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(54) **Title:** EXTRACTION OF HIGHLY UNSATURATED LIPIDS WITH LIQUID DIMETHYL ETHER

(57) **Abstract:** A process for obtaining lipids containing highly unsaturated fatty acids from plant or animal material, including contacting the material with liquid dimethyl ether to give a dimethyl ether solution containing lipids and a residue of plant or animal material, separating the solution from the residue of plant or animal material, and recovering lipids from the solution.

EXTRACTION OF HIGHLY UNSATURATED LIPIDS WITH LIQUID DIMETHYL ETHER

TECHNICAL FIELD

This invention relates to separation technology. In particular, the invention relates to the extraction of materials, such as dried or partially dried plants or seeds (including marine or terrestrial species), or animal products (including marine or terrestrial species), with liquid dimethyl ether (DME), and optionally also with near-critical carbon dioxide, to obtain an extract rich in highly unsaturated lipids, especially highly unsaturated complex lipids, and optionally, a residue that is useful as a nutraceutical or for extracting water soluble enzymes and/or proteins.

BACKGROUND

Highly unsaturated lipids (lipids having 3 or more sites of unsaturation, and 18 or more carbons in the fatty acid chain) have a variety of metabolic roles within the human body. They are essential in the development of the brain and eyesight for infants, and may also be beneficial for cardiovascular health, mental health, and immune and inflammatory conditions. The biological properties of these lipids are usually dependent on the type of fatty acids that are present, and those containing highly unsaturated fatty acids are the most bioactive. In general, these highly unsaturated fatty acids are only found in significant quantities in complex lipids of terrestrial plants and animals, but may also appear in both neutral and complex lipids of marine animals.

Phospholipids are a subset of complex lipids. They are essential components of all mammalian cell membranes, and play an important role in maintaining the fluidity of the cell membrane, and passage of molecules through the membrane. The highly unsaturated arachidonic acid (C20:4 w-6) is absent from, or present in very low concentrations in, secondary products derived from animals, such as phospholipids from non-human milk. Arachidonic acid is vital for the development of infants, and so infant formula made from non-human milk is supplemented with this fatty acid. There is a need, therefore, to obtain sources of this fatty acid for this purpose. The complex lipids of many animal tissues, especially organs and glands, are rich in arachidonic acid, as are eggs.

Mosses and ferns are also known to contain high levels of arachidonic acid in complex lipid form. It is therefore desirable to find an extraction technology which can recover this highly unsaturated fatty acid (HUFA) in a complex lipid form, especially since the complex lipid form of the fatty acid gives protection against oxidation.

Marine organisms (micro and macro algae, fish flesh, eggs and livers, molluscs, invertebrates) are rich sources of the HUFAs eicosapentaenoic (C20:5 w-3) and docosahexaenoic acid (C20:6

w-3) in neutral and/or complex lipid form. These fatty acids are also required for infant formula supplementation, and for use in controlling neurological disorders, cardiovascular disease, inflammation, and lipid content in the blood. It is also desirable to find an extraction technology which can recover these polyunsaturated fatty acids.

Similarly, seeds from certain plants, especially those from pinus and podocarp trees, contain complex lipids rich in non-methylene interrupted polyunsaturated fatty acids (C20:3 and C20:4). Non-methylene interrupted fatty acids are used for controlling satiety and as possible anti-inflammatory agents. There is a need therefore to find an extraction technology which can recover these polyunsaturated fatty acids.

The extraction of neutral lipids using supercritical CO_2 is well known, especially in the extraction of seed oils. A disadvantage of these processes in general is that large high pressure vessels (typically 300 bar or higher pressure is used) are required to contain the raw material, which makes the production plant very expensive. High flow rates and long extraction times are also required, as the oils have very low solubility in supercritical CO_2 (typically 1 g of oil per 100 g of solvent). There are fewer publications concerning the extraction of lipids from marine species. US 6,083,536 describes a process for the extraction of non-polar lipids from crude freeze-dried mussel powder to give a non-polar lipid fraction useful for the treatment of inflammatory conditions. Fresh mussel is stabilised with tartaric acid prior to freeze-drying and CO_2 extraction. No compositional data of the extract is given, and no complex lipids are extracted, as they are insoluble in CO_2.

US 4,367,178 describes a process for purifying crude soy lecithin by using supercritical CO_2 to extract neutral lipids and leave behind insoluble phospholipids, thereby concentrating the phospholipids in the lecithin. The crude lecithin had been produced by conventional degumming of soy oil. The use of co-solvents such as ethanol to increase the solvent power of supercritical CO_2 has been proposed to overcome the limitations of CO_2.

EP 1,004,245 A2 describes a process in which dried egg is first extracted with supercritical CO_2 to remove neutral lipids, and is then either extracted with supercritical CO_2 and an organic co-solvent (ethanol) that is a liquid at room temperature or the organic solvent (without CO_2) to extract the phospholipids. Both options have the disadvantage of incomplete phospholipid extraction. In addition, both leave solvent residues in the defatted egg powder, which results in denaturation of protein. The neutral egg lipids obtained by supercritical CO_2 extraction have negligible levels of highly unsaturated fatty acids, as shown in example 3.

Arntfield *et al.* (JAOCS, 69, 1992, 823 – 825) show that egg protein is substantially denatured after extraction with CO_2 and methanol as a co-solvent. The use of ethanol with supercritical CO_2 results in incomplete extraction of phospholipids. Phosphatidyl choline is the most readily extracted phospholipid, but all other phospholipids have very low or no solubility and are not extracted (Teberliker *et al.*, JAOCS, 78, 2002, 115 – 119). Schriener *et al.* (Journal of Food Lipids, 13, 2006, 36 – 56) show that the majority of highly unsaturated fatty acids in egg yolk lipids are in phosphatidyl ethanolamine, which is not extracted in this process.

PCT publication WO 02/092540 discloses medicinal uses of polar lipids containing HUFAs, and blends of polar lipids with other oils. The extraction method is disclosed as using alcohol and centrifugation, but no further details are given. It is also disclosed that the polar lipid-rich fraction could be obtained as a by-product of edible seed oil extraction by the industrial process of degumming.

A process for the extraction of phospholipids containing HUFA from wet phospholipid-containing material is described in PCT publication WO 2005/072477. An aliphatic alcohol, and in particular, isopropanol and/or n-propanol, is used. The material containing phospholipids is contacted with a water soluble aliphatic alcohol at a temperature sufficiently high that the phospholipids dissolve in the solvent, while the proteins, which become denatured, precipitate from solution.

DME has previously been used in the extraction of lipids from raw egg yolk (US 4,157,404) and dried egg powder (US 4,234,619). The process causes the fractionation of the lipid and protein components into separate streams. US 4,157,404 describes the extraction of lipids from raw egg yolk (50-55 % moisture content), but the proteins are denatured in the process. The described process also requires that the lipid and water mixture recovered is then dehydrated to a water content of 20 % or less, which then results in phase separation of neutral-rich and complex lipid/water-rich phases. US 4,234,619 discloses that proteins are not denatured if the egg is dry, but the phospholipids can then only be partially extracted. In the processes described, DME was used in a temperature range of -30°C to 40°C, spray dried whole egg powder was used and only a maximum 70 % yield of phospholipids was obtained. The desired product of the invention was an egg powder that contained at least 30 % of its original phospholipids content, and no cholesterol. A process for the recovery and concentration of highly unsaturated fatty acids is not disclosed. Further, the separation of neutral lipids and complex lipids in the total lipid extract into separate fractions was not discovered because of the low extraction and separation temperatures used.

PCT publication WO 2004/066744 describes the extraction of lipids from an aqueous dairy stream using near critical extraction where DME is the solvent. The publication also discloses that neither supercritical CO_2 nor liquid DME can extract lipids in useful yields from dry whey protein concentrate (WPC) dairy powders. The process does not disclose a method for extracting HUFA polar lipids from dry animal or plant tissue. Whey proteins are not found in animal or plant tissues, and the lipids obtained do not contain highly unsaturated fatty acids.

NZ 535894 describes the extraction of lipids from spray dried dairy products containing milk fat globular membrane proteins, which is a dairy lipoprotein/lipid/lactose mixture arising from the production of skim milk powder. The proteins are associated with the cream fraction of milk, and are not found in animal or plant tissue. Attempts to extract lipids from this dairy powder stream with high lactose contents (where high lactose content means at least 30% by mass of the total powder) by extraction using liquid DME were unsuccessful, and it was necessary to reduce the lactose content prior to production of the powder. There is no disclosure of a method for extracting HUFA lipids from dry animal or plant tissue, because the lipids contain no HUFAs. The residual powder after extraction still contains around 6 – 8 % complex lipids.

PCT publication WO 2006/058382 broadly describes a process for obtaining an extract from a range of materials using liquid DME. There is, however, no description of the extraction of HUFAs, nor the separation of complex lipids from neutral lipids. The process described is a simple conventional process which uses liquid DME. Indeed, the sole process described in any detail is a process that uses liquid DME for obtaining an extract from Jojoba seeds which do not contain HUFAs.

It is evident that the type of proteins and other complex carbohydrates present in products derived from animal and plant materials (and the method by which the material is dried) determines whether or not lipids can be successfully extracted. The proteins and complex carbohydrates that are present in plant or animal tissues differ substantially from those found in secondary products derived from animals, such as milk. It is therefore generally not possible to predict with any certainty whether extraction of lipids, and especially complex lipids containing highly unsaturated fatty acids, is possible from plant or animal tissue containing proteins and carbohydrates associated with cells and tissue using dimethyl ether.

Surprisingly, the applicant has discovered that liquid DME can be used as an efficient extractant for obtaining HUFAs from plant or animal material, and in particular that residual DME in a lipid extract consisting of neutral and complex lipids enables formation of a gum-like phase containing complex lipids which is then easily separated from the neutral lipids.

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