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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 20 April 2006 with an application for Letters Patent number 546681 made by OWEN JOHN CATCHPOLE and STEPHEN JOHN TALLON.

Dated 17 August 2007.

Neville Harris
Commissioner of Patents, Trade Marks and Designs

546681

NEW ZEALAND

PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

PRODUCT AND PROCESS

We, OWEN JOHN CATCHPOLE and STEPHEN JOHN TALLON, both New Zealand citizens c/- Industrial Research Limited, Gracefield Research Centre, Gracefield Road, Lower Hutt, New Zealand, do hereby declare this invention to be described in the following statement:

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, cerebrosides, sphingomyelin, or a combination thereof.

BACKGROUND

Phospholipids are a major component of all biological membranes, and include phosphoglycerides (phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, cardiolipin and phosphatidyl serine); and sphingolipids such as sphingomyelin.

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, cell differentiation.

Reasonable levels 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.

Both phospholipids and gangliosides have been implicated in conferring a number of health benefits including brain health, sports nutrition, 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.

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, or 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 IPA 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 conveys environmental benefits through reduced organic solvent use. The disadvantage of supercritical CO_2 processing is that the solubility of many compounds is low, and only neutral lipids are extracted.

It is known that the use of organic co-solvents such as ethanol allows extraction of some polar compounds. 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 PA. PC is selectively extracted under some conditions, but at higher temperatures and pressures a higher degree of extraction of the insoluble phospholipids is obtained. 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

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