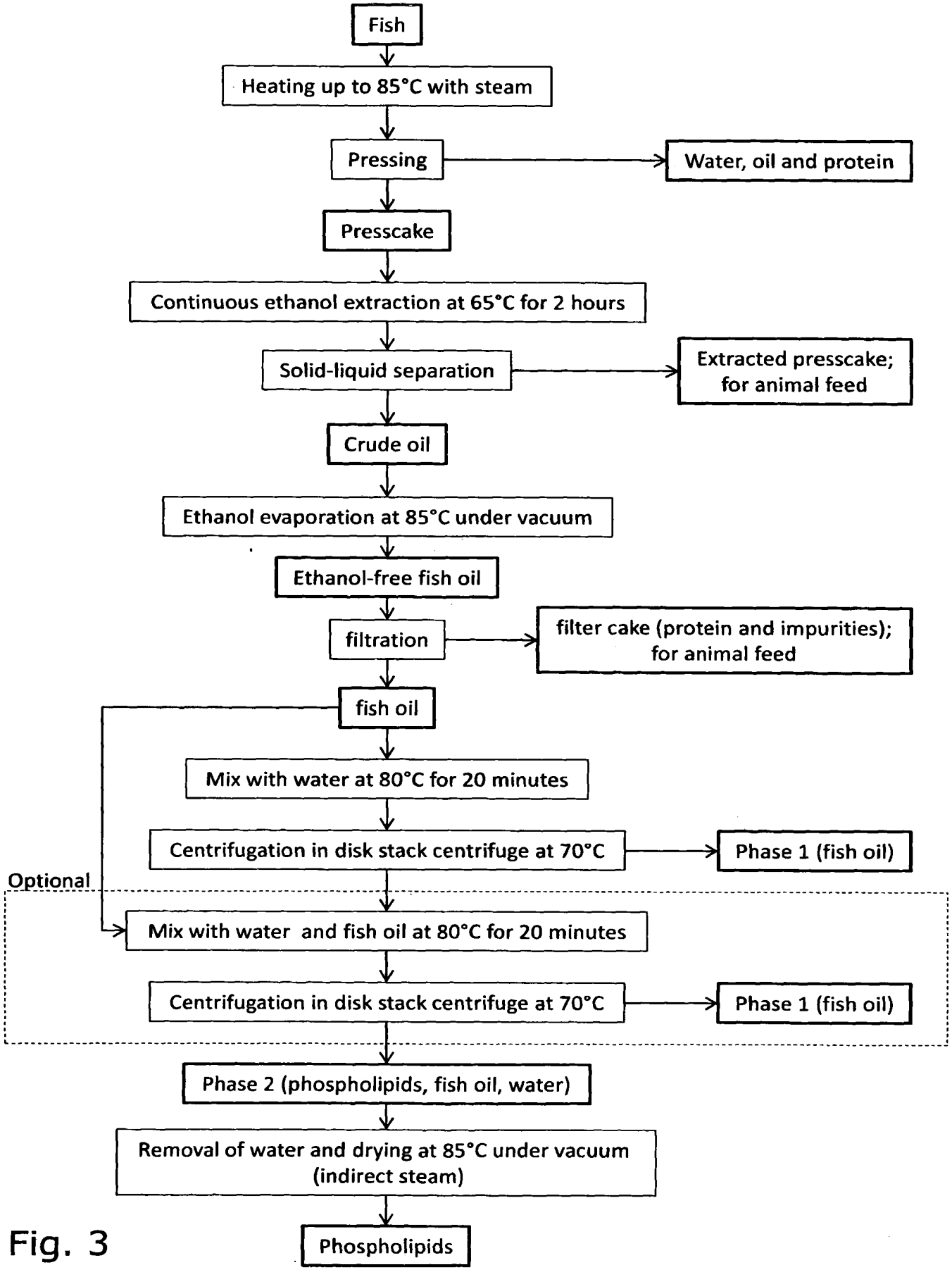

Fig. 3

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[Continued on next page]

(54) Title: A PROCESS FOR THE ISOLATION OF A PHOSPHOLIPID

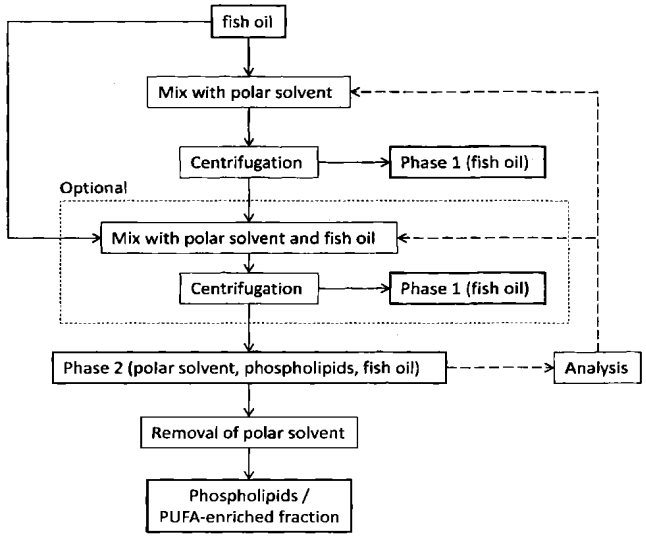

Fig. 1

(57) Abstract: The present invention relates to processes for the isolation of a phospholipid and for producing a polyunsaturated, long-chain fatty acids (PUFA)-enriched fraction from a fish oil comprising the steps of -providing a fish oil containing lipids and phospholipids; -mixing the fish oil with a polar solvent; -centrifuging the mixture of the fish oil and the polar solvent to separate a polar fraction from a lipid fraction; -isolating a phospholipid from the polar fraction or isolating a PUFA-enriched fraction from the polar fraction. The fish oil may be provided by -extracting a fish material with an extractant solvent; -removing the extractant solvent to provide the fish oil; -optionally subjecting the fish oil to a solid-liquid separation. The isolated phospholipids and PUFA's may be used as additives for functional foods, as a dietary supplement and for pharmaceutical application.

WO 2012/139588 A3

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
21 March 2013

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050124

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11B1/10 C11B3/00 A23D9/00 A23D9/04 A23J1/04
A23J7/00 A23L1/326 A23L1/30
ADD.
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)
C11B A23D A23J A23L

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)
EPO-Internal, BIOSIS, COMPENDEX, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 462 054 A (STATOILHYDRO ASA [NO]) 27 January 2010 (2010-01-27) page 13, line 27 - page 14, line 25; example 1	1-36
X	US 2005/129739 A1 (KOHN GERHARD [DE] ET AL) 16 June 2005 (2005-06-16) cited in the application paragraph [0037] - paragraph [0041]	1-36
X	GB 414 717 A (ALPHONSO THOMAS ARCHIBALD DOUG) 10 August 1934 (1934-08-10) claims; examples	34,35
X	US 4 584 141 A (PAULITZ BERNHARD G A [DE] ET AL) 22 April 1986 (1986-04-22) cited in the application claims 1,2,5,9,14,15,18,25; examples	1-36

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier 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
18 January 2013	25/01/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Saettel, Damien

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050124

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 172 247 B1 (COPELAND DICK [US] ET AL) 9 January 2001 (2001-01-09) cited in the application column 14, line 25 - line 56; figure 2	1-36
X	US 2006/110521 A1 (HEISE JERALD D [US] ET AL) 25 May 2006 (2006-05-25) cited in the application paragraph [0085] - paragraph [0088]	1-36
X	EP 0 269 277 A2 (CAMBRIAN ENG GROUP LTD [CA]) 1 June 1988 (1988-06-01) cited in the application page 3, line 34 - line 41; claims 1,8,11,12,20,22	1-36

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2012/050124

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2462054 A	27-01-2010	NONE	
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		AU 2009200194 A1	12-02-2009
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2012/050124

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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Form PCT/ISA/210 (patent family annex) (April 2005)

Electronic Patent Application Fee Transmittal

Application Number:	14020162
Filing Date:	06-Sep-2013
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Filer:	John Mitchell Jones/Mallory Checkett
Attorney Docket Number:	AKBM-14409/US-6/CON

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Claims in Excess of 20	1202	41	80	3280
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**4860**

Electronic Acknowledgement Receipt

EFS ID:	24566530
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:	08-JAN-2016
Filing Date:	06-SEP-2013
Time Stamp:	11:29:25
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$4860
RAM confirmation Number	8672
Deposit Account	504302
Authorized User	JONES, J. MITCHELL

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		14409US6CON_ROA_1-8-2016.pdf	132727 c479956c56c8a399bad1602da2fe6a5ec22a0ddb	yes	11
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	9	
	Applicant Arguments/Remarks Made in an Amendment		10	11	
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Information:					
2	Extension of Time	14409US6CON_Petition_EOT.pdf	163378 33ddc3acfff5b5e722a0286cce6710fd2e483c17	no	2
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Information:					
3	Information Disclosure Statement (IDS) Form (SB08)	14409US6CON_Suppl_IDS_1-8-2016.pdf	1036311 06a99eaf9b74c1a1c61612016af88504dcf4da95	no	5
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Information:					
4	Foreign Reference	WO0176385.pdf	749830 8cb76632f64d7bd81126af3c2c4601868ec08442	no	19
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Information:					
5	Foreign Reference	JP_S6323819_IncludesEngAbstract.pdf	3486089 e63b6d5ac1adb9ca1a6c9094f478da876c65f5b2	no	5
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Information:					
6	Foreign Reference	WO2012139588.pdf	1808091 c3d7918a78bdb27748105a38624346860ce5b4a1	no	43

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7	Non Patent Literature	KOLAKOWSKI_GAJOWIECKI_SeafoodScienceTechnology.pdf	2185274 3232078a0da026c2c4617ff95ef4f5493cca589c	no	8
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8	Non Patent Literature	Neptune_NOWFOODS_2011.pdf	176391 5a6b0c8e37ebd66e2cedec00e69de0232d168b9c	no	3
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9	Non Patent Literature	GIGLIOTTI_2010.pdf	831650 f114157b4dc3d5d5f3407e833ac6660decbc3aa5	no	9
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10	Non Patent Literature	ALI-NEHARI_2012.pdf	622325 85ef0de39ca2cff5c0231e487de1bf78efb910e8	no	7
Warnings:					
Information:					
11	Other Reference-Patent/App/Search documents	InterationalSearchReport_33382_PCTIB2014002130.pdf	5718367 5d8e5a0ebc981a1a7c07f2b3da0c1536d121fc76	no	107
Warnings:					
Information:					
12	Other Reference-Patent/App/Search documents	EP_OppositionFiled_2144618_05-08-2015_part1_uspto.pdf	25985298 75d002e15a0d7db3f9c20b695b21320aeb827d10	no	75
Warnings:					
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13	Other Reference-Patent/App/Search documents	EP_OppositionFiled__2144618_05-08-2015_part2.pdf	10543573 800454fe85fde9bf60bb04ab90b22d7a7755320a	no	76
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Warnings:	
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National Stage of an International Application under 35 U.S.C. 371
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Electronic Petition Request	**TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION AND TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT**
Application Number	14020162
Filing Date	06-Sep-2013
First Named Inventor	Inge Bruheim
Attorney Docket Number	AKBM-14409/US-6/CON
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

Owner	Percent Interest
Aker BioMarine Antarctic AS	100 %

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

14490204 filed on 09/18/2014

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

The owner(s) with percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

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9119864

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:
- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

(●) Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.

( ) I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.

Applicants claims the following fee status:

( ) Small Entity

( ) Micro Entity

(●) Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

(●) An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application

Registration Number 44174

( ) A sole inventor

( ) A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application

( ) A joint inventor; all of whom are signing this request

Signature	/J. Mitchell Jones/

Name	J. Mitchell Jones

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal

Application Number:	14020162
Filing Date:	06-Sep-2013
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Filer:	John Mitchell Jones/Mallory Checkett
Attorney Docket Number:	AKBM-14409/US-6/CON

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Statutory or Terminal Disclaimer	1814	1	160	160
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**160**

Doc Code: DISQ.E.FILE
Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 14020162

Filing Date: 06-Sep-2013

Applicant/Patent under Reexamination: Bruheim et al.

Electronic Terminal Disclaimer filed on January 8, 2016

☒ APPROVED

This patent is subject to a terminal disclaimer

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

Electronic Acknowledgement Receipt

EFS ID:	24566449
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:	08-JAN-2016
Filing Date:	06-SEP-2013
Time Stamp:	10:37:32
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$160
RAM confirmation Number	8682
Deposit Account	504302
Authorized User	JONES, J. MITCHELL

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Electronic Terminal Disclaimer-Filed	eTerminal-Disclaimer.pdf	36998 a17be2a9257fb9c678e841ac0c3dcc3a33f0f17c	no	3
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30477 e8840ce52341fe1b7d4e765ddd6e66c7a6ecbc4c	no	2
Warnings:					
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		Total Files Size (in bytes):	67475		

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

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

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

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/020,162	Filing Date 09/06/2013	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	01/08/2016	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 61	Minus	** 20	= 41	X $80 =	3280
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**3280**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** 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 number found in the appropriate box in column 1.

LIE
/KATRINA . TURNER/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/020,162	09/06/2013	Inge Bruheim	AKBM-14409/US-6/CON	4914

72960 7590 07/08/2015

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

EXAMINER
WARE, DEBORAH K

ART UNIT	PAPER NUMBER
1651	

NOTIFICATION DATE	DELIVERY MODE
07/08/2015	ELECTRONIC

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

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

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

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Office Action Summary	Application No. 14/020,162	Applicant(s) BRUHEIM ET AL.	
	Examiner DEBBIE K. WARE	Art Unit 1651	AIA (First Inventor to File) Status No

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

Period for Reply

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

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

Status

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

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

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

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

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

Disposition of Claims*

5)☒ Claim(s) *1-11* is/are pending in the application.

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

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-11* is/are rejected.

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

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

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

Application Papers

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

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

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

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

Priority under 35 U.S.C. § 119

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

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

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

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

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

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

Attachment(s)

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

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

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

4) ☐ Other: _____.

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

DETAILED ACTION

Claims 1-11 are presented for consideration on the merits.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted were received. The submission are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Claim Rejections - 35 USC § 112

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

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

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-11 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claims 1-11 are rendered grammatically indefinite for failing to recite --and-- after “phospholipids;” at line 4, of claims 1 and 11. In addition, in claims 1 and 11, lack

antecedent basis for “polar krill oil” in their respective preambles. lacks clear antecedent basis for “a polar krill oil” as recited in bridging lines 3-4 of claim 1 and line 4 of claim 15, wherein in step c) of both claims 1 and 11, it is the "said polar krill oil" formulated into oral consumption. This methods are directed to a method of production of polar krill oil and not a krill oil, per se which can be non-polar. Thus, it is suggested to insert the term --polar-- at line 1 of claim 1 and of claim 11, before recitation of "krill oil”. Furthermore, claims 2 and 11, lack antecedent basis for recitation of “said krill oil” at line 1, for reasons noted above for claim 1. Claims 2-10 are rejected under this statute as well as for being dependent upon a rejected base claim; so these claims also contain the same issue since they are dependent claims. Thus, when claim 1 is remedied and the rejection removed then the rejection of dependent claims will be removed as well.

Also, recitations of "w/w" at line 2 of each of claims 3-5, are rejected because it is uncertain what the units are intended to be. Weight by weight percent of what? Basically weight by weight of what per se, the polar krill oil, the krill oil or the krill? The metes and bounds of these claims cannot, therefore, be determined. It is suggested to insert –percent of total amount of the polar krill oil -- after each recitation of "w/w" in the claims wherein "w/w" is recited. These are suggestions by the Examiner.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double

patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-11 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 2, 3, and 6-7 of copending Application No. 14/490,204. Although the claims at issue are not identical, they are not patentably distinct from each other because the only difference between the instant claims and copending claims is a matter of scope of the claimed subject matter.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims of instant case are drawn to method of production of krill oil from krill comprising obtaining a krill oil and extracting the oil using supercritical fluid extraction. The krill is Euphausia superba. The krill oil comprises astaxanthin and the krill oil can be encapsulated.

Copending claims are drawn to method of extracting krill oil from krill comprising treating krill and extracting the oil using supercritical fluid extraction. The krill is Euphausia superba. The krill oil comprises astaxanthin and the krill oil can be encapsulated.

The claims differ from copending claims in that there are steps required by copending claims not required by instant claims.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to produce krill oil from krill as claimed herein comprising obtaining a krill oil and use of supercritical fluid extraction to provide krill oil and then to encapsulate for oral consumption. One of skill based on a reading of the copending claims would have been motivated to provide for the instant claimed method for

production of krill oil because each of the steps as instantly claimed are taught by the copending subject matter. Clearly encapsulating krill oil as taught by copending claimed process, claim 7, suggest formulating krill oil for oral consumption as instantly claimed . The instant claims are, therefore, considered to be prima facie obvious over the copending claimed subject matter.

Claims 1-11 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of copending Application No. 14/620,779. Although the claims at issue are not identical, they are not patentably distinct from each other because the only difference between the instant claims and copending claims is a matter of scope of the claimed subject matter.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims of instant case are drawn to methods of production of krill oil from krill. The krill is Euphausia superba. The krill oil comprises astaxanthin and the krill oil can be encapsulated. The steps of instant claim include denaturing wherease the step of claim 1 may include it but broadly recites obtaining krill oil. Both methods recite supercritical extraction, however, and include formulating step of krill oil for oral consumption. Encapsulation is also claimed.

Copending claims recite the same but clearly directed and recite denaturing step and include supercritical extraction and formulating step for oral consumption. Additionally, encapsulation can be performed.

The claims of instant case differ in terms of scope from copending claims 1-10.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to produce krill oil from krill as claimed herein comprising obtaining a krill oil, and include denaturing as required by instant claims 1-11, and use of supercritical fluid extraction to provide krill oil and then to encapsulate for oral consumption. One of skill based on a reading of the copending claims would have been motivated to provide for the instant claimed method for production of krill oil because each of the steps as instantly claimed are taught by the copending subject matter. Obtaining step can clearly include denaturing as the step is required by instant claim 15 and required by copending claims. Clearly encapsulating krill oil as taught by copending claimed process, claim 7, suggest formulating krill oil for oral consumption as instantly claimed . To obtain krill oil by denaturing the krill to provide a denatured krill product is clearly suggested by the copending claims. The instant claims are, therefore, considered to be prima facie obvious over the copending claimed subject matter.

Claims 1-11 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of U.S. Patent No. 9,034,388. Although the claims at issue are not identical, they are not patentably distinct from each other because the only difference between the instant claims and copending claims is a matter of scope of the claimed subject matter.

Claims of instant case are drawn to methods of production of krill oil from krill. The krill is Euphausia superba. The krill oil comprises astaxanthin and the krill oil can be encapsulated. The steps of instant claim include denaturing whereas the step of claim 1 may include it but broadly recites obtaining krill oil. Both methods recite

supercritical extraction, however, and include formulating step of krill oil for oral consumption. Encapsulation is also claimed.

Patent claims teach methods of production of krill oil which include steps of extraction (e.g. supercritical extraction) and denaturing all from krill and products thereof. Astaxanthin is comprised by the krill oil as well.

The claims of instant case differ in terms of scope from patent claims.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to produce krill oil from krill as claimed herein comprising obtaining a krill oil, and include denaturing, and use of supercritical fluid extraction to provide krill oil and then to encapsulate for oral consumption. One of skill based on a reading of the copending claims would have been motivated to provide for the instant claimed method for production of krill oil because each of the steps as instantly claimed are taught by the copending subject matter. Obtaining step can clearly include denaturing and treating as the steps are required by copending claims. Clearly encapsulating krill oil suggests formulating krill oil for oral consumption as instantly claimed . To obtain krill oil by denaturing the krill to provide a denatured krill product is clearly suggested by the copending claims. The instant claims are, therefore, considered to be prima facie obvious over the copending claimed subject matter.

Claims 1-11 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-9, 11, and 13-18 of U.S. Patent No. 9,028,877. Although the claims at issue are not identical, they are not patentably distinct from each other

because the only difference between the instant claims and copending claims is a matter of scope of the claimed subject matter.

Claims of instant case are drawn to methods of production of krill oil from krill. The krill is Euphausia superba. The krill oil comprises astaxanthin and the krill oil can be encapsulated. The steps of instant claim include denaturing whereas the step of claim 1 may include it but broadly recites obtaining krill oil. Both methods recite supercritical extraction, however, and include formulating step of krill oil for oral consumption. Encapsulation is also claimed.

Patent claims teach methods of production of krill oil which include steps of extraction (e.g. supercritical extraction) and denaturing all from krill and products thereof.

The claims of instant case differ in terms of scope from patent claims.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to produce krill oil from krill as claimed herein comprising obtaining a krill oil, and include denaturing, and use of supercritical fluid extraction to provide krill oil and then to encapsulate for oral consumption. One of skill based on a reading of the copending claims would have been motivated to provide for the instant claimed method for production of krill oil because each of the steps as instantly claimed are taught by the copending subject matter. Obtaining step can clearly include denaturing and treating as the steps are required by copending claims. Clearly encapsulating krill oil suggests formulating krill oil for oral consumption as instantly claimed . To obtain krill oil by denaturing the krill to provide a denatured krill product is

clearly suggested by the copending claims. The instant claims are, therefore, considered to be prima facie obvious over the copending claimed subject matter.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-11 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Patent Abstract of Japan 04-057853, dated Feb. 25, 1992, cited on enclosed PTO-1449 Form.

Claims drawn to method for producing oil and an oil produced thereby.

Abstract 04-057853 teaches method for extracting krill oil comprising a)providing krill meal; and extracting oil from the krill meal (powdered form of krill parts). The meal (powdered form of krill parts) can be provided from heat-treated (e.g. denaturing step) krill parts and is storable. The extracting is carried out by supercritical extraction. An oil is produced by the method.

The claims are identical to the abstract as discussed above and are considered to be clearly anticipated by the teachings therein. Krill shells are part of krill and oil is obtained from the krill parts. The krill parts are dried and hence subjected to heating to

provide for the krill meal which is subjected to supercritical extraction in two steps to obtain the oil.

Claim Rejections - 35 USC § 103

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

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

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1-11 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over JP4057853 (pages 1-7) (Tisueno et al) in view of USP 4814111 (Kearns et al),

USP US 2006/0078625 (Rockway) all these cited on enclosed PTO-1449 Form/829 Form(s), and USP 8,057,825 (Sampalis), cited on enclosed PTO-892 Form.

Claims are drawn to methods of production of krill oil from krill comprising obtaining krill oil (e.g. denaturing Euphausia superba krill to provided denatured krill product); extracting krill oil or denatured krill product with supercritical fluid extraction to provide krill oil comprising phospholipids and formulation of the same for oral consumption (e.g. capsule).

Tisueno et al teach method of production of krill oil from krill comprising a) obtaining a krill oil; and b) extracting krill oil with supercritical fluid extraction (e.g. using carbon dioxide-reading on instant claims 1-2) to provide krill oil for oral consumption. Triglycerides (reading on instant claims 1 and 5) are disclosed to be contained in the krill oil as well as astaxanthin (reading on instant claim 1 and claim 7) See pages 2 at col. 2, lines 1-4 and 12-26; and 3 at col. 1, last two lines and col. 2, lines 1-2 and lines 8-23. Denaturing (reading on step a) of claim 1 and 11 of instant case) is disclosed at page 2, col. 1, last paragraph under "Problems the Invention is to Solve" it is discussed that krill organisms are subjected to solvent and alkali which provides for a krill product, krill shells, for example. Also, see col. 2, lines 1-20.

Kearns et al teach process of purification of phospholipids using supercritical carbon dioxide fluid extraction, wherein this technique is disclosed to be one which avoids toxicity and flammability problems associated with many organic solvents. Note abstract and col. 3, lines 10-15. This teaching corresponds to step b) and phospholipids contained by krill oil of all of the instant claims (claims 1-11).

Rockway teaches krill extracts and krill oil that comprise EPA, DHA and phosphatidylcholine phospholipids, etc. See page 2, [0018], all lines and abstract. Note also, page 3, col. 1, all lines, wherein omega-3 fatty acids and phospholipids are disclosed to be contained in varied amounts in krill oil. This teaching corresponds to instant claims 6.

Sampalis teaches krill extracts krill oil, see abstract. Furthermore, phosphatidylcholine and varied ether phospholipids and triglycerides and omega-3 fatty acids are all disclosed to be comprised by krill oil. See col. 3, lines 1-50. The krill oil is disclosed to be formulated in capsule form for oral consumption by a human, see col. 4, lines 20-47. The krill can be selected from species Euphausia superba, see col.2 lines 52-53. This teaching corresponds to instant claims 1-11, and specifically claims 3-4 and 9-11.

The claims differ from Tisueno et al in that the formulating step c) of the krill oil in a capsule form and the specific phospholipids are not clearly disclosed.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to include a formulating step to provide for the krill oil of Tisueno et al in a capsule form as disclosed by Sampalis and to expect for the krill oil to contain varied percentage amounts of phospholipids, triglycerides, omega-3 fatty acids and astaxanthin since the secondary prior art teaches these components to be present in krill oil. Use of supercritical carbon dioxide fluid extraction would have been clearly expected to provide effective amounts of phospholipids in krill oil because Kearns et al clearly disclose the technique to be a useful process for phospholipid purification.

The steps are well known by those of ordinary skill in the art to provide successful results for production of krill oil from krill as disclosed by the cited prior art. Each of the dependent claims are encompassed and disclosed by the cited prior art as a whole and one of skill would have expected successful results for formulating the krill oil in a capsule for oral consumption by a human. The claims are, therefore, rendered prima facie obvious over the cited prior art. In the absence of persuasive evidence to the contrary the claims are properly rejected under 35 USC 103.

All claims fail to be patentably distinguishable over the state of the art discussed above and cited on the enclosed PTO-892 and/or PTO-1449. Therefore, the claims are properly rejected.

The remaining references listed on the enclosed PTO-892 and/or PTO-1449 are cited to further show the state of the art.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBBIE K. WARE whose telephone number is (571)272-0924. The examiner can normally be reached on 9:30-6:00.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR.

Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Deborah K. Ware/

Deborah K. Ware

Primary Examiner

Art Unit 1651

Notice of References Cited	Application/Control No. 14/020,162	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-8,057,825	11-2011	Sampalis, Tina	424/522
*	B	US-2006/0078625	04-2006	Rockway, Susie	424/538
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

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	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	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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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

	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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	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"	☐
	14	Certificate of translation of Ex. 1076: Japanese Patent Publication No. H08-231391, entitled "Medicine for Improvement of Dementia Symptoms"	☐
	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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	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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	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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	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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Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2014-01-14
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	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")	☐
	9	Lin et al., Effect of Dietary N-3 Fatty Acids Upon the PhospholipidMolecular Species of the Monkey Retina, Invest Ophthalmol Vis Sci. 1994;35:794-803	☐
	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	☐

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	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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	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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Name/Print	J. Mitchell Jones	Registration Number	44174

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

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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	2004/112767	WO		2004-12-29	Advanced Bionutrition Corp.		☐

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	1	European Search Report, EP Patent Application No. EP12187516, mailed June 10, 2013	☐

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	1	2002322233	AU		2003-02-17	Neptune Technologies & Bioressources, Inc.		☐
	2	04057853	JP		1992-02-25	CHLORINE ENG CORP LTD		☒

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	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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14020162 - GAU: 1651
Receipt date: 09/06/2013

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /D.W./

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

CERTIFICATION STATEMENT

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☒ A certification statement is not submitted herewith.

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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-			
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	Q					
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	U	
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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

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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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	Attorney Docket Number	NATNUT-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.	T[5]
	1	JP Office Action mailed February 23, 2012, JP Patent Application No. 2010-522444 (and English translation)	☐

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Examiner Signature	/Deborah Ware/	Date Considered	06/29/2015

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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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Doc code: IDS
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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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	1	5266564		1993-11-30	Modolell	
	2	8030348		2011-10-04	Sampalis, Fotni	
	3	7666447		2010-02-23	Rockway	

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	1	20080166419		2008-07-10	Sones	

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	1	2004-534800	JP		2004-11-18	Kohyo		☐

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	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	☐
	4	GORDEEV, K.Y., et al. "Fatty Acid Composition of the Main Phospholipids of the Antarctic Krill, Euphausia superba," Khim. Prirod. Soed. 2 (1990), pp. 181-187	☐

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Examiner Signature	/Deborah Ware/	Date Considered	06/29/2015

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Receipt date: 09/0

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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	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)	2012-01-24
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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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-			
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Doc code: IDS
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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, et al.
Art Unit	1651
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.		☐
	3	2002/102394	WO		2002-12-27	NEPTUNE TECHNOLOGIES & BIORESS		☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim, et al.
	Art Unit	1651
	Examiner Name	Susan Marie Hanley
	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.		☐
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	Examiner Name	Susan Marie Hanley
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	15	2004/047554	WO		2004-06-10	PHARES PHARM RES NV		☐
	16	0973532	EP		2005-09-07	I.B.R ISRAELI BIOTECHNOLOGY RESEARCH LTD.		☐
	17	1123368	EP		2008-04-09	UNIVERSITE DE SHERBROOKE		☐
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	19	2001/082928	WO		2001-11-08	HENDERSON		☐
	20	2003/013497	WO		2003-02-20	SUNTORY LIMITED		☐
	21	1419768	EP		2009-01-07	NEPTUNE TECHNOLOGIES & BIORESSOURCES, INC.		☐
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