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Beaudoin et al.

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(54) **METHOD OF EXTRACTING LIPIDS FROM MARINE AND AQUATIC ANIMAL TISSUES**

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(51) **Int. Cl.**⁷ **A61K 35/56**

(52) **U.S. Cl.** **424/522; 424/523**

(58) **Field of Search** **424/522, 523,**
424/94.1, 94.2

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,331,695 A 5/1982 Zosel
5,006,281 A 4/1991 Rubin et al.
6,055,936 A * 5/2000 Collin 11/215

FOREIGN PATENT DOCUMENTS

CA 1098900 4/1981
CA 2115571 5/1993
EP 0732378 9/1996
JP 53112195 9/1978
JP 60 035057 2/1985
JP 360035057 * 2/1985
JP 04 057853 2/1992
JP 04057853 2/1992
JP 08198754 6/1996
JP 08198754 8/1996
NO 147365 5/1982
WO WO 8401715 5/1984

OTHER PUBLICATIONS

Patent Abstracts of Japan, Jul. 2, 1976, JP 51 076467.
Patent Abstracts of Japan vo. 009, No. 059, Mar. 15, 1985, JP 59196032.

Thomas Carell, Edward A. Wintner, A. Bashir-Hashemi, Julius Rebek, Jr. "A Novel Procedure for the Synthesis of Libraries Containing Small Organic Molecules". *Angew. Chem. Int. Ed. Engl.* 1994, 33, No. 20; p. 2059.

Thomas Carell, Edward A. Wintner, Julius Rebek, Jr. "A Solution-Phase Screening Procedure for the Isolation of Active Compounds from a Library of Molecules". *Angew. Chem. Int. Ed. Engl.* 1994, 33, No. 20; p. 2061.

Marie-Thérèse Château, Céline Ginestier-Verne, Jean Chiesa, René Caravano, Jean Paul Bureau. "Dimethyl sulfoxide-induced apoptosis in human leukemic U937 cells". *Analytical Cellular Pathology* 10 (1996) 75-84.

Takashi Kojima, Masao Yamamoto, Chihiro Mochizuki, Toshihio Mitake, Norimasa Sawada and Yohichi Mochizuki. "Different Changes in Express and Function of Connexin 26 and Connexin 32 During DNA Synthesis and Redifferentiation in Primary Rat Hepatocytes Using a DMSO Culture System" *Hepatology*, Sep. 1997; vol. 26, No. 3, 1997.

Kit S. Lam. "Application of combinatorial library methods in cancer research and drug discovery". *Anti-Cancer Drug Design* (1997), 12, 145-167.

Jose Prados, Consolacion Melguizo, Juan Emilio Fernandez, Amelia Eva Aranega, Luis Alvarea and Antonia Arangea. "Actin, Tropomyosin and α -Actinin as Markers of Differentiation in Human Rhabdomyosarcoma Cell Lines Induced with Dimethyl Sulfoxide". *Cellular Molecular Biology*. 39(5) 525-536, 1993.

Howard M. Prentice, Stephen E. Moore, John G. Dickson, Patrick Doherty and Frank S. Walsh. "Nerve growth factor-induced changes in neural cell adhesion molecule (N-CAM) in PC12 cells". *The EMBO Journal*, vol. 6, No. 7, pp. 1859-1863, 1987.

Stefan Sjolander and Csaba Urbaniczky, "Integrated Fluid Handling System for Biomolecular Interaction Analysis". *Analytical Chemistry*, vol. 63, No. 29, Oct. 15, 1991.

Alexander Szabo, Lesley Stolz and Russ Granzow. "Surface plasmon resonance and its use in biomolecular interaction analysis (BIA)".

O. Trubiani, C. Peiri, M. Rapino, R. DiPrimo. "The c-myc gene regulates the polyamine pathway in DMSO-induced apoptosis". *Cell Proliferation*, 1999, 32, 119-129.

R.A. Houghten, J.R. Appel, S.E. Blondelle, J.H. Curevo, C.T. Dooler and C. Pinilla. "The Use of Synthetic Peptide Combinatorial Libraries for the Identification of Bioactive Peptides" *BioTechniques* vol. 13, No. 3 (1992).

* cited by examiner

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(57) **ABSTRACT**

Provided herein is a method for extracting lipid fractions from marine and aquatic animal material by acetone extraction. The resulting non-soluble and particulate fraction is preferably subjected to an additional solvent extraction with an alcohol, preferably ethanol, isopropanol or t-butanol or an ester of acetic acid, preferably ethyl acetate to achieve extraction of the remaining soluble lipid fraction from the marine and aquatic animal material. The remaining non-soluble particulate contents is also recovered since it is enriched in proteins and contains a useful amount of active enzymes. Also provided herein is a krill extract.

30 Claims, 20 Drawing Sheets

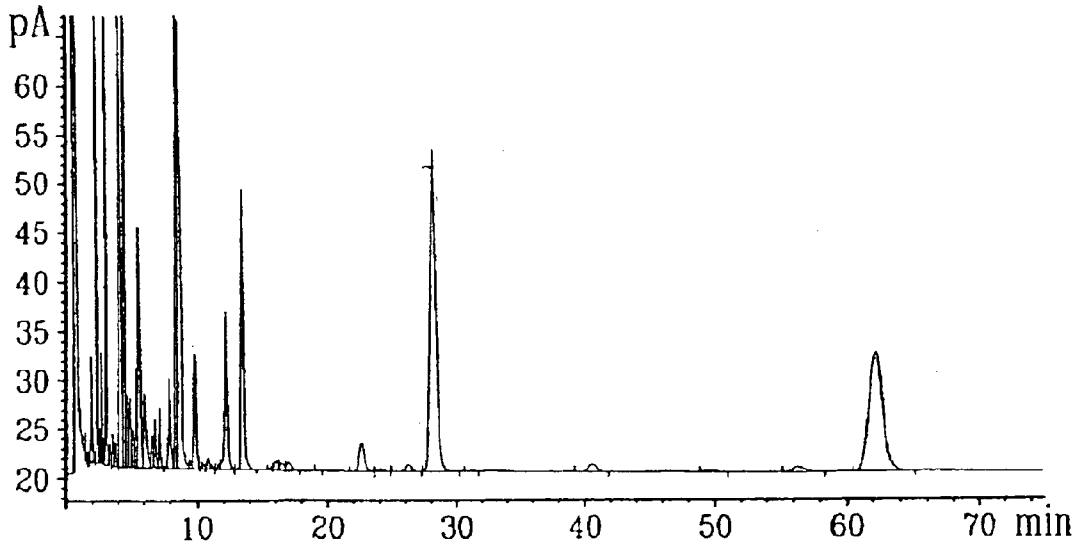
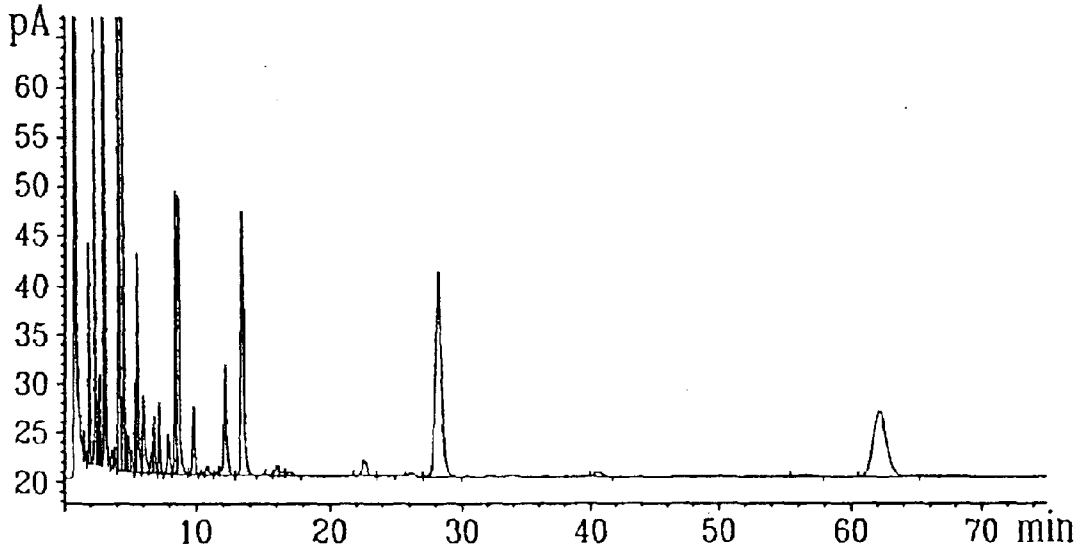


FIG. 1

1.263		4.521 - 16:1	11.637
1.455 - 12:0		4.684	12.145 - 18:3
1.625		4.891 - 16:1tr	13.458
1.812		5.121	15.626 - 20:0
1.876		5.426	16.045
2.056		5.570	16.482 - 20:1
2.173		6.037	17.017 - 20:1(cis11)
2.331 - 14:0		6.662	19.344 - 20:2
2.505		6.871	22.606 - 20:4(6,10,14,18)
2.591 - 14:1		7.235	24.103
2.682		7.925 - 18:0	26.247
2.802		8.439 - 18:1	28.287
2.855		8.640 - 18:1tr	31.295
3.078 - std 15:0		9.544	40.655
3.309		9.801 - 18:2	49.721
3.586		10.491	56.373
3.810		10.825	62.225
4.176 - 16:0		11.042	



1.217	3.806	9.786 - 18:2
1.264	4.157 - 16:0	10.484
1.454 - 12:0	4.515 - 16:1	10.813
1.624	4.680	11.590
1.812	4.891 - 16:1tr	12.136 - 18:3
1.876	5.028	13.447
2.055	5.109	15.623 - 20:0
2.171	5.421	16.025
2.330 - 14:0	5.562	16.466 - 20:1
2.505	6.031	17.021 - 20:1 (cis11)
2.591 - 14:1	6.642	22.585 - 20:4 (6,10,14,18)
2.680	6.870	24.100
2.800	7.230	26.217
2.854	7.910 - 18:0	28.241
3.077 - std 15:0	8.419 - 18:1	40.622
3.306	8.622 - 18:1tr	56.417
3.585	9.529	62.086

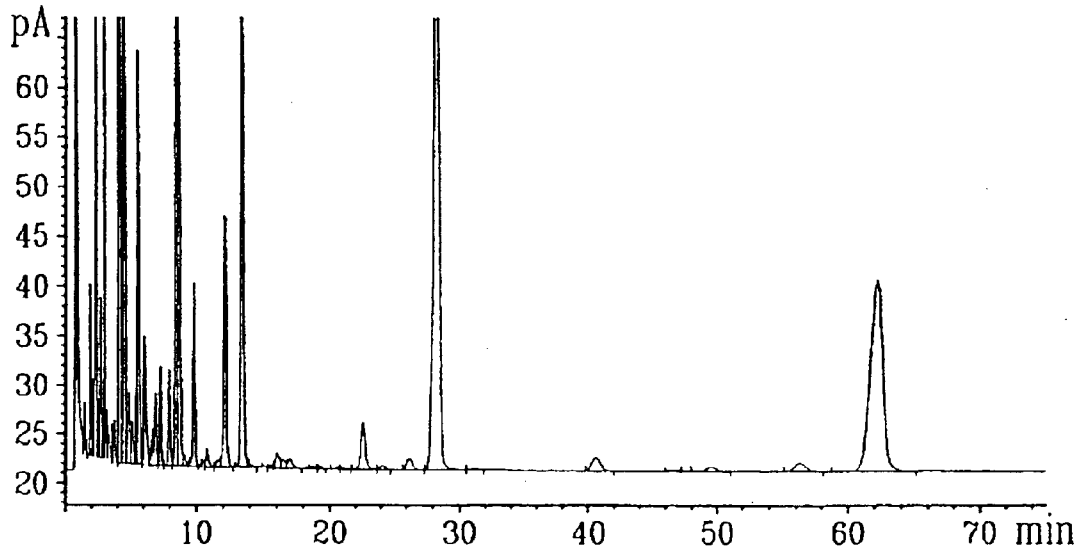


FIG. 3

1.216	4.520 - 16:1	13.457
1.262	4.683	13.943
1.454 - 12:0	4.884 - 16:1tr	15.053
1.624	5.030	15.572 - 20:0
1.811	5.111	16.016
1.875	5.420	16.486 - 20:1
2.016	5.561	16.999 - 20:1(cis11)
2.054	6.031	18.762
2.174	6.642	19.303 - 20:2
2.330 - 14:0	6.868	20.474
2.505	7.226	21.027 - 20:3
2.589 - 14:1	7.908 - 18:0	22.575 - 20:4 (6,10,14,18)
2.679	8.444 - 18:1	24.071
2.799	8.639 - 18:1tr	26.215
2.854	9.005	28.333
2.981	9.536	31.180
3.074 - std 15:0	9.788 - 18:2	40.560
3.304	10.267	46.595
3.580	10.481	49.513
3.804	10.807	56.292
4.169 - 16:0	11.626	62.250
4.296	12.140 - 18:3	

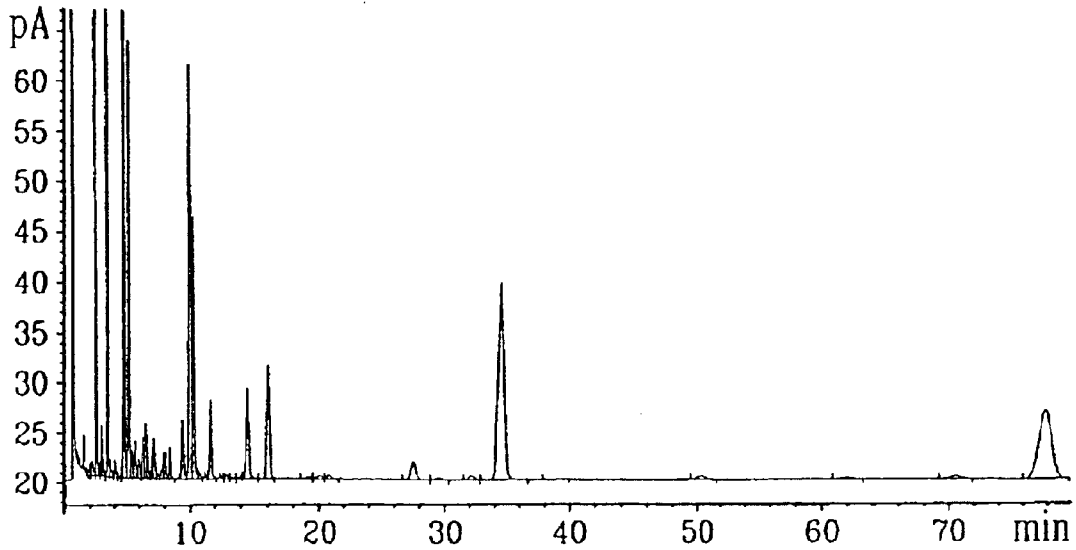


FIG. 4

1.552 - 12:0	5.675 - 16:1tr	12.888
1.749	5.964	13.388
1.968	6.284	14.017
2.095	6.533	14.524 - 18:3
2.262	6.655	16.107
2.485	7.009	19.275 - 20:0
2.582 - 14:0	7.159	20.112 - 20:1 (cis11)
2.784	7.440	20.781 - 20:1
2.886 - 14:1	7.874	27.553
3.004	8.019	29.529
3.145	8.462	32.161
3.478 - std 15:0	9.411 - 18:0	34.614
3.720	10.000 - 18:1	39.240
4.088	10.249 - 18:1tr	50.374
4.325	10.716	61.892
4.793 - 16:0	11.357	70.568
5.196 - 16:1	11.647 - 18:2	77.894
5.406	12.519	

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