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

	26	1406641	EP		2009-01-07	NEPTUNE TECHNOLOGIES & BIORESSOURCES, INC.		☐
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	29	2004/028529	WO		2004-04-08	SUNTORY LIMITED		☐
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	37	1706106	EP		2009-07-15	BRUZZESE		☐

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	20	ENGLISH ABSTRACT; JP 2007-509131; See abstract from corresponding WO 2005/037848 filed herewith	☐
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	1	4119619		1978-10-10	ROGOZHIN SERGEI VASILIEVICH et al.	
	2	5434183		1995-07-18	LARSSON-BACKSTROM	
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	1	8701265	BR		1987-03-12	SATO		☐
	2	1098900	CA		1981-04-07	ROGOZHIN, et al		☐
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	Attorney Docket Number	NATNUT-14409/US-5/ORD

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EXAMINER SIGNATURE

Examiner Signature	/Deborah Ware/	Date Considered	06/29/2015

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

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14020162 - GAU: 1651
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CONFIRMATION NO. 4914

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/020,162	09/06/2013	424	1651	AKBM-14409/US-6/CON

APPLICANTS

AKER BIOMARINE AS, Oslo, NORWAY

INVENTORS

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

**** CONTINUING DATA ***************************

This application is a CON of 12/057,775 03/28/2008 PAT 9034388
which claims benefit of 60/920,483 03/28/2007
and claims benefit of 60/975,058 09/25/2007
and claims benefit of 60/983,446 10/29/2007
and claims benefit of 61/024,072 01/28/2008

**** FOREIGN APPLICATIONS ***************************

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09/23/2013

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ Yes ☑ No	☐ Yes ☑ No	/DEBBIE K WARE/ Examiner's Signature	☐ Initials	NORWAY	19	11	2

ADDRESS

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

TITLE

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

PTO/SB/08a (01-10)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

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

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8697138		2014-04-15	Bruheim et al.	
	2	7488503		2009-02-10	Porzio et al	
	3	4749522		1988-06-07	Kamarei	
	4	4814111		1989-03-21	Kearns et al.	
	5	4133077		1979-01-09	Jasniewicz	

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20110130458		2011-06-02	Harald Breivik	

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

	2	20080166420		2008-07-10	Scott F. Sones		
	3	20060078625		2006-04-13	Susie Rockway		
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	7	20100160659		2010-06-24	Catchpole		
	8	20080166419		2008-07-10	Sones		

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	40348	CL		1997-07-08	Tepual S.A.		☐
	2	89/01031	WO		1989-02-09	Pharmacia AB		☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Examiner Name	NA
	Attorney Docket Number	AKBM-14409/US-6/CON

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

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NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	EP Opposition filed February 13, 2014 by Olympic Seafood AS, EP Patent Application No. EP087189l0l6	☐
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	Filing Date	2013-09-06
	First Named Inventor	Inge Bruheim
	Art Unit	1653
	Examiner Name	NA
	Attorney Docket Number	AKBM-14409/US-6/CON

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

EXAMINER SIGNATURE			
Examiner Signature	/Deborah Ware/	Date Considered	06/29/2015

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

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

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

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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

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

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

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Symbol	**Date**	**Examiner**

US CLASSIFICATION SEARCHED			
Class	**Subclass**	**Date**	**Examiner**

SEARCH NOTES		
Search Notes	**Date**	**Examiner**
CPC_WEST_INV_Searches: see search history print out	05/2015-06/2015	dkw

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

WEST Search History for Application 14020162

Creation Date: 2015062908:09

Interference Searches

Query	DB	Op.	Plur.	Thes.	Date
krill.clm. and oil.clm. and phosphatidylcholine.clm.	PGPB, USPT, UPAD	OR	YES		06-01-2015

Prior Art Searches

Query	DB	Op.	Plur.	Thes.	Date
9034388.pn.	USPT	OR	YES		06-01-2015
Inge.in. and Bruheim.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-01-2015
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(Inge.in. and Bruheim.in. and krill.clm.) and oil.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-01-2015
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(krill and oil and phosphatidylcholine and astaxanthin and triglycerides) and omega	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-01-2015
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and triglycerides and omega and fatty and acids and Euphausia and superba) and ((A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 | A61K31/683 | A61K31/202 | A61K45/06 | A61K31/6615 | A61K8/553 | A61K2800/70 | A61K31/047 | A61K35/63 | A61K35/64 | A61K8/925 | A61K9/4858 | A61K31/7036 | A61K2800/522 | A61K9/0019 | A61K31/07 | A61K31/133 | A61K31/198 | A61K31/232 | A61K31/352 | A61K31/353 | A61K31/575 | A61K31/661 | A61K36/05 | A61K36/535 | A61K36/537 | A61K36/55 | A61K38/1767 | A61K9/107 | A61K9/2009 | A61K9/2054 | A61K9/2866 | A61K9/4808 | A61K31/216 | A61K31/366 | A61K31/375 | A61K31/40 | A61K31/405 | A61K31/44 | A61K31/47 | A61K31/505 | A61K31/612 | A61K31/05 | A61K31/14 | A61K31/194 | A61K31/20 | A61K31/355 | A61K31/407 | A61K31/66 | A61K35/60 | A61K41/0028 | A61K47/46 | A61K8/4986 | A61K9/0095 | A61K9/08 | A61K9/1075 | A61K9/127 | A61K9/1271 | A23V2002/00 | A23V2250/187 | A23V2250/1846 | A23V2250/1868 | A23V2250/185 | A23V2250/1848 | A23V2250/1852 | A23V2250/70 | A23V2250/1882 | A23V2200/324 | A23V2200/326 | A23V2200/02 | A23V2200/322 | A23V2250/026 | A23V2250/1866 | A23V2250/211 | A23V2250/2116 | A23V2250/2136 | A23V2250/5432 | A23V2250/702 | A23V2250/708 | A23L1/3006 | A23L1/33 | A23L1/3008 | A23L1/302 | A23L1/305 | A23L1/30 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/0026 | A23L1/3002 | A23L1/0041 | A23L1/2753 | A23L1/303 | A23L1/304 | A23L1/29 | A23L1/326 | A23D9/013 | A23D9/007 | A23D9/02 | A23D7/011 | A23D9/00 | C11B1/06 | C11B1/10 | C11B3/006 | C11B13/00 | C11B1/02 | C11B1/16 | C11B1/025 | C11B1/104 | A61Q19/00 | A61Q1/06 | A61Q17/04 | A61Q19/007 | A61Q1/10 | A61Q1/12 | A23K1/164 | A23K1/103 | A23K1/1606 | A23K1/188 | A23K1/1755 | Y02W30/74 | A23J7/00 | A23J1/04 | A23J3/34 | C07F9/10 | C07F9/103 | C07F9/117 | C07F9/106 | C07D407/14 | C07K14/43509 | C07K19/00 | A23G3/40 | A23G3/364 | A23G3/368 | A23G3/54).CPC.)	USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS				
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8557297.pn.	USPT	OR	YES		06-01-2015
(krill and oil and phosphatidylcholine and astaxanthin and triglycerides and omega and fatty and acids and Euphausia and superba and ((A61K2300/00 | A61K31/122 | A61K35/612 | A61K31/685 | A61K31/23 |	PGPB, USPT, USOC, EPAB,	OR	YES		06-01-2015

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

**A61K31/612	A61K31/05	A61K31/14	A61K31/194	A61K31/20	A61K31/355	A61K31/407	A61K31/66	A61K35/60	A61K41/0028	A61K47/46	A61K8/4986	A61K9/0095	A61K9/08	A61K9/1075	A61K9/127	A61K9/1271	A23V2002/00	A23V2250/187	A23V2250/1846	A23V2250/1868	A23V2250/185	A23V2250/1848	A23V2250/1852	A23V2250/70	A23V2250/1882	A23V2200/324	A23V2200/326	A23V2200/02	A23V2200/322	A23V2250/026	A23V2250/1866	A23V2250/211	A23V2250/2116	A23V2250/2136	A23V2250/5432	A23V2250/702	A23V2250/708	A23L1/3006	A23L1/33	A23L1/3008	A23L1/302	A23L1/305	A23L1/30	A23L1/0152	A23L1/0153	A23L1/3053	A23L1/3252	A23L1/0026	A23L1/3002	A23L1/0041	A23L1/2753	A23L1/303	A23L1/304	A23L1/29	A23L1/326	A23D9/013	A23D9/007	A23D9/02	A23D7/011	A23D9/00	C11B1/06	C11B1/10	C11B3/006	C11B13/00	C11B1/02	C11B1/16	C11B1/025	C11B1/104	A61Q19/00	A61Q1/06	A61Q17/04	A61Q19/007	A61Q1/10	A61Q1/12	A23K1/164	A23K1/103	A23K1/1606	A23K1/188	A23K1/1755	Y02W30/74	A23J7/00	A23J1/04	A23J3/34	C07F9/10	C07F9/103	C07F9/117	C07F9/106	C07D407/14	C07K14/43509	C07K19/00	A23G3/40	A23G3/364	A23G3/368	A23G3/54).CPC.) and (capsule or encapsulated)) and Euphausia and superba**					
20040241249	PGPB	OR	YES		06-01-2015
(20040241249) and "omega"	PGPB	OR	YES		06-01-2015
(20040241249 and "omega") and "astaxanthin"	PGPB	OR	YES		06-01-2015
(20040241249 and "omega" and "astaxanthin") and "triglyceride"	PGPB	OR	YES		06-01-2015
(20040241249 and "omega" and "astaxanthin" and "triglyceride") and superba	PGPB	OR	YES		06-01-2015
(20040241249 and "omega" and "astaxanthin" and "triglyceride" and superba) and capsule	PGPB	OR	YES		06-01-2015
20040241249	PGPB	OR	YES		06-01-2015
(20040241249) and supercritical	PGPB	OR	YES		06-01-2015
(20040241249) and supercritical	PGPB	OR	YES		06-01-2015
9034388.pn.	USPT	OR	YES		06-29-2015
9034388.pn.	USPT	OR	YES		06-29-2015
9028877.pn.	USPT	OR	YES		06-29-2015

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

	FPRS				
(Snorre.in. and Tilseth.in.) and supercritical.clm. and denaturing.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-29-2015
Daniele.in. and Mancinelli.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-29-2015
(Daniele.in. and Mancinelli.in.) and krill.clm. and denaturing.clm. and supercritical.clm.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-29-2015
(krill and denaturing and supercritical) and ((A61K2300/00 | A61K31/122 | A61K31/23 | A61K31/683 | A61K31/685 | A61K31/202 | A61K31/232 | A61K31/355 | A61K31/663 | A61K35/74 | A61K9/4858 | A61K35/612 | A61K45/06 | A61K31/20 | A61K31/235 | A61K9/48 | A23L1/3006 | A23L1/0152 | A23L1/0153 | A23L1/3053 | A23L1/3252 | A23L1/33 | A23L1/0041 | A23L1/2753 | A23L1/302 | A23L1/303 | C11B3/006 | C11B1/025 | C11B1/10 | C11B1/104 | A23D9/007 | A23D9/013 | A23D9/02 | A23J1/04 | A23J3/34 | C07K14/43509 | C07K19/00 | A23K1/164).CPC.)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	OR	YES		06-29-2015

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                  Goes Live
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          0*  FILE CIN
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         27   FILE USPATFULL
          9   FILE USPAT2

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L1   QUE DENATUR? AND KRILL AND SUPERCRITICAL AND EXTRACTION AND OIL AND POLAR(
       P) KRILL(P) OIL

=> file uspat2 uspatfull ifiall
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                                                     ENTRY    SESSION
FULL ESTIMATED COST                                   1.70       1.95

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=> s L1
L2          40 L1

=> s L2 and phospholipid
L3          30 L2 AND PHOSPHOLIPID

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

=> d L4 1-30

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

L4   ANSWER 30 OF 30  USPATFULL on STN
AN     2008:312554  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Tilseth, Snorre, Bergen, NORWAY
       Banni, Sebastiano, Cagliari, ITALY
       Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 20080274203      A1  20081106
       US 9034388          B2  20150519
AI     US 2008-57775       A1  20080328 (12)
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 2199
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INCL   INCLM: 424/522.000
       INCLS: 514/121.000; 514/078.000; 514/114.000; 426/601.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685
              [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0035-56 [I]; A23D0009-00 [I]; A61K0031-66 [I]; A61K0031-661
              [I]; A61K0031-685 [I]; A61P0003-02 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> s L4 and phosphatidylcholine
L5          29 L4 AND PHOSPHATIDYLCHOLINE

=> d L4 1-30

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

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

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

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

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

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

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

L4   ANSWER 8 OF 30  USPATFULL on STN
AN     2015:4199  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20150004227      A1  20150101
AI     US 2014-14490221    A1  20140918 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1955
INCL   INCLM: 424/456.000
       INCLS: 424/522.000; 424/451.000
NCL    NCLM:  424/456.000
       NCLS:  424/451.000; 424/522.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
IPC    IPCI   A61K0035-56 [I]
       IPCR   A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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L4   ANSWER 9 OF 30  USPATFULL on STN
AN     2015:4195  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Tilseth, Snorre, Bergen, NORWAY
       Mancinelli, Daniele, Orsta, NORWAY
USPA   AKER BIOMARINE AS, Oslo, NORWAY
PI     US 20150004223      A1  20150101
       US 9028877          B2  20150512
AI     US 2014-14490176    A1  20140918 (14)
RLI    Continuation of Ser. No. US 2008-57775, filed on 28 Mar 2008, PENDING
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 1983
INCL   INCLM: 424/451.000
       INCLS: 424/522.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0031-23 [I], A61K2300-00; A61K0031-683 [I],
              A61K2300-00; A61K0031-685 [I], A61K2300-00; A61K0031-122 [I],
              A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
IPC    IPCI   A61K0035-56 [I]
       IPCI-2 A61K0045-06 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0009-48
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0031-202 [I]
       IPCR   A61K0035-56 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 10 OF 30  USPAT2 on STN
AN     2011:251469  USPAT2
TI     Solvent-free process for obtaining phospholipids and neutral enriched
       krill oils
IN     Katevas, Dimitri Sclabos, Santiage, CHILE
       Toro Guerra, Raul R., Santiage, CHILE
       Chiong Lay, Mario M., Santiage, CHILE
PA     Tharos. Ltd., Santiago, CHILE (non-U.S. corporation)
       Lonza, Ltd., Basel, SWITZERLAND (non-U.S. corporation)
PI     US 8772516          B2  20140708
AI     US 2011-13096644        20110428 (13)
RLI    Continuation-in-part of Ser. No. WO 2009-IB7269, filed on 30 Oct 2009,
       PENDING
DT     Utility
FS     GRANTED
LN.CNT 1996
INCL   INCLM: 554/023.000
       INCLS: 554/008.000; 554/078.000
NCL    NCLM:  554/023.000
       NCLS:  554/008.000; 554/078.000
CPC    CPCI   C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
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              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
       CPCI-2 C11B0001-16 [I]; A23D0009-007 [I]; A23D0009-013 [I];
              A23L0001-3006 [I]; A23L0001-33 [I]; A23V2002-00; A61K0008-553
              [I]; A61K0008-925 [I]; A61K0035-612 [I]; A61K0035-63 [I];
              A61K0035-64 [I]; A61K2800-70; A61Q0001-06; A61Q0001-10;
              A61Q0001-12 [I]; A61Q0017-04 [I]; A61Q0019-00 [I]; A61Q0019-007
              [I]; C11B0001-02 [I]; C11B0001-06 [I]; C11B0013-00 [I];
              Y02W0030-74
IPC    IPCI   C11B0001-00 [I]; C07F0009-10 [I]
       IPCI-2 C11B0001-00 [I]
       IPCR   C11B0001-00 [I]
EXF    554/8; 554/23; 554/78

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

L4   ANSWER 30 OF 30  USPATFULL on STN
AN     2008:312554  USPATFULL
TI     BIOEFFECTIVE KRILL OIL COMPOSITIONS
IN     Bruheim, Inge, Volda, NORWAY
       Griinari, Mikko, Espoo, FINLAND
       Tilseth, Snorre, Bergen, NORWAY
       Banni, Sebastiano, Cagliari, ITALY
       Cohn, Jeffrey Stuart, Camperdown, AUSTRALIA
       Mancinelli, Daniele, Orsta, NORWAY
PA     AKER BIOMARINE ASA, Oslo, NORWAY (non-U.S. corporation)
PI     US 20080274203      A1  20081106
       US 9034388          B2  20150519
AI     US 2008-57775       A1  20080328 (12)
PRAI   US 2007-60920483        20070328 (60)
       US 2007-60975058        20070925 (60)
       US 2007-60983446        20071029 (60)
       US 2008-61024072        20080128 (61)
DT     Utility
FS     APPLICATION
LN.CNT 2199
INCL   INCLM: 424/522.000
       INCLS: 514/121.000; 514/078.000; 514/114.000; 426/601.000
NCL    NCLM:  424/520.000
CPC    CPCI   A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-202 [I]; A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685
              [I]; A61K0045-06 [I]; C11B0003-006 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
       CPCI-2 A61K0035-612 [I]; A61K0009-4858 [I]; A61K0031-122 [I];
              A61K0031-23 [I]; A61K0031-683 [I]; A61K0031-685 [I]; A61K0045-06
              [I]; C11B0003-006 [I]; A61K0031-202 [I]; A61K0031-23 [I],
              A61K2300-00; A61K0031-683 [I], A61K2300-00; A61K0031-685 [I],
              A61K2300-00; A61K0031-122 [I], A61K2300-00
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IPC    IPCI   A61K0035-56 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0003-02
              [I]; A23D0009-00 [I]; A61K0031-66 [I]
       IPCI-2 A61K0009-48 [I]; A61K0031-23 [I]; A61K0031-122 [I]; A61K0031-202
              [I]; A61K0031-683 [I]; A61K0031-685 [I]; C11B0003-00 [I];
              A61K0045-06 [I]
       IPCR   A61K0035-56 [I]; A23D0009-00 [I]; A61K0031-66 [I]; A61K0031-661
              [I]; A61K0031-685 [I]; A61P0003-02 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


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     CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGU, EMBAL, EMBASE,
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               0*  FILE BIOTECHDS
               0*  FILE BIOTECHNO
               0*  FILE CEABA-VTB
               0*  FILE CIN
               0*  FILE FOMAD
               0*  FILE FROSTI
               4   FILE IFIALL
               0*  FILE KOSMET
               0*  FILE NTIS
               0*  FILE PASCAL
              27   FILE USPATFULL
               9   FILE USPAT2
L1              QUE DENATUR? AND KRILL AND SUPERCRITICAL AND EXTRACTION AND OIL
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     FILE 'USPAT2, USPATFULL, IFIALL' ENTERED AT 10:19:11 ON 29 JUN 2015
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L3           30 S L2 AND PHOSPHOLIPID
L4           30 DUP REM L3 (0 DUPLICATES REMOVED)
L5           29 S L4 AND PHOSPHATIDYLCHOLINE

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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
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www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/020,162	09/06/2013	1651	1740	AKBM-14409/US-6/CON	11	2

CONFIRMATION NO. 4914

CORRECTED FILING RECEIPT

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

OC000000075331796

Date Mailed: 06/04/2015

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

Inventor(s)

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

Applicant(s)

AKER BIOMARINE ANTARCTIC AS, Stamsund, NORWAY

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

Domestic Priority data as claimed by applicant

This application is a CON of 12/057,775 03/28/2008 PAT 9034388
which claims benefit of 60/920,483 03/28/2007
and claims benefit of 60/975,058 09/25/2007
and claims benefit of 60/983,446 10/29/2007
and claims benefit of 61/024,072 01/28/2008

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

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

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

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

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

BIOEFFECTIVE KRILL OIL COMPOSITIONS

Preliminary Class

424

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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Alexandria, Virginia 22313-1450
www.uspto.gov

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

CONFIRMATION NO. 4914

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

POA ACCEPTANCE LETTER

OC000000075531850

Date Mailed: 06/04/2015

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 06/01/2015.

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

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ttran/

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 11/30/2014. OMB 0651-0031
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
14/020,162	06-Sep-2013

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 72960

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

AKER BIOMARINE ANTARCTIC AS

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	
Name	Eduardo Turi		
Title	CEO		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office;U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of1995, no persons arerequired to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: AKER BIOMARINE AS

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

Titled: BIOEFFECTIVE KRILL OIL COMPOSITIONS

AKER BIOMARINE ANTARCTIC AS, a corporation

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

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

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

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

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

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

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

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

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

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

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

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

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

1. From: Inge Bruheim et al. To: AKER BIOMARINE AS

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

   Reel 031488, Frame 0138, or for which a copy thereof is attached.

2. From: AKER BIOMARINE AS To: AKER BIOMARINE ANTARCTIC AS

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

   Reel 034734, Frame 0841, or for which a copy thereof is attached.

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

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

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

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

4. From: _____ To: _____

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

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

5. From: _____ To: _____

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

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

6. From: _____ To: _____

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

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

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

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

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

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

/J. Mitchell Jones/	May 20, 2015
Signature	Date
J. Mitchell Jones	44174
Printed or Typed Name	Title or Registration Number

Privacy Act Statement

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

The informationprovided 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 informationin 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 InternationalApplication 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, arecord 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 thissystem of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	22499750
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Mallory Checkett
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON
Receipt Date:	01-JUN-2015
Filing Date:	06-SEP-2013
Time Stamp:	16:11:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	14409US6CON_RequestChangeApplicant_6-1.pdf	83701 63f4c5df26987d421758cc20b72b5776b9bd7d0b	no	1

Warnings:	
Information:	

2	Request for Corrected Filing Receipt	14409US6CON_MarkedUp_FilingReceipt.pdf	169444 129c01a2dab63c160261761b9a8713ab287221c4	no	3
Warnings:					
Information:					
3	Application Data Sheet	14409US6CON_MarkedUp_ApplicationDataSheet.pdf	83752 de94c964b71c2fa9bbf529c91e5889d66e6c4d4b	no	7
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Application Data Sheet	14409US6CONApplicationDataSheet_AKBM.pdf	1505651 f4b1583a472373be3ad50f72e7a757cef8a39223	no	7
Warnings:					
Information:					
5	Power of Attorney	14409US6CON_POA_AkerBiomarineAntarcticAS.pdf	146382 f96ea314035e6a2521349e2a9a0c58a09445eb79	no	1
Warnings:					
Information:					
6	Assignee showing of ownership per 37 CFR 3.73	14409US6CON_373Statement.pdf	119896 e06cd06dfcf9b6da8b8941550ad8f1a84e3c4a0d	no	3
Warnings:					
Information:					
		Total Files Size (in bytes):	2108826		

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.

JMJ

UNITED STATES PATENT AND TRADEMARK OFFICE

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

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

CONFIRMATION NO. 4914

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

FILING RECEIPT

OC000000063953065

Date Mailed: 09/26/2013

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

Inventor(s)

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

Applicant(s)

~~AKER BIOMARINE AS, Oslo, NORWAY~~ AKER BIOMARINE ANTARCTIC AS, Stamsund, Norway

Power of Attorney: None

Domestic Priority data as claimed by applicant

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

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

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

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

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

Projected Publication Date: 01/02/2014

Non-Publication Request: No

Early Publication Request: No
Title

BIOEFFECTIVE KRILL OIL COMPOSITIONS

Preliminary Class

435

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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

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

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Inge		Bruheim	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Volda	Country of Residence	NO

Mailing Address of Inventor:

Address 1	Storhagen 24		
Address 2			
City	Volda	**State/Province**	
Postal Code	6100	**Country**	NO

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Snorre		Tilseth	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Bergen	Country of Residence	NO

Mailing Address of Inventor:

Address 1	Fantoftasen 27 A		
Address 2			
City	Bergen	**State/Province**	
Postal Code	5027	**Country**	NO

Inventor 3 Remove

Legal Name

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniele		Mancinelli	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Orsta	Country of Residence [i]	NO

Mailing Address of Inventor:

Address 1	Vikegeila 15		
Address 2			
City	Orsta	State/Province	
Postal Code	6150	Country [i]	NO

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	72960		
Email Address	docketing@casimirjones.com	Add Email	Remove Email

Application Information:

Title of the Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS		
Attorney Docket Number	AKBM-14409/US-6/CON	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	19	Suggested Figure for Publication (if any)	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	72960		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12057775	2008-03-28
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60920483	2007-03-28
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60975058	2007-09-25
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60983446	2007-10-29
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	61024072	2008-01-28

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Remove

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

○ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	~~AKER BIOMARINE AS~~ AKER BIOMARINE ANTARCTIC AS		
Mailing Address Information For Applicant:			
Address 1	~~Fjordalleen 16~~ J.M. JOHANSENS VEI 99		
Address 2	~~P.O. Box 1423 Vika~~		
City	~~Oslo~~ STAMSUND	**State/Province**	
Country	NO	Postal Code	~~0115~~ 8340
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee is an Organization check here. ☐

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the Add button.

Signature:

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

Signature	/J. Mitchell Jones/			Date (YYYY-MM-DD)	2013-09-06
First Name	J. Mitchell	Last Name	Jones	Registration Number	44174

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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 records.

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 C o o p eration 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/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Inge		Bruheim	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Volda	Country of Residence i	NO

Mailing Address of Inventor:

Address 1	Storhagen 24		
Address 2			
City	Volda	**State/Province**	
Postal Code	6100	**Country** i	NO

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Snorre		Tilseth	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Bergen	Country of Residence i	NO

Mailing Address of Inventor:

Address 1	Fantoftasen 27 A		
Address 2			
City	Bergen	**State/Province**	
Postal Code	5027	**Country** i	NO

Inventor 3 Remove

Legal Name

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniele		Mancinelli	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Orsta	Country of Residence i	NO

Mailing Address of Inventor:

Address 1	Vikegeila 15		
Address 2			
City	Orsta	**State/Province**	
Postal Code	6150	**Country** i	NO

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	72960		
Email Address	docketing@casimirjones.com	Add Email	Remove Email

Application Information:

Title of the Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS		
Attorney Docket Number	AKBM-14409/US-6/CON	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	19	**Suggested Figure for Publication (if any)**	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	72960		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12057775	2008-03-28
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60920483	2007-03-28
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60975058	2007-09-25
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60983446	2007-10-29
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	61024072	2008-01-28

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i]the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code [i] (if applicable)

Remove

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

(◉) Assignee ( ) Legal Representative under 35 U.S.C. 117 ( ) Joint Inventor

( ) Person to whom the inventor is obligated to assign. ( ) Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. [X]

Organization Name	AKER BIOMARINE ANTARCTIC AS		
Mailing Address Information:			
Address 1	J.M. JOHANSENS VEI 99		
Address 2			
City	Stamsund	**State/Province**	
Country [i]	NO	Postal Code	8340
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee is an Organization check here. [ ]

PTO/AIA/14 (03-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information:

Address 1	
Address 2	

City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the Add button. Add

Remove

Signature:

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

Signature	/J. Mitchell Jones/			Date (YYYY-MM-DD)	2015-05-20
First Name	J. Mitchell	Last Name	Jones	Registration Number	44174

Additional Signature may be generated within this form by selecting the Add button. Add

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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 records.

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Inge Bruheim, et al.	Confirmation No.:	4914
Serial No.:	14/020,162	Group Art Unit:	1651
Filing Date:	06-Sep-2013	Examiner:	WARE, DEBORAH K
Title:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

REQUEST FOR CORRECTED FILING RECEIPT AND REQUEST FOR CHANGE OF APPLICANT UNDER 37 CFR 1.76

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

Dear Sir or Madam:

Applicants submit herewith a marked-up application data sheet in accordance with § 1.76 that identifies the Applicant's name and address and a Statement Under 37 CFR 3.73(c) showing the chain of title from the inventors to the current assignee. Applicants hereby request a replacement filing receipt for the above-referenced patent application.

No fees are believed due. However, should any fees be due, the Commissioner is hereby authorized to charge said fees or credit any overpayments to Deposit Account 50-4302, referencing Attorney Docket Number: AKBM-14409/US-6/CON.

Respectfully,

Dated: June 1, 2015

/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

UNITED STATES PATENT AND TRADEMARK OFFICE

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

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

CONFIRMATION NO. 4914

IMPROPER CPOA LETTER

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

Date Mailed: 05/26/2015

NOTICE REGARDING POWER OF ATTORNEY

This is in response to the power of attorney filed 05/20/2015. The power of attorney in this application is not accepted for the reason(s) listed below:

- The power of attorney has not been accepted because the party who is giving power has not been identified. Power of attorney may only be signed by the applicant for patent (37 CFR 1.42) or the patent owner. A party who is not the applicant must become the applicant in accordance with 37 CFR 1.46(c) and appoint any power of attorney in compliance with 37 CFR 3.71 and 3.73. For a reissue application, reexamination proceeding, or supplemental examination proceeding, a patent owner who was not the applicant under 37 CFR 1.46 must appoint any power of attorney in compliance with 37 CFR 3.71 and 3.73. See 37 CFR 1.32(b)(4).

/dtdinh/

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

UNITED STATES PATENT AND TRADEMARK OFFICE

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

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

CONFIRMATION NO. 4914

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

IMPROPER CFR REQUEST

Date Mailed: 05/26/2015

RESPONSE TO REQUEST FOR CORRECTED FILING RECEIPT

Power of Attorney, Claims, Fees, System Limitations, and Miscellaneous

In response to your request for a corrected Filing Receipt, the Office is unable to comply with your request because:

- The ADS submitted on 05/20/2015 was not properly marked up to show the desired changes. For information being changed relative to the information already of record, additions must be shown with underlining, and deletions must be shown with strike-through or brackets. See 37 CFR 1.76(c)(2).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/dtdinh/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Inge Bruheim, et al.	Confirmation No.:	4914
Serial No.:	14/020,162	Group Art Unit:	1651
Filing Date:	06-Sep-2013	Examiner:	WARE, DEBORAH K
Title:	BIOEFFECTIVE KRILL OIL COMPOSITIONS		

REQUEST FOR CORRECTED FILING RECEIPT AND REQUEST FOR CHANGE OF APPLICANT UNDER 37 CFR 1.76

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

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No fees are believed due. However, should any fees be due, the Commissioner is hereby authorized to charge said fees or credit any overpayments to Deposit Account 50-4302, referencing Attorney Docket Number: AKBM-14409/US-6/CON.

Respectfully,

Dated: May 20, 2015

/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/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Inge		Bruheim	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Volda	Country of Residence i	NO

Mailing Address of Inventor:

Address 1	Storhagen 24		
Address 2			
City	Volda	**State/Province**	
Postal Code	6100	**Country** i	NO

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Snorre		Tilseth	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Bergen	Country of Residence i	NO

Mailing Address of Inventor:

Address 1	Fantoftasen 27 A		
Address 2			
City	Bergen	**State/Province**	
Postal Code	5027	**Country** i	NO

Inventor 3 Remove

Legal Name

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniele		Mancinelli	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Orsta	Country of Residence i	NO

Mailing Address of Inventor:

Address 1	Vikegeila 15		
Address 2			
City	Orsta	**State/Province**	
Postal Code	6150	**Country** i	NO

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	72960		
Email Address	docketing@casimirjones.com	Add Email	Remove Email

Application Information:

Title of the Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS		
Attorney Docket Number	AKBM-14409/US-6/CON	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	19	**Suggested Figure for Publication (if any)**	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	72960		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12057775	2008-03-28
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60920483	2007-03-28
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60975058	2007-09-25
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	60983446	2007-10-29
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12057775	non provisional of	61024072	2008-01-28

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i]the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

PTO/AIA/14 (03-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code [i] (if applicable)

Remove

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

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PTO/AIA/14 (03-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Applicant 1 Remove

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Clear

(•) Assignee	( ) Legal Representative under 35 U.S.C. 117	( ) Joint Inventor
( ) Person to whom the inventor is obligated to assign.	( ) Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. [X]

Organization Name	AKER BIOMARINE ANTARCTIC AS

Mailing Address Information:

Address 1	J.M. JOHANSENS VEI 99		
Address 2			
City	Stamsund	**State/Province**	
Country [i]	NO	Postal Code	8340
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee is an Organization check here. [ ]

PTO/AIA/14 (03-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AKBM-14409/US-6/CON
	Application Number	
Title of Invention	BIOEFFECTIVE KRILL OIL COMPOSITIONS	

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information:

Address 1	
Address 2	

City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the Add button. Add

Remove

Signature:

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

Signature	/J. Mitchell Jones/			Date (YYYY-MM-DD)	2015-05-20
First Name	J. Mitchell	Last Name	Jones	Registration Number	44174

Additional Signature may be generated within this form by selecting the Add button. Add

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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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 records.

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

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

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

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

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

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
14/020,162	06-Sep-2013

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 72960

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

AKER BIOMARINE ANTARCTIC AS

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	
Name	Eduardo Turi		
Title	CEO		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. 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 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
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U.S. Patent and Trademark Office;U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: AKER BIOMARINE AS

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

Titled: BIOEFFECTIVE KRILL OIL COMPOSITIONS

AKER BIOMARINE ANTARCTIC AS, a corporation

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

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

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

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

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

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

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

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

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

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

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

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

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

1. From: Inge Bruheim et al. To: AKER BIOMARINE AS

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

   Reel 031488, Frame 0138, or for which a copy thereof is attached.

2. From: AKER BIOMARINE AS To: AKER BIOMARINE ANTARCTIC AS

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

   Reel 034734, Frame 0841, or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

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4. From: _____ To: _____

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

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

5. From: _____ To: _____

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

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

6. From: _____ To: _____

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

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

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

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

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

/J. Mitchell Jones/	May 20, 2015
Signature	Date
J. Mitchell Jones	44174
Printed or Typed Name	Title or Registration Number

Privacy Act Statement

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

The informationprovided 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 informationin 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 InternationalApplication filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from thissystem of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	22403645
Application Number:	14020162
International Application Number:	
Confirmation Number:	4914
Title of Invention:	BIOEFFECTIVE KRILL OIL COMPOSITIONS
First Named Inventor/Applicant Name:	Inge Bruheim
Customer Number:	72960
Filer:	John Mitchell Jones/Mallory Checkett
Filer Authorized By:	John Mitchell Jones
Attorney Docket Number:	AKBM-14409/US-6/CON
Receipt Date:	20-MAY-2015
Filing Date:	06-SEP-2013
Time Stamp:	17:11:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	14409US6CON_Request_ChangeApplicant_AkerBioMarineAntarcticAS.pdf	83698 2be06476406d147e7af768277cb9c691bbfc0a5b	no	1

Warnings:	
Information:	

2	Application Data Sheet	14409US6CONApplicationDataSheet_AKBM.pdf	1505651 f4b1583a472373be3ad50f72e7a757cef8a39223	no	7
Warnings:					
Information:					
3	Power of Attorney	14409US6CON_POA_AkerBiomarineAntarcticAS.pdf	146382 f96ea314035e6a2521349e2a9a0c58a09445eb79	no	1
Warnings:					
Information:					
4	Assignee showing of ownership per 37 CFR 3.73	14409US6CON_373Statement.pdf	119896 e06cd06dfcf9b6da8b8941550ad8f1a84e3c4a0d	no	3
Warnings:					
Information:					
		Total Files Size (in bytes):	1855627		

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New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PTO/SB/08a (01-10)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

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

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8697138		2014-04-15	Bruheim et al.	
	2	7488503		2009-02-10	Porzio et al	
	3	4749522		1988-06-07	Kamarei	
	4	4814111		1989-03-21	Kearns et al.	
	5	4133077		1979-01-09	Jasniewicz	

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20110130458		2011-06-02	Harald Breivik	

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

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

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

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

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NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	EP Opposition filed February 13, 2014 by Olympic Seafood AS, EP Patent Application No. EP0871891016	☐
	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)		
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	First Named Inventor	Inge Bruheim
	Art Unit	1653
	Examiner Name	NA
	Attorney Docket Number	AKBM-14409/US-6/CON

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

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

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

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

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

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

CERTIFICATION STATEMENT

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

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

OR

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

☐ See attached certification statement.

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

☐ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/J. Mitchell Jones/	Date (YYYY-MM-DD)	2014-12-16
Name/Print	J. Mitchell Jones	Registration Number	44174

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

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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.

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

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

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TITLE OR SUBJECT OF APPLICATION

KRILL OIL AND A METHOD TO OBTAIN IT BY THERMAL FRACTIONATION AND CENTRIFUGING.

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I/WE DECLARE THAT THE INFORMATION THAT APPEARS IN THE PINK COLORED BOXES IS TRUE AND I/WE ALSO ARE FAMILIAR WITH ART. 44 OF LAW No. 19.039 ON INDUSTRIAL PROPERTY AND THAT THIS DOCUMENT CONSTITUTES A FORMAL APPLICATION.

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ORIGINAL

INSTRUCTIONS:

1. — ONLY FILL OUT THE PINK BOXES WITH BOLD TYPING (NOT HANDWRITTEN)
2. — PRIORITY IS UNDERSTOOD TO BE THAT PROTECTION REQUESTED OR AWARDED PREVIOUSLY FOR THE SAME INVENTION, GENERALLY OVERSEAS

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(54)- Title of the Invention: (no longer than 330 characters)

Krill oil, method to obtain it by thermal fractionation and centrifuging: oil with high content of unsaturated fatty acids use for as a source of astaxanthin, a nutritional supplement, cosmetic, sunscreen, for use in the preparation of dermatological products, fat stabilizer.

(57) Abstract: (no longer than 1600 characters)

An oil from a marine crustacean known as krill and the method to produce it by thermal fractionation and centrifuging and/or decanting. An oil with a variable content of polyunsaturated fatty acids is described. This oil is characterized by having up to 95% of polyunsaturated fatty acids present in lipids from krill, and its saturated fatty acid composition is between 26 and 60%, monounsaturated fatty acids between 25 and 60% polyunsaturated fatty acids between 4 and 46%, and astaxanthin between 300 and 2,600 ppm. Further disclosed are uses of this oil as a pigmenting agent, nutritional supplement, stabilizer for other fats, an antioxidant, sunscreen, a source of astaxanthin, a palatability agent, solubilizing agent and/or vehicle for lipophilic compounds, a protective agent for fish eggs and shellfish eggs, a source of EPA (eicosapentaenoic acid), and a source of active ingredients for the preparation of dermatological products.
This invention's production of the oil is accomplished by cooking the krill at different temperatures, followed by pressing the precooked krill, collecting and accumulating the press liquor, removal of the suspended solids and centrifuging to separate the oil. The amount of polyunsaturated fatty acids may be regulated by varying the cooking temperature in the production process, as well as using low temperatures to obtain oils with high levels of polyunsaturated fatty acids.

40348

DESCRIPTIVE MEMORY

ABSTRACT OF THE INVENTION

This invention concerns an oil from a marine crustacean known as krill, and even a method to produce it. To date a process for the industrial production of krill oil has not been described. This invention describes an oil with a variable content of polyunsaturated fatty acids. The chemical composition of this oil is characterized by possessing up to 95% of polyunsaturated fatty acids content present in the lipids of krill. The amount of polyunsaturated fatty acids may be regulated at will by varying the cooking temperature in the production process for said oil. Further disclosed in this patent a description is given for the uses of this oil as a pigmenting agent, nutritional supplement, stabilizer for other fats, an antioxidant, sunscreen, a source of astaxanthin, a palatability agent, solubilizing agent and/or a vehicle for lipophilic compounds, a protective agent for fish eggs and shellfish eggs, a source of EPA (eicosapentaenoic acid), and a source of active ingredients for the preparation of dermatological products.

This invention's production of the oil is accomplished by cooking the krill at different temperatures, followed by pressing the precooked krill, collecting and accumulating the press liquor, removal of the suspended solids and centrifuging to separate the oil. When krill is cooked at low temperatures, oils are obtained which have high levels of polyunsaturated fatty acids.

GENERAL BACKGROUND

Krill belongs to the marine crustaceans group of the Euphasiaceae order. Antarctic krill, particularly those living in the Antarctic and sub-Antarctic, comprise 11 species of Euphausia, with species E. Superba and E. crystallorophia being the dominant species.

In recent years, krill has increasingly captured interest as a potential source of proteins and other active biological products (Ellingsen, T. and Mohr, V. 1979. Process Bioch. 14:14; Suzuki, T. 1987. Krill and Fish Protein Technology. Editorial Acribia S.A.). High hopes have been pinned on krill based on the large biomass that exists in the Antarctic oceans, which calculate their numbers at between 100 to 500 million tons. It is estimated that catches of this crustacean could amount to approximately 50-100 million tons/year, which is equivalent to the total annual catch of fish worldwide (Budzinski, E., Bykowski, P. and Dutkiewicz, D. 1986. Possibilities for processing and marketing of products made from Antarctic krill, FAO Fisheries Tech. Doc. (268): 47p).

There are several publications related to the content of lipids and the composition of the lipids of krill (Grantham, G.J. 1977. Uses of krill. Survey Fishery Program in the Southern Oceans. FAO GLO/SO/77/3:63p; Budzinski et al. 1986. Loc. cit.; Ellingsen and Mohr. 1979. Loc. cit.).

The lipid content varies from 10-26% of the dry weight, depending on the season, sexual maturity, and body size of the krill. The female krill contains about twice as much lipids as the male. Lipid concentrations increase with age and decrease rapidly after spawning. The study of lipid distribution in krill indicate that lipid-rich areas are located along the digestive tract, between the muscle bundles and beneath the exoskeleton

(Saether, O., Ellingsen, T. and Mohr, V. 1985. Comp. Biochem. Physiol. 81B:609).

The major lipid fractions correspond to triglycerides and phospholipids, as well as sterols and their esters (Grantham. 1977. Loc. cit.; Budzinski et al. 1986. Loc. cit.). Krill phospholipid fraction is rich in polyunsaturated fatty acids and especially 20:5 and 22:6, which account for approximately 50% of total phospholipids. The high proportion of polyunsaturated fatty acids in the phospholipid fraction may be needed to maintain the fluidity of the plasma membrane in the low temperatures of the Antarctic waters. It is also possible that the high grade of unsaturation is required to give sufficient plasticity to the deposits of phospholipids present in the krill, to allow the animal to flex and move itself at low temperatures.

An increase of total lipids is accompanied by a decrease of phospholipids and an increase of triglycerides. There is an almost linear correlation between the content of phospholipids and triglycerides and the total lipids, which may mean that both groups have a role in the reserve of energy. The content of polyunsaturated fatty acids decreases as the total content of lipids increases (Saether et al. 1985. Loc. cit.).

Changes which occur post-mortem in the lipids of krill show that the amount of polyunsaturated fatty acids (20:5, 22:6) in relation to the content of fatty acids (16:0) does not decrease when krill is stored at 0°C. This information appears to indicate that mayor oxidation does not occur in polyunsaturated lipids after the death of the crustacean.

In spite of many additional tests and operations, to date it has not been possible to obtain krill oil by centrifuging using traditional equipment. All lipid extraction procedures for krill described to date are based on successive extractions using different organic solvents

(Budzinski et al. 1986. Loc. cit.; Saether et al. 1985. Loc. cit.). Also, in the Chilean patent No. 33688, a procedure is described for obtaining the protein, oil, and shell from the krill. In said patent, obtaining krill oil takes place as a sub-product of obtaining a protein paste from the krill. The steps involved in said patent correspond to a mechanical disintegration of raw material, centrifuging, separating the meat fraction, coagulation of the protein through heat at a temperature of $70 - 95^{\circ}$ C and separation from the waste stream containing an oily product. There is no description in Patent No. 33688 of separation of the oil from the waste stream, nor are the characteristics of this oil described.

THE INVENTION

This invention comprises a krill oil with a composition of variable polyunsaturated fatty acids, a procedure to obtain by thermal fractionation and centrifuging, and uses for this oil for different purposes. Thermal fractionation makes it possible to regulate the degree of unsaturation of krill oil of this invention.

The krill oil described in this invention possesses a lower content of polyunsaturated fatty acids (between 4 and 46%) than the total lipids from krill (between 31 and 48%), and are able to reach up to 95% of the polyunsaturated fatty acids present in the total lipids from krill. This lower amount of polyunsaturated fatty acids is compensated by an increase in the proportion of saturated and monounsaturated fatty acids (see Table I).

Table I

Fatty acid composition of the total lipids from krill and the oil of this invention.

Content of fatty acids	**Total krill lipids***	**Oil of this invention**
Saturates	25 - 37 %	26 - 60%
Monounsaturates	24-33 %	25 - 60%
Polyunsaturates	31 - 48 %	4 - 46%

*Grantham, G.J. 1977. Uses of krill. Survey Fishery Program in the Southern Oceans. FAO GLO/SO/77/3:63p. Ellis, J. and Roch, M. 1984. Krill. Canadian Aquaculture. pp 45-46.

Studying the lipid fractions that make up the oil of this invention it was determined that it also possesses a lower content of phospholipids (between 4 and 28%) in comparison with the total lipids from krill (between 13 and 31%). This reduction in the content of the phospholipids is accompanied by a major increase in only the triglyceride fraction (see Table II).

Table II

Fractional composition of total lipids from krill and the oil of this invention.

Lipid Fractions	Total Lipids from Krill Lipids*	Oil of this Invention
Phospholipids	13 - 31%	4 - 28%
Monoglycerides	2 - 9%	3 - 10%
Diglycerides	1 - 4%	1 - 5%
Triglycerides	30 - 60%	35 - 96%
Sterols	5 - 9%	4 - 8%
Sterol esters	6 - 15%	6 - 15%

*Budzinski, E., Bykowski, P. and Dutkiewicz, D. 1986. Possibilities for processing and marketing of products made from Antarctic krill, FAO Fisheries Tech. Doc. (268): 47p.

The difference between both the chemical composition as well as the fractional distribution of the oil of this invention and that of the total lipids from krill is due to

production procedure of the oil described in this invention. This procedure extracts between 10 and 95% of the total lipids from the krill, favoring extraction of triglyceride fractions over those of the phospholipids. Due to the fact that the polyunsaturated fatty acids are mainly associated with the phospholipid fraction (Grantham. 1977. Loc. cit.; Budzinski et al. 1986. Loc. cit.) the procedure described in this invention produces oils with a lower content of polyunsaturated fatty acids than the total lipids from krill.

The oil of this invention is further characterized by having the carotenoid pigment astaxanthin, in amounts ranging between 300 and 2,600 ppm, more frequently between 450 and 1,200 ppm. This pigment is in free form (between 0 to 15%), monoesterified (between 25 and 50%) and diesterified (between 40 and 80%).

As indicated previously, the content and composition of the lipids in krill depend on several factors. For this reason, the composition of the oil, as well as its production yield depends, among other things, on the period in which the krill is caught. In this invention it was determined that the maximum levels of production can be reached capturing and processing krill in the period comprised between mating and spawning of the crustacean. This invention does not exclude the possibility of catching and processing krill to obtain oil in periods different from those indicated above, thus obtaining oil and fatty acid compositions different to those described in this invention.

The krill used in this invention is not limited to specific types and may correspond to any of the species of krill belonging to the genus Euphausia such as superba, crystallorophias, frigida, tricantha, vellantini, lougirostris, lucens, similis, spinifera, recurva , pacifica and others, and those belonging to the genus Thysanoessa, i.e., macrura, vicina, gregaria, raschii, inermis and others.

The method to produce krill oil is based on the following steps:

a) Cooking the krill.

b) Pressing the precooked krill.

c) Collection and accumulation of press liquor.

d) Removal of suspended solids.

e) Centrifugation and separation of oil.

f) Addition of stabilizers.

g) Storage.

a) Cooking krill: The whole krill, or part, such as the cephalothorax, is treated by cooking. Cooking can be performed by treating the krill or parts of the krill with steam, immersion in hot water or by direct treatment using heat or fire, not excluding other methods of heating and cooking. This process can be performed by a discontinuous batch system or a continuous flow system. In the case of the batch system, the batches should be of such size that the cooking temperature is reached in the entire mass in no more than 2 min. In the continuous process, the krill flow rate should allow the crustaceans to reach cooking temperature in no more than 2 minutes. Using the continuous process to cook the krill makes it possible to process from 5 to 200 kilos of material per minute. The required cooking temperature for this process ranges from 40 to 200° C. The duration of the cooking time can range between 2 to 80 minutes, more specifically between 5 and 25 minutes. The cooking temperature is regulated by controlling the temperature of the raw material as it exits the cooker by using a thermocouple, thermometer, or other instrument to measure temperatures.

The temperature of the krill upon exiting the cooker may range between 30 and 100° C.

In this invention does not exclude the possibility of performing in this first stage of production more than one cooking stage. Indeed, it is possible to perform the first cooking of the krill at a temperature ranging from between 30 to 200° C, and subsequently performing one or more cooking operations at temperatures which are equal to, or smaller than or larger than the first cooking, within a range of between 30 and 200° C. Each cooking step may or may not be separated by storage of the pre-cooked material, at room temperature, refrigerated or frozen, for variable time periods which range, for example, between 5 seconds and 2 years.

b) Pressed cooked krill. The cooked krill is pressed by discontinuous or continuous batches, using a double or single helical screw with a variable mesh. In press systems through presses helical screws, the pressing speed can be adjusted from 1 to 100 rpm, more specifically between 5 and 50 rpm, through independent motor-variators regulated at between 1 and 90° . Pressed volumes vary according to the size of the press. The solid product resulting from the press should have a moisture content of between 25 and 80%, more specifically about 50 to 60%.

By varying the pressing conditions mentioned above, it is possible to obtain pressed cakes with moisture values of between 25 to 50%, which method is also considered in this invention.

This pressing step can be performed separately from the cooking stage, or coupled, forming a fully continuous process.

c) Collection and accumulation of press liquor. The pressing liquid is collected through gravity or by pumps to a storage tank. During this storage, a decanting process is produced in which the larger solids stemming from contamination in the pressing process settle out. The pumps used should produce very low turbulence to prevent the later formation of an emulsion during collection. The storage tank is subject to heating between room temperature and 100° C.

It was also determined in this invention that the soluble protein in the press liquor could be removed through gradual heating up to the coagulation temperature of the soluble protein was reached. The coagulated protein could be separated by a new press treatment or through removal processes for suspended solids. Alternately the soluble protein may be removed through hydrolysis using protease. Examples of proteases that may be used include proteinases such as acrosin, alcalase, urokinase, uropepsina, elastase, enteropeptidase, cathepsin, kallikrein, kinase 2, chymotrypsin, chymopapain, collagenase, streptokinase, subtilisin, thermolysin, trypsin, thrombin, papain, and pancreatopeptidasa renin; peptidases such as aminopeptidases, for example, arginine aminopeptidase, leucine aminopeptidase and oxytocinase; angiotensinase, angiotensin converting enzyme, insulinase, carboxypeptidase, for example, arginine carboxypeptidase, kinase 1 and thyroid peptidase, dipeptidases, for example, carnosinase and prolinase and pronases as well as other proteases not listed. These proteases may be classified as exopeptidases, if they degrade the protein from the amino end or from the carboxyl end, or endopeptidases, if they degrade the interior portion of the protein. The latter is preferred for application in this invention. The protease may be applied between 4 and 90° C, preferably between 30 and 75° C, for 5 minutes to 80 hours,

preferably between 30 minutes an 8 hours. The enzyme may be employed in amounts ranging from 0.001 to 5% (w/v), depending on the enzyme used and the amount of protein contained in the press liquor.

The present invention also determined that it is possible to implement the method without subjecting the press liquor to heating or the subsequent proteolytic treatment during storage.

d) Removal of suspended solids. The press liquor obtained by the process of this invention possesses a maximum of 20% suspended solids, which can be removed by gravity decanting, filtration or a rotating drain. The press liquor submitted to the solid removal processes normally contains a maximum of 1% solids in suspension.

e) Centrifugation and separation of oil. Press liquor with suspended solids of less than or equal to 1% is submitted to a centrifuging process, which may be performed in discontinuous batches or in a continuous form using a centrifuge. Any type of centrifuge may be used and especially, a disc stack centrifuge. The speed of the centrifuging can range between 2,000 to 20,000 rpm, more specifically between 5,000 and 12,000 rpm. The temperature of centrifuging can range between room temperature and 100° C and more specifically between 50 and 98° C.

Oil extraction may also be achieved by decantation phase separation and the joint application of temperature ranging between room temperature and 100° C. Upper phases will correspond to krill oil. The decanter can be a continuous decanter such as a two-phase decanter.

f) Addition of stabilizers. After separating out the oil, you can add various stabilizers such as antioxidants, preservatives, among others, without excluding all those compounds that somehow or another increase the stability and useful life of the oil. Examples correspond to ethoxyquin, vitamin E (dl-alpha-tocopherol), ascorbic acid, sodium ascorbate, nordihydroguaiaretic acid, propyl gallate, guaiacum resin, butylhydroxyanisole, dibutylhydroxytoluene, butyl hydroquinone, tetrabutylhydroquinone, erythorbic acid, sodium erythorbate, among others, without excluding other possible additives.

g) Storage. The oil produced may be stored in plastic or metal tanks, preferably of stainless steel, refrigerated or at room temperature, and protected from light.

This invention determined that the chemical composition and the fractional distribution of the lipids from the oil of the invention may vary by alternating the cooking temperature of the krill. Low cooking temperatures produce press liquors with a higher content of soluble proteins and a higher fatty content than those obtained at higher cooking temperatures. This increase of fatty acids in the press liquor, caused by lower cooking temperatures, brings with it an increase in the yield in production of the oil of the invention. Parallel to this increase in yield is an increase in the content of phospholipids and polyunsaturated fatty acids. In this way it is possible to control the chemical composition and the fractional distribution of the oil of the invention by controlling the cooking temperature of the krill during the production process.

In general it is possible to obtain other chemical compositions and fractional distributions of the krill oil of this invention, by performing more than one cooking operation in the first stage if the production of krill oil. Successive cooking operations with or without storage at room temperature, refrigerated or frozen, using temperatures ranging from 30 to 200° C at any stage of cooking, makes it possible to vary the efficiency of extracting phospholipids, based on the same principle mentioned above.

The oil characterized in this invention and produced by the process described, has diverse uses. As an example, we will mention the following:

a) Pigmenting meat products. When included in fish diets, for example salmon and trout, crustaceans, mollusks, birds and their eggs, pigs, goats, sheep, cattle and other mammals, it is possible to pigment their flesh from a pale pink, orange, to an intense red color depending on the amounts of the carotenoid astaxanthin pigment added to the diet and retained by the animal.

b) Nutritional supplement for various animals, fish and even man. As a source of diverse essential fatty acids, as extra calories, and as a source of polyunsaturated fatty acids of the w3, w6 and w9 types, as well as phospholipids and triglycerides.

c) Stabilizers of other fatty materials. The presence of the pigment astaxanthin and other components of the krill oil, it provides great stability, especially to the lipoperoxidation phenomena that lead to oxidative rancidity of oils. Thus its addition to other oils, whether marine, animal, or plant, can increase their stability.

This procedure would be considered "natural" because it does not require other chemical additives like antioxidants.

d) Sunscreen. The krill oil described in this invention may be used for preparing pharmaceutical compounds having a photoprotective effect due to the ability that the pigment astaxanthin has to capture ultraviolet radiation. In this way the oil can be used for cosmetic or pharmaceutical formulations such as for example, suntan lotions, moisturizers, lipsticks, among other possibilities, without excluding other applications in this area.

e) Antioxidant. Krill oil can be used as a natural antioxidant, and as such may be used to prevent oxidation in various food, pharmaceutical, cosmetic products or other products requiring it, or to prepare pharmaceuticals or cosmetics that have an antioxidant activity.

f) Source of astaxanthin. Due to the high content of astaxanthin in the oil, this invention can be used as a natural source of the pigment, whether it is purified from it or used directly as pigmenting. Its uses in this area would be as food coloring for pinks, oranges, and red tones. It can also be used to color pharmaceutical and cosmetic preparations such as capsules, tablets, wafers, blisters, blushes, lipsticks, pressed powders, shadows, shampoos, and many others.

g) Agent for dissolution and/or a vehicle for lipophilic compounds. Krill oil can be used as a dissolving agent and/or a carrier for lipophilic compounds to be used in the chemical, pharmaceutical, food and cosmetic industries, due to their hydrophobic nature.

h) Palatability agent. The krill oil of this invention can be used to prepare diets as an agent that increases the palatability of food for human and animal nutrition.

i) Protection for fish eggs, and shellfish eggs. The antioxidant and photoprotective properties of krill oil allow the eggs of fish and shellfish that have been fed the krill oil of this invention, to improve their biological characteristics thus increasing their survival.

j) Source of EPA (eicosapentaenoic acid). The krill oil of the present invention possesses polyunsaturated fatty acids, whose content varies from 5 to 30% depending on the process. It has been determined that EPA can constitute 50% of the polyunsaturated fatty acids of the oil of this invention and is therefore an excellent source to obtain them. The EPA thus obtained may be used for dietary management of cardiovascular diseases.

k)Preparation of dermatological products. The essential fatty acid content, especially C18:2 w6 and C18:3 w3, as well as EPA makes it possible to use the oil of this invention for the preparation of topical or systemic pharmacological products for the treatment of diseases of the skin where there is deficiency of essential fatty acids. In this way, it allows for its use in diseases such as xerotic skin,

hyperkeratosis, ichthyosis, acne, psoriasis, skin ulcers, seborrheic eczema, vitiligo, atopic dermatitis, among others.

Example 1:

Production of Krill Oil.

Krill is cooked freshly caught on a continuous belt with steam for 15 minutes reaching a cooking temperature of between 90 and 95° C. The cooked krill is pressed through a double screw with variable mesh, producing a pressed cake with a moisture of between 50 to 60%. The press liquor is rapidly collected by gravity, and its suspended solids removed by decantation into a tank whose temperature is regulated at 95° C. The supernatant is conducted by a continuous flow to a disc stack centrifuge and is spun at 6000 rpm. The oil produced is collected and stored in stainless steel tanks at a refrigerated temperature of 4° C. This production process may be recovered as an oil with between 30 to 40% of the total lipids of the krill.

Example 2.

Krill Oil.

Krill oil obtained by the process described in Example 1. The chemical composition of the different oils is shown below:

Components	Oils	
	1	2
Saturated fatty acids (%)	52.9	37.2
Monounsaturated fatty acids (%)	41.6	33.6
Polyunsaturated fatty acids (%)	5.8	11.8
w3/w6 ratio	1.9	3.2
Astaxanthin (ppm)	550.0	643.0

The krill oils of this example have a moisture ranging from between 0 and 1%, a peroxide index of between 1 and 5 meq/Kg, free acidity (expressed as an oleic acid percentage) between 0.2 and 1.5%, an iodine index of between 20 and 160, unsaponifiable wastes of between 0.5 and 3.5%, a saponification index between 150 and 200, and ash between 0 and 1%.

The fatty acids profile is determined by gas chromatography. This oil sample was hydrolyzed by incubation with 0.5N sodium hydroxide in methanol at 85° C for 10 min. Subsequently the respective methyl esters are formed through the addition of boron trifluoride in 20% methanol and further incubation at 100° C for 10 min. After saturation with sodium chloride, esterified fatty acids were extracted with petroleum ether and dehydrated with sodium sulfate. The derivatized sample is injected into the gas chromatograph with a capillary column (column of fused silica, CP-Sil 88 WCOT, ID 0.1 cm. 50 meters) and is resolved by a temperature range which ranged from 100 to 240° C. A flame detector (FID) was used and peaks were identified using standards. From the chromatogram obtained, the compositions of the saturated, monounsaturated, polyunsaturated and the w3/w6 ratio fatty acids were calculated.

The determination of astaxanthin in the krill oil of this invention was made under light-shielded conditions. Acetone was added to the krill oil and it was agitated for 15 min. From the acetone phase, it was transferred to a decantation funnel and a volume of petroleum benzine was added. After vigorous agitation, the samples were allowed to rest until the separation phases were complete. The petroleum benzine phase was extracted, washed with water and dried with anhydrous sodium sulfate. Once graduated to 100 ml with petroleum, the absorbency was determined of the samples at 460 nm. The amount of astaxanthin was calculated using the molar extinction coefficient of the pigment.

Example 3.

Oils obtained by cooking the krill at different temperatures.

The oils obtained by the process described in Example 1, where the cooking temperature of the krill was the only variation.

In the following Table, 2 oils obtained by cooking the krill at different temperatures (75° C y 95° C) are described.

	Content of Krill Oil Obtained by Cooking at 75° C	95° C
Fatty Acids		
Saturated fatty acids	45.3%	50.7%
Monounsaturated fatty acids	34.1%	38.7%
Polyunsaturated fatty acids	20.6%	10.6%
Fraction		
Triglycerides	59.8%	60.0%
Phospholipids	13.3%	5.2%

It may be seen in the Table that upon increasing the krill cooking temperature from 75 to 95° C, the content of polyunsaturated fatty acids in the oil is reduced by about half. This phenomenon is accompanied by a reduction in the phospholipid content from 13.3% to 5.2%. This reduction in the content of polyunsaturated fatty acids is compensated by an increase in the levels of saturated and monounsaturated fatty acids.

The fatty acid profile was determined as described in Example 2. The lipid fraction was determined by thin-layer chromatography (Merck Article No. 5724, DC-Fertigplatten Kieselgel 60) and developed in chloroform: acetone: methanol (94 : 5.5 : 0.5). Lipids were detected by using iodine vapors. 200 ul of oil samples dissolved in hexane were used for the determinations.

Example 4.

Fish food with pigmenting capacity.

Composition of a diet for salmonids which allows pigmentation of their flesh through the incorporation of krill oil containing astaxanthin, prepared according to Example 1.

61.0%	Fishmeal
26.9%	Wheat
5.0%	Krill Oil
6.0%	Fish oil
0.1%	Minerals
1.0%	Vitamins

This formulation makes it possible to prepare a diet with about 50 ppm of astaxanthin.

To prepare the diet, micronutrients were mixed with the wheat and fishmeal previously ground to a particle size that allows complete homogeneity of the sample. Subsequently, part of the oil was added and it was introduced into a pelletizing machine. Afterwards, the rest of the oil is added. After making the pellets, they are divided by size through a mesh. The salmon are fed this diet once they have a body weight of at least 200 grams, twice daily for a variable period that can last until they are harvested.

Example 5.

Nutritional Supplement

A food rich in fatty material to supplement essential fatty acids is described. Said food was formulated in the form of crackers created based on krill oil.

400 g of oats, 100 g of flour, 50 g of sugar, 1 egg, 150 mL of krill oil of this invention and 10 mL of vanilla extract are mixed together in a bowl. Once fully homogenized, the crackers are molded. Each of them weighs about 25 g, and they are baked for 15 min. Higher baking times destroy the astaxanthin pigment.

The amount of the food used in the diets will depend on the formulation thereof, on the amount of essential fatty acids required such as 18:2 and 18:3, and the calories which must be provided.

Example 6.

Stabilizer and antioxidant of fatty material.

It is possible to stabilize vegetable oils by adding krill oil, prepared according to example 1, which range from 1 to 10% (v/v). The stabilizing effect is due in part to the antioxidant activity present in the krill oil. The oil mix thus prepared has a light orange color due to the astaxanthin; the final concentration of which ranges between 4 and 160 ppm.

When the krill oil is added at 5% (v/v) in vegetable oil as the only stabilizing agent, and it is heated to 45° C for 48 hours, it was determined that the free acidity and the value of the peroxide index did not increase significantly.

Example 7.

Sunscreen

When krill oil is prepared according to Example 1, it may be used for the preparation of suntan creams and oils for protection against the sun.

A) Suntan Creams

2 suntan creams are described below, one with a sun protection factor of 5 (SPF5) and another with a sun protection factor of 20 (SPF20).

	Amount for 100g	
Component	**SPF 5**	**SPF 20**
Krill Oil	5.00 g	5.00 g
Carbopol 940	0.25 g	0.25 g
Cetyl Alcohol	0.50 g	0.50 g
Methyl Paraben	0.15 g	0.15 g
Stearic Acid III pr.	2.00 g	2.00 g
Propyl Paraben	0.15 g	0.15 g
Panalene	5.00 g	5.00 g
Disodium EDTA	0.10 g	0.10 g
Triethanolamine	0.50 g	0.50 g
Propylene Glycol	2.50 g	2.50 g
Octyl Methoxycinnamate	-	7.50 g
Benzophenone 3	-	3.00 g
Titanium Dioxide	-	0.50 g
Imidazolidinyl Urea	0.30 g	0.30 g
Glycerol Monostearate	1.00 g	1.00 g
Natural Essences	c.s.	c.s.
Isopropyl Myristate	2.00 g	2.00 g
Water	c.s.	c.s.

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B) Suntan Oils:

The following describes a formula for suntan oil that possesses krill oil as the only blocking agent.

Compound	Amount for 100 gms of Oil
Cosmetic liquid petrolatum	80.0 g
Hydrogenated Polybutenes	5.0 g
Krill Oil	14.7 g
Essence	0.3 g

Example 8.

Cosmetic Products based on of Krill Oil

Because of the multiple biological activities of krill, i.e., pigmenting ability, antioxidant, and its content of essential EPA fatty acids, it is possible to design a series of high value cosmetic products.

A) Moisturizing Cream.

Component	Amount for 100 gms of Cream
xxx Pressed Stearic Acid	1.0 g
A.E. Anionic. Glycerol Monostearate	0.7 g
Neutral Glycerol Monostearate	0.5 g
Hydrogenated Polybutenes	3.2 g
Krill Oil	10.0 g
Propylene Glycol	2.0 g
Isopropyl Myristate	1.5 g
Carboxyvinyl Polymer	0.3 g
Propylparaben	0.1 g
Methylparaben	0.1 g
Essence	0.3 g
Disodium EDTA	0.2 g
Triethanolamine 99%	1.2 g
Demineralized Water	78.9 g

B) Powdered Blush.

A formula base for creating a powdered blush that possesses krill oil added at 10% (p/p) is described.

Component	Amount for 100 gms of Blush
Talc	52.56g
Krill Oil	10.00 g
Mica : Titanium Dioxide (2 : 1)	26.00 g
Magnesium Stearate	3.50 g
Isopropyl Myristate	1.60 g
Oleic Alcohol	2.60 g
Octyl Palmitate	3.40g
Methylparaben	0.17g
Propylparaben	0.17g

C) Powdered Eye Shadow

A formula base for creating a powdered eye shadow that possesses krill oil added at 10% (p/p) is described.

Component	Amount for 100 gms of Eye Shadow
Talc	51.46g
Krill Oil	10.00 g
Mica : Titanium Dioxide (3 : 1)	27.00 g
Magnesium Stearate	3.50 g
Isopropyl Myristate	1.60 g

Oleic Alcohol	2.60 g
Octyl Palmitate	3.50g
Methylparaben	0.17g
Propylparaben	0.17g

D) Cream Eye Shadow.

A formula base for creating a cream eye shadow that possesses krill oil added at 5.7% (p/p) is described.

Component	Amount for 100 gms of Eye Shadow
Talc	4.0 g
Stearic Acid	9.5 g
Isostearic Acid	1.9 g
Krill Oil	5.7 g
Titanium Dioxide	1.9 g
Aluminum and Magnesium Silicate	3.6 g
Propylene Glycol	16.1 g
Triethanolamine	11.0 g
Methylparaben	0.2 g
Propylparaben	0.2 g
Deionized Water	45.9 g

E) Compact Powder.

A formula base for creating a compact powder that possesses krill oil added at 10% (p/p) is described.

Component	Amount for 100 gms of Compact Powder
Kaolin	32.0 g
Krill Oil	10.0 g
Mica : Titanium Dioxide (2:1)	1.6 g
Magnesium Stearate	4.1 g
Octyl Palmitate	2.5 g
Propylparaben	0.5 g
Talc	49.3 g

F) Lipgloss.

A formula base for creating a lipgloss that possesses krill oil added at 3% (p/p) is described.

Component	Amount for 100 gms of Lipgloss
Castor Oil	42.04 g
Krill Oil	3.00 g
Oleic Alcohol	2.60 g

Lanoline	25.00 g
Sorbitan Monostearate	1.30 g
Ozokerite	4.50 g
Carnauba Wax	5.20 g
Bees Wax	5.60 g
Stearic Acid	4.90 g
Candelilla Wax	5.60 g
Methylparaben	0.13 g
Propylparaben	0.13 g

CLAIMS

1. A krill oil, that is equivalent to a fraction of the total of the krill lipids, **CHARACTERIZED** in that it is extracted by thermal fractionation and centrifuging and/or decanting which allows polyunsaturated fatty acids to reach up to 95% of the amount found in the total lipids of krill, and because it possesses between 26 and 60% in saturated fatty acids, between 25 and 60% in monounsaturated fatty acids, between 4 and 46% in polyunsaturated fatty acids, and the pigment carotenoid astaxanthin, in an amount ranging between 300 and 2,600 ppm.

2. **A krill oil** according to Claim 1, **CHARACTERIZED** in that content of saturated, monounsaturated and polyunsaturated fatty acids may be regulated by varying the krill cooking temperature in the production process for the oil.

3. Krill oil of Claim 1, **CHARACTERIZED** in that because within its fractional composition triglycerides stand out, from between 35 and 96%, and phospholipids, between 4 and 28%.

4. Krill oil of Claim 1, **CHARACTERIZED** in that the pigment of astaxanthin is in free form, between 0 to 15%, in a monoesterified state, between 25 and 50%, and in a diesterified stated, between 40 and 80%.

5. A procedure for the industrial production of the krill oil of Claim 1, **CHARACTERIZED** in that it includes the following stages:

a) Cooking the krill
b) Pressing the precooked krill
c) Collection and accumulation of press liquor
d) Removal of suspended solids
e) Centrifugation and separation of oil
f) Addition of stabilizers
g) Storage

6. A procedure for the industrial production of the krill oil of Claim 5, **CHARACTERIZED** in that the whole krill, or parts thereof, used for this process may be Euphasia superba, E. crystallorophias, E. frigid, E. tricantha, E. vellantini, E. loueirostris, E. lucens, E. similis, E. spinifera, E. recurva, E. pacifica, Thysanoessa macrura, T. vicinia, T. gregaria, T. raschii and/or T. inermis.

7. A procedure for the industrial production of the krill oil according to Claim 5, **CHARACTERIZED** in that the cooking step is a process, continuous or batch, where heat is applied in the form of steam, hot water immersion or by direct heat or fire for 2 to 80 minutes and more specifically from 5 to 25 minutes at a temperature of between 40 to 200° C, wherein the amount of polyunsaturated fatty acids of krill oil is regulated by varying the cooking temperature in the production process, obtaining oils with high levels of polyunsaturated fatty acids when using low temperatures

8. A procedure for the industrial production of the krill oil according to Claims 5 and 7, **CHARACTERIZED** in that more than one successive cooking operation of the krill may be performed, using temperatures ranging from 40 to 200° C at any of the stages of cooking, with or without storage at room temperature, refrigerated, or frozen between cooking.

9. A procedure for the industrial production of the precooked krill oil in accordance with Claim 5, **CHARACTERIZED** in that pressing is performed in discontinuous batches or in a continuous flow through any type of press, including those with single or double helical screw with a variable mesh, obtaining a pressed cake with moisture between 25 to 80%.

10. A procedure for the industrial production of krill oil in which the collection and accumulation stage of the press liquor in accordance with Claim 5, **CHARACTERIZED** in that the collection is performed by gravity or by using a pump with low turbulence and the liquor collected requires regulating the temperature to be between room temperature and 95° C.

11. A procedure for the industrial production of krill oil in which the accumulation stage of the press liquor in accordance with Claims 5 and 10, **CHARACTERIZED** in that the accumulated press liquor can be treated to remove soluble proteins through coagulation by heating in the accumulation tank followed by pressing, decanting, filtering or rotary drained.

12. A procedure for the industrial production of krill oil in which the accumulation stage of the press liquor in accordance with Claims 5 and 10, **CHARACTERIZED** in that the press liquor can be treated accumulated proteolytically to remove soluble proteins, with the addition of a protease, preferably an endoprotease, in amounts ranging from 0.001 to 5% (w/v), depending on the enzyme used and the amount of proteins contained in the press liquor, and incubated between 4 and 90° C, preferably between 30 and 75° C for 5 minutes to 80 hours, preferably between 30 minutes and 8 hours.

13. A procedure for the industrial production of krill oil in which the proteolytic treatment of the press liquor in accordance with Claim 12, **CHARACTERIZED** in that the protease may be selected from between proteases such as acrosin, alcalase, urokinase, uropepsina, elastase, enteropeptidase, cathepsin, kallikrein, kinase 2, chymotrypsin, chymopapain, collagenase, streptokinase, subtilisin, thermolysin, trypsin, thrombin, papain, and pancreatopeptidasa renin; peptidases such as aminopeptidases, for example, arginine aminopeptidase, leucine aminopeptidase and oxytocinase; angiotensinase, angiotensin converting enzyme, insulinase, carboxypeptidase, for example, arginine carboxypeptidase, kinase 1 and thyroid peptidase, dipeptidases, for example, carnosinase and prolinase and pronases.

14. A procedure for the industrial production of krill oil in which the removal stage for suspended solids in accordance with Claim 5, **CHARACTERIZED** in that it is performed by gravity decantation, filtration or a rotary drain.

15. A procedure for the industrial production of krill oil in which the centrifuging and separation stage for the oil in accordance with Claim 5, **CHARACTERIZED** in that it is performed, in batches or in a continuous flow through a disc stack centrifuge, at between 2,000 and 20,000 rpm, and the temperature of which can range from between room temperature and 100° C and more specifically between 50 and 98° C.

16. A procedure for the industrial production of krill oil in which the separation stage for the oil in accordance with Claim 5, **CHARACTERIZED** in that the centrifugation may be replaced by a decanter, either in batches or in a continuous flow, especially a two-phase decanter.

17. A procedure for the industrial production of krill oil in which the stabilization stage for the krill oil in accordance with Claim 5, **CHARACTERIZED** in that it adds antioxidants and/or preservatives.

18. A procedure for the industrial production of krill oil in which the storage stage for the krill oil in accordance with Claim 5, **CHARACTERIZED** in that it is carried out in plastic or metal tanks, especially stainless steel, at room temperature or refrigerated, and protected from the light.

19. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used to pigment meat products by adding it to diets of crustaceans, mollusks, fish, poultry and their eggs, pigs, goats, sheep, cattle, and other mammals.

20. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used to prepare a nutritional supplement in human or animals diets in formulation of foods since it supplements essential fatty acids, polyunsaturated fatty acids, triglycerides and phospholipids.

21. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used for the stabilization of fats due to its addition to oils or fats of animal and/or vegetable origin in varying amounts.

22. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used for the preparation of pharmaceutical or cosmetic products that have a photoprotective capacity, especially to block ultraviolet radiation.

23. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used as a natural antioxidant in foods or pharmaceutical and/or cosmetic products.

24. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used as a source of astaxanthin or it may be used directly as a natural pigment in foods and in pharmaceutical and/or cosmetic products.

25. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used as a dissolving agent and/or a carrier for lipophilic compounds to be used in chemicals, pharmaceuticals, foods, and cosmetics.

26. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used to prepare diets as an agent that increases the palatability of food for human and animal nutrition.

27. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used to prepare diets, additives and/or pharmaceutical products that are useful to improve the biological properties and survival of the eggs of fish and shellfish.

28. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used for the preparation of diets that are useful for treating cardiovascular diseases, as a source of eicosapentaenoic acid (EPA).

29. Use of the krill oil of Claim 1, **CHARACTERIZED** in that it may be used to prepare dermatological products, especially topical or systemic products for treating skin diseases where there is a deficiency in essential fatty acids, such as xerotic skin, hyperkeratosis, ichthyosis, acne, psoriasis, skin ulcers, seborrhoeic eczema, atopic dermatitis, vitiligo.

INSTRUCCIONES:
1.- LLENE SOLAMENTE LOS RECUADROS DE TONO ROSADO CON CARACTERES NEGROS DE MAQUINA (NO MANUSCRITO)
2.- SE ENTIENDE POR PRIORIDAD AQUELLA PROTECCION SOLICITADA O CONCEDIDA ANTERIORMENTE POR EL MISMO INVENTO, GENERALMENTE EN EL EXTRANJERO

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TITULO O MATERIA DE LA SOLICITUD

ACEITE DE KRILL Y UN PROCEDIMIENTO PARA OBTENERLO POR FRACCIONAMIENTO TERMICO Y CENTRIFUGACION.

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DECLARO/DECLARAMOS QUE LOS DATOS QUE APARECEN EN LOS RECUADROS DE TONO ROSADO SON VERDADEROS Y TAMBIEN CONOCER EL ART. 44 DE LA LEY Nº 19.039 SOBRE PROPIEDAD INDUSTRIAL Y QUE EL PRESENTE DOCUMENTO CONSTITUYE UNA SOLICITUD FORMAL.

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FIRMA Y R.U.T. REPRESENTANTE

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FIRMA Y R.U.T. SOLICITANTE

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(54) Título de la Invención: (máximo 330 caracteres)

Aceite de krill, procedimiento para su obtención por fraccionamiento térmico y centrifugación : aceite de alto contenido de ácidos grasos insaturados útil como fuente de astaxantina, complemento nutricional, cosmético, fotoprotector, en la preparación de productos dermatológicos, estabilizante de materias grasas.

(57) Resumen: (máximo 1600 caracteres)

Aceite proveniente de un crustáceo marino conocido como krill y su procedimiento para producirlo por fraccionamiento térmico y centrifugación y/o decantación. Se describe un aceite con un contenido variable de ácidos grasos poliinsaturados. Este aceite se caracteriza por poseer hasta un 95% del contenido de ácidos grasos poliinsaturados presente en los lípidos de krill, y su composición es ácidos grasos saturados entre un 26 y 60%, ácidos grasos monoinsaturados entre un 25 y 60%, ácidos grasos poliinsaturados entre un 4 y 46%, y astaxantina entre 300 y 2.600 ppm. Además, se describen usos de este aceite como pigmentante, complemento nutricional, estabilizantes de otras materias grasas, antioxidante, fotoprotector, fuente de astaxantina, agente palatable, agente disolvente y/o vehículo de compuestos lipofílicos, agente protector de ovas de peces y huevos de moluscos y crustáceos, fuente de EPA (ácido eicosapentaenoico), y fuente de principios activos para la preparación de productos dermatológicos.

La producción del aceite de la presente invención se logra cociendo el krill a distintas temperaturas, seguido de un prensado del krill precocido, una recolección y acumulación del licor de prensa, eliminación de sólidos suspendidos y una centrifugación para lograr la separación del aceite. La cantidad de ácidos grasos poliinsaturados puede regularse variando la temperatura de cocción en el proceso de producción, así al usar bajas temperaturas se obtienen aceites con altos niveles de ácidos grasos poliinsaturados.

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MEMORIA DESCRIPTIVA

RESUMEN DE LA INVENCION

La presente invención se refiere a un aceite proveniente de un crustáceo marino conocido como krill, y aun procedimiento para producirlo. Hasta ahora no se ha descrito un proceso para la producción industrial de aceite de krill. En esta invención se describe un aceite con un contenido variable de ácidos grasos poliinsaturados. La composición química de este aceite se caracteriza por poseer hasta un 95% del contenido de ácidos grasos poliinsaturados presente en los lípidos de krill. La cantidad de ácidos grasos poliinsaturados puede regularse a voluntad variando la temperatura de cocción en el proceso de producción de dicho aceite. Además, en esta patente se describen usos de este aceite como pigmentante, complemento nutricional, estabilizante de otras materias grasas, antioxidante, fotoprotector, fuente de astaxantina, agente palatable, agente disolvente y/o vehículo de compuestos lipofílicos, agente protector de ovas de peces y huevos de moluscos y crustáceos, fuente de EPA (ácido eicosapentaenoico), y fuente de principios activos para la preparación de productos dermatológicos.

La producción del aceite de la presente invención se logra cociendo el krill a distintas temperaturas, seguido de un prensado del krill precocido, una recolección y acumulación del licor de prensa, eliminación de sólidos suspendidos y una centrifugación para lograr la separación del aceite. Cuando la cocción del krill se realiza a bajas temperaturas se obtienen aceites con altos niveles de ácidos grasos poliinsaturados.

ANTECEDENTES GENERALES

El krill corresponde a un grupo de crustáceos marinos del orden Euphasiaceae. El krill Antártico, en particular aquel que habita en las zonas antárticas y sub-antárticas, está compuesto por 11 especies de Euphasia, siendo dominantes las especies E. superba y E. crystallorophias.

En años recientes el krill ha acaparado creciente interés como una fuente potencial de proteínas y otros productos biológicos activos (Ellingsen, T. y Mohr, V. 1979. Process Bioch. 14:14; Suzuki, T. 1987. Tecnología de las proteínas de pescado y krill. Editorial Acribia S.A.). Las grandes espectativas cifradas en el krill se basan en la gran biomasa que existe en los océanos antárticos, calculándose entre 100 a 500 millones de toneladas. Se ha estimado que las capturas de este crustáceo podrían ascender a unas 50-100 millones tons/año, cantidad que equivale al total anual de captura de pescado en todo el mundo (Budzinski, E., Bykowski, P. y Dutkiewicz, D. 1986. Posibilidades de elaboración y comercialización de producos preparados a partir de krill del Antártico, FAO Doc. Téc. Pesca (268):47p).

Existen numerosas publicaciones relacionadas con el contenido de lípidos y la composición de los lípidos de krill (Grantham, G.J. 1977. La utilización del krill. Programa de reconocimiento pesquero en los Mares Australes. FAO GLO/SO/77/3:63p; Budzinski y cols. 1986. Loc. cit.; Ellingsen y Mohr. 1979. Loc. cit.).

El contenido de lípidos oscila entre un 10-26% del peso seco, dependiendo de la estación del año, la madurez sexual y el tamaño corporal del krill. El krill hembra contiene alrededor del doble de lípidos que el macho. Las concentraciones de lípidos aumentan con la edad y después del desove disminuyen rápidamente. El estudio de distribución de los lípidos en krill indican que las áreas ricas en lípidos se encuentran localizadas a lo largo del tracto

digestivo, entre los haces musculares y debajo del exoesqueleto (Saether, O., Ellingsen, T. y Mohr, V. 1985. Comp. Biochem. Physiol. 81B:609).

Las principales fracciones de lípidos corresponden a triglicéridos y fosfolípidos, así como esteroles y sus ésteres (Grantham. 1977. Loc. cit.; Budzinski y cols. 1986. Loc. cit.). La fracción de fosfolípidos de krill es rico en ácidos grasos poliinsaturados y particularmente 20:5 y 22:6, que dan cuenta de aproximadamente de un 50 % del total de fosfolípidos. La alta proporción de ácidos grasos poliinsaturados en la fracción fosfolipídica podría ser necesaria para mantener la fluidez de las membranas plasmáticas en las bajas temperaturas de las aguas antárticas. Es también posible que el alto grado de insaturación se requiera para dar a los depósitos de fosfolípidos presentes en el krill, suficiente plasticidad para permitir al animal flectarse y moverse a bajas temperaturas.

Un aumento de los lípidos totales va acompañado de una disminución de los fosfolípidos y de un aumento de los triglicéridos. Existe una correlación casi lineal entre los contenidos de fosfolípidos y triglicéridos y el total de lípidos, lo que puede significar que ambos grupos tienen una función en la reserva de energía. El contenido de ácidos grasos polinsaturados disminuye al aumentar el contenido total de lípidos (Saether y cols. 1985. Loc. cit.).

Cambios que ocurren en los lípidos de krill post-mortem demuestran que la cantidad de ácidos grasos poliinsaturados (20:5, 22:6) en relación con el contenido de ácidos grasos (16:0) no disminuye durante el almacenamiento de krill a 0°C. Estos datos parecen indicar que no ocurre una gran oxidación de los lípidos poliinsaturados después de la muerte del crustáceo.

A pesar de muchos ensayos y de operaciones adicionales, hasta el momento no ha sido posible obtener aceite de krill por centrifugación con equipo tradicional. Todos los procedimientos de extracción de lípidos de krill hasta ahora descritos se basan en extracciones

suscesivas con distintos solventes orgánicos (Budzinski y cols. 1986. Loc. cit.; Saether y cols. 1985. Loc. cit.). Por otra parte, en la patente chilena N° 33688, se describe un procedimiento para la obtención de proteína, aceite y caparazón de krill. En dicha patente la obtención del aceite de krill se realiza como un subproducto de la obtención de una pasta proteica de krill. Las etapas involucradas en dicha patente corresponden a desintegración mecánica de la materia prima, centrifugación, separación de la fracción cárnea, coagulación de la proteína por calor a 70 - 95°C y separación de una corriente residual que contendría un producto oleoso. En la patente N°33688 no se describen la separación del aceite de la corriente residual, ni tampoco las características de este aceite.

LA INVENCIÓN

La presente invención comprende a un aceite de krill con una composición de ácidos grasos poliinsaturados variable, un procedimiento para obtenerlo por fraccionamiento térmico y centrifugación, y usos de este aceite para diversos fines. El fraccionamiento térmico permite regular el grado de insaturación del aceite de krill de la presente invención.

El aceite de krill descrito en esta invención posee un menor contenido de ácidos grasos poliinsaturados (entre un 4 a un 46%) que los lípidos totales de krill (entre un 31 a un 48%), pudiendo alcanzar hasta un 95% de los ácidos grasos poliinsaturados presentes en los lípidos totales de krill. Esta menor cantidad de ácidos grasos poliinsaturados es compensado por un aumento en la proporción de ácidos grasos saturados y monoinsaturados (ver tabla I).

Tabla I

Composición de ácidos grasos en los lípidos totales de krill y en el aceite de la presente invención.

Contenido de ácidos grasos	**Lípidos totales de krill***	**Aceite de la presente invención**
Saturados	25 - 37 %	26 - 60%
Monoinsaturados	24 - 33 %	25 - 60%
Poliinsaturados	31 - 48 %	4 - 46%

* Grantham, G.J. 1977. La utilización del krill. Programa de reconocimiento pesquero en los Mares Australes. FAO GLO/SO/77/3:63p. Ellis, J. y Roch, M. 1984. Krill. Canadian Aquaculture. pp 45-46.

Al estudiar las fracciones lipídicas que componen al aceite de la presente invención se determinó que además posee un menor contenido de fosfolípidos (entre un 4 a un 28%) al compararlo con los lípidos totales de krill (entre un 13 a un 31%). Esta disminución en el contenido de los fosfolípidos va acompañada de un aumento importante sólo en la fracción de triglicéridos (ver tabla II).

Tabla II

Composición fraccionaria de los lípidos totales de krill y del aceite de la presente invención.

Fracciones lipídicas	**Lípidos totales de krill***	**Aceite de la presente invención**
Fosfolípidos	13 - 31%	4 - 28%
Monoglicéridos	2 - 9%	3 - 10%
Diglicéridos	1 - 4%	1 - 5%
Triglicéridos	30 - 60%	35 - 96%
Esteroles	5 - 9%	4 - 8%
Esteres de esteroles	6 - 15%	6 - 15%

*Budzinski, E., Bykowski, P. y Dutkiewicz, D. 1986. Posibilidades de elaboración y comercialización de producos preparados a partir de krill del Antártico, FAO Doc. Téc. Pesca (268):47p.

La diferencia tanto de la composición química como de la distribución fraccionaria entre el aceite de la presente invención y la de los lípidos totales de krill se debe al

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procedimiento de producción del aceite descrito en la presente invención. Este procedimiento extrae entre un 10 a un 95% de los lípidos totales de krill, favoreciendo la extracción de las fracciones de triglicéridos por sobre las fosfolipídicas. Debido a que los ácidos grasos poliinsaturados están principalmente asociados a la fracción de fosfolípidos (Grantham. 1977. Loc. cit.; Budzinski y cols. 1986. Loc. cit.) el procedimiento descrito en la presente invención produce aceites con menor contenido de ácidos grasos poliinsaturados que los lípidos totales de krill.

El aceite de esta invención se caracteriza además por poseer el pigmento carotenoide astaxantina, en cantidades que varían entre un 300 y 2.600 ppm, más frecuentemente entre 450 y 1.200 ppm. Este pigmento se encuentra en forma libre (entre un 0 a un 15%), mono esterificado (entre un 25 y un 50%), y diesterificado (entre un 40 y un 80%).

Como se señaló anteriormente, el contenido y la composición de lípidos en el krill depende de varios factores. Por ello la composición del aceite, así como su rendimiento de producción depende, entre otros, de la época de captura del krill. En esta invención se determinó que los niveles máximos de producción se pueden alcanzar capturando y procesando krill en el período comprendido entre el apareamiento y el desove del crustáceo. En la presente invención no se excluye la posibilidad de capturar y procesar krill para la obtención de aceite en períodos diferentes a los antes señalados, obteniéndose rendimientos de aceites y composiciones de ácidos grasos diferentes a los descritos en esta invención.

El krill utilizado en la presente invención no está limitado a tipos específicos y puede corresponder a cualquiera de las especies de krill pertenecientes al género Euphasia, como por ejemplo superba, crystallorophias, frigida, tricantha, vellantini, lougirostris, lucens, similis, spinifera, recurva, pacifica y otros, y aquellas pertenecientes al género Thysanoëssa, por ejemplo macrura, vicina, gregaria, raschii, inermis y otros.

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El método de producción de aceite de krill se basa en las siguientes etapas:

a) Cocción del krill.

b) Prensado del krill precocido.

c) Recolección y acumulación del licor de prensa.

d) Eliminación de sólidos suspendidos.

e) Centrifugación y separación del aceite.

f) Adición de estabilizantes.

g) Almacenamiento.

a) Cocción del krill: El krill entero o parte del krill, como por ejemplo el cefalotórax, es tratado por una cocción. La cocción puede ser realizada a través del tratamiento del krill o partes del krill con vapor de agua, sumergiéndolo en agua caliente o por tratamiento directo con calor o fuego, no excluyéndose otros métodos de calentamiento y cocción. Este proceso puede ser realizado por un sistema de lotes discontínuos o por un sistema de flujo contínuo. En el caso de los lotes, éstos deben ser de tal tamaño que la temperatura de cocción se alcance en el total de la masa en no más de 2 min. En el proceso contínuo, la velocidad de flujo de krill debe permitir que el crustáceo alcance la temperatura de cocción en no más de 2 minutos. La realización de la cocción de krill por el proceso contínuo permite procesar 5 a 200 kilos de materia prima por minuto. La temperatura de cocción requerida para este proceso oscila entre 40 a 200°C. El tiempo que dura la cocción puede oscilar entre 2 a 80 minutos, más específicamente entre 5 y 25 minutos. La temperatura de cocción se regula controlando la temperatura de la materia prima a la salida del cocedor a través de una termocupla,

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termómetro u otro instrumento para medir temperaturas. La temperatura del krill a la salida del cocedor puede oscilar entre 30 y 100°C.

En esta invención no se excluye la posibilidad de realizar en esta primera etapa de producción más de una etapa de cocción. De hecho, es posible realizar una primera cocción del krill a una temperatura, cuyo rango puede oscilar entre 30 a 200°C, y realizar posteriormente una o más cocciones a temperaturas que sean iguales, menores o mayores que la primera cocción, en un rango que oscila entre 30 y 200°C. Cada etapa de cocción puede o no estar separada por almacenamientos del material precocido a temperatura ambiente, refrigerado o congelado, por tiempos variables que oscilan, por ejemplo, entre 5 segundos y 2 años.

b) Prensado del krill cocido. El krill cocido es prensado por lotes discontínuos o por lotes contínuos, a través de un doble o simple tornillo helicoidal de malla variable. En los sistemas de prensado a través de prensas con tornillos helicoidales, la velocidad de prensado pueden regularse desde 1 a 100 rpm, más específicamente entre 5 y 50 rpm, por medio de motovariadores independientes regulados entre 1 y 90°. Los volúmenes de prensado varían de acuerdo al tamaño de la prensa. El producto sólido resultante de la prensa debe tener un porcentaje de humedad entre un 25 y un 80%, más específicamente alrededor de un 50 a 60%.

Variando las condiciones de prensado anteriormente mencionadas, es posible obtener tortas de prensado con valores de humedad entre un 25 a un 50%, procedimiento que también está considerado en esta invención.

Esta etapa de prensado puede ser realizada en forma separada de la etapa de cocción, o bien acoplada, formando un proceso enteramente contínuo.

c) Recolección y acumulación del licor de prensa. El líquido de prensado es recolectado mediante gravedad o por bombas a un estanque de almacenamiento. Durante este almacenamiento se produce un proceso de decantación de los sólidos más grandes que hallan provenido como contaminación del proceso de prensado. Las bombas utilizadas deben producir muy baja turbulencia para impedir la formación posterior de emulsiones durante la recolección. El estanque de almacenamiento es sometido a un calentamiento entre temperatura ambiente y 100°C.

En esta invención también se determinó que se puede eliminar la proteina soluble del licor de prensa a través de un calentamiento gradual hasta alcanzar la temperatura de coagulación de la proteína soluble. La proteína coagulada puede separarse por un nuevo tratamiento de prensado o a través de los procesos de eliminación de sólidos suspendidos. Alternativamente la proteína soluble puede eliminarse a través de su hidrólisis mediante el uso de proteasas. Ejemplo de proteasas que pueden usarse incluyen a proteinasas tales como acrosina, alcalasa, uroquinasa, uropepsina, elastasa, enteropeptidasa, catepsina, kalicreina, kiniasa 2, quimotripsina, quimopapaína, colagenasa, estreptoquinasa, subtilisina, termolisina, tripsina, trombina, papaina, pancreatopeptidasa y renina; peptidasas tales como aminopeptidasas, por ejemplo, arginina aminopeptidasa, oxitocinasa y leucina aminopeptidasa; angiotensinasa, enzima convertidora de angiotensina, insulinasa, carboxipeptidasa, por ejemplo, arginina carboxipeptidasa, kiniasa 1 y tiroide peptidasa, dipeptidasas, por ejemplo, carnosinasa y prolinasa y pronasas; así como otras proteasas no mencionadas aquí. Estas proteasas pueden clasificarse como exopeptidasas, si degradan la proteína desde el extremo amino o desde el extremo carboxilo, o endopeptidasas, si degradan a la proteína en su parte interna, siendo estas últimas las preferidas para aplicarse en esta invención. La proteasa puede aplicarse entre 4 y 90°C, preferentemente entre 30 y 75°C, por 5 minutos a 80 horas,

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preferentemente entre 30 minutos a 8 horas. La enzima puede emplearse en cantidades que oscilan entre 0,001 a 5% (p/v), dependiendo de la enzima utilizada y de la cantidad de proteínas contenido en el licor de prensa.

En la presente invención se determinó además, que es posible realizar el proceso sin someter a calentamiento o tratamiento proteolítico posterior del licor de prensado durante el almacenamiento.

d) Eliminación de sólidos suspendidos. Los licores de prensa obtenidos por el procedimiento de esta invención posee sólidos suspendidos hasta un máximo de un 20%, que pueden ser eliminados a través de decantación por gravedad, filtración o un drenador rotatorio. El líquido de prensado sometido a los procesos de eliminación de sólidos poseen normalmente un máximo de un 1% de sólidos en suspensión.

e) Centrifugación y separación del aceite. El líquido de prensado con un sólido suspendido menor o igual a un 1% es sometido a un proceso de centrifugación, que puede realizarse en forma de lotes discontínuos o en forma contínua a través de una centrífuga. Se puede utilizar cualquier tipo de centrífuga y en especial una centrífuga de discos. La velocidad de centrifugación puede oscilar entre 2.000 a 20.000 rpm, más específicamente entre 5.000 y 12.000 rpm. La temperatura de centrifugación puede oscilar entre la temperatura ambiente y 100°C y más específicamente entre 50 y 98°C.

La obtención del aceite puede lograrse también por separación de fases por decantación y aplicación conjunta de temperatura en rangos que oscilarían entre ambiente y 100°C. Las fases susperiores corresponderían al aceite de krill. El decantador puede ser un decantador contínuo, por ejemplo un decantador de dos fases.

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f) Adición de estabilizantes. Una vez separado el aceite, es posible añadirle diversos estabilizantes como antioxidantes, preservantes, entre otros, sin excluir todos aquello compuestos que de alguna manera u otra aumentan la estabilidad y la vida útil del aceite. Algunos ejemplos corresponderían a etoxiquina, vitamina E (dl-alfa-tocoferol), ácido ascórbico, ascorbato de sodio, ácido norhidroguaiareico, propil galato, resina guaiaca, butil hidroxianisol, dibutilhidroxitolueno, butil hidroquinona, tetrabutilhidroquinona, ácido eritórbico, eritorbato de sodio, entre otros, sin excluir otros posibles aditivos.

g) Almacenamiento. El aceite producido puede ser almacenado en estanques de plásticos o metal, de preferencia acero inoxidable, ya sea refrigerado o a temperatura ambiente, y protegido de la luz.

En la presente invención se determinó que la composición química y la distribución fraccionaria de los lípidos del aceite de la invención puede variarse alterando la temperatura de cocción del krill. Las temperaturas bajas de cocción producen licores de prensa con mayor contenido de proteínas solubles y un mayor contenido de grasas que aquellos obtenidos a temperaturas de cocción elevadas. Este aumento de grasa en el licor de prensa, provocado por temperaturas menores de cocción, trae consigo un aumento en el rendimiento de producción de aceite de la invención. Paralelamente a este aumento de rendimiento se observa un aumento en su contenido de fosfolípidos y en ácidos grasos poliinsaturados. De esta manera es posible controlar la composición química y la distribución fraccionaria del aceite de la invención controlando la temperatura de cocción del krill durante el proceso de producción.

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En general es posible obtener otras composiciones químicas y distribuciones fraccionarias del aceite de krill de esta invención, realizando más de una cocción en la primera etapa de producción del aceite de krill. Cocciones sucesivas, con o sin eventos de almacenamiento a temperatura ambiente, refrigerado o congelado, usando de temperaturas que oscilan entre 30 y 200°C en cualquiera de las etapas de cocción, permiten variar la eficiencia de extracción de fosfolípidos, basado en el mismo principio antes señalado.

El aceite caracterizado en esta invención y producido por el procedimiento descrito, posee diversos usos. A modo de ejemplo se señalan los siguientes:

a) Pigmentante de productos cárneos. Al ser incluido en dietas de peces, por ejemplo salmones y truchas, crustáceos, moluscos, aves y sus huevos, cerdos, caprino, ovino, bovino y otros mamíferos, es posible pigmentar su carne desde un tono rosado pálido, naranjo, a un rojo intenso de acuerdo con las cantidades del pigmento carotenoide astaxantina añadida a la dieta y a la retenida por el animal.

b) Complemento nutricional para diversos animales, peces e incluido el hombre. Como fuente de diversos ácidos grasos esenciales, como aporte calórico extra, y como fuente de ácidos grasos poliinsaturados del tipo w3, w6 y w9, así como de fosfolípidos y triglicéridos.

c) Estabilizante de otras materias grasas. La presencia del pigmento astaxantina y otros componentes del aceite de krill le proporciona una gran estabilidad, en especial a los fenómenos de lipoperoxidación que conducen a la rancidez oxidativa de los aceites. De esta manera su adición a otros aceites, ya sea de origen marino, animal, o vegetal, puede aumentar

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sus respectivas estabilidades. Este procedimiento sería considerado "natural" debido a que no requeriría de otros aditivos químicos como antioxidantes.

d) Fotoprotección. El aceite de krill descrito en esta invención puede ser utilizado para preparar composiciones farmacéuticas que posean efectos fotoprotectores, debido a la capacidad que tienen el pigmento astaxantina de capturar la radiación ultravioleta. De esta manera el aceite se puede utilizar para formular preparados cosméticos o farmacéuticos tales como por ejemplo, bronceadores, cremas humectantes, lápices labiales, entre otras posibilidades, sin excluir otras aplicaciones en esta área.

e) Antioxidante. El aceite de krill puede ser utilizado como un antioxidante natural, y como tal se usaría para evitar oxidaciones en diversos productos alimenticios, productos farmacéuticos, cosméticos u otros productos que así lo requiera, o para preparar productos farmacéuticos o cosméticos que posean actividad antioxidante.

f) Fuente de astaxantina. Gracias al alto contenido de astaxantina en este aceite, la presente invención puede ser utilizada como una fuente natural del pigmento, ya sea para ser purificada a partir de él o para ser utilizada directamente como pigmentante. Sus usos en esta área sería para colorear alimentos desde tonos rosas, naranjas y rojos. Además se pueden utilizar para colorear preparados farmacéuticos y cosméticos tales como cápsulas, tabletas, obleas, ampollas, rubores, lápices labiales, polvos compactos, sombras, shampoos, y muchos otros.

g) Agente disolvente y/o vehículo de compuestos lipofílicos. El aceite de krill puede ser usado como agente disolvente y/o vehículo de compuestos lipofílicos de uso químico, farmacéutico,

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alimento y cosmético, gracias a su naturaleza hidrofóbica.

h) Agente palatable. El aceite de krill de esta invención puede ser usado para la elaboración de dietas como agente que aumenta la palatibilidad en alimentos destinados a nutrición animal y humana.

i) Protección de ovas de peces, y huevos de moluscos y crustáceos. Las propiedades antioxidantes y de fotoprotección del aceite de krill, permite que las ovas de peces y los huevos de moluscos y crustáceos que han sido alimentados con aceite de krill de la presente invención, mejoren sus características biológicas aumentando su sobrevida.

j) Fuente de EPA (ácido eicosapentaenoico). El aceite de krill de la presente invención posee ácidos grasos poliinsaturados, cuyo contenido varía entre un 5 a un 30%, dependiendo del proceso. Se ha determinado que EPA puede llegar a constituir el 50% de los ácidos grasos poliinsaturados del aceite de la presente invención y por ello es una excelente fuente para su obtención. El EPA así obtenido puede ser usado para la manipulación dietaria de enfermedades cardiovasculares.

k) Preparación de productos dermatológicos. El contenido de ácidos grasos esenciales, en especial C18:2 w6 y C18:3 w3, así como de EPA, permite que el aceite de la presente invención pueda ser usado para la preparación de productos farmacológicos tópicos o sistémicos para el tratamiento de enfermedades de la piel en donde exista deficiencia de ácidos grasos esenciales. De esta manera se prevee su uso en enfermedades como: piel xerótica,

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hiperqueratosis, ictiosis, acné, psoriasis, úlceras dérmicas, eczema serborreica, vitiligo, dermatitis atópica, entre otras.

Ejemplo 1.

Producción de aceite de krill.

Krill recién capturado es cocido en una banda contínua con vapor de agua por 15 minutos alcanzándose una temperatura de cocción entre 90 y 95°C. El krill cocido es prensado a través de un doble tornillo helicoidal de malla variable, produciendo una torta de prensa con una humedad entre un 50 a un 60%. El líquido de prensado es rápidamente recolectado por gravedad, y sus sólidos suspendidos eliminados por decantación en un estanque cuya temperatura es regulada a 95°C. El sobrenadante es conducido por flujo contínuo a una centrífuga de discos y centrifugado a 6000 rpm. El aceite producido es recolectado y almacenado en estanques de acero inoxidable en refrigeración a 4°C. Este proceso de producción permite recuperar como aceite entre el 30 al 40% de los lípidos totales del krill.

Ejemplo 2.

Aceite de krill.

Aceite de krill obtenido por el proceso descrito en el ejemplo 1. A continuación se muestra la composición química de dos aceites diferentes:

Componentes	Aceites	
	1	2
Acidos grasos saturados (%)	52,9	37,2
Acidos grasos monoinsaturados (%)	41,6	33,6
Acidos grasos poliinsaturados (%)	5,8	11,8
razón w3/w6	1,9	3,2
Astaxantina (ppm)	550,0	643,0

Los aceites de krill de este ejemplo poseen humedades que varían entre 0 y 1%, índice de peróxidos entre 1 y 5 meq/Kg, acidez libre (expresado como porcentaje de ácido oleico) entre 0,2 y 1,5%, índice de yodo entre 20 y 160, residuos insaponificables entre 0,5 y 3,5%, índice de saponificación entre 150 y 200, y cenizas entre 0 y 1%.

El perfil de ácidos grasos se determinó por cromatografía de gases. Para ello la muestra de aceite se hidrolizó por incubación con hidróxido de sodio 0,5N en metanol a 85°C por 10 min. Posteriormente se formaron los respectivos ésteres de metilo a través de la adición de trifluoruro de boro al 20% en metanol e incubación posterior a 100°C por 10 min. Después de saturar con cloruro de sodio, los ácidos grasos esterificados se extrajeron con éter de petróleo y se deshidrató con sulfato de sodio. La muestra derivatizada se inyectó al cromatógrafo de gases con una columna capilar (columna de sílica fundida, WCOT CP-Sil 88, ID 0,1 cm. 50 metros) y se resolvió por un rango de temperatura que varió desde 100 hasta 240°C. Se usó un detector de llama (FID) y los picos se identificacon con el uso de estándares. A partir del cromatograma obtenido se calcularon las composiciones de ácidos grasos saturados, monoinsaturados, poliinsaturados y la razón w3/w6.

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La determinación de astaxantina en el aceite de krill de la presente invención se realizó en condiciones protegidas de la luz. Al aceite de krill se le agregó acetona y se agitó por 15 min. La fase acetónica se traspasó a un embudo de decantación y le agregó un volúmen de bencina de petróleo. Después de agitar vigorosamente, las muestras se dejaron reposar hasta la separación completa de las fases. Se extrajo la fase de bencina de petróleo, se lavó con agua y se desecó con sulfato de sodio anhidro. Una vez aforado a 100 mL con bencina de petróleo, se determinó la absorbancia de las muestras a 460 nm. La cantidad de astanxantina se calculó usando el coeficiente de extinción molar del pigmento.

Ejemplo 3.

Aceites obtenidos por procesos con distintas temperaturas de cocción de krill.

Los aceites se obtuvieron por el proceso descrito en el ejemplo 1, en donde se varió solamente la temperatura de cocción del krill.

En la tabla siguiente se describen 2 aceites obtenidos usando distintas temperaturas de cocción (75°C y 95°C) de krill.

	Contenido en aceite de krill obtenido por cocción a 75°C	Contenido en aceite de krill obtenido por cocción a 95°C
Acidos grasos		
Saturados	45,3%	50,7%
Monoinsaturados	34,1%	38,7%
Poliinsaturados	20,6%	10,6%
Fracción		
Triglicéridos	59,8%	60,0%
Fosfolípidos	13,3%	5,2%

En la tabla se puede apreciar que al aumentar la temperatura de cocción del krill de 75 a 95°C, el contenido de ácidos grasos poliinsaturados del aceite disminuye a alrededor de la mitad. Este fenómeno es acompañado con una disminución del contenido de fosfolípidos de un 13,3% a un 5,2%. La disminución del contenido de ácidos grasos poliinsaturados se ve compensado por un aumento de los niveles de los ácidos grasos saturados y monoinsaturados.

El perfil de ácidos grasos se determinó tal como se describe en el ejemplo 2. La fracción de lípidos se determinó por cromatografía en placa fina (Merck artículo Nº 5724, DC-Fertigplatten Kieselgel 60) y se desarrollo en cloroformo : acetona : metanol (94 : 5,5 : 0,5). Los lípidos se detectaron a través del uso de vapores de yodo. Para las determinaciones se usaron 200 ul de las muestras de aceite disueltas en hexano.

Ejemplo 4.

Alimento para peces con capacidad pigmentante.

Composición de una dieta para salmonideos que permite pigmentar su carne a través de la incorporación de aceite de krill conteniendo astaxantina, preparado según el ejemplo 1.

61,0%	Harina de pescado
26,9%	Trigo
5,0%	Aceite de krill
6,0%	Aceite de pescado
0,1%	Minerales
1,0%	Vitaminas

Esta formulación permite preparar una dieta con alrededor de 50 ppm de astaxantina.

Para preparar la dieta, se mezclaron los micronutrientes con el trigo y la harina de pescado previamente molida hasta un tamaño de partícula que permita completa homogeneidad de la muestra. Posteriormente se añadió parte del aceite y se agregó a una máquina peletizadora. Posteriormente se adicionó el resto del aceite. Después de hacer los pellets, éstos se fraccionan por tamaño a través de una malla. Los salmones comienzan a ser alimentados con esta dieta una vez que tengan un peso corporal de al menos 200 gramos, dos veces al día por un período variable que puede prolongarse hasta la cosecha.

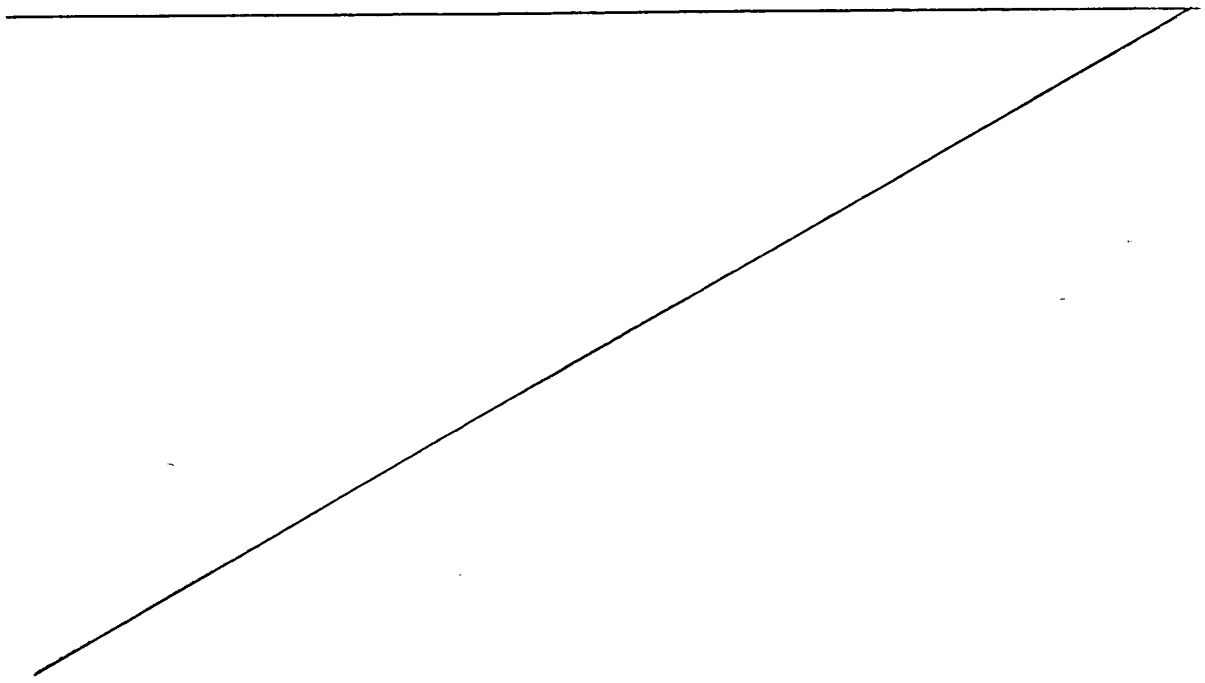
Ejemplo 5.

Complemento nutricional

Se describe un alimento rico en materia grasa para complementar ácidos grasos esenciales. Dicho alimento se ha formulado en forma de galletas elaboradas en base de aceite de krill.

Se mezclan en un bol 400 g de quaker, 100 g de harina, 50 g de azúcar, 1 huevo, 150 mL aceite de krill de la presente invención y 10 mL de extracto de vainilla. Una vez homogeneizado completamente se moldean las galletas pesando cada una de ellas alrededor de 25 g, y se hornea por 15 min. Tiempos de horneados superiores destruyen el pigmento astaxantina.

La cantidad de alimento utilizada en las dietas dependerá de la formulación de las mismas, de la cantidad de ácidos grasos esenciales como los 18:2 y 18:3 requeridos, y de las calorías que sean necesario aportar.



Ejemplo 6.

Estabilizante y antioxidante de materias grasas.

Es posible estabilizar aceites vegetales añadiendo aceite de krill, preparada según el ejemplo 1, que varían entre 1 a 10% (v/v). El efecto estabilizante se debería en parte a la actividad antioxidante que presenta el aceite de krill. La mezcla de aceite así preparada posee un color naranja claro debido a la astaxantina, cuya concentración final oscila entre 4 a 160 ppm.

Cuando el aceite de krill es añadido al 5% (v/v) en aceite vegetal como único agente estabilizante, y se calentó a 45°C por 48 horas, se determinó que la acidez libre y el valor del índice de peróxido no aumentaron significativamente.

Ejemplo 7.

Fotoprotector

El aceite de krill, preparado según el ejemplo 1, puede ser utilizado para la preparación de cremas bronceadoras y aceites bronceadores para la protección solar.

A) Cremas bronceadoras

A continuación se describen 2 bronceadores, uno con factor de protección solar 5 (SFP5) y otro con factor de protección solar 20 (SFP20).

	Cantidad para 100 g	
Componente	**SFP5**	**SFP20**
Aceite de krill	5,00 g	5,00 g
Carbopol 940	0,25 g	0,25 g
Alcohol cetílico	0,50 g	0,50 g
Metil parabeno	0,15 g	0,15 g
Acido esteárico III pr.	2,00 g	2,00 g
Propil parabeno	0,15 g	0,15 g
Panaleno	5,00 g	5,00 g
EDTA disódico	0,10 g	0,10 g
Trietanolamina	0,50 g	0,50 g
Propilénglicol	2,50 g	2,50 g
Octilmetoxicinamato	-	7,50 g
Benzofenona 3	-	3,00 g
Dióxido de titanio	-	0,50 g
Imidazolidinilurea	0,30 g	0,30 g
Monoesterato de gliceril	1,00 g	1,00 g
Esencias naturales	c.s.	c.s.
Miristato de isopropilo	2,00 g	2,00 g
Agua	c.s.	c.s.

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B) Aceites bronceadores:

A continuación se describe una fórmula de aceite bronceador solar que posee aceite de krill como único agente bloqueador.

Compuesto	**Cantidad para 100 grs de aceite**
Vaselina líquida cosmética	80,0 g
Polibutenos hidrogenados	5,0 g
Aceite de krill	14,7 g
Esencia	0,3 g

Ejemplo 8.

Productos cosméticos con base en aceite de krill.

Debido a las múltiples actividades biológicas del krill, por ejemplo su capacidad pigmentante, antioxidante, su contenido de EPA y ácidos grasos esenciales, es posible diseñar una serie de productos de gran valor cosmético.

A) Crema humectante.

Componente	**Cantidad para 100 grs de crema**
Ácido esteárico prensado xxx	1,0 g
Monoestearato de glicerilo A.E. Aniónic.	0,7 g
Monoestearato de glicerilo neutro	0,5 g
Polibutenos hidrogenados	3,2 g
Aceite de krill	10,0 g
Propilenglicol	2,0 g
Miristato de isopropilo	1,5 g
Polímero carboxivinilo	0,3 g
Propilparabeno	0,1 g
Metilparabeno	0,1 g
Esencia	0,3 g
EDTA disódico	0,2 g
Trietanolamina 99%	1,2 g
Agua desmineralizada	78,9 g

B) Rubor en polvo.

Se describe una fórmula base para la confección de un rubor en polvo que posee aceite de krill añadido en un 10% (p/p).

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Componente	Cantidad para 100 grs de rubor
Talco	52,56 g
Aceite de krill	10,00 g
Mica : dióxido de titanio (2:1)	26,00 g
Estearato de magnesio	3,50 g
Miristato de isopropilo	1,60 g
Alcohol oleico	2,60 g
Octilpalmitato	3,40 g
Metilparabeno	0,17 g
Propilparabeno	0,17 g

C) Sombra en polvo

Se describe una fórmula base para la confección de una sombra en polvo que posee aceite de krill añadido en un 10% (p/p).

Componente	Cantidad para 100 grs de sombra
Talco	51,46 g
Aceite de krill	10,00 g
Mica : dióxido de titanio (3:1)	27,00 g
Estearato de magnesio	3,50 g
Miristato de isopropilo	1,60 g

Alcohol oleico	2,60 g
Octilpalmitato	3,50 g
Metilparabeno	0,17 g
Propilparabeno	0,17 g

D) Sombra en crema.

Se describe una fórmula base para la confección de una sombra en crema que posee aceite de krill añadido en un 5,7% (p/p).

Componente	**Cantidad para 100 grs de sombra**
Talco	4,0 g
Ácido esteárico	9,5 g
Ácido isoesteárico	1,9 g
Aceite de krill	5,7 g
Dióxido de titanio	1,9 g
Silicato de aluminio y magnesio	3,6 g
Propilénglicol	16,1 g
Trietanolamina	11,0 g
Metilparabeno	0,2 g
Propilparabeno	0,2 g
Agua desionizada	45,9 g

E) Polvo compacto.

Se describe una fórmula base para la confección de un polvo compacto que posee aceite de krill añadido en un 10% (p/p).

Componente	**Cantidad para 100 grs de polvo compacto**
Caolín	32,0 g
Aceite de krill	10,0 g
Mica : dióxido detitanio (2:1)	1,6 g
Estearato de magnesio	4,1 g
Octilpalmitato	2,5 g
Propilparabeno	0,5 g
Talco	49,3 g

F) Brillo labial.

Se describe una fórmula base para la confección de un brillo labial que posee aceite de krill añadido en un 3% (p/p).

Componente	**Cantidad para 100 grs de brillo labial**
Aceite de ricino	42,04 g
Aceite de krill	3,00 g
Alcohol oleico	2,60 g

Lanolina	25,00 g
Monoestearato de sorbitán	1,30 g
Ozoquerita	4,50 g
Cera carnauba	5,20 g
Cera abejas	5,60 g
Ácido esteárico	4,90 g
Cera de candelilla	5,60 g
Metilparabeno	0,13 g
Propilparabeno	0,13 g

REIVINDICACIONES

1. Un aceite de krill, que corresponde a una fracción del total de los lípidos del krill, **CARACTERIZADO** porque se extrae por fraccionamiento térmico y centrifugación y/o decantación lo que permite a los ácidos grasos poliinsaturados llegar a alcanzar hasta un 95% de la cantidad encontrada en los lípidos totales de krill, y porque posee ácidos grasos saturados entre un 26 y 60%, ácidos grasos monoinsaturados entre un 25 y 60%, ácidos grasos poliinsaturados entre 4 y 46%, y el pigmento carotenoide astaxantina, en cantidad que varía entre 300 y 2.600 ppm.

2. Un aceite de krill según la reinvindicación 1, **CARACTERIZADO** el contenido de ácidos grasos saturados, monoinsaturados y poliinsaturados, es posible regularlo variando la temperatura de cocción de krill en el proceso de producción del aceite.

3. Aceite de krill de la reivindicación 1, **CARACTERIZADO** porque dentro de su composición fraccional se destacan los triglicéridos, entre un 35 a un 96%, y los fosfolípidos, entre un 4 y un 28%.

4. Aceite de krill de la reivindicación 1, **CARACTERIZADO** porque el pigmento de astaxantina está en estado libre, entre 0 y 15%, en estado de monoéster, entre 25 y 50%, y en estado de diéster, entre 40 y 80%.

5. Un procedimiento para la producción industrial del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque comprende las siguientes etapas:

a) Cocción del krill

b) Prensado del krill precocido

c) Recolección y acumulación del licor de prensa

d) Eliminación de los sólidos suspendidos

e) Centrifugación y separación del aceite

f) Adición de estabilizantes

g) Almacenamiento

6. Un procedimiento para la producción industrial de aceite de krill de la reivindicación 5, **CARACTERIZADO** porque el krill, entero o fracciones de él, utilizado para este proceso puede ser Euphasia superba, E. crystallorophias, E. frigida, E. tricantha, E. vellantini, E. lougirostris, E. lucens, E. similis, E. spinifera, E. recurva, E. pacífica, Thysanoëssa macrura, T. vicinia, T. gregaria, T. raschii y/o T. inermis.

7. Un procedimiento para la producción industrial de aceite de krill según la reivindicación 5, **CARACTERIZADO** porque la etapa de cocción es un proceso, continuo o por lotes, donde el calor se aplica en forma de vapor de agua, inmersión en agua caliente o por calor o fuego directo por 2 a 80 minutos y más específicamente entre 5 a 25 minutos, a una temperatura de entre 40 a 200°C, en donde la cantidad de ácidos grasos poliinsaturados del aceite de krill puede regularse variando la temperatura de cocción en el proceso de producción, obteniéndose aceites con altos niveles de ácidos grasos poliinsaturados al usar bajas temperaturas

8. Un procedimiento para la producción industrial de aceite de krill según las reivindicaciones 5 y 7, **CARACTERIZADO** porque se puede realizar más de una cocción sucesiva del krill, usando temperaturas que oscilan entre 40 y 200°C en cualquiera de las etapas de cocción, con o sin almacenamiento a temperatura ambiente, refrigerado o congelado entre las cocciones.

9. Un procedimiento para la producción industrial de aceite de krill precocido según la reivindicación 5, **CARACTERIZADO** porque el prensado se realiza, en lotes discontínuos o en flujo contínuo, a través de una prensa cualquiera, incluyendo aquellas de doble o simple tornillo helicoidal de malla variable, obteniendo una torta de prensa con humedad entre 25 a un 80%.

10. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de recolección y acumulación del licor de prensa según la reivindicación 5, **CARACTERIZADO** porque la recolección se realiza por gravedad o con ayuda de una bomba de baja turbulencia y el licor recolectado requiere regulación de temperatura entre la del ambiente y 95°C.

11. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de acumulación de licor de prensa según las reivindicaciones 5 y 10, **CARACTERIZADO** porque el licor de prensa acumulado puede ser tratado para eliminar las proteínas solubles a través de una coagulación por calentamiento en el estanque de acumulación seguido de un prensado, decantación, filtrado o drenado rotatorio.

12. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de acumulación de licor de prensa según las reivindicaciones 5 y 10, **CARACTERIZADO** porque el licor de prensa acumulado puede ser tratado proteolíticamente para eliminar las proteínas solubles, con la adición de una proteasa, de preferencia una endoproteasa, en cantidades que oscilan entre 0,001 a 5% (p/v), dependiendo de la enzima utilizada y de la cantidad de proteínas contenido en el licor de prensa, e incubado entre 4 y 90°C, preferentemente entre 30 y 75°C, por 5 minutos a 80 horas, preferentemente entre 30 minutos a 8 horas.

13. Un procedimiento para la producción industrial de aceite de krill en que el tratamiento proteolítico del licor de prensa según la reivindicación 12, **CARACTERIZADO** porque la proteasa puede seleccionarse entre las proteinasas tales como acrosina, alcalasa, uroquinasa, uropepsina, elastasa, enteropeptidasa, catepsina, kalicreina, kiniasa 2, quimotripsina, quimopapaína, colagenasa, estreptoquinasa, subtilisina, termolisina, tripsina, trombina, papaina, pancreatopeptidasa y renina; peptidasas tales como aminopeptidasas, por ejemplo, arginina aminopeptidasa, oxitocinasa y leucina aminopeptidasa; angiotensinasa, enzima convertidora de angiotensina, insulinasa, carboxipeptidasa, por ejemplo, arginina carboxipeptidasa, kiniasa 1 y tiroide peptidasa, dipeptidasas, por ejemplo, carnosinasa y prolinasa y pronasas.

14. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de eliminación de sólidos suspendidos según la reivindicación 5, **CARACTERIZADO** porque se realiza por decantación por gravedad, filtración o un drenador rotatorio.

15. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de centrifugación y separación del aceite según la reivindicación 5, **CARACTERIZADO** porque se realiza, por lotes o en flujo contínuo, a través de una centrífuga, incluyendo las centrífugas de discos, entre 2.000 y 20.000 rpm, y cuya temperatura puede oscilar entre la del ambiente y 100°C y más específicamente entre 50 y 98°C.

16. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de separación de aceite según la reivindicación 5, **CARACTERIZADO** porque la centrifugación puede ser reemplazada por un decantador, ya sea de lotes o de flujo continuo, en especial los decantadores de dos fases.

17. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de estabilización del aceite de krill según la reivindicación 5, **CARACTERIZADO** porque se adiciona antioxidantes y/o preservantes.

18. Un procedimiento para la producción industrial de aceite de krill en la que la etapa de almacenamiento del aceite de krill según la reivindicación 5, **CARACTERIZADO** porque se realiza en envases de plástico o metal, en especial de acero inoxidable, a temperatura ambiente o refrigerado, y protegido de la luz.

19. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para pigmentar productos cárneos añadidos a dietas de crustáceos, moluscos, peces, aves y sus huevos, cerdos, caprino, ovino, bovino y otros mamíferos.

20. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para preparar un complemento nutricional en dietas humanas o de animales en la formulación de alimentos ya que suplementa ácidos grasos esenciales, ácidos grasos poliinsaturados, triglicéridos y fosfolípidos.

21. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para la estabilización de materias grasas gracias a su adición a aceites o materias grasas de origen animal y/o vegetal en cantidades variables.

22. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para la preparación de productos farmacéuticos o cosméticos que poseen capacidad fotoprotectora, en especial a la radiación ultravioleta.

23. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para ser utilizado como antioxidante natural en alimentos o en productos farmacéuticos y/o cosméticos.

24. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para ser usado como fuente de astaxantina o ser usado directamente como pigmentante natural en alimentos y en productos farmacéuticos y/o cosméticos.

25. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para ser usado como agente disolvente y/o vehículo de compuestos lipofílicos de uso químico, farmacéutico, alimento y cosmético.

26. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para la preparación de dietas como agente que aumenta la palatibilidad en alimentos destinados a nutrición animal y humana.

27. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para la preparación de dietas, aditivos y/o productos farmacéuticos que son útiles para mejorar las propiedades biológicas y de sobrevida de las ovas de peces y los huevos de moluscos y crustáceos.

28. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para la preparación de dietas que son útiles para tratar enfermedades cardiovasculares, como una fuente de ácido eicosapentaenoico (EPA).

29. Uso del aceite de krill de la reivindicación 1, **CARACTERIZADO** porque sirve para preparar de productos dermatológicos, en especial productos tópicos o sistémicos, para tratar enfermedades de la piel en donde exista deficiencia de ácidos grasos esenciales, como la piel xerótica, hiperqueratosis, ictiosis, acné, psoriasis, úlceras dérmicas, eczema serborreica, dermatitis atópica, vitiligo.

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(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.

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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

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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]

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"&" 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

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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

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(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.

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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.

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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) [6]

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]

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"&" 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- 20
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)

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	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

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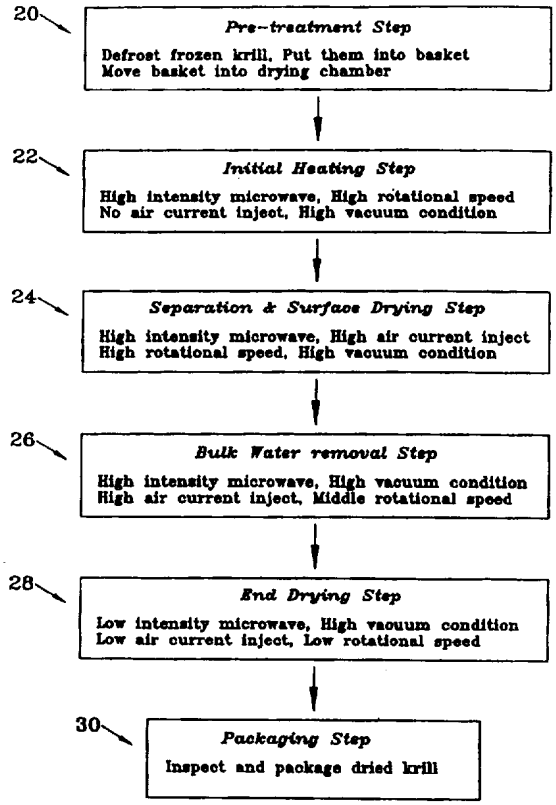
(54) Title: DEHYDRATED KRILL AND METHOD OF PRODUCING SAME

(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.



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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.

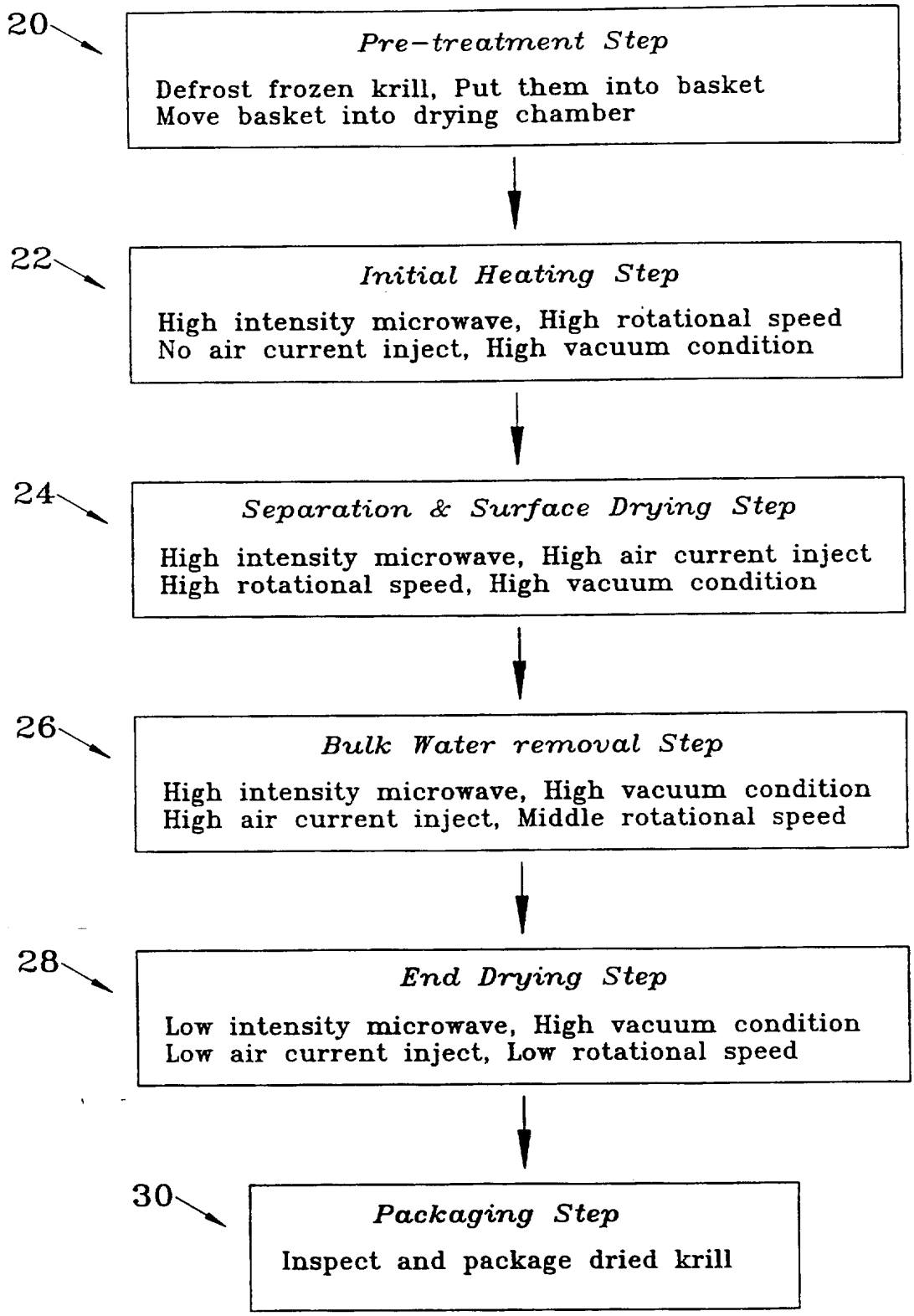

Figure 1

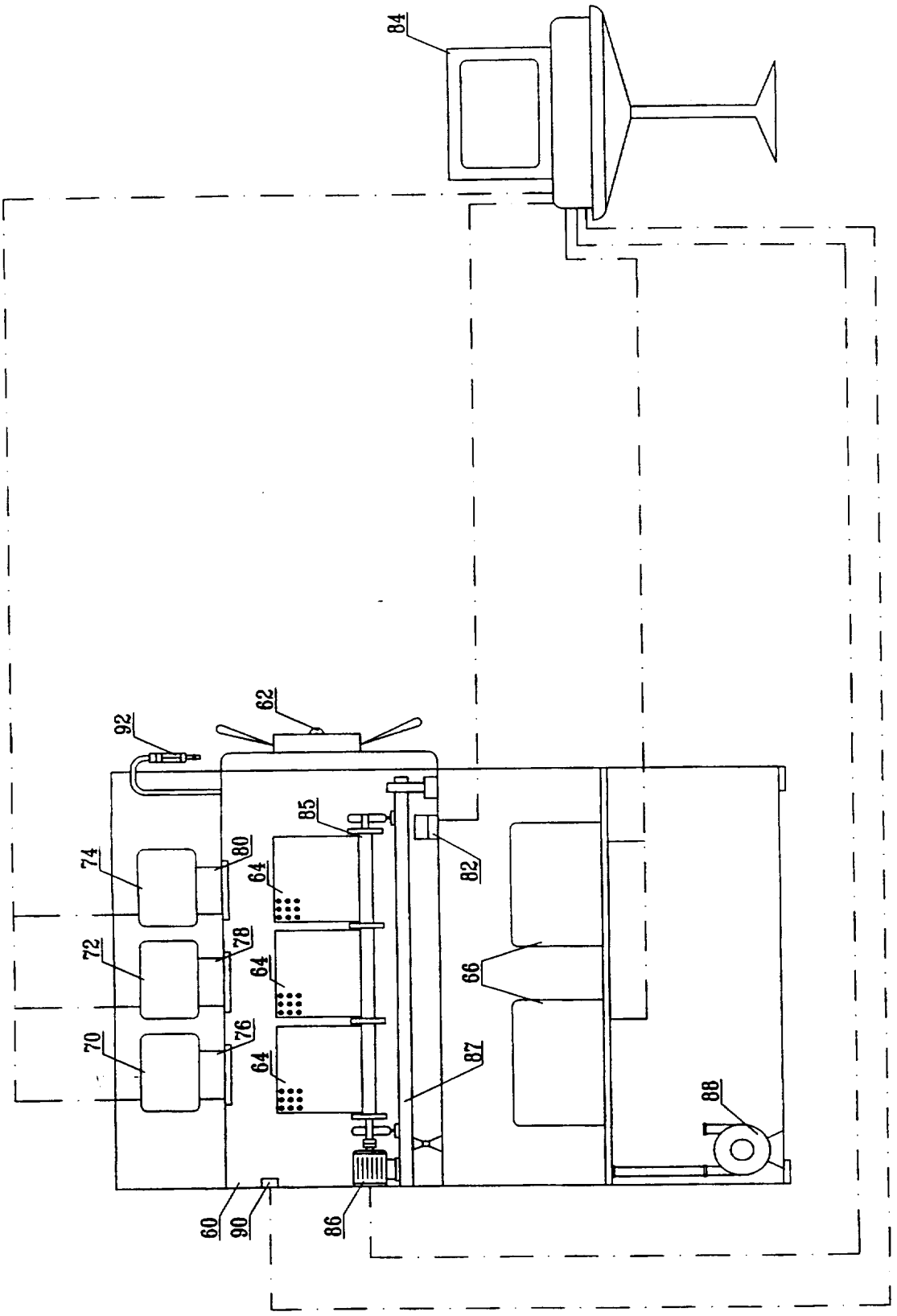

Figure 2

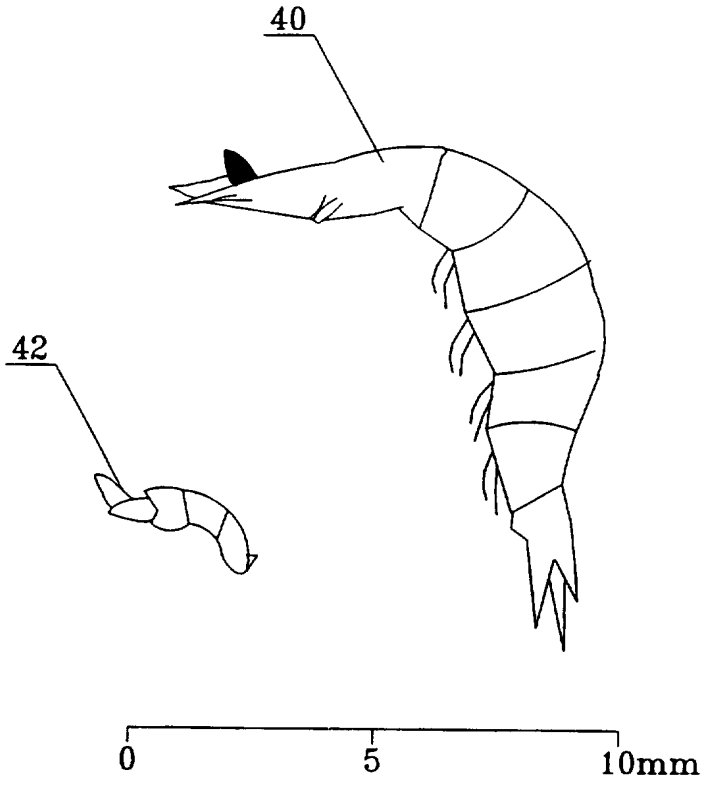

Figure 3

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.

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

International application No
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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

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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(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.

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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.

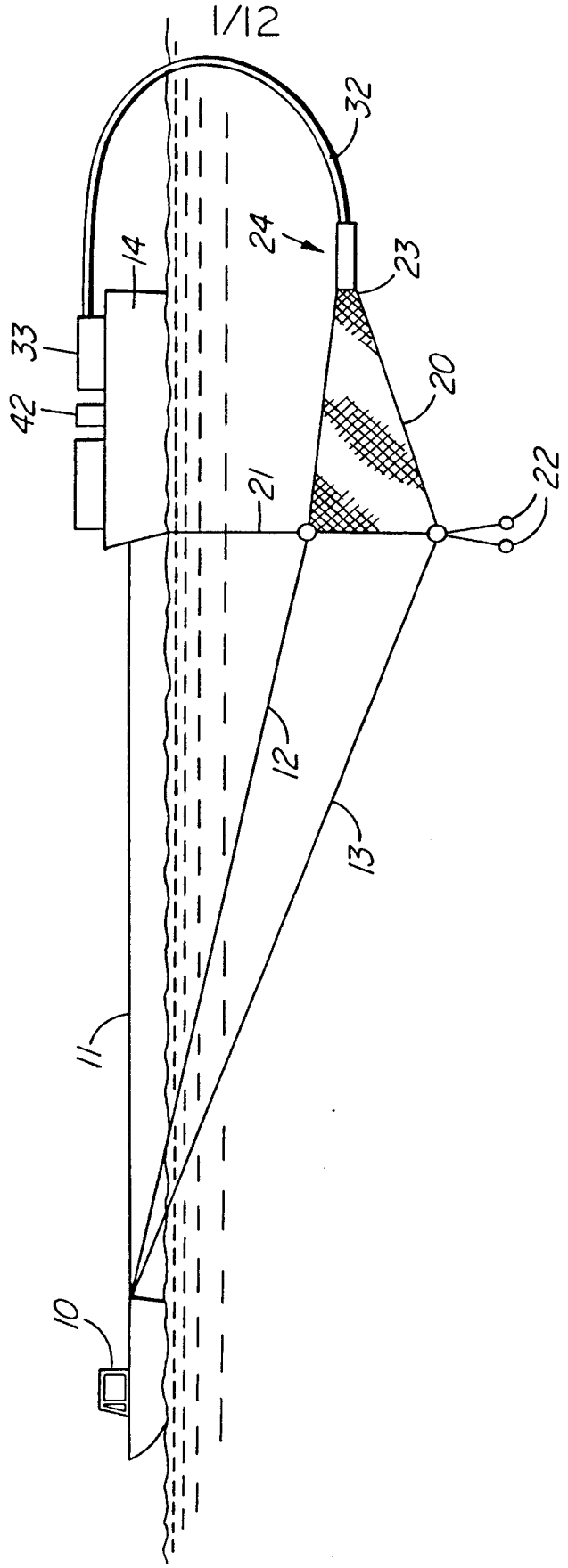

FIG. 1A

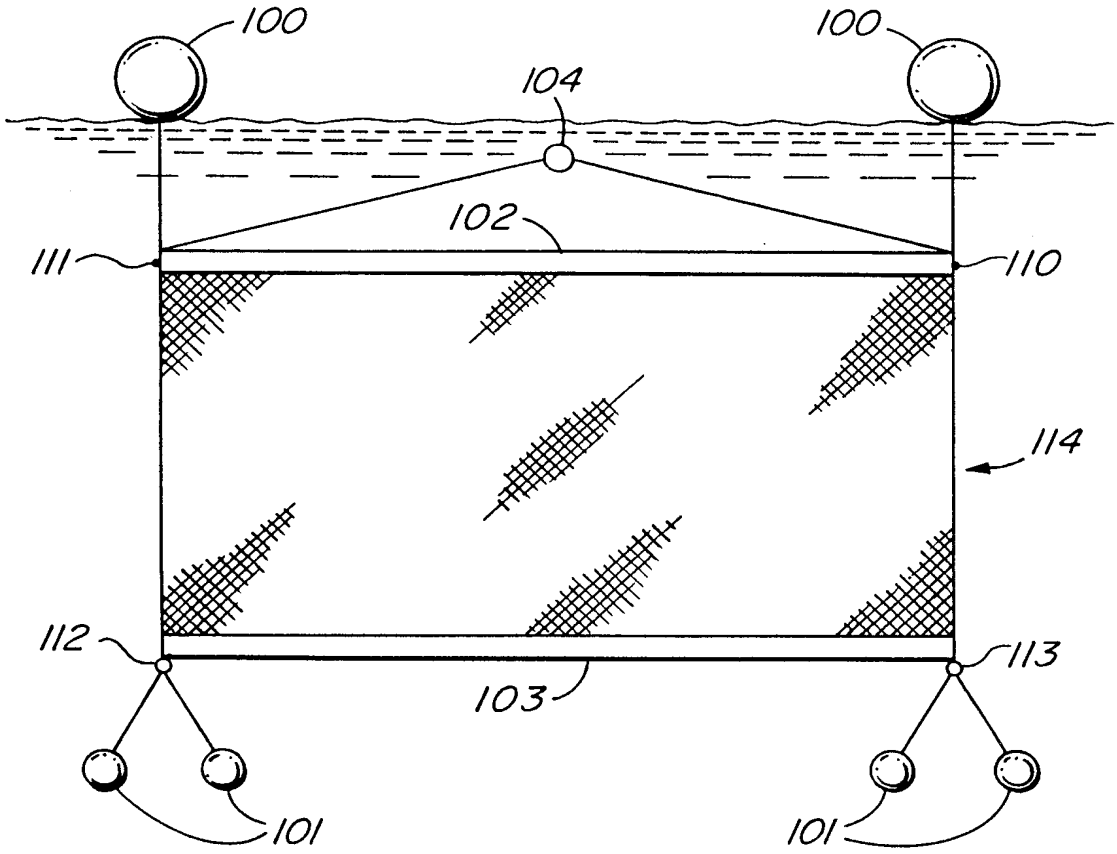

FIG. 1B

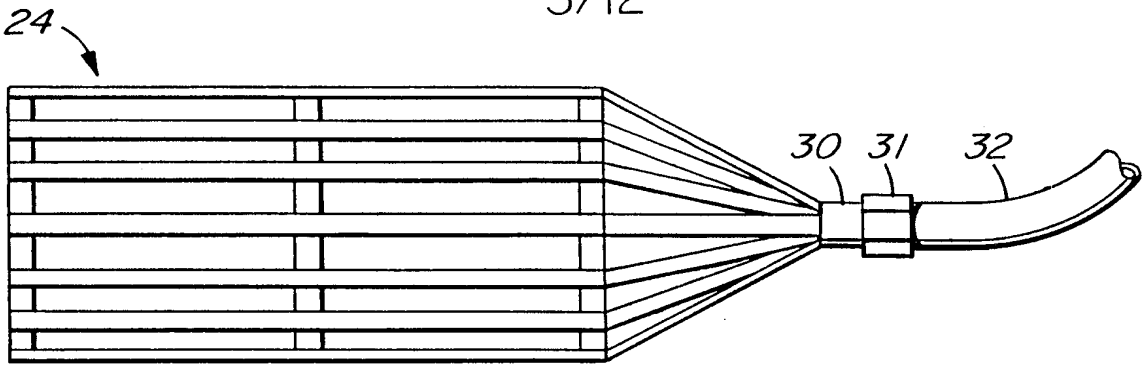

FIG. 2A

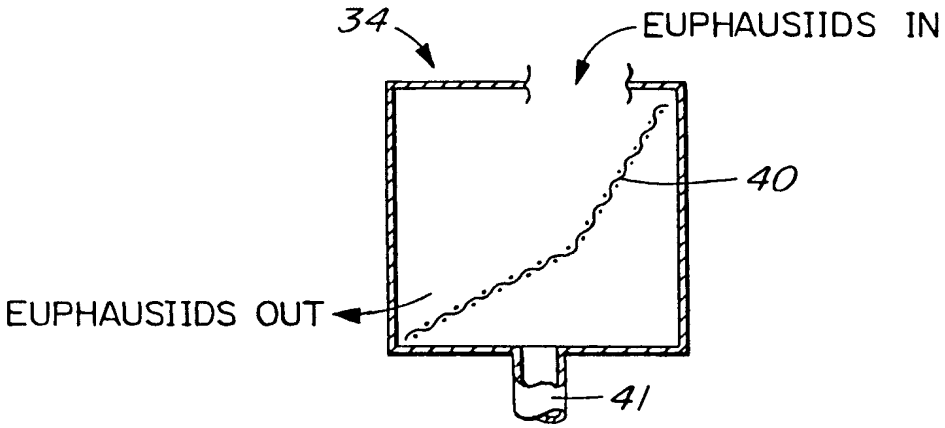

FIG. 2B

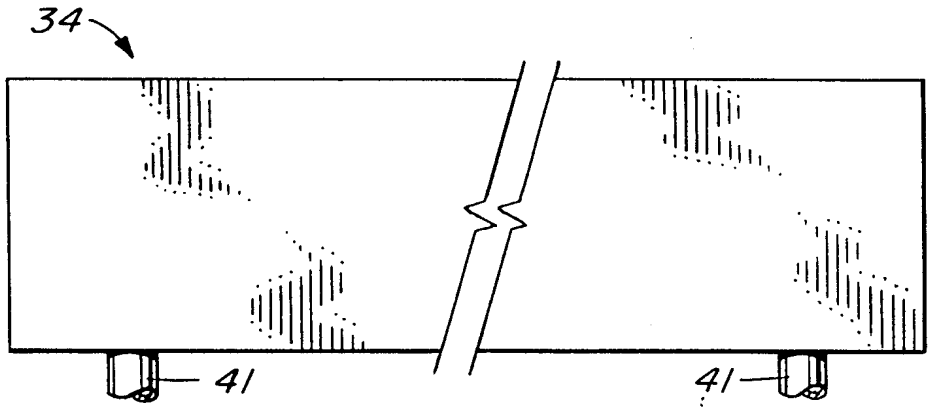

FIG. 2C

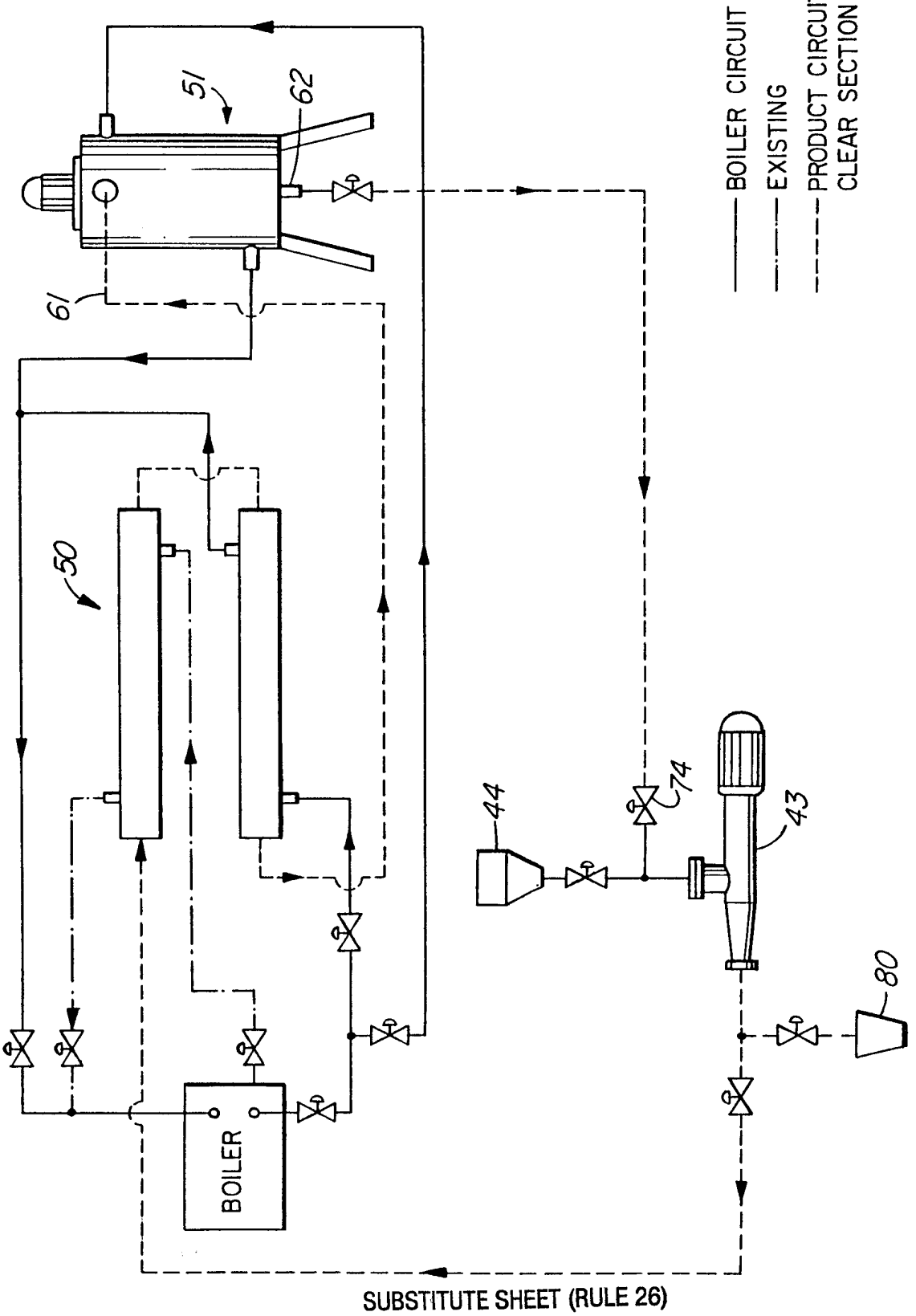
BOILER CIRCUIT
EXISTING
PRODUCT CIRCUIT
CLEAR SECTION
51
62
61
50
44
74
43
80
BOILER

FIG. 3

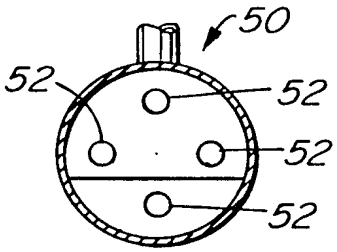

FIG. 4A

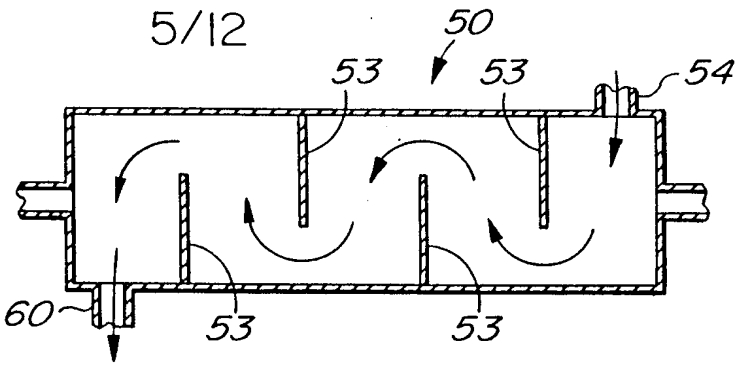

FIG. 4B

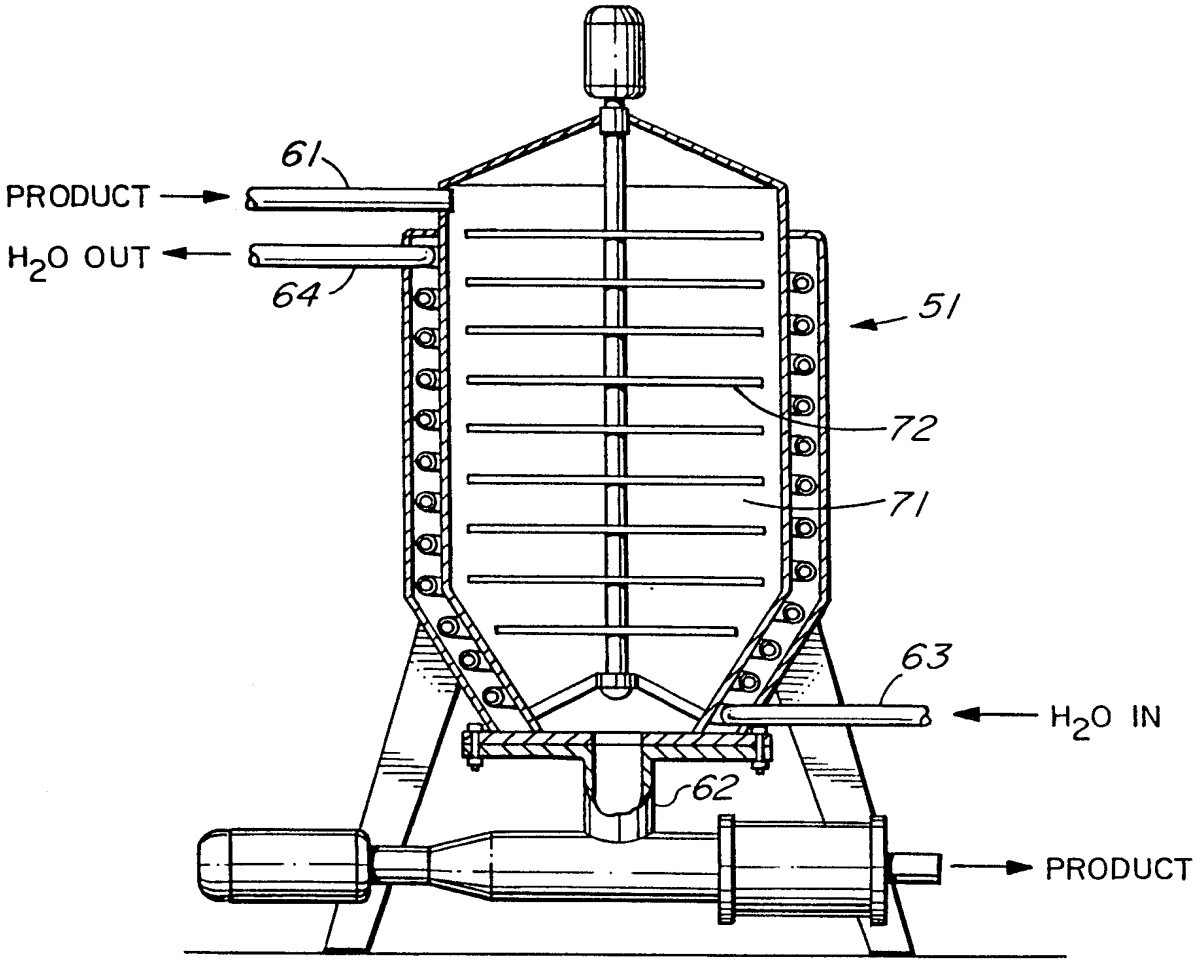

FIG. 5

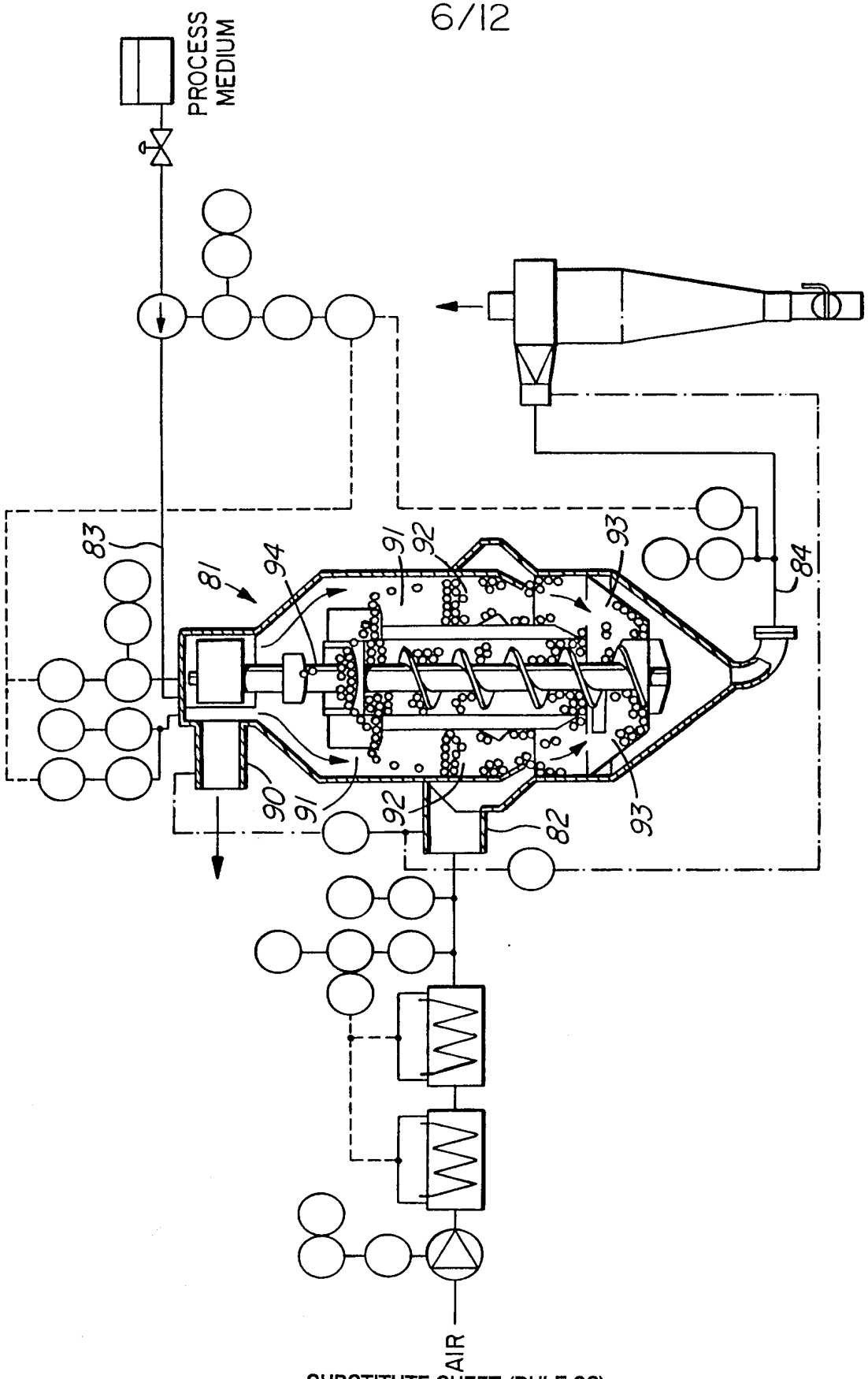

FIG. 6

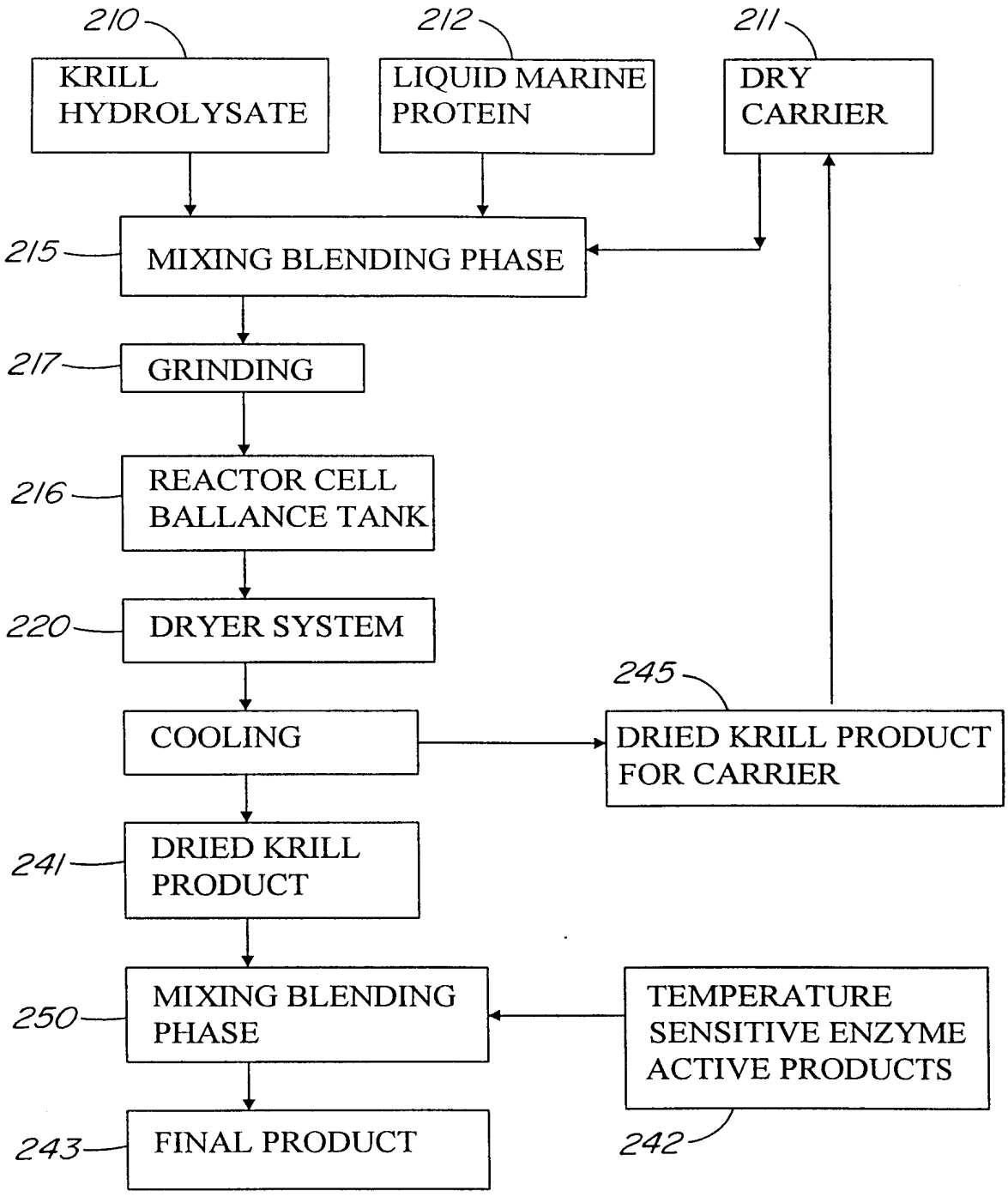
210
KRILL HYDROLYSATE
212
LIQUID MARINE PROTEIN
211
DRY CARRIER
215
MIXING BLENDING PHASE
217
GRINDING
216
REACTOR CELL BALLANCE TANK
220
DRYER SYSTEM
245
COOLING
DRIED KRILL PRODUCT FOR CARRIER
241
DRIED KRILL PRODUCT
250
MIXING BLENDING PHASE
TEMPERATURE SENSITIVE ENZYME ACTIVE PRODUCTS
243
FINAL PRODUCT
242

FIG. 7

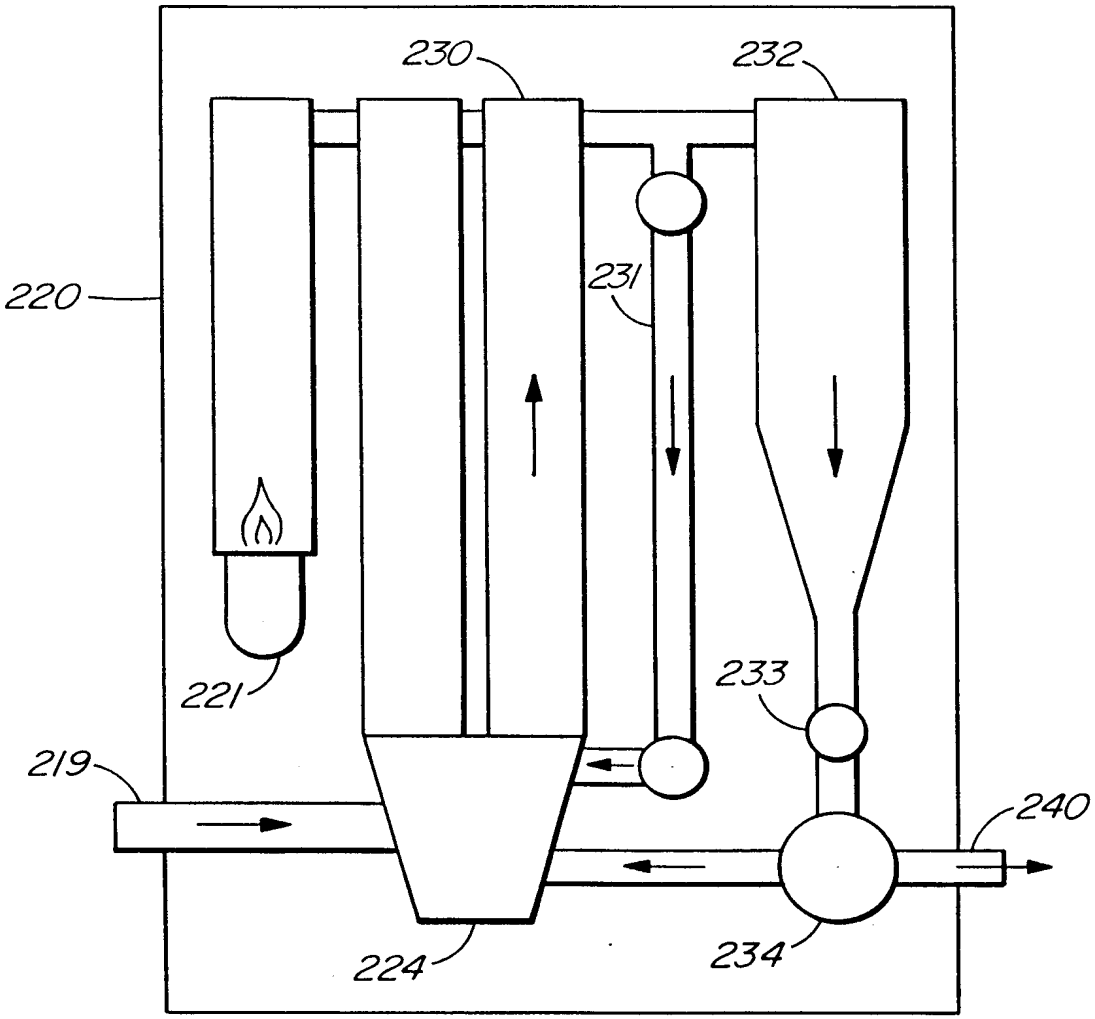

FIG. 8

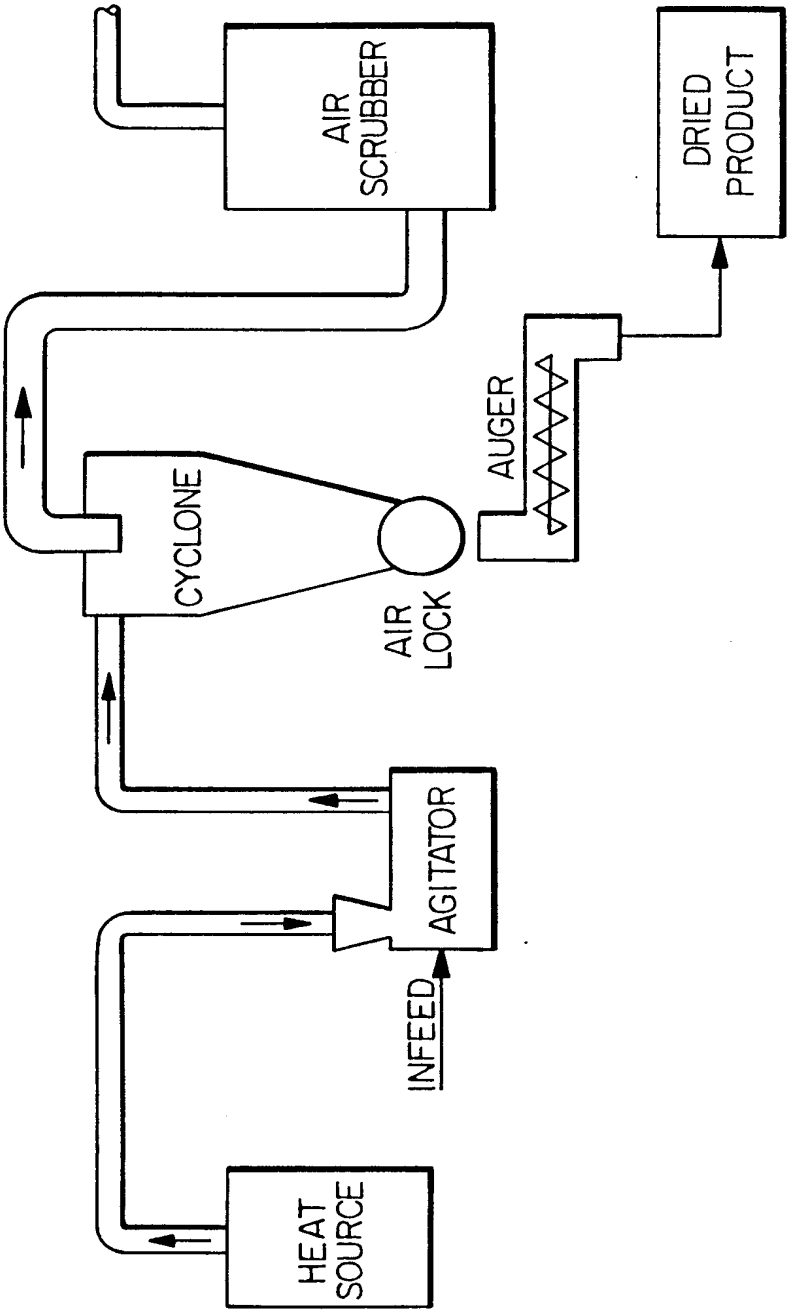
AIR SCRUBBER
DRIED PRODUCT
AUGER
CYCLONE
AIR LOCK
AGITATOR
INFEED
HEAT SOURCE

FIG. 9

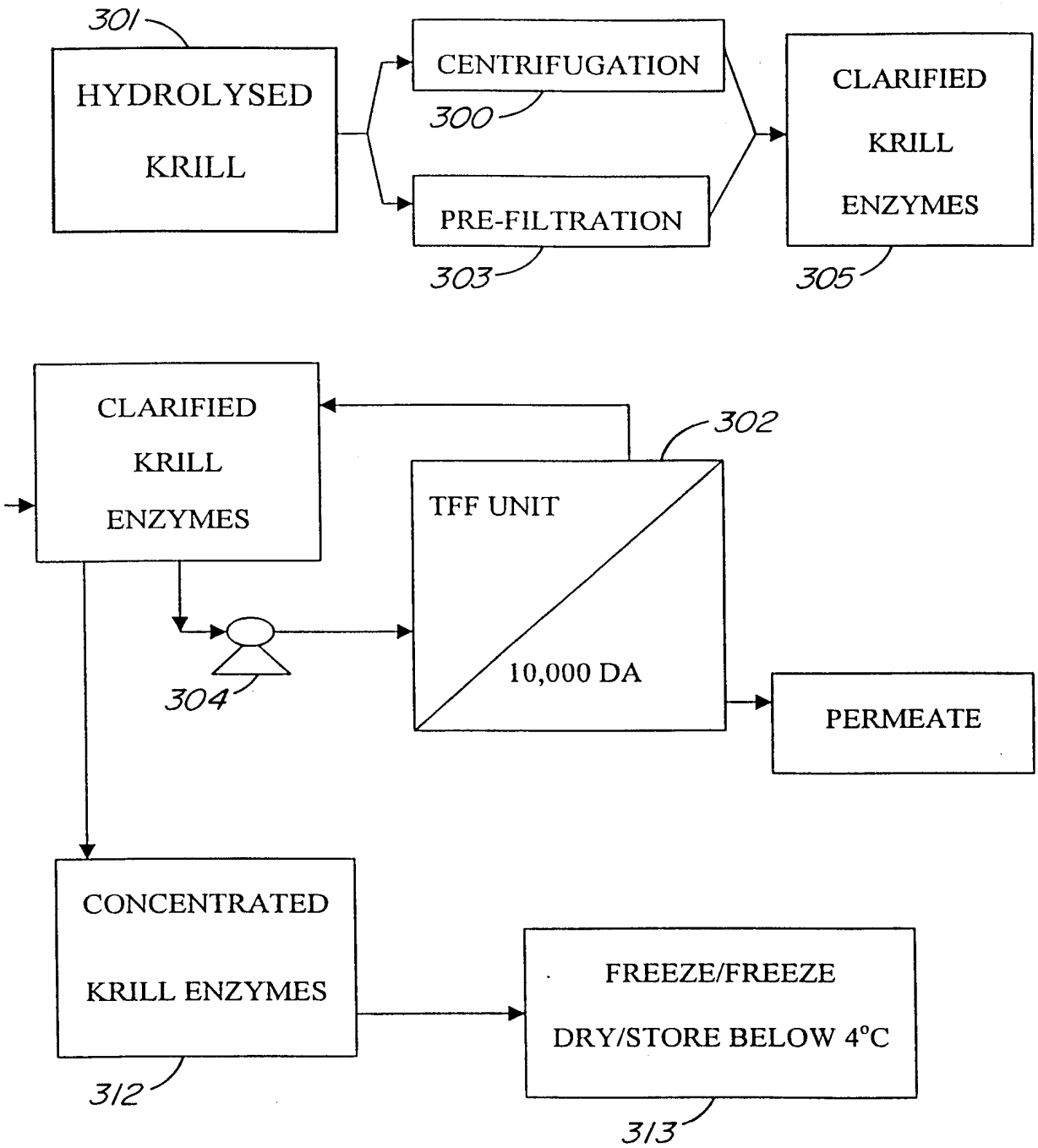

FIG. 10

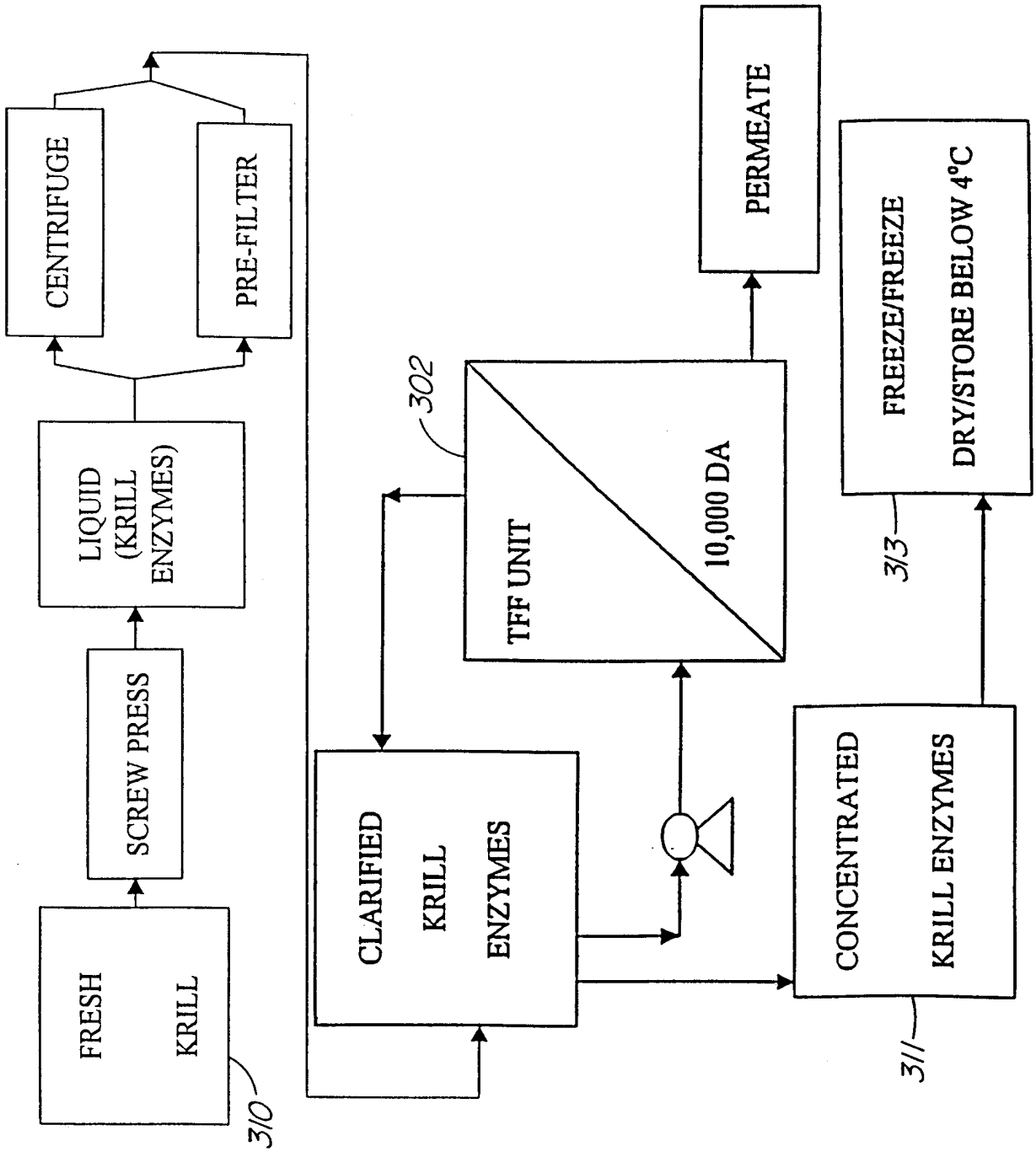

FIG. 11

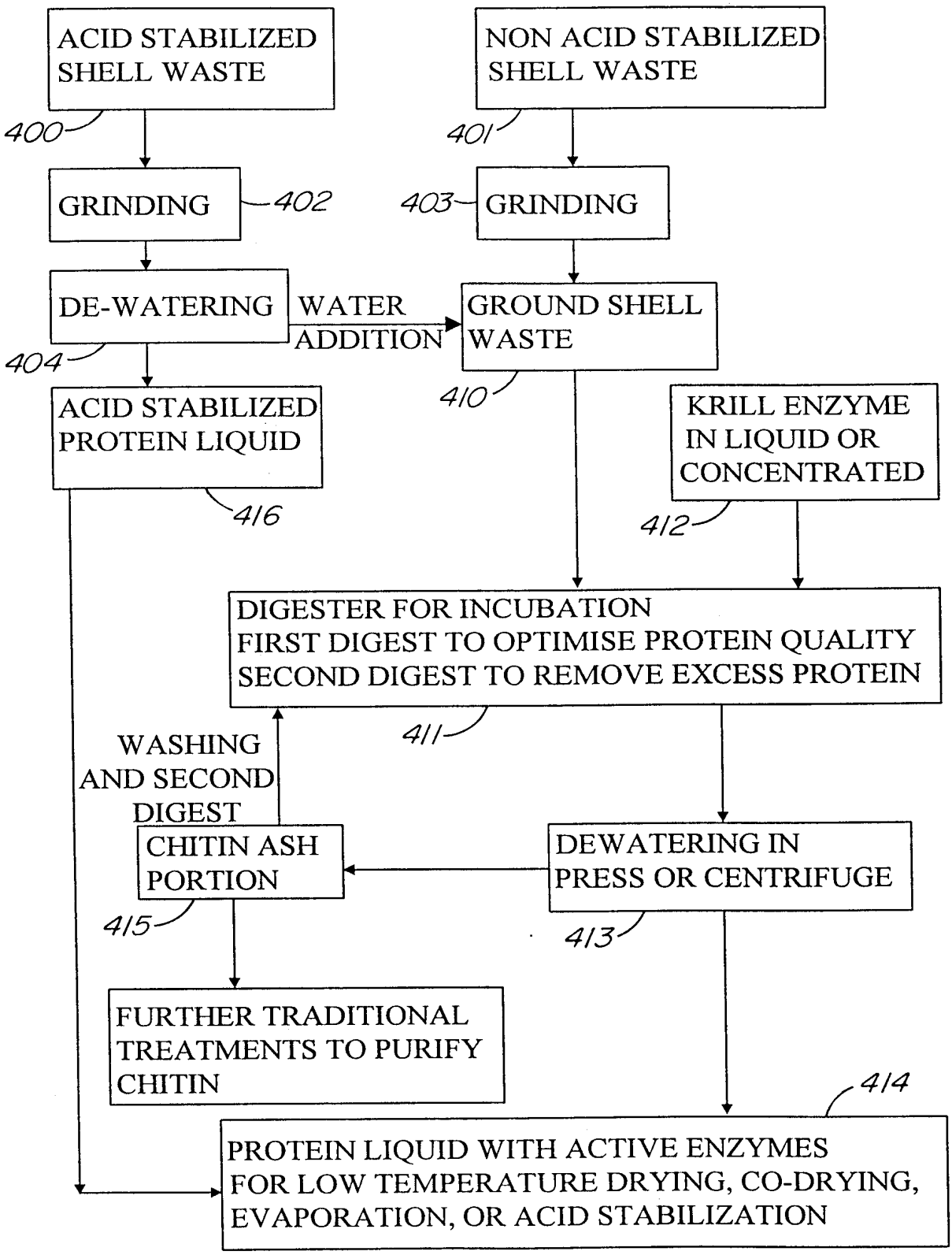

FIG. 12

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

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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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.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 July 1998	21/07/1998

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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.
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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
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/00082

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(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.

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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.

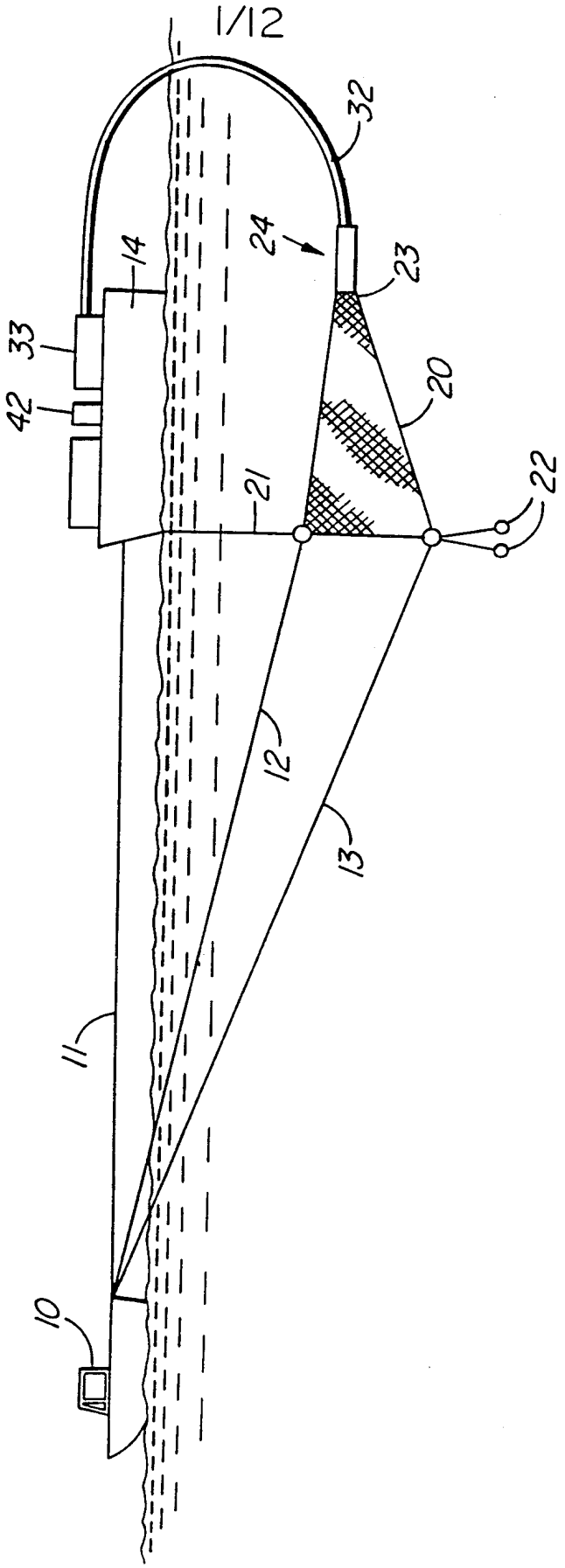

FIG. 1A

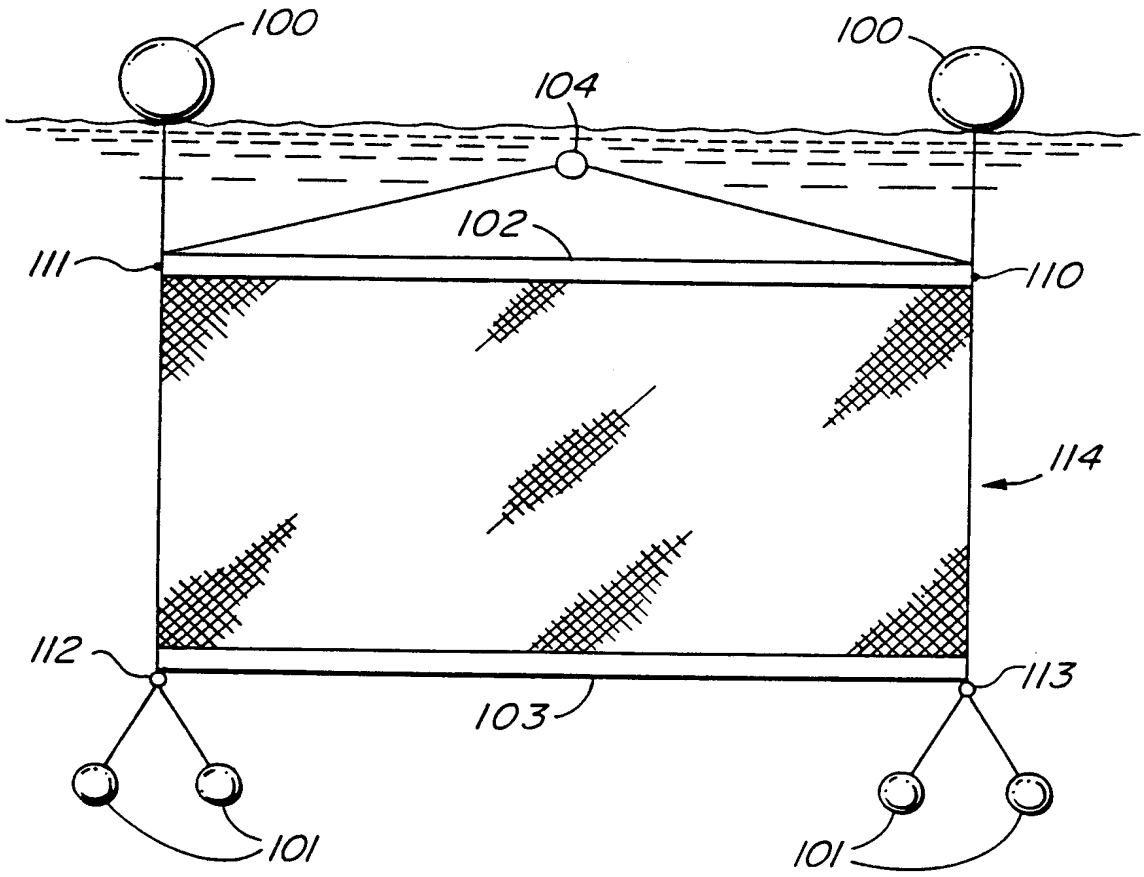

FIG. 1B

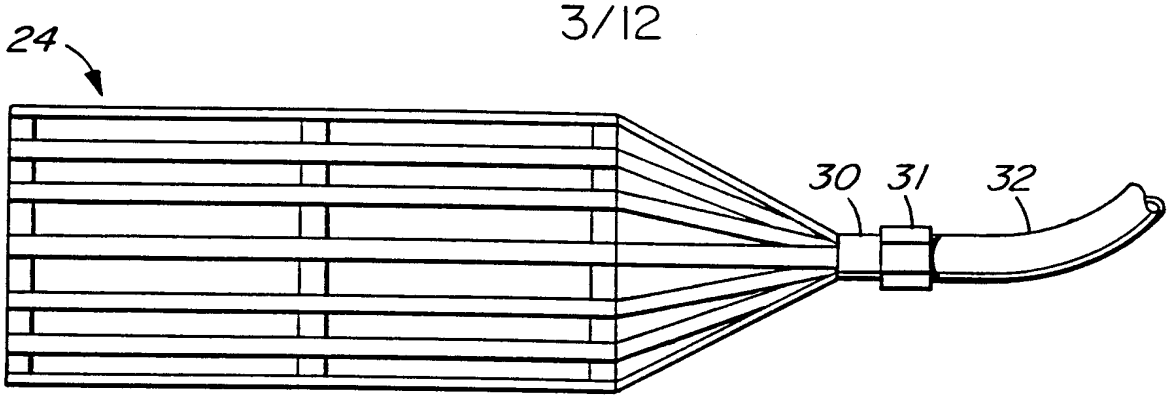

FIG. 2A

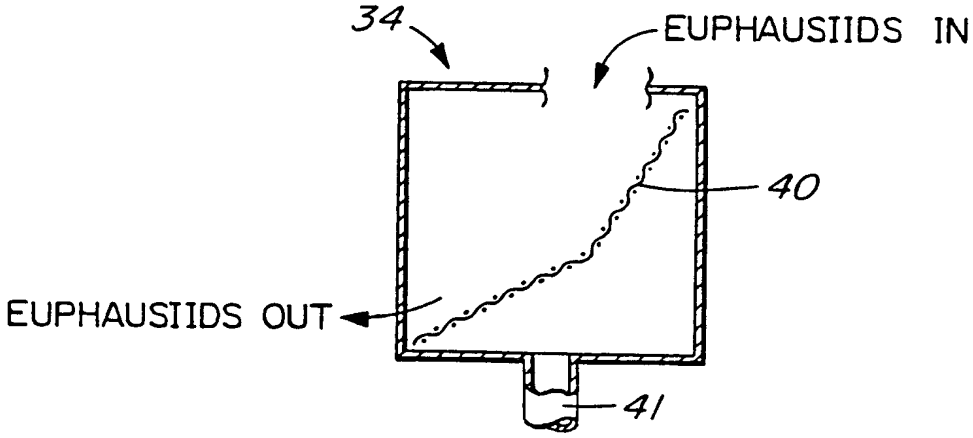

FIG. 2B

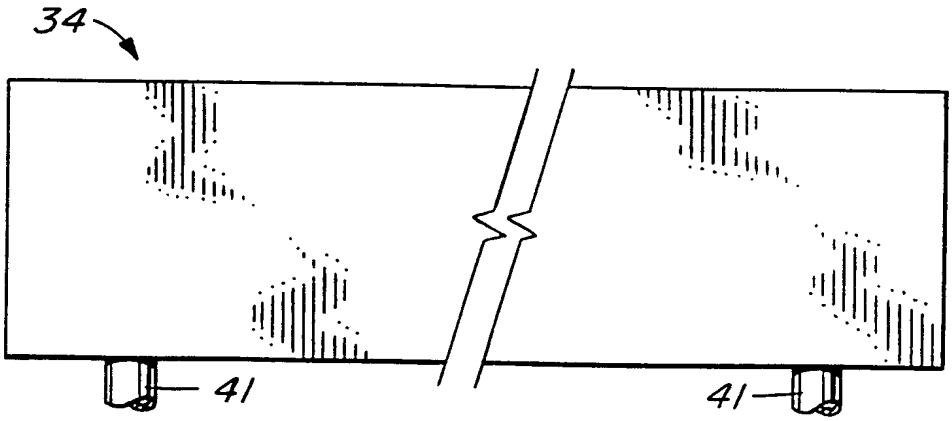

FIG. 2C

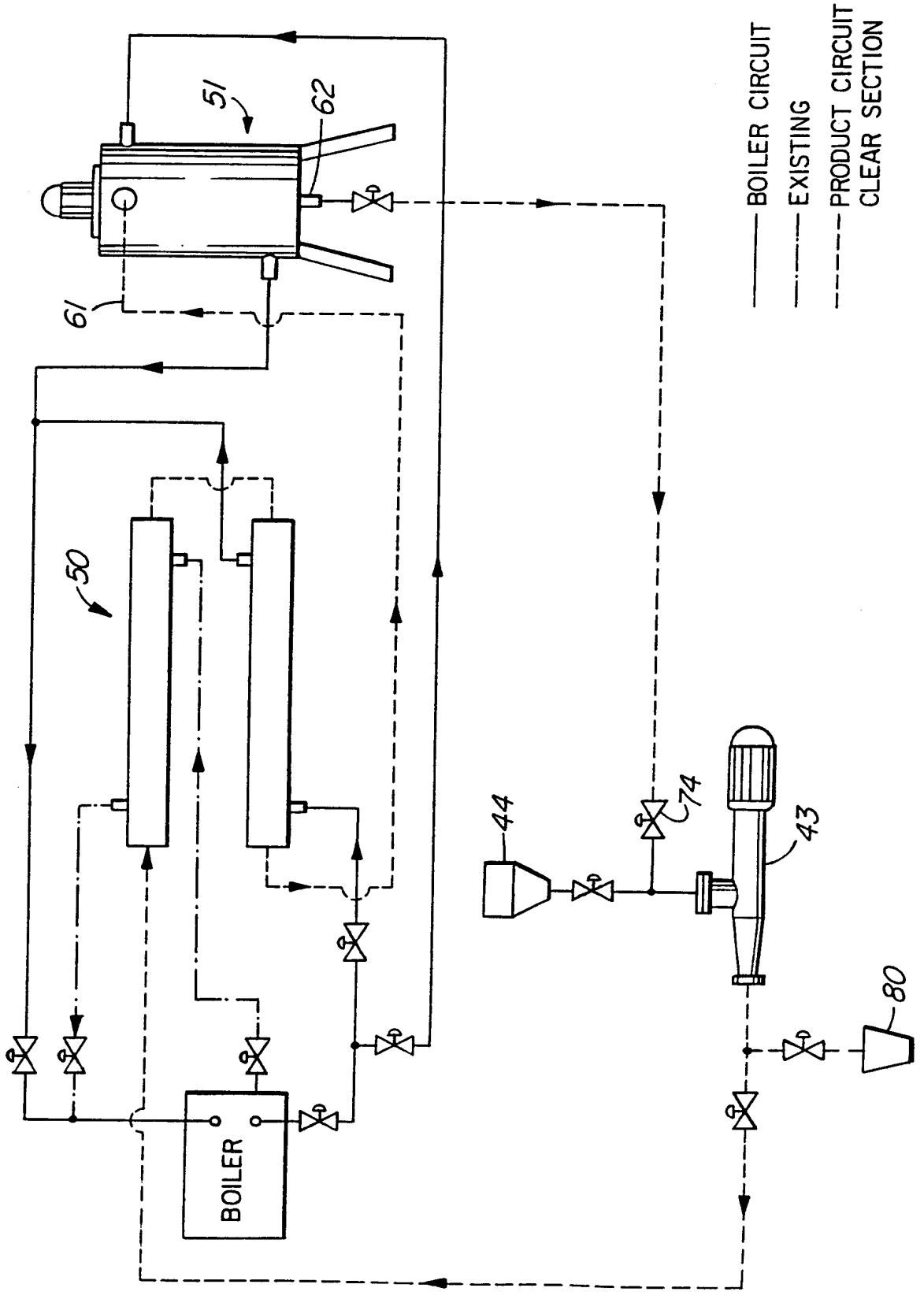

FIG. 3

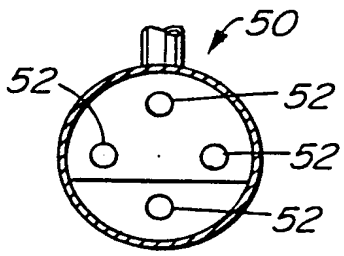

FIG. 4A

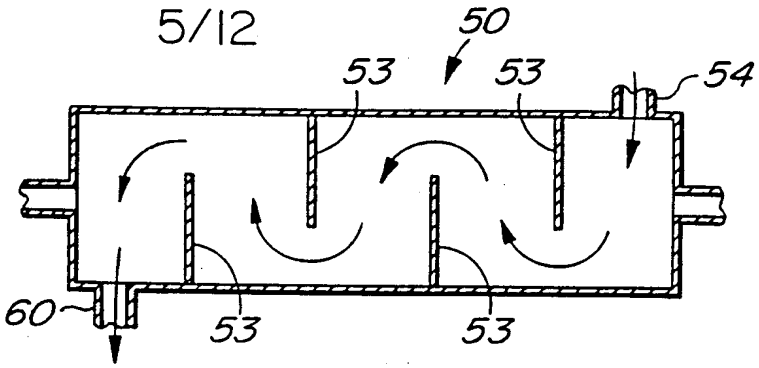

FIG. 4B

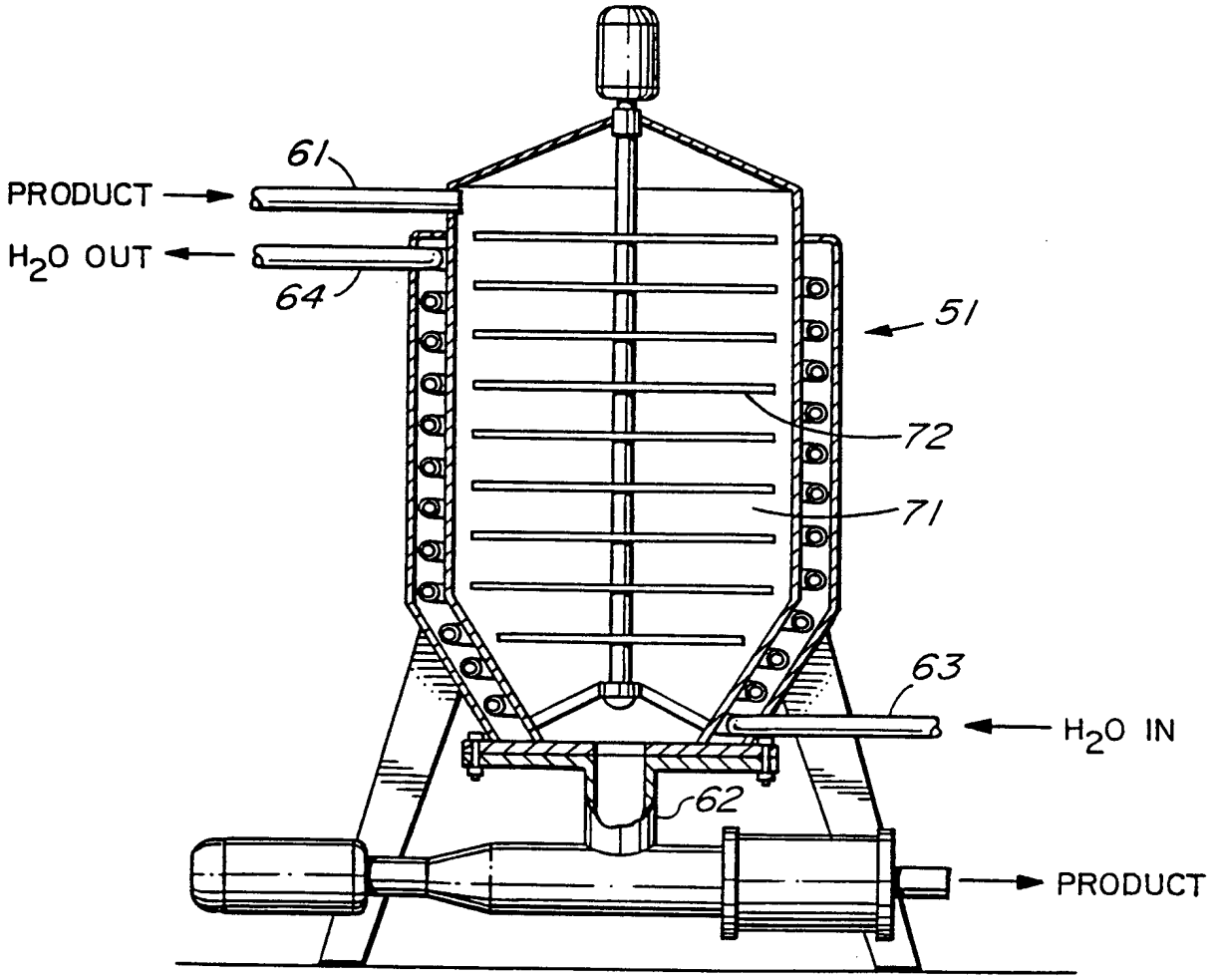

FIG. 5

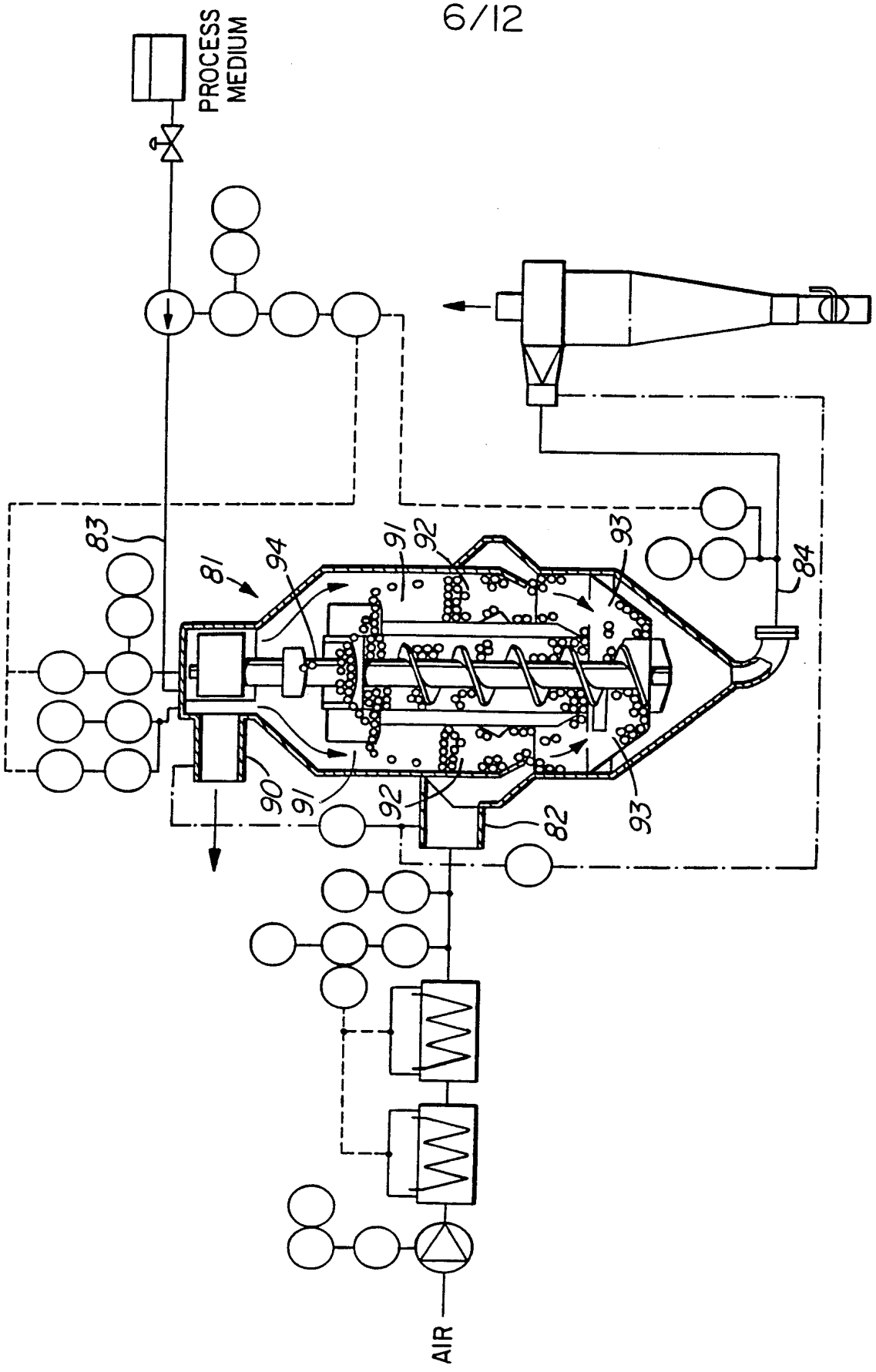

FIG. 6

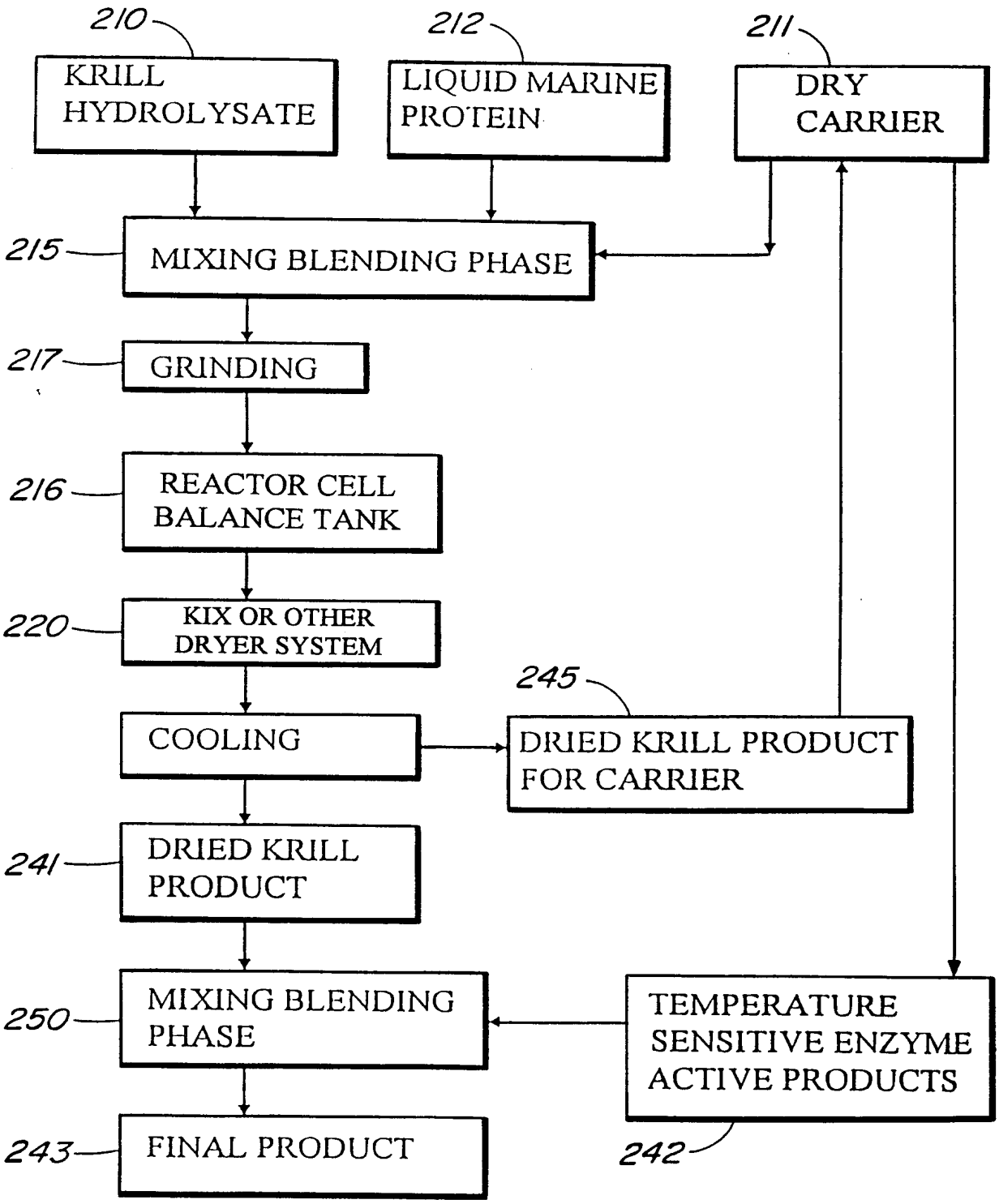
210
KRILL HYDROLYSATE
212
LIQUID MARINE PROTEIN
211
DRY CARRIER
215
MIXING BLENDING PHASE
217
GRINDING
216
REACTOR CELL BALANCE TANK
220
KIX OR OTHER DRYER SYSTEM
245
COOLING
DRIED KRILL PRODUCT FOR CARRIER
241
DRIED KRILL PRODUCT
250
MIXING BLENDING PHASE
TEMPERATURE SENSITIVE ENZYME ACTIVE PRODUCTS
243
FINAL PRODUCT
242

FIG. 7

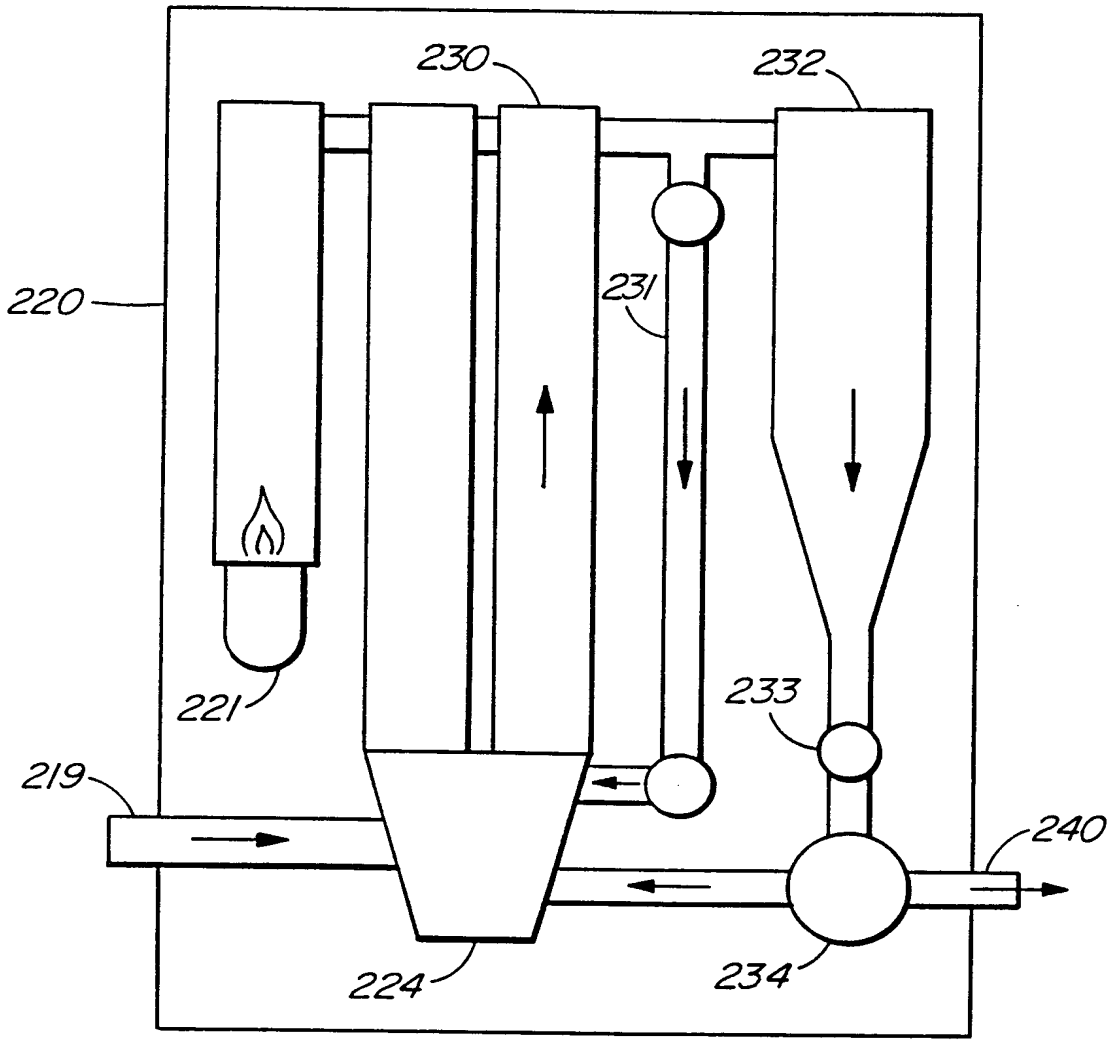

FIG. 8

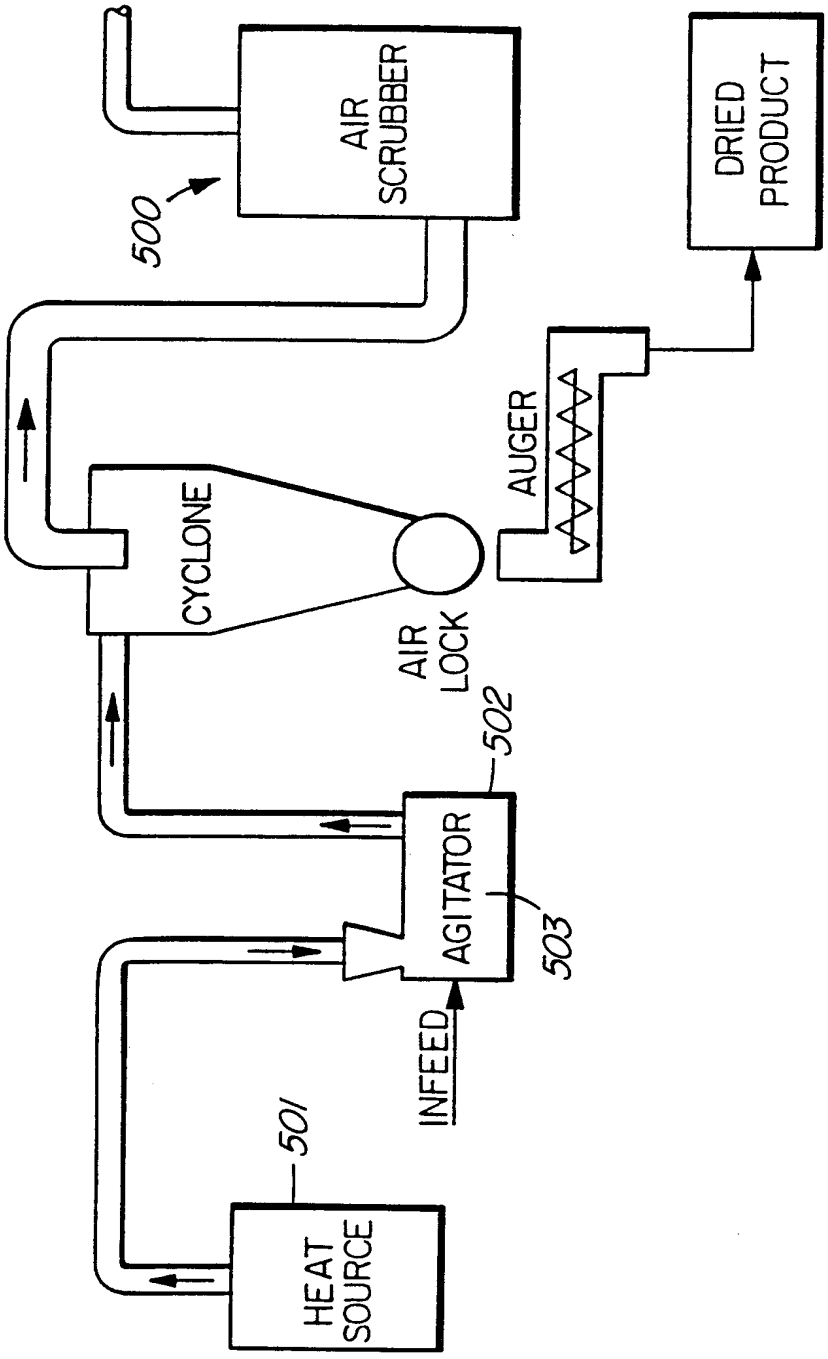

FIG. 9

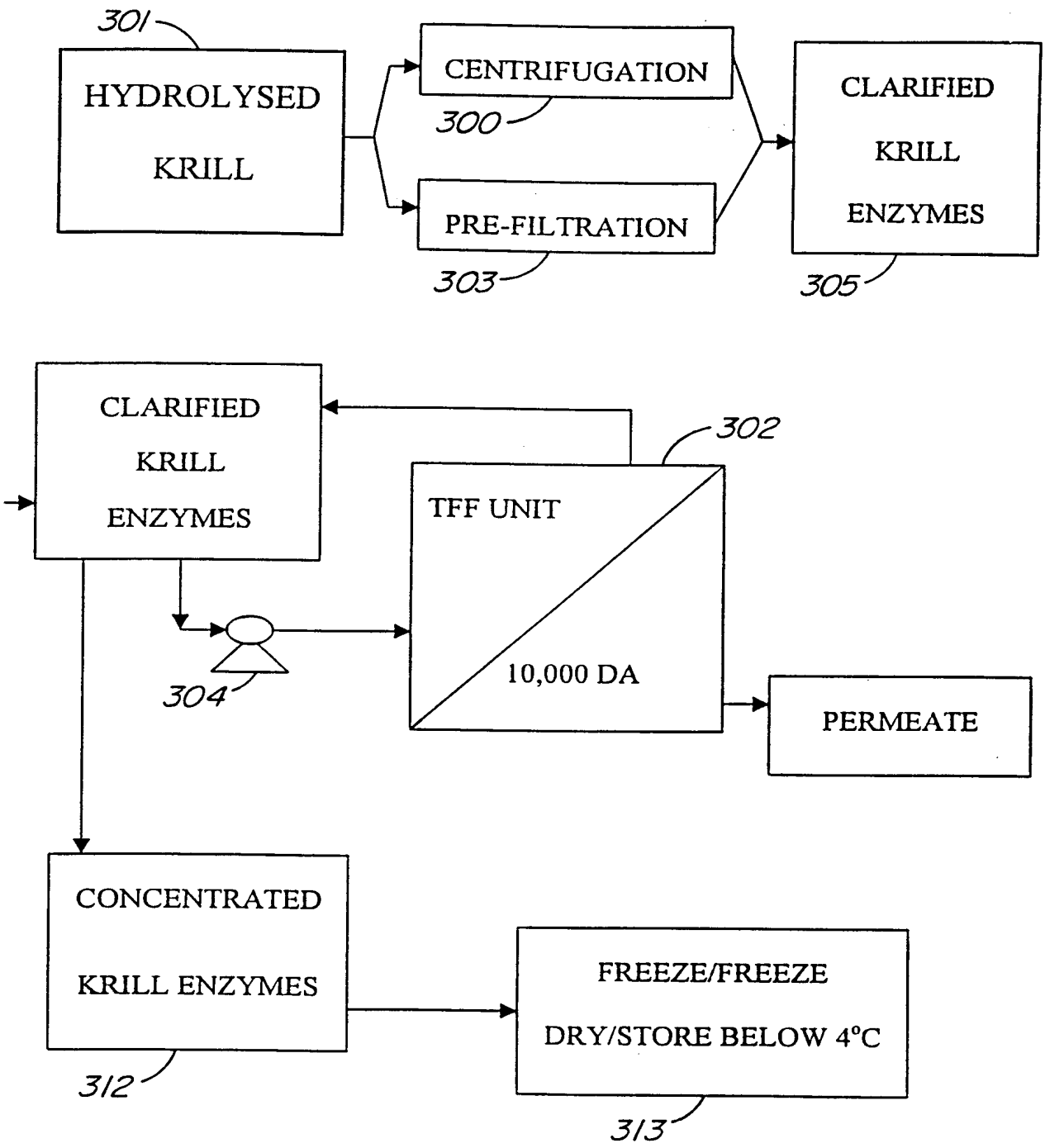

FIG. 10

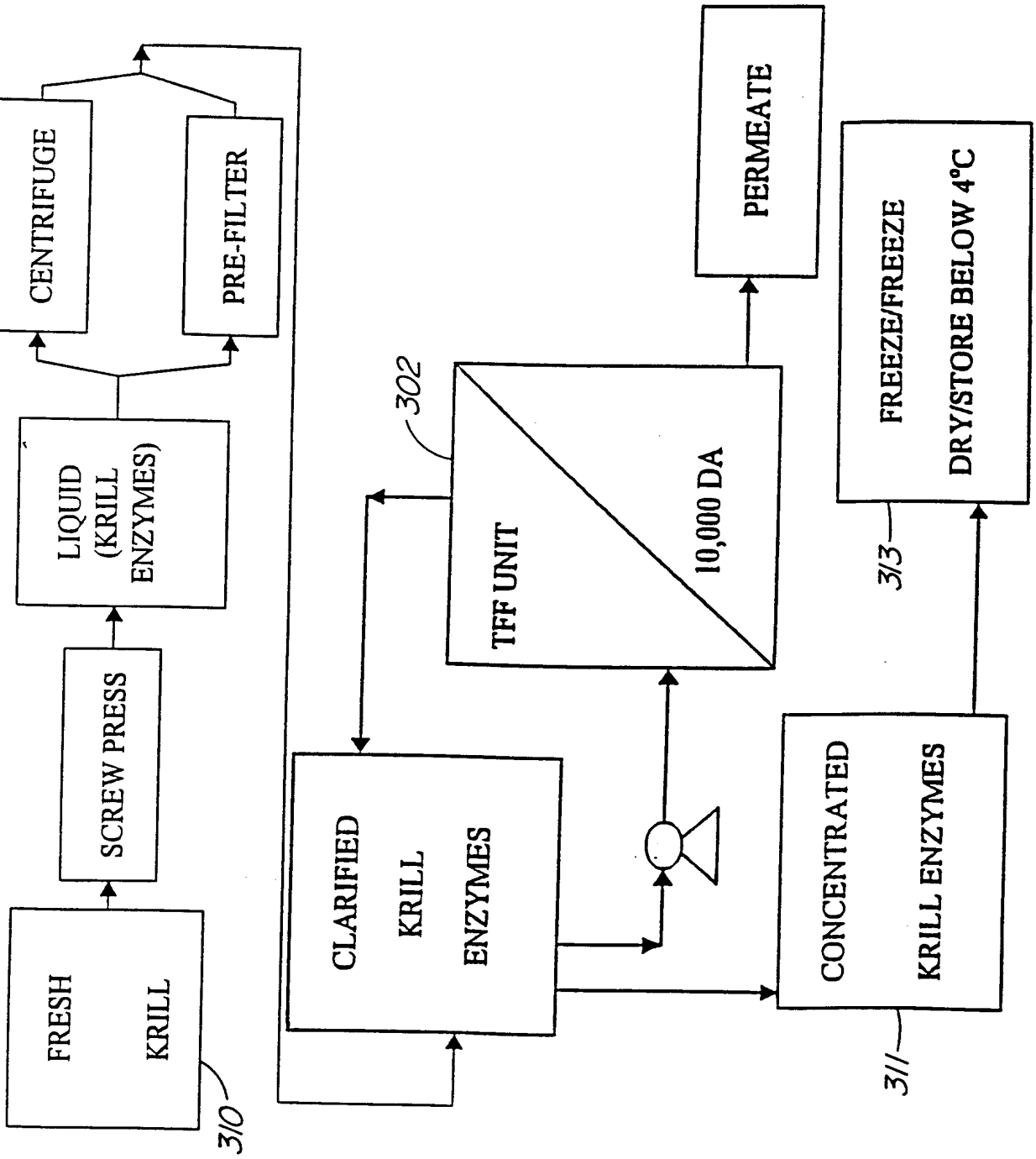

FIG. 11

12/12

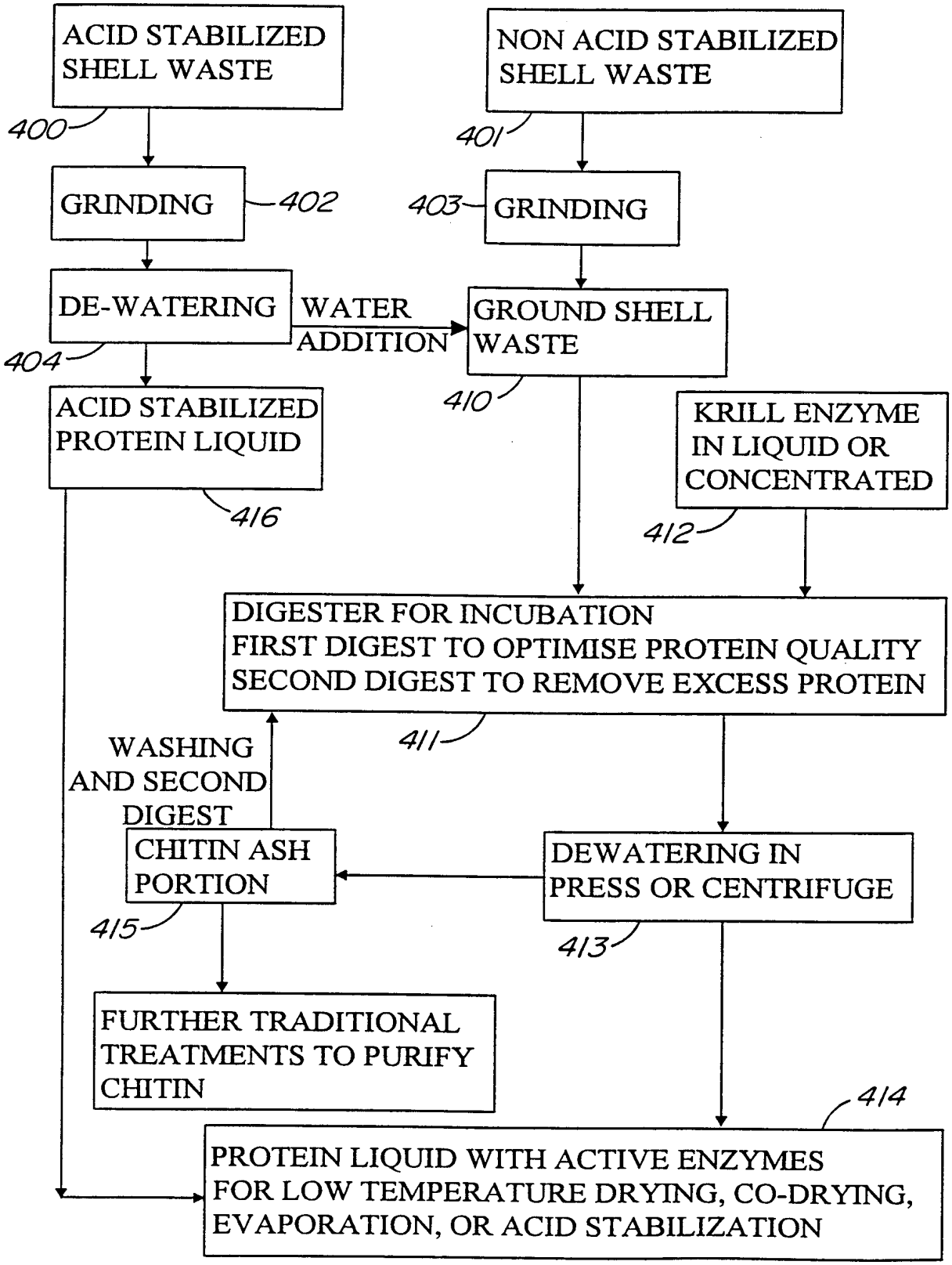
ACID STABILIZED SHELL WASTE
400
NON ACID STABILIZED SHELL WASTE
401
GRINDING
402
403
GRINDING
DE-WATERING
404
WATER ADDITION
GROUND SHELL WASTE
410
ACID STABILIZED PROTEIN LIQUID
416
KRILL ENZYME IN LIQUID OR CONCENTRATED
412
DIGESTER FOR INCUBATION
FIRST DIGEST TO OPTIMISE PROTEIN QUALITY
SECOND DIGEST TO REMOVE EXCESS PROTEIN
411
WASHING AND SECOND DIGEST
CHITIN ASH PORTION
415
DEWATERING IN PRESS OR CENTRIFUGE
413
FURTHER TRADITIONAL TREATMENTS TO PURIFY CHITIN
414
PROTEIN LIQUID WITH ACTIVE ENZYMES FOR LOW TEMPERATURE DRYING, CO-DRYING, EVAPORATION, OR ACID STABILIZATION

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.

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"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

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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

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/CA 99/00075

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		DE 3854050 D	27-07-1995
		EP 0393035 A	24-10-1990
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		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

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[X] See patent family annex.

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"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

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"&" 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

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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)
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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

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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

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Renseignements relatifs aux membres de familles de brevets

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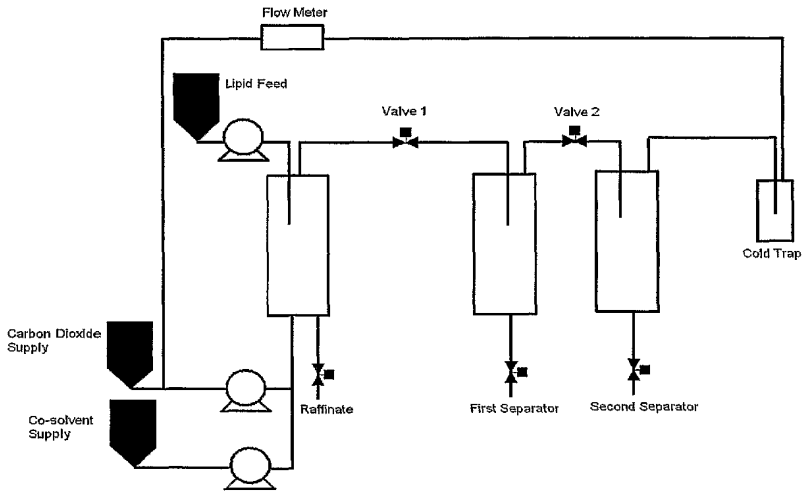
(54) **Title:** PROCESS FOR SEPARATING LIPID MATERIALS



(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.

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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.