Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2446059 C 2010/03/23

(11)(21) 2 446 059

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2002/05/14
(87) Date publication PCT/PCT Publication Date: 2002/11/21
(45) Date de délivrance/Issue Date: 2010/03/23
(85) Entrée phase nationale/National Entry: 2003/10/31
(86) N° demande PCT/PCT Application No.: US 2002/015479
(87) N° publication PCT/PCT Publication No.: 2002/092073
(30) Priorité/Priority: 2001/05/14 (US60/291,484)

(51) Cl.Int./Int.Cl. *A61K 31/22* (2006.01), *A23K 1/16* (2006.01), *A23L 1/30* (2006.01), *A61K 31/202* (2006.01), *A61K 31/66* (2006.01), *A61K 45/06* (2006.01), *A61K 8/36* (2006.01), *A61K 8/92* (2006.01), *A61Q 19/00* (2006.01)

(72) Inventeurs/Inventors:
KOHN, GERHARD, DE;
BANZHAF, WULF, DE;
ABRIL, JESUS RUBEN, US

(73) Propriétaire/Owner:
MARTEK BIOSCIENCES CORPORATION, US

(74) Agent: MBM INTELLECTUAL PROPERTY LAW LLP

(54) Titre : PRODUCTION ET UTILISATION D'UNE FRACTION POLAIRE RICHE EN LIPIDES CONTENANT L'ACIDE STEARIDONIQUE ET L'ACIDE GAMMA-LINOLENIQUE EXTRAITS DE GRAINES ET DE MICROORGANISMES

(54) Title: PRODUCTION AND USE OF A POLAR LIPID-RICH FRACTION CONTAINING STEARIDONIC ACID AND GAMMA LINOLENIC ACID FROM PLANT SEEDS AND MICROBES

(57) Abrégé/Abstract:
The production and use, and in particular, the extraction, separation, synthesis and recovery of polar lipid-rich fractions containing gamma linolenic acid (GLA) and/or stearidonic acid (SDA) from seeds and microorganisms and their use in human food applications, animal feed, pharmaceuticals and cosmetics.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2002 (21.11.2002)

PCT

(10) International Publication Number
WO 02/092073 A1

(51) International Patent Classification[7]: A61K 31/22

(21) International Application Number: PCT/US02/15479

(22) International Filing Date: 14 May 2002 (14.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/291,484 14 May 2001 (14.05.2001) US

(71) Applicant *(for all designated States except US)*: **MARTEK BIOSCIENCES BOULDER CORPORATION** [US/US]; 4909 Nautilus Court N. #208, Boulder, CO 80301-3242 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KOHN, Gerhard** [DE/DE]; Julius Caesar Street 29, 55286 Wöerrstadt (DE). **BANZHAF, Wulf** [DE/DE]; Wöllsteiner Street 26A, 55599 Siefersheim (DE). **ABRIL, Jesus, Ruben** [US/US]; 6638 West 98th Drive, Westminster, CO 80030 (US).

(74) Agents: **DALLAS, Angela, K.** et al.; Sheridan Ross P.C., 1560, Broadway, Suite 1200, Denvers, CO 80202-5141 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/092073 A1

(54) Title: PRODUCTION AND USE OF A POLAR LIPID-RICH FRACTION CONTAINING STEARIDONIC ACID AND GAMMA LINOLENIC ACID FROM PLANT SEEDS AND MICROBES

(57) Abstract: The production and use, and in particular, the extraction, separation, synthesis and recovery of polar lipid-rich fractions containing gamma linolenic acid (GLA) and/or stearidonic acid (SDA) from seeds and microorganisms and their use in human food applications, animal feed, pharmaceuticals and cosmetics.

PRODUCTION AND USE OF A POLAR LIPID-RICH FRACTION CONTAINING STEARIDONIC ACID AND GAMMA LINOLENIC ACID FROM PLANT SEEDS AND MICROBES

FIELD OF THE INVENTION

The present invention relates to the fields of production and use, and in particular, the extraction, separation, synthesis and recovery of polar lipid-rich fractions containing gamma linolenic acid (GLA) and/or stearidonic acid (SDA) from seeds and microorganisms and their use in human food applications, animal feed, pharmaceuticals and cosmetics.

BACKGROUND OF THE INVENTION

Polyunsaturated fatty acids of the omega-6 and omega-3 series represent a special class of bioactive lipids in that they are important structurally in membranes in the body, but also participate directly and indirectly in communication between cells through the eicosanoid pathways and by their influence of these fatty acids on gene expression. Two of these fatty acids GLA (gammalinolenic acid; C18:3n-6) and SDA (stearidonic acid; C18:4n-3) have been shown to be effective in treating inflammatory conditions, autoimmune conditions, women's health conditions (e.g. menopause and premenstrual disorders) and fatty acid imbalances in infants and animals. Recent evidence indicates that some polyunsaturated fatty acids may be more bioavailable when supplied in a phospholipid form than in a triglyceride form. GLA and SDA have historically been supplied to the nutritional supplement markets in the form of oil extracted from seeds. However recent evidence indicates that some polyunsaturated fatty acids may be more bioavailable in a phospholipid form rather than in a triglyceride form. This may be because of the bipolar nature of phospholipids making them readily solubilized in the gut and available for digestion and uptake. This same bipolar property of phospholipids additionally would make these fatty acids, such as GLA and SDA, more functional in topical applications such as creams and lotions because of their ability to participate in emulsification processes. The present inventors propose that there may be important advantages in supplying GLA and SDA in the form of phospholipids and improved processes for recovering polar lipids enriched in these fatty acids are also needed.

Examples of polar lipids include phospholipids (e.g. phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidyl-glycerol, diphosphatidylglycerols), cephalins, sphingolipids (sphingomyelins and glycosphingolipids), and glycoglycerolipids. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, and amino alcohols. They are generally considered to be structural lipids, playing important roles in the structure of the membranes of plants, microbes and animals. Because of their chemical structure, polar lipids exhibit a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents. The term polar lipid within the present description is not limited to natural polar lipids but also includes chemically modified polar lipids. Although the term oil has various meanings, as used herein, it will refer to the triacylglycerol fraction.

One of the important characteristics of polar lipids, and especially phospholipids, is that they commonly contain polyunsaturated fatty acids (PUFAs: fatty acids with 2 or more unsaturated bonds). In many plant, microbial and animal systems, they are especially enriched in the highly unsaturated fatty acids (HUFAs: fatty acids with 4 or more unsaturated bonds) of the omega-3 and omega-6 series. Although these highly unsaturated fatty acids are considered unstable in triacylglycerol form, they exhibit enhanced stability when incorporated in phospholipids.

The primary sources of commercial PUFA-rich phospholipids are soybeans and canola seeds. These biomaterials do not contain any appreciable amounts of GLA or SDA unless they have been genetically modified. The phospholipids (commonly called lecithins) are routinely recovered from these oilseeds as a by-product of the vegetable oil extraction process. For example, in the production of soybean or canola oil, the beans (seeds) are first heat-treated and then crushed, ground, and/or flaked, followed by extraction with a non-polar solvent such as hexane. Hexane removes the triacylglycerol-rich fraction from the seeds together with a varying amount of polar lipids (lecithins). The extracted oil is then de-gummed (lecithin removal) either physically or chemically as a part of the normal oil refining process and the precipitated lecithins recovered. This process however has two disadvantages: (1) the seeds must be heat-treated before extraction with hexane, both increasing the processing cost and denaturing the protein fraction, thereby decreasing its value as a by-product; and (2) the use of the non-polar

solvents such as hexane also presents toxicity and flammability problems that must be dealt with.

The crude lecithin extracted in the "de-gumming" process can contain up to about 33% oil (triacylglycerols) along with sterols and glucosides. One preferred method for separating this oil from the crude lecithin is by extraction with acetone. The oil (triacylglycerols) is soluble in acetone and the lecithin is not. The acetone solution is separated from the precipitate (lecithin) by centrifugation and the precipitate dried under first a fluidized bed drier and then a vacuum drying oven to recover the residual acetone as the product is dried. Drying temperatures of 50-70°C are commonly used. The resulting dried lecithins contain approximately 2-4% by weight of oil (triacylglycerols). Process temperatures above 70°C can lead to thermal decomposition of the phospholipids. However, even at temperatures below 70°C the presence of acetone leads to the formation of products that can impair the organoleptic quality of the phospholipids. These by-products can impart musty odors to the product and also a pungent aftertaste.

What is needed is an improved process for effectively recovering polar lipids and phospholipids rich in GLA and SDA from biomaterials that enables the use of these fatty acid in food, nutritional supplement, pharmaceutical and cosmetic applications. Furthermore the fractions are needed as an ingredient in feed for companion animals and in aquaculture.

In the first step of one embodiment of the process, a low-oil content material is selected or the material is de-oiled by any suitable de-oiling process, but preferably by a de-oiling process that does not cause denaturation of the proteins. This would include processes that do not utilize high temperatures (e.g. greater than about 65°C) or high concentrations of solvents (e.g. greater than about 50%). Preferably the de-oiling process outlined in WO 96/05278 (U.S. Patent No. 5,928,696) is utilized. Preferably, a key change is made to this de-oiling process. We have unexpectedly found that homogenizing the biomaterial prior to addition of the alcohol and water, or homogenization after the addition of the alcohol and water, but most preferably homogenization both prior to and after addition of alcohol and water, leads to improvements in oil recovery up to 85% higher than without homogenization (FIG. 2). As used herein, homogenization can include any high shear process such as processing the mixture under pressure through a small orifice, using a colloidal mill, or other high shear process, etc. Preferably, when the mixture is forced through a small orifice, the

homogenization is conducted at pressures from about 100 bars to about 1000 bars, and more preferably from about 150 bars to about 350 bars. This is an unexpected result, as one skilled in the art would expect that homogenization of this type of mixture would lead to formation of very strong emulsions which would be very difficult to break, making the process less efficient.

A lecithin recovery process utilizing low concentrations of alcohol throughout the entire process is outlined in FIG. 3. Liquid egg yolk is used as the polar-lipid rich biomaterial in this example. It is understood, however, that other polar lipid-containing biomaterials (e.g. fish, crustaceans, microbes, brain tissue, milk, meat and plant material including oilseeds) could also be processed in a similar manner with minor modifications to the process. In the first step of the process, the material is de-oiled by any well-known de-oiling process, but preferably by a de-oiling process that does not cause denaturation of the proteins. For a more efficient recovery of the oil, the material is sheared by means of homogenization to break up the fat-containing cellular particles so that the oil in the particles can be separated as well as the free oil in the biomaterial. Alcohol and water are then added to the yolk and the mixture is re-homogenized. The concentration of alcohol in the aqueous solution can be from about 5 to about 35% w/w, preferably from about 20 to about 35% w/w, and most preferably from about 25 to about 30% w/w. The free oil is then separated by means of centrifugal force due to a difference in density. This results in two fractions being recovered: (1) a fraction with approximately 50-70% protein (as % dry weight) and about 30-50% dry weight as polar lipids, the mixture containing a significantly lower cholesterol content that the egg yolk; and (2) an egg oil with approximately 85% of the triacylglycerols of the egg yolk. Additional dosing of the protein/lecithin fraction with low concentration alcohol disperses the lecithin that is then separated from the protein by means of centrifugal force. Counter-current washing/centrifugation or cross-current washing/separation of the protein and lecithin products can be employed to improve the purity of the products and economics of the overall process. The protein is not denatured in this process and retains high resale value (because of its functionality) as a by-product of the process thereby reducing overall costs of all products produced.

Because of the simplicity of the equipment required in the process, the entire process can very easily be conducted under a reduced-oxygen atmosphere (e.g., nitrogen,

a preferred embodiment of the process), further protecting any HUFAs in the polar lipids from oxidation. For example, a gas tight decanter can be used to separate oil from the mixture. A suitable decanter is model CA 226-28 Gas Tight available from Westfalia Separator Industry GmbH of Oelde Germany, which is capable of continuous separation of oil from suspensions with high solids content in a centrifugal field. A gas tight separator useful for separating polar lipids from proteins is model SC 6-06-576 Gas Tight available from Westfalia Separator Industry GmbH of Oelde Germany, which is capable of continuous separation of solids from suspensions with high solids content in a centrifugal field.

An improved version of this process has also been developed. In this process the de-oiling and lecithin washing steps employing low alcohol concentrations are similar to the process outlined above. However after the lecithin phase is dried, it is washed with concentrated alcohol. Since proteins are not soluble in high concentrations of alcohol, they precipitate (while the lecithin dissolves) and the precipitated proteins are separated by density separation, e.g., using gravity or centrifugal force. The protein-reduced lecithin is then concentrated by means of evaporation of water and alcohol. The advantage of this variation of the process is that it provides options for the production of both higher and lower quality lecithin fractions, and in providing the higher quality lecithin, only a very small portion of the protein is denatured.

The process has also been modified for use of high concentrations of alcohol after the de-oiling step. The process steps after de-oiling the biomaterials are similar to the low alcohol concentration process, but instead of diluted alcohol, concentrated alcohol is added. After de-oiling, concentration and drying of the polar lipid/protein intermediate product takes place. The concentration/drying step is necessary to reduce the amount of concentrated alcohol necessary to be added to re-dissolve the polar lipids. The dried polar lipid/protein phase is washed with concentrated alcohol and the protein precipitates. The precipitated protein is separated by density separation, e.g., using gravity or centrifugal force, in a counter-current washing system. The protein–reduced polar lipids are concentrated by means of evaporation of alcohol and water. The advantage of this process is that it requires lower thermal energy inputs. The major disadvantage is that all of the protein is denatured and is of lower value.

While not wishing to be bound by any theory, it is believed that several of the underlying mechanisms in the processes above are as discussed in further detail below.

With regard to homogenization it is believed that destruction of cellular material occurs here. An objective is to achieve homogeneous distribution of all components, i.e., to create a homogeneous polydisperse system (protein, oil, lipoproteins, continuous phase water), so that, when aqueous or pure alcohol is added, this can immediately be uniformly, i.e., homogeneously, distributed without causing local irreversible protein denaturation. The temperature is to be kept as low as possible, so that as little lecithin as possible is dissolved in the oil phase. The pressure employed in the homogenization process should preferably be less than 1000 bars, and more preferably less than 600 bars, in order to destroy the quaternary and tertiary structure of the proteins, but not the primary and secondary structure. The concentration of alcohol is preferably less than 30% w/w, more preferably about 28%. An unduly low alcohol concentration can lead to significant protein swelling, so that the free smaller fat globules can be incorporated in the protein. The percentage of fats bonded in the form of lipoproteins is not further considered here, since it may not interfere with liberation of the polar lipids (phospholipids).

In principle, it is believed that the higher the alcohol concentration, the stronger the protein contraction, but the more nonpolar the aqueous phase, more polar lipids may be dissolved in the oil phase. The appropriate concentration and temperature must therefore be found, for example, by conducting a few preliminary experiments (centrifuge tests), for each raw material.

Taking into consideration the natural moisture content of the raw material, aqueous alcohol is added to produce preferred final alcohol concentrations of about 25-30%, and the dispersion is homogenized again. The contracted protein molecules and fat droplets are separated from each other. The intermediate layer between both, the polar lipid layer present on the surface of the fat globules, is thus disrupted. The oil therefore has an easier opportunity to be present as free phase in the dispersion. In order to re-establish equilibrium in this oil-in-water emulsion, on the one hand, the polar lipid could surround the fat globules again or, on the other hand, the oil droplets could coagulate to larger drops. For this purpose, the additional force of the centrifugal field is employed. The now larger oil drops can then coalesce, i.e., forming a separable, continuous phase.

The procedure with a homogenizer is surprising for one skilled in the art as this produces very small oil droplets. In past methods, oil droplets were not reduced in size before being separated, because the degree of emulsion increases due to the larger internal

surface area. Quite the contrary, agitation or kneading was carefully carried out, so that the oil can coagulate into larger drops. Heat was helpful in this malaxation process in order to also reduce the viscosity, among other things. The surprising effect that more oil can also be separated by a homogenization pressure increase to about 300 bars or more may be explained by the interactions of the proteins, polar lipids and oil (actually, the nonpolar lipid phase) with the solvent layer.

Oil separation must therefore occur so that in general the surface tension and surface state of the droplets (destroyed as a result of shear) regain their original equilibrium. This means the homogenized slurry is preferably introduced immediately into a density separation device (preferably, a centrifuge of appropriate design and geometric considerations) and separated there into non-polar lipids (oil), and polar lipids with protein, water and alcohol. The viscosity reduction is not necessary to the degree it is necessary in oil recovery without homogenization (as described in WO 96/05278). Direct transfer of the homogenized slurry into the centrifugal field can be important in order to support coalescence.

After one- or two-stage oil separation, preferably in a decanter (other types of density separation devices, including centrifuges, are also successfully used for this purpose), all free oil fractions (lipids and nonpolar lipids) are ideally separated so that, by subsequent reduction of the alcohol concentration with water in the protein phase, no oil droplets are found in the free water/alcohol phase, although the polarity of the mixture is increased and lecithin is therefore bonded again in this phase and the oil therefore "liberated". Normally, the oil in this polar lipid/protein/alcohol mixture becomes free when the alcohol concentration is reduced; i.e., the oil solubility diminishes in the polar lipid phase. It was surprisingly found that, after two-fold homogenization and centrifuging, very little free oil was centrifugable, even if the alcohol concentration was only 15%.

Sterols including cholesterol may have a greater affinity for the polar lipid phase than for the oil phase, resulting in a higher sterol content in the polar lipid phase than in the oil phase. Movement of sterols into the oil or polar lipid phases can be manipulated by changing the pH of the mixture, altering temperature or by addition of processing aids such as salts to increase or decrease the polar nature of the aqueous phase. Another method to reduce the cholesterol in the polar lipid-rich fraction is to add oil with little or

no cholesterol to the polar lipid-rich fraction and repeat the de-oiling process. In this way, the cholesterol can be segregated into the oil phase.

EXAMPLE 1

Low Alcohol Extraction Process: One hundred kilograms of liquid egg yolk (containing 42 kg dry substance) was homogenized and then ethanol (35.4 kg of 96% purity) and 30.7 kg water were added to the egg yolk. The resulting alcohol concentration was about 20% w/w overall (27% w/w referring only to alcohol and water). The mixture was then re-homogenized and the mixture was centrifuged using a decanter centrifuge yielding an oil phase and an alcohol/water phase. This de-oiling step yielded 17 kg egg yolk oil and 149 kg of the alcohol/water phase. The alcohol/water phase was then washed 3 times with the same low concentration of alcohol using a counter-current wash process employing a separator centrifuge. The process yielded two fractions: (1) a phospholipid–rich fraction (the liquid phase) which was dried to yield a product containing a total of 17 kg dry substance (containing 8 kg of phospholipid); and (2) a protein-rich fraction which was dried to yield 12 kg of dry substance (containing 11 kg or protein and 0.3 kg of phospholipid). Using an approximate average weight of 16.0 g per yolk, each containing about 1.7 g phospholipid per yolk, 100 kg egg yolk should yield approximately 10.6 kg of phospholipids. The 8.0 kg of phospholipids recovered in the phospholipid-rich fraction by this process represented a recovery efficiency for the phospholipid fraction of approximately 76%.

EXAMPLE 2

Low Alcohol Extraction Process with High Alcohol Polishing Step: One hundred kilograms of liquid egg yolk (containing 42 kg dry substance) was homogenized and then ethanol and water were added to bring the mixture to a final alcohol concentration of 30% w/w in the alcohol/water phase. The mixture was then re-homogenized and the mixture was centrifuged using a decanter centrifuge yielding an oil phase and an alcohol/water phase. This de-oiling step yielded 16 kg egg yolk oil and 134 kg of the alcohol/water phase containing 26 kg dry substance. Seventy-two kg of ethanol and 170 kg water were then added to the alcohol/water phase, which was then mixed and centrifuged through a separator centrifuge. This yielded two fractions: (1) the liquid phase (299 kg) which contained 11 kg dry substance and (2) the solid phase (78 kg) which contained 15 kg dry

substance. Fraction 1 contained the phospholipids with a small amount of proteins and Fraction 2 contained primarily proteins. Fraction 1 was then dried to a weight of 11.2 kg and 20 kg ethanol (96%) was added to this fraction. The mixture was then processed through a separator centrifuge yielding a liquid phase containing 10 kg dry substance. The liquid phase was then dried yielding a final weight of 10.5 kg (10.0 kg dry substance - the phospholipid fraction). The 78 kg solids in Fraction 2 were also dried resulting in 16 kg total (or 15 kg dry substance -the protein fraction). Using an approximate average weight of 16.0 g per yolk, each containing about 1.7 g phospholipid per yolk, 100 kg egg yolk should yield approximately 10.6 kg of phospholipids. The 10.0 kg of phospholipids recovered in this process represents a minimal recovery efficiency for the phospholipid fraction of greater than approximately 90%.

EXAMPLE 3

Low Alcohol De-oiling Process with High Alcohol Polar Lipid Extraction Process: One hundred kilograms of liquid egg yolk (containing 45 kg dry substance) was homogenized and then ethanol and water were added to bring the mixture to a final alcohol concentration of 30% w/w in the alcohol/water phase. The mixture was then re-homogenized and the mixture was centrifuged using a decanter centrifuge yielding an oil phase and an alcohol/water phase. This de-oiling step yielded 17 kg egg yolk oil and 139 kg of the alcohol/water phase containing 28 kg dry substance. The alcohol/water phase was then dried (recovering 109 kg alcohol and water) yielding 30 kg material (containing 28 kg dry substance). Ninety kg ethanol (96% purity) was then added to this material and the mixture processed through a separator centrifuge yielding a liquid phase (containing the phospholipids) and a solid phase containing the proteins. The liquid phase (80 kg total containing 10.4 kg dry substance) was dried resulting in 10.6 kg of product containing 10.4 kg dry substance (phospholipids). The solid phase (40 kg total) was dried yielding 18.5 kg of product – protein (containing 17.6 kg of dry substance). Using an approximate average weight of 16.0 g per yolk, each containing about 1.7 g phospholipid per yolk, 100 kg egg yolk should yield approximately 10.6 kg of phospholipids. The 10.4 kg of phospholipids recovered in this process represents a minimum recovery efficiency for the phospholipid fraction of greater than approximately 90%.

SUMMARY OF THE INVENTION

In accordance with the present invention, an improved process is provided for recovering polar lipids enriched in gamma linolenic acid (GLA) and/or stearidonic acid (SDA) from native biomaterials such as seeds and microorganisms and the use thereof.

In one embodiment of the present invention, a method is provided for providing a human, animal or aquaculture organism diet supplement enriched with at least one of gamma linolenic acid (GLA) and stearidonic acid (SDA). The method includes the steps of producing a GLA- and/or SDA-enriched polar lipid-rich fraction from seeds or microbes; and providing the GLA- and/or SDA-enriched polar lipid-rich fraction in a form consumable by humans and animals. Preferably, the animals are companion animals.

In another embodiment of the present invention, a method is provided for treating a deficiency in at least one of gamma linolenic acid (GLA) and stearidonic acid (SDA).

The method includes the steps of producing a GLA- and/or SDA-enriched polar lipid-rich fraction from seeds or microbes; and providing the GLA- and/or SDA-enriched polar lipid-rich fraction to treat the deficiency. The deficiency can lead to an inflammatory condition, an autoimmune condition, a woman's health condition or an infant's health condition.

In another embodiment of the present invention, a method is provided for treating chronic inflammatory disease states of the lung, including but not limited to chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis. The method includes the steps of producing a GLA- and/or SDA-enriched purified phospholipid fraction from seeds or microbes; blending the GLA- and/or SDA-rich phospholipid fraction with at least one of EPA-, GLA- or SDA-rich oils; and producing an aerosol, such as by providing an aerosol delivery system, for the treatment of the disease states.

In another embodiment of the present invention, a method is provided for the treatment of skin lesions, induced burn, UV-irradiation or other skin disorders. The method includes the steps of producing a GLA- and/or SDA-enriched purified phospholipid fraction from seeds or microbes; blending the GLA- and/or SDA-rich phospholipid fraction with at least one EPA-, GLA- or SDA-rich oil; and producing a lotion or cream for the treatment of the skin disorders.

In another embodiment of the present invention, a method is provided for treating cachexia or fat malabsorption. The method includes the steps of producing a GLA- and/or SDA-enriched purified phospholipids; blending the GLA- and/or SDA-rich polar lipid fractions with at least one other purified phospholipid; blending the GLA- and/or SDA-rich polar lipid fractions with at least one DHA, EPA, GLA- or SDA-rich oil; and producing a liquid or dry dietetic product for the treatment of the disease states. The cachexia or fat malabsorption can result from the illnesses such as cancer and Crohn's disease. The at least one other purified phospholipid can be obtained from sources such as soybeans, rapeseed, canola, corn, peanuts, flax seed, linseed, sunflower, safflower, and eggs.

In another embodiment of the present invention, a method is provided for the treatment of *H. pylori*-infection of the gastrointestinal tract. The method includes the steps of producing a GLA- and/or SDA-enriched purified phospholipid fraction from seeds or microbes; blending the GLA- and/or SDA-rich phospholipid fraction with at

least one EPA-, GLA- or SDA-rich oil; and producing a fat emulsion or a dietetic product for the treatment of the disease.

In another embodiment of the present invention, a method is provided for providing a fat blend enriched with at least one of gamma linolenic acid (GLA) and stearidonic acid (SDA). The method includes the steps of extracting a GLA- and/or SDA-enriched polar lipid-rich fraction from seeds or microbes; and mixing the GLA- and/or SDA-enriched polar lipid-rich fraction with another oil. Preferably, the another oil is selected from the group consisting of fish oil, microbial oil, vegetable oil, GLA-containing oil, SDA-containing oil and mixtures thereof.

In another embodiment of the present invention, a method is provided for providing a blend of polar lipids enriched with at least one of gamma linolenic acid (GLA) and stearidonic acid (SDA). The method includes the steps of extracting a GLA- and/or SDA-enriched polar lipid-rich fraction from seeds or microbes; and mixing the GLA- and/or SDA-enriched polar lipid-rich fraction with another polar lipid. Preferably, the another polar lipid is selected from the group consisting of soy polar lipids, rapeseed polar lipids, sunflower polar lipids, safflower polar lipids, canola polar lipids, linseed polar lipids, flaxseed polar lipids, peanut polar lipids, egg yolk polar lipids and mixtures thereof.

In another embodiment of the present invention, a fat blend is provided that is enriched with at least one of gamma linolenic acid (GLA) and stearidonic acid (SDA) comprising a GLA- and/or SDA-enriched polar lipid-rich fraction from seeds or microbes; and another oil. Preferably, the another oil is selected from the group consisting of fish oil, microbial oil, vegetable oil, GLA-containing oil, SDA-containing oil and mixtures thereof.

In another embodiment of the present invention, a method is provided for providing a blend of polar lipids enriched with at least one of gamma linolenic acid (GLA) and stearidonic acid (SDA). The method includes the steps of extracting a GLA- and/or SDA-enriched polar lipid-rich fraction from seeds or microbes; and mixing the GLA- and/or SDA-enriched polar lipid-rich fraction with another polar lipid. Preferably, the another polar lipid is selected from the group consisting of soy polar lipids, rape seed polar lipids, sunflower polar lipids, safflower polar lipids, canola polar lipids, linseed polar lipids, flaxseed polar lipids, peanut polar lipids, egg yolk polar lipids and mixtures thereof.

In another embodiment of the present invention, purified phospholipids enriched with at least one gamma linolenic acid (GLA) and stearidonic acid (SDA) derived from polar lipid-rich fraction extracted from seeds or microbes are provided. Preferably, the GLA- and/or SDA-enriched phospholipid-fraction is in a form consumable by humans and animals.

Preferably, polar lipid-rich fractions of the methods or products of the present invention can be used as an ingredient of dietetic, pharmaceutical and cosmetic applications.

As used herein, the term dietetic includes nutritional supplements (in gel-cap, tablet, liquid, emulsion, powder or any other form) and food. The term pharmaceutical includes all compounds ingested (including special enteral and parenteral nutrition products) or injected or received intravenously, for the treatment of diseases or metabolic imbalances.

Preferably, fat blends of the methods or products of the present invention can be used as an ingredient of dietetic, pharmaceutical and cosmetic applications.

Preferably, blends of polar lipids of the methods or products of the present invention can be used as an ingredient of dietetic, pharmaceutical or cosmetic applications.

Preferably, purified phospholipids of the methods or products of the present invention can be used as an ingredient of dietetic, pharmaceutical or cosmetic applications.

Preferably, seeds useful in the methods and products of the present invention are from the plant families Boraginaceae, Onagraceae, Saxifragaceae, Scrophulariaceae or Cannabaceae, and more preferably, the seeds are selected from the group consisting of borage, echium, evening primrose and black currant.

Preferably, the microbes useful in the methods and products of the present invention are selected from fungi, microalgae and bacteria. More preferably, the microbes are selected from the group of genera consisting of *Mortierella, Mucor, Blastocladiella, Choanephora, Conidiobolus, Entomophthora, Helicostylum, Phycomyces, Rhizopus, Beauveria,* and *Pythium.*

Preferably, the GLA of the products and methods of the present invention makes up at least two weight percent of the total fatty acids of the polar lipid fraction.

Preferably, the SDA of the products and methods of the present invention makes up at least two weight percent of the total fatty acids of the polar lipid fraction.

Preferably, the plant seeds of the products and methods of the present invention have been genetically modified, and more preferably, the seeds have been genetically modified to increase the production of at least one of SDA and GLA.

Preferably, the seeds of the methods and products of the present invention are selected from the group consisting of canola, rapeseed, linseed, flaxseed, sunflower, safflower, soybeans, peanuts and corn.

Preferably, the polar lipid-rich fraction is extracted from the seeds or microbes using alcohol.

In an alternative embodiment of the present invention, the polar lipid-rich fraction is derived as a by-product of oil extraction, e.g. by de-gumming, from the seeds or microbes using hexane and other nonpolar solvents.

Preferably, the polar lipid-rich fraction is extracted from the seeds or microbes by use of gravity or centrifugal extraction technology.

DETAILED DESCRIPTION OF THE INVENTION

Because of their bipolar nature, polar lipids (including phospholipids) are of significant commercial interest as wetting and emulsifying agents. These properties may also help make PUFAs in the phospholipids more bioavailable, in addition to enhancing their stability. These properties make phospholipids ideal forms of ingredients for use in nutritional supplements, food, infant formula, pharmaceutical, and cosmetic applications. Dietary benefits of phospholipids include both improved absorption and improved incorporation. Phospholipids also have a broad range of functionality in the body in that they are important cell membrane constituents, they are good emulsifiers, they can act as intestinal surfactants, serve as a choline source and as a source of PUFAs.

GLA and SDA are normally produced for the nutritional supplement market through hexane extraction of seeds from the plant families Boraginaceae, Onagraceae, Saxifragaceae, Scrophulariaceae or Cannabaceae. These families include borage, echium, evening primrose and black currant. The phospholipids are removed in a degumming step that produces a waste material comprising a complex mixture of neutral lipids, sterols, glucosides and phospholipids. This material is normally sold to the domestic animal feed industry to dispose of it. To the best of the present inventors' knowledge,

there are no phospholipid forms of GLA and/or SDA available in the nutritional supplement, food, companion animal or aquaculture markets.

Besides oilseeds, there are also microbial sources of SDA and GLA but none of these is commercially available. Microorganisms known to contain GLA and/or SDA are found in yeast and the following genera of fungi: *Mortierella, Mucor, Blastocladiella, Choanephora, Conidiobolus, Entomophthora, Helicostylum, Rhizopus, Beauveria, Thamnidium, Lactarius, Cantherellus, Polyporus, Glomus, Zygorhynchus,* and *Pythium*; and genera of algae and algae-like microorganisms including: *Chlorella, Cyanidium, Scenedesmus, Chlamydomonas, Ankistrodesmus, Enteromorpha, Oocystis, Dunaliella, Heteromastix, Ochromonas, Prymnesium, Isochrysis, Dicrateria, Fucus, Gonlaulux, Amphidinium, Peridinium, Hemiselmis, Cryptomonas, Chroomonas, Rhodomonas, Hemiselmis, Thraustochytrium,* and *Schizochytium*. For the purposes of this application members of the former thraustochytrid genus *Ulkenia* are considered to be part of the genus *Thraustochytrium*. Microorganisms are good sources of phospholipids because they can be grown in culture in a manner that optimizes phospholipid production and minimizes triglyceride (oil) production. On the other hand the methods used in this invention allow both oil and phospholipids to be recovered separately in forms that can be used directly in food, feed, nutritional supplements, cosmetic or pharmaceutical application.

GLA and SDA phospholipids can be recovered from oilseeds through the degumming process described above. However, as noted, this produces a complex material containing many other compounds including neutral lipids, sterols, glucosides, etc

A preferred embodiment of the present invention is to use alcohol and centrifugation to recover the GLA- and SDA-rich phospholipids. Preferred methods for this recovery are described in the following references:

i. International Publication No. WO 01/76715, entitled "Method for the Fractionation of Oil and Polar Lipid-Containing Native Raw Materials" filed April 12, 2001;

ii. International Publication No. WO 01/76385, entitled " Method for the Fractionation Of Oil And Polar Lipid-Containing Native Raw Materials

Using Water-Soluble Organic Solvent And Centrifugation" filed April 12, 2001.

Although these are preferred extraction methods, any suitable extraction method can be employed with the present invention. Once the GLA- and SDA–rich phospholipids fractions have been extracted by these preferred processes, they can be used directly as ingredients or they can be purified further and even separated into phospholipid classes by well-known techniques such as different forms of chromatography, molecular distillation, and special refining techniques. The phospholipid rich polar lipids or the purified phospholipid rich fractions can also be mixed with another lipid or oil such as fish lipids, microbial lipids, vegetable lipids, GLA-containing lipids, SDA-containing lipids and mixtures thereof, or be mixed with another phospholipid fraction (lecithin) such as soy or egg yolk lecithin, sunflower lecithin, peanut lecithin or mixtures thereof prior to use as a nutritional supplement, feed or food ingredient. These mixtures of phospholipids can also be incorporated into creams or lotions for topical applications (e.g. treating of skin conditions) or skin lesions induced by burns, UV-irradiation or other skin damaging processes. The mixtures can also be processed to produce a liquid or spray-dried dietetic product or fat emulsion for treating cachexia and severe fat malabsorption or for treatment of *H. pylori* infection of the gastrointestinal tract, or be used to produce an aerosol (spray) for the treatment of chronic inflammatory disease states of the lung (COPD, asthma, cystic fibrosis).

Advantages of the present invention including providing GLA and SDA in a more bioactive and functional form (phospholipid) than the triglyceride form and include a better process: a) no need for heat treatment; b) no use of toxic solvents (like hexane) and c) no artifacts and off-flavors due to the use of acetone) for recovering these phospholipids from oilseeds and microbes.

The following example is provided for the purpose of illustration and are not intended to limit the scope of the present invention.

EXAMPLE

Example 1

Phospholipids were extracted from four types of oilseeds and the total fatty acid content of the phospholipids was determined by gas chromatography. The results are presented in Table 1. As can be observed the phospholipid fraction of these seeds can be

used to deliver GLA and or SDA and in this form these bioactive fatty acids should be more stable, more bioavailable, and more functional.

Table 1. Total fatty acid content of phospholipids extracted from four types of oilseeds containing GLA and SDA.

COMPOUND		Black Currant PL's % TFA	Borage PL's % TFA	EPO PL's % TFA	Echium PL's % TFA
MYRISTATE	C14:0	0,56	0,67	0,61	1,12
MYRISTOLEATE	C14:1	0,00	0,00	0,00	0,00
PALMITATE	C16:0	23,84	21,71	17,78	23,01
PALMITOLEATE	C16:1	0,43	0,00	0,46	1,75
STEARATE	C18:0	4,59	8,55	7,79	5,69
OLEATE	C18:1	18,70	25,52	27,67	24,56
LINOLEATE	C18:2n6	36,20	30,89	37,92	17,75
GAMMA LINOLENATE	C18:3n6	5,02	7,26	2,33	7,17
ARACHIDATE	C20:0	0,51	1,27	1,20	0,00
LINOLENATE	C18:3n3	7,30	4,15	1,00	14,09
OCTADECATETRAENOATE	C18:4	1,20	0,00	0,23	4,86
EICOSENOATE-11	C20:1	0,99	0,00	0,60	0,00
EICOSADIENOATE-11,14	C20:2	0,00	0,00	0,00	0,00
BEHENATE	C22:0	0,66	0,00	1,68	0,00
EICOSATRIENOATE	C20:3n3	0,00	0,00	0,00	0,00
ARACHIDONATE	C20:4 n6	0,00	0,00	0,00	0,00
ERUCATE	C22:1	0,00	0,00	0,00	0,00
EICOSAPENTAENOATE	C20:5n3	0,00	0,00	0,00	0,00
LIGNOCERATE	C24:0	0,00	0,00	0,72	0,00
NERVONATE	C24:1	0,00	0,00	0,00	0,00
DOCOSAPENTAENOATE n-6	C22:5n6	0,00	0,00	0,00	0,00
DOCOSAPENTAENOATE n-3	C22:5n3	0,00	0,00	0,00	0,00
DOCOSAHEXAENOATE	C22:6n3	0,00	0,00	0,00	0,00
		100,00	100,00	100,00	100,00

The present invention, in various embodiments, includes components, methods, processes, systems and/or apparatus substantially as depicted and described herein, including various embodiments, subcombinations, and subsets thereof. Those of skill in the art will understand how to make and use the present invention after understanding the present disclosure. The present invention, in various embodiments, includes providing devices and processes in the absence of items not depicted and/or described herein or in various embodiments hereof, including in the absence of such items as may have been used in previous devices or processes, *e.g.*, for improving performance, achieving ease and/or reducing cost of implementation.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of an effective amount of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-enriched polar lipid-rich fraction to supplement a human, an animal, or an aquaculture organism with GLA and/or SDA, wherein said GLA- and/or SDA-enriched polar lipid-rich fraction is produced from seeds or microbes.

2. Use of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-enriched polar lipid-rich fraction in the manufacture of a diet supplement for supplementing a human, an animal, or an aquaculture organism with GLA and/or SDA, wherein said diet supplement is enriched with GLA and/or SDA, and wherein said GLA- and/or SDA-enriched polar lipid-rich fraction is produced from seeds or microbes.

3. Use of an effective amount of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-enriched polar lipid-rich fraction for treatment of a deficiency in GLA and/or SDA, wherein said GLA- and/or SDA-enriched polar lipid-rich fraction is produced from seeds or microbes.

4. Use of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-enriched polar lipid-rich fraction in the manufacture of a medicament for treatment of a deficiency in GLA and/or SDA, wherein said GLA- and/or SDA-enriched polar lipid-rich fraction is produced from seeds or microbes.

5. The use according to Claim 3 or 4, wherein said deficiency results in an inflammatory condition, an auto-immune condition, menopause, premenstrual disorder, or a fatty acid imbalance in an infant.

6. Use of an effective amount of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction for treatment of a chronic inflammatory disease state of the lung, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

7. Use of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction in the manufacture of a medicament for treatment of a chronic inflammatory disease state of the lung, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

8. The use according to Claim 7, wherein said medicament further comprises at least one EPA-, GLA-, or SDA-rich oil.

9. The use according to Claim 6 or 7, wherein said chronic inflammatory disease state of the lung is COPD, asthma, or cystic fibrosis.

10. Use of an effective amount of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction for treatment of a skin condition, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

11. Use of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction in the manufacture of a medicament for treatment of a skin condition, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

12. The use according to Claim 11, wherein said medicament further comprises at least one EPA-, GLA, or SDA-rich oil.

13. The use according to Claim 10 or 11, wherein said skin condition is a skin lesion induced by skin damage.

14. The use according to Claim 13, wherein said skin lesion is induced by burns or UV-irradiation.

15. Use of an effective amount of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction for treatment of cachexia or fat malabsorption, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

16. Use of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction in the manufacture of a medicament for treatment of cachexia or fat malabsorption, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

17. The use according to Claim 16, wherein said medicament further comprises at least one additional purified phosphlipid fraction and at least one DHA, EPA, GLA- or SDA-rich oil.

18. Use of an effective amount of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction for treatment of *H. pylori*-infection of the gastrointestinal tract, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

19. Use of a gamma linolenic acid (GLA)- and/or a stearidonic acid (SDA)-rich purified phospholipid fraction in the manufacture of a medicament for treatment of *H. pylori*-infection of the gastrointestinal tract, wherein said GLA- and/or SDA-rich purified phospholipid fraction is produced from seeds or microbes.

20. The use according to Claim 19, wherein said medicament further comprises at least one EPA-, GLA- or SDA-rich oil.

21. The use according to any one of Claims 1 to 20, wherein said seeds are from the plant families Boraginaceae, Onagraceae, Saxifragaceae, Scrophulariaceae or Cannabaceae.

22. The use according to any one of Claims 1 to 20, wherein said seeds are selected from the group consisting of borage, echium, evening primrose and black currant.

23. The use according to any one of Claims 1 to 20, wherein said seeds are selected from the group consisting of canola, rapeseed, linseed, flaxseed, sunflower, safflower, soybeans, peanuts and corn.

24. The use according to any one of Claims 1 to 23, wherein said plant seeds have been genetically modified.

25. The use according to Claim 24, wherein said seeds have been genetically modified to increase the production of at least one of SDA and GLA.

26. The use according to any one of Claims 1 to 25, wherein said polar lipid fraction or phospholipid fraction is from microbes.

27. The use according to Claim 26, wherein said microbes are selected from fungi, microalgae and bacteria.

28. The use according to Claim 26, wherein said microbes are selected from the group of genera consisting of *Mortierella*, *Mucor*, *Blastocladiella*, *Choanephora*, *Conidiobolus*, *Entomophthora*, *Helicostylum*, *Phycomyces*, *Rhizopus*, *Beauveria*, and *Pythium*.

29. The use according to any one of Claims 1 to 28, wherein GLA comprises at least two weight percent of the total fatty acids of the polar lipid fraction or of the phospholipid fraction.

30. The use according to any one of Claims 1 to 28, wherein SDA comprises at least two weight percent of the total fatty acids of the polar lipid fraction or of the phospholipid fraction.

31. The use according to any one of Claims 1 to 30, wherein said polar lipid fraction or phospholipid fraction is extracted from said seeds or microbes using alcohol.

32. The use according to any one of Claims 1 to 30, wherein said polar lipid fraction or phospholipid fraction is extracted from said seeds or microbes by use of gravity or centrifugal extraction technology.