UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,034,388 B2
APPLICATION NO. : 12/057775
DATED : May 19, 2015
INVENTOR(S) : Bruheim et al.

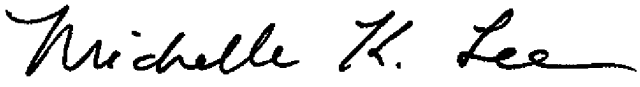
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the claims, column 38, lines 31-33 should be deleted and replaced with:

c) providing

i) dilapidated krill meal following said extraction comprising greater than 65% protein and less than 50 g/kg total fat.

Signed and Sealed this
Twelfth Day of April, 2016



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

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.
12/057,775	03/28/2008	Inge Bruheim	AKBM-14409/US-5/ORD	1945

72960 7590 03/17/2016

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
03/17/2016	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

Patent No. 9034388
Issued Date: 19 May, 2015
Appl. No: 12/057,775
Filed.: 28 March 2008

PART (A) RESPONSE FOR CERTIFICATES OF CORRECTION

This is a decision on the Certificate of Correction request filed 01 February 2016.

The request for issuance of Certificate of Correction for the above-identified correction(s) under the provisions of 37 CFR 1.322 and/or 1.323 is hereby:

(Check one)
☒ Approved ☐ Approved in Part ☐ Denied

Comments:

PART (B) PETITION UNDER 37 CFR 1.324 OR 37 CFR 1.48

This is a decision on the petition filed to correct inventorship under 37 CFR 1.324.

This is a decision on the request under 37 CFR 1.48, petition filed . In view of the fact that the patent has already issued, the request under 37 CFR 1.48 has been treated as a petition to correct inventorship under 37 CFR 1.324.

The petition is hereby: ☐ Granted ☐ Dismissed

The patented filed is being forwarded to Certificate of Corrections Branch for issuance of a certificate naming only the actual inventor or inventors.

/Renee Claytor/
Supervisory Patent Examiner, Art Unit 1651
Technology Center 1600
Phone: 571-272-8394

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of:	Inge Bruheim *et al.*	Confirmation No.: 1945
Patent No.:	9,034,388	
Application No.:	12/057,775	
Issued:	May 19, 2015	
Entitled:	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

REQUEST FOR CERTIFICATE OF CORRECTION OF PATENT

Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir or Madam:

Pursuant to 35 U.S.C. §255 and 37 C.F.R. §1.323, patentee respectfully request that the Director issue a Certificate of Correction in the above-referenced patent to correct a typographical error in claim 1.

Please correct the Letters Patent at Column 38, lines 31-33, as follows:

c) providing [[a]]

i) dilapidated krill meal following said extraction comprising greater than 65% protein and less than 50 g/kg total fat.

The above-noted correction does not involve such changes in the patent as would constitute new matter or would require reexamination.

A completed Form PTO/SB/44 accompanies this request, with the above-noted correction printed thereon. Accordingly, a Certificate of Correction is believed proper and issuance thereof is respectfully requested.

The director is hereby authorized to charge the $100 fee due with the filing of this Request to deposit account number 50-4302 referencing attorney docket number AKBM-14409/US-5/ORD.

Respectfully submitted,

CASIMIR JONES, S.C.

Dated: February 1, 2016

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

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. : 9,034,388

APPLICATION NO.: 12/057,775

ISSUE DATE : 19-May-2015

INVENTOR(S) : Inge Bruheim, Mikko Griinari, Snorre Tilseth, Sebastiano Banni, Jeffrey Stuart Cohn, 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:

In the claims, column 38, lines 31-33 should be deleted and replaced with:

c) providing
i) dilapidated krill meal following said extraction comprising greater than 65% protein and less than 50 g/kg total fat.

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.

Electronic Patent Application Fee Transmittal

Application Number:	12057775
Filing Date:	28-Mar-2008
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-5/ORD

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:				
Certificate of Correction	1811	1	100	100

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**100**

Electronic Acknowledgement Receipt

EFS ID:	24777203
Application Number:	12057775
International Application Number:	
Confirmation Number:	1945
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-5/ORD
Receipt Date:	01-FEB-2016
Filing Date:	28-MAR-2008
Time Stamp:	16:41:11
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$100
RAM confirmation Number	3564
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	Request for Certificate of Correction	14409US5ORD_RequestCertCorrection.pdf	78865 e102065c527c48713be6e1e0902a48cc10a1b5f7	no	2
Warnings:					
Information:					
2	Request for Certificate of Correction	14409US5ORD_CertCorrectionForm.pdf	164978 1fd095619afa4504a9f0df783ac38383053f5e21	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30235 a34f7428035f9d713876c4534a287c9a3e2cf6dc	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	274078		

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.
12/057,775	05/19/2015	9034388	AKBM-14409/US-5/ORD	1945

72960 7590 04/29/2015

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
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 1181 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):

Inge Bruheim, Volda, NORWAY;
Mikko Griinari, Espoo, FINLAND;
Snorre Tilseth, Bergen, NORWAY;
Sebastiano Banni, Cagliari, ITALY;
Jeffrey Stuart Cohn, Camperdown, AUSTRALIA;
Daniele Mancinelli, Orsta, NORWAY;

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, /C.C.B./ 4/17/2015	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	

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

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 04/08/2015
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.
12/057,775	03/28/2008	Inge Bruheim	AKBM-14409/US-5/ORD	1945

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	07/08/2015

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 April 10, 2015

Typed or printed name J. Mitchell Jones Registration No. 44174

RIMFROST EXHIBIT 1024 page 0014

Electronic Patent Application Fee Transmittal

Application Number:	12057775
Filing Date:	28-Mar-2008
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-5/ORD

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:	22027720
Application Number:	12057775
International Application Number:	
Confirmation Number:	1945
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-5/ORD
Receipt Date:	10-APR-2015
Filing Date:	28-MAR-2008
Time Stamp:	14:33:46
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	789
Deposit Account	504302
Authorized User	

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 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 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)	14409US5ORD_IssueFeeTransmittal.pdf	96594 9cc571f46d62e41920ac092236f683991b6cd33d	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30610 1ee84de6076c8f4dbaaab430995198fca0170829	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	127204		

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 04/08/2015

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

EXAMINER
WARE, DEBORAH K

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 04/08/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/057,775	03/28/2008	Inge Bruheim	AKBM-14409/US-5/ORD	1945

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	07/08/2015

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 04/08/2015
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.
12/057,775	03/28/2008	Inge Bruheim	AKBM-14409/US-5/ORD	1945

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	07/08/2015

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.
12/057,775	03/28/2008	Inge Bruheim	AKBM-14409/US-5/ORD	1945

72960 7590 04/08/2015

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

EXAMINER	
WARE, DEBORAH K	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 04/08/2015

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)	
	12/057,775	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: *13 March 2015*.

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*.

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...)

Discussed the claims and claims 50 and 52-54 previously indicated as allowed on the record. Applicants' Representative agreed to cancel claim 55 in order to place the case into condition for allowance. Therefore, this interview will serve as Applicants' response to the last final office action on record and the Applicants' Representative authorized changes by Examiner Amendment to allow the case. All withdrawn claims were authorized as well to be canceled by Examiner Amendment. Applicants maintain their right to file one or more divisonal applications to the non-elected claimed invention(s).

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of 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

/Deborah K. Ware/
Deborah K. Ware
Primary Examiner
Art Unit 1651

Notice of Allowability	Application No. 12/057,775	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 *3/13/2015*.
   ☐ 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 *50 and 52-54*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

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

   * Certified copies not received: _____.

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

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

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

Attachment(s)

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

/Deborah K. Ware/
Deborah K. Ware
Primary Examiner
Art Unit 1651

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 13, 2015.

The application has been amended as follows:

In the Claims

Claims 1-49 and 55-90, canceled .

Claims 50 and 52-54 are placed into condition for allowance.

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, Taeyoon Kim can be reached on 571-272-9041. 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

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	12/057,775	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: *13 March 2015*.

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*.

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...)

Discussed the claims and claims 50 and 52-54 previously indicated as allowed on the record. Applicants' Representative agreed to cancel claim 55 in order to place the case into condition for allowance. Therefore, this interview will serve as Applicants' response to the last final office action on record and the Applicants' Representative authorized changes by Examiner Amendment to allow the case. All withdrawn claims were authorized as well to be canceled by Examiner Amendment. Applicants maintain their right to file one or more divisonal applications to the non-elected claimed invention(s).

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of 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

/Deborah K. Ware/
Deborah K. Ware
Primary Examiner
Art Unit 1651

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12057775	BRUHEIM ET AL.
	Examiner	Art Unit
	DEBBIE K WARE	1651

CPC- SEARCHED		
Symbol	Date	Examiner
A61K2300/00 | A61K31/122 | A61K31/23 | A61K31/683 | A61K31/685 | A61K35/612 | A61K31/202 | A61K45/06 | A61K9/4858 | C11B3/006	1/2015	dkw
A61K2300/00 | A61K31/685 | A61K31/122 | A61K31/683 | A61K35/612 | A61K31/23 | A61K31/202 | A61K45/06 | A61K9/2054 | A61K9/4858 | A61K47/44 | A61K31/05 | A61K31/12 | A61K31/133 | A61K31/198 | A61K31/57	03/2015-04/2015	dkw

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
WEST, NPL and INV: see search history print out	12/2011-1/2012	DKW
WEST, NPL and INV: see search history print out	6/2012	DKW
CPC-WEST, NPL and INV: see search history print out	01/2015	dkw
CPC-WEST, NPL and INV: see search history print out	03/2015-04/2015	dkw

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	WEST Interference Search: see search history print out	04/2015	dkw

WEST Search History for Application 12057775

Creation Date: 2015040121:57

Interference Searches

Query	DB	Op.	Plur.	Thes.	Date
krill.clm. and oil.clm.	PGPB, USPT, UPAD	OR	YES		04-01-2015
(krill.clm. and oil.clm.) and meal.clm. and phospholipid	PGPB, USPT, UPAD	OR	YES		04-01-2015
(krill.clm. and oil.clm. and meal.clm. and phospholipid) and ether.clm.	PGPB, USPT, UPAD	OR	YES		04-01-2015

Prior Art Searches

Query	DB	Op.	Plur.	Thes.	Date
"krill oil"	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
("krill oil") and "krill meal"	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
("krill oil" and "krill meal") and "supercritical fluid"	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
extract? and krill and oil and meal and supercritical	PGPB, USPT, USOC, EPAB, JPAB,	OR	YES		01-03-2012

	DWPI, TDBD				
2004241249	PGPB	OR	YES		01-03-2012
200400241249	PGPB	OR	YES		01-03-2012
20040241249	PGPB	OR	YES		01-03-2012
(20040241249) and "supercritical"	PGPB	OR	YES		01-03-2012
(20040241249) and "solvent extraction"	PGPB	OR	YES		01-03-2012
(20040241249) and "extract"	PGPB	OR	YES		01-03-2012
(20040241249 and "extract") and "oil"	PGPB	OR	YES		01-03-2012
(20040241249 and "extract" and "oil") and "meal"	PGPB	OR	YES		01-03-2012
supercritical and extraction and krill	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
(supercritical and extraction and krill) and co-solvent	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
(supercritical and extraction and krill and co-solvent) and oil	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
supercritical and extraction and alcohol	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
(supercritical and extraction and alcohol) and monohydric	PGPB, USPT, USOC, EPAB,	OR	YES		01-03-2012

	JPAB, DWPI, TDBD				
(supercritical and extraction and alcohol and monohydric) and krill and meal	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		01-03-2012
krill and oil and cooking and drying	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
(krill and oil and cooking and drying) and extracting	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
20030113432	PGPB	OR	YES		05-29-2012
(20030113432) and extracting	PGPB	OR	YES		05-29-2012
(20030113432 and extracting) and cooking	PGPB	OR	YES		05-29-2012
(20030113432 and extracting) and drying	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying) and asta	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying) and oil	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil) and stickwater	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater) and meal	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater and meal) and stored	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater and meal and stored) and supercritical	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater and meal and stored) and extraction	PGPB	OR	YES		05-29-2012

(20030113432 and extracting and drying and oil and stickwater and meal and stored) and extracting	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater and meal and stored and extracting) and "oil"	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater and meal and stored and extracting and "oil") and oil	PGPB	OR	YES		05-29-2012
(20030113432 and extracting and drying and oil and stickwater and meal and stored and extracting and "oil" and oil) and extracting	PGPB	OR	YES		05-29-2012
supercritical and extraction and krill and oil	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
(supercritical and extraction and krill and oil) and ethanol	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
(supercritical and extraction and krill and oil and ethanol) carbon dioxide	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
(supercritical and extraction and krill and oil and ethanol) and dioxide	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
(supercritical and extraction and krill and oil and ethanol and dioxide) and (ethanol or butanol)	PGPB, USPT, USOC, EPAB, JPAB, DWPI,	OR	YES		05-29-2012

	TDBD				
(supercritical and extraction and krill and oil and ethanol and dioxide and (ethanol or butanol)) and "krill oil"	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD	OR	YES		05-29-2012
Inge.in. and Bruheim.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Inge.in. and Bruheim.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Inge.in. and Bruheim.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Inge.in. and Bruheim.in. and krill.clm. and oil.clm.) and dried.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Inge.in. and Bruheim.in. and krill.clm. and oil.clm. and dried.clm.) and meal.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI,	OR	YES		04-01-2015

	TDBD, FPRS				
8557297.pn.	USPT	OR	YES		04-01-2015
Mikko.in. and Griinari.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Mikko.in. and Griinari.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Mikko.in. and Griinari.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Mikko.in. and Griinari.in. and krill.clm. and oil.clm.) and dried.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
Snorre.in. and Tilseth.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Snorre.in. and Tilseth.in.) and krill.clm.	PGPB, USPT, USOC,	OR	YES		04-01-2015

	EPAB, JPAB, DWPI, TDBD, FPRS				
(Snorre.in. and Tilseth.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Snorre.in. and Tilseth.in. and krill.clm. and oil.clm.) and dried.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
Sebastiano.in. and Banni.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Sebastiano.in. and Banni.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Sebastiano.in. and Banni.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Sebastiano.in. and Banni.in. and krill.clm. and oil.clm.)	PGPB,	OR	YES		04-01-2015

and phospholipid.clm.	USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS				
(Sebastiano.in. and Banni.in. and krill.clm. and oil.clm. and phospholipid.clm.) and dried.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Sebastiano.in. and Banni.in. and krill.clm. and oil.clm. and phospholipid.clm.) and cook.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Sebastiano.in. and Banni.in. and krill.clm. and oil.clm. and phospholipid.clm.) and cooking.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Sebastiano.in. and Banni.in. and krill.clm. and oil.clm. and phospholipid.clm.) and extract.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
Jeffrey.in. and Cohn.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015

(Jeffrey.in. and Cohn.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
Daniele.in. and Mancinelli.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Daniele.in. and Mancinelli.in.) and krill.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Daniele.in. and Mancinelli.in. and krill.clm.) and extract.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(Daniele.in. and Mancinelli.in. and krill.clm. and extract.clm.) and dried.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
krill and oil and ether and phospholipids and cook? and delipi? and extract? and polar	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD,	OR	YES		04-01-2015

	FPRS				
krill and oil and ether and phospholipids and cook? and delipi? and extract?	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
krill and oil and ether and phospholipids	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(krill and oil and ether and phospholipids) and delipid?	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(krill and oil and ether and phospholipids) and extract?	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(krill and oil and ether and phospholipids and extract?) and cook?	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(krill and oil and ether and phospholipids and extract?) and dried and meal	PGPB, USPT, USOC, EPAB, JPAB,	OR	YES		04-01-2015

	DWPI, TDBD, FPRS				
(krill and oil and ether and phospholipids and extract? and dried and meal) and "krill oil"	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015
(krill and oil and ether and phospholipids and extract? and dried and meal and "krill oil") and ((A61K2300/00 | A61K31/685 | A61K31/122 | A61K31/683 | A61K35/612 | A61K31/23 | A61K31/202 | A61K45/06 | A61K9/2054 | A61K9/4858 | A61K47/44 | A61K31/05 | A61K31/12 | A61K31/133 | A61K31/198 | A61K31/575 | A61K31/7004 | A61K31/7048 | A61K31/714 | A61K33/06 | A61K38/02 | A61K38/1767 | A61K47/22 | A61K9/0019 | A61K9/06 | A61K9/12 | A61K9/2009 | A61K9/2018 | A61K9/2866 | A61K2800/10 | A61K31/232 | A61K31/352 | A61K31/66 | A61K35/60 | A61K36/00 | A61K47/26 | A61K47/36 | A61K47/48053 | A61K47/481 | A61K8/062 | A61K8/14 | A61K8/553 | A61K8/645 | A61K8/925 | A61K9/107 | A61K9/4816 | A61K9/5036 | A23V2002/00 | A23V2250/18 | A23V2250/194 | A23V2250/21 | A23V2250/2136 | A23V2250/1882 | A23V2200/306 | A23V2200/318 | A23V2250/2132 | A23V2250/314 | A23V2250/714 | A23V2200/322 | A23V2250/1842 | A23V2250/1942 | A23L1/3006 | A23L1/33 | A23L1/305 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/0026 | A23L1/3008 | A23L1/30 | A23L1/3002 | A23L1/3004 | A23L1/302 | C11B1/10 | C11B3/006 | C11B1/06 | C11B1/025 | C11B1/104 | C11B13/00 | A23K1/164 | A23K1/188 | A23K1/103 | A23K1/1606 | A23K1/1893 | A23D9/013 | A23D9/007 | A23D9/02 | A23D7/0053 | A23D7/013 | A23J1/04 | A23J3/34 | C07K14/43509 | C07K19/00 | C07F9/103 | C07F9/10 | A21D2/32 | A23G3/40 | A61Q19/00).CPC.)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		04-01-2015

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12057775	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
			/			
			/			
			/			
			/			
			/			
			/			

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		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	4	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651	04/02/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12057775	BRUHEIM ET AL.
	Examiner	**Art Unit**
	DEBBIE K WARE	1651

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				

INTERNATIONAL CLASSIFICATION																
CLAIMED						NON-CLAIMED										
A	6	1	K	9 / 48 (2006.01.01)												
A	6	1	K	31 / 23 (2006.01.01)												
A	6	1	K	31 / 122 (2006.01.01)												
A	6	1	K	31 / 202 (2006.01.01)												
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)	4	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651	04/02/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12057775	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		17		33		49		65		81				
	2		18		34	1	50		66		82				
	3		19		35		51		67		83				
	4		20		36	2	52		68		84				
	5		21		37	3	53		69		85				
	6		22		38	4	54		70		86				
	7		23		39		55		71		87				
	8		24		40		56		72		88				
	9		25		41		57		73		89				
	10		26		42		58		74		90				
	11		27		43		59		75						
	12		28		44		60		76						
	13		29		45		61		77						
	14		30		46		62		78						
	15		31		47		63		79						
	16		32		48		64		80						

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 4	
/DEBBIE K WARE/ Primary Examiner.Art Unit 1651 (Primary Examiner)	04/02/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

```
Connecting via Winsock to STN at pto-stn on port 23



Welcome to STN International!  Enter x:X

LOGINID:ssspt189dxw

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1  OCT 11  Instructor-led and self paced STN learning resources
                  available at https://cas.csod.com/Default.aspx?c=001
 NEWS  2  APR 26  Expanded Swedish Patent Application Coverage in CA/CAplus
                  Provides More Current and Complete Information
 NEWS  3  APR 28  The DWPI (files WPINDEX, WPIDS and WPIX) on STN have been
                  enhanced with thesauri for the European Patent Classifications
 NEWS  4  MAY 02  MEDLINE Improvements Provide Fast and Simple Access to DOI and
                  Chemical Name Information
 NEWS  5  MAY 12  European Patent Classification thesauri added to the INPADOC
                  files, PCTFULL, GBFULL and FRFULL
 NEWS  6  MAY 23  Enhanced performance of STN biosequence searches
 NEWS  7  JUN 20  STN on the Web Enhanced with New Patent Family Assistant and
                  Updated Structure Plug-In
 NEWS  8  JUN 20  INPADOC databases enhanced with first page images
 NEWS  9  JUN 20  PATDPA database updates to end in June 2011
 NEWS 10  JUN 26  MARPAT Enhancements Save Time and Increase Usability
 NEWS 11  JUL 25  STN adds Australian patent full-text database,
                  AUPATFULL, including the new numeric search feature.
 NEWS 12  AUG 01  CA Sections Added to ACS Publications Web Editions
                  Platform
 NEWS 13  AUG 16  INPADOC: Coverage of German Patent Data resumed,
                  enhanced legal status
 NEWS 14  AUG 18  Upgrade now to STN Express, Version 8.5
 NEWS 15  SEP 01  CAS Journal Coverage Now Includes Ahead-of-Print
                  Articles for More Than 100 Journal Titles
 NEWS 16  SEP 01  Older Versions of STN Express to be Discontinued
                  Beginning in March 2012
 NEWS 17  SEP 09  USAN Database Updates Offer Superior Currency on STN(R)
 NEWS 18  SEP 26  STN Adds Canadian Patent Full-text Database - CANPATFULL
 NEWS 19  SEP 26  GEOREF and ENCOMPLIT databases were reloaded on
                  September 24, 2011.
 NEWS 20  SEP 26  Updates to the IFIPAT/IFIUDB/IFICDB databases have resumed.
 NEWS 21  SEP 26  ECLA Thesaurus in CA/CAplus Improves Patent Searching on STN
 NEWS 22  SEP 26  Access AUPATFULL and CANPATFULL databases with STN Viewer
 NEWS 23  OCT 26  New STN Revolutionizes Patent Searching for Professionals
 NEWS 24  DEC  1  CA/CAplus Now Includes Examiner Citations for Japanese Patents
 NEWS 25  DEC  1  CAS Expands Global Patent Coverage - Intellectual Property
                  Corporation of Malaysia Becomes 62nd Authority on CA/CAplus
 NEWS 26  DEC  5  STN on the Web Enhancements Include Compatibility with
                  Microsoft Windows 7
 NEWS 27  DEC 14  Removal of ITRD and PATIPC databases from STN
 NEWS 28  DEC 15  Rolled-up IPC Core Codes Removed from IPC Reclassifications in
                  Patent Databases on STN
 NEWS EXPRESS 18 AUGUST 2011 CURRENT WINDOWS VERSION IS V8.5,
              AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2011.
```

```
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS TRAINING Find instructor-led and self-directed training opportunities

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN customer
  agreement.  This agreement limits use to scientific research.  Use
  for software development or design, implementation of commercial
  gateways, or use of CAS and STN data in the building of commercial
  products is prohibited and may result in loss of user privileges
  and other penalties.


* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 15:15:33 ON 03 JAN 2012

=> index bioscience
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.24       0.24

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
       AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
       CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
       DRUGU, EMBAL, EMBASE, ESBIOBASE, ...' ENTERED AT 15:15:47 ON 03 JAN 2012

56 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s krill and oil and meal and supercritical(p)extract? and solvent and heat? and
treat?
          0*  FILE ADISNEWS
          0*  FILE ANTE
          0*  FILE AQUALINE
          0*  FILE BIOENG
          0*  FILE BIOTECHABS
          0*  FILE BIOTECHDS
          0*  FILE BIOTECHNO
          0*  FILE CEABA-VTB
          0*  FILE CIN
  22 FILES SEARCHED...
          0*  FILE FOMAD
          0*  FILE FROSTI
          0*  FILE FSTA
  33 FILES SEARCHED...
          1   FILE IFIPAT
          0*  FILE KOSMET
          0*  FILE NTIS
          0*  FILE PASCAL
  48 FILES SEARCHED...
         28   FILE USPATFULL
          4   FILE USPAT2
  51 FILES SEARCHED...
          0*  FILE WATER
          1   FILE WPIDS
          1   FILE WPINDEX
```

```
   5 FILES HAVE ONE OR MORE ANSWERS,   56 FILES SEARCHED IN STNINDEX

L1   QUE KRILL AND OIL AND MEAL AND SUPERCRITICAL(P)EXTRACT? AND SOLVENT AND HE
        AT? AND TREAT?

=> file ifipat uspatfull uspat2
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       3.70       3.94

FILE 'IFIPAT' ENTERED AT 15:19:01 ON 03 JAN 2012
COPYRIGHT (C) 2012 IFI CLAIMS(R) Patent Services (IFI)

FILE 'USPATFULL' ENTERED AT 15:19:01 ON 03 JAN 2012
CA INDEXING COPYRIGHT (C) 2012 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPAT2' ENTERED AT 15:19:01 ON 03 JAN 2012
CA INDEXING COPYRIGHT (C) 2012 AMERICAN CHEMICAL SOCIETY (ACS)

=> s l1
L2          33 L1

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3           32 DUP REM L2 (1 DUPLICATE REMOVED)

=> s l3 andcarbon(p)dixoide
MISSING OPERATOR L3 ANDCARBON
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l3 and dioxide
L4          27 L3 AND DIOXIDE

=> d l4 1-27

L4   ANSWER 1 OF 27  IFIPAT  COPYRIGHT 2012 IFI on STN
AN    11934106  IFIPAT;IFIUDB;IFICDB
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

L4   ANSWER 2 OF 27  USPATFULL on STN
AN     2011:287830  USPATFULL
TI     Reducing the Risk of Pathological Effects of Traumatic Brain Injury
IN     Hadley, Kevin, Elkridge, MD, UNITED STATES
```

```
       Fealey, Terence, Marietta, GA, UNITED STATES
       Bailes, Julian E., Morgantown, WV, UNITED STATES
PI     US 20110257267      A1  20111020
AI     US 2010-904049      A1  20101013 (12)
PRAI   US 2009-251230P         20091013 (61)
DT     Utility
FS     APPLICATION
LN.CNT 2397
INCL   INCLM: 514/547.000
       INCLS: 514/560.000; 514/549.000
NCL    NCLM:  514/547.000
       NCLS:  514/549.000; 514/560.000
IPC    IPCI   A61K0031-232 [I,A]; A61P0025-00 [I,A]; A61K0031-202 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 3 OF 27  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
AI     US 2011-96644       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
IPC    IPCI   C11B0001-00 [I,A]; C07F0009-10 [I,A]
       IPCR   C11B0001-00 [I,A]; C07F0009-10 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 4 OF 27  USPATFULL on STN
AN     2011:212256  USPATFULL
TI     METHOD FOR PRODUCING LIPIDS
IN     Yoshikawa, Kazuhiro, Tokyo, JAPAN
       Mikajiri, Akihiro, Tokyo, JAPAN
PA     NIPPON SUISAN KAISHA, LTD., Tokyo, JAPAN (non-U.S. corporation)
PI     US 20110189760      A1  20110804
AI     US 2009-120842      A1  20090924 (13)
       WO 2009-JP66530         20090924
                               20110425  PCT 371 date
PRAI   JP 2008-248986          20080926
DT     Utility
FS     APPLICATION
LN.CNT 1345
INCL   INCLM: 435/271.000
       INCLS: 554/020.000
NCL    NCLM:  435/271.000
       NCLS:  554/020.000
IPC    IPCI   C11C0001-00 [I,A]; C11B0001-00 [I,A]
       IPCR   C11C0001-00 [I,A]; C11B0001-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 5 OF 27  USPATFULL on STN
```

```
AN     2011:211870  USPATFULL
TI     METHOD FOR CONCENTRATING LIPIDS
IN     Yoshikawa, Kazuhiro, Tokyo, JAPAN
PA     NIPPON SUISAN KAISHA, LTD., Tokyo, JAPAN (non-U.S. corporation)
PI     US 20110189374      A1  20110804
AI     US 2009-120875      A1  20090924 (13)
       WO 2009-JP66529         20090924
                               20110425  PCT 371 date
PRAI   JP 2008-248986          20080926
DT     Utility
FS     APPLICATION
LN.CNT 961
INCL   INCLM: 426/601.000
       INCLS: 554/008.000
NCL    NCLM:  426/601.000
       NCLS:  554/008.000
IPC    IPCI   A23D0009-00 [I,A]; C11B0001-06 [I,A]
       IPCR   A23D0009-00 [I,A]; C11B0001-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 6 OF 27  USPATFULL on STN
AN     2011:198158  USPATFULL
TI     METHODS OF TREATING AND PREVENTING NEUROLOGICAL DISORDERS USING
       DOCOSAHEXAENOIC ACID
IN     AISEN, Paul S., Solana Beach, CA, UNITED STATES
       Quinn, Joseph F., Portland, OR, UNITED STATES
       Yurko-Mauro, Karin, Silver Spring, MD, UNITED STATES
PA     MARTEK BIOSCIENCES CORPORATION, Columbia, MD, UNITED STATES (U.S.
       corporation)
PI     US 20110177061      A1  20110721
AI     US 2010-833913      A1  20100709 (12)
PRAI   US 2009-224836P         20090710 (61)
       US 2010-359792P         20100629 (61)
DT     Utility
FS     APPLICATION
LN.CNT 2653
INCL   INCLM: 424/133.100
       INCLS: 514/560.000; 514/120.000; 514/547.000; 514/549.000; 514/297.000;
              514/319.000; 514/479.000; 514/215.000; 424/184.100; 424/172.100;
              424/152.100; 514/458.000
NCL    NCLM:  424/133.100
       NCLS:  424/152.100; 424/172.100; 424/184.100; 514/120.000; 514/215.000;
              514/297.000; 514/319.000; 514/458.000; 514/479.000; 514/547.000;
              514/549.000; 514/560.000
IPC    IPCI   A61K0031-202 [I,A]; A61K0031-661 [I,A]; A61K0031-232 [I,A];
              A61K0031-473 [I,A]; A61K0031-445 [I,A]; A61K0031-27 [I,A];
              A61K0031-55 [I,A]; A61K0039-00 [I,A]; A61K0039-395 [I,A];
              A61K0031-355 [I,A]; A61P0025-28 [I,A]; A61P0025-00 [I,A]
       IPCR   A61K0031-202 [I,A]; A61K0031-232 [I,A]; A61K0031-27 [I,A];
              A61K0031-355 [I,A]; A61K0031-445 [I,A]; A61K0031-473 [I,A];
              A61K0031-55 [I,A]; A61K0031-661 [I,A]; A61K0039-00 [I,A];
              A61K0039-395 [I,A]; A61P0025-00 [I,A]; A61P0025-28 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 7 OF 27  USPATFULL on STN
AN     2011:146375  USPATFULL
TI     KRILL OIL PROCESS
IN     Breivik, Harald, Porsgrunn, NORWAY
       Thorstad, Olav, Porsgrunn, NORWAY
PA     PRONOVA BIOPHARMA NORGE AS, Lysaker, NORWAY (non-U.S. corporation)
PI     US 20110130458      A1  20110602
AI     US 2009-992365      A1  20090515 (12)
```

```
       WO 2009-NO184           20090515
                               20110211  PCT 371 date
PRAI   US 2008-53455P          20080515 (61)
DT     Utility
FS     APPLICATION
LN.CNT 688
INCL   INCLM: 514/560.000
       INCLS: 426/608.000; 426/417.000
NCL    NCLM:  514/560.000
       NCLS:  426/417.000; 426/608.000
IPC    IPCI   A61K0031-202 [I,A]; A61P0003-06 [I,A]; A61P0003-00 [I,A];
              A61P0009-00 [I,A]; A61P0009-04 [I,A]; A61P0009-10 [I,A];
              A23D0007-00 [I,A]; A23D0009-00 [I,A]
       IPCR   A61K0031-202 [I,A]; A23D0007-00 [I,A]; A23D0009-00 [I,A];
              A61P0003-00 [I,A]; A61P0003-06 [I,A]; A61P0009-00 [I,A];
              A61P0009-04 [I,A]; A61P0009-10 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 8 OF 27  USPATFULL on STN
AN     2011:117434  USPATFULL
TI     POWDERED COMPOSITION CONTAINING OIL-SOLUBLE COMPONENT, FUNCTIONAL FOOD
       USING THE SAME, AND PACKAGED PRODUCT THEREOF
IN     Suzuki, Keiichi, Kanagawa, JAPAN
       Sasaki, Hidemi, Kanagawa, JAPAN
       Serizawa, Shinichiro, Kanagawa, JAPAN
       Arakawa, Jun, Kanagawa, JAPAN
PA     FUJIFILM CORPORATION, Minato-ku, Tokyo, JAPAN (non-U.S. corporation)
PI     US 20110104340      A1  20110505
AI     US 2008-673977      A1  20080819 (12)
       WO 2008-JP65061         20080819
                               20100218  PCT 371 date
PRAI   JP 2007-213712          20070820
       JP 2007-230582          20070905
DT     Utility
FS     APPLICATION
LN.CNT 2345
INCL   INCLM: 426/096.000
       INCLS: 426/654.000; 426/590.000
NCL    NCLM:  426/096.000
       NCLS:  426/590.000; 426/654.000
IPC    IPCI   A21D0002-16 [I,A]; A23L0002-52 [I,A]
       IPCR   A21D0002-16 [I,A]; A23L0002-52 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 9 OF 27  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
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 2007-975058P         20070925 (60)
```

```
       US 2007-983446P         20071029 (60)
       US 2008-24072P          20080128 (61)
       US 2009-181743P         20090528 (61)
       US 2007-920483P         20070328 (60)
DT     Utility
FS     APPLICATION
LN.CNT 2547
INCL   INCLM: 424/522.000
       INCLS: 426/002.000
NCL    NCLM:  424/522.000
       NCLS:  426/002.000
IPC    IPCI   A61K0035-56 [I,A]; A61P0009-10 [I,A]; A61P0003-04 [I,A];
              A61P0003-00 [I,A]
       IPCR   A61K0035-56 [I,A]; A61P0003-00 [I,A]; A61P0003-04 [I,A];
              A61P0009-10 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 10 OF 27  USPATFULL on STN
AN     2011:97925  USPATFULL
TI     Methods for Treating Traumatic Brain Injury
IN     Bailes, Julian E., Morgantown, WV, UNITED STATES
PI     US 20110086914      A1  20110414
AI     US 2010-904045      A1  20101013 (12)
PRAI   US 2009-251234P         20091013 (61)
DT     Utility
FS     APPLICATION
LN.CNT 2356
INCL   INCLM: 514/549.000
       INCLS: 514/560.000
NCL    NCLM:  514/549.000
       NCLS:  514/560.000
IPC    IPCI   A61K0031-232 [I,A]; A61K0031-20 [I,A]; A61P0025-00 [I,A]
       IPCR   A61K0031-232 [I,A]; A61K0031-20 [I,A]; A61P0025-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 11 OF 27  USPATFULL on STN
AN     2011:92475  USPATFULL
TI     Docosahexaenoic Acid Gel Caps
IN     PANKER, Cynthia A., Jessup, MD, UNITED STATES
       Billard, Michael Ames, Laurel, MD, UNITED STATES
       Ryan, Alan, Ellicott City, MD, UNITED STATES
       Dangi, Bindi, Elkridge, MD, UNITED STATES
PI     US 20110082205      A1  20110407
AI     US 2010-896763      A1  20101001 (12)
PRAI   US 2009-247944P         20091001 (61)
DT     Utility
FS     APPLICATION
LN.CNT 2444
INCL   INCLM: 514/549.000
NCL    NCLM:  514/549.000
IPC    IPCI   A61K0031-232 [I,A]; A61P0003-06 [I,A]
       IPCR   A61K0031-232 [I,A]; A61P0003-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 12 OF 27  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
AI     US 2010-711822      A1  20100224 (12)
```

PRAI   US 2009-155758P   20090226 (61)
DT   Utility
FS   APPLICATION
LN.CNT 3112
INCL   INCLM: 514 2
NCL   NCLM: 514/005.500
       NCLS: 514/691.000
IPC   IPCI   A61K0038-02 [I,A]
       IPCR   A61K0038-02 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 13 OF 27 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 2009-155767P   20090226 (61)
       US 2007-968765P   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
IPC   IPCI   A61K0031-685 [I,A]; A23D0009-00 [I,A]; A23D0009-02 [I,A];
              A61K0009-48 [I,A]; A61P0009-00 [I,A]; A61P0019-00 [I,A];
              A61P0029-00 [I,A]
       IPCR   A61K0031-685 [I,A]; A23D0009-00 [I,A]; A23D0009-02 [I,A];
              A61K0009-48 [I,A]; A61P0009-00 [I,A]; A61P0019-00 [I,A];
              A61P0029-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 14 OF 27 USPATFULL on STN
AN   2010:228249 USPATFULL
TI   METHODS FOR IMPROVING COGNITIVE FUNCTION AND DECREASING HEART RATE
IN   YURKO-MAURO, Karin, Silver Spring, MD, UNITED STATES
PA   MARTEK BIOSCIENCES CORPORATION, Columbia, MD, UNITED STATES (U.S.
       corporation)
PI   US 20100203123   A1 20100812
AI   US 2010-699009   A1 20100202 (12)
PRAI   US 2009-149310P   20090202 (61)
       US 2009-183548P   20090602 (61)
DT   Utility
FS   APPLICATION
LN.CNT 2358
INCL   INCLM: 424/456.000
       INCLS: 514/560.000; 514/549.000; 514/458.000
NCL   NCLM: 424/456.000
       NCLS: 514/458.000; 514/549.000; 514/560.000
IPC   IPCI   A61K0009-64 [I,A]; A61K0031-20 [I,A]; A61K0031-22 [I,A];
              A61K0031-355 [I,A]; A61P0025-00 [I,A]; A61P0009-00 [I,A]
       IPCR   A61K0009-64 [I,A]; A61K0031-20 [I,A]; A61K0031-22 [I,A];
              A61K0031-355 [I,A]; A61P0009-00 [I,A]; A61P0025-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 15 OF 27 USPATFULL on STN
AN   2010:161551 USPATFULL

```
TI     PROCESS FOR PRODUCTION OF OMEGA-3 RICH MARINE PHOSPHOLIPIDS FROM KRILL
IN     Breivik, Harald, Porsgrunn, NORWAY
PI     US 20100143571      A1  20100610
AI     US 2007-515098      A1  20071115 (12)
       WO 2007-NO402           20071115
                               20100217  PCT 371 date
PRAI   US 2006-859289P         20061116 (60)
DT     Utility
FS     APPLICATION
LN.CNT 537
INCL   INCLM: 426/643.000
       INCLS: 426/417.000; 554/021.000; 568/366.000; 536/020.000
NCL    NCLM:  426/643.000
       NCLS:  426/417.000; 536/020.000; 554/021.000; 568/366.000
IPC    IPCI   A23L0001-325 [I,A]; A23K0001-10 [I,A]; A23K0001-18 [I,A];
              C11B0001-10 [I,A]; C07C0045-78 [I,A]; C08B0037-08 [I,A]
       IPCR   A23L0001-325 [I,A]; A23K0001-10 [I,A]; A23K0001-18 [I,A];
              C07C0045-78 [I,A]; C08B0037-08 [I,A]; C11B0001-10 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 16 OF 27  USPATFULL on STN
AN     2009:109974  USPATFULL
TI     Polyunsaturated Fatty Acid-Containing Solid Fat Compositions and Uses
       and Production Thereof
IN     Namal Senanayake, S.P. Janaka, Lexington, KY, UNITED STATES
       Ahmed, Naseer, Lexington, KY, UNITED STATES
       Fichtali, Jaouad, Lexington, KY, UNITED STATES
PA     Martek Biosciences Corporation, Columbia, MD, UNITED STATES (U.S.
       corporation)
PI     US 20090099260      A1  20090416
AI     US 2008-201728      A1  20080829 (12)
PRAI   US 2007-969536P         20070831 (60)
DT     Utility
FS     APPLICATION
LN.CNT 2660
INCL   INCLM: 514/560.000
       INCLS: 426/601.000; 426/072.000
NCL    NCLM:  514/560.000
       NCLS:  426/072.000; 426/601.000
IPC    IPCI   A61K0031-20 [I,A]; A23D0007-005 [I,A]; A23L0001-30 [I,A]
       IPCR   A61K0031-20 [I,A]; A23D0007-005 [I,A]; A23L0001-30 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 17 OF 27  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 2007-968765P         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
IPC    IPCI   A23D0007-005 [I,A]; A23D0007-02 [I,A]; A23D0007-04 [I,A];
```

A23L0001-29 [I,A]; B01F0015-06 [I,A]; A23L0001-33 [I,A];
A23L0001-326 [I,A]; B01D0021-30 [I,A]
IPCR A23D0007-005 [I,A]; A23D0007-02 [I,A]; A23D0007-04 [I,A];
A23L0001-29 [I,A]; A23L0001-326 [I,A]; A23L0001-33 [I,A];
B01D0021-30 [I,A]; B01F0015-06 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 18 OF 27 USPATFULL on STN
AN 2006:254989 USPATFULL
TI Natural astaxanthin extract reduces dna oxidation
IN Chew, Boon P., Pullman, WA, UNITED STATES
Park, Jean Soon, Pullman, WA, UNITED STATES
PI US 20060217445 A1 20060928
AI US 2004-565717 A1 20040726 (10)
WO 2004-US24314 20040726
20060123 PCT 371 date
PRAI US 2003-490121P 20030725 (60)
DT Utility
FS APPLICATION
LN.CNT 1366
INCL INCLM: 514/690.000
INCLS: 514/763.000; 514/560.000
NCL NCLM: 514/690.000
NCLS: 514/560.000; 514/763.000
IPC IPCI A61K0031-12 [I,A]; A61K0031-015 [I,A]
IPCR A61K0031-12 [I,A]; A61K0031-015 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 19 OF 27 USPATFULL on STN
AN 2006:227598 USPATFULL
TI Preventive or remedy for arthritis
IN Kamiya, Toshikazu, Ibaraki, JAPAN
Nakagiri, Ryusuke, Chapel Hill, NC, UNITED STATES
PA Kyowa Hakko Kogyo Co., Ltd., Tokyo, JAPAN, 100-8185 (non-U.S.
corporation)
PI US 20060193962 A1 20060831
AI US 2004-552526 A1 20040409 (10)
WO 2004-JP5115 20040409
20051011 PCT 371 date
PRAI JP 2003-107405 20030411
DT Utility
FS APPLICATION
LN.CNT 1047
INCL INCLM: 426/615.000
NCL NCLM: 426/615.000
IPC IPCI A23L0001-212 [I,A]
IPCR A23L0001-212 [I,A]; A23K0001-14 [I,A]; A23K0001-16 [I,A];
A23L0001-30 [I,A]; A61K0031-7008 [I,A]; A61K0031-726 [I,A];
A61K0036-00 [I,A]; A61K0036-185 [I,A]; A61P0019-02 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 20 OF 27 USPATFULL on STN
AN 2004:209092 USPATFULL
TI Process for producing a plant extract containing plant powder
IN Sakai, Yasushi, Tsukuba-shi, JAPAN
Yokoo, Yoshiharu, Sagamihara-shi, JAPAN
PI US 20040161524 A1 20040819
US 7521079 B2 20090421
AI US 2003-481519 A1 20031219 (10)
WO 2002-JP6226 20020621
PRAI JP 2001-188480 20010621
DT Utility

```
FS     APPLICATION
LN.CNT 1479
INCL   INCLM: 426/655.000
NCL    NCLM:  426/655.000
       NCLS:  426/433.000; 426/594.000; 426/597.000
IPC    [7]
       IPCI   A23L0001-28 [ICM,7]
       IPCI-2 A23L0001-28 [I,A]
       IPCR   A23L0001-28 [I,A]; A23K0001-14 [I,A]; A23K0001-16 [I,A];
              A23L0001-30 [I,A]; A61K0036-185 [I,A]

L4   ANSWER 21 OF 27  USPATFULL on STN
AN     2004:209046  USPATFULL
TI     Preventives or remedies for arthritis
IN     Nakagiri, Rysuke, Tokyo, JAPAN
       Kamiya, Toshikazu, Tsuchiura-shi, JAPAN
       Suda, Toshio, Sunto-gun, JAPAN
       Miki, Ichiro, Mishima-shi, JAPAN
PI     US 20040161478      A1  20040819
AI     US 2003-480044      A1  20031209 (10)
       WO 2002-JP5790          20020611
PRAI   JP 2001-181947          20010615
       JP 2002-70702           20020314
DT     Utility
FS     APPLICATION
LN.CNT 1301
INCL   INCLM: 424/725.000
NCL    NCLM:  424/725.000
IPC    [7]
       IPCI   A61K0035-78 [ICM,7]
       IPCR   A21D0002-36 [I,A]; A21D0013-08 [I,A]; A23K0001-14 [I,A];
              A23K0001-16 [I,A]; A23L0001-30 [I,A]; A61K0036-185 [I,A];
              A61P0019-02 [I,A]; A61P0029-00 [I,A]

L4   ANSWER 22 OF 27  USPATFULL on STN
AN     2004:159281  USPATFULL
TI     Liver funcion protecting or ameliorating agent
IN     Sakai, Yasushi, Tsukuba-shi, JAPAN
       Kayahashi, Shun, Tsukuba-shi, JAPAN
       Hashizume, Erika, Tsukuba-shi, JAPAN
       Nakagiri, Ryusuke, Tokyo, JAPAN
PI     US 20040122085      A1  20040624
       US 7332522          B2  20080219
AI     US 2003-473867      A1  20031003 (10)
       WO 2002-JP3098          20020328
DT     Utility
FS     APPLICATION
LN.CNT 1146
INCL   INCLM: 514/470.000
NCL    NCLM:  514/457.000; 514/470.000
       NCLS:  514/470.000; 549/283.000
IPC    [7]
       IPCI   A61K0031-365 [ICM,7]
       IPCI-2 A61K0031-34 [I,A]; A61K0031-343 [I,A]
       IPCR   A61K0031-34 [I,A]; A23L0001-30 [I,A]; A61K0031-343 [I,A];
              A61K0031-365 [I,A]; A61K0031-366 [I,A]; A61P0001-16 [I,A];
              C07D0307-88 [I,A]; C07D0311-76 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 23 OF 27  USPATFULL on STN
AN     2003:64375  USPATFULL
TI     Processes for extracting carotenoids and for preparing feed materials
```

```
IN     Kagan, Michael, Jerusalem, ISRAEL
       Braun, Sergei, Zur Hadassa, ISRAEL
PI     US 20030044495      A1  20030306
       US 6818239          B2  20041116
AI     US 2002-172747      A1  20020617 (10)
RLI    Continuation of Ser. No. WO 2000-IL846, filed on 18 Dec 2000, UNKNOWN
PRAI   GB 1999-30194           19991221
DT     Utility
FS     APPLICATION
LN.CNT 526
INCL   INCLM: 426/250.000
NCL    NCLM:  426/429.000; 426/250.000
       NCLS:  426/250.000; 426/253.000; 426/431.000; 426/478.000; 426/540.000
IPC    [7]
       IPCI   A23L0001-27 [ICM,7]
       IPCI-2 A23L0001-28 [ICM,7]; A23L0001-27 [ICS,7]
       IPCR   A23L0001-27 [I,A]; A23L0001-275 [I,A]; C07C0403-00 [I,A];
              C07C0403-24 [I,A]; C09B0061-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 24 OF 27  USPATFULL on STN
AN     2002:205917  USPATFULL
TI     Liver function protecting or improving agent
IN     Nakagiri, Ryusuke, Tsukuba-shi, JAPAN
       Kamiya, Toshikazu, Tsukuba-shi, JAPAN
       Hashizume, Erika, Tsukuba-shi, JAPAN
       Sakai, Yasushi, Inashiki-gun, JAPAN
       Kayahashi, Shun, Tsukuba-shi, JAPAN
PI     US 20020110605      A1  20020815
AI     US 2001-10154       A1  20011210 (10)
PRAI   JP 2000-375510          20001211
DT     Utility
FS     APPLICATION
LN.CNT 1786
INCL   INCLM: 424/725.000
NCL    NCLM:  424/725.000
IPC    [7]
       IPCI   A61K0035-78 [ICM,7]
       IPCR   A21D0002-36 [I,A]; A21D0013-08 [I,A]; A23K0001-14 [I,A];
              A23K0001-16 [I,A]; A23L0001-212 [I,A]; A23L0001-30 [I,A];
              A61K0036-185 [I,A]; A61P0001-16 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 25 OF 27  USPAT2 on STN
AN     2004:209092  USPAT2
TI     Process for producing an extract of Hydrangea containing plant powder
IN     Sakai, Yasushi, Tsukuba, JAPAN
       Yokoo, Yoshiharu, Sagamihara, JAPAN
PA     Kyowa Hakko Kogyo Co., Ltd., Tokyo, JAPAN (non-U.S. corporation)
PI     US 7521079          B2  20090421
       WO 2003000074           20030301
AI     US 2002-481519          20020621 (10)
       WO 2002-JP6226          20020621
                               20031219  PCT 371 date
PRAI   JP 2001-188480          20010621
DT     Utility
FS     GRANTED
LN.CNT 1371
INCL   INCLM: 426/655.000
       INCLS: 426/594.000; 426/597.000; 426/433.000
NCL    NCLM:  426/655.000
       NCLS:  426/433.000; 426/594.000; 426/597.000
```

```
IPC    IPCI   A23L0001-28 [ICM,7]
       IPCI-2 A23L0001-28 [I,A]
       IPCR   A23L0001-28 [I,A]; A23K0001-14 [I,A]; A23K0001-16 [I,A];
              A23L0001-30 [I,A]; A61K0036-185 [I,A]
EXF    426/597; 426/433; 426/594

L4   ANSWER 26 OF 27  USPAT2 on STN
AN     2004:159281  USPAT2
TI     Liver function protecting or ameliorating agent
IN     Sakai, Yasushi, Tsukuba, JAPAN
       Kayahashi, Shun, Tsukuba, JAPAN
       Hashizume, Erika, Tsukuba, JAPAN
       Nakagiri, Ryusuke, Tokyo, JAPAN
PA     Kyowa Hakko Kogyo Co., Ltd., Tokyo, JAPAN (non-U.S. corporation)
PI     US 7332522          B2  20080219
       WO 2002080904           20021017
AI     US 2002-473867          20020328 (10)
       WO 2002-JP3098          20020328
                               20031003  PCT 371 date
PRAI   JP 2001-106600          20010405
DT     Utility
FS     GRANTED
LN.CNT 1099
INCL   INCLM: 514/457.000
       INCLS: 514/470.000; 549/283.000
NCL    NCLM:  514/457.000; 514/470.000
       NCLS:  514/470.000; 549/283.000
IPC    IPCI   A61K0031-365 [ICM,7]
       IPCI-2 A61K0031-34 [I,A]; A61K0031-343 [I,A]
       IPCR   A61K0031-34 [I,A]; A23L0001-30 [I,A]; A61K0031-343 [I,A];
              A61K0031-365 [I,A]; A61K0031-366 [I,A]; A61P0001-16 [I,A];
              C07D0307-88 [I,A]; C07D0311-76 [I,A]
EXF    549/283; 549/290; 549/307; 549/289; 514/457; 514/470
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 27 OF 27  USPAT2 on STN
AN     2003:64375  USPAT2
TI     Processes for extracting carotenoids and for preparing feed materials
IN     Kagan, Michael, Jerusalem, ISRAEL
       Braun, Sergei, Zur Hadassa, ISRAEL
PA     Fermentron Ltd., Jerusalem, ISRAEL (non-U.S. corporation)
PI     US 6818239          B2  20041116
AI     US 2002-172747          20020617 (10)
RLI    Continuation of Ser. No. WO 2000-IL846, filed on 18 Dec 2000
PRAI   GB 1999-30194           19991221
DT     Utility
FS     GRANTED
LN.CNT 501
INCL   INCLM: 426/429.000
       INCLS: 426/431.000; 426/478.000; 426/250.000; 426/253.000; 426/540.000
NCL    NCLM:  426/429.000; 426/250.000
       NCLS:  426/250.000; 426/253.000; 426/431.000; 426/478.000; 426/540.000
IPC    [7]
       IPCI   A23L0001-27 [ICM,7]
       IPCI-2 A23L0001-28 [ICM,7]; A23L0001-27 [ICS,7]
       IPCR   A23L0001-27 [I,A]; A23L0001-275 [I,A]; C07C0403-00 [I,A];
              C07C0403-24 [I,A]; C09B0061-00 [I,A]
EXF    426/807; 426/250; 426/253; 426/635; 426/425; 426/429; 426/430; 426/431;
       426/478; 426/540; 424/439; 424/451
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

```
=> d hist

     (FILE 'HOME' ENTERED AT 15:15:33 ON 03 JAN 2012)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
     AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
     CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
     DRUGU, EMBAL, EMBASE, ESBIOBASE, ...' ENTERED AT 15:15:47 ON 03 JAN 2012
               SEA KRILL AND OIL AND MEAL AND SUPERCRITICAL(P)EXTRACT? AND SOL
              ---------
              0*  FILE ADISNEWS
              0*  FILE ANTE
              0*  FILE AQUALINE
              0*  FILE BIOENG
              0*  FILE BIOTECHABS
              0*  FILE BIOTECHDS
              0*  FILE BIOTECHNO
              0*  FILE CEABA-VTB
              0*  FILE CIN
              0*  FILE FOMAD
              0*  FILE FROSTI
              0*  FILE FSTA
              1   FILE IFIPAT
              0*  FILE KOSMET
              0*  FILE NTIS
              0*  FILE PASCAL
             28   FILE USPATFULL
              4   FILE USPAT2
              0*  FILE WATER
              1   FILE WPIDS
              1   FILE WPINDEX
L1             QUE KRILL AND OIL AND MEAL AND SUPERCRITICAL(P)EXTRACT? AND SOL
              ---------

     FILE 'IFIPAT, USPATFULL, USPAT2' ENTERED AT 15:19:01 ON 03 JAN 2012
L2           33 S L1
L3           32 DUP REM L2 (1 DUPLICATE REMOVED)
L4           27 S L3 AND DIOXIDE

=> logoff
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                                  43.57      47.51

STN INTERNATIONAL LOGOFF AT 15:21:58 ON 03 JAN 2012



Connecting via Winsock to STN at pto-stn on port 23




Welcome to STN International!  Enter x:X

LOGINID:ssspt189dxw

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2
```

```
* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1  FEB  1  Instructor-led and on-demand STN training options available
                  from CAS
 NEWS  2  AUG 01  CA Sections Added to ACS Publications Web Editions
                  Platform
 NEWS  3  AUG 16  INPADOC: Coverage of German Patent Data resumed,
                  enhanced legal status
 NEWS  4  AUG 18  Upgrade now to STN Express, Version 8.5
 NEWS  5  SEP 01  CAS Journal Coverage Now Includes Ahead-of-Print
                  Articles for More Than 100 Journal Titles
 NEWS  6  SEP 01  Older Versions of STN Express to be Discontinued
                  Beginning in March 2012
 NEWS  7  SEP 09  USAN Database Updates Offer Superior Currency on STN(R)
 NEWS  8  SEP 26  STN Adds Canadian Patent Full-text Database - CANPATFULL
 NEWS  9  SEP 26  GEOREF and ENCOMPLIT databases were reloaded on
                  September 24, 2011.
 NEWS 10  SEP 26  Updates to the IFIPAT/IFIUDB/IFICDB databases have resumed.
 NEWS 11  SEP 26  ECLA Thesaurus in CA/CAplus Improves Patent Searching on STN
 NEWS 12  SEP 26  Access AUPATFULL and CANPATFULL databases with STN Viewer
 NEWS 13  OCT 26  New STN Revolutionizes Patent Searching for Professionals
 NEWS 14  DEC  1  CA/CAplus Now Includes Examiner Citations for Japanese Patents
 NEWS 15  DEC  1  CAS Expands Global Patent Coverage - Intellectual Property
                  Corporation of Malaysia Becomes 62nd Authority on CA/CAplus
 NEWS 16  DEC  5  STN on the Web Enhancements Include Compatibility with
                  Microsoft Windows 7
 NEWS 17  DEC 14  Removal of ITRD and PATIPC databases from STN
 NEWS 18  DEC 15  Rolled-up IPC Core Codes Removed from IPC Reclassifications in
                  Patent Databases on STN
 NEWS 19  JAN 12  Structure Graphics Have Been Added to Abstracts for
                  MARPAT and CA/CAplus on STN
 NEWS 20  JAN 15  Online Access to Very Large Chemical Structure Images
                  Enhanced on STN
 NEWS 21  JAN 26  IFICLS Updates Resume on STN
 NEWS 22  JAN 31  MEDLINE Reload - Updated MeSH Vocabulary and Two New
                  Fields on STN
 NEWS 23  FEB  1  INPADOC Databases Enhanced with Japanese Patent
                  Classifications, Current U.S. Classification and Japanese
                  Legal Status.
 NEWS 24  FEB  3  Access More Than 32,000 Harmonized Tariff Codes Now in
                  CHEMLIST on STN
 NEWS 25  FEB 13  PCTFULL Documents with Non-Latin Filing Language Enhanced
                  with English Machine Translations
 NEWS 26  FEB 28  REACH List of Registered Substances Now in CHEMLIST on STN
 NEWS 27  MAR 12  RTECS Database on STN Enhanced with Aquatic and In Vitro
                  Exposure Toxicity Data
 NEWS 28  MAR 12  MARPAT Database Enhanced with Additional Markush Backfile
                  Content for STN
 NEWS 29  MAR 19  The 1MOBILITY and 2MOBILITY databases were reloaded on
                  March 18, 2012
 NEWS 30  MAR 19  STN Adds Chinese Patent Full Text Database - CNFULL
 NEWS 31  MAR 19  Get the Content You Need Sooner with ePub Ahead of Print
                  Records Available in MEDLINE on STN!
 NEWS 32  MAR 30  NAPRALERT Updated with More Natural Products Information
 NEWS 33  APR  9  CAS Expands Global Patent Coverage - The Eurasian Patent
                  Organization Becomes 63rd Authority on CA/CAplus
 NEWS 34  APR 16  DWPI Database (WPINDEX, WPIDS, WPIX) Enhanced with
                  Numerical Property Search Feature
 NEWS 35  APR 23  RSS Delivery for STN Alerts (SDIs) is Now Available on STN
 NEWS 36  MAY  9  INIS, BABS and GMELIN97 Databases Removed from STN
 NEWS 37  MAY 21  Reload of FSTA Database
```

```
 NEWS 38  MAY 21  Search DOIs in CA/CAplus on STN
 NEWS 39  MAY 23  Get the Latest Version of STN Express, Version 8.5.1!

 NEWS EXPRESS 23 MAY 2012 CURRENT WINDOWS VERSION IS V8.5.1,
              AND CURRENT DISCOVER FILE IS DATED 19 APRIL 2012.

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS TRAINING Find instructor-led and self-directed training opportunities

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN customer
  agreement.  This agreement limits use to scientific research.  Use
  for software development or design, implementation of commercial
  gateways, or use of CAS and STN data in the building of commercial
  products is prohibited and may result in loss of user privileges
  and other penalties.


* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:50:46 ON 29 MAY 2012

=> index bioscience
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.24       0.24

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
       AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
       CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
       DRUGU, EMBAL, EMBASE, ESBIOBASE, ...' ENTERED AT 14:51:15 ON 29 MAY 2012

56 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s krill and oil and cook? and dry?(p)krill and krill(p)meal and
extract?(p)krill(p)oil
          0*  FILE ADISNEWS
          0*  FILE ANTE
          0*  FILE AQUALINE
          0*  FILE BIOENG
          0*  FILE BIOTECHABS
          0*  FILE BIOTECHDS
          0*  FILE BIOTECHNO
          0*  FILE CEABA-VTB
          0*  FILE CIN
  29 FILES SEARCHED...
          0*  FILE FOMAD
          0*  FILE FROSTI
          3   FILE IFIPAT
          0*  FILE KOSMET
          0*  FILE NTIS
          0*  FILE PASCAL
          9   FILE USPATFULL
  50 FILES SEARCHED...
          3   FILE USPAT2
          0*  FILE WATER
```

```
          4   FILE WPIDS
          4   FILE WPINDEX

   5 FILES HAVE ONE OR MORE ANSWERS,   56 FILES SEARCHED IN STNINDEX

L1   QUE KRILL AND OIL AND COOK? AND DRY?(P)KRILL AND KRILL(P)MEAL AND EXTRACT?
       (P)KRILL(P)OIL

=> file ifipat uspatfull uspat2
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       1.48       1.72

FILE 'IFIPAT' ENTERED AT 14:52:35 ON 29 MAY 2012
COPYRIGHT (C) 2012 IFI CLAIMS(R) Patent Services (IFI)

FILE 'USPATFULL' ENTERED AT 14:52:35 ON 29 MAY 2012
CA INDEXING COPYRIGHT (C) 2012 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPAT2' ENTERED AT 14:52:35 ON 29 MAY 2012
CA INDEXING COPYRIGHT (C) 2012 AMERICAN CHEMICAL SOCIETY (ACS)

=> s l1
L2          15 L1

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3           13 DUP REM L2 (2 DUPLICATES REMOVED)

=> d l3 1-13

L3   ANSWER 1 OF 13  IFIPAT  COPYRIGHT 2012 IFI on STN DUPLICATE 1
AN    12887434  IFIPAT;IFIUDB;IFICDB
TI    SOLVENT-FREE PROCESS FOR OBTAINING PHOSPHOLIPIDS AND NEUTRAL ENRICHED
      KRILL OILS
IN    Sclabos Katevas Dimitri (CL); Toro Guerra Raul R (CL); Chiong Lay Mario M
      (CL)
PA    THAROS LTD CL
      LONZA LTD CH
      (50035)
PI    US 20110224450  A1  20110915
AI    US 2011-96644       20110428  (13)
RLI   WO 2009-IB7269      20091030 CONTINUATION-IN-PART   PENDING
FI    US 20110224450      20110915
DT    Utility; Patent Application - First Publication
FS    CHEMICAL
      APPLICATION
ED    Entered STN: 21 Oct 2011
      Last Updated on STN: 13 Jan 2012
CLMN  25

L3   ANSWER 2 OF 13  USPAT2 on STN
AN     2007:272601  USPAT2
TI     Gels, gel composites, and gel articles
IN     Chen, John Y., Hillsborough, CA, UNITED STATES
PA     Applied Elastomerics, Inc., South San Francisco, CA, UNITED STATES (U.S.
       corporation)
PI     US 7930782          B2  20110426
AI     US 2007-810584          20070605 (11)
RLI    Continuation-in-part of Ser. No. US 2007-787257, filed on 12 Apr 2007,
       Pat. No. US 7661164 Continuation-in-part of Ser. No. US 2004-912464,
       filed on 4 Aug 2004, Pat. No. US 7226484 Continuation-in-part of Ser.
```

```
       No. US 2002-420489, filed on 21 Apr 2002, Pat. No. US 7222380
       Continuation-in-part of Ser. No. US 2003-420492, filed on 21 Apr 2003,
       Pat. No. US 7344568 Continuation-in-part of Ser. No. US 2000-721213,
       filed on 21 Nov 2000, Pat. No. US 6867253
DT     Utility
FS     GRANTED
LN.CNT 5886
INCL   INCLM: 005/655.500
       INCLS: 005/636.000; 005/652.000; 005/654.000; 005/909.000; 602/041.000;
              602/061.000; 602/062.000; 602/063.000; 623/016.110; 623/020.140;
              623/021.110; 623/023.400; 623/027.000; 623/033.000; 623/036.000;
              524/270.000; 524/284.000; 524/490.000; 524/491.000; 524/549.000;
              524/571.000; 524/575.000; 521/050.000; 521/054.000; 521/139.000;
              521/140.000; 521/148.000
NCL    NCLM:  005/655.500; 525/240.000
       NCLS:  005/636.000; 005/652.000; 005/654.000; 005/909.000; 521/050.000;
              521/054.000; 521/139.000; 521/140.000; 521/148.000; 524/270.000;
              524/284.000; 524/490.000; 524/491.000; 524/549.000; 524/571.000;
              524/575.000; 602/041.000; 602/061.000; 602/062.000; 602/063.000;
              623/016.110; 623/020.140; 623/021.110; 623/023.400; 623/027.000;
              623/033.000; 623/036.000
IPC    IPCI   C08L0023-16 [I,A]
       IPCI-2 B29C0067-20 [I,A]; B60R0021-26 [I,A]; A61F0002-80 [I,A];
              B60K0028-00 [I,A]; A47C0007-00 [I,A]
       IPCR   B29C0067-20 [I,A]; A47C0007-00 [I,A]; A61F0002-80 [I,A];
              B60K0028-00 [I,A]; B60R0021-26 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 3 OF 13  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
AI     US 2010-711822      A1  20100224 (12)
PRAI   US 2009-155758P         20090226 (61)
DT     Utility
FS     APPLICATION
LN.CNT 3112
INCL   INCLM: 514 2
NCL    NCLM:  514/005.500
       NCLS:  514/691.000
IPC    IPCI   A61K0038-02 [I,A]
       IPCR   A61K0038-02 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 4 OF 13  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 2009-155767P         20090226 (61)
       US 2007-968765P         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
IPC    IPCI   A61K0031-685 [I,A]; A23D0009-00 [I,A]; A23D0009-02 [I,A];
              A61K0009-48 [I,A]; A61P0009-00 [I,A]; A61P0019-00 [I,A];
              A61P0029-00 [I,A]
       IPCR   A61K0031-685 [I,A]; A23D0009-00 [I,A]; A23D0009-02 [I,A];
              A61K0009-48 [I,A]; A61P0009-00 [I,A]; A61P0019-00 [I,A];
              A61P0029-00 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 5 OF 13  IFIPAT  COPYRIGHT 2012 IFI on STN DUPLICATE 2
AN    12061067  IFIPAT;IFIUDB;IFICDB
TI    METHOD FOR MAKING KRILL MEAL
IN    Hostmark Oistein (NO); Tilseth Snorre (NO)
PA    Aker BioMarine ASA NO (79725)
PI    US 20090061067  A1  20090305
AI    US 2008-201325      20080829  (12)
PRAI  US 2007-968765P      20070829 (Provisional)
FI    US 20090061067      20090305
DT    Utility; Patent Application - First Publication
FS    CHEMICAL
      APPLICATION
ED    Entered STN: 10 Mar 2009
      Last Updated on STN: 9 Apr 2009
CLMN  51

L3   ANSWER 6 OF 13  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
AI     US 2008-57775       A1  20080328 (12)
PRAI   US 2007-920483P         20070328 (60)
       US 2007-975058P         20070925 (60)
       US 2007-983446P         20071029 (60)
       US 2008-24072P          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:  424/522.000
       NCLS:  426/601.000; 514/078.000; 514/114.000; 514/121.000
IPC    IPCI   A61K0035-56 [I,A]; A61K0031-661 [I,A]; A61K0031-685 [I,A];
              A61P0003-02 [I,A]; A23D0009-00 [I,A]; A61K0031-66 [I,A]
       IPCR   A61K0035-56 [I,A]; A23D0009-00 [I,A]; A61K0031-66 [I,A];
              A61K0031-661 [I,A]; A61K0031-685 [I,A]; A61P0003-02 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 7 OF 13  USPATFULL on STN
AN     2007:272601  USPATFULL
TI     Gels, gel composites, and gel articles
IN     Chen, John Y., Hillsborough, CA, UNITED STATES
PI     US 20070238835      A1  20071011
       US 7930782          B2  20110426
```

AI US 2007-810584 A1 20070605 (11)
RLI Continuation-in-part of Ser. No. US 2007-787257, filed on 12 Apr 2007, PENDING Continuation-in-part of Ser. No. US 2004-912464, filed on 4 Aug 2004, GRANTED, Pat. No. US 7226484 Continuation-in-part of Ser. No. US 2003-613567, filed on 2 Jul 2003, GRANTED, Pat. No. US 7093316 Continuation-in-part of Ser. No. US 2003-420489, filed on 21 Apr 2003, GRANTED, Pat. No. US 7222380 Continuation-in-part of Ser. No. US 2003-420487, filed on 21 Apr 2003, GRANTED, Pat. No. US 7193002 Continuation-in-part of Ser. No. US 2003-420488, filed on 21 Apr 2003, GRANTED, Pat. No. US 7134929 Continuation-in-part of Ser. No. US 2003-420490, filed on 21 Apr 2003, GRANTED, Pat. No. US 7105607 Continuation-in-part of Ser. No. US 2003-420491, filed on 21 Apr 2003, GRANTED, Pat. No. US 7093599 Continuation-in-part of Ser. No. US 2003-420492, filed on 21 Apr 2003, PENDING Continuation-in-part of Ser. No. US 2003-420493, filed on 21 Apr 2003, GRANTED, Pat. No. US 7067583 Continuation-in-part of Ser. No. US 2004-896047, filed on 22 Jul 2004, PENDING Continuation-in-part of Ser. No. US 2002-273828, filed on 17 Oct 2002, GRANTED, Pat. No. US 6909220 Continuation-in-part of Ser. No. US 2002-334542, filed on 31 Dec 2002, GRANTED, Pat. No. US 7159259 Continuation-in-part of Ser. No. US 2002-299073, filed on 18 Nov 2002, ABANDONED Continuation-in-part of Ser. No. US 2002-199364, filed on 20 Jul 2002, GRANTED, Pat. No. US 6794440 Continuation-in-part of Ser. No. US 2002-199361, filed on 20 Jul 2002, GRANTED, Pat. No. US 7134236 Continuation-in-part of Ser. No. US 2002-199362, filed on 20 Jul 2002, GRANTED, Pat. No. US 7208184 Continuation-in-part of Ser. No. US 2002-199363, filed on 20 Jul 2002, GRANTED, Pat. No. US 7108873 Continuation-in-part of Ser. No. US 2000-721213, filed on 21 Nov 2000, GRANTED, Pat. No. US 6867253 Continuation-in-part of Ser. No. US 1998-130545, filed on 8 Aug 1998, GRANTED, Pat. No. US 6627275 Continuation-in-part of Ser. No. US 1999-230940, filed on 3 Feb 1999, GRANTED, Pat. No. US 6161555 Continuation-in-part of Ser. No. US 1997-863794, filed on 27 May 1997, GRANTED, Pat. No. US 6117176
PRAI JP 2003-204428 20030731
WO 1994-US4278 19940419
WO 1994-US7314 19940627
DT Utility
FS APPLICATION
LN.CNT 5757
INCL INCLM: 525/240.000
NCL NCLM: 005/655.500; 525/240.000
NCLS: 005/636.000; 005/652.000; 005/654.000; 005/909.000; 521/050.000; 521/054.000; 521/139.000; 521/140.000; 521/148.000; 524/270.000; 524/284.000; 524/490.000; 524/491.000; 524/549.000; 524/571.000; 524/575.000; 602/041.000; 602/061.000; 602/062.000; 602/063.000; 623/016.110; 623/020.140; 623/021.110; 623/023.400; 623/027.000; 623/033.000; 623/036.000
IPC IPCI C08L0023-16 [I,A]
IPCI-2 B29C0067-20 [I,A]; B60R0021-26 [I,A]; A61F0002-80 [I,A]; B60K0028-00 [I,A]; A47C0007-00 [I,A]
IPCR B29C0067-20 [I,A]; A47C0007-00 [I,A]; A61F0002-80 [I,A]; B60K0028-00 [I,A]; B60R0021-26 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 8 OF 13 USPAT2 on STN
AN 2004:24434 USPAT2
TI Gelatinous food elastomer compositions and articles for use as fishing bait
IN Chen, John Y., Pacifica, CA, UNITED STATES
PA Applied Elastomerics, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)
PI US 7208184 B2 20070424
AI US 2002-199362 20020720 (10)

DT Utility
FS GRANTED
LN.CNT 4932
INCL INCLM: 426/001.000
INCLS: 043/042.000; 043/042.240; 424/084.000
NCL NCLM: 426/001.000
NCLS: 043/042.000; 043/042.240; 424/084.000
IPC IPCI A23L0001-00 [ICM,7]
IPCI-2 A23L0001-00 [I,A]
IPCR A23L0001-00 [I,A]; A01K0085-01 [I,A]; A01K0097-04 [I,A]
EXF 426/1; 043/42; 043/42.24; 424/84
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 9 OF 13 USPAT2 on STN
AN 2004:24385 USPAT2
TI Gelatinous food elastomer compositions and articles
IN Chen, John Y., Pacifica, CA, UNITED STATES
PA Applied Elastomerics, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)
PI US 7108873 B2 20060919
AI US 2002-199363 20020720 (10)
RLI Continuation-in-part of Ser. No. US 2001-721213, filed on 21 Nov 2001, Pat. No. US 6867253 Continuation-in-part of Ser. No. US 2001-896047, filed on 30 Jun 2001, PENDING Continuation-in-part of Ser. No. US 1999-421886, filed on 5 Oct 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-285809, filed on 1 Apr 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-274498, filed on 23 Mar 1999, Pat. No. US 6420475 Continuation-in-part of Ser. No. US 1998-130545, filed on 8 Aug 1998, Pat. No. US 6627275 Continuation-in-part of Ser. No. US 1997-984459, filed on 3 Dec 1997, Pat. No. US 6324703 Continuation-in-part of Ser. No. WO 1997-US17534, filed on 30 Sep 1997, Pat. No. WO 6161555 Continuation-in-part of Ser. No. US 1997-909487, filed on 12 Jul 1997, Pat. No. US 6050871 Continuation-in-part of Ser. No. US 1997-863794, filed on 27 May 1997, Pat. No. US 6117176 Continuation-in-part of Ser. No. US 1996-719817, filed on 30 Sep 1996, Pat. No. US 6148830 Continuation-in-part of Ser. No. US 1996-665343, filed on 17 Jun 1996, PENDING Continuation-in-part of Ser. No. US 1996-612586, filed on 8 Mar 1996, Pat. No. US 6552109 Continuation-in-part of Ser. No. US 1995-581191, filed on 29 Dec 1995, Pat. No. US 5760117 Continuation-in-part of Ser. No. US 1995-581188, filed on 29 Dec 1995, ABANDONED Continuation-in-part of Ser. No. US 1995-581125, filed on 29 Dec 1995, Pat. No. US 5962572 Continuation-in-part of Ser. No. US 1994-288690, filed on 11 Aug 1994, Pat. No. US 5633286 Continuation-in-part of Ser. No. WO 1994-US7314, filed on 27 Jun 1994, Pat. No. WO 5868597 Continuation-in-part of Ser. No. WO 1994-US4278, filed on 19 Apr 1994, Pat. No. WO 6033383
DT Utility
FS GRANTED
LN.CNT 3521
INCL INCLM: 426/001.000
INCLS: 426/573.000; 524/505.000
NCL NCLM: 426/001.000; 424/439.000
NCLS: 426/573.000; 524/505.000
IPC IPCI A61K0047-00 [ICM,7]
IPCI-2 A01K0097-04 [I,A]; A23L0001-05 [I,A]
IPCR A01K0097-04 [I,A]; A23L0001-05 [I,A]; A23L0001-317 [I,A]; A23L0001-325 [I,A]; A61K0047-00 [I,A]
EXF 524/505; 424/486; 426/1; 426/648; 426/656; 426/534; 426/555; 426/573
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 10 OF 13 IFIPAT COPYRIGHT 2012 IFI on STN
AN 04308583 IFIPAT;IFIUDB;IFICDB

TI Protein and lipid sources for use in aquafeeds and animal feeds and a process for their preparation; Subjecting oilseed to heat treatment to reduce concentration of antinutritional components to obtain heat-treated seed; dehulling seed to produce a meat fraction, a hull fraction or a mixture; cold pressing to obtain plant oils andm meals
IN Shand Ian (CA); Cairns Robert E (CA); Higgs David (CA)
PA Canada Fisheries and Oceans Minister of CA (51835)
PI US 6955831 B2 20051018 (CITED IN 002 LATER PATENTS)
    US 20030072866 A1 20030417
AI US 2002-76499 20020219 (10)
RLI US 2000-566728 20000509 CONTINUATION-IN-PART ABANDONED
PRAI CA 2001-2334745 20010213
    WO 2001-CA663 20010508
    CA 2001-2351903 20010626
FI US 6955831 20051018
    US 20030072866 20030417
DT Utility; Granted Patent - Utility, with Pre-Grant Publication
FS CHEMICAL
    GRANTED
ED Entered STN: 19 Oct 2005
    Last Updated on STN: Jan 2011
MRN 012837 MFN: 0842
CLMN 32

L3 ANSWER 11 OF 13 USPATFULL on STN
AN 2004:24434 USPATFULL
TI Gelatinous food elastomer compositions and articles for use as fishing bait
IN Chen, John Y., Pacifica, CA, UNITED STATES
PI US 20040018272 A1 20040129
    US 7208184 B2 20070424
AI US 2002-199362 A1 20020720 (10)
DT Utility
FS APPLICATION
LN.CNT 4354
INCL INCLM: 426/001.000
NCL NCLM: 426/001.000
    NCLS: 043/042.000; 043/042.240; 424/084.000
IPC [7]
    IPCI A23L0001-00 [ICM,7]
    IPCI-2 A23L0001-00 [I,A]
    IPCR A23L0001-00 [I,A]; A01K0085-01 [I,A]; A01K0097-04 [I,A]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 12 OF 13 USPATFULL on STN
AN 2004:24385 USPATFULL
TI Gelatinous food elastomer compositions and articles
IN Chen, John Y., Pacifica, CA, UNITED STATES
PI US 20040018223 A1 20040129
    US 7108873 B2 20060919
AI US 2002-199363 A1 20020720 (10)
DT Utility
FS APPLICATION
LN.CNT 3229
INCL INCLM: 424/439.000
NCL NCLM: 426/001.000; 424/439.000
    NCLS: 426/573.000; 524/505.000
IPC [7]
    IPCI A61K0047-00 [ICM,7]
    IPCI-2 A01K0097-04 [I,A]; A23L0001-05 [I,A]
    IPCR A01K0097-04 [I,A]; A23L0001-05 [I,A]; A23L0001-317 [I,A];
          A23L0001-325 [I,A]; A61K0047-00 [I,A]

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 13 OF 13 USPATFULL on STN
AN 2003:165578 USPATFULL
TI Process for making dried powdery and granular krill
IN Yoshitomi, Bunji, Tokyo, JAPAN
Shigematsu, Yoshiaki, Tokyo, JAPAN
PA NIPPON SUISAN KAISHA, LTD., Tokyo, JAPAN (non-U.S. corporation)
PI US 20030113432 A1 20030619
AI US 2002-283063 A1 20021030 (10)
RLI Continuation of Ser. No. US 2001-807953, filed on 25 Apr 2001, PENDING
PRAI JP 1998-311730 19981102
DT Utility
FS APPLICATION
LN.CNT 481
INCL INCLM: 426/643.000
NCL NCLM: 426/643.000
IPC [7]
IPCI A23L0001-325 [ICM,7]
IPCR A23B0004-03 [I,A]; A23L0001-325 [I,A]; A23L0001-326 [I,A];
A23L0001-33 [I,A]

=> d l3 13 kwic

L3 ANSWER 13 OF 13 USPATFULL on STN
TI Process for making dried powdery and granular krill
AB A dried powdery and granular krill product containing all components of krill. The proteolytic enzymes originally contained in krill materials are perfectly disabled. The product is produced by a process including only heating as means for denaturing protein and disabling the proteolytic enzymes originally contained in krill materials. The product is produced by a process including no chemicals treatment to remove water and disable or inactivate the proteolytic enzymes in any production steps, and generating no wastewater. The production process comprises the steps of lightly dehydrating krill, coarsely crushing the krill, and drying the coarsely crushed krill under heating. Thus, water is removed from the krill by only heating, and degradation of the lipid in the krill product is prevented without using an anti-oxidant. Application fields are enlarged and the preservation characteristic is improved. The so-called zero-emission. . .
SUMM [0002] The present invention relates to a dried powdery and granular krill product which contains all components of krill and in which lipid degradation is sufficiently prevented with no need of an anti-oxidant.
SUMM [0004] Krill are animal plankton living primarily in the Arctic and Antarctic Oceans, and about 80 kinds of krill have been known up to date. Of those many kinds of krill, Antarctic Krill (Euphasia superba) living in the Antarctic Ocean are found in abundance as one of natural resources. Therefore, survey of the resource and development of the method of catching the krill have been extensively conducted in the period of 1970 to 1985, including studies for developing methods of processing the krill to be useful in practical applications.
SUMM [0005] Krill are comparable to fish, flesh and fowl in point of nutritive value, but there are several problems in processing the krill for practical applications. One of the problems is that krill lose freshness in short time. If krill are left to stand after being caught, the heads and chests of the krill start changing into black color in 1-2 hours even at a low atmospheric temperature of about 0° C. Further, shells of the heads and chests of krill are so vulnerable to external pressure that the krill are easily broken down upon impacts applied at the time of catching, whereupon the enzymes

present in the internal organs flow out and decompose muscles. Those phenomena occur under actions of the enzymes present in krill. It is thought that tyrosinase is responsible for the former color-changing phenomenon, and protease is responsible for the latter muscle-decomposing. . .

SUMM [0006] Accordingly, those enzymes require to be disabled or inactivated when processing krill. In other words, it has been required immediately after catching krill to quickly freeze the krill down to below -40° C., thereby inactivating the enzymes, or to heat the krill up to above 80° C., thereby disabling the enzymes, followed by preserving the krill.

SUMM [0007] Known krill products include raw frozen and peeled krill products which are subjected to quick freezing and then preserved in a frozen condition, boiled krill products which are heated and then preserved in a frozen condition, and krill meal which is heated and dried and then preserrved at the normal temperature. The following Tables 1 and 2 list classifications of those products depending on how krill are processed, and features and points to be improved of the products.

SUMM . . . Japan, the product price greatly depends on the transportation cost. There is hence a desire for extracting excellent characteristics of krill more efficiently and realizing krill products having a higher value added.

TABLE 1

Processing	Processing Object	Product Examples
Quick freezing, Preserve in frozen condition	Inactivate enzymes	Raw frozen and stripped krill
Heating, Preserve in frozen condition	Disable enzymes	Boiled krill
Heating & drying, Preserve at normal temperature	Disable enzymes	Krill meal

SUMM . . . Points to be improved

Raw frozen and stripped krill	Products have flavor, taste and feeling of raw krill.	Remaining high water content and activity of enzymes necessitate storage and distribution in frozen state. Enzymes are activated upon thawing and product quality degrades. Drips flow out.
Boiled krill	Heating disables enzymes and makes protein stable to give meat-like feeling.	Flavor and taste components flow out during boiling. Cold chain is required because of high water content.
Krill meal	Heating disables enzymes and makes protein stable. Meal can be stored at normal temp. because of low water content.	Digestibility lowers due to protein denaturation during heating. Water-soluble components flow out into stickwater.

SUMM [0010] Japanese Unexamined Patent Publication No. 57-11876 discloses a method of impeding activity of the proteolytic enzymes in krill and utilizing the krill as protein materials. With the disclosed method, a krill paste is degenerated with alcohol to effect fixation (denaturation) of protein and degeneration of the enzymes at the same time. The processed krill paste is then washed with water to remove alcohol. The disclosed method however has the following problems.

SUMM [0013] 3. Polar lipid is removed together with alcohol during washing with water. Most of the lipid in krill is phospholipid and is rich in polyunsaturated fatty acids (PUFAs). Thus these PUFAs are removed.

SUMM . . . square. The shrimp materials thus processed are dried under heating to thereby provide dried shrimp granules. Considering specific properties of krill, however, it is inferred that even if krill are dried under heating after being processed in a similar manner as in the prior art, ground krill are very difficult to dry into a satisfactory condition.

SUMM [0018] From intensive studies, the inventors found that when krill are processed in a similar manner as in the prior art, lipid, protein and water contained in the krill are brought into an emulsified state, and the processed krill are very difficult to dry even with a heating and drying machine. Such a difficulty is related to the fact that most of the lipid in krill is phospholipid, as described above, and therefore emulsification is further increased. In other words, water in the krill is stabilized in structure with emulsification and becomes still harder to evaporate under heating.

SUMM [0019] In addition, when krill are crushed into the form of ground meat, the proteolytic enzymes present in the internal organs of the krill develop activity, and a temperature rise during the grinding process increases the activity of those enzymes. As a consequence, proteolysis in the krill is promoted and specific taste is deteriorated.

SUMM [0021] An object of the present invention is therefore to effectively utilize krill as one of valuable aquatic resources, and to provide a dried powdery and granular krill product and a method of producing the dried powdery and granular krill product, which contains all components of krill and has a good preservation ability while activity of the enzymes in the krill is totally disabled.

SUMM [0022] The present invention resides in a dried powdery and granular krill product that contains all components of krill. Because of containing all components of krill, the present product has a function capable of sufficiently preventing degradation of the lipid in the krill product without using an anti-oxidant. In the dried powdery and granular krill product, the proteolytic enzymes originally contained in krill materials are perfectly disabled. Accordingly, the present invention also resides in a dried powdery and granular krill product which contains all components of krill and in which the proteolytic enzymes originally contained in krill materials are perfectly disabled. The present product is produced by a process including only heating as means for denaturing protein and disabling the proteolytic enzymes originally contained in krill materials. Accordingly, the present invention further resides in a dried powdery and granular krill product which contains all components of krill, in which the proteolytic enzymes originally contained in krill materials are perfectly disabled, and which is produced by a process including only heating as means for denaturing protein and disabling the proteolytic enzymes originally contained in krill materials.

SUMM [0023] The dried powdery and granular krill product of the present invention is produced by a process including no chemicals treatment to remove water and disable or. . . the proteolytic enzymes in any production steps, and generating no wastewater. The production process comprises the steps of lightly dehydrating krill, coarsely crushing the krill, and drying the coarsely crushed krill under heating.

SUMM [0024] The dried powdery and granular krill product of the present invention is subjected to no chemical treatment using chemicals, etc. in any production steps, and is. . . Also, there is no step in the production process in which wastewater is generated. Thus, water is removed from the krill by only heating. Moreover, application fields are enlarged and the preservation characteristic is improved. The so-called zero-emission method and product,. . .

SUMM [0025] The production method of the present invention comprises steps of

removing seawater from krill, coarsely crushing the krill, and drying the coarsely crushed krill under heating. In the conventional process of producing krill meal, krill are first boiled in water in the same amount as the krill, and are then subjected to separation into solid and liquid components. The solid component is heated and dried using a. . . drier. The liquid component obtained from the solid/liquid separation is called stickwater and preserved separately. For this reason, the conventional krill meal contains less water-soluble components than the krill product of the present invention, and therefore has disadvantages in not providing satisfactory flavor and taste in the extracted form,. . . the conventional production process is disadvantageous in that protein is excessively denatured by heating applied in both the boiling and heating/drying steps, and digestibility of the product is reduced.

DRWD [0026] FIG. 1 is a graph showing activity of the proteolytic enzymes remaining in raw krill and the product of the present invention; and

DETD [0028] There are 80 or more kinds of krill as described above, but the kind of krill used in the present invention is not restricted. In addition to krill, mysids are also usable.

DETD [0029] Krill primarily used in an embodiment are Antarctic Krill (Euphasia superba) which have been employed in industrial fields.

DETD [0031] Krill used as materials are put into a fish tank at once after being caught. The krill are then put in a dehydrator to remove seawater, etc. attaching to the krill surfaces. The type of the dehydrator is not particularly restricted, but outer shells of krill are so fragile that the shells are easily broken down under pressure of 40-140 g/cm.sup.2 and the internal components flow. . . Therefore, the type of the dehydrator is preferably selected so that an excessive physical load will not be applied to krill.

DETD [0032] The dehydrated krill are chopped to improve thermal efficiency in the heating and drying process. The type of a machine used for chopping the krill is not particularly restricted. The grain size of the chopped krill is selected to a coarsely crushed state, i.e., about 1.5-2.5 cm square, at which outer shells and muscular tissues of the krill materials remain. This process can be performed with, e.g., a known mincing apparatus, which is usually employed for grinding meat. . .

DETD [0033] The chopped krill are dried under heating. The type of a machine for use in this process is also not particularly restricted. While a known heating and drying machine such as a steam type disk dryer, for example, can be used, the machine is preferably adjustable in heating time, heating temperature, degree of agitation, and so forth. Because the internal components of krill as one of natural resources change depending on the season, it is desired to adjust the parameters of the machine in match with the change of the internal components of krill for obtaining products with constant quality.

DETD [0034] The heating time and the heating temperature are set to such an extent that the muscular protein of krill and the proteolytic enzymes in krill are denatured and degenerated under heating, and that the water content is reduced down to below 10% from a point of ensuring good preservation. It is important that the heating and drying process is not performed at overly high temperatures and for an overly long time, and is performed at the necessary. . . values to satisfy the above-described conditions. Excessive heating lowers digestibility due to extreme denaturation, reduces astaxanthin, natural dye, present in krill, reduces vitamins, and oxidizes lipid. On the other hand, if heating is insufficient, activity of the proteolytic enzymes in krill remains, which leads to a deterioration of product quality. If the water content is over ten and several percents, the krill product gathers mold during preservation.

DETD [0035] The dried krill are very fragile, including the shells, and therefore can be easily crushed any desired grain size.

DETD [0036] The krill product of the present invention can be used as a main material of feed for cultured fish in place of. . .

DETD . . . above in connection with the prior art is attributable to crushing of raw materials into the form of ground meat, krill materials are first chopped into pieces having a size of 20-30% of the body length (about 1.5-2.5 cm square) and are then put into a heating and drying machine in the present invention. As a result, the krill materials are avoided from being emulsified and the drying efficiency is enhanced. Further, strong activity of the proteolytic enzymes present in the internal organs of krill is suppressed and an adverse influence upon flavor and taste of the krill product is reduced. In addition, the chopped krill do not adhere to the heating surface and can be heated appropriately, thus greatly contributing to improvement of product quality.

DETD [0038] Moreover, since the dried krill product obtained in accordance with the method of the present invention has a large grain size and maintains a fair part of shapes of the krill materials, it is also possible to produce products utilizing the shapes of the krill materials advantageously. Additionally, the dried krill can be simply crushed into a desired grain size as required.

DETD [0040] FIG. 1 shows comparatively activity of the proteolytic enzymes remaining in raw krill and the krill product of the present invention.

DETD . . . as a substrate. As will be seen from FIG. 1, the activity of the remaining proteolytic enzymes in the raw krill is increased with lapse of the reaction time, while the activity of the remaining proteolytic enzymes in the krill product of the present invention is hardly changed. This suggests that the proteolytic enzymes remain not alive in the krill product of the present invention and they are perfectly disabled in the production process, and that a possibility of quality deterioration of the krill product during the preservation is low.

DETD [0042] Preservation characteristics of the krill product of the present invention will be described with reference to Tables 3 and 4 below.

DETD [0043] For comparison, the results listed in Table 3 were obtained by preparing two groups of the krill product of the present invention, in one of which ethoxyquin that is most generally used as an anti-oxidant in meal, etc. was added to the krill product and in the other of which no ethoxyquin was added, and then measuring a change of product quality by. . .

DETD [0045] There are several indexes indicating a degree of lipid degradation. About the lipid in krill, particularly, the krill lipid having been extracted and refined, it is known that, during the preservation, a peroxide value hardly increases and only a carbonyl value increases. In other words, it is pointed out that degradation of the krill lipid differs in creation of oxides and progress rate of the decomposing reaction from those in general fish oil, etc.

TABLE 3

Acid value					
	with	Peroxide value		Carbonyl value	
no anti-oxidant	anti-oxidant	with		with	
		no. . .			

DETD . . . from Table 4, a phenomenon of the lipid degrading at apparently different rates during the preservation was found between the krill product of the present invention and a control prepared by perfectly removing all the water-soluble components originally present in krill from the krill product of the present invention. Although the material responsible for the above phenomenon is not yet known, it is believed that the water-soluble components originally present in krill have some anti-oxidizing action. For this reason, in the krill product of

the present invention which contains all the components of krill in an enriched condition, lipid degradation can be prevented satisfactorily without using any anti-oxidant.

TABLE 4

Peroxide value Carbonyl. . .

DETD [0048] 1. Process Flow Including Plant for Drying Krill

DETD [0049] An outline of the process flow is as shown in FIG. 2. Krill materials are first conveyed by a krill supply apparatus from a fish tank to a material tank, and are then supplied to a dehydrator in a proper lot. The use of a dehydrator basically intends to remove seawater contained in the krill materials. Since it is expected that the amount of water contained in krill varies depending on the materials, a diaphragm is adjusted to provide a proper dehydration rate, taking into account the performance. . . are then supplied to a drier. The materials are boiled in the drier under heating with vapor, followed by further drying. At the time when reaching a predetermined water content, the drying is stopped and a resulting dried semifinished product is ejected. The dried semifinished product is conveyed to a product tank,. . .

DETD [0050] The conventional production process for krill meal is represented by raw krill→boiling→centrifugal separation or solid/liquid separation→extraction of solid→ drying→crushing→packaging. The liquid component was removed in the centrifugal separation step, and the useful components of krill contained in the liquid component were discarded. It can be said from one aspect that the krill meal was a product resulted from drying the sludge.

DETD [0051] By contrast, the process flow for producing the krill product of the present invention is represented by raw krill→removal of water attached to krill→boiling→ drying→crushing →packaging. The centrifugal separation step is not included. In the boiling and drying steps, the enzymes in krill are disabled and the krill components are stabilized through thermal degeneration. Thus, the components originally contained in the krill are all kept in the product without being discarded externally. An apparatus for implementing the above process is featured in omitting a step of squeezing boiled krill using a decanter or a press. The krill drying apparatus used in the present invention differs from the conventional meal producing apparatus in that a cooker and a drier are combined in an integral structure.

DETD [0053] Table 5 lists component analytical values of the krill product of the present invention. For comparison, Table 5 also lists component analytical values of the krill meal produced by the conventional process. In particular, the krill product of the present invention contains free amino acids as much as more than twice the amount contained in the conventional krill meal. The free amino acids deeply take part in developing flavor and taste of the product when eaten, attractant of feed. . .

DETD [0054] Since the squeezing step subsequent to boiling of the krill materials is omitted, the components developing flavor and taste are not lost and the krill product of the present invention has good flavor. Further, the production process of the present invention generates no appreciable wastewater and provides a high yield.

TABLE 5

	Krill meal	Product of invention
Water	6.5	8.3

Coarse protein 64.0 65.1
(Free amino acid) (2.9) (7.54)
Coarse fat 7.0 7.0
Coarse. . .

DETD [0055] According to the present invention, a method is provided which can effectively utilize krill, as one of important aquatic resources, in a perfect manner without any loss due to efflux of krill components. The dried powdery and granular krill product obtained by the present invention contains all the components originally contained in the krill, and strong activity of the enzymes specific to the krill is disabled. Therefore, the krill product of the present invention can be widely applied to not only the feed industry, but also the food industry.

CLM What is claimed is:
1. A dried powdery and granular krill product containing all components of krill.

CLM What is claimed is:
2. A dried powdery and granular krill product according to claim 1, wherein the proteolytic enzymes originally contained in krill materials are perfectly disabled.

CLM What is claimed is:
3. A dried powdery and granular krill product according to claim 1 or 2, wherein said product is produced by a process including only heating as means for denaturing protein and disabling the proteolytic enzymes originally contained in krill materials.

CLM What is claimed is:
4. A dried powdery and granular krill product according to claim 1, 2 or 3, wherein said product is produced by a process including no chemicals treatment. . .

CLM What is claimed is:
5. A dried powdery and granular krill product according to any one of claims 1 to 4, wherein said product is produced by a process comprising the steps of lightly dehydrating krill, coarsely crushing the krill, and drying the coarsely crushed krill under heating.

=> d hist

(FILE 'HOME' ENTERED AT 14:50:46 ON 29 MAY 2012)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGU, EMBAL, EMBASE, ESBIOBASE, ...' ENTERED AT 14:51:15 ON 29 MAY 2012
SEA KRILL AND OIL AND COOK? AND DRY?(P)KRILL AND KRILL(P)MEAL A

0* FILE ADISNEWS
0* FILE ANTE
0* FILE AQUALINE
0* FILE BIOENG
0* FILE BIOTECHABS
0* FILE BIOTECHDS
0* FILE BIOTECHNO
0* FILE CEABA-VTB
0* FILE CIN
0* FILE FOMAD
0* FILE FROSTI
3 FILE IFIPAT

```
                0*  FILE KOSMET
                0*  FILE NTIS
                0*  FILE PASCAL
                9   FILE USPATFULL
                3   FILE USPAT2
                0*  FILE WATER
                4   FILE WPIDS
                4   FILE WPINDEX
L1               QUE KRILL AND OIL AND COOK? AND DRY?(P)KRILL AND KRILL(P)MEAL A
                 ---------

     FILE 'IFIPAT, USPATFULL, USPAT2' ENTERED AT 14:52:35 ON 29 MAY 2012
L2            15 S L1
L3            13 DUP REM L2 (2 DUPLICATES REMOVED)

=> logoff
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                                      29.49      31.21

STN INTERNATIONAL LOGOFF AT 14:55:32 ON 29 MAY 2012



Connecting via Winsock to STN at pto-stn on port 23



Welcome to STN International!  Enter x:X

LOGINID:ssspt189dxw

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1  JAN 29  Instructor-led and on-demand STN training options available
                  from CAS
 NEWS  2  MAY 27  Get the Latest Version of STN Express, Version 8.5.2.1,
                  Available May 2014
 NEWS  3  JAN 09  Updated Enzyme Nomenclature Improves Access to Biological
                  Information in CAS REGISTRY
 NEWS  4  JAN 09  DEFULL - German (Deutschland, DE) Patents Full-text Database
                  New on STN
 NEWS  5  JAN 27  STN on the Web Now Compatible with Microsoft Windows 8.1 and
                  current Versions of Internet Explorer and Google Chrome
 NEWS  6  JAN 27  Annual MEDLINE Reload on STN Introduces New Searching
                  Capabilities and the Updated 2014 MeSH Thesaurus
 NEWS  7  FEB 03  DWPI: Latest Manual Code Revision goes live
 NEWS  8  FEB 03  DWPI: New coverage of Singapore PCT-transfers and grants
 NEWS  9  FEB 24  INFULL and DEFULL databases Now Available via STN Viewer
 NEWS 10  MAR 28  New STN Platform Enhancements Available, Increase Efficiency
                  of Search Workflow.
 NEWS 11  APR 25  New Format Adopted for Taiwanese Granted Patent Numbers
                  in CAS Databases and INPADOC.
 NEWS 12  MAY 2   New STN Global Value Pricing Empowers You to Maximize the
                  Value of STN
```

```
 NEWS 13  MAY 9   STN AnaVist, Version 2.1, Improves Operating System
                  Compatibility and Performance
 NEWS 14  MAY 19  Availability of Digital Object Identifiers (DOIs) Enhanced in
                  STN Databases
 NEWS 15  MAY 20  New Cluster NPS available for all Databases with the Numeric
                  Property Search feature
 NEWS 16  MAY 29  CAS REGISTRY BLAST Upgrade Improves Search Capabilities and
                  Results Ranking
 NEWS 17  JUN 10  MEDLINE on STN Now Updated Daily
 NEWS 18  JUL 1   CHEMCATS (Chemical Catalogs Online) on STN Enhanced with New
                  Search and Display Fields and More Frequent Updates
 NEWS 19  JUL 24  Batch search results for DGENE, USGENE and PCTGEN now
                  available for 30 days
 NEWS 20  JUL 28  Latest release of new STN now available, expands global
                  patent coverage and enhances search capabilities
 NEWS 21  SEP 4   KRFULL: New Full-text Database for Korean Patent
                  Publications Now Available on new STN
 NEWS 22  OCT 1   Cooperative Patent Classification (CPC) Combination Set Data
                  Now Available in CAplus, INPADOCDB and USPAT Databases
 NEWS 23  OCT 23  CPC Thesaurus based on official CPC Scheme
 NEWS 24  DEC 22  2015 MeSH Thesaurus Installed in MEDLINE with a Special
                  Message for Customers Doing Pharmacovigilance Research
 NEWS 25  DEC 24  CAS Expands Coverage of Reactions from Dissertations in
                  CASREACT
 NEWS 26  DEC 24  Additional Experimental Spectra Now Available in CAS REGISTRY
                  in STN
 NEWS 27  JAN 8   Latest Version of Emtree Introduces 937 New Terms
 NEWS 28  JAN 9   Derwent World Patents Index: Latest Manual Code Revision
                  Goes Live
 NEWS 29  JAN 26  Revision of DWPI Fragmentation Codes for 2015
 NEWS 30  JAN 26  Annual MEDLINE Reload on STN Features Enhanced Clinical Trial
                  Information and the 2015 MeSH Thesaurus
 NEWS 31  MAR 23  Enhanced Coverage of Latin America (AR, MX) in Derwent World
                  Patent Index

 NEWS EXPRESS 27 MAY 2014 CURRENT WINDOWS VERSION IS V8.5.2.1,
              AND CURRENT DISCOVER FILE IS DATED 26 JANUARY 2015.

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS TRAINING Find instructor-led and self-directed training opportunities

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN customer
  agreement.  This agreement limits use to scientific research.  Use
  for software development or design, implementation of commercial
  gateways, or use of CAS and STN data in the building of commercial
  products is prohibited and may result in loss of user privileges
  and other penalties.

* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:09:18 ON 02 APR 2015

=> index bioscience
FILE 'WPIDS' ACCESS NOT AUTHORIZED
FILE 'WPINDEX' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.25       0.25
```

```
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 13:09:33 ON 02 APR 2015

46 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s krill and meal and krill(p)oil and cook? and delip? and extract? and
polar(p)solvent and ether(p)phospholipids
          0*  FILE ADISNEWS
          0*  FILE BIOTECHABS
          0*  FILE BIOTECHDS
          0*  FILE BIOTECHNO
  10 FILES SEARCHED...
          0*  FILE CEABA-VTB
          0*  FILE CIN
          0*  FILE FOMAD
          0*  FILE FROSTI
          0*  FILE KOSMET
          0*  FILE NTIS
  33 FILES SEARCHED...
          0*  FILE PASCAL
          7   FILE USPATFULL
          1   FILE USPAT2

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

L1   QUE KRILL AND MEAL AND KRILL(P) OIL AND COOK? AND DELIP? AND EXTRACT? AND
        POLAR(P) SOLVENT AND ETHER(P) PHOSPHOLIPIDS

=> file uspatfull uspat2
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     2.55       2.80

FILE 'USPATFULL' ENTERED AT 13:11:32 ON 02 APR 2015
CA INDEXING COPYRIGHT (C) 2015 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPAT2' ENTERED AT 13:11:32 ON 02 APR 2015
CA INDEXING COPYRIGHT (C) 2015 AMERICAN CHEMICAL SOCIETY (ACS)

=> s L1
L2           8 L1

=> dup rem L2
PROCESSING COMPLETED FOR L2
L3            8 DUP REM L2 (0 DUPLICATES REMOVED)

=> d L3 1-8

L3   ANSWER 1 OF 8  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
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:  424/522.000; 424/451.000
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]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 2 OF 8  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
PI     US 20150004223      A1  20150101
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:  424/451.000
       NCLS:  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]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 3 OF 8  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.

L3   ANSWER 4 OF 8  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
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.

L3   ANSWER 5 OF 8  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
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 6 OF 8  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]

L3   ANSWER 7 OF 8  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.

L3   ANSWER 8 OF 8  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
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:  424/522.000
       NCLS:  426/601.000; 514/078.000; 514/114.000; 514/121.000
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
IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [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.


=> d hist

     (FILE 'HOME' ENTERED AT 13:09:18 ON 02 APR 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 13:09:33
     ON 02 APR 2015
               SEA KRILL AND MEAL AND KRILL(P)OIL AND COOK? AND DELIP? AND EXT
               ---------
               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
                0*  FILE KOSMET
                0*  FILE NTIS
                0*  FILE PASCAL
                7   FILE USPATFULL
                1   FILE USPAT2
L1               QUE KRILL AND MEAL AND KRILL(P) OIL AND COOK? AND DELIP? AND EX
                 ---------

     FILE 'USPATFULL, USPAT2' ENTERED AT 13:11:32 ON 02 APR 2015
L2              8 S L1
L3              8 DUP REM L2 (0 DUPLICATES REMOVED)

=> logoff
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                                  14.96      17.76

STN INTERNATIONAL LOGOFF AT 13:12:58 ON 02 APR 2015
```

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.
12/057,775	03/28/2008	Inge Bruheim	AKBM-14409/US-5/ORD	1945

72960 7590 02/20/2015

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

EXAMINER
WARE, DEBORAH K

ART UNIT	PAPER NUMBER
1651	

MAIL DATE	DELIVERY MODE
02/20/2015	PAPER

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.

Office Action Summary	Application No. 12/057,775	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 *9/7/2012*.

☐ 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) *1-50 and 52-90* is/are pending in the application.

5a) Of the above claim(s) *1-49 and 56-90* is/are withdrawn from consideration.

6)☐ Claim(s) *50 and 52-54* is/are allowed.

7)☒ Claim(s) *55* 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

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on September 7, 2012, has been entered.

Claims 1-50 and 52-90 are pending.

Response to Amendment

The Amendments filed September 7, 2012, were received and entered. Claims 50 and 52-55 are considered on the merits.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted after the filing of the RCE have been received.

Election/Restrictions

Applicant's election without traverse of Group VIII, claims 50-55, 51, now canceled so remaining elected, claims 50 and 52-55, original election in the reply filed on October 31, 2011, and was acknowledged.

Claims 1-49 and 56-90 are hereby withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on October 31, 2011.

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.

Claim 55 is 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 previously enclosed PTO-1449 Form or cited US 2003/0113432 (US), cited on previously enclosed PTO-892 form.

Claim drawn to 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 krill parts and is storable. The extracting is carried out by supercritical extraction. An oil is produced by the method.

US teaches Krill lipid (e.g. krill oil) at [0045], lines 1-8. An apparatus comprising a cooker and a drier is disclosed at [0051], at page 5, col. 1, lines 1-2. Heating which is akin to cooking is disclosed at page 5, line 17 at col. 2. Cooking and drying step is disclosed at [0051], lines 1-12, to provide dried krill meal. Extraction of Krill oil is disclosed at [0045], line 3. US clearly teaches that the krill meal contains all of its components and is not extracted and since it is dried it can inherently be stored before extraction.

The claims are identical to the abstract and US 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. US clearly teaches cooking and drying and extraction is disclosed as well which will be carried out on a prepared product having all the contents including oil or lipid. The krill lipid or oil is not different than any krill oil or lipid as disclosed in the art or Applicants have not shown a single difference. The krill oil as claimed must be different than the oil or lipid as disclosed, no matter how it is prepared. Krill meal can be stored before it is desired to extract an oil therefrom. The claims are anticipated by the cited references.

Response to Arguments

Applicant's arguments filed September 7, 2012, have been fully considered but they are not persuasive. The argument that there are specified phospholipid content is noted, however, the krill oil is only claimed to contain phospholipids. The JP abstract clearly

teaches that krill oil is extracted from treated krill shells and will contain phospholipids. No specified amounts are claimed in claim 55. US teaches [0002] that the krill product contains all components of krill. Thus, phospholipids will inherently be present in krill oil. No matter how the krill oil is made it will contain phospholipids. The composition of claim 55 is not limited necessarily to the lipid content of defined by claim 50.

Claim 55 fails 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 claim is 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.

Claims 50 and 52-54 are allowed.

All claims are drawn to the same invention claimed in the application prior to the entry of the submission under 37 CFR 1.114 and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the application prior to entry under 37 CFR 1.114. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action after the filing of a request for continued examination and the submission under 37 CFR 1.114. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the

shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

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, Taeyoon Kim can be reached on 571-272-9041. 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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	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.	

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

U.S.PATENT APPLICATION PUBLICATIONS Remove

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

Receipt date: 06/12/2014

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775 12057775 - GAU: 1651
Filing Date	2008-03-28
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

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					

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

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	1098900	CA		1981-04-07	Inst. Elementoorganicheskikh So, et al.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	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	☐

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	01/2015

*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.

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
Attorney Docket Number	NATNUT-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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

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					

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

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2004/112767	WO		2004-12-29	Advanced Bionutrition Corp.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

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

Receipt date: 08/01/2013	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	NATNUT-14409/US-5/ORD

	1	European Search Report, EP Patent Application No. EP12187516, mailed June 10, 2013	☐

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

EXAMINER SIGNATURE

Examiner Signature	/Deborah Ware/	Date Considered	01/2015

*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./

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12057775	BRUHEIM ET AL.
	Examiner	Art Unit
	DEBBIE K WARE	1651

CPC- SEARCHED		
Symbol	Date	Examiner
A61K2300/00 | A61K31/122 | A61K31/23 | A61K31/683 | A61K31/685 | A61K35/612 | A61K31/202 | A61K45/06 | A61K9/4858 | C11B3/006	1/2015	dkw

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
WEST, NPL and INV: see search history print out	12/2011-1/2012	DKW
WEST, NPL and INV: see search history print out	6/2012	DKW
CPC-WEST, NPL and INV: see search history print out	01/2015	dkw

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	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	

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	20110130458		2011-06-02	Harald Breivik	

Receipt date: 12/17/2014	Application Number	12057775	12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28	
	First Named Inventor	Inge Bruheim	
	Art Unit	1651	
	Examiner Name	Ware	
	Attorney Docket Number	AKBM-14409/US-5/ORD	

	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		

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

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./

Receipt date: 12/17/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	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./

Receipt date: 12/17/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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

Receipt date: 12/17/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

EXAMINER SIGNATURE			
Examiner Signature	/Deborah Ware/	Date Considered	01/2015

*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	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	D. K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	4714571		1987-12-22	Kearns et al.	
	2	8278351		2012-10-02	Sampalis	
	3	8383675		2013-02-26	Sampalis	

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					

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

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	2251265	CA		2000-04-21	Beaudoin		☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2008-03-28
(Not for submission under 37 CFR 1.99)	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button **Add**

NON-PATENT LITERATURE DOCUMENTS **Remove**

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	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)	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.")	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.")	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

EXAMINER SIGNATURE			
Examiner Signature	/Deborah Ware/	Date Considered	01/2015

*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	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	D. K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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					

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

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							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2008-03-28
(Not for submission under 37 CFR 1.99)	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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)	☐

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

Receipt date: 01/15/2014	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2008-03-28
(Not for submission under 37 CFR 1.99)	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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")	☐

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	01/2015

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware, Deborah K.
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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					

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

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	2002322233	AU		2003-02-17	Neptune Technologies & Bioressources, Inc.		☐
	2	04057853	JP		1992-02-25	CHLORINE ENG CORP LTD		☒

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Receipt date: 11/15/2012	Application Number	12057775 12057775 - GAU: 1651
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2008-03-28
(Not for submission under 37 CFR 1.99)	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware, Deborah K.
	Attorney Docket Number	AKBM-14409/US-5/ORD

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	CN Office Action mailed April 27, 2012, JP Patent Application No. 200880112125.6 (and English translation)	☒
	2	FRICKE, et al., Lipid, Sterol and Fatty Acid Composition of Antarctic Krill (Euphausia superba Dana), Lipids (1984) 19 (11): 821-827.	☐
	3	FRICKE, et al., 1-O-Alkylglycerolipids in Antarctic Krill (Euphausia Superba Dana), Comp. Biochem. Physiol. (1986) 85B(1): 131-134	☐
	4	GORDEEV, K.Y., et al. "Fatty Acid Composition of the Main Phospholipids of the Antarctic Krill, Euphausia superba," Chem. Nat. Cmpds. (1990) 26(2), pp. 143-147	☐
	5	GRANTHAM (1977) Southern Ocean Fisheries Survey Programme, FAO Rome, GLO/SO/77/3: 1-61.	☐
	6	RAVENTOS et al., Application and Posssibilities of Supercritical CO2 Extraction in Food Processing Industry: An Overview, Food Science and Technology International (2002) 8: 269-284	☐
	7	TANAKA, T., et al., Platelet-activating Factor (PAF)-like Phospholoipds Formed during Peroxidation of Phosphatidylcholines from Different Foodstuffs, Biosci. Biotech. Biochem. (1995) 59 (8), pp. 1389-93	☐
	8	WINTHER, et al., Elucidation of Phosphatidylcholine Composition in Krill Oil Extracted from Euphausia superba, Lipids (2011) 46: 25-36	☐

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	01/2015

*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.

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	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	

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	20110130458		2011-06-02	Harald Breivik	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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		

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

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		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	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	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	Ware
	Attorney Docket Number	AKBM-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)	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.**

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

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

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

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

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

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

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :		(11) International Publication Number:
C12N 9/00, 9/14, A61K 37/48	A1	WO 89/ 01031
		(43) International Publication Date: 9 February 1989 (09.02.89)

(21) International Application Number: PCT/SE88/00374

(22) International Filing Date: 8 July 1988 (08.07.88)

(31) Priority Application Number: 8703064-9

(32) Priority Date: 6 August 1987 (06.08.87)

(33) Priority Country: SE

(71) Applicant *(for all designated States except US)*: PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : MOHR, Viggo [NO/NO]; S:t Jørgensveita 6A, N-7000 Trondheim (NO). VINCENT, Jan [SE/SE]; Ulvsundavägen 49, S-161 35 Bromma (SE). HELLGREN, Lars [SE/SE]; Bronsgjutaregatan 13, S-421 63 V:A Frölunda (SE). KARLSTAM, Björn [SE/SE]; Spiltvägen 7, S-740 30 Björklinge (SE). BERGLINDH, Thomas [SE/SE]; Ripvägen 5, S-752 52 Uppsala (SE).

(74) Agents: BERGANDER, Håkan et al.; Pharmacia AB, S-751 82 Uppsala (SE).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: METHOD FOR THE ISOLATION OF ACTIVE ENZYME(S) FROM KRILL TISSUE

(57) Abstract

Method for the isolation of active enzyme(s) from an animal of the order *Euphausiaceae*, characterized in that the animals, or parts of the animals are induced to autolyse to the formation of distinct oil and aqueous phases, whereupon the phases are separated and the active enzyme(s) is(are) isolated from the appropriate phases by conventional methods.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**ML**	Mali
AU	Australia	**GA**	Gabon	**MR**	Mauritania
BB	Barbados	**GB**	United Kingdom	**MW**	Malawi
BE	Belgium	**HU**	Hungary	**NL**	Netherlands
BG	Bulgaria	**IT**	Italy	**NO**	Norway
BJ	Benin	**JP**	Japan	**RO**	Romania
BR	Brazil	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SN**	Senegal
CH	Switzerland	**LI**	Liechtenstein	**SU**	Soviet Union
CM	Cameroon	**LK**	Sri Lanka	**TD**	Chad
DE	Germany, Federal Republic of	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America
FI	Finland	**MG**	Madagascar		

Method for the isolation of active enzyme(s) from krill tissue.

Field of invention

This invention relates to an improvement in the isolation of active enzymes from aquatic animals of the order Euphausiaceae, commonly called krill. The method of the invention is adapted for enzymes and enzyme precursors from different krill tissues, particularly for those enzymes that originate from the digestive apparatus. The enzymes to be isolated may be different hydrolases, such as proteases, lipases, nucleaser, polysaccharidases etc, and other enzymes that effect breakdown of biologic substances e.g. protein, lipid, polysaccharides and nucleic acids, or their constituents.

General background

Animals of the order Euphausiaceae, and in particular Antarctic krill represented by Euphausia superba and Euphausia crystallorophias, and North Atlantic krill represented by Meganyctiphanes norvegica and Thyssanoessa species, have become increasingly promising as a source of biologically active substances. They are known to contain very effective hydrolases. The high efficiency of mixtures of endo- and exopeptidases of krill in degrading proteinaceous substrates has been demonstrated (Ellingsen, 1982; Saether, 1986; Ellingsen & Mohr, 1987; and Saether, Ellingsen & Mohr, 1987). The peptide hydrolases of krill effect an extensive breakdown of krill tissues and proteins post mortem, resulting in the release of free amino acids and shorter peptides. Visually this is observed as an autolysis. The phenomenon has been suggested to be employed for the preparation of free amino acids on an industrial scale (Ellingsen & Mohr, 1979).

The conditions effecting autolysis of krill have been extensively studied (Ellingsen & Mohr, 1979; Ellingsen, 1982; Ellingsen & Mohr, 1987 and Saether, Ellingsen & Mohr, 1987). The latter publication relates to North Atlantic krill, whereas the former publications deal with Antarctic krill. The rate of autolysis, as measured by the amount of free amino acids released into solution, depends on a series of factors, e.g. temperature, time of incubation, pH and whether whole krill or homogenate is being autolyzed (Ellingsen, 1982).

Substantial evidence is now starting to accumulate that peptide hydrolases from Antarctic krill are superior as debriding agents for wound compared to the enzyme preparations currently in clinical use for this purpose. The general picture which emerges from studies in vitro and clinically (Hellgren, Mohr & Vincent, 1986 and 1987, respectively) is that the debridement with the krill enzymes proceeds at a higher rate, and results in a more complete breakdown of the necrotic tissue than that obtained by other clinically used enzymatic debriders.

The peptide hydrolases of krill which are of particular interest therapeutically have recently been isolated and studied in considerable detail (Osnes & Mohr, 1985a ; Osnes & Mohr, 1985b; Osnes & Mohr, 1986; Osnes, Ellingsen & Mohr, 1986). As shown in these studies, the major krill peptide hydrolases include three different trypsin-like serine proteinases, two carboxypeptidase A-type of enzymes, two carboxypeptidase B-type of enzymes and one aminopeptidase. The enzymes seem to originate almost exclusively from the digestive tract of the krill, and thus seem to constitute enzymes of the digestive apparatus of the animals (Ellingsen, 1982; Grundseth & V.Mohr, unpublished).

Among other enzyme activities that have been measured in different preparations of krill are various polysaccharidase

activities (Karlstam, 1988 and Chen & Gau 1981), lipase activity (Nagayama 1979), ribonuclease activity (Van 1982) etc.

The krill peptide hydrolases seem to possess a property which is highly valuable and essential for the use of the mixture of these enzymes for practical applications. The simple digestive system in krill probably implies that the individual enzymes are mutually protected against the degrading effect of each other. Thus, in contrast to enzymes from the mammalian digestive tract, it has been demonstrated conclusively that the krill peptide hydrolases show considerable inertness to breakdown and loss of activity when they are mixed (Osnes & Mohr, 1985ab; Osnes, 1986; Osnes, Ellingsen & Mohr, 1986).

The krill enzymes which are of particular interest for medical and technical applications are usually water soluble, and can be isolated by extracting whole krill, homogenized krill or parts of krill with either water, or buffered, aqueous solutions, followed by isolation and purification of the individual enzymes or groups of enzymes by suitable, established methods (Osnes & Mohr, 1985a). Although such procedures may be satisfactory for laboratory work, large scale industrial processes based on this procedure may represent a problem. The problems relate to the fact that important species of krill usually have a high lipid content, of which glycerophospholipids may make up a considerable proportion (Ellingsen, 1982; Saether, 1986). When extracting different forms of krill with aqueous solvents glycerophospholipids tend to associate with protein, and after centrifugation such extracts may typically consist of a top layer containing oil, below which is a layer rich in glycerophospholipids and protein, below which is an aqueous phase and, finally, at the bottom, an insoluble sediment.

Due to the protein-glycerophospholipid association the phase separation will be far from distinct. Efficient separation of the aqueous phase containing the enzymes therefor represents a problem in an industrial process. Furthermore, in addition to the enzymes, the aqueous phase contains large amounts of soluble proteins, including muscle proteins of the krill. The separation of active enzymes from other proteins require expensive processing technology and, in addition, the non-enzymatic proteins obtained as by-products in this type of process occur in a form which generally has a low market value. Thus, large-scale isolation procedures based on extraction of fresh krill with water may not secure that the therapeutically and technically important enzymes can be isolated in a way which is economically feasible. These problems may be partly overcome by defatting the unhomogenized krill and/or the homogenized with a lipophilic/hydrophobic solvent (e.g. carbon tetrachloride). However, this way of processing krill will give at least one or two extra steps.

The promising prospects of using the digestive enzymes of krill as novel preparations for medical and technical use, stress the need for effective methods aimed at isolating and purifying the krill enzymes.

The invention

The present invention proposes a novel procedure for isolating active enzymes from krill without facing the drawbacks mentioned above. The invention utilizes the well-documented fact that the digestive enzymes of krill effect an extensive breakdown of the krill tissues post mortem, yielding a liquefied system, comprising an oil, an aqueous and an insoluble phase (= sediment). The invention takes advantage of the fact that the system formed after autolysis under efficient conditions may form distinct phases (phase boundaries). A physical separation can effectively be performed by simple process technology, e.g. centrifugation, and without the

problems in particular caused by the protein/glycerophospholipid layer described when extracting fresh krill as outlined previously. Prior art methods have aimed at avoiding autolysis. The invention employs autolysis as a prestep to facilitate separation and extraction. The need for separate defatting steps is minimized.

The method of the invention accordingly is characterized by whole krill, homogenized krill, squeezed krill or similarly treated parts of krill (preferably containing the digestive tract) being permitted to autolyse so that a system comprising an oil phase and an aqueous phase is formed having a distinct phase boundary therebetween. High levels of enzyme activity is retained in the aqueous phase after autolysis. The efficient phase separation is probably due to the degradation of glycerophosphólipid by krill phopholipase. After the autolysis step, the enzyme-containing aqueous phase is separated from other phases present, followed by the isolation of the enzyme(s) contemplated by conventional procedures. In case the enzyme(s) to be isolated is partitioned to the oil phase conventional isolation procedures known per se is applied to the oil phase. The separation of the individual phases of the autolysate can be carried out be several different methods. Particularly well suited are those exposing the autolysate to centrifugal forces, but other procedures e.g. sedimentation and flotation may also be applicable.

The yield of enzymes obtained depends on time of incubation (autolysis), temperature, pH, type of krill preparation (whole, homogenized or squeezed krill) and the specific enzyme(s) to be isolated.

Generally the conditions for the autolysis to proceed properly should be as below.

Temperature: The lower limit is 15 °C with a preference for temperatures above 20 °C. The upper limit is 70 °C, perferably below 45 °C. Certain krill enzymes have been shown to be heat-sensitive so that when such enzymes are to be retained in the end product, the temperature has to be carefully selected. For instance, if krill hyaluronidase or krill amylase is to be isolated it is recommended to run the autolysis below 45 °C. The krill proteases are quite heat-stable with a temperature optimum around 55-60 °C. This means that if krill proteases are the important enzymes in the end product, the autolysis can be performed up to 70 °C. If a mixture of enzyme(s) are to be isolated and one of them is heat-sensitive, the temperature should be considerable lower, e.g. below 45 °C. In conclusion the temperature should be selected in the range 15-70 °C, preferably 20-45 °C.

pH: This value should be selected in the range of 6-8,5, although autolysis may also be performed down to pH = 5. The preferred range is 6-7,5. We have performed experiments at the pH-optimum for the proteases (pH = 8,2), in order to work effectively. However, at this pH-value the phase separation after autolysis was not satisfactory. This might indicate that enzymes other than the proteases are important to obtain an efficient autolysis (e.g. phospholipases).

Time for incubation: This variable should be selected so as to result in the most economic feasible process. By selecting pH and temperature within the ranges given above incubation times of 1 h - 2 weeks, with preference for 5-48 hours, can be accomplished.

It is important to investigate in pretrial experiments that the combination of temperature, pH and time of incubation will not lead to significant degradation and/or inactivation of the enzyme(s) intended to be isolated. Accordingly each enzyme or enzyme mixture has its own optimal conditions within the above-mentioned limits in order to reach the best quality and yield.

Depending on the particular enzyme or group of enzymes to be isolated, and the purity required, methods well known to the specialist can be used to isolate samples of active enzymes from the autolysate, or from appropriate, individual fractions (phases) concentration and/or separation according to molecular size and shape, electrical charge, functional groups, solubility characteristics, or on a combination of these principles, e.g. membrane technology such as ultrafiltration, gel chromatography, ion exchange chromatography, affinity chromatography, electrophoresis, electrodialysis, precipitation by salts or acids, or selective extraction. Final concentration and removal of solvents from the preparations may be achieved by appropriate methods which do not affect enzyme activity adversly, e.f., membrane technology and freeze drying, respectively.

For the purification of specific enzymes see for instance (peptide hydrolases Osnes & Mohr 1985a, 1985b, 1986, Osnes, Ellingsen & Mohr 1986, Chen et al 1978, and Hellgren, Mohr & Vincent 1985; hyaluronidase, endo-(1,3)-beta-D-glucanase and beta-glucuronidase Karlstam 1987; ribonuclease Van 1982). Our study in the krill field has revealed that the trypsin-like krill proteases can be affinity purified on benzamidine adsorbents and most probably also on adsorbents to which trypsin inhibitors are bound, the krill carboxypeptidases on arginine or phenylalanine (hydrophobic) adsorbents and some polysaccharidases on ConA adsorbents (krill hyaluronidase, beta-glucuronidase and endo-(1,3)-beta-D-glucanase).

Example 1

25 g of frozen, Antarctic krill (Euphausia superba) were thawed at room temperature, and homogenized together with 25 ml of deionised water for 45 sec at room temperature using a Janke & Kunkel Homogenizer TP 18. The homogenate was highly viscous and contained a considerable proportion of particulate material. An aliquot of the homogenate was removed for determination of enzyme activity. The homogenate was incubated at 50 ^{o}C for 20 h at the natural pH (about 7) of the homogenate.

After incubation the homogenate was centrifuged at 13 000 g for 40 min in the cold. The centrifuged homogenate consisted of three distinct, and well-separated phases: a top layer of oil red in colour due to carotenoids, a clear, aqueous middle layer, and a particulate bottom layer. The aqueous, middle layer was removed with a pipette in the form of a clear solution with low viscosity. An aliquot of the aqueous phase was taken for determination of enzyme activity.

The proteolytic activity of the homogenized krill and of the aqueous phase of the autolysate, was determined with TAME as a substrate according to the method of Rick, 1974 (Methods of Enzymatic Analysis (H.Bergmeyer ed.)2nd.edn., Vol2,pp. 1013-1023. Academic Press, New York). In accordance with the claims of the invention, the aqueous fraction after autolysis at 50 ^{o}C for 20 h contained an enzymic activity corresponding to 95 % of that of the original homogenate prior to autolysis.

Example 2

10 ml of the aqueous phase of the krill autolysate prepared according to Example 1 were subjected to ultrafiltration in order to separate the enzyme preparation from low-molecular weight substances. The separation was carried out in an Amicon ultrafiltration unit, using an Amicon Diaflo Ultra-filter type PM 10. The filter effects retention of material with a molecular weight exceeding 10 000. The ultrafiltration was run at a rate of 2.5 ml per sq.cm per hour at room temperature, using a pressure of 1,4 atm. of nitrogen.

Due to the low viscosity of the aqueous fraction of the autolysate, the ultrafiltration proceeded very effectively. Ultrafiltration was continued until the volume of the autolysate had been reduced to one tenth of the original volume. The high-molecular weight fraction after ultra-filtration contained the enzyme activity, whereas the permeate contained low-molecular weight material, mainly free amino acids and other break-down products after autolysis.

Example 3

Squeezed krill was obtained from whole fresh or frozen krill by pressing and centrifuging the raw material. In brief frozen krill, stored at minus 20-30 °C, was allowed to thaw at room temperature for 20 hrs and then centrifuged for 10-30 min at 1 500-3 000 xg to remove insoluble substances. The viscous liquid was collected and defined as squeezed krill. The fresh krill was processed in the same way without thawing.

1 000 ml of squeezed krill were subjected to spontaneous autolysis by storing at different temperatures (20-45 °C) and times (10-48 hrs) at different pH-values 6,8-7,0. After terminated incubation the mixtures were centrifuged at 3 500 xg for 60 min. This resulted in partitioning the material into three distinct phases for pH below 8. The middle phase represented by a clear aqueous liquid contained, as in example 2, high levels of hydrolytic enzymes degrading proteins, polysaccharides and polynucleotides. This was removed by sucking and subjected to concentration/purification by membrane filtration. The high molecular weight substances (>10 000 Daltons) were further purified by ion exchange chromatography (e.g. Q-Sepharose®, Pharmacia AB, Sweden) or hydrophobic interaction chromatography (e.g. Phenyl Sepharose® or Alkyl Sepharose®) using continuous or discontinuous salt gradients when eluting different enzymes/proteins. The enzymatic activity was collected for different enzyme groups and desalted by gel filtration or dialysis procedures. In this matter one or several bulk enzyme mixtures were obtained for further isolation and purification of individual hydrolytic enzymes.

The protein content, total proteolytic activity, trypsin-like activity and hyaluronidase activity were followed during the process, see table I-IV. In addition amylase, beta-glucuronidase, endo-(1,3)-beta-D-glucanase and carboxypeptidase activities were measured.

Table I

Summary of autolysis (25°C; 20 h) and partial purification of squeezed krill by membrane filtration and anion exchange chromatography on Q-Sepharose FF

Sample	Volume	Protein		Caseinolytic activity			Trypsin-like activity		
				Total units	Recovery (%)	Purification (fold)	Total units	Recovery (%)	Purification (fold)
	(ml)	(g)	(%)						
Squeezed krill	1000	40	100	1500	100	1	32000	100	1
Autolysate	760	17	42	1250	83	2	32000	100	2.4
Protein concentrate	95	6	15	1150	77	5	29600	92	6
Enzyme pool after ion exchange chromatography	250	1.5	4	900	60	16	21700	68	18

Table II

Summary of further purification of serine proteinases from a bulk enzyme mixture isolated from autolysed squeezed krill

Step	Volume (ml)	Protein		Trypsin-like activity			Recovery (%)	Purification (fold)
		(mg/ml)	Total mg	(U/ml)	Total units	Spec. act. (U/mg protein)		
Bulk enzyme	9	6.5	58.5	68	612	10.5	100	1
Benzamidine-Sepharose 6B	21	0.5	10.5	25	525	50	86	4.8
Sephadex G-25	43	0.24	10.3	10	430	42	70	4
Protein concentrate	8.6	0.8	6.9	43	370	54	60	5.1

Table III

Protein degradation

Effect of temperature

Temperature (°C)	Protein residues (%)
8	100 (no autolysis)
20	12-21
35	12-17
45	8-17

Experiments performed for 20 h.

Effect of time

Time (h)	Protein residues (%)
0	100
10	20-25
20	10-20
48	10-20

Experiments performed at 25°C

Table IV

Summary of further purification of hyaluronidase from a bulk enzyme mixture isolated from autolysed squeezed krill

Step	Volume (ml)	Protein (mg/ml)	Protein Total mg	Hyaluronidase activity (U/ml)	Hyaluronidase activity Total units	Hyaluronidase activity Spec.act. (U/mg protein)	Recovery (%)	Purification (fold)
Bulk enzyme	77	5	385	140	10780	28	100	1
Con A-Sepharose	43	1.2	52	68	2924	57	27	2
Protein concentrate YM 10 filter	10	4.1	41	291	2910	71	27	2.5
Superose 6	26	0.6	16	59	1534	98	14	3.5
FPLC-Mono Q HR 10/10 after concentration YM 10 filter	22	0.47	10	63	1386	134	13	4.8

Referenslista

* Chen et al., 1978: J. Food Biochem. 2, p 349-66.

* Chen & Gau, 1981: J. Food Biochem. 5, p 63-8.

* Ellingsen & Mohr, 1979: Process Biochem. 14 (10), p 14.

* Ellingsen, 1982: Biochemical Studies on Antarctic Krill (Ph. D. Thesis) Department of Biochemistry, The Norwegian Institute of Technology, The University of Trondheim.

* Ellingsen & Mohr, 1987: Biochem. J. In press.

* Hellgren, Mohr & Vincent, 1986: Experentia 42, p 403-4.

* Hellgren, Mohr & Vincent, 1987: 17th World Congr. Dermatol., Berlin, p 196.

* Hellgren, Mohr & Vincent, 1985: WO-A-85/04809.

* Karlstam, 1988: EP-A-252,044 (Pharmacia AB).

* Nagayama et al., 1979: Transactions of the Tokyo University of Fisheries 3, p 153-159.

* Osnes & Mohr, 1985a: Comp. Biochem. Physiol. 82B, p 599-606.

* Osnes & Mohr, 1985b: Comp. Biochem. Physiol. 82B, p 607-19.

* Osnes & Mohr, 1986: Comp. Biochem. Physiol. 83B, p 445-458.

* Osnes, Ellingsen & Mohr, 1986: Comp. Biochem. Physiol. 83B, p 802-5.

* Rick, 1974: Methods of Enzymatic Analysis (ed.: H. Bergmeyer) 2nd edn., vol 2 pp 1013, 1023.

* Saether, 1986: Biochemistry of North Atlantic Krill. (Ph. D. Thesis) Department of Biochemistry, The Norwegian Institute of Technology, The University of Trondheim.

* Saether, Ellingsen & Mohr, 1987: Comp. Biochem. Physiol. In press.

* Van, 1982: Agric. Biol. Chem. 46, p 691-6.

CLAIMS

1. Method for the isolation of active enzyme(s) from an animal of the order Euphausiaceae, characterized in that the animals, or parts of the animals are induced to autolyse to the formation of distinct oil and aqueous phases, whereupon the phases are separated and the active enzyme(s) is(are) isolated from the appropriate phases by conventional methods.

2. Method according to claim 1, characterized in that the animals or part of the animals prior to the autolysis may have been unfrozen, frozen and/or homogenized, or squeezed,

3. Method according to claim 1, characterized in that autolysis is achieved by incubating the animals or part of the animals at a temperature in the range 15 to 70 ^{o}C, for a period of time ranging from 1 hour to 2 weeks.

4. Method according to anyone of claim 1-3, characterized in that autolysis is carried out at pH-values in the range pH 6 to 8.

5. Method according to anyone of claim 1-5, characterized in that the enzyme(s) is(are) isolated from the aqueous phase.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE88/00374

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

C 12 N 9/00, C 12 N 9/14, A 61 K 37/48

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	C 12 N 9/00, /14; A 61 K 37/48; A 61 K 37/54

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above. Data base search: WPI,WPIL,CA, Biosis.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Comp.Biochem.Physiol. Vol 82B, No 4, pp. 599-606, 1985, (KNUT KR. OSNES AND VIGGO MOHR), "Peptide hydrolases of antarctic krill, ëuphausia superba", see page 601, right column and table 3, page 602, left column lines 1-6	1-2,4-5
Y	WO, A1, 85/04809 (HELLGREN) 7 November 1985 see example 1 & SE, 8402238 EP, 0177605 JP, 61501918 US, 4695457 SE, 454566	1-2,4-5
A	Journal of Food Biochemistry 2 (1978) 349-366, (CHING-SAN CHEN), "Purification and properties of tryspsin-like enzymes and a carboxypeptidase a from euphausia superba"	1-5

.../...

* Special categories of cited documents: [10]

"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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1988-10-10	1988 -10- 19
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Yvonne Siösteen

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Comp.Biochem.Physiol.Vol 83B, No 4, pp 801 805, 1986, (KNUT KR. OSNES), "Hydrolysis of proteins by peptide hydrolases of antarctic krill, euphausia superba"	1-5

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C12N 9/00, 9/50, A61K 37/54

A1

(11) International Publication Number: **WO 89/10960**

(43) International Publication Date: 16 November 1989 (16.11.89)

(21) International Application Number: PCT/SE89/00235

(22) International Filing Date: 27 April 1989 (27.04.89)

(30) Priority data:
8801701-7 5 May 1988 (05.05.88) SE

(71) Applicant *(for all designated States except US):* PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* MOHR, Viggo [NO/NO]; Tegelbrennervn. 8, N-7013 Trondheim (NO). HELLGREN, Lars [SE/SE]; Bronsgjutaregatan 13, S-421 63 V:A Frölunda (SE). VINCENT, Jan [SE/SE]; Ulvsundavägen 49, S-161 35 Bromma (SE).

(74) Agents: ENGHOLM, Carl et al.; Pharmacia AB, S-751 82 Uppsala (SE).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: METHOD FOR MODIFYING PROTEINS, PEPTIDES AND/OR LIPIDS BY ENZYMES FROM EUPHAUCIACEAE

(57) Abstract

The invention relates to the use of enzymes selected from animals belonging to the order Euphauciaceae. The enzymes are used to modificate protein, peptide and/or lipid constituents of biological material in industrial processes.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MR	Mauritania
BB	Barbados	GA	Gabon	MW	Malawi
BE	Belgium	GB	United Kingdom	NL	Netherlands
BF	Burkina Fasso	HU	Hungary	NO	Norway
BG	Bulgaria	IT	Italy	RO	Romania
BJ	Benin	JP	Japan	SD	Sudan
BR	Brazil	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic			SN	Senegal
CG	Congo	KR	Republic of Korea	SU	Soviet Union
CH	Switzerland	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany, Federal Republic of	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		
ES	Spain	MG	Madagascar		

Method for modifying proteins, peptides and/or lipids by enzymes from Euphauciaceae

FIELD OF THE INVENTION

The present invention relates to novel applications of enzymes in the area of production technology. The invention specifies how preparations of active enzymes or enzyme systems selected from animals belonging to the order Euphauciaceae can be used in a novel way in industrial processes.

According to the methods specified in the invention, the unique properties of the enzymes of this order of animals are utilized in a way which opens up new perspectives in the area of enzyme technology. The invention explicitly documents how the preparations of said enzymes can be applied in clearly defined processes and with the purpose of manufacturing specific products.

GENERAL BACKGROUND

Animals belonging to the order Euphauciaceae, and in particular Antarctic krill represented by Euphausia superba, Euphausia crystallorophias and other species, and North Atlantic krill represented by Meganyctiphanes norvegica, Thysanoessa inermis and other species, have become increasingly interesting as a source of unique enzyme systems. This is clearly underlined by the fact that groups of enzymes from these animals hold considerable promise as new therapeutic agents for treating wounds, burns, and dermatoses (Hellgren, Mohr & Vincent, 1986), and as an aid for digestive processes in both animals and man. The effectiveness of the enzymes in debriding wounds is presently being verified by large-scale clinical tests in several countries.

The unique effects exhibited by the enzyme systems of krill are intimately related to the unusual ecological situation facing both Antarctic- and Arctic krill. During a substantial part of the year the krill in these regions have a very limited food supply. Food becomes available in plenty only during the short summer season when phytoplankton production in the sea is abundant.

As a consequence, the krill have developed an unusually effective digestive apparatus which secures that food, when available, is quickly digested and deposited as storage lipid. The digestive enzymes of this apparatus are of considerable interest therapeutically and industrially.

Apart from their highly effective digestive apparatus, krill are also unusual in another sense. Few other groups of animals are adapted to life at such low temperatures as Antarctic- and Arctic krill which may frequently experience temperatures approaching that of freezing sea water (-1.9 °C). Thus, the enzyme systems of krill are designed to exhibit activity at such low temperatures. When studied in vitro, activity can be observed at even lower temperatures, provided that freezing is prevented by the addition of antifreeze agents.

In conclusion, krill are characterized by having not only an unusually high level of digestive enzymes, but the enzymes are also adapted to function effectively at low temperatures. At present few, if any enzyme systems with such characteristics are generally available commercially, despite the fact that enzymes of this type would open up a completely new field of considerable commercial importance, namely low temperature enzyme technology.

The present invention makes a systematic contribution to this area by pointing out specific industrial applications which depend on the unique properties of the enzymes of

krill, and which utilize the unusual effectiveness of these enzymes and/or their particular temperature relationships.

As a background for the novel applications presented and the claims made, an overview over the krill digestive enzymes and their properties is given below.

THE DIGESTIVE ENZYMES OF KRILL

Peptide hydrolases

Krill contain an array of enzymes required to break down the polymeric substances making up the food of the animals. Of particular interest in the context of industrial application are the peptide hydrolases and the lipolytic enzymes of krill.

The peptide hydrolases of krill have been studied in considerable detail, and it has been shown that krill rely on a system of both endo- and exopeptidases to degrade protein. The krill peptide hydrolases include three trypsin-like enzymes, two carboxypeptidase A-type of enzymes, two carboxypeptidase B-type of enzymes and one aminopeptidase (Osnes & Mohr, 1985a; Osnes & Mohr, 1985b; Osnes & Mohr, 1986). These enzymes seem to originate almost exclusively from the digestive tract of the krill, and thus seem to constitute enzymes of the digestive apparatus of the animals. When acting in combination the peptide hydrolases of krill effect an extensive breakdown of proteins to shorter peptides and amino acids (Osnes, Ellingsen & Mohr, 1986; Saether, Ellingsen & Mohr, 1987; Ellingsen & Mohr, 1987).

The peptide hydrolases give rise to a rapid autolyses of the krill tissues post mortem, resulting in the production of large amounts of free amino acids (Ellingsen & Mohr, 1987;

Saether, Ellingsen & Mohr, 1987). Other work has shown that the krill peptide hydrolases have a similar effect on milk and meat proteins (Osnes, Ellingsen & Mohr, 1986), and on the protein constituents of necrotic wounds (Hellgren, Mohr & Vincent, 1986).

In sum, the studies by the present authors and others provide substantial scientific documentation showing that the natural mixture of the endo- and exopeptidases of krill constitute an unusually effective system for degrading common proteins to soluble peptides and amino acids. The krill enzymes surpass most purified peptide hydrolases of microbial, plant or animal origin in this respect, because the krill enzyme system combines in a very effective way both endo- and exopeptidase activity.

This is illustrated by the fact that a highly specific protease such as e.g. trypsin will, when acting alone, only cleave proteins at the comparatively few peptide bonds involving basic amino acids and, hence, produce comparatively large peptides which are not likely to be readily soluble. This effect can clearly be seen when examining the effect of various proteolytic enzymes in cleaning wounds, in which case most purified enzymes including trypsin exhibit very limited effect compared to that of the mixture of krill peptide hydrolases (Hellgren, Mohr & Vincent, 1986).

Detailed studies of the krill peptide hydrolases have also provided interesting insight into the temperature relationships of these enzymes. Examination of highly purified preparations of the enzymes reveal that both the endo- and exopeptidases of krill have a temperature optimum in the range from 35-50 °C. However, as mentioned previously, the enzymes exhibit considerable activity also at lower temperatures.

The striking temperature relationships of the krill enzymes have been clearly demonstrated in the case of the trypsin-like enzymes of krill, which have been shown to exhibit far lower activation energies for the hydrolysis of peptide bonds than comparable trypsin from warm-blooded animals (Osnes & Mohr, 1986). This provides insight into the fundamental mechanism which enables the krill enzymes to operate effectively at temperatures far below their optimum.

The krill peptide hydrolases possess yet another property which is highly valuable and essential for the use of the mixture of krill enzymes for practical purposes. Krill are animals which are characterized by having a simple digestive system, in which the different endo- and exopeptidases apparently act together in the digestive tract. This contrasts the situation in higher animals, in which the individual digestive enzymes operate in anatomically distinct portions of the digestive system.

The simple digestive system of krill probably implies that the individual enzymes are mutually protected against the degrading effect of each other. Thus, in contrast to the enzymes from the mammalian digestive tract, it has been demonstrated conclusively that the krill peptide hydrolases show considerable inertness to breakdown and loss of activity when they are mixed (Osnes & Mohr, 1985ab; Osnes, Ellingsen & Mohr, 1986).

This property makes the mixture of krill peptide hydrolases unusually valuable as an enzyme composition for practical application in industrial processes.

Lipolytic enzymes

In addition to peptide hydrolases, krill contain a number of other enzyme systems required for breaking down polymeric

substances in the food ingested. Of these enzymes lipases and phosholipases are of particular interest from an industrial point of view.

Although less precise knowledge is available on the lipolytic enzymes of krill, it is clear that such enzymes, and in particular phospholipases are present in considerable amounts, and that they operate effectively at temperatures down to freezing (Ellingsen, 1982; Saether, Ellingsen & Mohr, 1986a).

These enzyme systems are evidently responsible for the degradation of lipids and the production of free fatty acids when krill are stored post mortem.

THE INVENTION

The present invention specifies procedures which utilize the unique properties of specific enzyme systems of krill in industrial processes in a completely novel way. Compared with enzyme processes previously known, the effectiveness of the procedures based on krill enzymes is striking and surprising.

The procedures specified in the invention utilize one or more of the following properties of the krill enzymes:

- The high efficiency of the mixture of krill peptide hydrolases in breaking down proteins to peptides and free amino acids.

- The high efficiency of krill trypsin type I in breaking down proteins to peptides and free amino acids.

- The high efficiency of the mixture of krill peptide hydrolases, and in particular the exopeptidases, in breaking down peptides to free amino acids.

- The high efficiency of the krill lipolytic enzymes in breaking down lipids, and in particular phospholipids, to free fatty acids.

- The high efficiency of the krill enzymes at low temperatures.

- The high stability of krill enzymes when mixed.

The procedures specified in the invention may be carried out using several different types of compositions of active krill enzymes. The simplest procedure is to utilize the krill enzymes as they occur in situ, i.e., to let whole

krill autolyse under proper conditions with the formation of e.g. free amino acids and free fatty acids.

Another method may depend on using macerated, whole krill as a source of enzymes in the process or, alternatively, an aqueous extract of krill which should preferably be defatted, and if necessary, also concentrated.

The appropriate enzyme systems of krill or, alternatively, specific enzymes of the animals, may be isolated and purified by methods well known to the specialist, for instance by procedures based on differences in molecular size, electric charge, types of active sites etc (Osnes & Mohr, 1985a; Osnes & Mohr, 1985b; Osnes & Mohr, 1986; Osnes et al., 1986).

APPLICATION OF THE KRILL ENZYMES IN INDUSTRIAL PROCESSES ACCORDING TO THE INVENTION

1. Production of protein concentrates

The peptide hydrolases of krill can be used as a very effective means of manufacturing protein concentrates from suitable raw materials of either microbial-, plant- or animal origin. The objective is to anchieve removal of unwanted parts of the raw material, and at the same time secure a concentration of protein and other desirable constituents. It is often an objective to obtain a protein concentrate which is water-soluble.

Enzyme technology has been applied to a certain extent within this area, and usually in the form of a partial hydrolysis using proteolytic enzymes of mammalian-, plant- or microbial origin (Mohr, 1978; Mohr, 1980). Krill peptide hydrolases hold particular promise in this context, and in particular as regards production of protein concentrates from cheap fish or from fish- or abbatoir by-products.

The advantage of the krill peptide hydrolases as compared to the enzyme systems tested so far, depends on the high efficiency of the mixture of the krill peptide hydrolases as pointed out above, and the fact that they can be used at comparatively low temperatures if necessary.

When the hydrolytic process is run at low temperature and/or with a low enzyme concentration, a limited breakdown of protein will accur. However, such treatment may be sufficient to allow bones to be effectively removed from e.g. fish products, and at the same time yield a protein concentrate with good functional properties. By increasing the temperature and enzyme concentration a soluble protein concentrate can be obtained in good yields.

2. Production of protein hydrolysates for dietary applications

Dietary protein hydrolysates represent a small, but important market segment. Such preparations are used for postoperative patients or for individuals with an impaired digestive system. The hydrolysates may be administered as comparatively crude preparations per os (Clegg, 1978), or as highly purified mixtures of amino acids for intravenous administration.

Enzyme hydrolysates of milk proteins have been applied as dietary preparations. However, when using conventional enzymes of either microbial-, plant- or animal origin serious problems are encountered, both because the yield of free amino acids may be low, and because a large amount of bitter peptides are formed (Clegg, 1978).

Dietary protein hydrolysates made by the application of krill peptide hydrolases represent an important, new area,

both due to the high yield of amino acids obtained in the process, and because a very limited amount of bitter peptides is produced.

3. Production of free amino acids

The peptide hydrolases of krill may be applied in effective processes for the manufacture of free amino acids from cheap protein sources. The free amino acids produced in the process may either be prepared as a crude mixture, or further separated into the individual amino acids or groups of individual amino acids by methods well known to the specialist.

The present process provides a new alternative to amino acid production by fermentation, and should hold considerable promise, both because the process economy is favourable, and because the process yields the entire range of free amino acids. The essential amino acids are of particular interest in this context, but also other amino acids, e.g. glutamic acid and others, may be of considerable commercial interest.

4. Production of a growth medium for fermentation

By applying the technique of krill autolysis or, alternatively, by using purified krill enzymes to hydrolyse a suitable protein source, it is possible to produce a crude preparation of free amino acids and peptides which is highly suitable as a substrate for microorganisms that have a specific requirement for amino acids for growth.

This is the case of a considerable number of the microorganisms used in industrial fermentations. The supply of the necessary amino acids often represent an important factor for process economy in such fermentations.

The preparation of amino acids produced by applying krill enzymes has properties similar to that of Trypton and Pepton, and is suitable as a substrate both in laboratory- and large scale industrial fermentations.

5. Improvement of nutritional- and physical/chemical properties of food- and feedstuffs

Partial hydrolysis of proteinaceous feed- or foodstuffs can lead to significant improvements in either their digestibility or functional properties. Such improvements are of considerable importance from a practical point of view, for instance in the aquaculture industry.

After hatching it is in many cases essential to provide the fish fry with a feed which can easily be utilized by the young progeny before their digestive apparatus has been fully developed. Partial enzymic hydrolysis of suitable feedstuffs, e.g. from marine sources, can make several protein raw materials suitable as a "start feed" for fish fry.

Enzymes from krill are particularly well suited for this application. A particularly interesting aspect of this application is the fact that the krill enzymes can be added to the feed and allowed to act during low-temperature storage at chill temperatures.

Partial hydrolyses of protein constituents can also confer improved functional properties to feed- and foodstuffs, by increasing water solubility, emulsifying capacity, foaming ability or texture. In such cases the conditions of hydrolyses have to be specifically adapted to achieve the desired effects.

6. Tenderizing of muscle foods

Enzymic tenderization of muscle foods, and in particular meat, represents a large market segment, which is presently dominated by plant proteases and certain microbial enzymes. Enzymic maturation and tenderization of fish muscle is also of considerable importance in many countries (Mohr, 1980). Krill enzymes provide an interesting alternative to present enzymic practices within this area.

A particularly interesting aspect of krill proteases, as opposed to the enzymes presently used, is the ability of the former to act at temperatures down to freezing. This opens the possibility of achieving artificial tenderizing of meat or fish during chill storage, which is a completely new concept, which has so far not been explored due to the lack fo suitable enzymes.

7. Debittering of peptides

Enzymic hydrolysis of protein raw materials frequently leads to the formation of bitter peptides as mentioned above (Clegg, 1978). The bitter peptides occurring in protein hydrolysates may represent a considerable practical problem, as is the case e.g. during the ripening of different types of cheese and in the production of dietary protein hydrolysates.

The bitterness of hydrolysates is usually due to particular peptides, and expecially those which contain a high proportion of hydrophobic amino acids. Bitterness can be effectively reduced by complete or partial hydrolyses of the bitter peptides.

The natural mixture of krill peptide hydrolases, and in particular the exopeptidases of such mixtures, are excellently suited to remove bitter peptides from hydrolysates.

In contrast to the enzymes presently used for this purpose, debittering based on krill enzymes can also be carried out at low temperature.

8. Antihaze treatment

Protein precipitates may present a considerable problem in certain products such as e.g. beer, because the precipitate causes the product to be hazy. In beer the haziness arises when soluble proteins precipitate during chill storage of the beer.

The problem is of considerable economic importance and, apart from selecting suitable raw materials for the manufacture of beer, the main way of avoiding the problem today is to add proteolytic enzymes to the beer. Since it is desirable that hydrolysis, if necessary, can take place during chill storage, the use of krill enzymes represents a unique, new possibility in this area.

9. Viscosity reduction

Industrial processing of proteinaceous raw materials with the purpose of manufacturing protein concentrates frequently involves treatment of solutions or suspensions containing high concentrations of proteins, for instance during extraction, centrifugation, evaporation or concentration steps. The high viscosity of such systems often causes serious problems with respect to the efficiency and economy of these unit operations.

Reduction of the viscosity by partial enzymic hydrolyses of the protein constituents can provide a very effective solution to such problems. One example is the treatment of the stickwater during fishmeal production with proteolytic enzymes.

Due to the presence of both endo- and exopeptidases the digestive enzymes of krill can provide a far more efficient viscosity reduction than the bacterial proteases which are presently used for this purpose. Furthermore, the fact that the enzymic step can be carried out at low temperatures offers yet another interesting possibility in the case of the krill enzymes.

10. Dehairing of hides

Industrial leather manufacture relies on a series of steps involving cleaning, dehairing and finally tanning and dying of the hides. Enzyme treatment plays an important part in the dehairing step, which is achieved by the application of proteolytic enzymes. Krill peptide hydrolases can provide an effective alternative to the mammalian proteases presently used in leather manufacture, both because of their high proteolytic activity, and their efficiency at low temperatures.

11. Separation and removal of tissues

In the food industry physical separation and removal of tissues constitute essensial parts of many processes. These steps often rely on manual or complex mechanical procedures which may have a strong, negative influence on process economy. Examples of such procedures are fileting of fish, deskinning of fish filets, and removal of shells from shrimps, just to mention a few in the area of marine products.

So far enzymic methods have been employed only to a limited extent in this area, but such procedures hold considerable promise. Peptide hydrolases from krill are particularly interesting in this respect because of their high activity at low temperature. This is particularly important since many of the products in question do not tolerate elevated

temperatures because of the adverse effect of heating on sensory properties and hygienic standard.

12. Dissociation of tissues and cells

Dissociation of tissues and individual cells represents an important step in tissue culture procedures. Proteolytic enzymes are used routinely for this purpose. The effectiveness and high activity at low temperatures should make the krill peptide hydrolases particularly attractive for such applications.

13. Industrial oil manufacture

Industrial production of oil from animal- or plant sources depends on either mechanical pressing or solvent extraction of the raw material. Prior to these steps the raw material is often pre-treated to faciliate removal of the oil from the cells in which the lipid is stored. The mechanism of release of lipid from fatty cells has been discussed by Mohr (1979), with particular reference to the production of fish meal and oil.

The release of oil from fatty tissues can be significantly improved if the walls of the cells storing the fat are weakened or punctured prior to pressing or extraction. Enzymic treatment constitutes such a method. The application of the effective endo- and exopeptidases of krill is of particular interest.

In order to achieve the goal, the enzymes should be added to the macerated fatty raw material, and allowed to act for a comparatively short time at low temperature in order to cause a weakening of the cell walls, but without causing extensive hydrolysis of the protein phase, in which case problems may be encountered in the pressing stage.

14. Production of free fatty acids

Antarctic krill, as well as krill species in the North Atlantic, are characterized by containing high proportions of lipids, including phospholipids. The phospholipids of krill have an unusually high proportion of long-chain w-3 fatty acids (Ellingsen, 1982; Saether et al., 1986b). Furthermore, as mentioned above, krill possess a very efficient enzyme apparatus for degrading lipids to free fatty acids.

The application of krill lipases and phospholipases presents a highly interesting, new method for producing free fatty acids, either by a process based on controlled autolysis of krill itself, or by letting purified preparations of krill lipases and phospholipases act on a suitable lipid raw material. Such processes present a new, competitive avenue to the manufacture of highly concentrated preparations of fatty acids, and it particular the w-3 fatty acids from marine organisms.

REFERENCES

K.M. Clegg (1978), In "Biochemical Aspects of New Protein Food", J. Adler-Nissen, B.O. Eggum, L. Munck & H.S. Olsen eds., p. 109-117, Pergamon, Oxford.

T.E. Ellingsen (1982), Dr.ing. thesis, Department of Biotechnology, The Norwegian Institute of Technology, Trondheim.

T.E. Ellingsen & V. Mohr (1987), Biochem. J., 246, 295-305.

V. Mohr (1978), In "Biochemical Aspects of New Protein Food", J. Adler-Nissen, B.O. Eggum, L. Munck & H.S. Olsen eds., p. 53-62, Pergamon, Oxford.

V. Mohr (1979), Proc. Third Symp. Fish Meal and Oil, p. 90. Int. Assoc. Fish Meal Manufacturers, London.

V. Mohr (1980), Process Biochem. 15, 18-21.

K.K. Osnes & V. Mohr (1985a), Comp. Biochem. Physiol. 82B, 599-606.

K.K. Osnes & V. Mohr (1985b), Comp. Biochem. Physiol. 82B, 607-619.

K.K. Osnes & V. Mohr (1986), Comp. Biochem. Physiol. 83B, 445-458.

K.K. Osnes, T.E. Ellingsen & V. Mohr (1986), Comp. Biochem. Physiol. 83B, 801-805.

W.I. Rick (1974), In "Methods of Enzymatic Analysis", Bergmeyer ed., Academic Press, New York.

O. Saether, T.E. Ellingsen & V. Mohr (1986a), Comp. Biochem. Physiol. 83B, 51-55.

O. Saether, T.E. Ellingsen & V. Mohr (1986b), J. Lipid Res. 27, 274-285.

O. Saether, T.E. Ellingsen & V. Mohr (1987), Comp. Biochem. Physiol. 88B, 165-176.

EXAMPLES

EXAMPLE 1. ENZYME PREPARATIONS

A. Enzyme preparation made from macerated krill

Frozen Antarctic krill (Euphausia superba) were thawed, and subsequently treated in a partly thawed state for two periods of each 30 s in a MSE Homogeniser. The macerated krill was used as a source of enzymes according to the invention. Such material will be referred to as "macerated krill".

B. Enzyme preparation in the form of an aqueous extract of krill

Frozen Antarctic krill (Euphausia superba) were thawed and macerated as described above. 25 g of the macerated krill were mixed with 50 ml of water, homogenised and centrifuged in the cold (0 °C) for 30 min at 12 500 g. The sediment was resuspended in 50 ml water, homogenised and centrifuged as described above.

The combined extracts were added 20 ml of tetrachloromethane and homogenised in the cold. The mixture was centrifuged for 15 min at 2 500 g. The water-phase was removed and extracted once more with tetrachloromethane and centrifuged.

The combined, defatted aqueous extracts were freeze dried and used as an enzyme preparation according to the invention.

Such preparations will be referred to as "freeze dried krill extract".

C. Preparation of partly purified krill enzymes

25 g of macerated krill (Example 1A) were mixed with 25 ml deionised water and incubated at 50 °C for 20 h at the

natural pH of the homogenate. During incubation a major proportion of the krill proteins are broken down, leaving the digestive enzymes intact.

After incubation the mixture was centrifuged at 13 000 g for 40 min in the cold. The aqueous phase was removed and subjected to ultrafiltration using an Amicon Diaflo Ultrafilter type PM 10. The filter effects retention of material with a molecular weight exceeding 10 000. The high - molecular weight fraction after ultrafiltration containing the digestive enzymes including peptide hydrolases was concentrated, freeze dried and used according to the claims of the invention. The present preparation will be referred to as "purified krill enzymes".

D. Preparation of chromatographed krill enzymes

20 ml of defatted, aqueous krill extract as described in Example 2B were chromatographed on Sephadex G-100 (dextran crosslinked with epichlorhydrin, Pharmacia Fine Chemicals AB, Uppsala, Sweden) in a column having a diameter of 3.1 cm and a height of 69 cm. The column was equilibrated and eluted (30 ml/h) with Tris-HCl buffer (0.05 M, pH 7.5) at 5 °C.

The elution profile was monitored spectrophotometrically at 280 nm. Fractions were collected and enzymatically active fractions pooled, dialysed, freeze dried and used as enzyme preparations according to the invention.

The proteolytic activity was determined using hemoglobin or casein as substrates according to the method of Rick (1974). The fractions collected during gel chromatography corresponded to molecular weights of roughly 20 000-40 000 dalton. Such preparations were used in accordance with the claims of the invention, and will be referred to as "chromatographed preparations of krill enzymes".

EXAMPLE 2. PROTEIN CONCENTRATES

A. Fish protein concentrate

3 g dry weight of fresh cod filet with skin and bones were suspended in 100 ml of deionised water in a vessel equipped with a stirrer and connected to an automatic pH-control unit maintaining pH at 7.5. The temperature was raised to, and maintained at 50 °C. 30 mg of "purified krill enzymes" were added and the hydrolyses carried out for 120 min. Muscle tissue and skin were broken down during the process, and bones easily sedimented.

The mixture was afterwards cooled to 4 °C and centrifuged at 12 000 g for 15 min. The water soluble fraction after centrifugation accounted for approximately 60 % of the dry weight of the cod muscle hydrolysed. The present fish protein concentrate exhibited very good sensory and functional properties.

B. Beef protein concentrate

3 g dry weight of beef muscle with adhering tendon and small fragments of bone were hydrolysed with 30 mg of "purified krill enzymes" as outlined in Example 2A. The muscle tissue and tendon were effectively broken down during hydrolysis. The water soluble fraction after hydrolysis accounted for approximately 55 % of the dry weight of the starting material.

The water soluble fraction was freeze dried. The present beef protein concentrate exhibited very good sensory and functional properties.

C. Milk protein concentrate

3 g dry weight of casein were suspended in 100 ml of deionised water and hydrolysed with 30 mg of "purified krill enzymes" for 120 min at 40 °C and pH 7.0 as outlined in Example 2A.

The hydrolysed casein was freeze dried. The present milk protein concentrate exhibited very good sensory properties, particularly from the point of view of bitterness.

D. Plant protein concentrate

3 g of soy protein isolate were suspended in 100 ml of deionised water and hydrolysed with 30 mg of "purified krill enzymes" for 120 min at 50 °C and pH 7.5 as outlined in Example 2A. The hydrolysed soy protein preparation was freeze dried. The present soy protein concentrate exhibited very good functional properties, and was very satisfactory from the point of view of bitterness.

EXAMPLE 3. DIETARY PROTEIN HYDROLYSATE

3 g dry weight of casein were suspended in 100 ml deionised water and hydrolysed with 20 mg of "chromatographed krill enzymes" for 5 h at 50 °C and pH 7.5 as outlined in Example 2A. After hydrolysis approximately 10 % of the casein had been converted to free amino acids.

After centrifugation at 12 000 g for 15 min the clear supernatant was treated in an Amicon Diaflo Ultrafiltration Unit using a Diaflo Ultrafilter Type YC having a 500 MW cutoff. The low-molecular fraction containing the free amino acids was concentrated and freeze dried. The product exhibited almost no bitterness and was suitable as a dietary protein hydrolysate.

EXAMPLE 4. PRODUCTION OF FREE AMINO ACIDS

A. Free amino acids produced by autolysis of krill

Frozen Antarctic krill (Euphausia superba) were thawed and macerated as described in Example 1A. 50 g of macerated krill were placed in a plastic bottle with screw cap and incubated at 50 °C for 20 h at the natural pH of the krill.

During this period a considerable proportion of the total protein of the krill was converted to free amino acids.

After incubation the mixture was centrifuged at 13 000 g for 40 min in the cold. The aqueous phase containing a high proportion of free amino acids was removed and treated as described in Example 4C.

B. Production of free amino acids from fish protein

3 g dry weight of macerated capelin (Mallotus villosus) were suspended in 100 ml deionised water. 0.20 g of "freeze dried krill extract" (Example 1B) were added, and the hydrolysis allowed to proceed at pH 7.0 for 20 h at 50 °C. After hydrolysis the mixture was centrifuged at 13 000 g for 40 min in the cold. The aqueous phase after centrifugation was removed and treated as described in Example 4C.

C. Production of a crude preparation of free amino acids

25 ml of the aqueous phase arising from autolysis of krill (Example 4A) or from hydrolysis of the fish protein (Example 4B) were treated in an Amicon Diaflo Ultrafiltration Unit using a Diaflo Ultrafilter type YC having a 500 MW cutoff.

The low-molecular fraction containing a high proportion of free amino acids was concentrated and freeze dried. The present sample contains a mixture of free amino acids.

EXAMPLE 5. PRODUCTION OF A GROWTH MEDIUM FOR FERMENTATION

A. Growth medium from krill protein

Frozen Antarctic krill (Euphausia superba) were thawed, macerated and allowed to autolyse as described in Example 4A. After autolysis the mixture was centrifuged at 13 000 g

for 40 min. The aqueous phase was removed, concentrated in a rotary evaporator and freeze dried.

The preparation is suitable as a source of amino acids in microbial media. This was demonstrated by including the preparation at a concentration of 0.5 % w/v in a microbiological medium instead of Trypton. In separate experiments a Lactobacillus sp. and a Bacillus sp. was inoculated into the medium. The rate of growth was of the same order in the medium containing krill autolysate as in that with Trypton.

B. Growth medium from fish protein

Frozen capelin (Mallotus villosus) were thawed, macerated and 3 g dry weight suspended in 100 ml deionised water. 0.20 g of "freeze dried krill extract" were added, and the hydrolysis carried out at 50 °C for 20 h at pH 7.0. After hydrolysis the mixture was centrifuged at 13 000 g for 40 min. The aqueous phase was concentrated in a rotary evaporator and freeze dried.

The preparation served as a good source of amino acids for the growth of a Lactobacillus sp. and a Bacillus sp.

EXAMPLE 6. ENZYMIC TREATMENT OF ANIMAL FEEDSTUFFS

A. Improvement of the digestibility of fish protein

Commercial fishmeal was subjected to partial hydrolysis in order to improve its digestibility. 10 g of fish meal were added 10 ml of deionised water and 0.5 g of "freeze dried krill extract". The slurry was dried under vacuum to a water content of approx. 30 %, and subsequently stored at 10 °C for 3 weeks. During this period a partial proteolysis took place as evidenced by an increase in solubility of the fish meal. The preparation was suitable as a component in fish feed.

B. Improvement of the functional properties of fish protein

10 g of fishmeal were added 10 ml of deionised water and 0.5 g of "freeze dried krill extract". The slurry was kept at a temperature of 25 °C for 3 days, and subsequently dried under vacuum at 60 °C. The preparation exhibited improved functional properties compared to untreated fishmeal, as evidenced by a higher swelling- and fat emulsifying capacity.

EXAMPLE 7. TENDERIZING OF MUSCLE FOODS

A. Tenderizing of beef muscle

A piece of beef longissimus dorsi muscle weighing approximately 100 g was injected with approximately 10 ml of a 1 % solution of "purified krill enzymes" in water using a syringe. The enzyme was distributed throughout the sample by injecting small amounts enzyme solution into various parts of the muscle.

The sample was kept at 10 °C for 1 week. After heating to 80 °C for 10 min to inactivate the enzyme, the piece of meat treated with enzyme was noticeably more tender than a control injected with just water, and stored under the same conditions.

B. Tenderizing of herring muscle

A piece of filet of herring (Clupea harengus) weighing approximately 100 g was injected with 10 ml of a 1 % solution of "purified krill enzyme" using a syringe as described above under Example 7A. The filet was kept at 10 °C for 1 week, during which time a noticable tenderization of the flesh took place.

EXAMPLE 8. DEBITTERING OF A MILK PROTEIN HYDROLYSATE

3 g of casein were suspended in 100 ml of deionised water and hydrolysed with 15 mg of Papain (Merck) at 60 °C for 3 hours. The resulting hydrolysate was heated to 95 °C for 10 min to inactivate the enzyme. The hydrolysate had a distinctly bitter taste.

The hydrolysate was added 15 mg of "chromatographed krill enzyme", and incubated at 30 °C for 3 hours. The treatment with the krill enzyme significantly reduced the bitterness of the hydrolysate.

EXAMPLE 9. ANTIHAZE TREATMENT OF BEER

Commercial lager beer in cans was stored at 0 °C for several weeks, and subsequently taken through a cycle of cooling to approximately -5 °C, followed by heating to approximately +5 °C in order to develop haziness. After the treatment, the beer was centrifuged at 13 000 g for 60 min.

The sediment after centrifugation was suspended in 25 ml of lager beer, giving a clearly turbid sample. 0.1 g of "purified krill enzymes" were added, and the beer incubated at 10 °C or one week. During this period haziness was noticeably reduced.

EXAMPLE 10. VISCOSITY REDUCTION OF A PROTEIN SOLUTION

A 5 % (w/v) solution of bovine serum albumin in water was prepared. 0.05 g of "purified krill extract" were added to 25 ml of the albumin solution, and the solution kept for one week at 10 °C. During this period the viscosity of the solution was noticeably reduced.

EXAMPLE 11. DEHAIRING OF HIDES

A sample of raw cows hide was treated with a 2 % solution of "purified krill extract" for 24 h at 30 °C. The treatment caused effective depilation of the hide.

EXAMPLE 12. REMOVAL OF TISSUES

A. Removal of scales from fish

A frozen herring (Clupea harengus) was thawed and placed in a solution containing 0.5 % (w/v) of "freeze dried krill extract". The herring was left in the solution for one week at 5 °C. After this treatment the scales of the fish could easily be brushed off.

B. Removal of skin from fish

Frozen capelin (Mallotus villosus) were thawed and placed in a solution containing 2 % (w/v) of "freeze dried krill extract". The fish were left in the solution for one week at 5 °C. The treatment effected a partial breakdown of the skin, and allowed the remainder of the skin on the fish to be easily removed.

C. Removal of shells from shrimps

Frozen shrimps (Pandalus borealis) were thawed and placed in a solution containing 2 % (w/v) of "freeze dried krill extract". The shrimps were left in the solution for four days at 5 °C. The shrimps were subsequently heated briefly in hot water. After the present treatment the shells could easily be removed from the shrimps.

D. Weakening of fish roe membrane

The entire roe of a 2 kg cod (Gadus morhua) was placed in a solution containing 2 % (w/v) of "freeze dried krill extract"

and left for one week at 5 °C. During this period the roe membrane was weakened.

EXAMPLE 13. DISSOCIATION AND DISPERSION OF CELLS

The kidney from a rat was removed and cut into small pieces. The pieces of the kidney were placed in buffer containing 0.25 % (w/v) of "chromatographed krill enzymes" and 0.01 M EDTA prewarmed to 37 °C. The suspension was gently shaken during the incubation which lasted for 60 min. The procedure resulted in the liberation of individual kidney cells.

EXAMPLE 14. ISOLATION OF FISH OIL

Frozen herring (Clupea harengus) was thawed and ground in a meat grinder. 25 g of the ground herring were mixed with an equal weight of water. 0.15 g of "freeze dried krill extract" were added, and the mixture incubated for three days at 5 °C. After incubation the mixture was heated to 90 °C for 10 min, and subsequently centrifuged at 13 000 g for 30 min. A distinct oil phase was formed during centrifugation.

EXAMPLE 15. PRODUCTION OF FREE FATTY ACIDS

A. Production of free fatty acids by autolysis of krill

Frozen Antarctic krill (Euphausia superba) were thawed and macerated as described in Example 1A. 50 g of macerated krill were placed in a plastic bottle with screw cap and incubated for 20 h at 40 °C at the natural pH of the krill. During this period a considerable proportion of the lipid, and in particular the phospholipid, was hydrolysed with the formation of free fatty acids. Free fatty acids were isolated by the procedure given in Example 15C.

B. Production of free fatty acids from fish lipid

Frozen herring (Clupea harengus) was thawed and ground in a meat grinder. 25 g of the ground herring were mixed with an equal weight of deionised water, and added 2 g of "freeze dried krill extract". The mixture was incubated at 40 °C for 24 h, during which period free fatty acids were produced. A preparation of free fatty acids was isolated as discribed in Example 15C.

C. Isolation of free fatty acids

10 g of autolysed krill (Example 15A) or hydrolysed herring (Example 15B) were acidified with mineral acid to a pH-value of 2-3. The acidified material was extracted several times with diethyl ether. The ether was removed from the extracts on a rotary evaporator. The residues contained a high proportion of free fatty acids, including the long-chain w-3 fatty acids of marine origin.

CLAIMS

1. Method for modifying the protein-, peptide- and/or lipid constituents of biological material in industrial processes based on the application of enzymes from an animal selected from the group of animals belonging to the order Euphausiaceae. The term modification does not include the application of said enzymes in cleaning procedures or their action inside living organisms.

2. Method according to claim 1, characterized in that the enzymes are peptide hydrolases and/or lipolytic enzymes.

3. Method according to claim 1, characterized in that the enzymes in their monomeric form have apparent molecular weights in the range from 10 000 to 400 000 dalton.

4. Method according to claim 1, characterized in that the substrates hydrolysed are proteins, peptides and/or lipids.

5. Method according to claim 1, characterized in that the enzymic modification takes place at temperatures ranging from - 60 to 250 °C, in particular -5 to 70 °C, for periods ranging from 1 sec to three years, in particular 1 min to 3 months.

6. Method according to claim 1, characterized in that the enzymic modification takes place at the natural pH of the biological material, or at pH-values adjusted artificially in the range from 1 to 13, in particular pH 3 to 10.

7. Method according to claim 1, characterized in that the modification consists in the production of a protein concentrate from a raw material of animal-, plant- or microbial origin.

8. Method according to claim 1, characterized in that the modification consists in the production of a protein hydrolysate for dietary applications.

9. Method according to claim 1, characterized in that the modification consists in the production of free amino acids from a raw material of animal-, plant- or microbial origin.

10. Method according to claim 1, characterized in that the modification consists in the production in an amino acid supplement for microbiological media.

11. Method according to claim 1, characterized in that the modification consists in improving the nutritional and/or functional properties of food- and feedstuffs.

12. Method according to claim 1, characterized in that the modification consists in tenderizing muscle foods.

13. Method according to claim 1, characterized in that the modification consists in the debittering of peptides.

14. Method according to claim 1, characterized in that the modification consists in antihaze treatment of solutions.

15. Method according to claim 1, characterized in that the modificaton consists in reducing the viscosity of protein solutions.

16. Method according to claim 1, characterized in that the modification consists in the dehairing of hides.

17. Method according to claim 1, characterized in that the modification consists in facilitating the removal of skin, membranes, scales or exoskeletons from organisms.

18. Method according to claim 1, characterized in that the modification consists in the dissociation and dispersion of cells in cell culture processes.

19. Method according to claim 1, characterized in that the modification consists in improving the release of oil from fatty raw materials.

20. Method according to claim 1, characterized in that the modification consists in the production of free fatty acids from raw materials of animal-, plant- or microbial origin.

21. Method according to claim 1, characterized in that the modification consists in the production of free fatty acids by autolysis of animals belonging to the order Euphausiaceae.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE89/00235

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [5]

According to International Patent Classification (IPC) or to both National Classification and IPC[4]

C 12 N 9/00, C 12 N 9/50, A 61 K 37/54

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	C 12 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE,NO, DK,FI classes as above. Data base search WPI/L, CA.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	WO, A1, 84/01715 (HELLGREN, LARS) 10 May 1984 see page 2 lines 5-8, page 6, lines 24-25 page 7 lines 16-20, page 5 line 25, page 7 lines 16-20 & EP, 0107634 CA, 1220740 AU, 573730 SE, 8302268 US, 4801451	1-20
X	Chemical Abstracts, Vol 105 (1986), abstract No 72633t, Experientia 1986, 42(4), 403-4 (Eng).	1-20
X	WO, A1, 85/04809 (HELLGREN, LARS) 7 November 1985 see page 6 lines 24-28 and example 4, page 16 & SE, 8402238 EP, 0177605 JP,T, 61501918	1-20

.../...

* Special categories of cited documents: [10]

"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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1989-06-14	1989 -06- 2[illegible]
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Yvonne Siösteen

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	US, 4695457 SE, 454566	
A	Process Biochemistry, Vol 14, October 1979, pages 17-19, (T Ellingsen and V Mohr), "A new process for the utilization of antarctic krill"	1-6
A	Comp.Biochem.Physiol., Vol 83B, No 4, 1986, pages 801-805, (Knut Kr. Osnes et al), "Hydrolysis of proteins by peptide hydrolases of antarctic krill, euphausia superba", see page 805	1-6

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A23B 4/03 — A1

(11) International Publication Number: **WO 97/38585**

(43) International Publication Date: 23 October 1997 (23.10.97)

(21) International Application Number: PCT/CA97/00238

(22) International Filing Date: 9 April 1997 (09.04.97)

(30) Priority Data:
08/632,832 16 April 1996 (16.04.96) US

(71) Applicant: THE UNIVERSITY OF BRITISH COLUMBIA [CA/CA]; 2075 Wesbrook Mall, Vancouver, British Columbia V6T 1Z1 (CA).

(72) Inventors: DURANCE, Timothy, Douglas; 3656 Point Grey Road, Vancouver, British Columbia V6R 1A9 (CA). LIU, Fang; 109 - 2730 Acadia Road, Vancouver, British Columbia V6T 1R9 (CA).

(74) Agent: ROWLEY, C., A.; MacMillan Bloedel Research, Patents & Licensing, 4225 Kincaid Street, Burnaby, British Columbia V5G 4P5 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

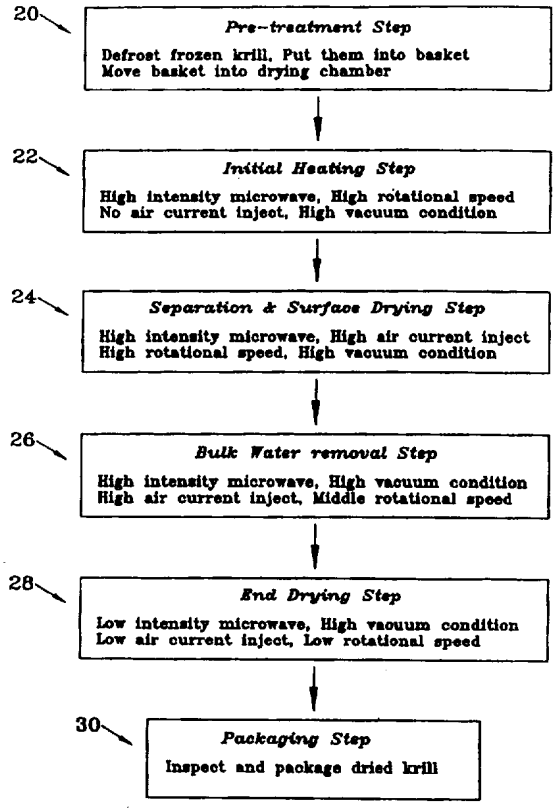
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DEHYDRATED KRILL AND METHOD OF PRODUCING SAME

(57) Abstract

A new form of dried krill is provided by a plurality of substantially separate whole dried krill carcasses substantially all of which have a natural red color and sufficient strength and integrity to withstand normal handling without crumbling into small pieces and retain a strong wholesome fish aroma and flavor. The krill are dried by a method wherein a sequence of energy applications are applied at pressures below atmospheric and the surface of the product simultaneously swept by air to remove moisture. During the process the krill are subjected to a tumbling action. The energy applications are preferably microwave energy applications.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Dehydrated Krill and Method of Producing Same

Field of Invention

The present invention relates to a new form of dried krill and to a process and apparatus for producing such substantially dry krill.

Background

Krill are small marine crustaceans belong to the family Euphasiacea. They are closely related, but distinct from the shrimp family, Decapoda. More than 80 species of Euphasiacea are known but only about six species are commercially important, particularily *Eupahasia pacifica, E. superba, Thysanoessa spinifera, T. inspinata, T. longipes* and *T. rashii*. Frozen and dried krill and krill products are consumed as human food. Substantial quantities of krill are also caught and processed for animal feeds, especially fish feed.

The main current market for dried krill product is for fish food with another important market being human food where it is used as a flavorant. The texture, color, flavor and aroma are important characteristics of the dried krill and generally reflect the quality of the product.

Currently there are two known methods of drying krill to produce the product for the market. Both processes produce a dried krill with poor coloring and generally of small particle size i.e. broken pieces or ,more likely in powder form.

Freeze drying of krill is one of the process use to produce dried krill. In this process the krill are frozen shortly after they are caught and then freeze dried at a convenient time. The dried product is usually in block form. The krill are brittle and easily broken and are in many cases crushed into a powder. Freeze dried krill have a very low moisture content due to the nature of the drying process, exhibit a pale red color, initially has a mild aroma, but oxidizes quickly to take on a fishy odor and has a flat or oxidized flavor. Protein retention of freeze dried krill is excellent.

Another method of drying krill is air drying wherein the fresh krill is immediately blanched and then dried in trays or ground and spray dried. Obviously with this technique the krill is treated immediately. The resultant product has a high moisture content (greater than about 12%), may be in whole or broken form if tray dried or in powder form if spray dried, has a yellow to pale red color, very mild weak aroma and little flavor. Blanching and air drying of krill significantly reduces its protein content.

It will be apparent that the dried product formed by either of the two methods is not high quality in that the color aroma and flavor, which are some of the most important characteristics of the product have been significantly deteriorated.

Brief description of the Invention

It is the main object of the invention to provide a new dried krill product that has a natural red color, is largely unbroken, has a strong, desirable characteristic odor and good taste and to provide a method and apparatus for producing same.

Broadly the present invention relates to a dried krill product comprising a plurality of substantially separate whole dried krill carcasses substantially all of which have a natural red color and sufficient strength and integrity to withstand normal handling without crumbling into small pieces and retain a strong wholesome fish aroma and flavor.

The present invention also relates to a method and apparatus for producing dried krill products in the form of whole but separate carcasses comprising arranging raw krill in an at least partially separated arrangement in a microwave transparent carrier, partially drying said raw krill to provide a partially dried product substantially free of surface moisture but containing a first amount of unbound moisture within its structure, heating said partially dried product by means of electromagnetic radiation, subjecting said partially dried predate to a reduced pressure below atmospheric pressure during at least a portion of a period of time in which said product is subjected to electromagnetic radiation coordinated to provide a heated dried product containing unbound within its structure a second amount of moisture sufficient to generate flexibility and strength in the product, such that the form of whole krill is maintained during the drying process and subjecting said krill to a tumbling action during said partially drying and said heating by means of electromagnetic energy.

Preferably said partially drying includes defrosting said raw krill prior to said heating said partially dried product by means of electromagnetic radiation,

Preferably said subjecting to reduce pressure below atmospheric pressure includes sweeping surfaces of said product with moisture unsaturated air.

Preferably said below atmospheric pressure will be less than 120 Torr preferably less than 100 Torr and said pressure will be attained in less than 2 minutes preferably less than 1.7 minutes.

Preferably said second amount of moisture comprises between 10 and 40 % by weight of the separate dried product.

Preferably said dried product will be at a temperature of between 40 and 90 °C

Preferably said electromagnetic radiation comprise microwaves

Brief Description of the Drawing

Further features, objects and advantages will be apparent from the detailed description of the preferred embodiments of the present invention taken in conjunction with the accompanying drawing in which;

Fig. 1 is a flow chart of the method of the present invention.

Fig. 2 is a diagram of one embodiment of the apparatus of the present invention.

Figure 3 is an illustration of a typical whole dried krill carcass as produced using the present invention.

Description of the preferred Embodiment

The method of the present invention is suitable for the preparation of dried krill and other sea foods for either fresh krill or frozen krill which may be defrosted and pressed to remove some of the free water. In this description, the term wet product shall mean fresh or frozen krill or other sea foods to which the invention may be applied for example shrimp, algae, small fish, etc.

The following description will deal primarily with krill, but it is intended that the term krill to read where reasonable as any of other similar materials that may be treated or processed to advantage using the present invention. It will be apparent that when a different material is to be dried to provide the dried product the conditions will have to be tuned to obtain the desired natural color and high quality in the dried product.

As shown Figure 1, initial preparation of fresh or frozen krills as designated by the box 20 includes the steps of defrosting, if required, and weighting the fresh krill and arranging them in or on a microwave transparent carrier such as a basket or the like for transport. Preferably the fresh krill will also be treated to drain excess surface moisture by a pressing or centrifugation method.

In carrying out the method of the invention as part of the preparation stage 20 of Figure 1 fresh krill are preferably placed in a suitable transport system such as the plastic basket drum and, if desired treated with suitable seasoning. If krill was frozen before drying, it will preferably be defrosted before drying, although defrosting can be achieved in the vacuum microwave chamber during a heating stage, if desired. It is

believed that predrying of previously frozen and defrosted krill enhances the drying rate because some water is removed as drip loss and need not be evaporated.

After such treatment, the treated product is subjected to an initial heating step as indicated at 22 which at least partially dries the krills preferably by application of microwave energy under partial vacuum conditions with reduced oxygen concentration. During this initial heating step 22 water releases from the krill, drips from the baskets 64 (see Figure 2) and is removed from the vacuum chamber 60 (Figure 2) as liquid, through the vacuum pump 88 or through an optional draining system (not shown). The time to complete step 22 depends on the weight of fresh krill in the chamber and microwave power density and is set so that at the end of the initial heating step 22, the moisture content of the krill is about 70 % to 78 % by weight.

The initial heating step 22 is followed by a moisture separation and surface drying step 24. wherein a high intensity microwave field (more than about 0.6 kW/kg of krill) is applied. The intensity of the field in step 24 is preferably selected to raise the temperature of the krill to about 60 °C in about 10 minutes, thereby to rapidly convert a major portion of the moisture within the krills into a heated vapor. While typically raw krill have a moisture content of approximately 80 % by weight and it is slightly reduced to about 70 to 78 % in step 22, the expose of the krills to the high intensity microwave field in the moisture separation and drying at 24 applies sufficient heat to heat the krill the required temperature to substantially prevent enzyme reaction and also to reduce the moisture content, yet not so high as to damage the krill. The separation and surface drying step 24 is carried out preferably at a pressure of about 80 to 120 Torr and a temperature of about 47 °C to 55 °C. The drying step 24 may take up to about 15 minutes.

In the preferred embodiment of the present invention, total moisture content of the krill leaving the stage 24 is about 60 % to 75 % by weight of the krills with desired optimum of about 73 %.

The separation and surface drying step 24 serves to vaporize a substantial portion of the tissue moisture and flush the water vapor out of the chamber. It also serves to dry the shell surface of the krill, thereby allowing the subsequent bulk water drying step 26 of the present invention.

If the krill are insufficiently dried in the separation and surface drying step 24, the shell of the krill will be sticky and the krill will tend to form a ball if placed together in a revolving basket.

Air flow rates for these air currents in step 24 are preferably between about $2.8x10^{-5}$ and $5.6x10^{-5}$ $m^3/kg.s$ fresh krill. Because a larger amount of moisture escapes from the krill during expose to the high intensity microwave field, the air injection method preferably is used to minimize condensation within the chamber. Such condensation would decrease the amount of microwave energy available for heating and drying krill because the condense again absorbs microwave energy in the chamber, is vaporized and may again condense on the chamber wall. This is called the "heat pump effect" and it greatly reduces microwave energy usage efficiency and increases the processing time if not minimized or prevented.

Next the partially dried krill are subjected to a bulk water drying step as indicated at 26 wherein further moisture is preferably removed by evaporation under below atmospheric pressure conditions and the use of air jets which spray dry air over the partially dried krill product i.e. the product is swept by air currents which pick up moisture from the surface of the product while it is simultaneously subjected to the application of high intensity microwave energy under below atmospheric pressure conditions.

In the bulk water removal step 26 the at least partially dried krill are exposed to a middle intensity microwave field for a period of time to raise their temperature to at least 50 °C within about 10 minutes and under a pressure of about 100 Torr to reduce the moisture content of krill to about 65 % to 30 % by weight. The temperature of krill is higher because in part of the increased mass flow resistance of the krill surface increases the vapor pressure inside the krill body thereby effecting the vapor temperature by thermodynamic relationship between vapor pressure and temperature

The intensity of the microwave field and the duration of exposure is coordinated with the weight of fresh krill to achieve the desired dehydration and heating rates.

Preferably heat is being applied in the stage 22 and the separation and surface drying and bulk water removing steps 24 and 26 are carried out in the same closed vessel.

Obviously any step requiring pressure and/or a controlled atmosphere other than atmospheric must be carried out in some form of closed container which in some stages must also contain microwave energy when used. Where such conditions are not applied the krill need not to be so contained.

After a bulk removal step 26, the substantially dehydrated krill are finish dried in end drying step 28 to the desired moisture content by applying a low microwave intensity (about 0.4 kW/kg krill), high vacuum (less than 80 Torr) and a low air injection flow rate e.g. $2.8x10^{-5}$ $m^3/kg.s$. If desired, this end drying step 28 may alternatively be achieve using hot air drying at elevated temperature about 45 °C and at atmosphere air pressure, but finish drying in a conventional air dryer or oven is slower. With either option after end drying in step 28 the resultant product is a dried krill composed of substantially whole carcasses with natural red color, a moisture content of about 10 % to 15 % by weight and retaining its wholesome seafood aroma and flavor.

The krill is subjected to a tumbling action applied thereto by rotation of the basket or the like in which it is contained during the stages or steps 22, 24, 26 and 28 to facilitate the escape of moisture from the load of krill, permit more uniform drying and to impede the individual krill from sticking together.

The dried krill product so produced is shown in Figure 3 and will consist mainly of whole krill 40 and a significant portion of krill pieces similar to those shown at 42, and will not contain a substantial amount of powdered krill.

Turning to Figure 2, equipment for carrying the process of the present invention is illustrated schematically. The equipment includes a microwave and vacuum chamber 60 having an inlet door 62. The krill product in suitable, substantially cylindrical shaped (for rotation within the chamber 60 as will be described below) containers (baskets) 64 of is delivered to the chamber 60. The baskets 64 are substantially right cylindircal containing the product are introduced into the chamber 60 at the appropriate point in the process (depending on where microwave power is to be first applied, for example to defrost frozen krill) and are sealed within the chamber 60 for the application of energy, reduced pressure and sweeping of surfaces with dry air as described above.

The microwave energy is provided in the illustrated system by three magnetrons 70, 72 and 74 which inject the microwaves into the chamber 60 through sealing windows 76, 78 and 80 and hence into the basket(s) 64 within the chamber 60.

The baskets 64 are shown supported within the chamber on a rolling system 86 formed by a plurality of horizontal rollers 85 (only one shown) that in turn is preferably supported by a suitable platform 87 on side of which is supported by a load cell 82 which measures the weight in the chamber 60 and delivers this information to the control computer 84. The rolling of the basket 64 during the process applies a tumbling action to the krill.

Suitable temperature and pressure gauges schematically indicated at 90 measure the temperature and pressure in the chamber 60 and provide this information to the control computer 84.

Below atmospheric pressure is applied by vacuum pump 88 controlled by computer 84 to reduce ambient pressure within the chamber to the appropriate level, at the appropriate time in the process and air is bled into the chamber 60 at the appropriate times under control of the flow meter 92 which in turn regulates the air flow based on the commands from the computer 84.

After completion of the operation to be carried out in the chamber 60 the baskets 64 are removed through the door 62.

Destructive enzyme reactions take place within a few hours at temperatures above freezing, especially when oxygen content is high around the krill and these reactions change the natural red color of fresh krill to black, and also cause a loss of protein content due to enzyme catalyzed hydrolysis during drying. The vacuum condition, the elevated temperature during the initial heating step 22 and the rapid drying rate during steps 24, 26, 28 and 30 substantially prevent these reactions.

Example 1:

The frozen krill (*Euphausia pacifica*) are defrosted first and drained of free water. 5.0 kilograms of krill with initial moisture content of 80 % by weight are placed into plastic rolling (cylindrical) basket (Fig. 1) then moved into the microwave vacuum dehydration system 60. High intensity microwave power (above defined), high rate of rotation of the basket on a horixontasl axis (4 RPM) and 120 Torr of ambient pressure are used in step 22. There is no air injection flow during the step 22.. The temperature of the krill is about 60°C in the initial heating step 22. The chamber

pressure in the step 22 is 100 Torr and the time is 10 minutes. At end of step 22, the moisture content of krill is 78 % by weight.

After the initial heating step 22, the krill are next subjected to a separation and surface drying step 24 wherein high intensity microwave energy is applied. The air injection flow rate is $5.6x10^{-5}$ $m^3/kg.s$ with air temperature 20 °C. The chamber pressure in step 24 is 120 Torr. The separation and surface drying step 24 is 15 minutes long. High air flow rate quickly sweeps water vapor out of the chamber and the surface of krill dry without sticking to each other. The moisture content of krill at end of the separation and surface drying step 24 is 74.6 % by weight.

In the bulk water removal step 26 following step 24 the ambient pressure is 100 Torr and air injection flow rate is $2.8x10^{-5}$ $m^3/kg.s$. The rotational speed of basket is 2 RPM. and the temperature of krill is 65 °C. High intensity of microwave is used in this step. At end of step 26, the krill weigh 2.2 kilograms with moisture content of 63 % by weight. End drying step drying step 28 was finished by air dryer in this example.

The final krill product after above treatment has a natural red color which was measured by LabScan Color Meter (Hunter Associate Laboratory, Inc.), L = 30.92, a = 12.94, and b = 6.81. The protein content of dried krills is about 54 % by weight. The final moisture content is 12% by weight.

Example 2:

Following the flow chart in Fig. 1, the six kilograms of fresh krill with initial moisture content of 77 % by weight are placed into plastic cylindrical basket then moved in microwave vacuum dehydration system in the pre-treatment step 20. The initial heating step 22 following step 20 applies high intensity microwave energy (0.6 kW/kg krill), high rotation rate (4 RPM) and 15.95 kPa (120 Torr) of ambient pressure. No air injection is used in the step 22. The temperature of krill is 60 °C in the initial heating step 22 and heating time is 10 minutes long. At end of the step 22, the krill weigh is reduced to 4.38 kilograms and the moisture content is 70 % by weight.

High intensity microwave energy (0.65 kW/kg.krill), high rotation speed (4 RPM), high air injection flow rate, 5.6×10^{-5} $m^3/kg.s$, and 15.95 kPa absolute (120 Torr) ambient pressure are applied in surface drying step 24 after the step 22. The surface of krill is dried quickly. At end of separation drying step 24, the krill are separated from each other and krill surfaces are more dry than inside the body. The

drying time during the separation and drying step 24 is ten minutes. The weight of krill at end of the step 24 is 3.5 kg with 62 percent of moisture content by weight.

In the bulk water removal step 26 following the step 24 the ambient pressure is 13.28 kPa and air injection flow rate is $2.8x10^{-5}$ $m^3/kg.s$. The rotational speed of the plastic basket is 2 RPM. The moisture content of krill at end of bulk water removal step 26 is 35 % by weight and the total weight of krill is reduced to 2.04 kilograms. Time is 45 minutes from the beginning of drying.

After the bulk water removal step 26, krill are finish dried in low intensity, high vacuum and low air current injection rate, in the finish drying step 28, i.e. 0.4 kW per kilogram of krill, 10.63 kPa ambient pressure, $2.8x10^{-5}$ $m^3/kg.s$ of air injection flow rate (air temperature is 20°C) and the basket is revolving at one RPM. The duration of the finish drying step 28 is 20 minutes. The dehydrated krill leaving the step 28 has a weight of 1.48 kilograms with a moisture content of 11.5 % by weight. The color of the dried krill product was measured by LabScan Color Meter (Hunter Association Laboratory, Inc.). The results of measure are L = 33.16, a = 16.36 and b = 6.81. The protein content was about the same as the last example.

Having described the invention modifications will be evident to those skilled in the art without departing from the invention as defined in the appended claims.

We claim

1. A dried krill product comprising a plurality of substantially separate whole dried krill carcasses substantially all of which have a natural red color and sufficient strength and integrity to withstand normal handling without crumbling into small pieces and retain a strong wholesome fish aroma and flavor.

2. A method for producing dried krill products in the form of whole but separate carcasses comprising arranging raw krill in an at least partially separated arrangement in a microwave transparent carrier, partially drying said raw krill to provide a partially dried product substantially free of surface moisture but containing a first amount of unbound moisture within its structure, heating said partially dried product by means of electromagnetic radiation, subjecting said partially dried product to a reduced pressure below atmospheric pressure during at least the portion of a period of time in which said product is subjected to electromagnetic radiation to provide a heated dried product containing unbound within its structure a second amount of moisture sufficient to generate flexibility and strength in the product, such that the form of whole krill is maintained during the drying process, and subjecting said krill to a tumbling action during said partial drying and said heating by electromagnetic radiation.

3. A method as defined in claim 2 wherein said partially drying includes defrosting said raw krill prior to heating said partially dried product by means of electromagnetic radiation.

4. A method as defined in claim 2 wherein said subjecting said partially dried product to reduce pressure below atmospheric pressure includes sweeping surfaces of said product with moisture unsaturated air.

5. A method as defined in claim 2, 3 or 4 wherein said second amount of moisture comprises between 10 and 40 % by weight of the dried product.

6. A method as defined in claim 2, 3 or 4 wherein said pressure below atmospheric pressure is less than 120 Torr and said pressure is attained in less than 2 minutes.

7. A method as defined in claim 2, 3 or 4 wherein said pressure below atmospheric pressure is less than 100 Torr and said pressure is attained in less than 1.7 minutes.

8. A method as defined in claim 2, 3 or 4 wherein said dried product is heated to a temperature of between 40 and 90 °C.

9. A method as defined in claim 2, 3 or 4 wherein said electromagnetic radiation comprise microwaves.

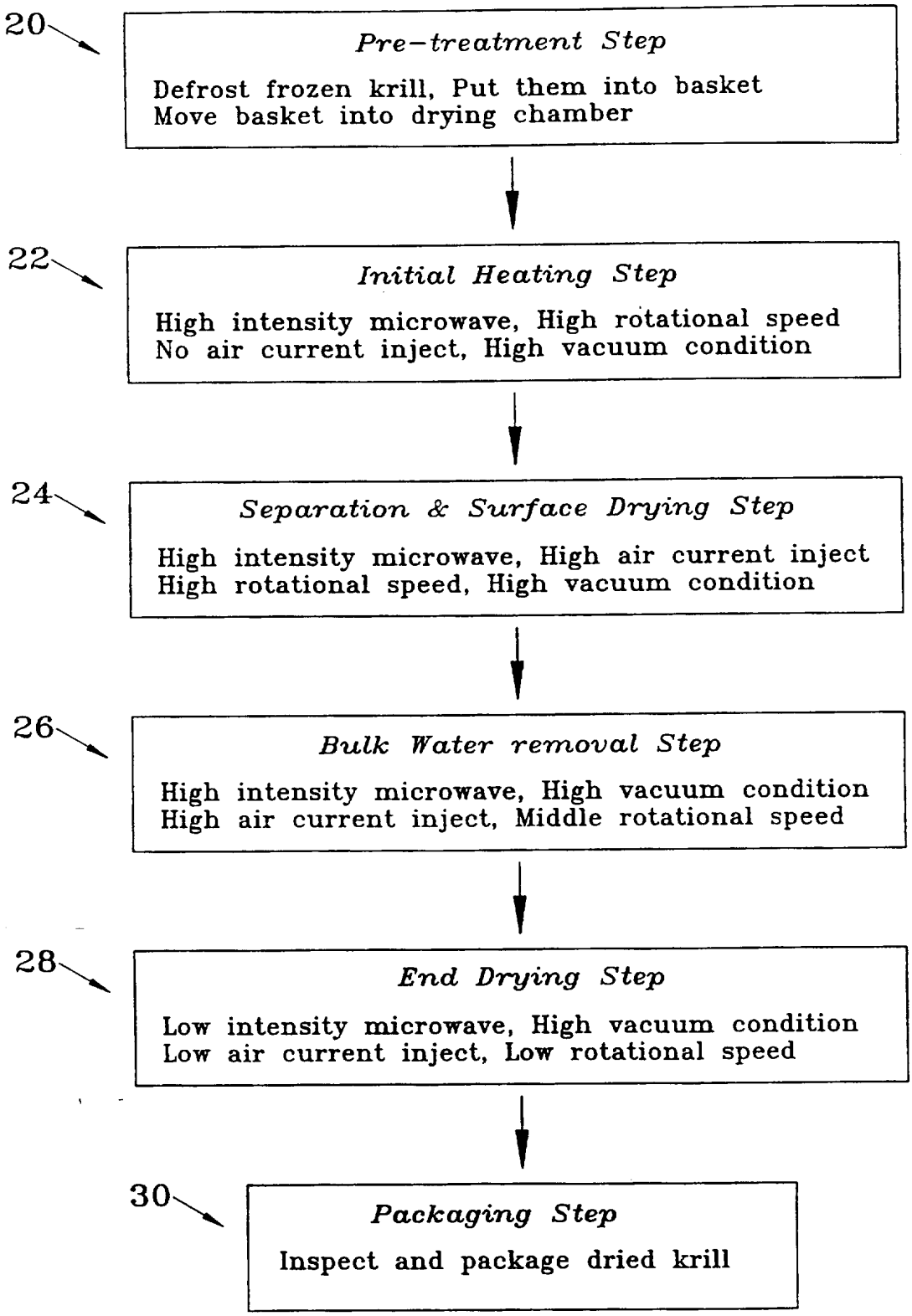


Figure 1

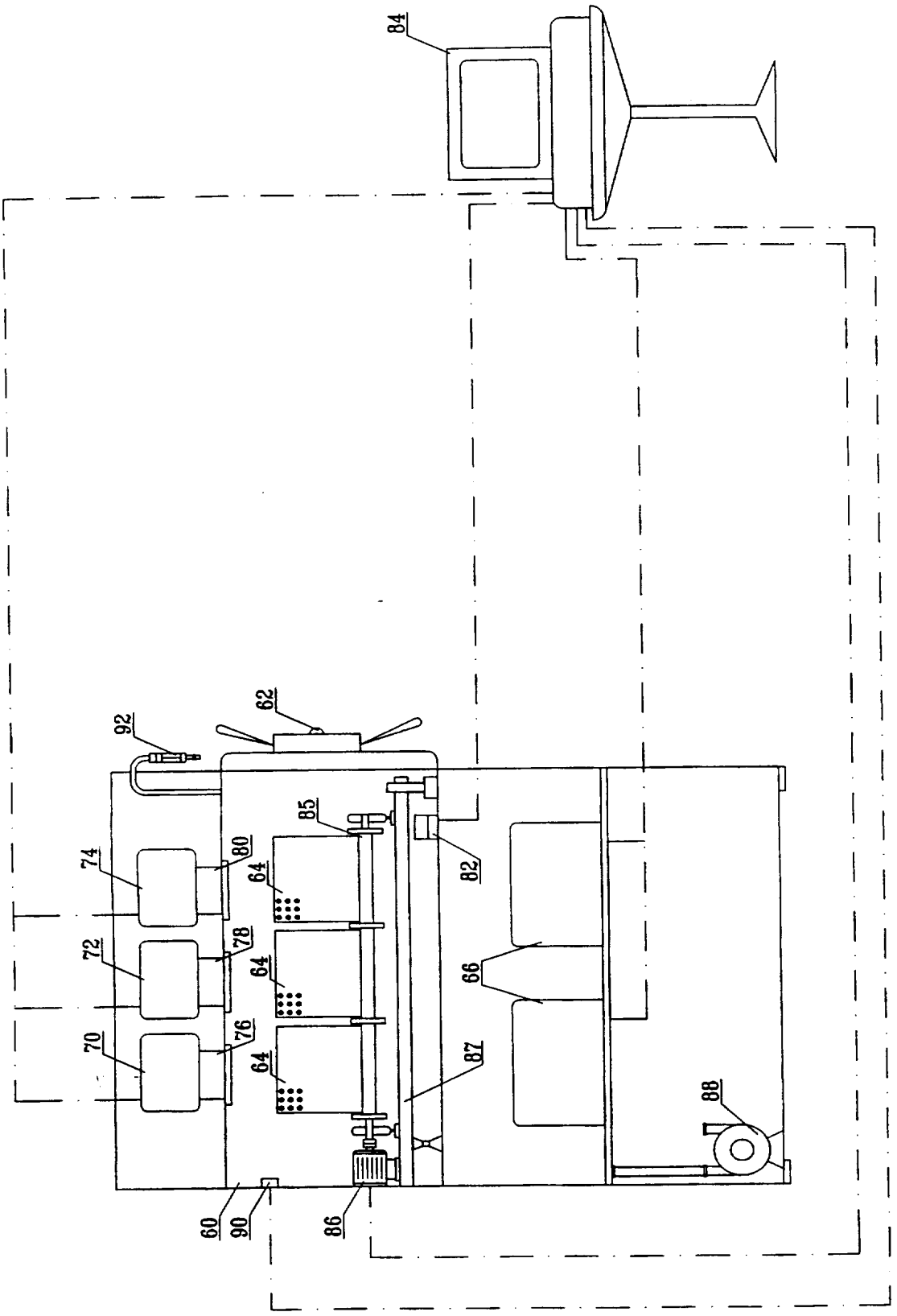


Figure 2

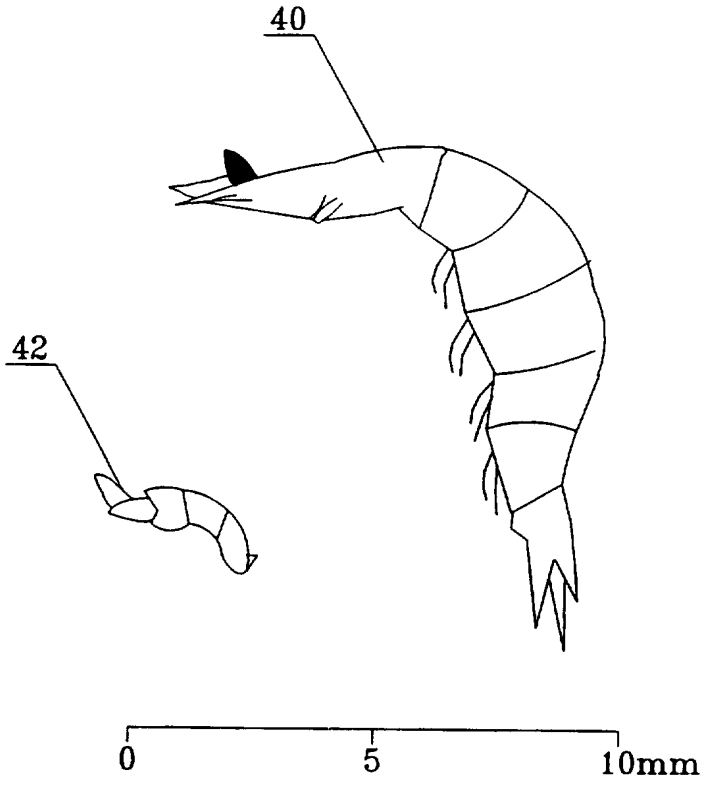


Figure 3

INTERNATIONAL SEARCH REPORT

International ..pplication No
PCT/CA 97/00238

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23B4/03

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 6 A23B

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 112 438 A (D. J. GODSON) 8 May 1968 see claims 1-8; example 2 ---	1,2,4,5, 8,9
A	US 5 467 694 A (MEIJI SEIKA) 21 November 1995 see column 1, line 13 - line 16; figures see column 2, line 11 - line 16 ---	1
A	CA 2 118 159 A (PRAWNTO SHRIMP MACHINE) 7 December 1995 see the whole document ---	1
A	PATENT ABSTRACTS OF JAPAN vol. 11, no. 63 (C-406), 26 February 1987 & JP 61 224966 A (AKABOSHI RYOICHI ET AL.), 6 October 1986, see abstract ---	1
	-/--	

[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

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 1997	10.09.97

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	Guyon, R

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/CA 97/00238

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 13, no. 211 (C-597), 17 May 1989 & JP 01 030562 A (TAGUCHI YUKITOSHI), 1 February 1989, see abstract ---	1
A	GB 1 025 959 A (UNILEVER) 14 April 1966 see claims 1-4,9 -----	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CA 97/00238

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 1112438 A		NONE	
US 5467694 A	21-11-95	JP 7274922 A GB 2288718 A,B	24-10-95 01-11-95
CA 2118159 A	07-12-95	NONE	
GB 1025959 A		NONE	

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A23K 1/10, 1/16, 1/18, A23N 17/00, C12N 9/00, A23J 1/04

A1

(11) International Publication Number: **WO 98/34498**

(43) International Publication Date: 13 August 1998 (13.08.98)

(21) International Application Number: PCT/CA98/00082

(22) International Filing Date: 9 February 1998 (09.02.98)

(30) Priority Data:
2,197,137 7 February 1997 (07.02.97) CA

(71) Applicant *(for all designated States except US)*: BIOZYME SYSTEMS INC. [CA/CA]; 4160 Marine Drive, West Vancouver, British Columbia V7V 1A7 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SAXBY, David, J. [CA/CA]; 4727 South Piccadily, West Vancouver, British Columbia V6Z 2R1 (CA). SPENCE, John, A. [CA/CA]; 2025 Gisby Street, West Vancouver, British Columbia V7V 4N2 (CA). SAXBY, Gregor [CA/CA]; 4727 South Piccadily, West Vancouver, British Columbia V6Z 2R1 (CA). ALOISE, Pedro [CA/CA]; 202–1725 Osoyoos Crescent, Vancouver, British Columbia V6T 1X7 (CA).

(74) Agent: UREN, John, Russell; #202–1590 Bellevue Avenue, West Vancouver, British Columbia V7V 1A7 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD AND APPARATUS FOR PROCESSING KRILL HYDROLYSATES

(57) Abstract

Method and apparatus used in producing a feed product or premix and the products made by the method. A predetermined quantity of krill hydrolysate is added to a predetermined quantity of dry carrier with or without a predetermined quantity of liquid marine protein. The mixture is subject to evaporation and drying steps in which relatively heavier particles are separated from relatively lighter particles. The mixture may be blended, ground and subject to chemical reaction in a balance tank prior to entering a dryer. The dryer utilises a warm air source, a tower and a cyclone to dry the mixture following its entry into the dryer. Temperature sensitive enzymes or other bioactive products may be added to the product produced from the dryer. A method for obtaining enzymes from a fresh krill extract or an autolysed krill preparation and the product are also disclosed. A method for separating the bound protein and pigments from crustacean waste using krill enzymes and a product produced by the method are also described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TITLE OF THE INVENTION

METHOD AND APPARATUS FOR PROCESSING KRILL HYDROLYSATES

INTRODUCTION

This invention relates to a method and apparatus used in producing a feed product or premix and the product made by the method and, more particularly, to a process using co-drying to dry a mixture of krill hydrolysate and dry carrier or a mixture of krill hydrolysate, fish hydrolysate and dry carrier. The invention further relates to recovering enzymes from krill and, more particularly, to recovering enzymes from both freshly harvested and hydrolyzed krill. The invention further relates to utilising krill enzymes for removing protein from marine and biological wastes and, more particularly, for removing

protein, chitin and other constitutents from crustacean and other marine wastes.

BACKGROUND OF THE INVENTION

With the advent of increasing activity in aquaculture or fish farming in the early to mid-1980s, research has been ongoing into increasing productivity or growth rate and reducing the mortality rate of fish raised in aquaculture conditions since survival of such fish is important. One such factor relates to enhancing the nutritional value and palatability of feed used in raising such fish. In addition to the nutritional value, it is desirable to reduce the cost of feed to such fish since, typically, the feed totals approximately 40 to 50% of the cost of raising the fish. Such feed should be a high quality feed to meet the objectives of having high nutritional value to maximize growth and to reduce fish mortality.

The requirement for feed products in aquaculture is projected to grow substantially and, as a result, there is and will be pressure to obtain the necessary ingredients for fish food. The possibility of using zooplankton and, in particular, euphausiids, as a fish feed, appetizer or food product has been investigated and has been found to be possible and desirable, particularly as a feed product.

In addition, blends of krill hydrolysates and fish hydrolysates or any one of these with a dry carrier, can

povide alternatives to fish meals in aquaculture and other animal feed diets. Euphausiids are a natural feed harvested directly from coastal waters and have a high nutritional value but, previously, the cost of harvesting and processing such zooplankton for a feed product has been prohibitively expensive.

As well, the questions of the availability of the biomass of such zooplankton and its harvesting, handling, storage and processing are parameters that must be investigated in order to determine whether the product would be appropriate as a feed product.

Through papers written by Fulton and other authors, the use of zooplankton as a food or feed product has been contemplated for some time. In particular, antarctic krill (Euphausia superba) for human consumption have been investigated, although relatively little work has been investigated related to aquaculture. The use of Euphausia pacifica in the coastal waters of British Columbia, Canada has been considered in relation to its use in aquaculture and other animal feeds.

It appears, from those investigations, that the necessary biomass is available in coastal waters. Previously, euphausiids have been used as a pet food ingredient and some aquaculture operators have used euphausiids as a feed product. The euphausiids were used for such purposes in a frozen form after being harvested and

in some cases, the euphausiids were freeze dried following harvesting. This is an expensive procedure.

In processing feed products, it has typically been the case that the ingredients used in such feed products are heated to a high temperature around 100°C when the product is processed and dried. By heating the product to such a high temperature, it is believed that the enzymes and other proteins in the product are denatured. If, however, it is intended to utilize the product for early stage or juvenile aquaculture, which young fish have relatively undeveloped digestive systems, it is desirable that in some application, the euphausiid products maintain a certain proportion of enzymes which will assist the digestive process in juvenile and other life stages. If the theory that enzymes are advantageous in nutrition is correct, such destruction of the enzymes during the aforementioned drying process is disadvantageous.

It is also desirable to have a natural product, where the proteins are not denatured, available for early stage juvenile or larvae feed. In some previous products, exogenous enzymes have been added to the zooplankton mix. However, the addition of such enzymes is difficult to control and can result in a complete hydrolysis of the proteins to amino acids. The presence of free amino acids in the feed needs to be controlled since they can create an inferior product of substantially reduced value as a feed product.

It has been shown, surprisingly, that the degree of enzyme activity which results in determining the digestibility of a product, reaches a relatively constant value after a certain period of time in a natural product. Recent investigations conducted by the applicant have confirmed this characteristic for Euphausia pacifica. This characteristic was first discovered in relation to Euphausia superba by Kubota and Sakai in a report entitled "Autolysis of Antarctic Krill Protein and Its Inactivation by Combined Effects of Temperature and pH", Transactions of the Tokyo University of Fisheries, number 2, page 53-63, March 1978. However, the antarctic krill study done by Messrs. Kubota and Sakai had the objective of limiting enzyme activity which was deleterious to obtaining a food as opposed to a feed product. Messrs. Kubota and Sakai wished to inhibit the enzymatic activity by certain processing techniques which they considered desirable when the product was intended as a food product.

An appropriate degree of hydrolysis is obtained during the digestion of the euphausiids. The approximate degree of hydrolysis will vary depending on the final application and it can be monitored by measuring the apparent viscosity in the final product. Further processing may then take place in order to make a useful product for commercial feed. Such processes may include adding acid to obtain an acid stabilized product concentrating fractionating or drying the product. A variety of drying techniques such as freeze drying, spray drying, or vacuum and air drying. Spray drying, as well as some other drying

processes, however, are done at temperatures that will permanently inactivate the enzymes in the euphausiids which, as earlier mentioned, may be undesirable for aquaculture purposes although it is acceptable for purposes where the product is intended to be used as a carotenoid biopigment for coloring purposes in both feed and food products or as a source of protein, fatty acids, minerals or other nutrients.

SUMMARY OF THE INVENTION

According to one aspect of the invention, there is provided a method of producing a feed product comprising the steps of adding a predetermined quantity of krill hydrolysate to a quantity of dry carrier to produce a mixture and co-drying said mixture to obtain an end product. The dry carrier may conveniently be a plant protein, dry krill, fish meal, byproduct meal or other dry ingredient suitable for inclusion in a diet.

According to a further aspect of the invention, there is provided a product produced by adding a predetermined quantity of krill hydrolysate to a quantity of liquid marine protein and a quantity of dry carrier to produce a mixture and co-drying said mixture.

According to a further aspect of the invention, there is provided a co-drying apparatus for drying a mixture of krill hydrolysate with or without an evaporator and liquid marine product and a dry carrier comprising a dryer

for concentrating, mixing, agitating, heating and separating particles of said mixture.

According to still a further aspect of the invention, there is provided a method of obtaining an enzyme extract from a liquid krill hydrolysate comprising the steps of subjecting said hydrolysate to decanting and then to centrifugation to obtain a clarified liquid and further subjecting said clarified liquid to ultrafiltration using a membrane with a capacity to retain said enzymes having a molecular weight greater than 10,000 daltons and the product produced by the method.

According to still a further aspect of the invention, there is provided a method of obtaining an enzyme extract from fresh krill comprising the steps of squeezing said krill to obtain an aqueous extract and subjecting said aqueous extract to ultrafiltration with a membrane adapted to retain enzymes having molecular weights above 10,000 daltons and the product produced by the method.

According to still yet a further aspect of the invention, there is provided a method for removal of protein from non-stabilized or fresh crustacean shell wastes comprising grinding said crustacean wastes and water, transferring said product to a digester, adding a predetermined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively

enzymatically active and relatively high in protein and a second portion of shell material relatively high in chitin and low in protein.

According to still yet a further aspect of the invention, there is provided a method for removal of protein from acid stabilized shell wastes comprising grinding said crustacean wastes, transferring said small particulate size shell wastes to a digester, adding a predetemined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively enzymatically active and relatively high in protein and a second portion of shell material relatively high in chitin and low in protein.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Specific embodiments of the invention will now be described, by way of example only, with the use of drawings in which:

Figure 1A is a diagrammatic isometric view of a fishing vessel with an attached net which utilizes the euphausiid harvesting technique according to the invention;

Figure 1B is a diagrammatic front view of a net in an alternative harvesting technique according to the invention;

Figure 2A is a diagrammatic side view of a cage which is used to maintain the cod end of the fishing net illustrated in Figure 1 in an open position and which is further used to transport the harvested euphausiids to the harvesting vessel;

Figures 2B and 2C are side and rear views, respectively, of the dewatering trough used to remove water from the harvested euphausiids;

Figure 3 is a diagrammatic process chart illustrating the processing of the euphausiids subsequent to the dewatering steps illustrated in Figure 2 and prior to the drying step;

Figures 4A and 4B are end and side sectional views of the heat exchanger used to raise the temperature of the harvested euphausiids prior to the digester process;

Figure 5 is a diagrammatic side sectional view of the digester used to create the desired enzyme activity within the euphausiids;

Figure 6 is a diagrammatic side sectional view of a ball drier used to dry the euphausiids following removal of the euphausiids from the surge tank located downstream from the digester;

Figure 7 is a flow chart illustrating the process of co-drying the product according to the invention;

Figure 8 is a diagrammatic view of the dehydrator used in the co-drying process according to the invention;

Figure 9 is a diagrammatic view of the codrying process according to a further aspect of the present invention;

Figure 10 is a diagrammatic flow chart illustrating the enzyme extraction process utilising hydrolysed krill;

Figure 11 is a diagrammatic flow chart illustrating the enzyme extraction process utilising fresh krill; and

Figure 12 is a diagrammatic flow chart illustrating the removal of protein and other constitutents from crustacean wastes using krill enzymes according to a further aspect of the present invention.

DESCRIPTION OF SPECIFIC EMBODIMENT

Referring now to the drawings, a towing vessel 10 is illustrated in Figure 1. A plurality of towing ropes 11, 12, 13 are connected to the towing vessel 10 in order to tow a barge 14 and a net 20. A plurality of ropes 21 (only one of which is shown) are connected to the net 20 and extend downwardly from the barge 14. Weights 22 are connected to the bottom of the open forward facing portion of the net 20 in order to maintain the net 20 at a desired and

predetermined depth where the concentration of zooplankton is satisfactory.

The cod or rearward end 23 of the net 20 is maintained in an open condition by the use of a cage generally illustrated at 24 in Figure 2. Cage 24 is of cylindrical configuration and is positioned within the cod end of net 20. It is made from aluminum and is preferably corrosion resistant. A fitting 30 is welded to the downstream end of the cage 24 and one end of a swivel connection 31 is joined to the fitting 30 to prevent fouling the net in the event components become unstable under adverse harvesting conditions. A hose 32 is connected to the other end of the connection 31.

Referring again to Figure 1, hose 32 extends upwardly from the cod end of the net 20 to the barge 14. A pump of a variety of configurations but, conveniently, a diaphragm sump pump 33, is located at the other end of the hose 32 on barge 14. A dewatering trough is generally shown at 34 and is illustrated in Figures 2B and 2C. Dewatering trough 34 has a lengthwise generally rectangular configuration and is also located on barge 14. Dewatering trough conveniently takes the configuration of a "lazy L". A set of screens 40 positioned at obtuse angles are utilised to allow water to drain from the pumped euphausiids and exit the trough 34 through drain pipes 41 while the euphausiids accumulate within the dewatering trough 34.

A blast freezer 42 was also located on the barge 14 to stabilize the harvested euphausiids. The blast freezer 42 subjects the euphausiids to a temperature of approximately +9° to -17°C and is used to freeze the dewatered euphausiids and stabilize the product for further processing. The euphausiids accumulate within the dewatering trough 34 and which are periodically removed from the trough 34 from time to time for freezing. Thereafter, the frozen euphausiids are transported to a processing location and processed as described hereafter. Alternatively, the euphausiids may conveniently be processed aboard a vessel.

In prototype demonstrations, the net 20 utilised for the harvesting operation was a specially designed 13 ft. by 21 ft. plankton net suspended from a 46 ft. aluminum barge. The pumping action was by a three inch diaphragm pump located on the barge 14 and the freezing action occurred within a minus seventeen (-17°C) degree centigrade blast freezer 42.

As earlier described, the frozen euphausiids are transported to a processing location in order to transform the euphausiids into the desired feed product. Reference is now made to the flow chart of Figure 3.

A pump 43 is connected to a hopper 44 which receives the euphausiids which are now in a thawed condition. Pump 43 is connected to a heat exchanger generally illustrated at 50 and diagrammatically illustrated

in Figure 3. The heat exchanger 50 is intended to raise the temperature of the euphausiids to a temperature of approximately 40°C to 60°C which will more closely approximate the temperature maintained in the digester which is generally lower than 70°C and which digester is generally illustrated at 51. Digester 51 is located downstream of the heat exchanger 50 in the process illustrated in Figure 3.

Although several different types of heat exchangers may be used, heat exchanger 50 conveniently comprises a plurality of pipes 52 (Figure 4A) in which the euphausiids are conveyed through the heat exchanger. Heated water enters the inlet 54 of the heat exchanger 50 and is circulated through the heat exchanger 50 generally following the flow path seen in Figure 4B which utilizes a plurality of baffles 53. The heated water exits the heat exchanger at outlet 61. Following the increase of temperature created in the euphausiids by the heat exchanger 50, the euphausiids pass to the digester 51.

Digester 51 is seen is greater detail in Figure 5. It comprises a product inlet 61 and a product outlet 62. A water inlet 63 and a water outlet 64 are provided. A water jacket 70 through which the heated water circulates surrounds the cylindrical cavity area 71 of the digester 51 which contains the euphausiids. A plurality of stirring discs 72 are located vertically within the cavity area 71 of the digester 51 and are used to stir the euphausiids when they are positioned within the digester 51. A valve 73 is used to close the product outlet 62 so as to maintain the

euphausiids within the digester 51 until the proper temperature and time for the desired enzyme action within the euphausiids has taken place. The time period has conveniently extended between thirty (30) minutes and two (2) hours.

It is thought that a degree of hydrolysis will enhance digestibility of the feed product particularly for early stage larvae or juveniles but also for virtually all fish. This degree of hydrolysis is detemined by the applications and will be monitored by measuring the apparent viscosity in the final product. In utilising the digester 51 illustrated in Figure 5, a batch process is currently being used with a volume of euphausiids of 250 lb./hr being used.

The valve 62 is then opened and the quantity of euphausiids within the digester 51 pass through the valve 62 and are transported through valve 74 to the surge tank or heated batch storage vessel 80 where they await treatment in the dryer, conveniently a ball dryer generally illustrated at 81 (Figure 6) where relatively low and controlled temperatures can be applied to the euphausiids such that any enzymes existing within the euphausiids are not inactivated as would otherwise be the case in a normal drying process.

The euphausiids pass from the storage vessel 80 to the ball dryer 81 through product inlet 83 and, thence, about the periphery of the dryer 81 initially through the application zones 91 where the balls initially contact the

euphausiids and begin the drying process. The ball dryer 81 performs a "soft" drying process which reduces damage to the euphausiids because of its gentle action by way of controlled temperature. The ball drying process utilises a continuous feed into the ball dryer 81 and a product flow of 15 lb./hr. is available.

As the balls and euphausiids move downwardly through the drying zones 92, they meet a counter-current flow of controlled-temperature drying air at less than 50°C which air enters the ball dryer 81 through air inlet 82. Air flow, temperature and dwell time are precisely controlled and monitored within this zone. All of these are variable factors which depend upon whether the product is wet or dried and what period of time the product is intended to stay in the dryer 81.

In the separation zone 93 at the bottom of the dryer 81, the ball and euphausiids meet a co-current flow of controlled temperature air for final drying and separation. The dried euphausiids leave the ball dryer 81 through the product outlet 84 and pass to the packaging step. The drying balls are elevated by rotating helix 94 and recycled to the application zone 91 and the process continues.

One of many commercial and known dryers may be used for the air drying of the euphausiids.

It is contemplated that although the processing of the euphausiids has been described as taking place at a land

location, such processing steps may take place at the harvesting location on board either the harvesting vessel or another vessel conveniently located nearby. This results in advantages in that the euphausiids need not be frozen following harvesting and need not be transported to a land based processing plant thereby resulting in considerable cost savings and quality improvement. In addition, the euphausiids may be introduced directly to a low tempeature dryer on board a vessel following harvesting or to an evaporator. The dried or concentrated euphausiids, after being subjected to the digester and/or the drying processes, may then be stored on the vessel until a substantial quantity of krill hydrolysate concentrate has been obtained at which time they may be transferred to another vessel for transport to the processing vessel itself which, when full, will transport the euphausiids to the shore.

Likewise and while it is desirable for the digester and drying steps to take place concurrently and sequentially in the event the euphausiids are intended to be used as a feed product for juvenile and early stage larvae.

A further harvesting technique is contemplated in Figure 1B. In this technique, weights 101 are connected to the mouth end of the net generally illustrated at 114 at the ends of the lower horizontal beam 103. Floats 100 are connected to the top horizontal beam 102 of the mouth end of the net 114. Depending on the size of the net 114, lines are connected on one end to attachment points 104, in the first instance or, alternatively, to points 110, 111, 112,

113 and, on the other end, to the towing vessel. The net 114 is pulled through the water gathering the zooplankton which enter the net 114 through the mouth.

Many applications for the hydrolysed krill and hydrolysed krill concentrate products are also contemplated because of the desirable characteristics of the of the krill hydrolysate in which the proteins and nutritional value is retained and improved through the partial digestions of the proteins. For example, fish under stress, which is common with cultivated species raised with aquacultural techniques, are reluctant to eat and, accordingly, therapeutic drug delivery and special diets used for such marine species are difficult to use because the fish do not find such products palatable. The hydrolysed krill products and other zooplankton products according to the invention may be used with such special diets and drug delivery by creating an enhanced flavour and enhanced assimilation when the medicinal product such as a pellet is coated or mixed with the hydrolysed zooplankton product in a liquid or paste form. Likewise, while other such products may include specially added amino acids and other compounds to enhance the flavour of the product, the hydrolysed krill according to the present invention preserves, enhances and optimises the level of certain free amino acids and other flavourants thereby allowing flavour enhancement with a natural product and without the addition of amino acids or other flavourants. Likewise, the krill hydrolysates retain the protein and nutrient quality inlcuding the original pigments, fatty acids, other nutrients and mineral elements.

The activity of the enzymes, which are contained in the krill, is also retained in the hydrolysed natural product according to the invention. Such enzymes allow for enhanced digestion of feed by certain cultivated marine species by increasing the availability of peptides and free amino acids without creating additional harmful stress on such species.

Yet a further application contemplated by the present invention is the use of hydrolysed krill that is blended and codried in association with plant or vegetable protein and other dry carriers such as soymeal, corn gluten meal and canola meal in fish feed mixtures. The range of co-drying cariers used in the blending process include a wide range of dry animal or vegetable protein and feed ingeedients including soy conola and other soil seed meals, coarse ground cereal gains and flours, oil seed concentrates and isolates, corn and cereal glutens, pea and pulse meals, oil seed and cereal processing by products and brans, dried yeasts, algae and other single cell organisms, milk powders, blood meal and other body fluid products, namial and poultry by products, fish and shellfish meals, and vitaminised mineral premixes. Such applications would increase the palatability, amino acid balance and other nutrient levels in the dry blended meal so that it can be used to replace fish meal in aquaculture feeds and other applications. Further enzymes in the hydrolysed krill products according to the invention are preserved following he hydrolysis and can be allowed to act on the plant proteins. The enhanced digestibility of a product combination of plant protein and hydrolysed krill is also contemplated to improve the

efficiency of the feed and decrease the fecal load in the environment by fish fed with diets containing such combination. This can be an important feature with the rearing of cultivated marine and freshwater species. Likewise, the palatability of such non-fish meal proteins, in particular, plant proteins such as canola, corn gluten or soy meal is enhanced.

Experiments conducted to date utilize the enzymes in krill to carry out a limited hydrolysis of soy, canola and other plant proteins. For example, one part of dry canola or soy meal which has added ten percent (10%) wheat bran is blended with five (5) parts of hydrolysed krill. The hydrolysate is pumped from the digester to the feed stock hopper and the dry blend is added. The mixture is brought to the desired temperature while agitated in the digester for approximately one (1) hour. Measurements of phytic acid and the levels of the amino acids and ammonia are then taken. For example, 250 lbs. of krill is hydrolysed by bringing the krill to approximately 45° Celsius. The temperature is held for one (1) hour and is then blended with 5 lbs. of wheat bran with 45 lbs. of canola concentrate. The use of wheat bran is necessary to provide phytase, an enzyme which is absent in canola meal and krill. The phytic acid is dephosphorylated by phytase from the wheat bran. The phytic acid is acted on by the phytase enzyme. It is noted that the blend may be retained in the digester for an extended period, up to a period of four (4) hours or even longer.

In yet a further embodiment of the invention, it is contemplated that the wet krill hydrolysate product is evaporated and then mixed with and co-dried with other wet and dry products. Various predetermined ratios of wet krill hydrolysate and liquid marine products may be concentrated and tehn mixed with dry carrier conveniently in the form of dried krill products, dried vegetable protein and/or dried fish product, used in combination or singly. The resulting moist blend is subject to concentration, processing and co-drying in a dehydrator such as a dryer. A dehydrator system with the following characteristics has been found to work well, namely a type of flash and fluidized drier or combination thereof with an agitator and vertical or tangential flow of heated air. Although the temperature of the inflowing air may be high at impact (the impact temperature), the temperature of the product is not significantly increased in the dryer. This is an important element in the drying system. Following hot air impact and agitation, the water evaporates rapidly and the duration of the drying process is greatly reduced as set out in greater detail hereafter.

Co-drying the mixture of the krill hydrolysate, liquid marine product and the dry carrier product mixture has been found to be relatively economical at relatively low temperatures. Under such conditions, the krill poteins, pigments and other constitutents are substantially preserved. Thus produced, the product has unique benefits for dietary uses in aquaculture and animal feeds. These blended and agglomerated dry products are uniquely different

from other product mixes. The unique sequences and control of the process provides initimate agglomeration and adsorption of the krill hydrolysate with the dry carrier. It also preserves the unique nutient quality of the krill hydrolysate in the blend without significant losses due to excess heat or oxidation during the drying process. Further, cost savings and economic advantages in the manufacture of the product are improved.

Depending on the moisture content of the dry carrier, liquid marine protein, and the krill hydrolysate, and the proportion of each in the mixture to be co-dried, the removal of moisture can be accomplished by a drying process at relatively low temperatures thereby to preserve the temperature and oxidation sensitive constituents including the krill constitutents and the krill pigments. Particles of the dry carrier are coated with, adsorbed and absorbed with the wet hydrolysate thereby facilitating the drying process by exposing a greater surface area of wet hydrolysate and/or liquid fish product for heated air to act upon. The mixture may then be fractured into smaller particles which further increases the available surface area to expedite the drying process. At the outset, the mixture may be placed in a reactor cell balance tank to permit chemical interactions between components of the mixture, such reactions including enzymatic activity of a wide range of enzymes including proteolytic, lipolytic and carbohydrate splitting enzyme prior to drying. A well-mixed, homogeneous mixture is prepared to reduce and to eliminate high moisture pockets. Water is then removed from this mixture by an

evaporator and a subsequent dehydrator such as is described above and the endproduct is a dried krill premix or feedstuff blended with the aforementioned carrier. Temperature sensitive enzymes, flavorants or other bioactive products may be added to the cooled endproduct after the drying step. Alternatively, the krill hydrolysate may be combined with wet fish products and other carriers such as dry fish meal, corn meal, canola meal, oil seed meal, or other vegetable meals, used in combination or taken singly.

Referring now to the drawings, Figure 7 illustrates the steps of the co-drying process in its entirety according to the present invention. A predetermined quantity of wet krill hydrolysate product 210 is mixed with a predetermined quantity of liquid marine protein 212 and a predetermined amount of dry carrier 211, conveniently dried krill product, dried fish product and/or dried vegetable protein used in combination or taken singly. The resulting mixture is placed in a mixing blender 215, where the various ratios of hydrolysate, marine protein and dry carrier are thoroughly blended. The blending required will vary with the constitution of the mixture. The blended mixture is then ground within a grinder 217 where the mixture is reduced to particles of substantially uniform size. The ground mixture is then transferred to reactor cell balance tank 216 where the continuously stirred blended mixture is allowed to chemically react and/or undergo enzymatic action prior to the drying process. After the intended reaction has taken place in the tank 216, the mixture is conveyed to the dehydrator 220 for drying.

The dehydrator 220 is illustrated in greater detail in Figure 8 and with reference thereto, the mixture enters the agitator bowl 224 of the dehydrator 220 through inlet 219 where the mixture is agitated into smaller particles which is intended to prevent clumping of the mixture. A continuous feed of mixture into the dehydrator 220 is intended through inlet 219.

Directly heated air from the burner 221 or indirectly heated air is directed to the agitator bowl 224 of the dehydrator 220 by way of fans (not illustrated) where the air mixes with particles of the mixture in the bowl 224. The particles are carried up the drying tower 230 by the column of hot air. The classifier 231 sorts the particles at the top of tower 230. Drier mixture consists of lighter, individual particles which proceed along the column of hot air into a cyclone 232. The classifier 231 redirects larger and heavier masses of more damp mixture back to the agitator bowl 224 for further agitation and drying.

The particles are drawn downwards along a spiralling column of heated air in cyclone 232 and centrifugal action removes further moisture from the particles. At the bottom of the cyclone 232, the particles are isolated from the air column by airlock 233 and are sorted by a rotary screen 234. Smaller, lighter particles of dried product pass through the rotary screen 234 and exit the dehydrator 220 at outlet 240 for further processing. Larger, heavier particles of damp mixture are redirected to

the agitator bowl 224 from outlet 241 for further agitation and drying within several seconds.

With reference again to Figure 7, heated product 241 exiting the dehydrator 220 from outlet 240. The average transit time through the dryer is between 60 and 90 seconds and the end moisture content below 10% moisture may then be permitted to cool. Some of this dried product 245 may be further used in the co-drying process as a quantity of the dry carrier 211 so as to increase the fluid content of marine constitutents. Temperature sensitive enzyme active products 242 or other bioactive products, which might be denatured by the drying process, may be introduced to the dried product 241 after the product has passed through the dehydrator 220 as illustrated. The dried product 241 then undergoes further mixing and blending at mixing step 250 to ensure the homogenous addition of the temperature sensitive enzyme active products 242. The final product 243 may then proceed to a packaging step such as a bagger 244 or to a storage bin 245 prior to further use in aquaculture or animal feeds.

Concentration and Co-Drying or Krill with Vegetable proteins Trials

The objectives were the concentration of liquid krill hydrolysate to 42%DM in a rising film plate evaporator. (Alfa Vap). The drying of a krill concentrate blend with soya meal and corn gluten meal in a flash dryer (drier with performance characteristics as defined), to determine the

maximum amount of krill concentate that can be added to the dry vegetable protein meal.

Raw material hydrolysed krill with 18-20% DM including approximately 0.3% oil.

Evaporator. The hydrolysed krill was concentrated in an Alfa Vap evaporator from 18-20% DM to 42% DM. The 42% level was not obtained with any difficulty.

Mixing

The mixing was done in 100 kg batches using a cylindrical container with a vertical shaft paddle. This was accomplished without unusual difficulties.

Drying

Drying and mising was caried out in two steps: Step 1 was mixing the krill concentrate and carrier (vegetable and protein) and drying to about 90% DM. Step 2 was mixing the dried product from step 1 with more krill concentrate and drying a second time.

Flash Drying

The mixtures were dried in a flash dryer. This was done by feeding the mixture into a chamber containing a fast rotating agitator. Through intake air ducts hot air was led through the chamber and agitator.

Impact Temperature was 165-175 deg. C.

Drying Temperature (set point) is 110 deg. C to 125 deg C.

Capacity

The flow to the dryer for all three test vegetable protein products was 600-700 kg/hr. This gave an evaporation rate of approximately 500 kg/hr. in the dryer.

Results

The temperature of the product is not increased in the dryer by any significatnt ammount. The evaporation of the water on the product keeps the temperature low. The rapid transit of the product through the dryer also minimizes the temperature and time effects that can reduce the value of the product as a feed.

A third or fourth step is also contemplated and considered possible with this type of dryer.

Other driers besides those of ball dryer 81 (Figure 6) are contemplated. For example, dryers such as direct heated flash driers or fluidized bed driers that cause rapid drying of the particles within a few seconds are well known. With reference to Figure 9, a built in air scrubber generally illustrated at 500 is used for odour control. A burner or indirect heating system 501 heats the air to the required level with impact temperatures not

exceeding 450 deg. C before the air enters agitator 502. the product is augered tangentially into the agitator chamber 503 where most of the water in the product is evaporated. Agitator 502 rotates with a high tangential speed of the agitator blades concurrent with the tangential air flow. The motion of the agitator 502 causes mechanical fluidization of the particles and comminutes the particles, thus accelerating evaporation. The acceleration of the drying velocity reduces the adverse effect of heat or the heat burden on the product during the drying process.

In yet a further embodiment of the invention, it is contemplated that a process for obtaining enzymes from the Euphausia superba species of krill and other krill species is of interest. Euphasia superba ("E.s.") is a small crustacean from the Antarctic that contains numerous enzymes that are principally but not exclusively represented by proteases, amylases, chitinases, carboxymethy cellulases, lipases, etc. This enzymatic cocktail as a whole or in a partial purified form can be used for a number of industrial applications such as aquaculture and other general feed manufacturing and the further process of marine and other proteins. The inclusion rate of enzymes in the feed would vary depending on the target species and the composition of the diet. For example, these krill enzyme cocktails can be added to aquaculture diets containing large quantities of vegetable proteins which would otherwise be difficult to process by the animals and which could also be part of specialty diets for larval stages of shrimp and starter diets for salmonids where higher survival rates are

required. Krill enzymes may also conveniently be used to produce protein hydrolysates from other proteins to incorporate into diets or to improve the functional properties of these diets. Other potential applications would include the production of flavors, protein and peptide extraction from marine by products, protein and pigment recovery from shrimp and crab shell offal, the production of free amino acids and other benefits relating to the actions of these krill enzymes on biological materials.

Using the processes previously disclosed, it was desired to obtain enzymes from the previously autolysed krill preparations.

With reference to Figures 9 and 10, ultrafiltration membrane 303 was used with the krill hydrolysate 301 and with fresh krill 310. Since most of the krill-derived enzymes have molecular weights above 20,000 daltons, experiments were conducted to determine the most appropriate molecular weight cut-off ultrafiltration membrane to attempt a concentration of the aqueous phase enzyme-rich E.s. and E.p. extracts. It was revealed during experiments that total protease activity begins to become apparent in the filtrates at the 50,000 molecular weight cut off and up. On the other hand, trypsin-like activity is present in filtrates at 30,000 molecular weight cut off. It is therefore desirable to use a 10,000 dalton cut off membrane for filtration purposes.

In order to handle larger volumes of krill hydrolysate and to concentrate the enzyme extracts, a tangential flow filtration ("TFF") cartridge 302 was used using a 10,000 dalton molecular weight cut-off. One such cartridge commercially available is a Millipore Preparative Scale Tangential Flow Filtration cartridge. Such cartridges are intended to handle volumes from 100 ml to 100 liters, although it is readily possible to scale up such techniques to handle larger volumes, if desired. Before subjecting the krill extracts to TFF, they were centrifuged at 4000-10000 x G for twenty(20) minutes in a Beckman centrifuge 300 to clarify from solids and eliminate part of the fat. Rather than centrifugation, this clarification step can be replaced by prefiltration 303 with a larger pore filter. After centrifugation, the aqueous phase 305 containing the enzymes of interest was recover and stored at 4 deg. C. The autolysed krill extracts were run through a one square foot TFF cartridge 302 using a Hoechst displacement pump 304. The initial extract volume was about two(2) liters and was brought down to approximately 250-300 ml after four(4) to five(5) hours of operation (below 20 psi of pressure). It was revealed that enzymatic activity recovery differed significantly between the two samples (i.e., autolysed and freshly squeezed extracts).

By measuring the trpysin-like activity ("TLA"), it was found that the recovery of krill enzymes from the fresh frozen krill 310 was relatively smaller than the recovery from hydrolysed krill 301. However, the total units recovered after ultrafiltration were higher for fresh frozen

extracts. Accordingly, TLA could be recovered from either freshly squeezed or autolysed krill preparations. Since there was little or no enzymatic activity associated with the filtrate, it is apparent the proteins of interest were not leaching out through the membrane filter.

The resultant enzyme cocktail obtained by the ultrafiltration technique from both the hydrolysed and fresh krill 301, 310, respectively, could then be coupled with freeze drying 313 which would reduce the amount of water associated with the enzymes significantly which would reduce transportation costs. Subsequent processing could then be performed on the enzyme cocktails to further increase the purity and quality of the enzymes present.

Yet a further aspect of the invention relates to a method for removal of protein from crustacean wastes using the aforementioned krill enzyme extracts. With reference to Figure 12, a quantity of crustacean wastes 400, 401 is ground to dried particulate size by grinders 402, 403, respectively, with a portion of water added to facilitate this grinding. Various of a plurality of grinders which will accomplish this include a piranha pump, a macerator or cerator, all of which are known. Acid stabilized shell waste 400 is then de-watered through a de-watering system 404, many of which are readily known to be available, such as the Vincent screw press, wine presses or centrifuges. Non acid stabilized shell waste 401 has no need to be de-watered prior to the addition of enzymes. Water is conveniently added to the de-watered acid stabilized shell

waste 410 to facilitate enzymatic reaction. The shell waste 410 is transferred to a digesting tank 411 where an amount of krill enzyme cocktail 412 is added. The enzyme cocktail can be in either a concentrated or non-concentrated form consistent with squeezed extractions from the whole animal as has been described. The squeezed fractions are in the range of 25-75% of the whole animal depending on the amount of enzyme desired and the need to keep the enzyme with the krill to facilitate autolysis. The shell enzyme mixture is subjected to digestion in the digester 411 for a time period in the range of one(1) to forty-eight(48) hrs at a temperature in the range of 0 to 70 Celsius with an optimum temperature being approximately 45 deg. Celsius. Following the digestive process, the mixture is subjected to water removal 413 as has been described. Two fractions will result, a protein rich enzymatically active portion 414 and a shell material portion 415 high in chitin and low in protein. The liquid high protein portion 414 is low temperature dried or co-dried as earlier described or acid stablized. The shell portion 415 can then be further processed by the addition of more enzyme cocktail to facilitate further protein removal in further steps or can be subjected to traditional deproteinization or demineralization techniques as illustrated generally at 420. The extent of de-mineralization necessary can be greatly reduced by the storing of the shell waste for long periods of time while stabilized with acids, preferably formic.

In experiments which have been conducted to date, 70kg of water was added to 210 kg of mechanically peeled

shrimp shell wastes. The slurry was subjected to grinding with a piranha pump to a suitable particle size. 60kg of this slurry was combined with 15 kg of Euphasia superba juice obtained by squeezing whole krill through a screw press 315 (Figure 11) to obtain 50% by weight of the animal in a liquid form. The shell juice mixture was subjected to digestion for six(6) hours at 45 deg. C. The mixture was dewatered by pressing through a Vincent screw press to obtain the protein rich enzymatically active portion and the shell ash portion 415, as described. The shell portion was approximately 7.5% by weight and the liquid portion made up the remainder. The liquid portion was acid stabilized with 3% by weight formic acid. The shell portion was washed and dried.

In a second trial conducted to establish the efficacy of using krill enzymes for the removal of protein from shrimp shell wastes and the benefit of reincorporating the superba squeezed solids, 26 kg of squeezed superba juice, obtained through the procedures described, was incubated with 10 kg water and 70 kg of ground shrimp shell for six(6) hours at 45 deg C. Samples were taken every hour and squeezed through a screw press. After six(6) hours, 14 kg of squeezed superba solids compising the remainder of the whole animal after enzyme liquid removal were added into the mixture and hydrolyzed for an additional one and one-half (1.5) hours. The remaining slurry was squeezed and the separate fractions were frozen.

While specific embodiments of the invention have been described, such descriptions should be taken as illustrative of the invention only and not as limiting its scope as defined in accordance with the accompanying claims.

5

WE CLAIM:

1. Method of producing a feed product comprising the steps of adding a predetermined quantity of krill hydrolysate to a quantity of liquid marine protein and a quantity of dry carrier to produce a mixture and co-drying said mixture to obtain an end product.

2. Method as in claim 1 wherein said mixture is mixed prior to co-drying said mixture.

3. Method as in claim 2 wherein said mixture is subjected to chemical and/or enzymatic reaction for a predetermined time period prior to co-drying said mixture.

4. Method as in claim 3 wherein said mixture is co-dryed in a dryer or other dehydrator.

5. Method as in claim 4 wherein said mixture is ground prior to being subject to said chemical reaction.

6. Method as in claim 5 wherein said mixture is cooled following drying of said mixture in said dryer.

7. Method as in claim 6 wherein said dry carrier may be one or a combination of dry marine protein

meals, dried krill products, dried vegetable and dried fish product.

8. Method as in claim 7 wherein said liquid marine protein may be liquid fish product.

9. Method as in claim 8 wherein temperature sensitive enzyme active or other bioactive dry products are added or readded to said mixture following said drying of said mixture.

10. Method as in claim 9 and further comprising mixing said temperature sensitive enzyme active products with said mixture.

11. Method as in claim 1 wherein said mixture is co-dryed in a dryer or other dehydrator.

12. Method as in claim 11 wherein said dryer includes an agitator to agitate said mixture entering said dryer.

13. Method as in claim 12 wherein said dryer further includes a drying tower downstream from said agitator and a heat source to provide heat to said tower.

14. Method as in claim 13 and further comprising a classifier downstream of said tower for separating said mixture, said mixture comprising relatively

lighter and relatively heavier particles, said classifier separating said lighter from said heavier particles.

15. Method as in claim 14 wherein said relatively heavier particles are returned to said agitator.

16. Method as in claim 14 and further comprising a cyclone downstream from said classifier.

17. Method as in claim 16 wherein said cyclone removes further moisture from said relatively lighter particles.

18. Method as in claim 17 wherein said relatively lighter particles are separated into relatively smaller and relatively larger particles.

19. Method as in claim 18 wherein said relatively larger particles are returned to said agitator.

20. A feed product or additive produced by the method as in any one of claims 1 to 19.

21. Co-drying apparatus for drying a mixture of krill hydrolysate, liquid marine product and a dry carrier comprising a dryer for agitating, heating and separating particles of said mixture.

22. Co-drying apparatus as in claim 21 and further comprising a mixer for blending said mixture prior to said mixture entering said dryer.

23. Co-drying apparatus as in claim 22 and further comprising a reactor cell for treating said mixture prior to said mixture entering said dryer.

24. Co-drying apparatus as in claim 23 and further comprising a grinder for grinding said mixture prior to said mixture entering said reactor cell.

25. Co-drying apparatus as in claim 24 wherein said dryer produces a product.

26. Co-drying apparatus as in claim 25 and further comprising a mixer for mixing said product following said product exiting said dryer.

27. Co-drying apparatus as in claim 21 wherein said dryer comprises a source of warm air, an agitator for agitating said mixture following entry of said mixture into said dryer, a tower to expose said mixture to said warm air, a first classifier to separate the relatively lighter particles of said mixture from the relatively heavier particles of said mixture, a cyclone for drying said relatively lighter particles separated from said relatively heavier particles, and a second classifier to separate relatively lighter particles and relatively heavier

particles constituting said relatively lighter particles in said cyclone.

28. Co-dryer as in claim 27 and further comrising a fan to move said warm air within said dryer.

29. Method of obtaining an enzyme extract from a liquid krill hydrolysate comprising the steps of subjecting said hydrolysate to centrifugation to obtain a clarified liquid and further subjecting said clarified liquid to ultrafiltration using a membrane with a capacity to retain said enzymes having a molecular weight greater than 10,000 daltons.

30. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 29 and further comprising the step of storing said clarified liquid at a reduced temperature for a predetermined time period.

31. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 30 wherein said ultrafiltration is achieved using a tangential flow filtration system.

32. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 31 wherein said enzyme extract obtained from said ultrafiltration is freeze dried.

33. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 32 wherein said krill is Euphausia superba.

34. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 32 wherein said krill is Euphausia pacifica.

35. Method of obtaining an enzyme extract from fresh krill comprising the steps of squeezing said krill to obtain an aqueous extract and subjecting said aqueous extract to ultrafiltration with a membrane adapted to retain enzymes having molecular weights above 10,000 daltons.

36. Method of obtaining an enzyme extract from fresh krill as in claim 35 wherein said ultrafiltration is achieved using a tangential flow filtration system allowing enzymes to retain which have molecular weights above 10,000 daltons.

37. Method of obtaining an enzyme extract from fresh krill as in claim 36 and further including the step of centrifuging said aqueous extract prior to subjecting said extract to ultrafiltration.

38. Method of obtaining an enzyme extract from fresh krill as in claim 37 and further comprising the step of storing said aqueous extract at a reduced temperature following said centrifuging.

39. Method of obtaining an enzyme extract from fresh krill as in claim 38 wherein said reduced temperature is approximately 4 degrees Celsius.

40. Method of obtaining an enzyme extract from fresh krill as in claim 39 and further comprising subjecting said enzyme extract obtained from said ultrafiltration to low temperature drying.

41. Product produced by the method as in any one of claims 29 to 39.

42. Method for removal of protein from non-stabilized crustacean shell wastes, comprising grinding said crustacean wastes and water to a relatively small particulate size, transferring said small particulate size product to a digester, adding a predetermined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively enzymatically active and relatively high in protein and a second portion of shell material relatively high in chitin and low in protein.

43. Method for removal of protein from acid stabilized shell wastes comprising grinding said crustacean wastes to a described small particulate size, transferring desired size shell wastes to a digester, adding a predetemined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined

time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively enzymatically active and relatively high in protein and a second portion of shell ash relatively high in chitin and low in protein.

44. Method as in claim 42 and further comprising drying said liquid portion by means of low temperature drying to preserve the enzymatic activity.

45. Method as in claim 44 wherein said drying is by way of a flash drier.

46. Method as in claim 45 wherein said drying is by way of a fluidized bed drier.

47. Method as in claim 42 and further comprising adding krill enzyme material to said shell material portion.

48. Method as in claim 43 and further comprising adding krill enzyme material to said shell material portion.

49. Method as in claim 42 wherein said product is subject to digestion between approximately 0-70 degrees Celsius and for times between 30 minutes and several hours.

50. Method as in claim 43 wherein said product is subject to digestion between approximately 0-70 degrees Celsius.

51. Method of producing a concentrated krill hydrolysate comprising the steps of harvesting, digesting and evaporating the krill hydrolysate to provide a partial hydrolysis for a predetermined time and temperature so as to enhance the nutrient characteristics of said krill.

52. Method of producting a dry krill premix or feedstuff comprising the steps of producing a predetermined amount of concentrated krill hydrolysate, producing a predetermined amount of dry matter and mixing said concentrated krill hydrolysate and said dry carrier matter and co-drying said mixture.

54. Method as in claim 52 wherein the dry matter is selectted from the group of vegetable and/or vegetable and/or animal protein meals and by products.

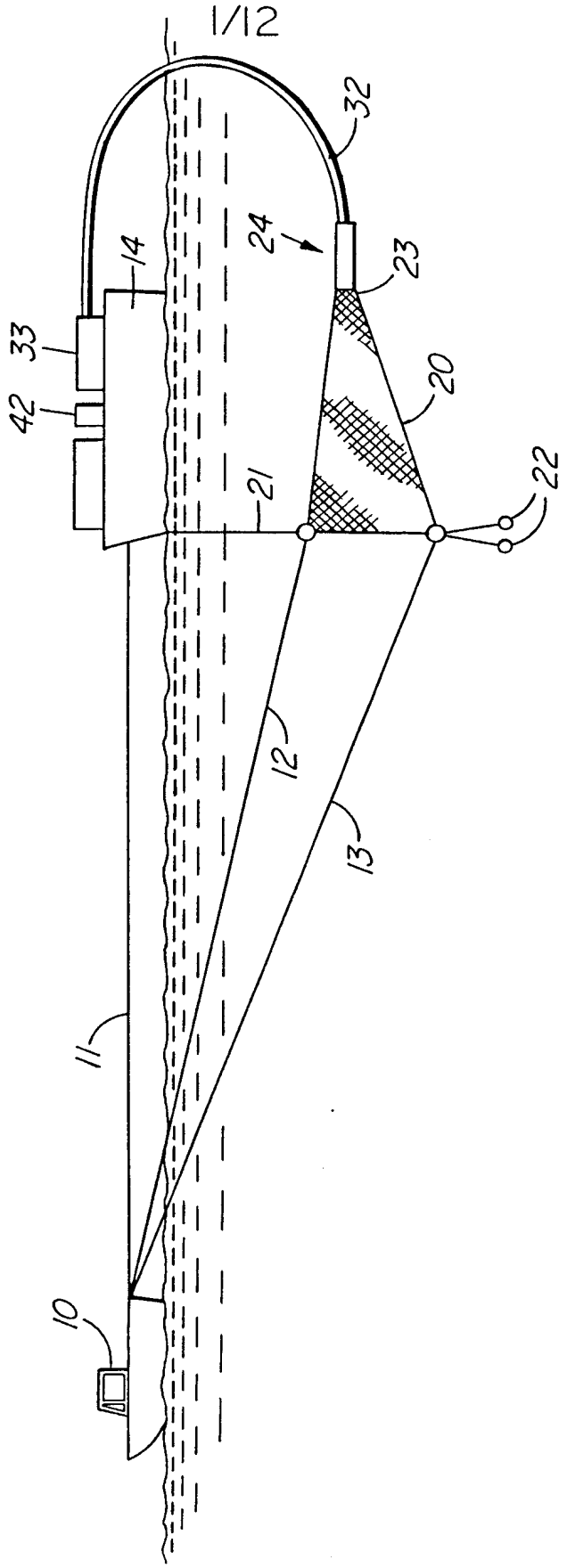


FIG. 1A

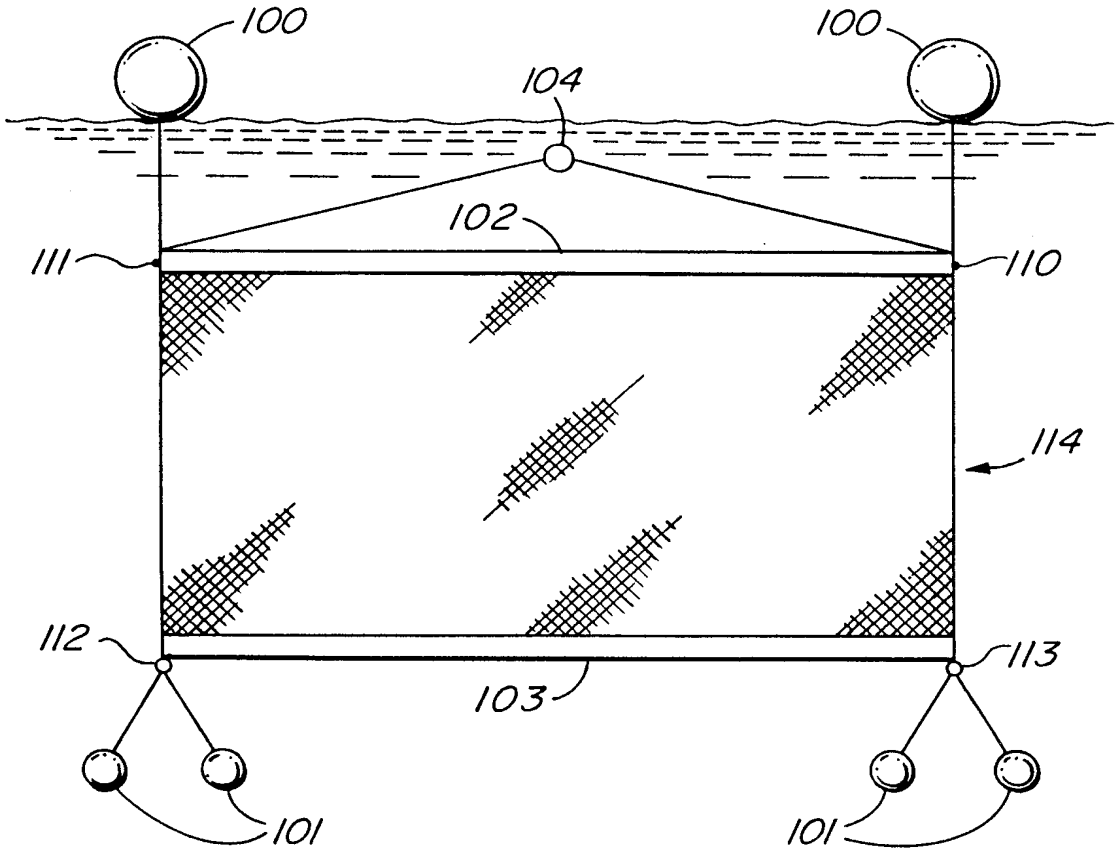


FIG. 1B

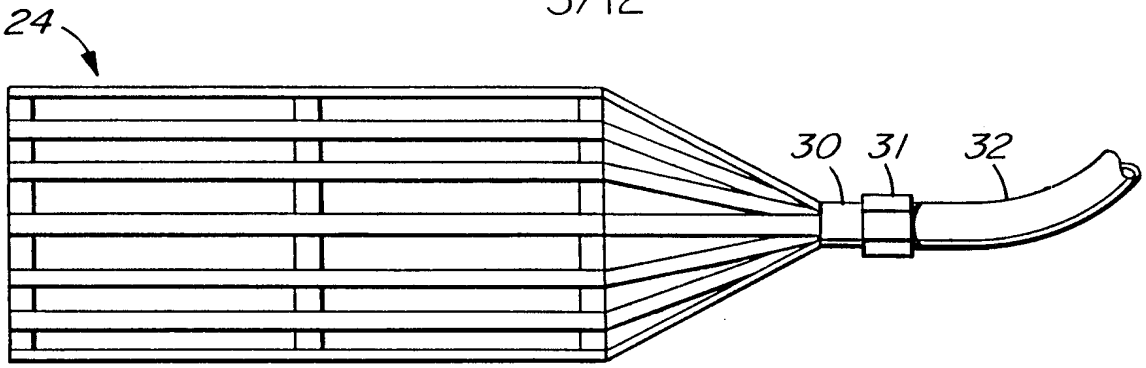


FIG. 2A

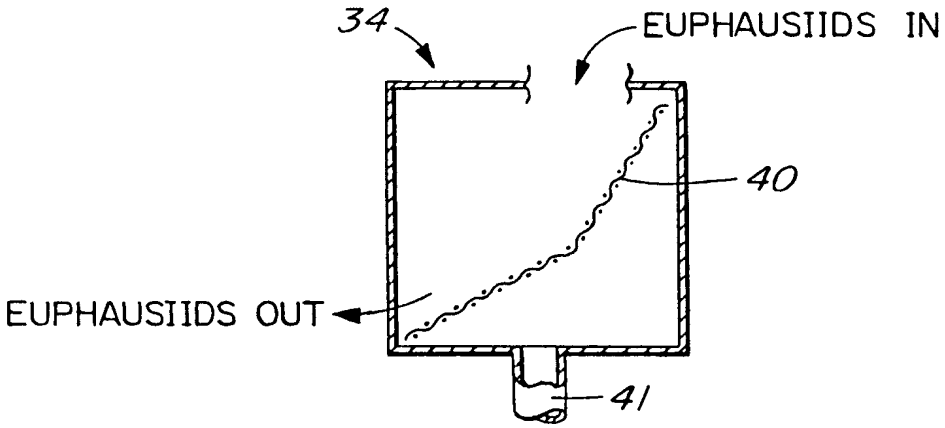


FIG. 2B

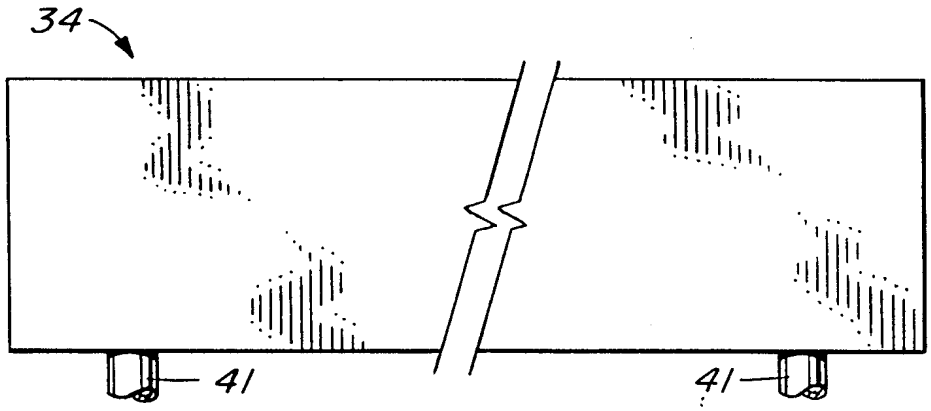


FIG. 2C

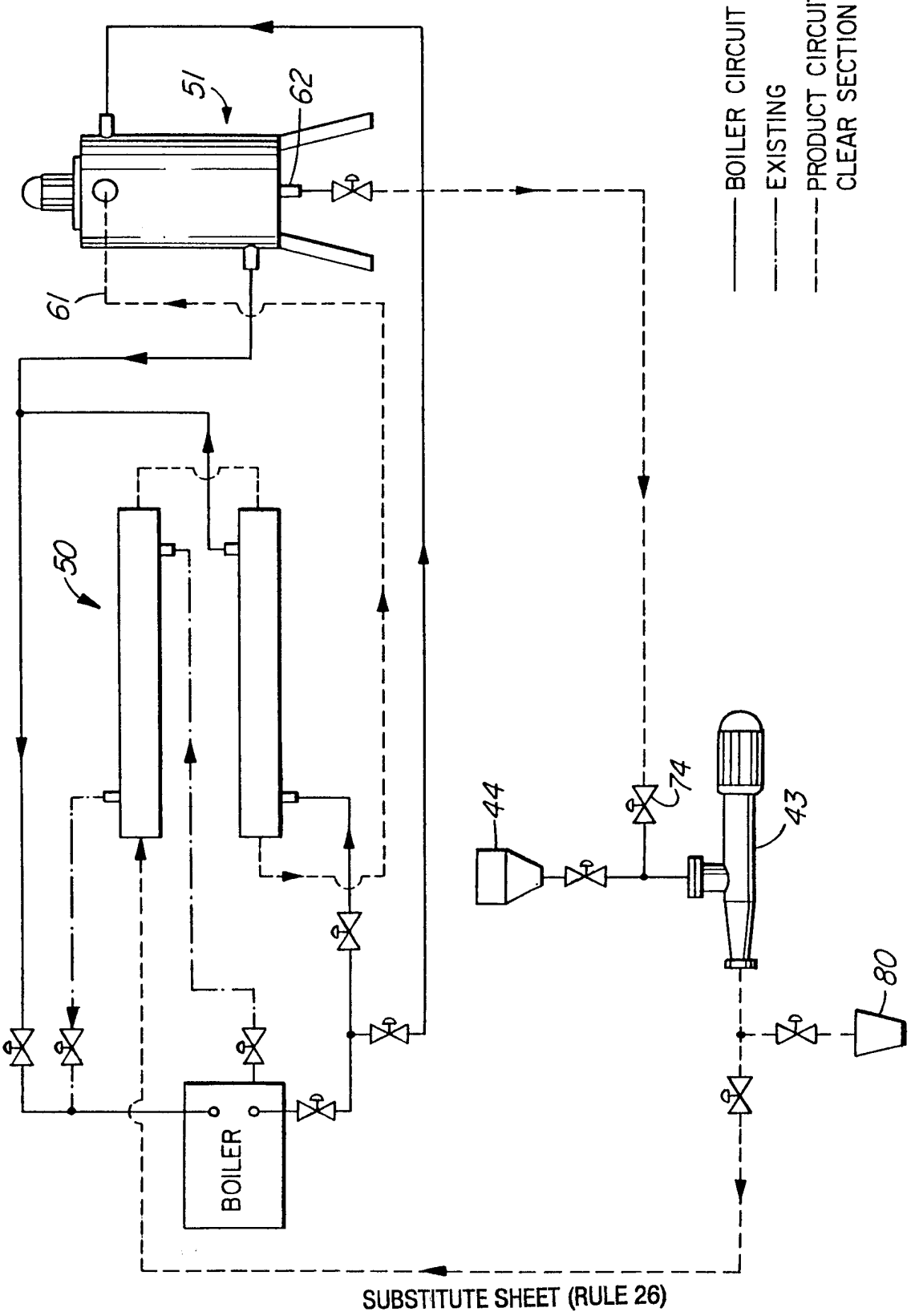

BOILER CIRCUIT
EXISTING
PRODUCT CIRCUIT
CLEAR SECTION
51
62
61
50
44
74
43
80
BOILER

FIG. 3

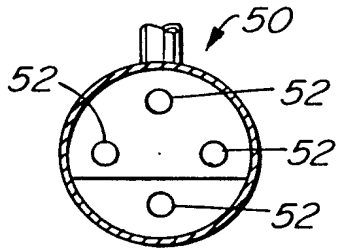


FIG. 4A

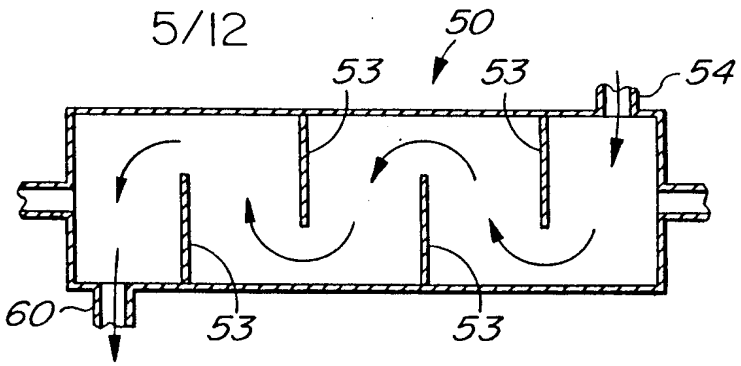


FIG. 4B

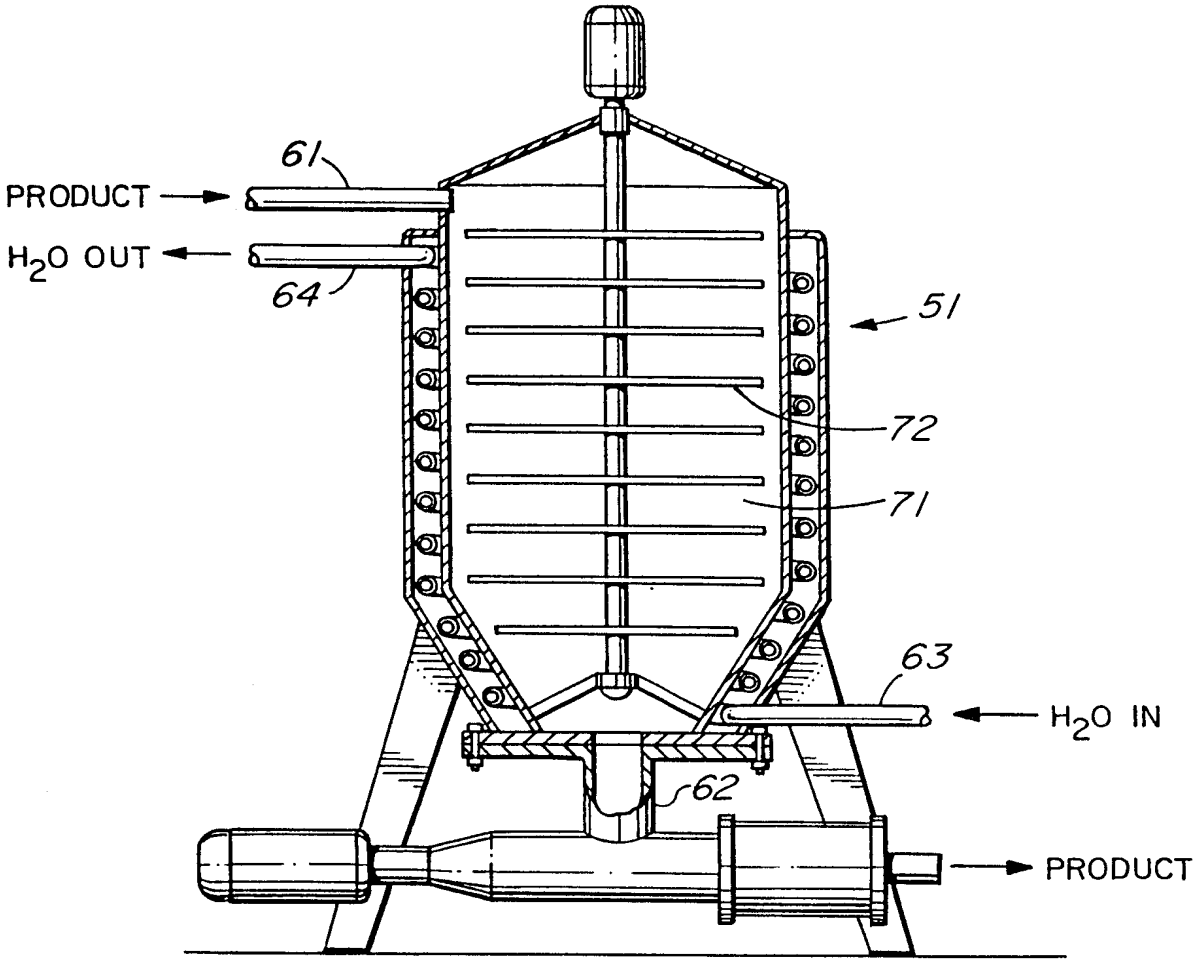


FIG. 5

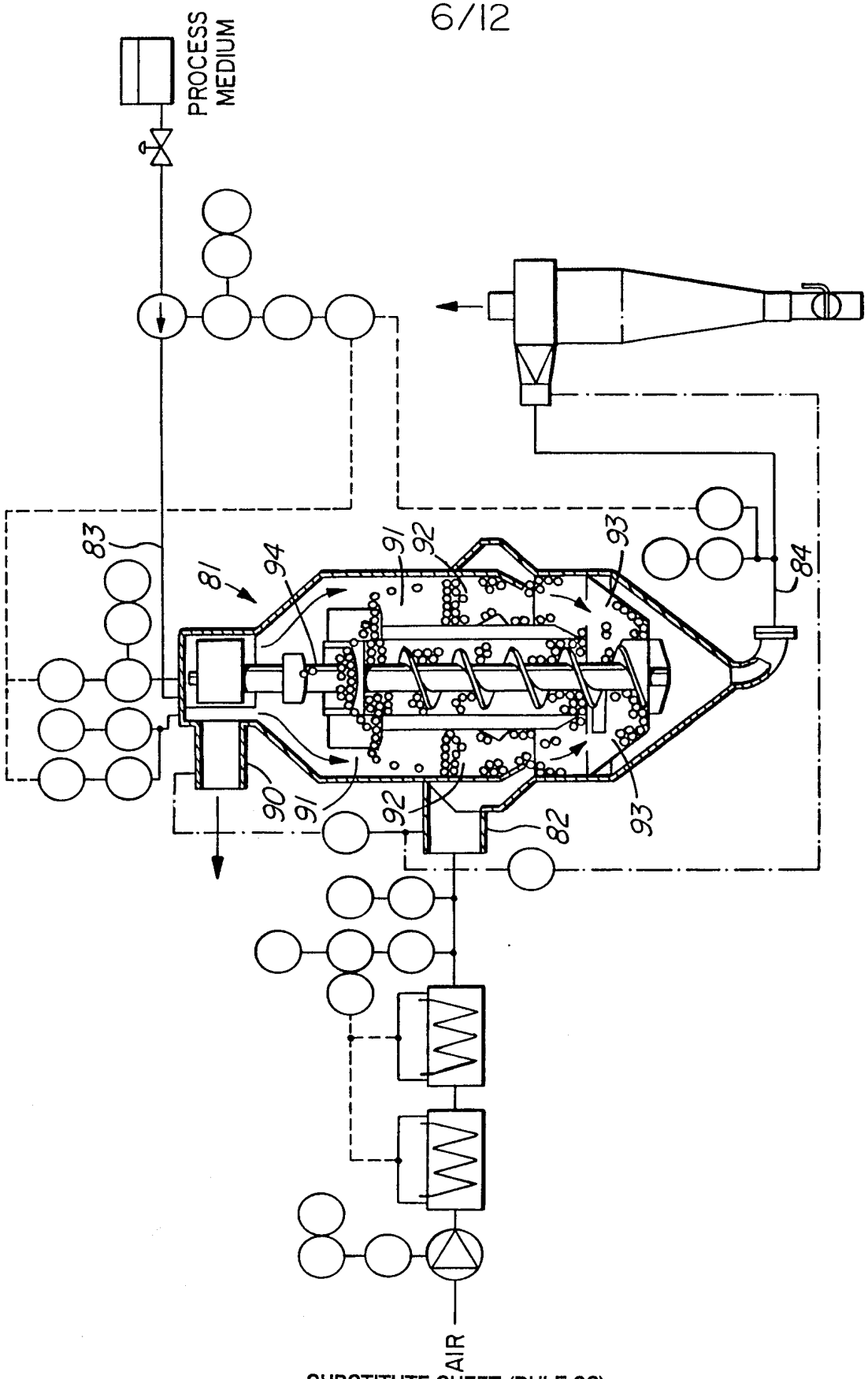


FIG. 6

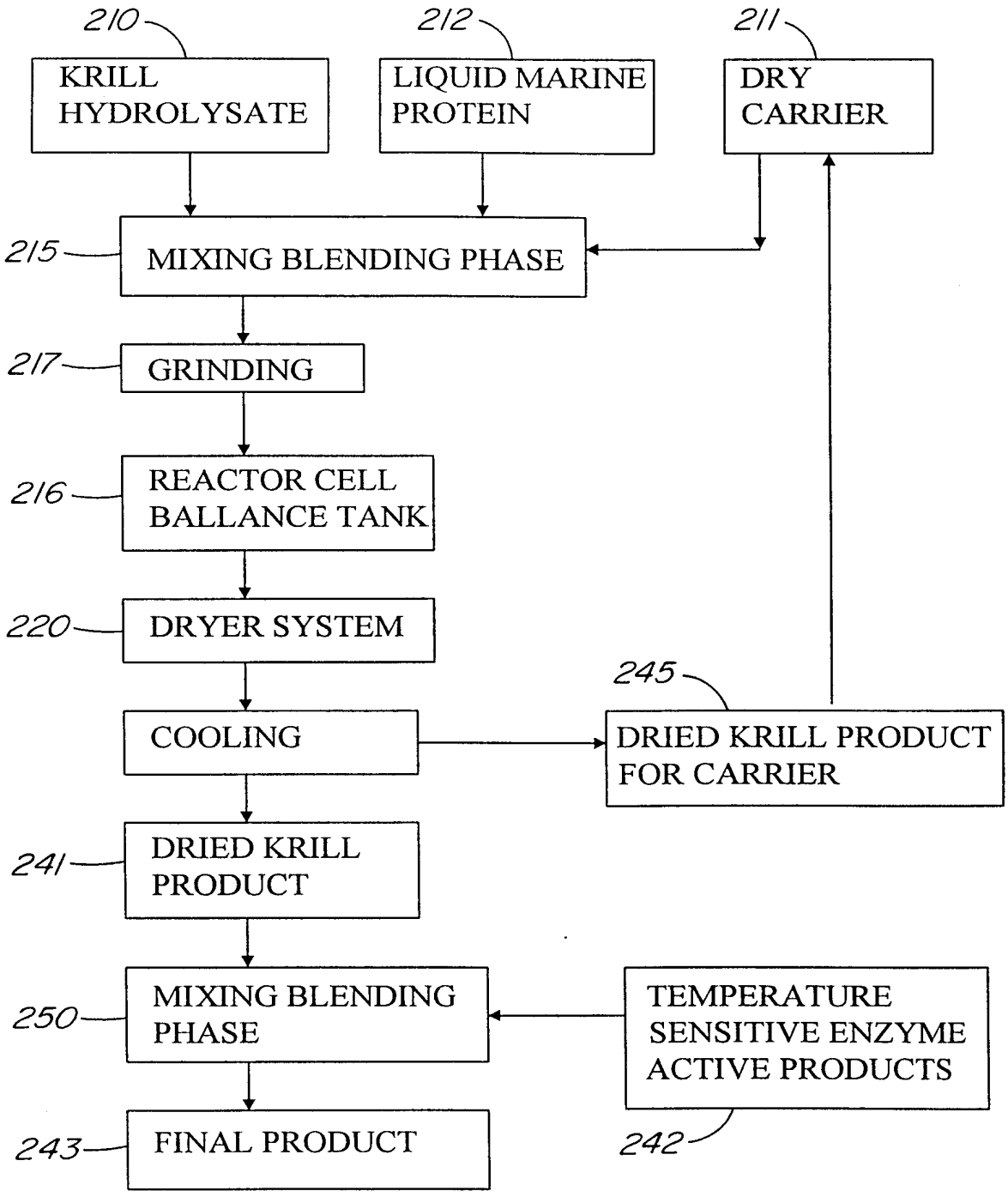

210
KRILL HYDROLYSATE
212
LIQUID MARINE PROTEIN
211
DRY CARRIER
215
MIXING BLENDING PHASE
217
GRINDING
216
REACTOR CELL BALLANCE TANK
220
DRYER SYSTEM
245
COOLING
DRIED KRILL PRODUCT FOR CARRIER
241
DRIED KRILL PRODUCT
250
MIXING BLENDING PHASE
TEMPERATURE SENSITIVE ENZYME ACTIVE PRODUCTS
243
FINAL PRODUCT
242

FIG. 7

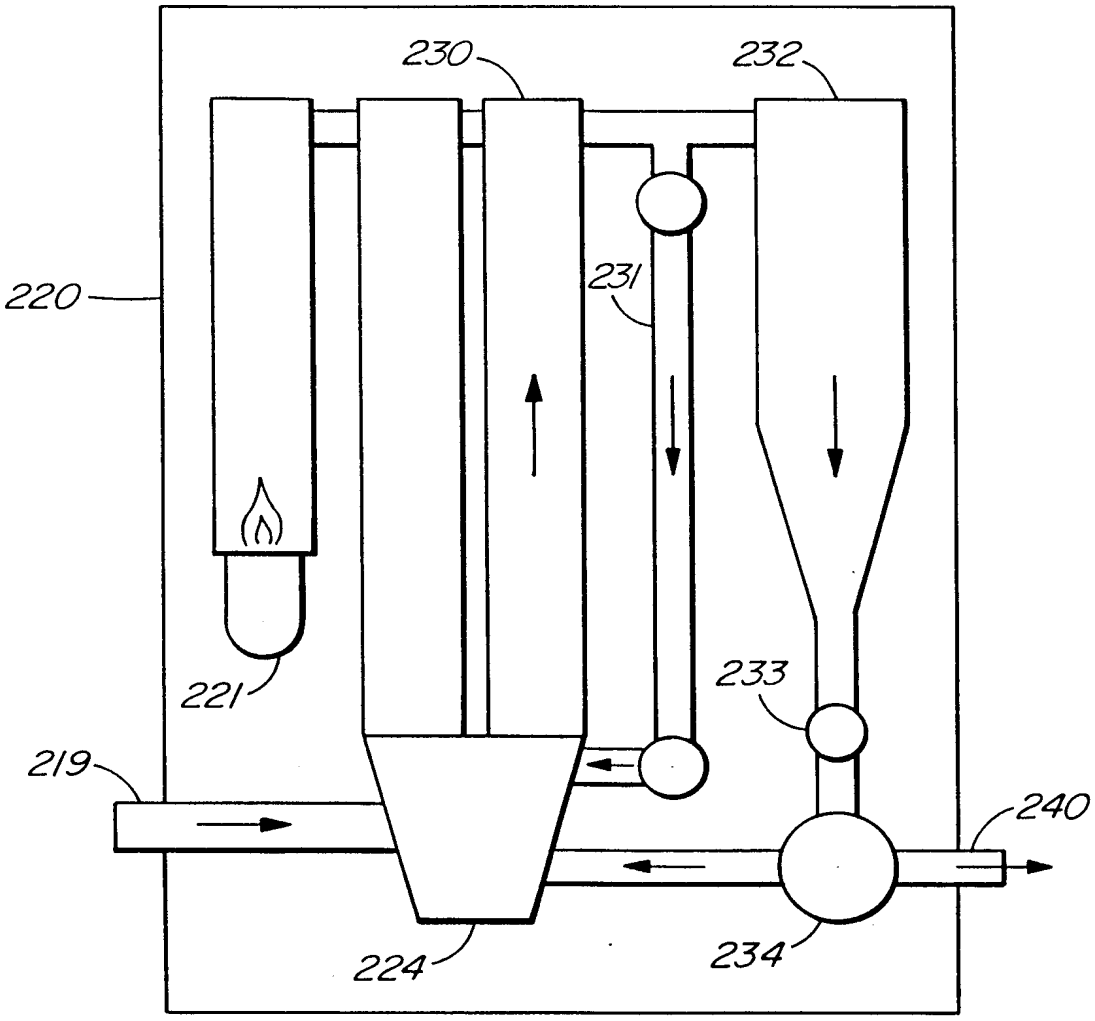


FIG. 8

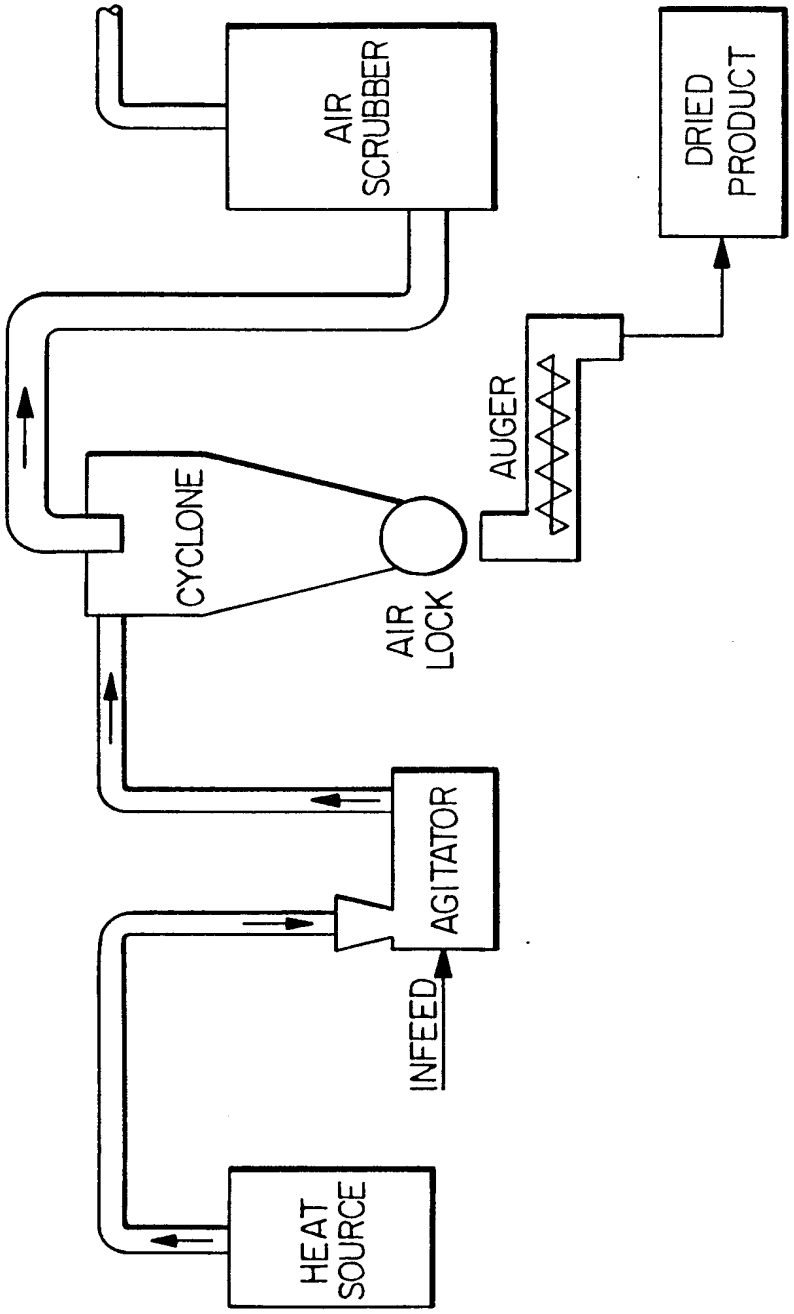

AIR SCRUBBER
DRIED PRODUCT
AUGER
CYCLONE
AIR LOCK
AGITATOR
INFEED
HEAT SOURCE

FIG. 9

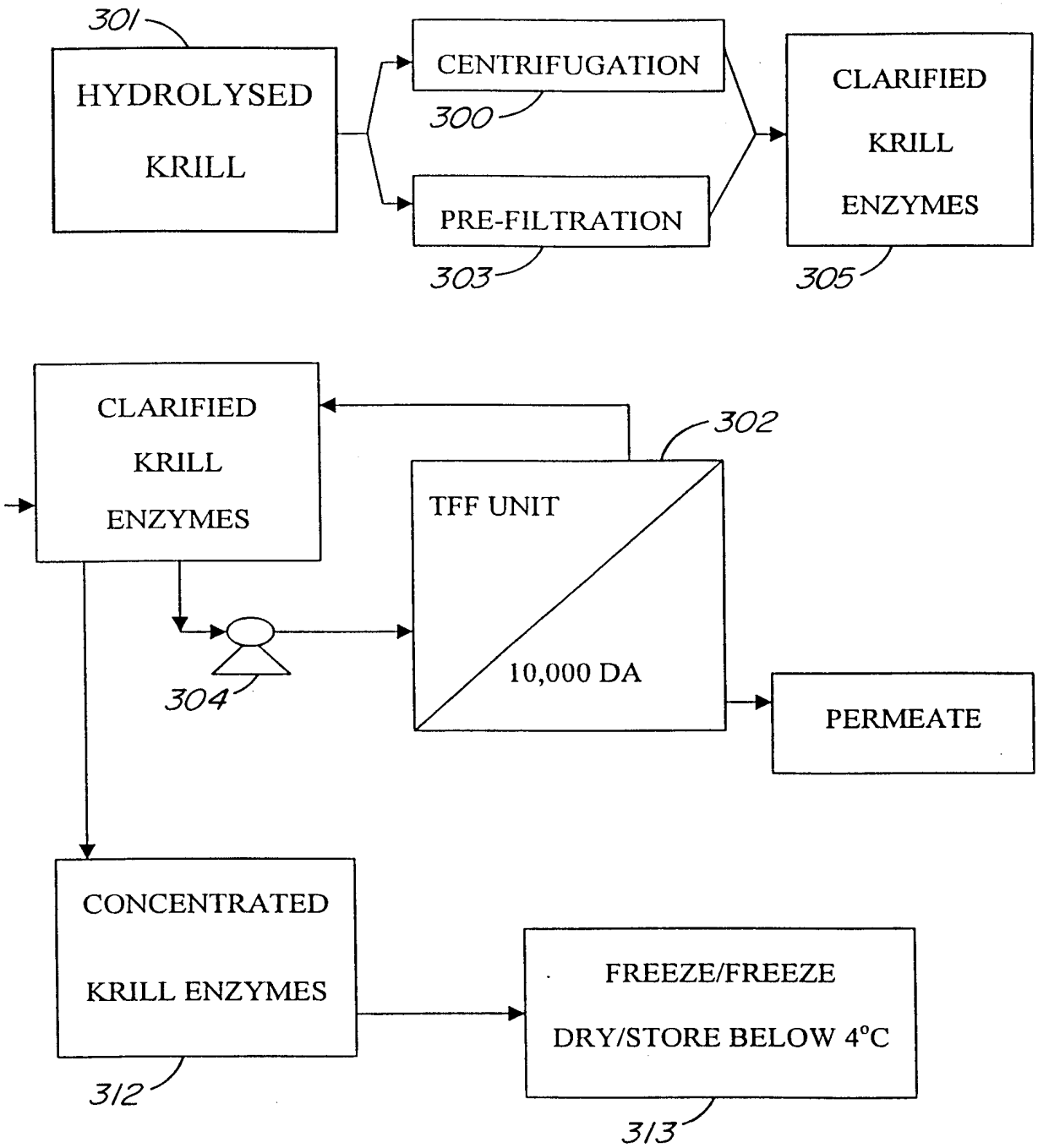


FIG. 10

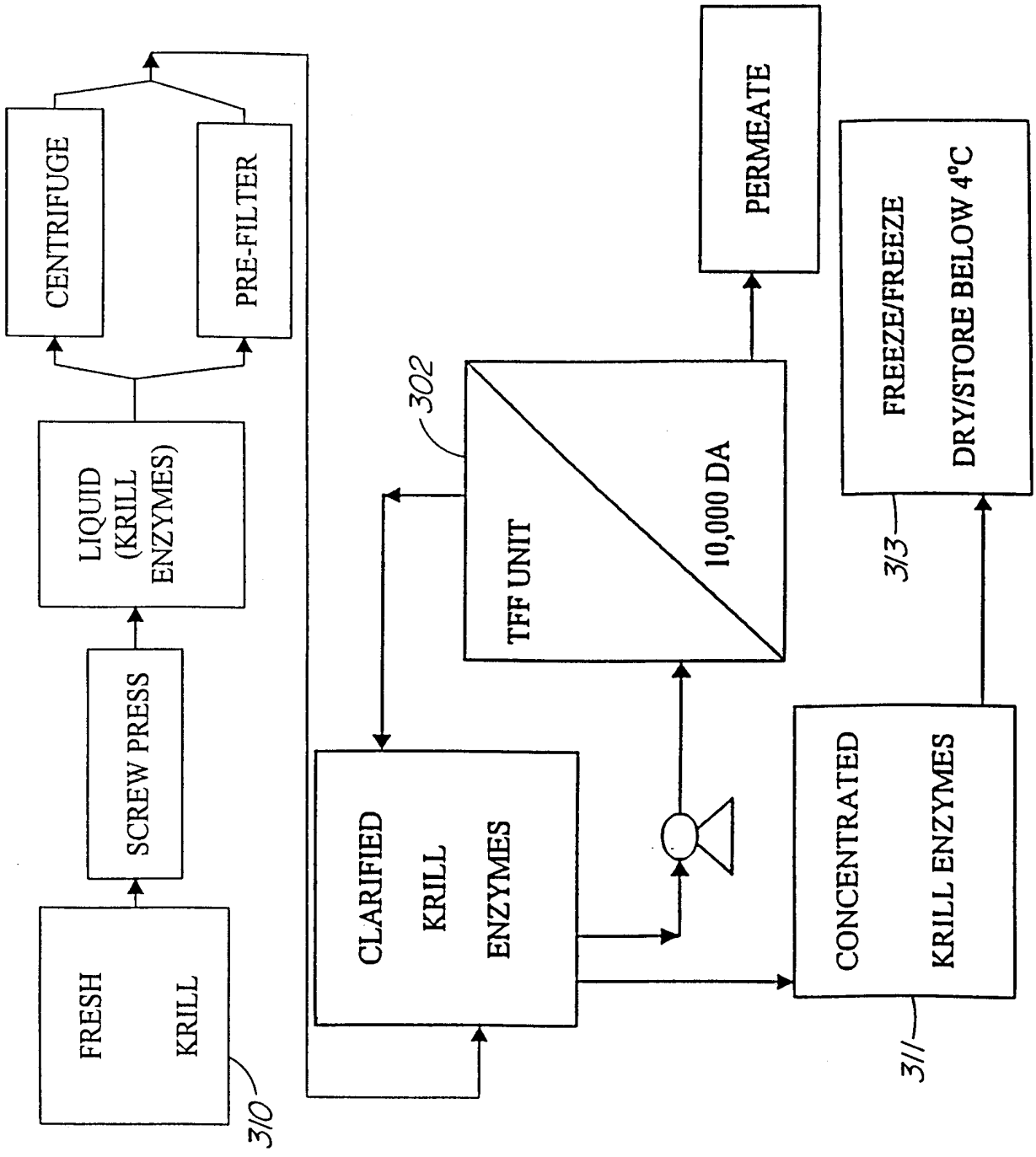


FIG. 11

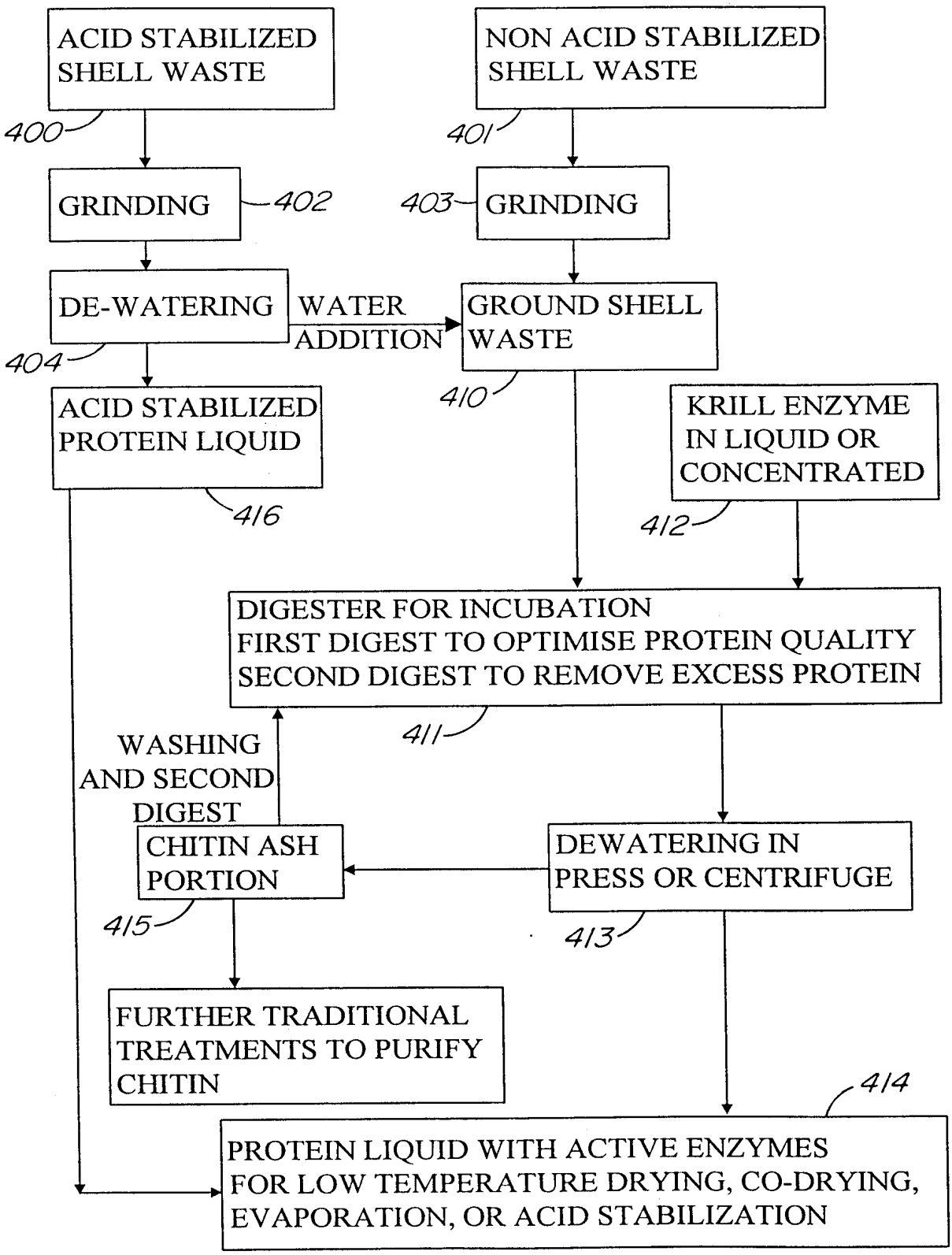


FIG. 12

INTERNATIONAL SEARCH REPORT

Ir. .ational Application No
PCT/CA 98/00082

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23K1/10 A23K1/16 A23K1/18 A23N17/00 C12N9/00
A23J1/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23K C12N A23J A23N

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 8447 Derwent Publications Ltd., London, GB; Class C03, AN 84-293719 XP002070859 & SU 1 084 005 A (N BASSIN FISHING IND) see abstract ---	1,20,52
X	WO 95 22893 A (SPECIALTY MARINE FEEDS INC) 31 August 1995 see page 15, line 19 - page 17, line 19 see claims 11-28,30-46 --- -/--	51

[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
8 July 1998	21/07/1998

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	Dekeirel, M

1

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

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00082

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 89 01031 A (PHARMACIA AB) 9 February 1989 see page 5, paragraph 2 see page 7, paragraph 1 see examples 1-3 see claim 1	29-34,41
A	---	35-40
X	WO 89 10960 A (PHARMACIA AB) 16 November 1989 see page 8, last paragraph see page 11, paragraph 3 - paragraph 5 see page 14, paragraph 3 see page 27, paragraph 4 see claims 1,7,17	42,43
X	---	21,27
	WO 90 05026 A (AKT CONSULTANTS) 17 May 1990 see figure 1	
A	---	1,20,52
	PATENT ABSTRACTS OF JAPAN vol. 017, no. 315 (C-1071), 16 June 1993 & JP 05 030923 A (RIKEN VITAMIN CO LTD), 9 February 1993, see abstract	
A	---	1,20,52
	DATABASE WPI Section Ch, Week 9602 Derwent Publications Ltd., London, GB; Class D13, AN 96-018544 XP002070860 & RU 2 034 492 C (TROITSKII B N) see abstract	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/00082

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9522893 A	31-08-1995	CA 2134515 A	28-04-1996
		AU 1802695 A	11-09-1995
		CN 1146709 A	02-04-1997
		EP 0758842 A	26-02-1997
		FI 963343 A	27-08-1996
		JP 10500004 T	06-01-1998
WO 8901031 A	09-02-1989	AT 124085 T	15-07-1995
		AU 2259788 A	01-03-1989
		DE 3854050 D	27-07-1995
		EP 0393035 A	24-10-1990
		JP 2504465 T	20-12-1990
WO 8910960 A	16-11-1989	NONE	
WO 9005026 A	17-05-1990	AU 4518389 A	28-05-1990
		CA 2002193 A	03-05-1990
		DK 158990 A	22-08-1990
		EP 0403608 A	27-12-1990
		GR 89100729 A	31-12-1990
		PT 92192 A,B	31-05-1990
		US 5105560 A	21-04-1992

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:	WO 99/39589
A23K 1/10, 1/16, 1/18, A23J 1/04, A23N 17/00, C12N 9/00	A1	(43) International Publication Date:	12 August 1999 (12.08.99)

(21) International Application Number: PCT/CA99/00075

(22) International Filing Date: 9 February 1999 (09.02.99)

(30) Priority Data:
09/020,695 9 February 1998 (09.02.98) US

(71) Applicant *(for all designated States except US)*: BIOZYME SYSTEMS INC. [CA/CA]; 4160 Marine Drive, West Vancouver, British Columbia V7V 1N6 (CA).

(71)(72) Applicants and Inventors: SAXBY, David, J. [CA/CA]; 4727 South Piccadily, West Vancouver, British Columbia V7W 1J8 (CA). SPENCE, John, A. [CA/CA]; 2025 Gisby Street, West Vancouver, British Columbia V7V 4N2 (CA). SAXBY, Gregar [CA/CA]; 4727 South Piccadily, West Vancouver, British Columbia V7W 1J8 (CA). ALOISE, Pedro [CA/CA]; 202–1725 Osoyoos Crescent, Vancouver, British Columbia V6T 1X7 (CA).

(74) Agent: UREN, John, Russell; Suite 202, 1590 Bellevue Avenue, West Vancouver, British Columbia V7V 1A7 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD AND APPARATUS FOR PROCESSING KRILL HYDROLYSATES

(57) Abstract

Method and apparatus used in producing a feed product or premix and the products made by the method. A predetermined quantity of krill hydrolysate is added to a predetermined quantity of dry carrier with or without a predetermined quantity of liquid marine protein. The mixture is subject to evaporation and drying steps in which relatively heavier particles are separated from relatively lighter particles. The mixture may be blended, ground and subject to chemical reaction in a balance tank prior to entering a dryer. The dryer utilises a warm air source, a tower and a cyclone to dry the mixture following its entry into the dryer. Temperature sensitive enzymes or other bioactive products may be added to the product produced from the dryer. A method for obtaining enzymes from a fresh krill extract or an autolysed krill preparation and the product are also disclosed. A method for separating the bound protein and pigments from crustacean waste using krill enzymes and a product producted by the method are also described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TITLE OF THE INVENTION

METHOD AND APPARATUS FOR PROCESSING KRILL HYDROLYSATES

INTRODUCTION

This invention relates to a method and apparatus used in producing a feed product or premix and the product made by the method and, more particularly, to a process using co-drying to dry a mixture of krill hydrolysate and dry carrier or a mixture of krill hydrolysate, fish hydrolysate and dry carrier. The invention further relates to recovering enzymes from krill and, more particularly, to recovering enzymes from both freshly harvested and hydrolyzed krill. The invention further relates to utilising krill enzymes for removing protein from marine and biological wastes and, more particularly, for removing

protein, chitin and other constitutents from crustacean and other marine wastes.

BACKGROUND OF THE INVENTION

With the advent of increasing activity in aquaculture or fish farming in the early to mid-1980s, research has been ongoing into increasing productivity or growth rate and reducing the mortality rate of fish raised in aquaculture conditions since survival of such fish is important. One such factor relates to enhancing the nutritional value and palatability of feed used in raising such fish. In addition to the nutritional value, it is desirable to reduce the cost of feed to such fish since, typically, the feed totals approximately 40 to 50% of the cost of raising the fish. Such feed should be a high quality feed to meet the objectives of having high nutritional value to maximize growth and to reduce fish mortality.

The requirement for feed products in aquaculture is projected to grow substantially and, as a result, there is and will be pressure to obtain the necessary ingredients for fish food. The possibility of using zooplankton and, in particular, euphausiids, as a fish feed, appetizer or food product has been investigated and has been found to be possible and desirable, particularly as a feed product.

In addition, blends of krill hydrolysates and fish hydrolysates or any one of these with a dry carrier, can

povide alternatives to fish meals in aquaculture and other animal feed diets. Euphausiids are a natural feed harvested directly from coastal waters and have a high nutritional value but, previously, the cost of harvesting and processing such zooplankton for a feed product has been prohibitively expensive.

As well, the questions of the availability of the biomass of such zooplankton and its harvesting, handling, storage and processing are parameters that must be investigated in order to determine whether the product would be appropriate as a feed product.

Through papers written by Fulton and other authors, the use of zooplankton as a food or feed product has been contemplated for some time. In particular, antarctic krill (Euphausia superba) for human consumption have been investigated, although relatively little work has been investigated related to aquaculture. The use of Euphausia pacifica in the coastal waters of British Columbia, Canada has been considered in relation to its use in aquaculture and other animal feeds.

It appears, from those investigations, that the necessary biomass is available in coastal waters. Previously, euphausiids have been used as a pet food ingredient and some aquaculture operators have used euphausiids as a feed product. The euphausiids were used for such purposes in a frozen form after being harvested and

in some cases, the euphausiids were freeze dried following harvesting. This is an expensive procedure.

In processing feed products, it has typically been the case that the ingredients used in such feed products are heated to a high temperature around 100°C when the product is processed and dried. By heating the product to such a high temperature, it is believed that the enzymes and other proteins in the product are denatured. If, however, it is intended to utilize the product for early stage or juvenile aquaculture, which young fish have relatively undeveloped digestive systems, it is desirable that in some application, the euphausiid products maintain a certain proportion of enzymes which will assist the digestive process in juvenile and other life stages. If the theory that enzymes are advantageous in nutrition is correct, such destruction of the enzymes during the aforementioned drying process is disadvantageous.

It is also desirable to have a natural product, where the proteins are not denatured, available for early stage juvenile or larvae feed. In some previous products, exogenous enzymes have been added to the zooplankton mix. However, the addition of such enzymes is difficult to control and can result in a complete hydrolysis of the proteins to amino acids. The presence of free amino acids in the feed needs to be controlled since they can create an inferior product of substantially reduced value as a feed product.

It has been shown, surprisingly, that the degree of enzyme activity which results in determining the digestibility of a product, reaches a relatively constant value after a certain period of time in a natural product. Recent investigations conducted by the applicant have confirmed this characteristic for Euphausia pacifica. This characteristic was first discovered in relation to Euphausia superba by Kubota and Sakai in a report entitled "Autolysis of Antarctic Krill Protein and Its Inactivation by Combined Effects of Temperature and pH", Transactions of the Tokyo University of Fisheries, number 2, page 53-63, March 1978. However, the antarctic krill study done by Messrs. Kubota and Sakai had the objective of limiting enzyme activity which was deleterious to obtaining a food as opposed to a feed product. Messrs. Kubota and Sakai wished to inhibit the enzymatic activity by certain processing techniques which they considered desirable when the product was intended as a food product.

An appropriate degree of hydrolysis is obtained during the digestion of the euphausiids. The approximate degree of hydrolysis will vary depending on the final application and it can be monitored by measuring the apparent viscosity in the final product. Further processing may then take place in order to make a useful product for commercial feed. Such processes may include adding acid to obtain an acid stabilized product concentrating fractionating or drying the product. A variety of drying techniques such as freeze drying, spray drying, or vacuum and air drying. Spray drying, as well as some other drying

processes, however, are done at temperatures that will permanently inactivate the enzymes in the euphausiids which, as earlier mentioned, may be undesirable for aquaculture purposes although it is acceptable for purposes where the product is intended to be used as a carotenoid biopigment for coloring purposes in both feed and food products or as a source of protein, fatty acids, minerals or other nutrients.

SUMMARY OF THE INVENTION

According to one aspect of the invention, there is provided a method of producing a feed product comprising the steps of adding a predetermined quantity of krill hydrolysate to a quantity of dry carrier to produce a mixture and co-drying said mixture to obtain an end product. The dry carrier may conveniently be a plant protein, dry krill, fish meal, byproduct meal or other dry ingredient suitable for inclusion in a diet.

According to a further aspect of the invention, there is provided a product produced by adding a predetermined quantity of krill hydrolysate to a quantity of liquid marine protein and a quantity of dry carrier to produce a mixture and co-drying said mixture.

According to a further aspect of the invention, there is provided a co-drying apparatus for drying a mixture of krill hydrolysate with or without an evaporator and liquid marine product and a dry carrier comprising a dryer

for concentrating, mixing, agitating, heating and separating particles of said mixture.

According to still a further aspect of the invention, there is provided a method of obtaining an enzyme extract from a liquid krill hydrolysate comprising the steps of subjecting said hydrolysate to decanting and then to centrifugation to obtain a clarified liquid and further subjecting said clarified liquid to ultrafiltration using a membrane with a capacity to retain said enzymes having a molecular weight greater than 10,000 daltons and the product produced by the method.

According to still a further aspect of the invention, there is provided a method of obtaining an enzyme extract from fresh krill comprising the steps of squeezing said krill to obtain an aqueous extract and subjecting said aqueous extract to ultrafiltration with a membrane adapted to retain enzymes having molecular weights above 10,000 daltons and the product produced by the method.

According to still yet a further aspect of the invention, there is provided a method for removal of protein from non-stabilized or fresh crustacean shell wastes comprising grinding said crustacean wastes and water, transferring said product to a digester, adding a predetermined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively

enzymatically active and relatively high in protein and a second portion of shell material relatively high in chitin and low in protein.

According to still yet a further aspect of the invention, there is provided a method for removal of protein from acid stabilized shell wastes comprising grinding said crustacean wastes, transferring said small particulate size shell wastes to a digester, adding a predetemined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively enzymatically active and relatively high in protein and a second portion of shell material relatively high in chitin and low in protein.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Specific embodiments of the invention will now be described, by way of example only, with the use of drawings in which:

Figure 1A is a diagrammatic isometric view of a fishing vessel with an attached net which utilizes the euphausiid harvesting technique according to the invention;

Figure 1B is a diagrammatic front view of a net in an alternative harvesting technique according to the invention;

Figure 2A is a diagrammatic side view of a cage which is used to maintain the cod end of the fishing net illustrated in Figure 1 in an open position and which is further used to transport the harvested euphausiids to the harvesting vessel;

Figures 2B and 2C are side and rear views, respectively, of the dewatering trough used to remove water from the harvested euphausiids;

Figure 3 is a diagrammatic process chart illustrating the processing of the euphausiids subsequent to the dewatering steps illustrated in Figure 2 and prior to the drying step;

Figures 4A and 4B are end and side sectional views of the heat exchanger used to raise the temperature of the harvested euphausiids prior to the digester process;

Figure 5 is a diagrammatic side sectional view of the digester used to create the desired enzyme activity within the euphausiids;

Figure 6 is a diagrammatic side sectional view of a ball drier used to dry the euphausiids following removal of the euphausiids from the surge tank located downstream from the digester;

Figure 7 is a flow chart illustrating the process of co-drying the product according to the invention;

Figure 8 is a diagrammatic view of the dehydrator used in the co-drying process according to the invention;

Figure 9 is a diagrammatic view of the codrying process according to a further aspect of the present invention;

Figure 10 is a diagrammatic flow chart illustrating the enzyme extraction process utilising hydrolysed krill;

Figure 11 is a diagrammatic flow chart illustrating the enzyme extraction process utilising fresh krill; and

Figure 12 is a diagrammatic flow chart illustrating the removal of protein and other constitutents from crustacean wastes using krill enzymes according to a further aspect of the present invention.

DESCRIPTION OF SPECIFIC EMBODIMENT

Referring now to the drawings, a towing vessel 10 is illustrated in Figure 1. A plurality of towing ropes 11, 12, 13 are connected to the towing vessel 10 in order to tow a barge 14 and a net 20. A plurality of ropes 21 (only one of which is shown) are connected to the net 20 and extend downwardly from the barge 14. Weights 22 are connected to the bottom of the open forward facing portion of the net 20 in order to maintain the net 20 at a desired and

predetermined depth where the concentration of zooplankton is satisfactory.

The cod or rearward end 23 of the net 20 is maintained in an open condition by the use of a cage generally illustrated at 24 in Figure 2. Cage 24 is of cylindrical configuration and is positioned within the cod end of net 20. It is made from aluminum and is preferably corrosion resistant. A fitting 30 is welded to the downstream end of the cage 24 and one end of a swivel connection 31 is joined to the fitting 30 to prevent fouling the net in the event components become unstable under adverse harvesting conditions. A hose 32 is connected to the other end of the connection 31.

Referring again to Figure 1, hose 32 extends upwardly from the cod end of the net 20 to the barge 14. A pump of a variety of configurations but, conveniently, a diaphragm sump pump 33, is located at the other end of the hose 32 on barge 14. A dewatering trough is generally shown at 34 and is illustrated in Figures 2B and 2C. Dewatering trough 34 has a lengthwise generally rectangular configuration and is also located on barge 14. Dewatering trough conveniently takes the configuration of a "lazy L". A set of screens 40 positioned at obtuse angles are utilised to allow water to drain from the pumped euphausiids and exit the trough 34 through drain pipes 41 while the euphausiids accumulate within the dewatering trough 34.

A blast freezer 42 was also located on the barge 14 to stabilize the harvested euphausiids. The blast freezer 42 subjects the euphausiids to a temperature of approximately +9° to -17°C and is used to freeze the dewatered euphausiids and stabilize the product for further processing. The euphausiids accumulate within the dewatering trough 34 and which are periodically removed from the trough 34 from time to time for freezing. Thereafter, the frozen euphausiids are transported to a processing location and processed as described hereafter. Alternatively, the euphausiids may conveniently be processed aboard a vessel.

In prototype demonstrations, the net 20 utilised for the harvesting operation was a specially designed 13 ft. by 21 ft. plankton net suspended from a 46 ft. aluminum barge. The pumping action was by a three inch diaphragm pump located on the barge 14 and the freezing action occurred within a minus seventeen (-17°C) degree centigrade blast freezer 42.

As earlier described, the frozen euphausiids are transported to a processing location in order to transform the euphausiids into the desired feed product. Reference is now made to the flow chart of Figure 3.

A pump 43 is connected to a hopper 44 which receives the euphausiids which are now in a thawed condition. Pump 43 is connected to a heat exchanger generally illustrated at 50 and diagrammatically illustrated

in Figure 3. The heat exchanger 50 is intended to raise the temperature of the euphausiids to a temperature of approximately 40°C to 60°C which will more closely approximate the temperature maintained in the digester which is generally lower than 70°C and which digester is generally illustrated at 51. Digester 51 is located downstream of the heat exchanger 50 in the process illustrated in Figure 3.

Although several different types of heat exchangers may be used, heat exchanger 50 conveniently comprises a plurality of pipes 52 (Figure 4A) in which the euphausiids are conveyed through the heat exchanger. Heated water enters the inlet 54 of the heat exchanger 50 and is circulated through the heat exchanger 50 generally following the flow path seen in Figure 4B which utilizes a plurality of baffles 53. The heated water exits the heat exchanger at outlet 61. Following the increase of temperature created in the euphausiids by the heat exchanger 50, the euphausiids pass to the digester 51.

Digester 51 is seen is greater detail in Figure 5. It comprises a product inlet 61 and a product outlet 62. A water inlet 63 and a water outlet 64 are provided. A water jacket 70 through which the heated water circulates surrounds the cylindrical cavity area 71 of the digester 51 which contains the euphausiids. A plurality of stirring discs 72 are located vertically within the cavity area 71 of the digester 51 and are used to stir the euphausiids when they are positioned within the digester 51. A valve 73 is used to close the product outlet 62 so as to maintain the

euphausiids within the digester 51 until the proper temperature and time for the desired enzyme action within the euphausiids has taken place. The time period has conveniently extended between thirty (30) minutes and two (2) hours.

It is thought that a degree of hydrolysis will enhance digestibility of the feed product particularly for early stage larvae or juveniles but also for virtually all fish. This degree of hydrolysis is detemined by the applications and will be monitored by measuring the apparent viscosity in the final product. In utilising the digester 51 illustrated in Figure 5, a batch process is currently being used with a volume of euphausiids of 250 lb./hr being used.

The valve 62 is then opened and the quantity of euphausiids within the digester 51 pass through the valve 62 and are transported through valve 74 to the surge tank or heated batch storage vessel 80 where they await treatment in the dryer, conveniently a ball dryer generally illustrated at 81 (Figure 6) where relatively low and controlled temperatures can be applied to the euphausiids such that any enzymes existing within the euphausiids are not inactivated as would otherwise be the case in a normal drying process.

The euphausiids pass from the storage vessel 80 to the ball dryer 81 through product inlet 83 and, thence, about the periphery of the dryer 81 initially through the application zones 91 where the balls initially contact the

euphausiids and begin the drying process. The ball dryer 81 performs a "soft" drying process which reduces damage to the euphausiids because of its gentle action by way of controlled temperature. The ball drying process utilises a continuous feed into the ball dryer 81 and a product flow of 15 lb./hr. is available.

As the balls and euphausiids move downwardly through the drying zones 92, they meet a counter-current flow of controlled-temperature drying air at less than 50°C which air enters the ball dryer 81 through air inlet 82. Air flow, temperature and dwell time are precisely controlled and monitored within this zone. All of these are variable factors which depend upon whether the product is wet or dried and what period of time the product is intended to stay in the dryer 81.

In the separation zone 93 at the bottom of the dryer 81, the ball and euphausiids meet a co-current flow of controlled temperature air for final drying and separation. The dried euphausiids leave the ball dryer 81 through the product outlet 84 and pass to the packaging step. The drying balls are elevated by rotating helix 94 and recycled to the application zone 91 and the process continues.

One of many commercial and known dryers may be used for the air drying of the euphausiids.

It is contemplated that although the processing of the euphausiids has been described as taking place at a land

location, such processing steps may take place at the harvesting location on board either the harvesting vessel or another vessel conveniently located nearby. This results in advantages in that the euphausiids need not be frozen following harvesting and need not be transported to a land based processing plant thereby resulting in considerable cost savings and quality improvement. In addition, the euphausiids may be introduced directly to a low tempeature dryer on board a vessel following harvesting or to an evaporator. The dried or concentrated euphausiids, after being subjected to the digester and/or the drying processes, may then be stored on the vessel until a substantial quantity of krill hydrolysate concentrate has been obtained at which time they may be transferred to another vessel for transport to the processing vessel itself which, when full, will transport the euphausiids to the shore.

Likewise and while it is desirable for the digester and drying steps to take place concurrently and sequentially in the event the euphausiids are intended to be used as a feed product for juvenile and early stage larvae.

A further harvesting technique is contemplated in Figure 1B. In this technique, weights 101 are connected to the mouth end of the net generally illustrated at 114 at the ends of the lower horizontal beam 103. Floats 100 are connected to the top horizontal beam 102 of the mouth end of the net 114. Depending on the size of the net 114, lines are connected on one end to attachment points 104, in the first instance or, alternatively, to points 110, 111, 112,

113 and, on the other end, to the towing vessel. The net 114 is pulled through the water gathering the zooplankton which enter the net 114 through the mouth.

Many applications for the hydrolysed krill and hydrolysed krill concentrate products are also contemplated because of the desirable characteristics of the of the krill hydrolysate in which the proteins and nutritional value is retained and improved through the partial digestions of the proteins. For example, fish under stress, which is common with cultivated species raised with aquacultural techniques, are reluctant to eat and, accordingly, therapeutic drug delivery and special diets used for such marine species are difficult to use because the fish do not find such products palatable. The hydrolysed krill products and other zooplankton products according to the invention may be used with such special diets and drug delivery by creating an enhanced flavour and enhanced assimilation when the medicinal product such as a pellet is coated or mixed with the hydrolysed zooplankton product in a liquid or paste form. Likewise, while other such products may include specially added amino acids and other compounds to enhance the flavour of the product, the hydrolysed krill according to the present invention preserves, enhances and optimises the level of certain free amino acids and other flavourants thereby allowing flavour enhancement with a natural product and without the addition of amino acids or other flavourants. Likewise, the krill hydrolysates retain the protein and nutrient quality inlcuding the original pigments, fatty acids, other nutrients and mineral elements.

The activity of the enzymes, which are contained in the krill, is also retained in the hydrolysed natural product according to the invention. Such enzymes allow for enhanced digestion of feed by certain cultivated marine species by increasing the availability of peptides and free amino acids without creating additional harmful stress on such species.

Yet a further application contemplated by the present invention is the use of hydrolysed krill that is blended and codried in association with plant or vegetable protein and other dry carriers such as soymeal, corn gluten meal and canola meal in fish feed mixtures. The range of co-drying cariers used in the blending process include a wide range of dry animal or vegetable protein and feed ingeedients including soy conola and other soil seed meals, coarse ground cereal gains and flours, oil seed concentrates and isolates, corn and cereal glutens, pea and pulse meals, oil seed and cereal processing by products and brans, dried yeasts, algae and other single cell organisms, milk powders, blood meal and other body fluid products, namial and poultry by products, fish and shellfish meals, and vitaminised mineral premixes. Such applications would increase the palatability, amino acid balance and other nutrient levels in the dry blended meal so that it can be used to replace fish meal in aquaculture feeds and other applications. Further enzymes in the hydrolysed krill products according to the invention are preserved following he hydrolysis and can be allowed to act on the plant proteins. The enhanced digestibility of a product combination of plant protein and hydrolysed krill is also contemplated to improve the

efficiency of the feed and decrease the fecal load in the environment by fish fed with diets containing such combination. This can be an important feature with the rearing of cultivated marine and freshwater species. Likewise, the palatability of such non-fish meal proteins, in particular, plant proteins such as canola, corn gluten or soy meal is enhanced.

Experiments conducted to date utilize the enzymes in krill to carry out a limited hydrolysis of soy, canola and other plant proteins. For example, one part of dry canola or soy meal which has added ten percent (10%) wheat bran is blended with five (5) parts of hydrolysed krill. The hydrolysate is pumped from the digester to the feed stock hopper and the dry blend is added. The mixture is brought to the desired temperature while agitated in the digester for approximately one (1) hour. Measurements of phytic acid and the levels of the amino acids and ammonia are then taken. For example, 250 lbs. of krill is hydrolysed by bringing the krill to approximately 45° Celsius. The temperature is held for one (1) hour and is then blended with 5 lbs. of wheat bran with 45 lbs. of canola concentrate. The use of wheat bran is necessary to provide phytase, an enzyme which is absent in canola meal and krill. The phytic acid is dephosphorylated by phytase from the wheat bran. The phytic acid is acted on by the phytase enzyme. It is noted that the blend may be retained in the digester for an extended period, up to a period of four (4) hours or even longer.

In yet a further embodiment of the invention, it is contemplated that the wet krill hydrolysate product is evaporated and then mixed with and co-dried with other wet and dry products. Various predetermined ratios of wet krill hydrolysate and liquid marine products may be concentrated and tehn mixed with dry carrier conveniently in the form of dried krill products, dried vegetable protein and/or dried fish product, used in combination or singly. The resulting moist blend is subject to concentration, processing and co-drying in a dehydrator such as a dryer. A dehydrator system with the following characteristics has been found to work well, namely a type of flash and fluidized drier or combination thereof with an agitator and vertical or tangential flow of heated air. Although the temperature of the inflowing air may be high at impact (the impact temperature), the temperature of the product is not significantly increased in the dryer. This is an important element in the drying system. Following hot air impact and agitation, the water evaporates rapidly and the duration of the drying process is greatly reduced as set out in greater detail hereafter.

Co-drying the mixture of the krill hydrolysate, liquid marine product and the dry carrier product mixture has been found to be relatively economical at relatively low temperatures. Under such conditions, the krill poteins, pigments and other constitutents are substantially preserved. Thus produced, the product has unique benefits for dietary uses in aquaculture and animal feeds. These blended and agglomerated dry products are uniquely different

from other product mixes. The unique sequences and control of the process provides initimate agglomeration and adsorption of the krill hydrolysate with the dry carrier. It also preserves the unique nutient quality of the krill hydrolysate in the blend without significant losses due to excess heat or oxidation during the drying process. Further, cost savings and economic advantages in the manufacture of the product are improved.

Depending on the moisture content of the dry carrier, liquid marine protein, and the krill hydrolysate, and the proportion of each in the mixture to be co-dried, the removal of moisture can be accomplished by a drying process at relatively low temperatures thereby to preserve the temperature and oxidation sensitive constituents including the krill constitutents and the krill pigments. Particles of the dry carrier are coated with, adsorbed and absorbed with the wet hydrolysate thereby facilitating the drying process by exposing a greater surface area of wet hydrolysate and/or liquid fish product for heated air to act upon. The mixture may then be fractured into smaller particles which further increases the available surface area to expedite the drying process. At the outset, the mixture may be placed in a reactor cell balance tank to permit chemical interactions between components of the mixture, such reactions including enzymatic activity of a wide range of enzymes including proteolytic, lipolytic and carbohydrate splitting enzyme prior to drying. A well-mixed, homogeneous mixture is prepared to reduce and to eliminate high moisture pockets. Water is then removed from this mixture by an

evaporator and a subsequent dehydrator such as is described above and the endproduct is a dried krill premix or feedstuff blended with the aforementioned carrier. Temperature sensitive enzymes, flavorants or other bioactive products may be added to the cooled endproduct after the drying step. Alternatively, the krill hydrolysate may be combined with wet fish products and other carriers such as dry fish meal, corn meal, canola meal, oil seed meal, or other vegetable meals, used in combination or taken singly.

Referring now to the drawings, Figure 7 illustrates the steps of the co-drying process in its entirety according to the present invention. A predetermined quantity of wet krill hydrolysate product 210 is mixed with a predetermined quantity of liquid marine protein 212 and a predetermined amount of dry carrier 211, conveniently dried krill product, dried fish product and/or dried vegetable protein used in combination or taken singly. The resulting mixture is placed in a mixing blender 215, where the various ratios of hydrolysate, marine protein and dry carrier are thoroughly blended. The blending required will vary with the constitution of the mixture. The blended mixture is then ground within a grinder 217 where the mixture is reduced to particles of substantially uniform size. The ground mixture is then transferred to reactor cell balance tank 216 where the continuously stirred blended mixture is allowed to chemically react and/or undergo enzymatic action prior to the drying process. After the intended reaction has taken place in the tank 216, the mixture is conveyed to the dehydrator 220 for drying.

The dehydrator 220 is illustrated in greater detail in Figure 8 and with reference thereto, the mixture enters the agitator bowl 224 of the dehydrator 220 through inlet 219 where the mixture is agitated into smaller particles which is intended to prevent clumping of the mixture. A continuous feed of mixture into the dehydrator 220 is intended through inlet 219.

Directly heated air from the burner 221 or indirectly heated air is directed to the agitator bowl 224 of the dehydrator 220 by way of fans (not illustrated) where the air mixes with particles of the mixture in the bowl 224. The particles are carried up the drying tower 230 by the column of hot air. The classifier 231 sorts the particles at the top of tower 230. Drier mixture consists of lighter, individual particles which proceed along the column of hot air into a cyclone 232. The classifier 231 redirects larger and heavier masses of more damp mixture back to the agitator bowl 224 for further agitation and drying.

The particles are drawn downwards along a spiralling column of heated air in cyclone 232 and centrifugal action removes further moisture from the particles. At the bottom of the cyclone 232, the particles are isolated from the air column by airlock 233 and are sorted by a rotary screen 234. Smaller, lighter particles of dried product pass through the rotary screen 234 and exit the dehydrator 220 at outlet 240 for further processing. Larger, heavier particles of damp mixture are redirected to

the agitator bowl 224 from outlet 241 for further agitation and drying within several seconds.

With reference again to Figure 7, heated product 241 exiting the dehydrator 220 from outlet 240. The average transit time through the dryer is between 60 and 90 seconds and the end moisture content below 10% moisture may then be permitted to cool. Some of this dried product 245 may be further used in the co-drying process as a quantity of the dry carrier 211 so as to increase the fluid content of marine constitutents. Temperature sensitive enzyme active products 242 or other bioactive products, which might be denatured by the drying process, may be introduced to the dried product 241 after the product has passed through the dehydrator 220 as illustrated. The dried product 241 then undergoes further mixing and blending at mixing step 250 to ensure the homogenous addition of the temperature sensitive enzyme active products 242. The final product 243 may then proceed to a packaging step such as a bagger 244 or to a storage bin 245 prior to further use in aquaculture or animal feeds.

Concentration and Co-Drying or Krill with Vegetable proteins Trials

The objectives were the concentration of liquid krill hydrolysate to 42%DM in a rising film plate evaporator. (Alfa Vap). The drying of a krill concentrate blend with soya meal and corn gluten meal in a flash dryer (drier with performance characteristics as defined), to determine the

maximum amount of krill concentate that can be added to the dry vegetable protein meal.

Raw material hydrolysed krill with 18-20% DM including approximately 0.3% oil.

Evaporator. The hydrolysed krill was concentrated in an Alfa Vap evaporator from 18-20% DM to 42% DM. The 42% level was not obtained with any difficulty.

Mixing

The mixing was done in 100 kg batches using a cylindrical container with a vertical shaft paddle. This was accomplished without unusual difficulties.

Drying

Drying and mising was caried out in two steps: Step 1 was mixing the krill concentrate and carrier (vegetable and protein) and drying to about 90% DM. Step 2 was mixing the dried product from step 1 with more krill concentrate and drying a second time.

Flash Drying

The mixtures were dried in a flash dryer. This was done by feeding the mixture into a chamber containing a fast rotating agitator. Through intake air ducts hot air was led through the chamber and agitator.

Impact Temperature was 165-175 deg. C.

Drying Temperature (set point) is 110 deg. C to 125 deg C.

Capacity

The flow to the dryer for all three test vegetable protein products was 600-700 kg/hr. This gave an evaporation rate of approximately 500 kg/hr. in the dryer.

Results

The temperature of the product is not increased in the dryer by any significatnt ammount. The evaporation of the water on the product keeps the temperature low. The rapid transit of the product through the dryer also minimizes the temperature and time effects that can reduce the value of the product as a feed.

A third or fourth step is also contemplated and considered possible with this type of dryer.

Other driers besides those of ball dryer 81 (Figure 6) are contemplated. For example, dryers such as direct heated flash driers or fluidized bed driers that cause rapid drying of the particles within a few seconds are well known. With reference to Figure 9, a built in air scrubber generally illustrated at 500 is used for odour control. A burner or indirect heating system 501 heats the air to the required level with impact temperatures not

exceeding 450 deg. C before the air enters agitator 502. the product is augered tangentially into the agitator chamber 503 where most of the water in the product is evaporated. Agitator 502 rotates with a high tangential speed of the agitator blades concurrent with the tangential air flow. The motion of the agitator 502 causes mechanical fluidization of the particles and comminutes the particles, thus accelerating evaporation. The acceleration of the drying velocity reduces the adverse effect of heat or the heat burden on the product during the drying process.

In yet a further embodiment of the invention, it is contemplated that a process for obtaining enzymes from the Euphausia superba species of krill and other krill species is of interest. Euphasia superba ("E.s.") is a small crustacean from the Antarctic that contains numerous enzymes that are principally but not exclusively represented by proteases, amylases, chitinases, carboxymethy cellulases, lipases, etc. This enzymatic cocktail as a whole or in a partial purified form can be used for a number of industrial applications such as aquaculture and other general feed manufacturing and the further process of marine and other proteins. The inclusion rate of enzymes in the feed would vary depending on the target species and the composition of the diet. For example, these krill enzyme cocktails can be added to aquaculture diets containing large quantities of vegetable proteins which would otherwise be difficult to process by the animals and which could also be part of specialty diets for larval stages of shrimp and starter diets for salmonids where higher survival rates are

required. Krill enzymes may also conveniently be used to produce protein hydrolysates from other proteins to incorporate into diets or to improve the functional properties of these diets. Other potential applications would include the production of flavors, protein and peptide extraction from marine by products, protein and pigment recovery from shrimp and crab shell offal, the production of free amino acids and other benefits relating to the actions of these krill enzymes on biological materials.

Using the processes previously disclosed, it was desired to obtain enzymes from the previously autolysed krill preparations.

With reference to Figures 9 and 10, ultrafiltration membrane 303 was used with the krill hydrolysate 301 and with fresh krill 310. Since most of the krill-derived enzymes have molecular weights above 20,000 daltons, experiments were conducted to determine the most appropriate molecular weight cut-off ultrafiltration membrane to attempt a concentration of the aqueous phase enzyme-rich E.s. and E.p. extracts. It was revealed during experiments that total protease activity begins to become apparent in the filtrates at the 50,000 molecular weight cut off and up. On the other hand, trypsin-like activity is present in filtrates at 30,000 molecular weight cut off. It is therefore desirable to use a 10,000 dalton cut off membrane for filtration purposes.

In order to handle larger volumes of krill hydrolysate and to concentrate the enzyme extracts, a tangential flow filtration ("TFF") cartridge 302 was used using a 10,000 dalton molecular weight cut-off. One such cartridge commercially available is a Millipore Preparative Scale Tangential Flow Filtration cartridge. Such cartridges are intended to handle volumes from 100 ml to 100 liters, although it is readily possible to scale up such techniques to handle larger volumes, if desired. Before subjecting the krill extracts to TFF, they were centrifuged at 4000-10000 x G for twenty(20) minutes in a Beckman centrifuge 300 to clarify from solids and eliminate part of the fat. Rather than centrifugation, this clarification step can be replaced by prefiltration 303 with a larger pore filter. After centrifugation, the aqueous phase 305 containing the enzymes of interest was recover and stored at 4 deg. C. The autolysed krill extracts were run through a one square foot TFF cartridge 302 using a Hoechst displacement pump 304. The initial extract volume was about two(2) liters and was brought down to approximately 250-300 ml after four(4) to five(5) hours of operation (below 20 psi of pressure). It was revealed that enzymatic activity recovery differed significantly between the two samples (i.e., autolysed and freshly squeezed extracts).

By measuring the trpysin-like activity ("TLA"), it was found that the recovery of krill enzymes from the fresh frozen krill 310 was relatively smaller than the recovery from hydrolysed krill 301. However, the total units recovered after ultrafiltration were higher for fresh frozen

extracts. Accordingly, TLA could be recovered from either freshly squeezed or autolysed krill preparations. Since there was little or no enzymatic activity associated with the filtrate, it is apparent the proteins of interest were not leaching out through the membrane filter.

The resultant enzyme cocktail obtained by the ultrafiltration technique from both the hydrolysed and fresh krill 301, 310, respectively, could then be coupled with freeze drying 313 which would reduce the amount of water associated with the enzymes significantly which would reduce transportation costs. Subsequent processing could then be performed on the enzyme cocktails to further increase the purity and quality of the enzymes present.

Yet a further aspect of the invention relates to a method for removal of protein from crustacean wastes using the aforementioned krill enzyme extracts. With reference to Figure 12, a quantity of crustacean wastes 400, 401 is ground to dried particulate size by grinders 402, 403, respectively, with a portion of water added to facilitate this grinding. Various of a plurality of grinders which will accomplish this include a piranha pump, a macerator or cerator, all of which are known. Acid stabilized shell waste 400 is then de-watered through a de-watering system 404, many of which are readily known to be available, such as the Vincent screw press, wine presses or centrifuges. Non acid stabilized shell waste 401 has no need to be de-watered prior to the addition of enzymes. Water is conveniently added to the de-watered acid stabilized shell

waste 410 to facilitate enzymatic reaction. The shell waste 410 is transferred to a digesting tank 411 where an amount of krill enzyme cocktail 412 is added. The enzyme cocktail can be in either a concentrated or non-concentrated form consistent with squeezed extractions from the whole animal as has been described. The squeezed fractions are in the range of 25-75% of the whole animal depending on the amount of enzyme desired and the need to keep the enzyme with the krill to facilitate autolysis. The shell enzyme mixture is subjected to digestion in the digester 411 for a time period in the range of one(1) to forty-eight(48) hrs at a temperature in the range of 0 to 70 Celsius with an optimum temperature being approximately 45 deg. Celsius. Following the digestive process, the mixture is subjected to water removal 413 as has been described. Two fractions will result, a protein rich enzymatically active portion 414 and a shell material portion 415 high in chitin and low in protein. The liquid high protein portion 414 is low temperature dried or co-dried as earlier described or acid stablized. The shell portion 415 can then be further processed by the addition of more enzyme cocktail to facilitate further protein removal in further steps or can be subjected to traditional deproteinization or demineralization techniques as illustrated generally at 420. The extent of de-mineralization necessary can be greatly reduced by the storing of the shell waste for long periods of time while stabilized with acids, preferably formic.

In experiments which have been conducted to date, 70kg of water was added to 210 kg of mechanically peeled

shrimp shell wastes. The slurry was subjected to grinding with a piranha pump to a suitable particle size. 60kg of this slurry was combined with 15 kg of Euphasia superba juice obtained by squeezing whole krill through a screw press 315 (Figure 11) to obtain 50% by weight of the animal in a liquid form. The shell juice mixture was subjected to digestion for six(6) hours at 45 deg. C. The mixture was dewatered by pressing through a Vincent screw press to obtain the protein rich enzymatically active portion and the shell ash portion 415, as described. The shell portion was approximately 7.5% by weight and the liquid portion made up the remainder. The liquid portion was acid stabilized with 3% by weight formic acid. The shell portion was washed and dried.

In a second trial conducted to establish the efficacy of using krill enzymes for the removal of protein from shrimp shell wastes and the benefit of reincorporating the superba squeezed solids, 26 kg of squeezed superba juice, obtained through the procedures described, was incubated with 10 kg water and 70 kg of ground shrimp shell for six(6) hours at 45 deg C. Samples were taken every hour and squeezed through a screw press. After six(6) hours, 14 kg of squeezed superba solids compising the remainder of the whole animal after enzyme liquid removal were added into the mixture and hydrolyzed for an additional one and one-half (1.5) hours. The remaining slurry was squeezed and the separate fractions were frozen.

While specific embodiments of the invention have been described, such descriptions should be taken as illustrative of the invention only and not as limiting its scope as defined in accordance with the accompanying claims.

WE CLAIM:

1. Method of producing a feed product comprising the steps of adding a predetermined quantity of krill hydrolysate to a quantity of liquid marine protein and a quantity of dry carrier to produce a mixture and co-drying said mixture to obtain an end product.

2. Method as in claim 1 wherein said mixture is mixed prior to co-drying said mixture.

3. Method as in claim 2 wherein said mixture is subjected to chemical and/or enzymatic reaction for a predetermined time period prior to co-drying said mixture.

4. Method as in claim 3 wherein said mixture is co-dryed in a dryer or other dehydrator.

5. Method as in claim 4 wherein said mixture is ground prior to being subject to said chemical reaction.

6. Method as in claim 5 wherein said mixture is cooled following drying of said mixture in said dryer.

7. Method as in claim 6 wherein said dry carrier may be one or a combination of dry marine protein

meals, dried krill products, dried vegetable and dried fish product.

8. Method as in claim 7 wherein said liquid marine protein may be liquid fish product.

9. Method as in claim 8 wherein temperature sensitive enzyme active or other bioactive dry products are added or readded to said mixture following said drying of said mixture.

10. Method as in claim 9 and further comprising mixing said temperature sensitive enzyme active products with said mixture.

11. Method as in claim 1 wherein said mixture is co-dryed in a dryer or other dehydrator.

12. Method as in claim 11 wherein said dryer includes an agitator to agitate said mixture entering said dryer.

13. Method as in claim 12 wherein said dryer further includes a drying tower downstream from said agitator and a heat source to provide heat to said tower.

14. Method as in claim 13 and further comprising a classifier downstream of said tower for separating said mixture, said mixture comprising relatively

lighter and relatively heavier particles, said classifier separating said lighter from said heavier particles.

15. Method as in claim 14 wherein said relatively heavier particles are returned to said agitator.

16. Method as in claim 14 and further comprising a cyclone downstream from said classifier.

17. Method as in claim 16 wherein said cyclone removes further moisture from said relatively lighter particles.

18. Method as in claim 17 wherein said relatively lighter particles are separated into relatively smaller and relatively larger particles.

19. Method as in claim 18 wherein said relatively larger particles are returned to said agitator.

20. A feed product or additive produced by the method as in any one of claims 1 to 19.

21. Co-drying apparatus for drying a mixture of krill hydrolysate, liquid marine product and a dry carrier comprising a dryer for agitating, heating and separating particles of said mixture.

22. Co-drying apparatus as in claim 21 and further comprising a mixer for blending said mixture prior to said mixture entering said dryer.

23. Co-drying apparatus as in claim 22 and further comprising a reactor cell for treating said mixture prior to said mixture entering said dryer.

24. Co-drying apparatus as in claim 23 and further comprising a grinder for grinding said mixture prior to said mixture entering said reactor cell.

25. Co-drying apparatus as in claim 24 wherein said dryer produces a product.

26. Co-drying apparatus as in claim 25 and further comprising a mixer for mixing said product following said product exiting said dryer.

27. Co-drying apparatus as in claim 21 wherein said dryer comprises a source of warm air, an agitator for agitating said mixture following entry of said mixture into said dryer, a tower to expose said mixture to said warm air, a first classifier to separate the relatively lighter particles of said mixture from the relatively heavier particles of said mixture, a cyclone for drying said relatively lighter particles separated from said relatively heavier particles, and a second classifier to separate relatively lighter particles and relatively heavier

particles constituting said relatively lighter particles in said cyclone.

28. Co-dryer as in claim 27 and further comrising a fan to move said warm air within said dryer.

29. Method of obtaining an enzyme extract from a liquid krill hydrolysate comprising the steps of subjecting said hydrolysate to centrifugation to obtain a clarified liquid and further subjecting said clarified liquid to ultrafiltration using a membrane with a capacity to retain said enzymes having a molecular weight greater than 10,000 daltons.

30. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 29 and further comprising the step of storing said clarified liquid at a reduced temperature for a predetermined time period.

31. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 30 wherein said ultrafiltration is achieved using a tangential flow filtration system.

32. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 31 wherein said enzyme extract obtained from said ultrafiltration is freeze dried.

33. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 32 wherein said krill is Euphausia superba.

34. Method of obtaining an enzyme extract from a liquid krill hydrolysate as in claim 32 wherein said krill is Euphausia pacifica.

35. Method of obtaining an enzyme extract from fresh krill comprising the steps of squeezing said krill to obtain an aqueous extract and subjecting said aqueous extract to ultrafiltration with a membrane adapted to retain enzymes having molecular weights above 10,000 daltons.

36. Method of obtaining an enzyme extract from fresh krill as in claim 35 wherein said ultrafiltration is achieved using a tangential flow filtration system allowing enzymes to retain which have molecular weights above 10,000 daltons.

37. Method of obtaining an enzyme extract from fresh krill as in claim 36 and further including the step of centrifuging said aqueous extract prior to subjecting said extract to ultrafiltration.

38. Method of obtaining an enzyme extract from fresh krill as in claim 37 and further comprising the step of storing said aqueous extract at a reduced temperature following said centrifuging.

39. Method of obtaining an enzyme extract from fresh krill as in claim 38 wherein said reduced temperature is approximately 4 degrees Celsius.

40. Method of obtaining an enzyme extract from fresh krill as in claim 39 and further comprising subjecting said enzyme extract obtained from said ultrafiltration to low temperature drying.

41. Product produced by the method as in any one of claims 29 to 39.

42. Method for removal of protein from non-stabilized crustacean shell wastes, comprising grinding said crustacean wastes and water to a relatively small particulate size, transferring said small particulate size product to a digester, adding a predetermined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively enzymatically active and relatively high in protein and a second portion of shell material relatively high in chitin and low in protein.

43. Method for removal of protein from acid stabilized shell wastes comprising grinding said crustacean wastes to a described small particulate size, transferring desired size shell wastes to a digester, adding a predetemined quantity of krill enzymes to said digester, subjecting said mixture to digestion for a predetermined

time period at a predetermined temperature, dewatering said digested product to obtain a first portion being relatively enzymatically active and relatively high in protein and a second portion of shell ash relatively high in chitin and low in protein.

44. Method as in claim 42 and further comprising drying said liquid portion by means of low temperature drying to preserve the enzymatic activity.

45. Method as in claim 44 wherein said drying is by way of a flash drier.

46. Method as in claim 45 wherein said drying is by way of a fluidized bed drier.

47. Method as in claim 42 and further comprising adding krill enzyme material to said shell material portion.

48. Method as in claim 43 and further comprising adding krill enzyme material to said shell material portion.

49. Method as in claim 42 wherein said product is subject to digestion between approximately 0-70 degrees Celsius and for times between 30 minutes and several hours.

50. Method as in claim 43 wherein said product is subject to digestion between approximately 0-70 degrees Celsius.

51. Method of producing a concentrated krill hydrolysate comprising the steps of harvesting, digesting and evaporating the krill hydrolysate to provide a partial hydrolysis for a predetermined time and temperature so as to enhance the nutrient characteristics of said krill.

52. Method of producting a dry krill premix or feedstuff comprising the steps of producing a predetermined amount of concentrated krill hydrolysate, producing a predetermined amount of dry matter and mixing said concentrated krill hydrolysate and said dry carrier matter and co-drying said mixture.

54. Method as in claim 52 wherein the dry matter is selectted from the group of vegetable and/or vegetable and/or animal protein meals and by products.

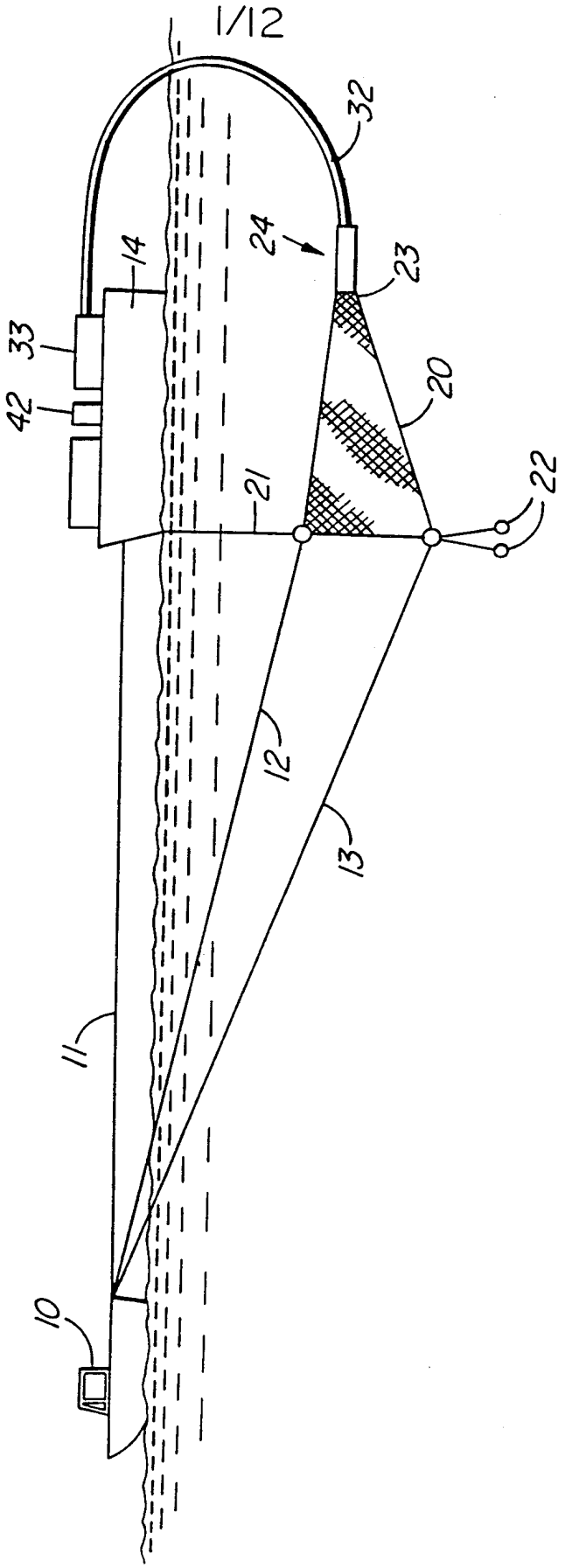


FIG. IA

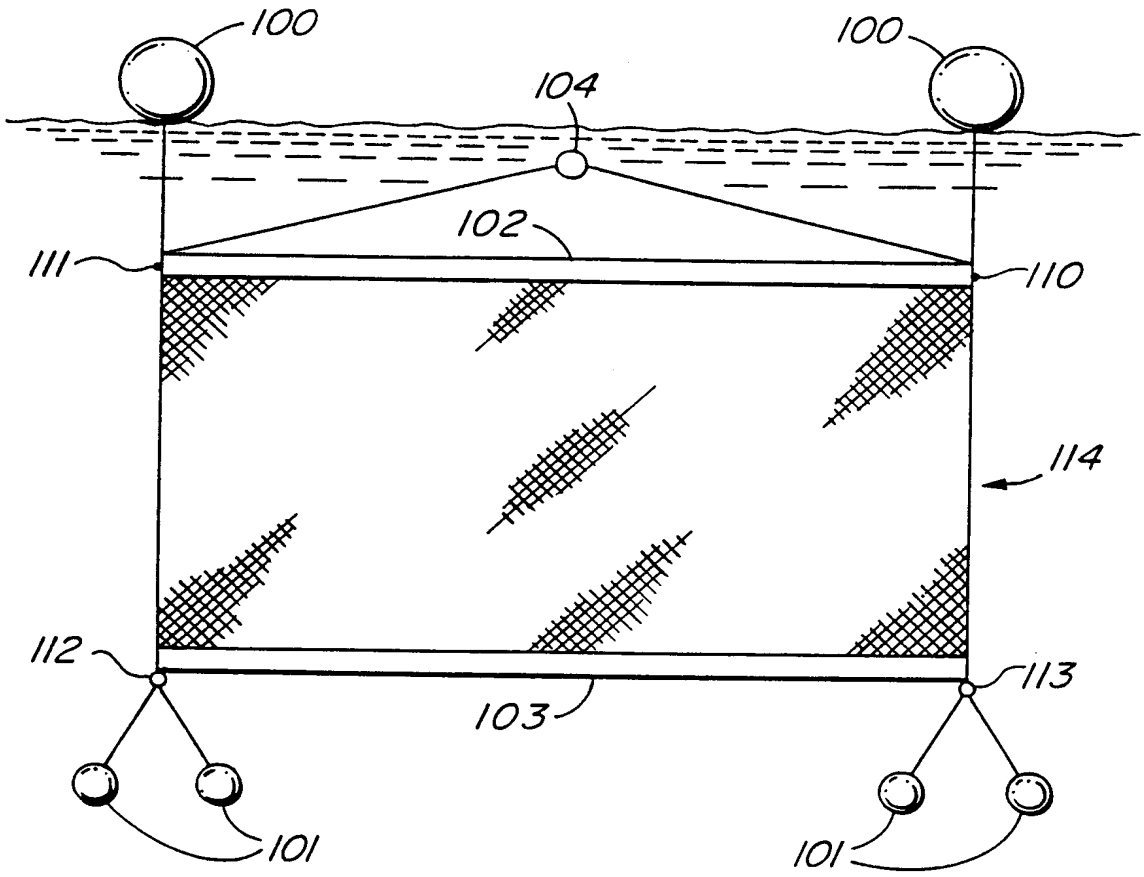


FIG. 1B

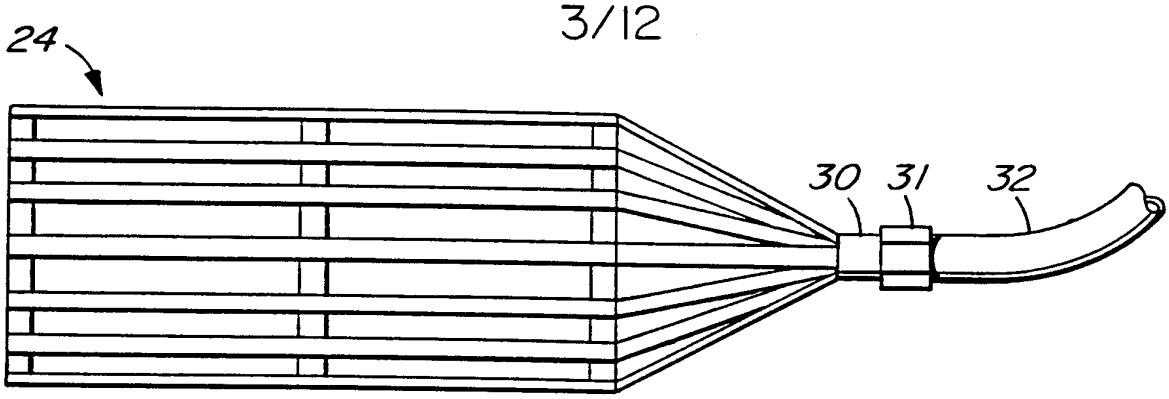


FIG. 2A

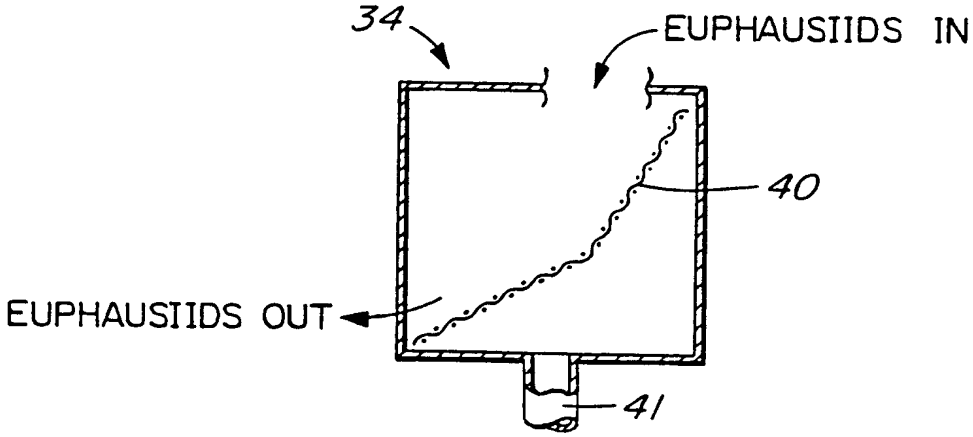


FIG. 2B

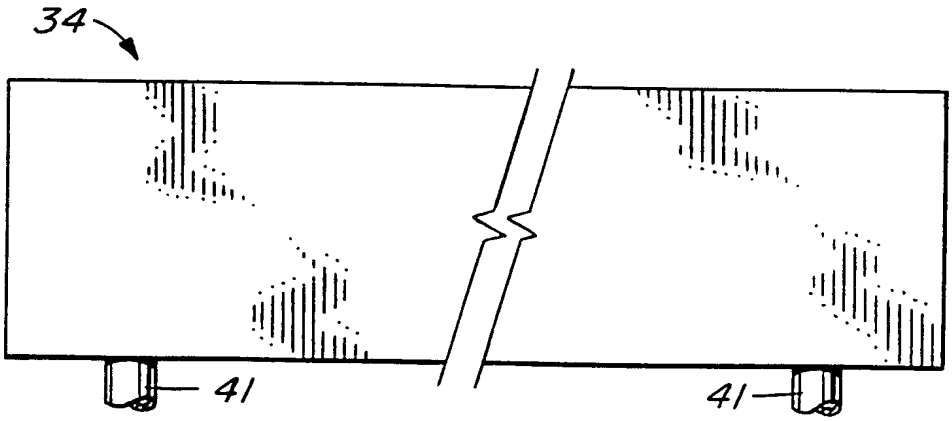


FIG. 2C

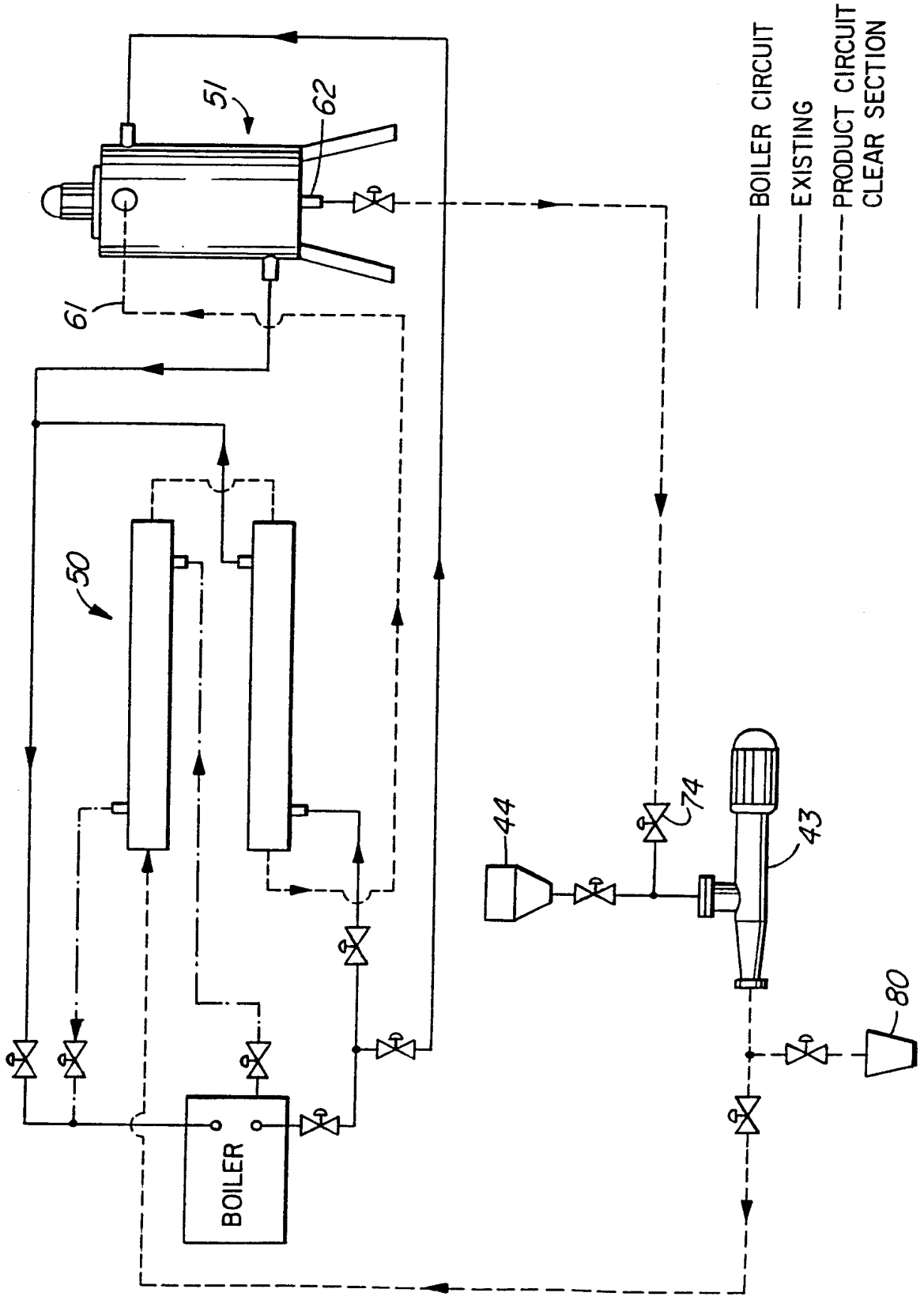


FIG. 3

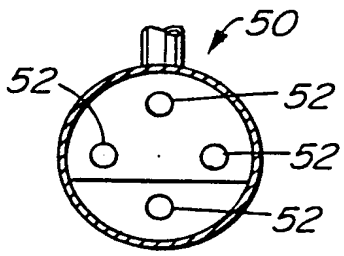


FIG. 4A

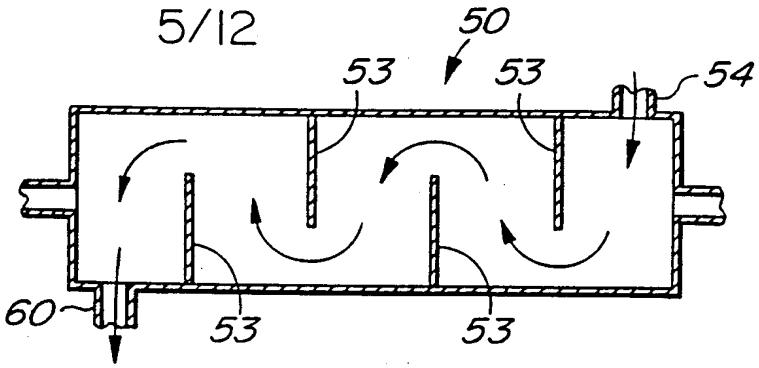


FIG. 4B

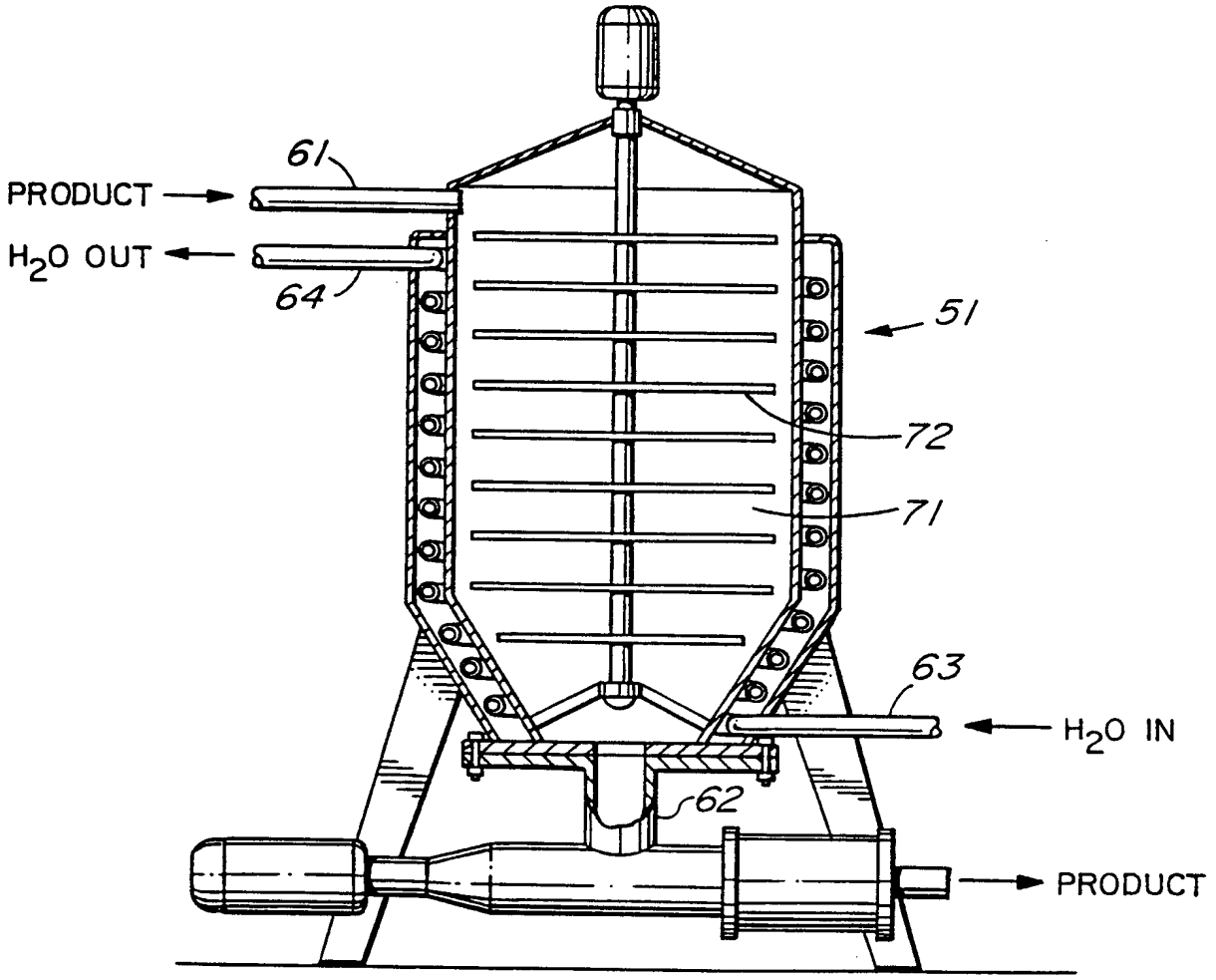


FIG. 5

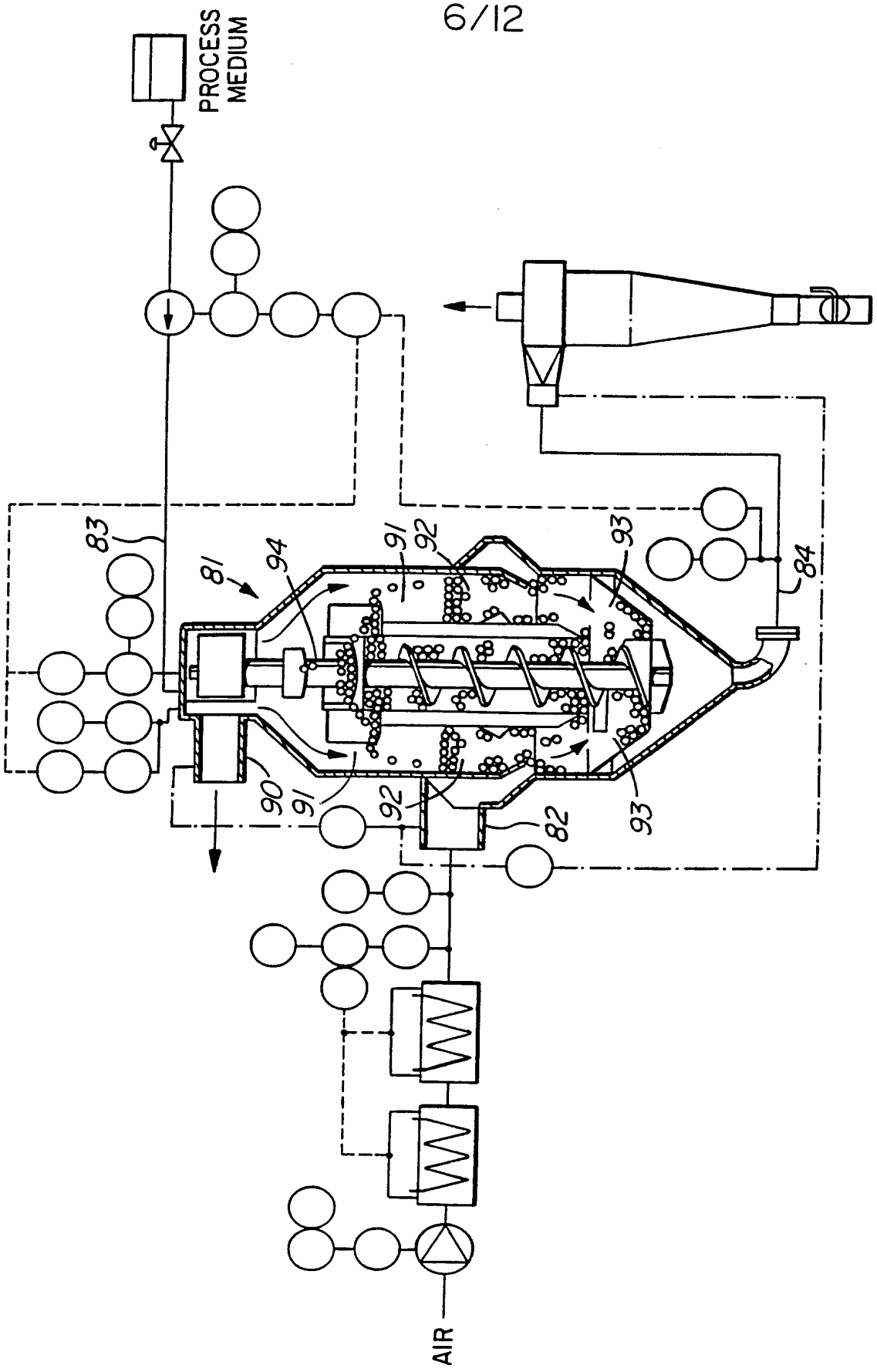


FIG. 6

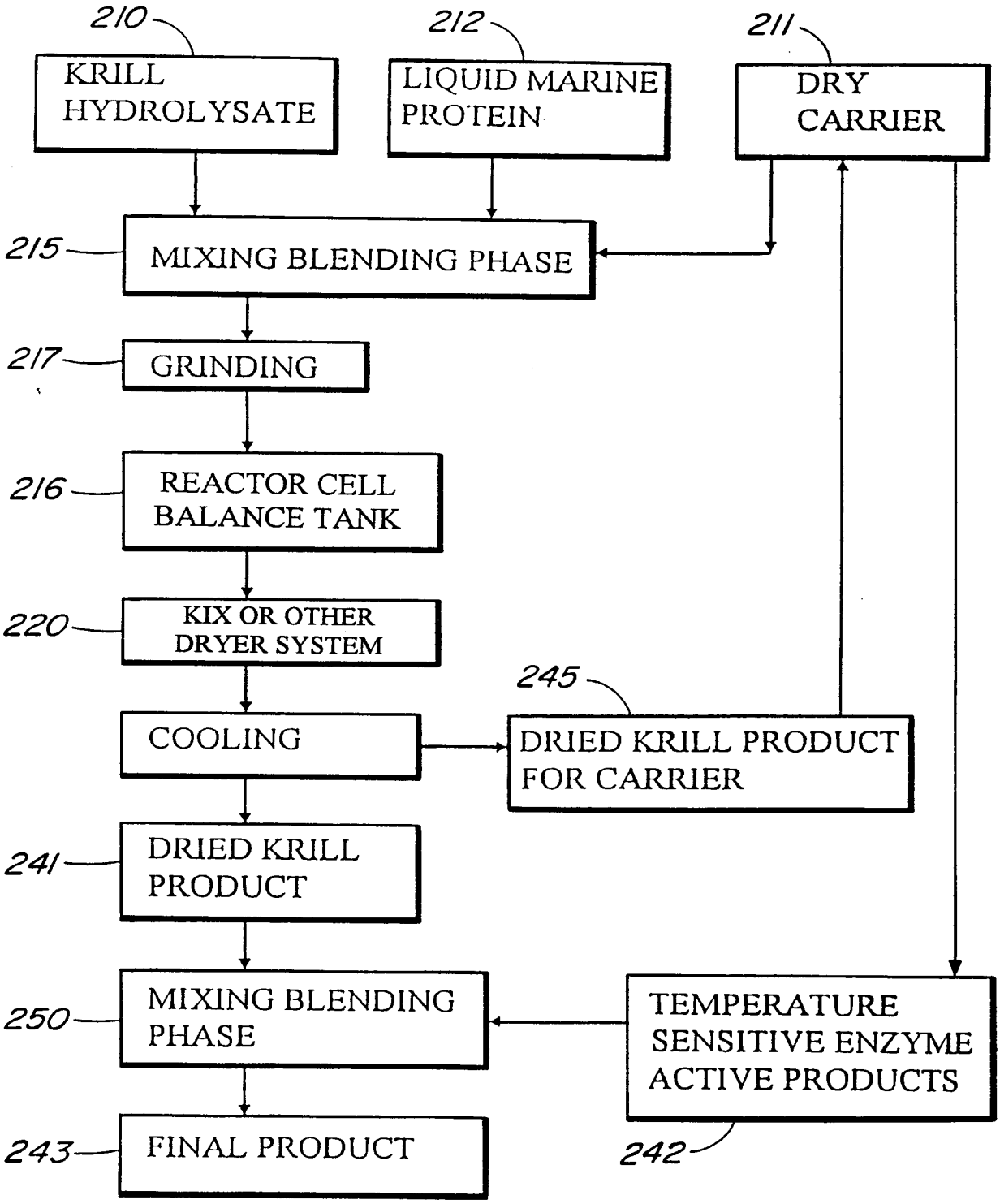


FIG. 7

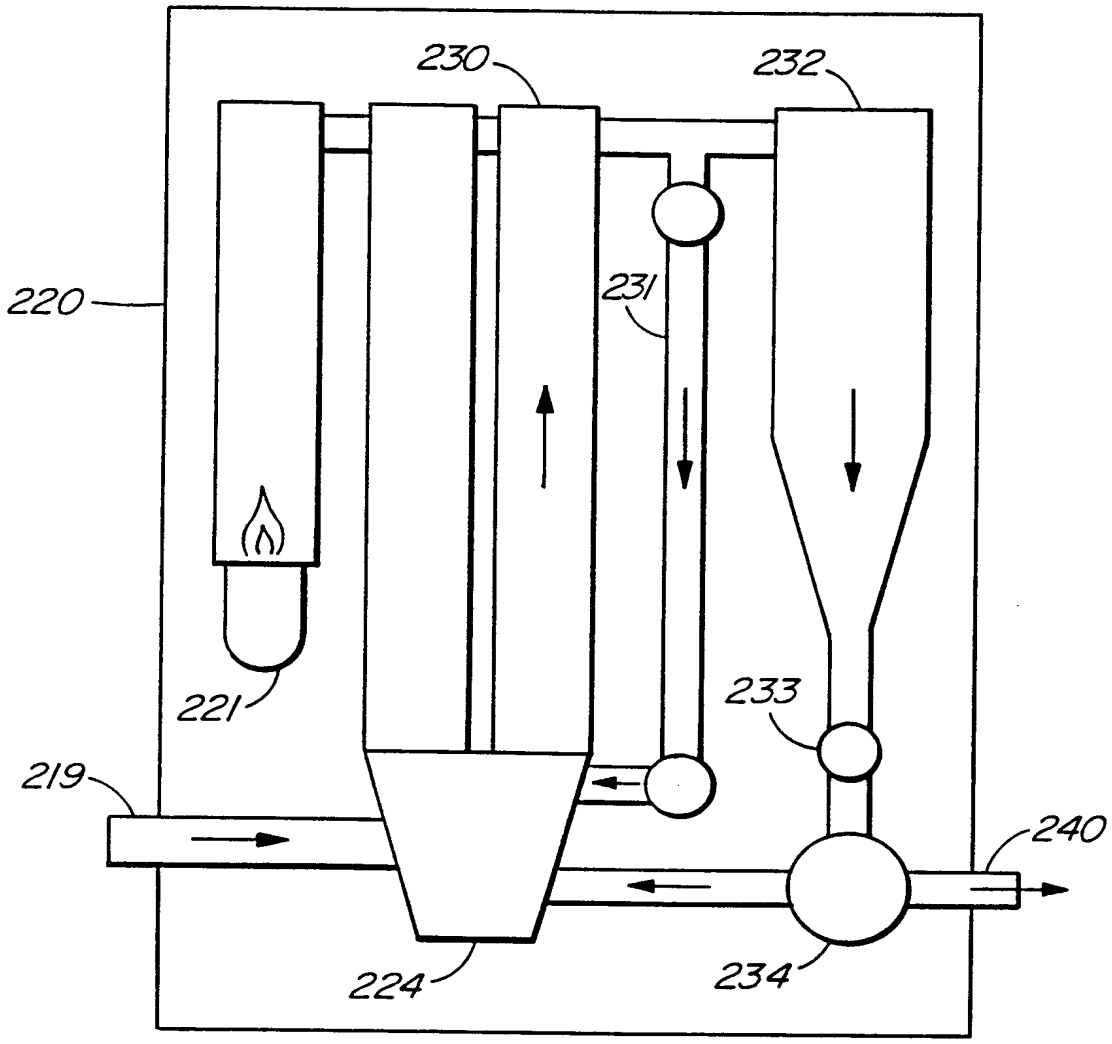


FIG. 8

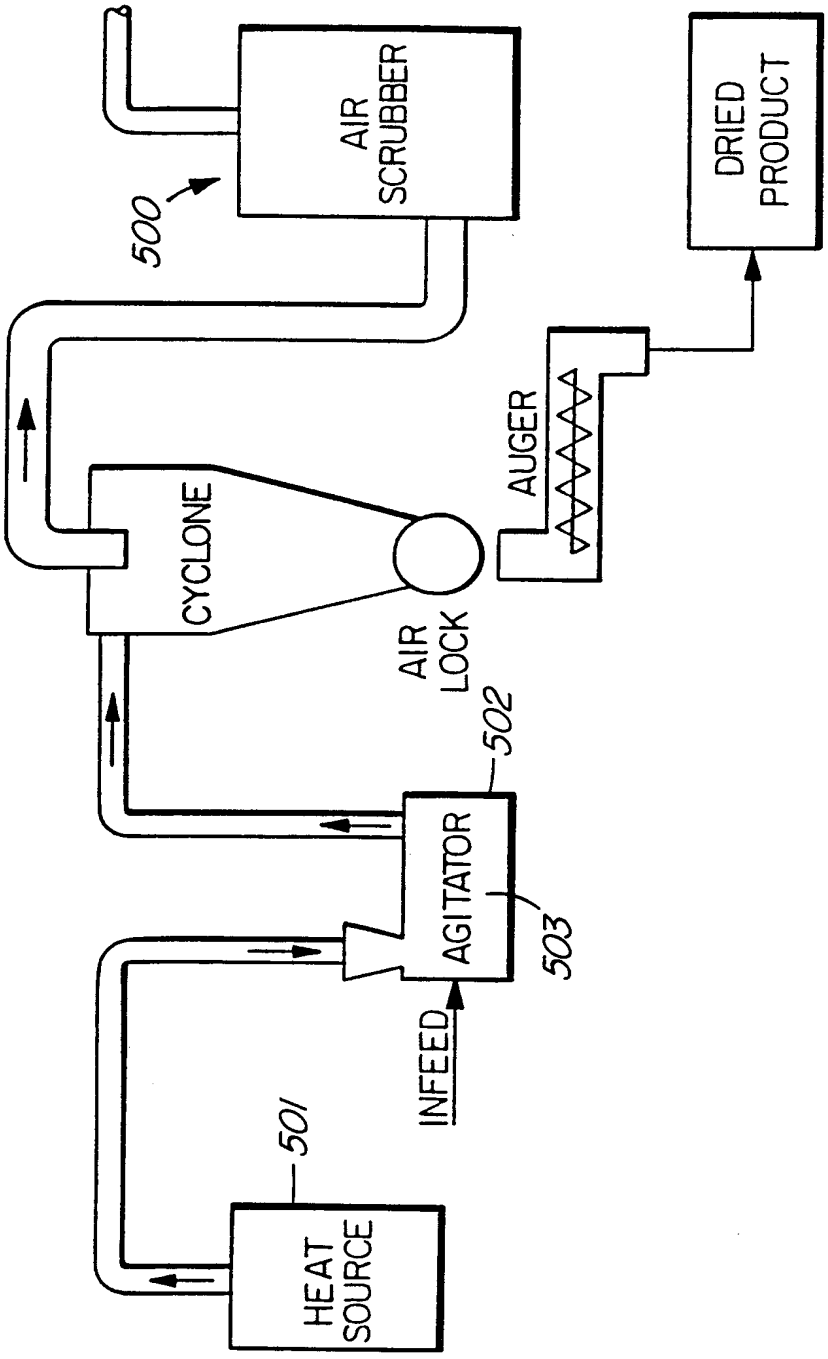


FIG. 9

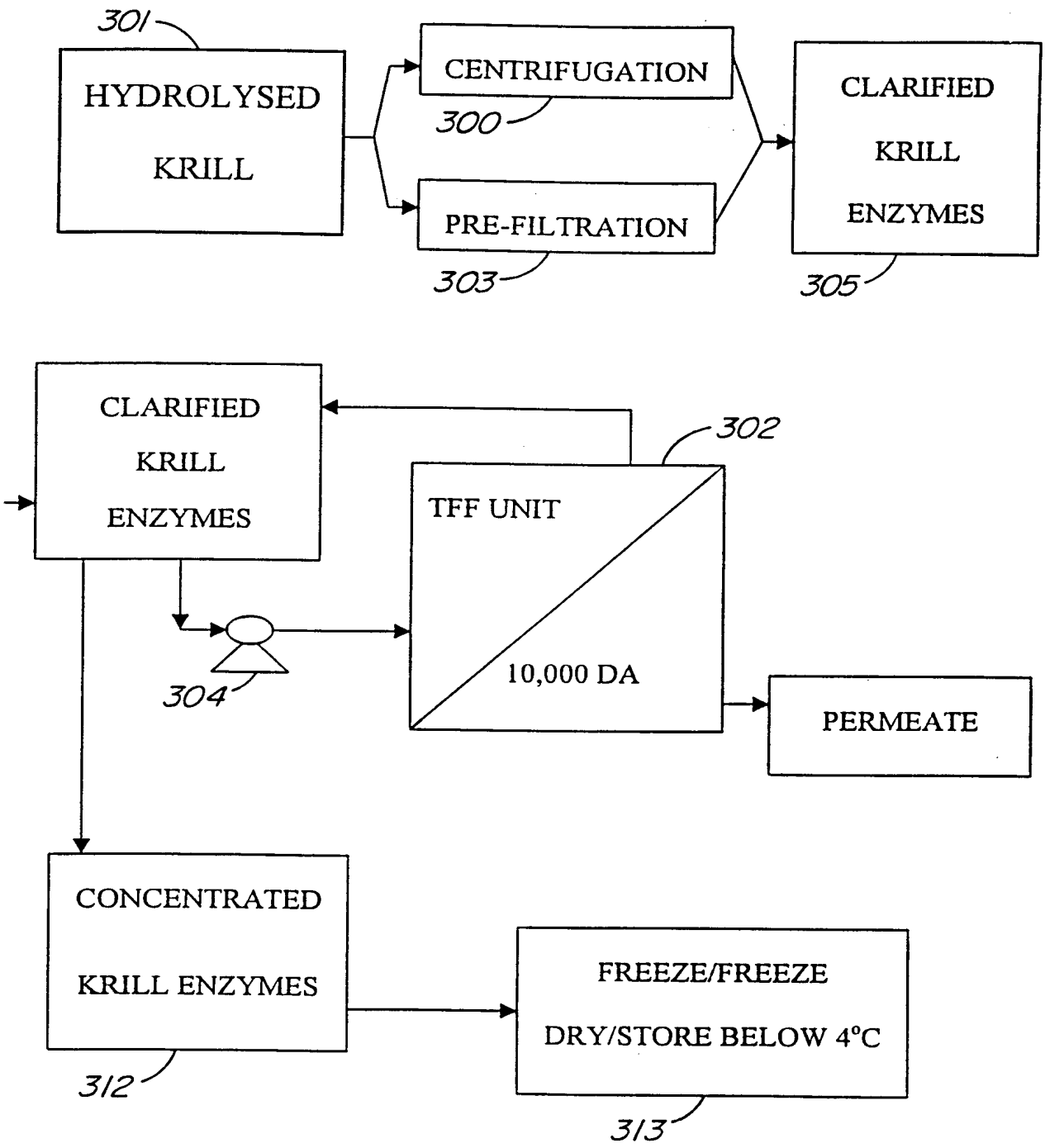


FIG. 10

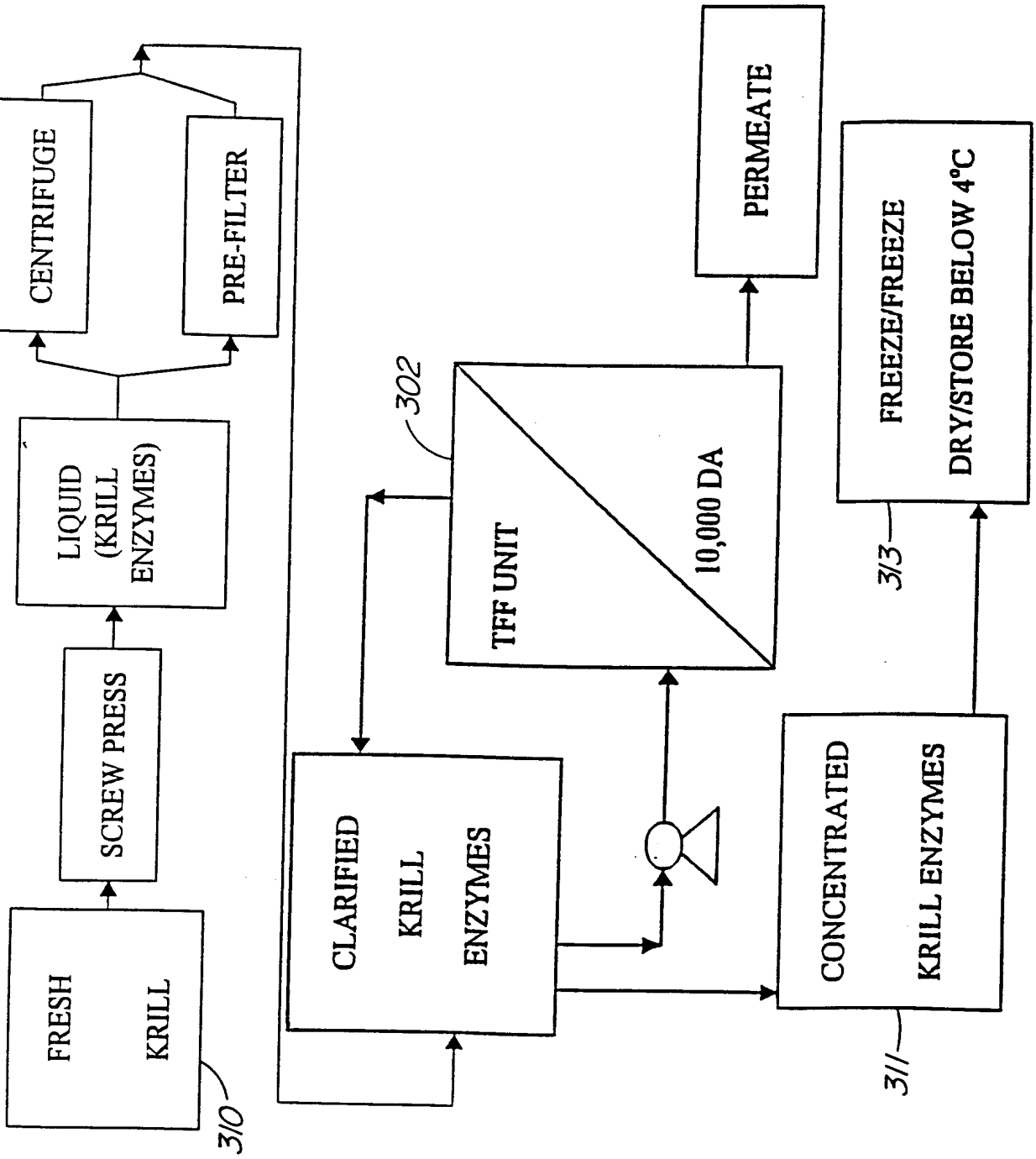


FIG. 11

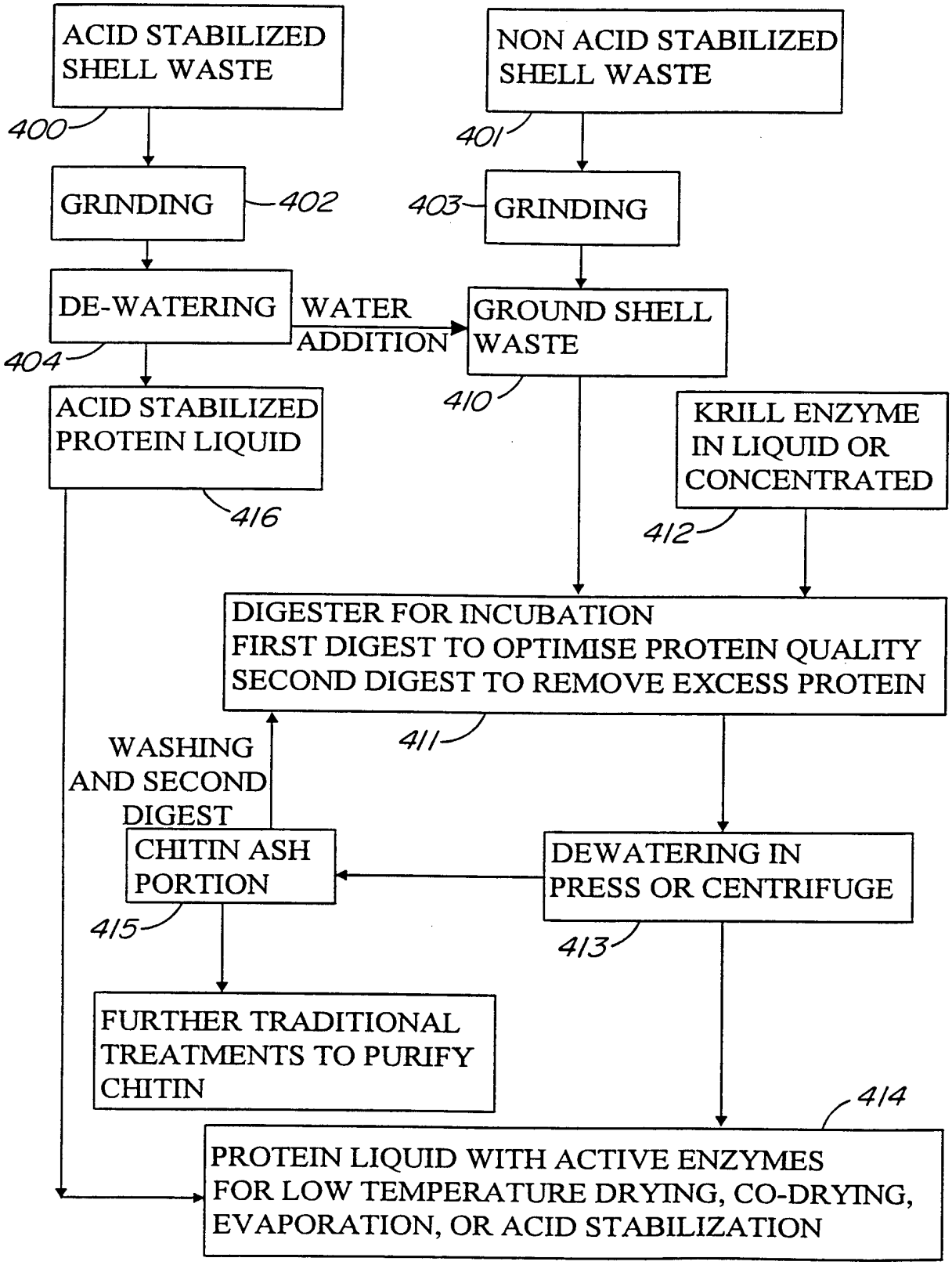


FIG. 12

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/CA 99/00075

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23K1/10 A23K1/16 A23K1/18 A23J1/04 A23N17/00
C12N9/00

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 6 A23K A23J C12N

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 34498 A (BIOZYME SYSTEMS INC ;SAXBY DAVID J (CA); SPENCE JOHN A (CA); ALOIS) 13 August 1998 see the whole document ---	1-54
X	DATABASE WPI Section Ch, Week 8447 Derwent Publications Ltd., London, GB; Class C03, AN 84-293719 XP002070859 & SU 1 084 005 A (N BASSIN FISHING IND) , 7 April 1984 see abstract ---	1,20,52
X	WO 95 22893 A (SPECIALTY MARINE FEEDS INC) 31 August 1995 see page 15, line 19 - page 17, line 19 see claims 11-28,30-46 --- -/--	51

[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
9 June 1999	29/06/1999

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	Dekeirel, M

1

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

INTERNATIONAL SEARCH REPORT

Inte .ional Application No
PCT/CA 99/00075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 89 01031 A (PHARMACIA AB) 9 February 1989 see page 5, paragraph 2 see page 7, paragraph 1 see examples 1-3 see claim 1	29-34,41
A	---	35-40
X	WO 89 10960 A (PHARMACIA AB) 16 November 1989 see page 8, last paragraph see page 11, paragraph 3 - paragraph 5 see page 14, paragraph 3 see page 27, paragraph 4 see claims 1,7,17	42,43
X	--- WO 90 05026 A (AKT CONSULTANTS) 17 May 1990 see figure 1	21,27
A	--- PATENT ABSTRACTS OF JAPAN vol. 017, no. 315 (C-1071), 16 June 1993 & JP 05 030923 A (RIKEN VITAMIN CO LTD), 9 February 1993 see abstract	1,20,52
A	--- DATABASE WPI Section Ch, Week 9602 Derwent Publications Ltd., London, GB; Class D13, AN 96-018544 XP002070860 & RU 2 034 492 C (TROITSKII B N) , 10 May 1995 see abstract	1,20,52

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/CA 99/00075

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9834498 A	13-08-1998	CA 2197137 A	07-08-1998
		AU 5976698 A	26-08-1998
WO 9522893 A	31-08-1995	CA 2134515 A	28-04-1996
		AU 1802695 A	11-09-1995
		CN 1146709 A	02-04-1997
		EP 0758842 A	26-02-1997
		FI 963343 A	27-08-1996
		JP 10500004 T	06-01-1998
WO 8901031 A	09-02-1989	AT 124085 T	15-07-1995
		AU 2259788 A	01-03-1989
		DE 3854050 D	27-07-1995
		EP 0393035 A	24-10-1990
		JP 2504465 T	20-12-1990
WO 8910960 A	16-11-1989	NONE	
WO 9005026 A	17-05-1990	AU 4518389 A	28-05-1990
		CA 2002193 A	03-05-1990
		DK 158990 A	22-08-1990
		EP 0403608 A	27-12-1990
		GR 89100729 A	31-12-1990
		PT 92192 A,B	31-05-1990
		US 5105560 A	21-04-1992

(12) DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

(19) Organisation Mondiale de la Propriété Intellectuelle
Bureau international

PCT

(43) Date de la publication internationale
26 octobre 2006 (26.10.2006)

(10) Numéro de publication internationale
WO 2006/111633 A3

(51) Classification internationale des brevets :
A61K 35/60 (2006.01) *A61P 17/00* (2006.01)

(21) Numéro de la demande internationale :
PCT/FR2006/000792

(22) Date de dépôt international : 11 avril 2006 (11.04.2006)

(25) Langue de dépôt : français

(26) Langue de publication : français

(30) Données relatives à la priorité :
0503827 18 avril 2005 (18.04.2005) FR
0506496 27 juin 2005 (27.06.2005) FR

(71) Déposant *(pour tous les États désignés sauf US)* : **SC DICOPHAR** [FR/FR]; 10 Allée de Corrèze, F-31770 Colomiers (FR).

(72) Inventeur; et

(75) Inventeur/Déposant *(pour US seulement)* : **DUPONT, Paul** [FR/FR]; 10 Allée de Corrèze, F-31770 Colomiers (FR).

(74) Mandataire : **MORELLE, Guy**; Cabinet Morelle & Bardou, SC, Parc Technologique du Canal, BP 72253, F-31522 Ramonville Saint Agne Cedex (FR).

(81) États désignés *(sauf indication contraire, pour tout titre de protection nationale disponible)* : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) États désignés *(sauf indication contraire, pour tout titre de protection régionale disponible)* : ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), européen (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Déclarations en vertu de la règle 4.17 :
— *relative au droit du déposant de revendiquer la priorité de la demande antérieure (règle 4.17.iii))*
— *relative à la qualité d'inventeur (règle 4.17.iv))*

Publiée :
— *avec rapport de recherche internationale*

(88) Date de publication du rapport de recherche internationale: 5 avril 2007

En ce qui concerne les codes à deux lettres et autres abréviations, se référer aux "Notes explicatives relatives aux codes et abréviations" figurant au début de chaque numéro ordinaire de la Gazette du PCT.

(54) **Title:** USE OF LECITHIN AS A MEDICAMENT FOR TREATING PSORIASIS

(54) **Titre :** UTILISATION DE LA LECITHINE COMME MEDICAMENT DANS LE TRAITEMENT DU PSORIASIS

(57) **Abstract:** The invention relates to the use of lecithin or of an extract rich in lecithin for preparing a pharmaceutical composition that is useful in the prevention and therapeutic treatment of new or previous dermatites, particularly psoriasis. The invention also relates to therapeutic compositions containing lecithin or an extract rich in lecithin. According to one advantageous characteristic, the phospholipids that compose the lecithin are esterified by omega-3 polyunsaturated fatty acids, in particular, by docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) or by an alkyl glycerol. The lecithin can be a marine lecithin , i.e. an extract of a marine organism selected among fish, shrimp, krill, zooplankton, algae, phytoplankton or a mixture thereof, of which the advantage resides in the fact that its phospholipids, particularly the phosphatidylcholine, are naturally esterified by omega-3 fatty acids, and essentially by DHA and EPA.

(57) **Abrégé :** La présente invention se rapporte à l'emploi de la lécithine ou d'un extrait riche en lécithine pour la préparation d'une composition pharmaceutique utile dans la prévention et le traitement thérapeutique des dermatoses récentes ou anciennes, notamment du psoriasis. Les compositions thérapeutiques comprenant de la lécithine ou d'un extrait riche en lécithine sont également objet de la présente invention. Selon une caractéristique avantageuse de la présente invention, les phospholipides composant la lécithine sont estérifiés par des acides gras polyinsarurés du type oméga3, en particulier par l'acide docosahexanoïque (DHA), l'acide eicosapentanoïque (EPA), l'acide docosapentanoïque (DPA) ou par un alkyl-glycérol. La lécithine peut être une "lécithine marine" c'est-à-dire qu'elle est extraite d'un organisme marin choisi parmi les poissons, les crevettes, le krill, le zooplancton, les algues, le phytoplancton ou d'un mélange de ceux-ci, dont l'avantage réside dans le fait que ses phospholipides, notamment la phosphatidylcholine, sont naturellement estérifiés par des acides gras de type oméga3, et essentiellement par le DHA et l'EPA.

WO 2006/111633 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/FR2006/000792

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/60 A61P17/00

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)
A61K A61P

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, FSTA, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/42348 A (CRANDALL, WILSON, TRAFTON) 1 October 1998 (1998-10-01) page 3, lines 26-34 page 5, lines 9,10 page 6, lines 7-27	1-4,11, 12
X	WO 2005/002591 A (HASSANIN, FOUAD, ABDELAZIZ, AHMED) 13 January 2005 (2005-01-13) page 1, lines 13-25	1-3,11
X	US 2002/012648 A1 (ORTHOEFER FRANK T) 31 January 2002 (2002-01-31) paragraph [0024]; claims 1-3,10; table 1	1-4,6, 11,12,14

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2006	05/10/2006

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	ESCOLAR BLASCO, P

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.
A	ESCOBAR S O ET AL: "TOPICAL FISH OIL IN PSORIASIS-A CONTROLLED AND BLIND STUDY" CLINICAL AND EXPERIMENTAL DERMATOLOGY, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 17, no. 3, 1992, pages 159-162, XP009030105 ISSN: 0307-6938 abstract page 161, left-hand column, paragraphs 3,4; figure 1	1-18
A	MAYSER P ET AL: "[omega]-3 Fatty acid-based lipid infusion in patients with chronic plaque psoriasis: Results of a double-blind, randomized, placebo-controlled, multicenter trial" JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY 1998 UNITED STATES, vol. 38, no. 4, 1998, pages 539-547, XP002360544 ISSN: 0190-9622 abstract; table 1	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/FR2006/000792

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9842348 A	01-10-1998	AU 2550397 A	20-10-1998
WO 2005002591 A	13-01-2005	NONE	
US 2002012648 A1	31-01-2002	US 6312703 B1	06-11-2001

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale n°
PCT/FR2006/000792

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
INV. A61K35/60 A61P17/00

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
A61K A61P

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si cela estréalisable, termes de recherche utilisés)
EPO-Internal, WPI Data, FSTA, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie*	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X	WO 98/42348 A (CRANDALL, WILSON, TRAFTON) 1 octobre 1998 (1998-10-01) page 3, ligne 26-34 page 5, ligne 9,10 page 6, ligne 7-27	1-4,11, 12
X	WO 2005/002591 A (HASSANIN, FOUAD, ABDELAZIZ, AHMED) 13 janvier 2005 (2005-01-13) page 1, ligne 13-25	1-3,11
X	US 2002/012648 A1 (ORTHOEFER FRANK T) 31 janvier 2002 (2002-01-31) alinéa [0024]; revendications 1-3,10; tableau 1	1-4,6, 11,12,14

-/--

[X] Voir la suite du cadre C pour la fin de la liste des documents

[X] Les documents de familles de brevets sont indiqués en annexe

* Catégories spéciales de documents cités:

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document antérieur, mais publié à la date de dépôt international ou après cette date

"L" document pouvant jeter un doute sur une revendication de priorité ou cité pour déterminer la date de publication d'une autre citation ou pour une raison spéciale (telle qu'indiquée)

"O" document se référant à une divulgation orale, à un usage, à une exposition ou tous autres moyens

"P" document publié avant la date de dépôt international, mais postérieurement à la date de priorité revendiquée

"T" document ultérieur publié après la date de dépôt international ou la date de priorité et n'appartenenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'inven tion revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive par rapport au document considéré isolément

"Y" document particulièrement pertinent; l'inven tion revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant évidente pour une personne du métier

"&" document qui fait partie de la même famille de brevets

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
27 septembre 2006	05/10/2006

Nom et adresse postale de l'administration chargée de la recherche internationale	Fonctionnaire autorisé
Office Européen des Brevets, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESCOLAR BLASCO, P

C(suite). DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie*	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
A	ESCOBAR S O ET AL: "TOPICAL FISH OIL IN PSORIASIS-A CONTROLLED AND BLIND STUDY" CLINICAL AND EXPERIMENTAL DERMATOLOGY, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 17, no. 3, 1992, pages 159-162, XP009030105 ISSN: 0307-6938 abrégé page 161, colonne de gauche, alinéas 3,4; figure 1 -----	1-18
A	MAYSER P ET AL: "[omega]-3 Fatty acid-based lipid infusion in patients with chronic plaque psoriasis: Results of a double-blind, randomized, placebo-controlled, multicenter trial" JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY 1998 UNITED STATES, vol. 38, no. 4, 1998, pages 539-547, XP002360544 ISSN: 0190-9622 abrégé; tableau 1 -----	1-18

RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

Demande internationale n°
PCT/FR2006/000792

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
WO 9842348 A	01-10-1998	AU 2550397 A	20-10-1998
WO 2005002591 A	13-01-2005	AUCUN	
US 2002012648 A1	31-01-2002	US 6312703 B1	06-11-2001

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 November 2007 (01.11.2007)

PCT

(10) International Publication Number
WO 2007/123424 A1

(51) International Patent Classification:
C11B 7/00 (2006.01) *A23L 1/48* (2006.01)
C11B 1/10 (2006.01)

(21) International Application Number:
PCT/NZ2007/000087

(22) International Filing Date: 20 April 2007 (20.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
546681 20 April 2006 (20.04.2006) NZ

(71) Applicants and
(72) Inventors: **CATCHPOLE, Owen John,** [NZ/NZ]; C/- Industrial Research Limited, Gracefield Research Centre, Gracefield Road, Lower Hutt (NZ). **TALLON, Stephen John,** [NZ/NZ]; C/- Industrial Research Limited, Gracefield Research Centre, Gracefield Road, Lower Hutt (NZ).

(74) Agents: **ADAMS, Matthew, D** et al.; A J Park, 6th Floor Huddart Parker Building, Post Office Square, P O Box 949, Wellington, 6015 (NZ).

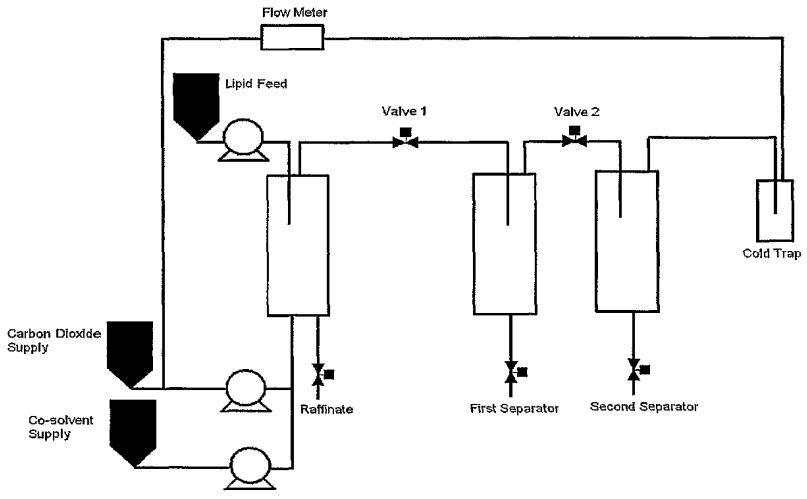
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

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.

(54) Title: PROCESS FOR SEPARATING LIPID MATERIALS



(57) Abstract: The present invention relates to processes for separating a feed material into soluble and insoluble components, by contacting a feed material and a solvent and subsequently separating the solvent containing the soluble components from the insoluble components, wherein the feed material comprises one or more of: at least 1% by mass phosphatidyl serine, at least 1% by mass sphingomyelin, at least 0.3 % by mass acylalkylphospholipids and/or plasmalogens, at least 0.5 % by mass aminoethylphosphonate and/or other phosphonolipids, at least 1% by mass cardiolipin, and at least 0.3% by mass gangliosides; and wherein the solvent comprises: supercritical or near-critical CO_2, and a co-solvent comprising one or more C_1-C_3 monohydric alcohols, and water, wherein the co-solvent makes up at least 10% by mass of the CO_2, and the water content of the co-solvent is 0 to 40 % by mass. The present invention also relates to processes for separating a feed material into soluble and insoluble components, comprising contacting a feed material and a first solvent and subsequently separating the first solvent containing the first soluble components from the first insoluble components, wherein the feed material comprises one or more of: at least 1 % by mass phosphatidyl serine, at least 1% by mass sphingomyelin, at least 0.3 % by mass acylalkylphospholipids and/or plasmalogens, at least 0.5 % by mass aminoethylphosphonate and/or other phosphonolipids, at least 1% by mass cardiolipin, or at least 0.3% by mass gangliosides; and wherein the first solvent comprises supercritical or near-critical CO_2. The process then provides contacting the first insoluble components with a second solvent and subsequently separating the second solvent containing the second soluble components from the second insoluble components, wherein the second solvent comprises supercritical or near- critical CO_2, and a co-solvent comprising one or more C_1-C_3 monohydric alcohols, and water, wherein the co-solvent makes up at least 10% by mass of the CO_2, and the water content of the co-solvent is 0 to 40% by mass.

WO 2007/123424 A1

PRODUCT AND PROCESS

FIELD OF INVENTION

This invention relates to a separation process. More particularly it relates to a process for separating lipid materials containing phospholipids and/or glycolipids, including for example phosphatidyl serine, gangliosides, cardiolipin, sphingomyelin, plasmalogens, alkylacylphospholipids, phosphonolipids, cerebrosides or a combination thereof.

BACKGROUND

Phospholipids are a major component of all biological membranes, and include phosphoglycerides (phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), cardiolipin (CL), phosphatidyl serine (PS)), plasmalogens (PL), phosphonolipids (PP), alkylacylphospholipids (ALP); and sphingolipids such as sphingomyelin (SM) and ceramide aminoethylphosphonate (CAEP).

Gangliosides are glycolipid components in the cell plasma membrane, which modulate cell signal transductions events. They are implicated as being important in immunology and neurodegenerative disorders. Cerebrosides are important components in animal muscle and nerve cell membranes.

Both phospholipids and gangliosides are involved in cell signalling events leading to, for example, cell death (apoptosis), cell growth, cell proliferation, and cell differentiation.

Reasonable levels of some of these components can be found in milk, soy products, eggs, animal glands and organs, marine animals, plants and other sources. A source of these components is the bovine milk fat globule membrane (MFGM) which is known to contain useful quantities of sphingomyelin, ceramides, gangliosides, and phosphatidyl serine. Another source of these components is the green-shell mussel, which is known to contain useful quantities of plasmalogens, alkylacylphospholipids and ceramide aminoethylphosphonate

Both phospholipids and gangliosides have been implicated in conferring a number of health benefits including brain health, skin health, eczema treatment, anti-infection, wound healing, gut microbiota modifications, anti-cancer activity, alleviation of arthritis, improvement of

cardiovascular health, and treatment of metabolic syndromes. They can also be used in sports nutrition.

Cardiolipin is an important component of the inner mitochondrial membrane. It is typically present in metabolically active cells of the heart and skeletal muscle. It serves as an insulator and stabilises the activity of protein complexes important to the electron transport chain.

Existing methods for isolation of these compounds rely on the use of chromatographic techniques, which are slow and costly processes to operate. These techniques can also require the use of solvents that are unsuitable and/or undesirable in products for nutritional or human use. For example, Palacios and Wang [1] describe a process for extraction of phospholipids from egg yolks using acetone and ethanol extractions, followed by a methanol/chloroform separation. Kang and Row [2] describe a liquid chromatography process for separation of soybean derived PC from PE and PI. This process may be expensive to carry out on an industrial scale, and also uses hexane, methanol, and isopropyl alcohol as solvents. Kearns et al [3] describe a process for purification of egg yolk derived PC from PE using mixtures of acetonitrile, hydrocarbons, and fluorocarbons. Again, these solvents are undesirable for nutritional or pharmaceutical use.

Supercritical fluid extraction processes using CO_2 are becoming increasingly popular because of a number of processing and consumer benefits. CO_2 can be easily removed from the final product by reducing the pressure, whereupon the CO_2 reverts to a gaseous state, giving a completely solvent free product. The extract is considered to be more 'natural' than extracts produced using other solvents, and the use of CO_2 in place of conventional organic solvents also confers environmental benefits through reduced organic solvent use. The disadvantage of supercritical CO_2 processing is that the solubility of many compounds in CO_2 is low, and only neutral lipids can be extracted.

It is known that the use of CO_2 with organic co-solvents such as ethanol allows extraction of some phosphatidyl choline and to a much lesser extent phosphatidyl ethanolamine. For example, Teberikler et al [4] describe a process for extraction of PC from a soybean lecithin. Using 10% ethanol in CO_2 at 60°C they found that PC was easily extracted, while PE and PI were extracted to a very low extent. Extraction at 12.5 % ethanol at 80°C gave a four-fold increase in solubility of PC. Montanari et al [5] describe a process for extracting phospholipids from soybean flakes. After first extracting neutral lipids using only CO_2 at 320 bar, they found that using 10 % ethanol co-solvent at pressures of 194 to 689 bar resulted in

some extraction of PC, PE, PI, and phosphatidic acid (PA). PC is selectively extracted under some conditions, but at higher temperatures and pressures some extraction of PE and PI was achieved. The pressures required to achieve good extraction were impractically high for industrial application, and the high temperatures used (80°C) could cause polyunsaturated fatty acids to be degraded. Taylor et al [6] describe a process in which soybean flakes are first extracted using only CO_2, followed by CO_2 with 15% ethanol at 80°C and 665 bar. A mixture of phospholipids is obtained which were fractionated by alumina column. Again, the temperatures and pressures are too high for practical application. In these works, the soybean-derived feed materials do not contain detectable levels of SM, CL, GS or PS.

Tanaka and Sakaki [7] describe a method for extracting phospholipids from waste tuna shavings using CO_2 and ethanol as a co-solvent. They describe extraction of DHA-containing phospholipids using 5 % ethanol in CO_2, and by presoaking the tuna flakes in straight ethanol and then extracting using CO_2. The phospholipids obtained in this process are not specified and no fractionation of the different phospholipids is described. In addition, the phospholipids fraction makes up a relatively small proportion of the total processed material, requiring use of large pressure vessels to produce a small yield of phospholipids.

Bulley et al [8] describe extraction of frozen egg yolks using CO_2 and 3 % ethanol, and CO_2 with up to 5 % methanol. Higher rates of triglyceride extraction were obtained with the use of the co-solvent. Extraction of small amounts of phospholipids, up to 17% concentration in the extract, was also achieved. Fractionation of the phospholipids is not described.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents or such sources of information is not to be construed as an admission that such documents or such sources of information, in any jurisdiction, are prior art or form part of the common general knowledge in the art.

It is an object of this invention to provide a process for producing a product that contains desirable levels of particular phospholipids and/or gangliosides and/or cerebrosides, or at least to offer the public a useful choice.

SUMMARY OF INVENTION

Accordingly the present invention provides a process for separating a feed material into soluble and insoluble components, comprising:

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

(i) at least 1% by mass phosphatidyl serine

(ii) at least 1% by mass sphingomyelin

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

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

(v) at least 1% by mass cardiolipin

(vi) at least 0.3% by mass gangliosides

(b) providing a solvent comprising:

(i) supercritical or near-critical CO_2, and

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

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

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

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

Preferably the feed material comprises greater than 1% phosphatidyl serine. More preferably the feed material comprises greater than 2% phosphatidyl serine. Most preferably the feed material comprises greater than 5% phosphatidyl serine.

Alternatively the feed material comprises greater than 1% sphingomyelin. More preferably the feed material comprises greater than 5% sphingomyelin. Most preferably the feed material comprises greater than 15% sphingomyelin.

Alternatively the feed material comprises greater than 1% cardiolipin. More preferably the feed material comprises greater than 2% cardiolipin. Most preferably the feed material comprises greater than 5% cardiolipin.

Alternatively the feed material comprises greater than 0.3% gangliosides. More preferably the feed material comprises greater than 1% gangliosides. Most preferably the feed material comprises greater than 2% gangliosides.

Alternatively the feed material comprises greater than 0.5% acylalkyphospholipids and/or plasmalogens. More preferably the feed material comprises greater than 2% acylalkyphospholipids and/or plasmalogens. Most preferably the feed material comprises greater than 10% acylalkyphospholipids and/or plasmalogens.

Alternatively the feed material comprises greater than 0.5% aminoethylphosphonate and/or other phosphonolipids. More preferably the feed material comprises greater than 5% aminoethylphosphonate and/or other phosphonolipids. Most preferably the feed material comprises greater than 20% aminoethylphosphonate and/or other phosphonolipids.

The present invention also provides a process for separating a feed material into soluble and insoluble components, comprising

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

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

(ii) at least 1% by mass sphingomyelin,

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

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

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

(vi) at least 0.3% by mass gangliosides

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

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

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

(e) providing a second solvent comprising:

(i) supercritical or near-critical CO_2, and

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

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

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

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

Preferably the first solvent comprises a mixture of supercritical or near-critical CO_2 and less than 10% C_1-C_3 monohydric alcohol.

The feed material preferably comprises greater than 1% phosphatidyl serine. More preferably the feed material comprises greater than 2% phosphatidyl serine. Most preferably the feed material comprises greater than 5% phosphatidyl serine.

Alternatively the feed material comprises greater than 1% sphingomyelin. Preferably the feed material comprises greater than 5% sphingomyelin. More preferably the feed material comprises greater than 15% sphingomyelin.

Alternatively the feed material comprises greater than 1% cardiolipin. Preferably the feed material comprises greater than 2% cardiolipin. More preferably the feed material comprises greater than 5% cardiolipin.

Alternatively the feed material comprises greater than 0.3% gangliosides. Preferably the feed material comprises greater than 1% gangliosides. More preferably the feed material comprises greater than 2% gangliosides.

Alternatively the feed material comprises greater than 0.5% acylalkyphospholipids and/or plasmalogens. Preferably the feed material comprises greater than 2% acylalkyphospholipids and/or plasmalogens. More preferably the feed material comprises greater than 10% acylalkyphospholipids and/or plasmalogens.

Alternatively the feed material comprises greater than 0.5% aminoethylphosphonate and/or other phosphonolipids. Preferably the feed material comprises greater than 5% aminoethylphosphonate and/or other phosphonolipids. More preferably the feed material comprises greater than 20% aminoethylphosphonate and/or other phosphonolipids.

The feed material of the present invention may be derived from terrestrial animals, marine animals, terrestrial plants, marine plants, or micro-organisms such as microalgae, yeast and bacteria. Preferably the feed material is derived from sheep, goat, pig, mouse, water buffalo, camel, yak, horse, donkey, llama, bovine or human.

Optionally the feed material is selected from: tissue, a tissue fraction, organ, an organ fraction, milk, a milk fraction, colostrum, a colostrum fraction, blood and a blood fraction.

Preferably the feed material is derived from dairy material, soy material, eggs, animal tissue, animal organ or animal blood. More preferably the feed material is selected from: a composition comprising dairy lipids, a composition comprising egg lipids, and a composition comprising marine lipids.

Most preferably the feed material used in the process of the present invention is a bovine milk fraction. Preferably the feed material is selected from: buttermilk, a buttermilk fraction, beta serum, a beta serum fraction, butter serum, a butter serum fraction, whey, a whey fraction, colostrum, and a colostrum fraction.

The feed material may comprise milk fat globule membrane.

Preferably, the feed material is in solid form. When solid, the feed material may be cryomilled before contact with the solvent.

The solvent of the present invention preferably comprises:

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

(b) 0 – 40% v/v water

More preferably the solvent comprises between 0 and 20% v/v water. Most preferably the solvent comprises between 1 and 10% v/v water.

Preferably the alcohol is ethanol.

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

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

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

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

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

Preferably the co-solvent is recycled for further use.

Preferably the CO_2 is recycled for further use.

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

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

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

The lipid and solvent streams may be fed continuously.

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

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

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

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

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

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

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

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

BRIEF DESCRIPTION OF THE DRAWINGS

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

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

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

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

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

ABBREVIATIONS AND ACRONYMS

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

"CL" means cardiolipin

"PC" means phosphatidyl choline

"PI" means phosphatidyl inositol

"PS" means phosphatidyl serine

"PE" means phosphatidyl ethanolamine

"PA" means phosphatidic acid

"PL" means plasmalogen

"PP" means phosphonolipid

"ALP" means alkylacylphospholipid

"SM" means sphingomyelin

"CAEP" means ceramide aminoethylphosphonate

"GS" means ganglioside

"N/D" means not detected

"CO_2" means carbon dioxide

GENERAL DESCRIPTION OF THE INVENTION

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

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

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

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

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

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

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

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

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

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

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

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

EXAMPLES

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

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

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

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

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

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

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

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

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

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

Table 1

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

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

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

Table 2

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

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

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

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

Table 3

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

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

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

Table 4

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

Example 5: Fractionation of dairy phospholipid concentrate

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

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

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

Table 5

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

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

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

Table 6

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

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

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

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

Table 7

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

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

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

Table 8

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

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

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

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

Example 10: Fractionation of egg yolk lecithin

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

Table 9

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

Example 11: Fractionation of egg yolk phospholipid extract

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

Table 10

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

Example 12: Fractionation of Hoki head lipid extract

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

Table 11

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

Example 13: Fractionation of bovine heart lipid extract

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

Table 12

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

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

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

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

Table 13

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

Example 15: Fractionation of soy lecithin

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

Table 14

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

Example 16: Continuous fractionation of egg yolk lipids

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

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

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

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

Example 17: Fractionation of green-lipped mussel lipid extract

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

Table 15

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

Example 18: Fractionation of krill lipids

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

Table 16

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

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

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

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

Table 17

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

INDUSTRIAL APPLICATION

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

REFERENCES

1. Palacios, L.E., Wang, T., Extraction of egg-yolk lecithin, JAOCS 82,8,2005

2. Kang, D.H., Row, K.H., Fractionation of soybean phospholipids by preparative high-performance liquid chromotography with sorbents of various particle size, Journal of Chromatography A, 949, 2002

3. Kearns, J.J., Tremblay, P.A., Robey, R.J., Sunder, S., Process for purification of phospholipids, US patent 4814111, 1989

4. Teberikler, L., Koseoglu, S., Akgerman, A., Selective extraction of phosphatidylcholine from lecithin by supercritical carbon dioxide/ethanol mixture, JAOCS 78,2,2001

5. Montanari, L., Fantozzi, P., Snyder, J.M., King, J.W., Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol, J Supercritical Fluids, 14, 1999

6. Taylor, S.L., King, J.W., Montanari, L., Fantozzi, P., Blanco, M.A., Enrichment and fractionation of phospholipid concentrates by supercritical fluid extraction and chromatography, Ital J Food Sci, 1,12,2000

7. Tanaka, Y., Sakaki, I., Extraction of phospholipids from unused natural resources with supercritical carbon dioxide and an entrainer, Journal of oleo science, 54, 11, 2005

8. Bulley, N.R., Labay, L., Arntfield, S.D., Extraction/Fractionation of egg yolk using supercritical CO2 and alcohol entrainers, J supercritical fluids, 5, 1992.

WHAT WE CLAIM IS:

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

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

(i) at least 1% by mass phosphatidyl serine

(ii) at least 1% by mass sphingomyelin

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

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

(v) at least 1% by mass cardiolipin

(vi) at least 0.3% by mass gangliosides

(f) providing a solvent comprising:

(i) supercritical or near-critical CO_2, and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(ii) at least 1% by mass sphingomyelin,

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

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

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

(vi) at least 0.3% by mass gangliosides

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

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

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

(l) providing a second solvent comprising:

(iii) supercritical or near-critical CO_2, and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(a) 1% phosphatidyl serine, and

(b) 0.3% gangliosides.

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

(a) 1% phosphatidyl serine,

(b) 1% sphingomyelin, and

(c) 0.3% gangliosides.

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

(a) 1% phosphatidyl serine,

(b) 1% sphingomyelin,

(c) 1% cardiolipin, and

(d) 0.3% gangliosides.

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

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

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

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

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

(b) 0-40% by mass water.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure 1

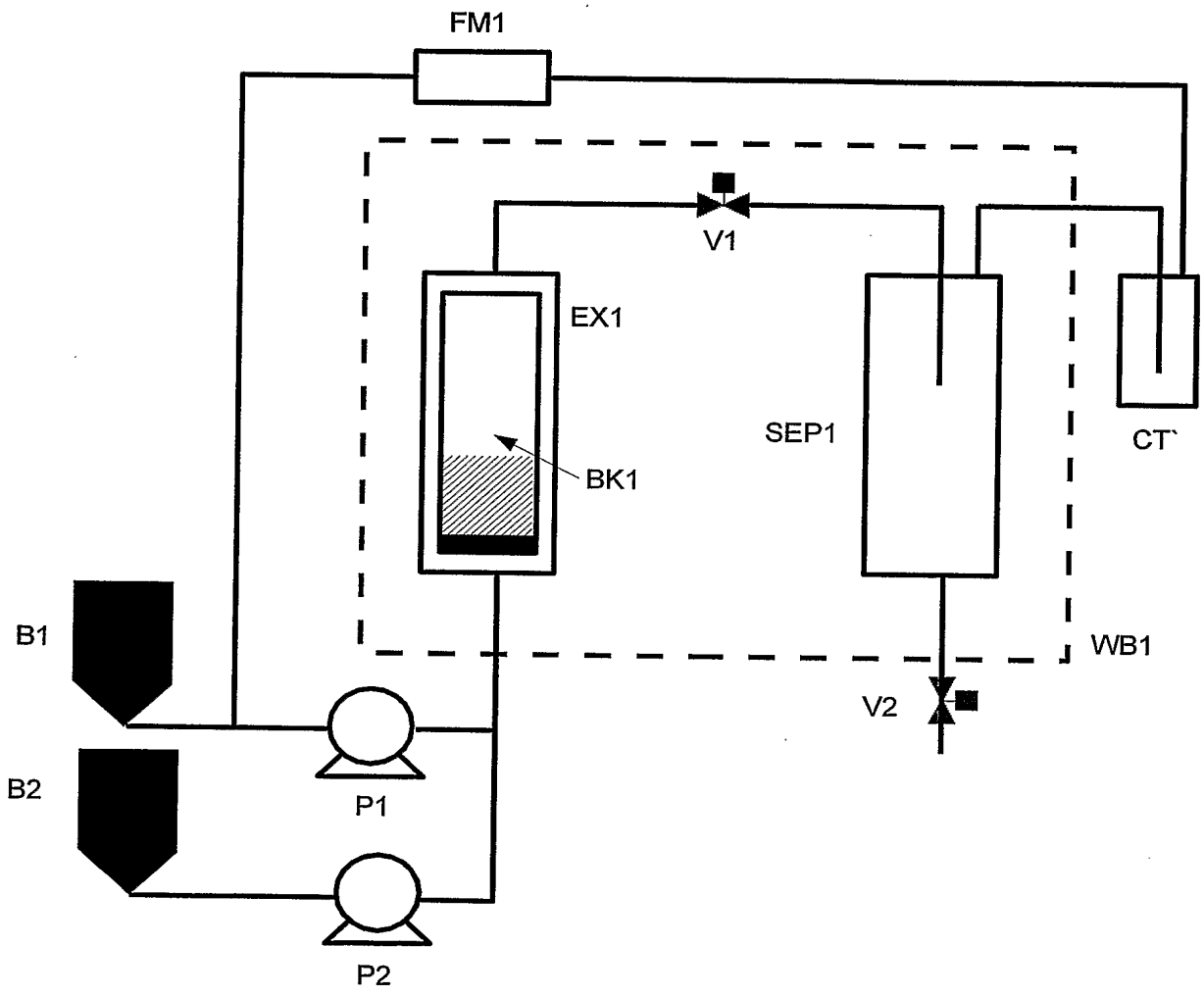


Figure 2

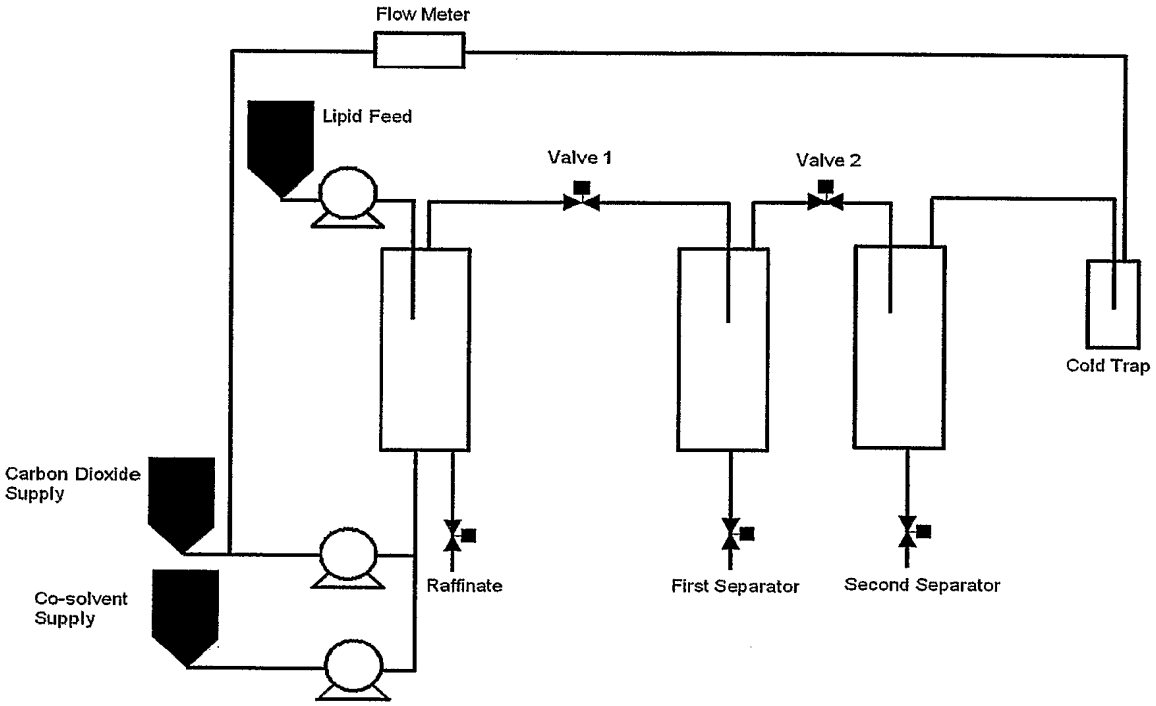


Figure 3

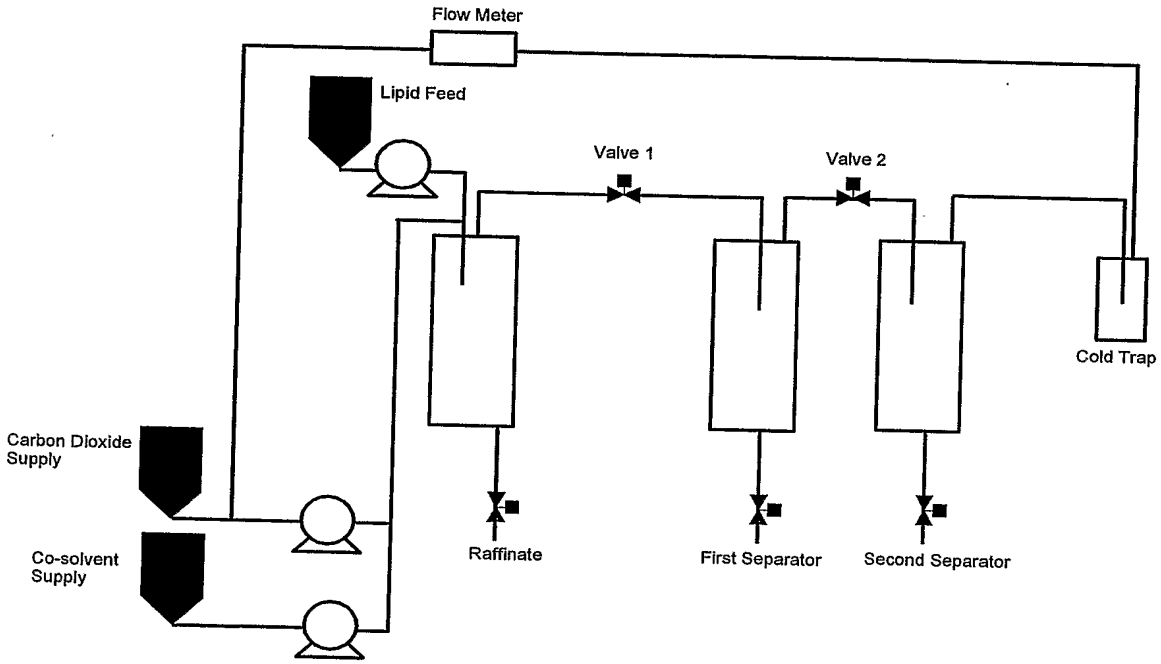
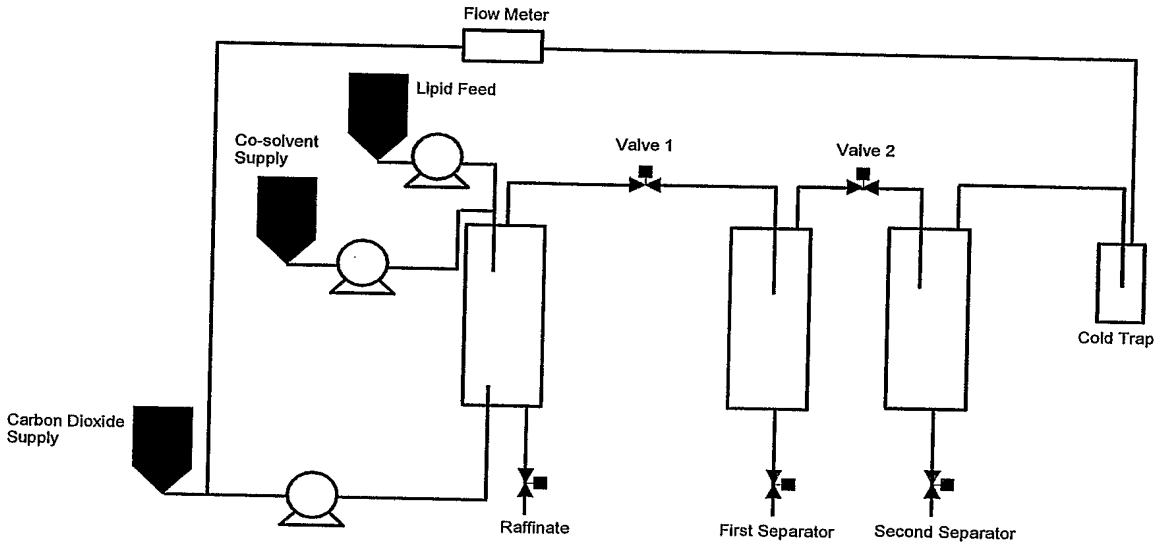


Figure 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2007/000087

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

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

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

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

* 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
21 June 2007	28 JUN 2007

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer
ALBERT S. J. YONG
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No : (02) 6283 2160

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ2007/000087

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

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

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

END OF ANNEX

(12) 特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局

(43) 国際公開日
2008 年6 月19 日 (19.06.2008)

PCT

(10) 国際公開番号
WO 2008/072563 A1

(51) 国際特許分類:
A23K 1/10 (2006.01) *A23K 1/18* (2006.01)

(21) 国際出願番号: PCT/JP2007/073669

(22) 国際出願日: 2007 年12 月7 日 (07.12.2007)

(25) 国際出願の言語: 日本語

(26) 国際公開の言語: 日本語

(30) 優先権データ:
特願 2006-333329
2006 年12 月11 日 (11.12.2006) JP

(71) 出願人 (米国を除く全ての指定国について): 日本水産株式会社 **(NIPPON SUISAN KAISHA, LTD.)** [JP/JP]; 〒1008686 東京都千代田区大手町２丁目６番２号 Tokyo (JP).

(72) 発明者; および
(75) 発明者/出願人 (米国についてのみ): 吉富 文司 **(YOSHITOMI, Bunji)** [JP/JP]; 〒1920906 東京都八王子市北野町５５９－６ 日本水産株式会社中央研究所内 Tokyo (JP).

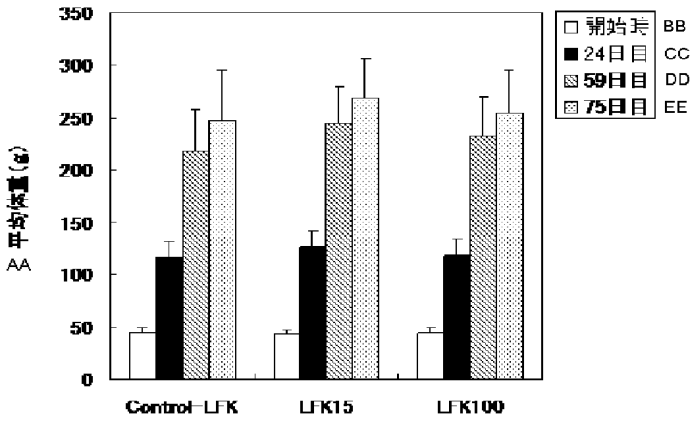
(81) 指定国 (表示のない限り、全ての種類の国内保護が可能): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) 指定国 (表示のない限り、全ての種類の広域保護が可能): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), ユーラシア (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), ヨーロッパ (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

添付公開書類:
— 国際調査報告書

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

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



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

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

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

WO 2008/072563 A1

明 細 書

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

技術分野

[0001] 本発明は、飼料のタンパク原料である魚粉の代替物に関する。

背景技術

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

非特許文献7：J. Fish. Res. Bd. Can. 36, p1414-1416 (1979), Soevik, T. andBraekkan, O. R., “Fluoride in Antarctic krill (Euphausia superba) and Atlantickrill (Meganoctiphanes norvegica).”

発明の開示

発明が解決しようとする課題

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

課題を解決するための手段

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

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

[0008] 本発明は、(1)～(6)の飼料を要旨とする。

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

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

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

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

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

(6)飼料が養魚用飼料である(1)ないし(5)いずれかの飼料。

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

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

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

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

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

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

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

発明の効果

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

図面の簡単な説明

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

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

[図3]比較例1のニジマスの成長率を示す図である。

[図4]比較例2のブリの成長率を示す図である。

[図5]実施例3のブリの成長率を示す図である。

発明を実施するための最良の形態

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

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

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

されて用いられてきた。

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

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

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

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

実施例

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

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

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

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

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

[0015] [表1]

オキアミミールと低フッ素オキアミミールの比較(乾燥物ベース(重量%))

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

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

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

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

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

[0017] [表2]

試験飼料の配合と化学組成（乾燥物ベース（重量%））

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

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

[0018] [表3]

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

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

[0019] [表4]

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

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

[0020] ＜実施例2＞

1．試験方法

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

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

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

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

[0022] 2. 分析方法

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

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

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

Japan)を用いて解析した。

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

[0023] 3. 統計解析

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

P<0.05を有意差ありとした。

[0024] 4. 結果

(1)成長性

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

[0025] [表5]

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

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

[0026] (2)フッ素濃度

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

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

(3)組織学的研究

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

[0027] [表6]

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

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

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

[0028] ＜比較例1＞

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

[0029] ＜比較例2＞

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

[0030] ＜実施例3＞

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

産業上の利用可能性

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

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

請求の範囲

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

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

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

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

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

[6] 飼料が養魚用飼料である請求項1ないし5いずれかの飼料。

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

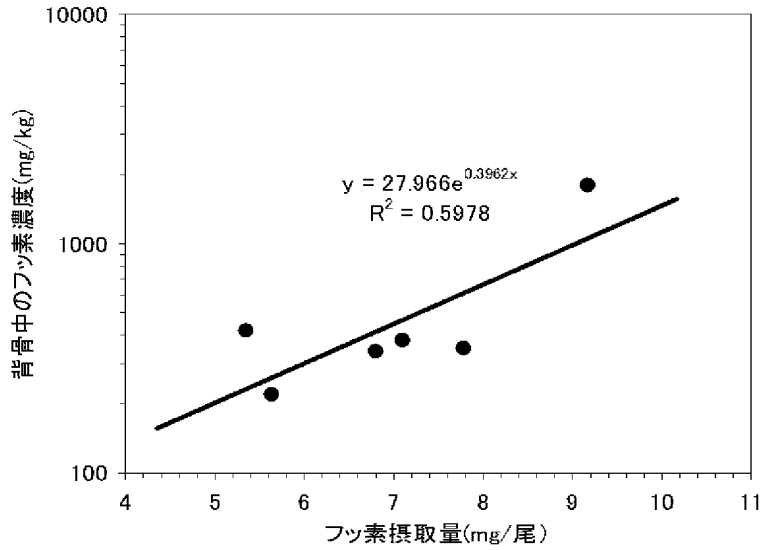
[8] 外殻を除去したオキアミを魚粉の代替として用いることを特徴とする、請求項7のオキアミを飼料原料として用いることによる魚類の成長率の低下を抑制する方法。

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

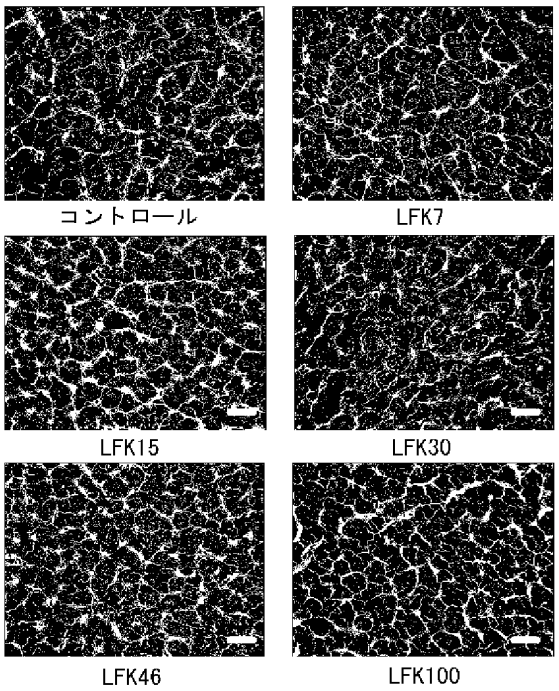
[10] 外殻を除去したオキアミが、外殻を除去したことによりフッ素含有量が250mg／kg乾燥重量以下に減少したものである請求項7、8又は9の飼料。

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

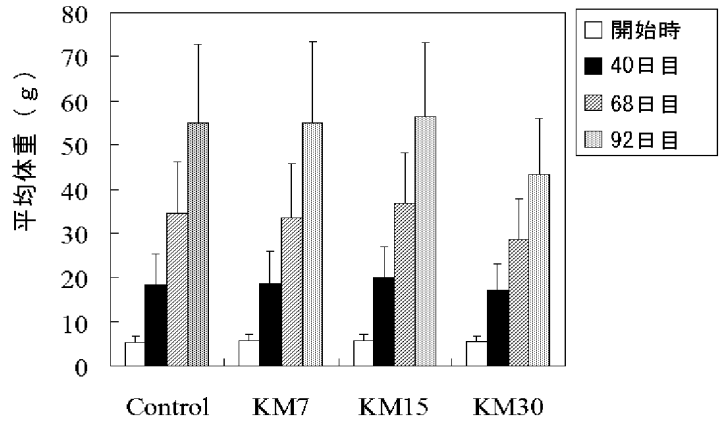
[図1]


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

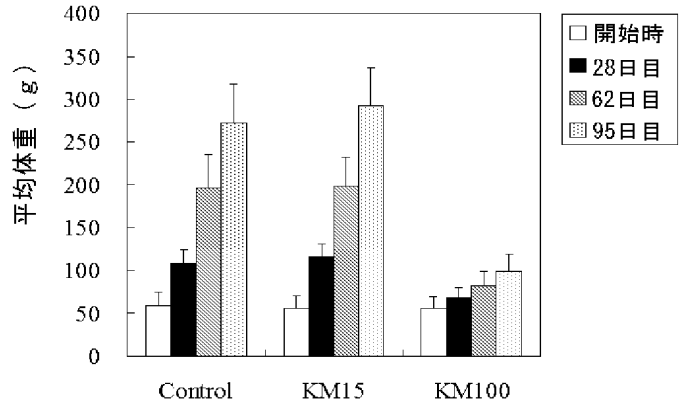
[図2]


コントロール
LFK7
LFK15
LFK30
LFK46
LFK100

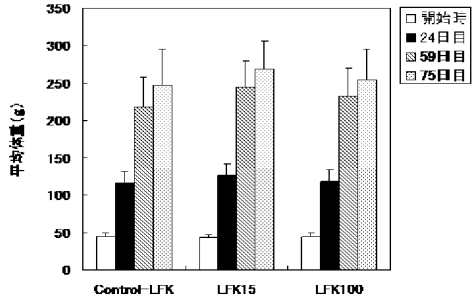
[図3]


80
70
60
50
40
30
20
10
0
平均体重（g）
Control
KM7
KM15
KM30
開始時
40日目
68日目
92日目

[図4]


400
350
300
250
200
150
100
50
0
平均体重（g）
Control
KM15
KM100
開始時
28日目
62日目
95日目

[図5]


350
300
250
200
150
100
50
0
Control-LFK
LFK15
LFK100
24日目
59日目
75日目

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2007/073669

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

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

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

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

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2007/073669

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2007/073669

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

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

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

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

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

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

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

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

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

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

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

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

Remark on Protest

the

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

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

☐ No protest accompanied the payment of additional search fees.

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２００７／０７３６６９

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

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

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　A23K1/00-3/04, A23L1/33

最小限資料以外の資料で調査を行った分野に含まれるもの

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

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

Ｃ．　関連すると認められる文献

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

☑　Ｃ欄の続きにも文献が列挙されている。

☐　パテントファミリーに関する別紙を参照。

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

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

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（２００７年４月）

Ｃ（続き）．　関連すると認められる文献

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

第Ⅱ欄　請求の範囲の一部の調査ができないときの意見（第１ページの２の続き）

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

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

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

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

第Ⅲ欄　発明の単一性が欠如しているときの意見（第１ページの３の続き）

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

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

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

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

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

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

追加調査手数料の異議の申立てに関する注意

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

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

☐ 追加調査手数料の納付はあったが、異議申立てはなかった。

Electronic Patent Application Fee Transmittal

Application Number:	12057775
Filing Date:	28-Mar-2008
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Filer:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-5/ORD

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:	20979444
Application Number:	12057775
International Application Number:	
Confirmation Number:	1945
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Vickie Hoeft
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-5/ORD
Receipt Date:	17-DEC-2014
Filing Date:	28-MAR-2008
Time Stamp:	17:30:36
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	3922
Deposit Account	504302
Authorized User	

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

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	14409US5IDSLetter12152014.pdf	81261 d26dbcf2f715eb11344fc6602d1e93f5abbfcd50	no	1
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	14409_US5_IDs.pdf	614317 4f0f62bdc278bf43a0de9bd4eed8ade627fb9dde	no	7
Warnings:					
Information:					
3	Other Reference-Patent/App/Search documents	CL_40348_App102_1995_patente_EnglishTrans.pdf	1977383 885ace055996975c639f26eb30f3c3bb2d3e186f	no	76
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
4	Foreign Reference	WO001031A1.pdf	2443714 59cc3f6ddbbe4d8b438fd9818e42a988518a175f	no	20
Warnings:					
Information:					
5	Foreign Reference	WO010960A1.pdf	4457079 73fa13a7c452be58aa0da6d7f5427d57c0a367bb	no	36
Warnings:					
Information:					
6	Foreign Reference	WO1997038585A1.pdf	499494 c70abd9354e0fd615f0a0a3c64b51f40a2ec9e0e	no	19
Warnings:					
Information:					
7	Foreign Reference	WO1998034498A1.pdf	1088473 d878be3a1f2ad4cf4828d431c7723b748213579d	no	59
Warnings:					
Information:					

8	Foreign Reference	WO1999039589A1.pdf	1149668 9bf1ac2abcbabde04358ac34952ac43114217310	no	59
Warnings:					
Information:					
9	Foreign Reference	WO2006111633A3.pdf	147703 064a2f4ffc7be7d0c1769cd6e56deb9305aaa75c	no	7
Warnings:					
Information:					
10	Foreign Reference	WO2007123424A1.pdf	1133186 a072a421fcfc9ed84d7ce845e19405dd199166f8	no	42
Warnings:					
Information:					
11	Foreign Reference	WO2008072563A1.pdf	386379 b480125948fca9fe6a8cdde7db2edfc8f6537 9cf	no	25
Warnings:					
Information:					
12	Other Reference-Patent/App/Search documents	14409EP1PCT_NoticeofOppositionFiled02-14-2014.pdf	15222775 47f9023abc353c46c7fb262fbe1bb27220623db3	no	131
Warnings:					
Information:					
13	Non Patent Literature	BrzustowiczBiochemistry2002.pdf	3329266 ffe2d20672459 92f4b093f68af56161ef7be2aaf	no	13
Warnings:					
Information:					
14	Non Patent Literature	Jong_Ho_Lee_D1_cute.pdf	1105425 70c8335c47f80c2a45355281e5b4f3eb35b883dc	no	8
Warnings:					
Information:					
15	Non Patent Literature	Ki_woong_Cho_D2_cute.pdf	1245361 3d1649e40ba0f981b85a2ebadfd531f03374e700	no	8
Warnings:					
Information:					
16	Non Patent Literature	HvattumJournalofChromatography2000.pdf	1888912 1786ba65bcbbe031cfc31aa3934eb7f17f5789c4	no	15
Warnings:					
Information:					

17	Non Patent Literature	IGARASHI2001_English.pdf	429031 53b1ca1ba56a69d280d913a69ed5bde80dc42a85	no	12
Warnings:					
Information:					
18	Non Patent Literature	TOCHIZAWAJpnOilChemSoc1997.pdf	609363 76bb7f5bd1ae2944be3f540162b569131cdc7f9e	no	9
Warnings:					
Information:					
19	Non Patent Literature	Zerouga1995.pdf	1057697 e7fb4e1e888a02b223d6e1604ce2b2b6394161de	no	9
Warnings:					
Information:					
20	Non Patent Literature	Eung_Ho_Lee_D8cute.pdf	1465563 93e7e748bd7215dca4c38f9dfd4246f48a6d7a39	no	10
Warnings:					
Information:					
21	Non Patent Literature	Hyun_ku_Kim_D5_cute.pdf	910799 69dd8dca935835d6eb6f6b5ab3cb59204f3877de	no	6
Warnings:					
Information:					
22	Non Patent Literature	Mi_Yae_Shon_D3_cute.pdf	571990 593230ffc0d00efdc719f755fa8450b73e362840	no	6
Warnings:					
Information:					
23	Other Reference-Patent/App/Search documents	SummonsMaterials_EP08718910_6.pdf	428722 ee9960446697a4c01871a6dd2d6fb727f7989b39	no	12
Warnings:					
Information:					
24	Fee Worksheet (SB06)	fee-info.pdf	30517 65f5ad899b26c2771d308f0159e83ce9cb31efea	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	42274078	

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bruheim, et al	Confirmation:	1945
Serial No.:	12/057775	Group No.:	1651
Filed:	03-28-2008	Examiner:	D.K. 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-5/ORD.

Dated: December 16, 2014

/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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	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.	

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

U.S.PATENT APPLICATION PUBLICATIONS Remove

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Bruheim
	Art Unit	1651
	Examiner Name	D.K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

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					

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

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	1098900	CA		1981-04-07	Inst. Elementoorganicheskikh So, et al.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	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	☐

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

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Bruheim
	Art Unit	1651
	Examiner Name	D.K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Bruheim
Art Unit	1651
Examiner Name	D.K. Ware
Attorney Docket Number	AKBM-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)	2014-06-12
Name/Print	J. Mitchell Jones	Registration Number	44174

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

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

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

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

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

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

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

(11) (A) No. 1098900

(45) ISSUED 810407

(52) CLASS 260-129
C.R. CL. 260-219;
260-497

(51) INT. CL.[2] C07G 7/00, C08B 37/08

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

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

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

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

(21) APPLICATION No. 293,095

(22) FILED 771214

No. OF CLAIMS 3 - NO DRAWING

X93095

1098900 ABSTRACT OF THE DISCLOSURE APR 7 1981

A1

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

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

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

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

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

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

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

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

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

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

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

According to the invention, the first stage of the

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

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

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

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

For a better understanding of the present invention

examples are presented below.

Example 1

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

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

Example 2

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

chitin integuments.

Examples 3

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

Example 4

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

Example 5

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

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

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

A

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

Example 2

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

Example 3

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

of chitin integuments.

Example 4

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

Example 5

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

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

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

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

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

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

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente

Electronic Acknowledgement Receipt

EFS ID:	19284188
Application Number:	12057775
International Application Number:	
Confirmation Number:	1945
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Amanda Jones
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-5/ORD
Receipt Date:	12-JUN-2014
Filing Date:	28-MAR-2008
Time Stamp:	16:09:04
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	14409US5_IDSLetter.pdf	82780 e3aa67bf19b94f48d0c0eeae8bf8d8be1dfa3e84	no	1

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	14409US5_IDS_June2014.pdf	612659 e74cd32d083b3427adf8a9d39a4ce697e4b1914a	no	5
Warnings:					
Information:					
3	Foreign Reference	CA1098900.pdf	445086 f00e547133aaaf3cc4adea4f3843b70636d720ab	no	11
Warnings:					
Information:					
4	Non Patent Literature	VALERI.pdf	360428 0fd6bc3596f268ee25b716c9ad89f2934bab79ec	no	6
Warnings:					
Information:					
5	Non Patent Literature	CRC.pdf	261666 b0e6f92385faf3c61201f41ac79428bcff150b56	no	5
Warnings:					
Information:					
			Total Files Size (in bytes):	1762619	

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bruheim, et al	Confirmation:	1945
Serial No.:	12/057775	Group No.:	1651
Filed:	03-28-2008	Examiner:	D.K. 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.

CERTIFICATION STATEMENT

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

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

The Commissioner is hereby authorized to charge any required fees or credit any overpayments to Attorney Deposit Account No.: **50-4302**, referencing Attorney Docket No.: AKBM-14409/US-5/ORD.

Dated: June 12, 2014

/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

Electronic Acknowledgement Receipt

EFS ID:	17912463
Application Number:	12057775
International Application Number:	
Confirmation Number:	1945
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Thomas Vita
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-5/ORD
Receipt Date:	15-JAN-2014
Filing Date:	28-MAR-2008
Time Stamp:	14:57:05
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

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

Warnings:

Information:

2	Non Patent Literature	Henderson_1994_pp311-317.pdf	6645569 c65db91ec3d35a2aff33c1817732b17747afe22d	no	7
Warnings:					
Information:					
3	Non Patent Literature	Herman-Groves_1993_pp774-776.pdf	244315 e2f691b02deaa18b8980e60dd40d1d87e280abf4	no	3
Warnings:					
Information:					
4	Non Patent Literature	Itano_1994_pp1-16.pdf	1295233 9441357ccb70d9e5cf09b6f03170e7008ade2dab	no	16
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
5	Non Patent Literature	Johnson_1983_pp229-242.pdf	2198687 c8f4ab46c281d535004a072f71eb55ba80efda07	no	14
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
6	Non Patent Literature	LeGrandois_2009_pp6014-20.pdf	934258 8f7e7a9b738636197bf95e47ac4f63798cfc3004	no	7
Warnings:					
Information:					
7	Non Patent Literature	Lin_1994_pp794-803.pdf	1329895 f96890bfa276072d6851e8bdd0562c3058e03380	no	10
Warnings:					
Information:					
8	Non Patent Literature	Medina_1994_pp479-82.pdf	789751 67db1a33d4aeb19c8d956cd04d27f4777c01b94c	no	4
Warnings:					
Information:					
9	Non Patent Literature	October-24-2012-Office-Action-675-patent.pdf	268862 6c8bee479f01a8cd3edcc5c9adc3382c9be28c3d	no	7
Warnings:					
Information:					

10	Non Patent Literature	OfficeAction-Jan-5-2012-351-patent.pdf	543851 2e0c04a3848be427a36a60cd25f5004ac22612df	no	14
Warnings:					
Information:					
11	Non Patent Literature	Provisional-Application-60307842.pdf	1580888 42b2ae5ee9877fe7e1daf7de750a00e5adcfb3a4	no	37
Warnings:					
Information:					
12	Non Patent Literature	Supplemental-Declaration-Bjorn-Ole-Haugsgjerd-8030348.pdf	948103 c9ae46045ddbc460a4ab1f5c767787857b00f006	no	4
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
13	Non Patent Literature	Supplemental-Declaration-Earl-White-8030348.pdf	1503773 85940899180baf6d3b6a07423db92247480a5341	no	19
Warnings:					
Information:					
14	Non Patent Literature	Supplemental-Declaration-Earl-White-8278351.pdf	487925 a2c3da8c882191cfddd2af9cc427507b8f443e52	no	7
Warnings:					
Information:					
15	Non Patent Literature	Suzuki_1990_119-147.pdf	5529614 287be569a5e1715327e9e52d71bf085c5c85ff15	no	30
Warnings:					
Information:					
16	Non Patent Literature	Takahashi_1986_pp80-84.pdf	299970 fcc068b9d51aeb13777b4e2d2b91e92a022f434d	no	6
Warnings:					
Information:					
17	Non Patent Literature	Takahashi_1982_pp1803-1814.pdf	575928 9741092eb72162a77696b88b3ff9d4beabfa7936	no	12
Warnings:					
Information:					
18	Non Patent Literature	Takahashi_1987_pp398-404.pdf	333964 [illegible]	no	8

Warnings:					
Information:					
19	Non Patent Literature	Tanaka_1999_pp189-194.pdf	335180 5caad1b7c9a6f68d2fb54048ccd3906eea22d3d0	no	6
Warnings:					
Information:					
20	Non Patent Literature	Supplemental-Declaration-Thomas-Gundersen-8030348.pdf	3042898 7d1c0f18a6a48056f8b479e72e983ca2afcbf13e	no	42
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
21	Non Patent Literature	Tocher_1995_Chapter-6.pdf	2980058 7d0fc14bb86a79e671c5114cd9a2c1f0f07508ea	no	39
Warnings:					
Information:					
22	Non Patent Literature	Watanabe_1991_pp681-694.pdf	788087 1203c5f19a27e567b8bf41614d0697fccbc69756	no	14
Warnings:					
Information:					
23	Non Patent Literature	WHO-News_1995_pp547-51.pdf	4533905 ba3db6f08be4d5c0db86ebe4d7e82e6e98cea3e1	no	11
Warnings:					
Information:					
			Total Files Size (in bytes):	37832372	

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.

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	D. K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	4714571		1987-12-22	Kearns et al.	
	2	8278351		2012-10-02	Sampalis	
	3	8383675		2013-02-26	Sampalis	

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					

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

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2251265	CA		2000-04-21	Beaudoin		☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	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)	☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.")	☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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.")	☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-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)	2014-01-14
Name/Print	J. Mitchell Jones	Registration Number	44174

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

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

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

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

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

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

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	D. K. Ware
Attorney Docket Number	AKBM-14409/US-5/ORD

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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					

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

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							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12057775
	Filing Date	2008-03-28
	First Named Inventor	Inge Bruheim
	Art Unit	1651
	Examiner Name	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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	☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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)	☐

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	D. K. Ware
	Attorney Docket Number	AKBM-14409/US-5/ORD

	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")	☐

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

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12057775
Filing Date	2008-03-28
First Named Inventor	Inge Bruheim
Art Unit	1651
Examiner Name	D. K. Ware
Attorney Docket Number	AKBM-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)	2014-01-14
Name/Print	J. Mitchell Jones	Registration Number	44174

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

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

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

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

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

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

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

OPIC
OFFICE DE LA PROPRIÉTÉ INTELLECTUELLE DU CANADA

CIPO
CANADIAN INTELLECTUAL PROPERTY OFFICE

(12) (19) (CA) **Demande-Application**

(21) (A1) **2,251,265**
(22) 1998/10/21
(43) 2000/04/21

(72) BEAUDOIN, Adrien, CA
(72) MARTIN, Geneviève, CA
(71) UNIVERSITÉ DE SHERBROOKE, CA

(51) Int.Cl.[6] C11B 1/10, A23J 1/04, A23D 9/02

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

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

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

TITLE OF THE INVENTION

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

FIELD OF THE INVENTION

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

SUMMARY OF THE INVENTION

Extraction process

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

Variations of the process

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

Methods

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

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

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

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

Applications

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

1-Aquaculture

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

2-Nutraceuticals

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

3-Animal food

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

4-Cosmetic industry

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

5-Medical applications

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

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

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

DESCRIPTION OF THE PREFERRED EMBODIMENT

Results

Note on experimental conditions

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

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

Interpretation of results

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

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

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

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

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

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

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

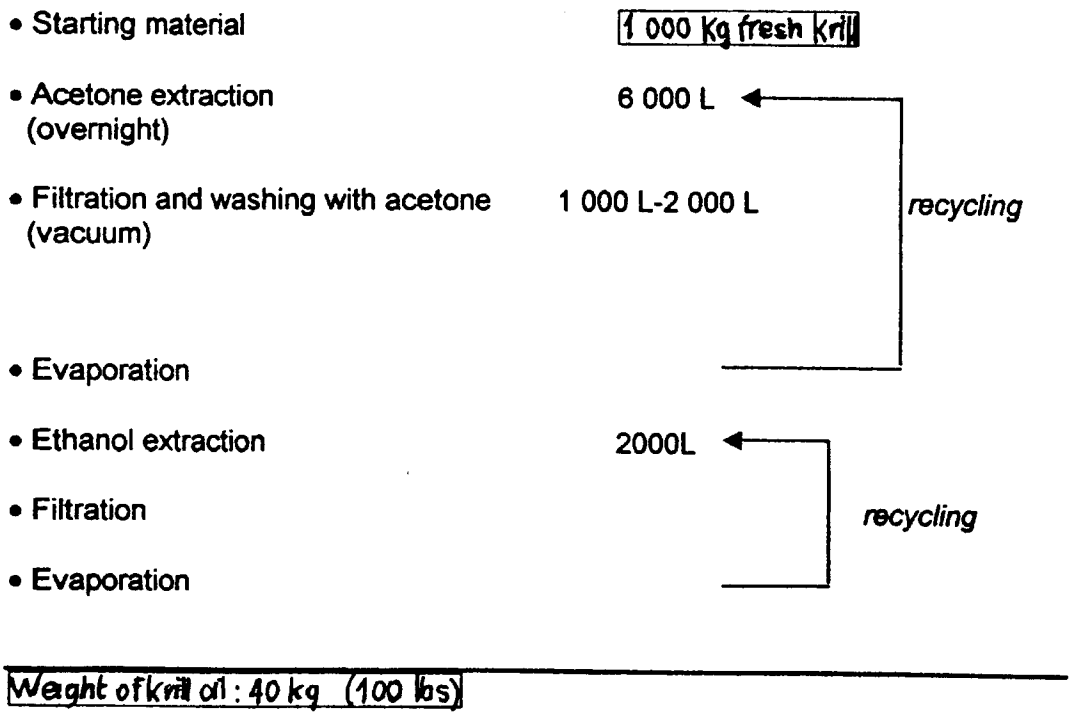
Figures 1 to 4 show chromatograms of fatty acid composition of *E. pacifica* lipids. On each of them, high proportions of 20:5 and 22:6 fatty acids (characteristic of marine oils) are noticeable and represented by two distinct peaks. The concentration of the sample on Figure 4 was lower than the others,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

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

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

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

DIAGRAM 1. KRILL LIPID EXTRACTION PROCESS



Weight of krill oil : 40 kg (100 lbs)

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

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

Determinations in triplicates (variation < 5 %).

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

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

[c)] :Folch et al. 1957

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

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

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

Determinations in triplicates (variation < 5 %).

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

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

[c] :See Table 4 for total composition.

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

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

Determinations in triplicates (variation < 5 %).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[b] :Extraction made with a sample-ethanol ratio of 1:4 (w/v), incubated 1h at 4°C.
[c] :Folch et al. 1957.

TABLE 8. EXTRACTION OF FRESH FISH LIPIDS (Trout)

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

Determinations in triplicates (variation < 5 %).

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

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

[c] :Folch et al. 1957.

TABLE 9. EXTRACTION OF FRESH FISH LIPIDS (Herring)

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

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