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<p>(54) Title: METHOD OF EXTRACTING LIPIDS FROM MARINE AND AQUATIC ANIMAL TISSUES</p> <p>(57) Abstract</p> <p>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 <i>t</i>-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.</p> <div data-bbox="1133 1774 1437 1942" style="border: 1px solid black; padding: 5px; text-align: center;"> Petition for Inter Partes Review Of U.S. Patent 8,278,351 Exhibit ENZYMOTEC - 1002 </div>		

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METHOD OF EXTRACTING LIPIDS FROM MARINE AND AQUATIC ANIMAL TISSUES

BACKGROUND OF THE INVENTION

5 This invention relates to the extraction of lipid fractions from marine and aquatic animals such as krill, *Calanus*, fish and sea mammals. More specifically, this invention relates to an improved method of extracting lipid fractions by dehydration with solvents and recovering a solid residue rich in active enzymes.

10 Lipid fractions obtained from marine and aquatic animals such as krill, *Calanus*, fish and sea mammals have various applications:

Medical applications

Marine and aquatic animal oils and fractions thereof contain various therapeutic agents. For example, it is reported that various marine and aquatic animal oils have
15 anti-inflammatory properties. Marine and aquatic animal oils are also reported as helpful in reducing the incidence of cardiovascular disease. Also, some marine and aquatic animal oils are reported as suppressing the development of certain forms of lupus and renal diseases. As a further example, krill may be used as a source of enzymes for debridement of ulcers and wounds or to facilitate food digestion. Also
20 marine and aquatic oils contain various antioxidants, which may have potential therapeutic properties.

Nutraceuticals

Considering the beneficial effects of omega-3 fatty acids, oils from krill, *Calanus* and fish could be used as dietary supplements to human diet. These fatty acids are
25 essential for proper development of the brain and the eye. Marine and aquatic animal oils are also rich in liposoluble vitamins A, D and E and carotenoids.

Cosmetics

Various marine and aquatic animal oils are used for the production of moisturizing creams.

Fish farming

Among the lipids found in krill, *Calanus* and fish, high concentrations of fatty acids 20:5 (eicosapentaenoic acid) and 22:6 (docosahexaenoic acid) are present. These fatty acids are essential nutrients and are beneficial as fish feed. Furthermore, these essential nutrients are carried over in human diet by eating the fish grown on such diets.

Animal feed

Animal feed diets rich in omega-3 fatty acids may increase the level of unsaturated fatty acids and decrease cholesterol levels of meat. This property is already exploited in the poultry industry to improve the quality of eggs.

Various methods for extracting marine and aquatic animal oils are known. For example, it is known to extract fish oil using organic solvents such as hexane and ethanol. It is also known to measure the fat content in fish muscle tissue using solvents such as acetone.

USP 4,331,695 describes a method using pressurized solvents which are gaseous at room temperature, such as propane, butane or hexane. The extraction is performed at preferred temperatures of 15 to 80°C on shredded vegetable or finely divided animal products. The extracted oils are then made to precipitate under high pressure and elevated temperatures of 50 to 200°C. However, hexane is a poor extraction solvent for marine animals such as krill. Furthermore, the high temperatures used in the precipitation step negatively alters the lipids.

Canadian Patent Application 2,115,571 describes a method for extracting oils from various brown and red algae species. The method provides for example Soxhlet extraction using nearly pure ethanol for 40 hours.

USP 5,006,281 describes a method for extracting oil from marine and aquatic animals such as fish. The marine and aquatic animal is first treated with an antioxidant compound, finely divided and centrifuged to separate the oil phase from

the aqueous phase and solid phase. The oil phase is then further treated with antioxidant to remove undesirable odour or taste.

5 Canadian Patent 1,098,900 describes a method for extracting oils from krill. The method involves emulsifying fresh or defrosted krill in an aqueous medium. The oil fraction is recovered by centrifugation.

10 Folch in the article published in the year 1957 in J. biol. Chem. 226: 497-509 "A simple method for the isolation and purification of total lipids from animal tissues" proposes an extraction method using chloroform and methanol. This method is not commercially feasible because of the toxicity of the solvents involved.

15 However, prior art processes are generally commercially unfeasible or provide low quantitative yields. Thus, it is an object of the present invention to provide an improved marine and aquatic animal oil extraction method allowing recovery of a valuable lipid fraction and separate recovery of a valuable protein rich solid residue that comprises active enzymes.

20 Other objects and further scope of applicability of the present invention will become apparent from the detailed description given hereinafter. It should be understood, however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

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BRIEF DESCRIPTION OF THE DRAWINGS

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

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

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

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

Figure 5. Gas-liquid chromatography of fatty acids from frozen krill (*t*-butanol)

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