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(54) A METHOD FOR PURIFYING FATTY ACID GLYCERIDES, COMPOSITIONS DERIVED THEREFROM, AND USE THEREOF

(57) The present invention relates to a new method of purification of fatty acid mono- and/or di-glycerides, of any origin, and in particular those characterized by an at least partial esterification with long chain polyunsaturated fatty acids (PUFAs).

This purification method consists in the treatment of said mono- and/or di-glycerides with an amount of urea suitable for their total complexation, and in the subsequent separation by filtration or centrifugation of the urea inclusion complex from the solvent phase containing the other products that are structurally unrelated to the mono- and/or di-glycerides, followed by the isolation from the complex of the glycerides, which are purified and therefore free from the many "Persistent" Organic Pollutants (POPs) of the environment, such as polychlorinated dibenzodioxins and polychlorinated dibenzo-furans, polychlorinated biphenyls, polybrominated- diphenyl ethers, polycyclic aromatic hydrocarbons, and other pollutants that are usually present and extremely toxic too.

The invention also relates to the purified compositions of mono- and/or di-glycerides thus obtained, as well as those derivable from the same with processes of hydrolysis or transesterification, by either chemical or enzymatic route, as well as the use of such compositions as a food, food for special medical use, food and dietary supplement, and as a drug.

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Description

[0001] The present invention relates to a new method of purification of fatty acid mono- and/or diglycerides, of any origin and in particular those characterized by an at least partial esterification with long chain polyunsaturated fatty acids (PUFAs), in particular C18 - C22, preferably C20 - C22, and comprising 2-6 double bonds, preferably 5-6 double bonds.

[0002] The purification method according to the invention allows to remove from such glycerides products that are structurally unrelated to them. These foreign products, especially in the case of natural glycerides, essentially consist, certainly not from the point of view of their weight but for their extreme toxicity, of so-called "Persistent" Organic Pollutants (POPs) now ubiquitously present in the environment (land, agricultural land, marine and river waters, etc.).

[0003] Among the numerous POPs known from the literature and present in compositions of glycerides there may be mentioned, for example, the polychlorinated-dibenzo-dioxins and polychlorinated dibenzo-furans, polychlorinated biphenyls, polybrominated-diphenyl ethers, polycyclic aromatic hydrocarbons, and other pollutants that are also usually present and extremely toxic.

BACKGROUND OF THE INVENTION

[0004] The glycerides of fatty acids (esters with glycerol) object of the purification method of the invention, can be mono- and/or di- glycerides and are so called in function of their degree of substitution. They are also reported in the literature as acyl-glycerols and distinguished according to their position and their steric structure into monoacyl-glycerols in position sn-1 or sn-2 and diacyl-glycerols in position *sn*-1,2 or *sn*-1, 3.

[0005] The physico-chemical and biological properties of glycerides depend essentially on the number, position and type of the acyl radical present as a glycerol ester.

[0006] It is well known that the fatty acids contained in the glycerides may be short-chain or more often medium- or long-chain; by "medium- or long-chain fatty acids", fatty acids with 16 or more carbon atoms are hereby meant. It is also known that said acids may be saturated (such as stearic acid C18:0), or monounsaturated (such as oleic acid C18:1 n-9), or polyunsaturated, often containing from 2 to 6 double bonds, and generally belonging to the class of omega-6 (or n-6) acids such as arachidonic acid (ARA, C20:4 n-6) or to that of omega-3 (or n-3) acids such as e.g. eicosapentaenoic acid (EPA, C20:5 n-3, all cis) and docosahexaenoic acid (DHA, C22:6 n-3, all cis), or even the docosapentaenoic acid (DPA, C22:5 n- 3).

[0007] In particular, these omega-3 acids and their ethyl esters have acquired in recent decades an increasing importance for their biological properties and their use as nutraceuticals, food and dietary supplements, foods for special medical purposes, and as drugs. The same is happening at present for their glycerides, considered more "natural" and better absorbed in the human body, which have the added advantage of being their predominant source of production.

[0008] In fact, in relation to their origin, all of these acids are found in nature in widely varying ratios among them and in the form of various chemical structures, such as e.g. in the phospholipids, etc.. The omega-6 acids are especially abundant in plant oils and seeds, while the omega-3 acids and in particular EPA and DHA have predominantly marine origin and are derived essentially from fish oils, also from aquaculture, or from "krill oils" or even from algae and other oleaginous microorganisms, or from "single cell fermentation" from selected strains of algae or other microorganisms.

[0009] The mono-glycerides (and also the di-glycerides) are instead mainly industrial products, used for their excellent emulsifying properties, and represent 70-80% of the world emulsifiers used in food products, cosmetics and pharmaceuticals. They are obtained usually, although not with high yields and with low purity, by chemical glycerolysis of triglycerides (natural oils and fats) at high temperatures and by alkaline catalysis.

[0010] In recent times, the glycerides substituted with polyunsaturated acyl groups are preferably subjected to hydrolysis or alcoholysis by enzymatic route, by means of lipases - also immobilized - selective for the structure of the acyl and for its position on the glycerol: it is thus possible to operate in very mild reaction conditions and - by virtue of the higher resistance to enzymatic cleavage of polyunsaturated acyl groups, compared to saturated and monounsaturated acyls - it is also possible to isolate compositions of glycerols that are still partially acylated and particularly enriched in polyunsaturated acids.

[0011] It is also known that said hydrolysis or alcoholysis by enzymatic route, as well as the more traditional cleavage by chemical means, can be brought to completeness with total separation of the PUFA polyunsaturated acids (or their esters) from glycerol, polyunsaturated acids which can be enriched in concentrated compositions by means of various technologies, also coupled, but these phases concern the production technology of polyunsaturated acids and their ethyl esters (see monograph 07/2012: 1250 of EP 8.0 and monograph p. 4059-61 of USP 37), and not the present glyceride purification process. They will still be applicable, as we shall see, even to glycerides that have already been purified by the method of the present invention.

[0012] Thousands of scientific publications are currently available on the various processes and biological effects involving polyunsaturated acids and the various "concentrated" PUFA oils and esters obtainable therefrom. Whenever possible, we will make reference, however, to the excellent volume of Breivik H, "Long-Chain Omega-3 Specialty Oils"

(H Breivik, ed.), The Oily Press, Bridgwater, (2007), hereinafter referred to as "Breivik 2007" .

[0013] All that said, in conclusion, the purification method of the present invention preferably relates, in addition to the saturated and monounsaturated glycerides, to the purification of mono- and di-glycerides of any origin and obtained by any route, or their mixtures, including natural oils, partially substituted with radicals of long-chain fatty acids, comprising 18-22 carbon atoms, preferably 20-22 carbon atoms, and of polyunsaturated type, i.e. comprising 2-6 double bonds, preferably 5-6 double bonds . Particular reference is made to acids belonging to the class of omega-6 and/or omega-3 acids, more preferably to the class of omega-3 acids, in particular to EPA and / or DHA.

[0014] The content of a polyunsaturated fatty acid as defined above, e.g. EPA or DHA, or of their sum, is between 15-45%, more often 15-30%, and 90%, or between 30-60%, more often 45-60%, and 90%, of the weight of the composition , being 15-30% the average content in EPA or DHA or of their sum in a natural oil (fish oil or the like) used for the production of PUFAs, being 45% and 60% the minimum content of the sum of EPA and DHA and respectively of the sum of omega-3 acids - - in accordance with the monograph 01/2009: 1352 relative to the EP7.0 glycerides of omega-3 acids, reconstituted by chemical or enzymatic route and enriched in PUFAs, and being 90% around the maximum possible content for a glyceride having as substituents only polyunsaturated acids as described above, preferably EPA and/or DHA.

[0015] In more general terms, we will summarize the content in polyunsaturated acids as described above, such as between 15 and 90%, more frequently between 15 and 60%, of the composition weight.

[0016] The fact that glycerides derive, directly or indirectly, from "natural" and thus apparently reassuring sources, however poses serious health hazards in all uses mentioned above, due to the known presence of many environmental pollutants ("dioxins", polychlorinated biphenyls, etc..). Many of these are particularly fat-soluble and tend to concentrate together with the various fat components (from which the definition of Persistent Organic Pollutants, POPs). The main features of these substances are persistence over time, bio-accumulation through the food chain, the potential for dissemination and long-range environmental transport, and their toxicity. Many of these substances are clearly teratogenic, mutagenic and carcinogenic.

[0017] The danger posed by these substances to humans and animals has led to a growing concern about the content of toxic substances in food and the food chain. Food products that do not contain, or have limited content of pollutants, are gaining greater popularity and market capacity. Therefore, the elimination or reduction of pollutants in foodstuffs has a great potential to substantially increase their chances of selling and their added value, but also has a high ethical value with particular reference to their use in the field of "baby foods" and "infant formulas" and the risk of transmission to the baby with breast milk.

[0018] The first official act against these substances derived from the "Stockholm Convention on POPs" held in May 2001 in Sweden, which individuated 12 distinct chemical classes as more aggressive and dangerous to human health and the environment, including agriculture and livestock farming. Many of these substances were in the past used as pesticides (herbicides, insecticides, fungicides, rodenticides, etc.), for others the production was unaware and not intentional, being undesirable byproducts of a series of chemical processes and/or combustion. The conclusion of the Convention was to ban the production and use of many of these substances and the aim to check all of the environmental pollutants was then pursued in subsequent years by the various POPs Review Committees as well as from various Regional Agencies for environmental protection.

[0019] In detail, the "dioxins" are in fact constituted by two chemical families of polychloro-dibenzo-para-dioxins (PCDDs) and of polychloro-dibenzo-furans (PCDFs): of these only 7 PCDDs and 10 PCDFs are of particular concern from a toxicological point of view.

[0020] Generally, the PCDD/F are detected as mixtures of these toxic congeners, having attributed to each of them an appropriate "toxicity equivalent factor" (TEF), more precisely the WHO-TEF (European Commission Regulation (EC) 1881/2006, Off.J.EU, L364/5; 20 December 2006, p.20). The maximum level of WHO PCDD/PCDF TEQ (Toxicity equivalent) for oils of marine origin intended for consumption in man according to the above Regulation (EC) 1881/2006, p. 18, is equal to 2 pg/g oil. According to USP 37, acceptability criterion corresponds to "not more than" (NMT) 1 pg/g of WHO toxic equivalents.

[0021] Another group of chemical contaminants is constituted by industrial agents as the polychloro-biphenyls (PCBs), consisting of molecules of variously chlorinated biphenyl: only 12 have toxicological properties similar to the "dioxins" and to "furans", and are therefore defined dioxin-like PCBs (DL-PCBs). Also for the DL-PCBs the toxicity equivalent factors TEF were determined and their toxicity equivalent TEQ is usually given cumulatively with that of dioxins. Their maximum level is equal to 10.0 pg/g oil.

[0022] The sum of 6 other congeners defined "indicators" or "markers" is considered by the EFSA (European Food Security Agency) an adequate indicator of the presence of not dioxin-like PCBs (NDL-PCBs) and of the human exposure to the same. According to USP 37, acceptability of PCBs markers - also including PCB 118 (IUPAC name) - is limited to NMT 0.5 ppm (0.5 micrograms/g).

[0023] The PBDEs are polybromo- diphenyl ethers, constituted by molecules of diphenyl ether that are variously brominated, and some of them are highly neurotoxic and even carcinogenic. They are used as flame retardants, and are considered as persistent chemical pollutants of "emerging" type. The industrial production of some terms has already been banned, but their presence has been demonstrated in polluted waters and in landfill sites and in many samples of fish oils (Zennegg M et al, Organohalogen Compounds, 68,1967,2006; US 7,732,488).

[0024] Polycyclic aromatic hydrocarbons (IPAs or PAHs) are compounds formed by two or more fused aromatic rings and are of toxicological interest as they are considered possible carcinogenic. PAHs are usually expressed as marker substance benzo[a]pyrene, with a maximum permissible amount of 2 ng/g in oils and fats (Regulation (EC) 1881/2006, Section 6, p.18).

[0025] Among other POPs that are hereby not discussed in detail, we cite still by way of not exhaustive example 2,2 bis-(p-chlorophenyl)-ethane (DDE), 2,2 bis-(p-chlorophenyl)-1,1-dichloroethane (DDD), and 2,2 bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT), the latter having been traced everywhere in the global environment, the polybromo- biphenyls (PBB), hexachlorobenzene, isomers of hexacloro-cyclohexane, and others.

[0026] The current methods for attenuating the content of these environmental pollutants in the glycerides, e.g. in the triglycerides of fish oils, content which is relatively very high if the fish comes from the seas very polluted, fall within basis routine processes on raw materials, such as the "whitening" (treatment with active carbon) and the deodorization (stripping in a steam current).

[0027] These standard procedures are in any case of modest efficacy (Breivik 2007, page 133), and in a more advanced stage the treatment is therefore made by distillation, either molecular or short path distillation, which currently represents the most usual method for the purification from environmental pollutants.

[0028] In more recent times, patent application WO04/007654 (EP1523541 and US 7,732,488) described a method for decreasing the amount of environmental pollutants in a mixture of oils or fats, by which a "working" volatile fluid is added to the mixture, then the mixture is subjected to at least one step of "stripping", during which a part of environmental pollutants together with the "working " volatile fluid is distilled off.

[0029] This method seems to be an improvement, but it is not decisive with respect to the prior art, since it involves high temperatures for an efficient stripping phase, long heating time, formation of byproducts, complex equipments as the molecular distiller, and thus overall high costs. Moreover, the presence of many classes of POPs and inside them of different molecular species of POPs, each one with its own chemical-physical characteristics and a wide range of boiling points in dependence on their degree of substitution (chlorination, bromination, etc.), makes this work very difficult and generally only able to reduce the presence of a pollutant practically without ever deleting it in its totality, and in the favorable case for a single product, without being able to extend the purification to the totality of the POPs.

[0030] We therefore concluded that none of the current techniques is able to purify the compositions at issue from all impurities described above, and only if used in combination, repeatedly and with serious yield losses such techniques may approach the limits imposed by the various legislations without ever reaching a substantial absence of each pollutant, as it would be ethically desirable, or even imperative at least in the use of the compositions in early childhood or during breast-feeding.

[0031] The systematic elimination of each and all POPs appears instead easily obtainable with the method of the invention.

SUMMARY OF THE INVENTION

[0032] In a first aspect, the present invention relates to a method for the purification from persistent organic pollutants (POPs) of a composition consisting of mono-glycerides and/or di-glycerides of any origin, wherein the glycerol is at least partially esterified with long chain saturated or unsaturated fatty acids having 16-22 carbon atoms, this composition containing said POPs, wherein the method comprises the steps of:

a) forming an inclusion urea complex that contains essentially all of said composition by treating one part by weight of the above composition with at least 3 parts by weight of urea in a polar solvent, such as a lower alcohol or ketone, optionally containing up to 5% of water, at a temperature comprised between 20°C and the boiling temperature of the solvent;

b) cooling until precipitation of the above urea complex, and isolating it, by filtration or centrifugation, from the mother waters containing the above mentioned environmental persistent organic pollutants (POPs) and washing it with said polar solvent, previously saturated with urea and preferably cooled to a temperature of about 4-5°C;

c) obtaining a purified composition having a reduced content of POPs by dissolution in water of the inclusion urea complex and separation of the oily phase formed following the dissolution, or by extraction of the oily phase with an organic solvent unmixable with water, typically hexane or the like, followed by evaporation of the solvent to dryness, or by direct extraction from the urea inclusion complex by means of fluids in supercritical state, in particular carbon dioxide.

[0033] Preferably, the mother waters and the washing waters of the above isolated urea complex can be pooled and treated, as a second purification step, with further 2-3 parts by weight of urea (with reference to the starting composition), repeating the steps from a) to c) of formation of the urea inclusion complex, cooling, isolating and obtaining the purified composition.

[0034] Optionally it is possible to collect and to pool even the mother waters obtained from the second step of precipitation and isolation, as well as the washing waters of the second isolated urea inclusion complex, and repeat the steps from a) to c) (third purification step).

[0035] In one aspect of the method according to the present invention, the aforesaid long chain fatty acids have 18-22 carbon atoms, preferably 20-22 carbon atoms, and are of the polyunsaturated type (PUFA), i.e. containing 2-6 double bonds, preferably 5-6 double bonds, belonging to the class of omega-6 and/or omega-3 acids, preferably to the class of the omega-3 acids, more preferably represented by eicosapentaenoic acid (EPA, C20:5 n-3, all cis) and/or docosahexaenoic acid (DHA, C22:6 n-3, all cis).

[0036] In a further aspect of the method according to the present invention, the composition comprising the abovementioned mono-, and/or diglycerides is obtained by chemical and/or enzymatic route, by means of lipases selective for the fatty acid and its position on the glycerol, starting from natural triglycerides, or from glycerides or glycerol and polyunsaturated fatty acids, through known procedures.

[0037] Preferably, step a) of the method according to the present invention, i.e. the treatment of the above-mentioned composition with urea, is performed by using as polar solvent a C_1-C_4 alcohol or at least one C_1-C_6 alkyl ketone or a mixture thereof.

[0038] Preferably, said polar solvent is selected from the group consisting of methanol, ethanol, n-butanol, i-butanol, acetone, methyl ethyl ketone and methyl isobutyl ketone.

[0039] Preferably, step a) of the method according to the present invention, i.e. the treatment of the above-mentioned composition with urea, is performed by using as a polar solvent methanol in an amount equal to 4.5-7 parts by weight, or ethanol in an amount equal to 45-65 parts by weight.

[0040] The environmental persistent organic pollutants (POPs) mentioned above comprise mainly polychloro-dibenzo-para-dioxins (PCDDs) and polychloro-dibenzo-furans (PCDFs), and/or polychloro-biphenyls (PCBs) dioxin-like (DL-PCBs) and markers, and/or polybromo- diphenyl ethers (PBDEs), and/or polycyclic aromatic hydrocarbons (PAHs), and their presence in the purified composition obtained in step (c) is at least 2 times reduced, preferably at least 5 times reduced, conveniently at least 10 times reduced, and in any case lower - for each component - than the respective limit of quantification (LOQ) with standard GC-MS method.

[0041] In other aspects, the present invention relates to a composition consisting of mono-glycerides and/or di-glycerides , as defined in claims 8, 9 and 17, as well as the use of such a composition for the preparation, from mono-glycerides and/or di-glycerides , of the corresponding polyunsaturated fatty acids or their salts, by chemical or enzymatic hydrolysis, or for the preparation of the related C1-C3 alkyl esters, as specified in claims 11 and 12. The present invention further relates to the use of a composition according to any of claims 8 and 9 for the preparation from said monoglycerides and/or diglycerides of the corresponding triglycerides, by means of chemical or enzymatic esterification or transesterification, in presence of said PUFAs as defined in claim 3 or of C_1-C_3 alkyl esters thereof, preferably ethyl esters thereof.

[0042] Moreover, the present invention relates to a composition comprising these polyunsaturated fatty acids or their salts or C1-C3 alkyl esters obtained by chemical or enzymatic hydrolysis, as specified in claims - 13-15.

[0043] In another aspect thereof, the present invention relates to the use of a composition consisting of the above mentioned mono-glycerides and/or di-glycerides or the corresponding polyunsaturated fatty acids or their salts or C1-C3 alkyl esters for the preparation of formulations useful as food ingredients, food and dietary supplements, foods for special medical purposes (functional foods), food for animal consumption and for aquaculture, infant food formulas, cosmetic and pharmaceutical preparations, as specified in claim 16.

[0044] When the term "glyceride" is referred to the present invention, only mono-and diglycerides are hereby meant by this term.

DETAILED DESCRIPTION

[0045] It is remarked that the use of urea in the chemistry of lipids is absolutely known.

[0046] A standard procedure provides that an oil or fat is hydrolyzed to give the acid components or transesterified in alcoholic medium to give the corresponding esters, by chemical or enzymatic route, and then one proceeds to the concentration of the polyunsaturated components: this generally occurs by molecular distillation, extraction with fluids under supercritical condition, or other methods, but more often by complexation precisely with urea ("inclusion" in urea). The use of urea however is only presented as a means suitable for isolating and removing saturated and monounsaturated fatty acids, and therefore for the concentration of polyunsaturated acids in the solvent of the reaction: this is thus coupled - inter alia - to an increased concentration of extraneous impurities together with polyunsaturated components. The inclusion of polyunsaturated components in urea appears from the literature of increasing difficulty with the growth of their concentration and of their relative degree of unsaturation: in the treatment of a mixture of fatty acids, the PUFAs appear to be so the "passive" component of the complexation.

[0047] There is therefore a profound difference with the method of the present invention, whereby e.g.:

- "complexation" is carried out directly on the glycerides (including natural oils and fats, or other glycerides particularly selected), instead of fatty acids or esters thereof;
- glycerides are the "active" components of the complexation, while the PUFAs of literature in their essence are not suitable for the complexation, if not marginally, and remain unchanged in the solvent;
- the literature procedure is directed to increase the concentration of PUFAs, enriching them in the mother liquor of the complexation, while it remains essentially constant during the present purification process;
- the purpose of the complexation according to the new process is to reduce markedly, essentially to cancel, the presence of the environmental pollutants that are all officially declared teratogenic, mutagenic and carcinogenic or generically toxic, instead of just leading to the concentration of PUFA and their esters, according to the current standard purposes.

[0048] On the use of urea as a complexing agent and on the related procedures, there are many publications, among which there are cited the extensive reviews of Schlenk H, "Urea inclusion compounds of fatty acids", in: Progress in the chemistry of fats and other lipids Vol II (RT Holmar, ed.), Pergamon Press, New York, p. 243-267 (1954), and Swern D, "Techniques of separation. Urea complexes", in: Fatty Acids, Part 3 (KS Markley, ed.), Interscience, New York, p. 2309-2358 (1963). In particular, it is noted the already mentioned "Breivik 2007" in the chapter "Concentrates", pages 111-130.

[0049] In relation to the glycerides, subject of the present application, Aylward F and Wood PDS, Chem&Ind. (London),53 ((1956); Nature, 177, 146 (1956) (Ref. 127 and Ref. 129, Swern, above mentioned, pages 2337-8), and others, report the urea complex of mono-glyceride in position 1 of the stearic acid (C18:0), as well as a series of lower homologous, from caprylic acid C8:0 to palmitic acid C16:0. Apparently the monoglycerides in position 2 do not form complexes.

[0050] It is always a case, however, of glycerides of saturated fatty acids and attempts of fractionation and enrichment of various components and not of purification from environmental pollutants.

[0051] The same authors and others (Martinez Moreno JM et al, Grasas y Aceites (Seville, Spain), 7, 285 (1956), Ref. 131, Swern, above mentioned) reported that the di-glycerides in position 1,3 are able to give complexes with urea, while the tri-glycerides do not form complexes. The lack of complexation of tri-glycerides, already anticipated by Schlenk, above mentioned, page 251, is then confirmed in the review of "Breivik 2007", page 118, as an expert in the art.

[0052] Other authors cited by Swern, page 2338 (ref. 128, 132, 133), have studied the formation of monoglycerides by glycerolysis of vegetable oils, usually containing acyls of length up to C18 and up to 3 double bonds and have tried with poor results their separation from other glycerides by complexation with urea under certain conditions. In particular, Heckles J.S. and Dunlap L.H., J Am Oil Chemists' Soc, 32, 224, 1955 (ref. 133) reported that the monoglycerides of saturated and monounsaturated (oleic acid) acids form complexes with urea, while those with 2 or 3 unsaturation points (resp. linoleic and linolenic acid) do not form complexes. In admixture with saturated and unsaturated diglycerides, a separation by complexation is obtainable for the glycerides of saturated acids, while only a slight enrichment is obtainable with the glycerides of unsaturated acids. This conclusion is not shared by Mehta T.N. and Shah S.N., J Am Oil Chemists' Soc, 34, 587, 1957 (ref. 128).

[0053] Even in these cases, and within the limits of the specified products, the discussion focuses only on the possibility of fractionation and enrichment by complexation.

[0054] In recent times, Hayes D.G. et al., JAOCS, 77,207, 2000 reported that the mono-, di-, and tri-acylglycerols are poor templates for the formation of urea complexes (UC) with respect to the free fatty acids (FFA) and that their inclusion, in mixtures of acylglycerols containing FFA, even reduces the formation of UC (see Abstract), a conclusion which has then been resumed even by those skilled in the art, as exemplified by "Breivik 2007", page 118.

[0055] From the examination of the literature, the data reported on acylglycerols appear to us absolutely poor, often conflicting, limited (since they only occasionally, if ever, comprise the PUFAs of highest interest, with longer chain and more unsaturated), and almost always bound to specific cases and therefore not generalizable. The ability of complexation is often attributed to a single or a few factors, while in our opinion it depends on all the reaction parameters in their entirety, as e.g. the degree of substitution of the glyceride, the length and the degree of unsaturation of the acyl, the ratio with the urea, the type and volume of solvent, the precipitation temperature of the solvent, and so forth, each factor interfering with all the others.

[0056] The problem can be solved only experimentally, keeping well in mind the objective to be achieved and the products to be investigated.

[0057] We have thus found, through a series of experiments, that all glycerides in the broadest sense and in essentially total manner, and under certain conditions in a way that is poorly related to their structure, can be complexed with urea. This then allows the precipitation of the complex and the total removal - as they are dissolved in the solvent in non-complexed form - of all persistent organic pollutants (as the feared dioxins and related substances, the pesticides used in agriculture, etc.) that today increasingly are widely found in the animal and plant environment and in many foods, in particular in natural oils and fats and in substances derived therefrom, representing a continuous source of danger to human health, although present within the limits imposed by the present laws and by the various Pharmacopoeias .

[0058] A first aspect of the invention provides a method of purification which consists in treating the glyceride of a fatty acid - in a suitable solvent - with an excess amount of urea, sufficient for an essentially total complexation of the glyceride, generally greater than or equal to 3 parts by weight and up to 6 parts by weight or more, optionally subdividing the treatment in 2-3 consecutive steps, if necessary, preferably in 2 consecutive steps, and in any case until reaching an essentially total complexation. The division into multiple steps is not particularly harmful to the economy of the process since it involves only one additional filtration, in so far as it is possible to add the additional aliquot of urea directly to the filtered solution and the washing waters of the urea inclusion complex without further manipulation.

[0059] The treatment is followed by a precipitation of the complex, optionally divided into the respective 1-3 fractions, then the complex is collected by filtration or centrifugation and washed thoroughly with the same solvent saturated with urea and precooled around 4-5°C or less, while the solvent containing the totality of the environmental pollutants (POPs) as described above and other POPs reported in the specialist literature, and any other impurities not covalently linked to the glyceride and not able to complex, are deleted. From the urea complex, or from the pooled fractions, the glyceride, which is now purified and essentially devoid of the abovementioned POPs, is then easily recovered.

[0060] Within the scope of the present method, the glyceride may be a mono-and/or di-glyceride - (all also named as monoacyl- and diacyl-glycerols), and they will be at least partially esterified - in the case by far more preferred - with long-chain fatty acids, comprising 18-22 carbon atoms, preferably 20-22 carbon atoms, and of polyunsaturated type, i.e. comprising 2-6 double bonds, preferably 5-6 double bonds. This refers in particular to the acids belonging to the class of omega-6 and/or omega-3 acids, more preferably to the class of the acids omega-3, in particular to the EPA and/or DHA.

[0061] These glycerides may have any origin.

[0062] The omega-6 acids are particularly abundant in vegetable oils and seeds, while the omega-3 acids and in particular EPA and DHA have predominantly marine origin and derive essentially from fish oils, even from aquaculture, or from "krill oils" or still from algae and other oleaginous microorganisms, or from "single cell fermentation" starting from selected strains of algae or other microorganisms.

[0063] The content of polyunsaturated fatty acids as defined above, especially EPA and/or DHA is higher than or equal to 15%, or is comprised between 15 and 90%, or in particular is comprised between 15 and 65%.

[0064] By way of example let us remind that a typical content of a natural fish oil used for the production of PUFAs is represented by EPA 18% and DHA 12%, while 90% is around the maximum possible content for a glyceride having as substituents only polyunsaturated acids as described, e.g. EPA and/or DHA.

[0065] The mono-glycerides and di-glycerides are instead mainly industrial products, and all glycerides enriched in polyunsaturated acids are obtained with chemical and/or enzymatic processes too. For this purpose, lipases that are selective for the fatty acid and its position on the glycerol are often used, starting from natural triglycerides to hydrolyze the saturated and monounsaturated acids, or from various glycerides or glycerol and reacting with concentrated polyunsaturated acids, and using known procedures.

[0066] In these cases, the content of polyunsaturated acids will usually be greater than or equal to 30%, or between 30 and 90%. These contents of the raw material do not usually increase in a substantial way in the course of the purification in question, except in special cases.

[0067] From an operational point of view, this purification process shows some similarities with the process of enrichment of PUFA and their esters by complexation with urea, since it is possible to distinguish:

--a) a reaction step by which one part by weight of glyceride is treated with 3-6 parts by weight or more of urea (divided into 1-3 steps) in a polar solvent, preferably a protic solvent such as a lower alcohol, such as methanol or ethanol, optionally containing up to 20% of water (or others, as indicated by Swern D, already cited), at a temperature close to the boiling point, to form a ureic complex of inclusion of the glyceride;

--b) a step of cooling to about 4 - 5°C until precipitation of this urea complex (or complexes), isolation by filtration or centrifugation (with elimination of the filtered mother liquors) and careful washing of the complex or pooled complexes with the same solvent saturated with urea and pre-cooled to about 4-5°C;

--c) a step of obtaining a purified composition by dissolution in water of this inclusion urea complex and direct isolation of the oily phase formed as a result of said dissolution or by extraction of said oily phase with an organic solvent unmixable with water, typically hexane or the like, and subsequent evaporation of said solvent to dryness, or still by direct extraction from such inclusion urea complex through fluids in supercritical state, in particular carbon dioxide, operations followed by optional molecular distillation.

[0068] On the other hand it will be readily apparent to the skilled person that the purpose of the whole process is totally different, as well as it will be evident in step a) the use of urea in a strong excess and possibly in more steps, in step b) the careful washing of the complex to eliminate the pollutants contained in the mother liquors incorporated, in step c) the recovery of the purified product from the solid complex, instead of the recovery of the composition enriched in PUFA performed on mother waters as taught by the "prior art".

[0069] The purified composition according to the invention will have essentially the same composition in glycerides and then in fatty acids as in the raw material, but will be substantially free from the totality of toxic environmental pollutants described above, such as dioxins and furans, polychlorinated biphenyls, polybrominated diphenyl ethers, cyclic and polycyclic hydrocarbons, simple and condensed, and other POPs known in the specialized literature.

[0070] In particular it is understood that each of the above-mentioned substances and their sum will be at least 2 times, or at least 5 times reduced, or preferably at least ten times reduced (reduction of at least 90%) with respect to the compositions from which they derive, if initially present in concentrations correspondingly higher than their limit of quantification (LOQ), or even each substance will be less than the limit of quantification (LOQ) with standard methodology GC-MS, whereby, by assimilating its value to "zero" (lower bound), we shall define their sum "essentially zero". A value of "zero" instead corresponds to a sum of data less than the limit of detection (LOD).

[0071] As shown by the experimental tests, the purified compositions of glycerides may be characterized by the contents of POPs that follow, although in most cases each congener will be generally lower than its limit of quantification LOQ (essentially zero).

- polychloro-dibenzo-para-dioxins (PCDDs, n=7) and polychloro-dibenzo-furans (PCDFs, n=10) in an overall concentration of less than or equal to 1.0 pg/g, value determined in accordance with the toxic equivalent factors (TEFs) of WHO and expressed as toxic equivalents (TEQs);
- PCDDs, PCDFs and polychloro-biphenyls (PCBs) dioxin-like (DL-PCBs, n=12) in an overall concentration of less than or equal to 5.0 pg/g, value determined as defined above (TEQs);
- PCBs markers (n=6) in an overall concentration of less than or equal to 5.0 ng/g;
- polybromo- diphenyl ethers (PBDEs, tested n=9) in an overall concentration of less than or equal to 5.0 ng/g;
- a sum of polycyclic aromatic hydrocarbons (PAHs, tested n=4), expressed as marker substance benzo[a]pyrene, of less than or equal to 1.0 ng/g;
- other environmental persistent organic pollutants (POPs) comprising 2,2 bis-(p-dichlorophenyl)-ethane (DDE), and/or 2,2 bis-(p-dichlorophenyl)-1,1-dichloroethane (DDD), and/or 2,2 bis-(p-dichlorophenyl)-1,1,1-trichloroethane (DDT) in an overall concentration of less than or equal to 2.0 ng/g, polybromo- biphenyls (PBB) in an overall concentration of less than or equal to 5.0 ng/g; hexachlorobenzene in concentration of less than or equal to 0.1 ng/g, and the isomers of hexacloro-cyclohexane in an overall concentration of less than or equal to 0.1 ng/g.

[0072] Also all other POPs listed by the Stockholm Convention of 2001 and by the successive review Committees, may be cited here as it is in all cases a matter of sterically bulky molecules, not suitable for the complexation with urea, and easily eliminated quantitatively in the course of this purification process.

[0073] It will then be obvious that also other impurities can be eliminated in the process, if not covalently linked to the glyceride, and if unsuitable to complex with urea, as some long chain aliphatic hydrocarbons having branched structures (typically squalene), some oligomers and polymers of fatty acids, some sterol substances, and the like.

[0074] A second aspect of the invention relates to the compositions of glycerides themselves, as obtained according to the method of purification described above.

[0075] Among these compositions, we explicitly mention a purified composition of mono- and/or di- esters of omega-3 acids with glycerol.

[0076] A third aspect of the invention is that easily deducible from the availability itself of glycerides, free from environmental pollutants and highly purified: this aspect relates therefore to the use of said glycerides for the production by chemical or enzymatic route of other compositions based on PUFAs and omega-3 acids and derivatives, including PUFA-enriched triglycerides, in more or less concentrated form, according to standard procedures of the literature described in the field of the chemistry of these substances.

[0077] Already the current technology in fact provides, starting from natural oils of various origin and then from complex mixtures, for proceeding to the isolation of the acid components more interesting for pharmaceutical use or as food or diet supplements, just as e.g. EPA and/or DHA and then to their concentration.

[0078] For this purpose, in a first step the natural oils (triglycerides) more suitable for the intended purpose are subjected to a mild hydrolytic process, e.g. by alcoholic potassium hydroxide, thus obtaining the corresponding potassium salts and then the free acids and - if desired - the alkyl esters; or more often to a transesterification process e.g. in the presence of excess aliphatic alcohols, preferably C1-C3, and alkaline or acid catalyst, thus obtaining directly the corresponding alkyl esters of fatty acids, and from these - if desired - the related acids or salts.

[0079] The subsequent step of concentration of the more valuable components, and/or of adjustment of their ratio according to the request, and/or isolation of a single component, is carried out with different methods, also combined, among which mainly:

- distillation under high vacuum, usually the molecular or short path distillation more suitable to limit the thermal processes of degradation, providing of course to the appropriate fractionations;

- some chromatographic processes, even under high pressure (HPLC), more suitable however for a laboratory scale or analytical purpose;

- extraction with supercritical fluids (SFE), usually with CO_2, also in combination with chromatographic processes (SFC) on preparative scale and with a suitable stationary phase, more specific for the separation of individual components;

- in the more general and industrially accepted case, it is however resorted to a complexation reaction with urea in various solvents, such as alcohols. It is known that urea under particular conditions can crystallize in hexagonal crystals, with formation of channels able to include the linear chains of fatty acids, mainly those of acids and esters saturated and/or monounsaturated or with the lower degree of unsaturation. The inclusion complex formed precipitates by cooling from the alcoholic solution and is isolated and eliminated, thus allowing to recover from the solution a composition enriched in polyunsaturated components such as e.g. EPA and DHA ("passive" complexation).

[0080] To this concentration step, a final molecular distillation to remove small low- and high-boiling fractions, usually follows.

[0081] All these processes, easily adoptable also with the purified glycerides of the invention, are extensively described e.g. by "Breivik 2007", Chapter "Concentrates", p. 111-130 and cited references, and do not require further description.

[0082] From recent years, said hydrolysis or alcoholysis procedures, apart from the chemical route, are also carried out enzymatically by means of selective lipases, also immobilized ones: this allows to operate under reaction conditions still milder, moreover - by virtue of the greater resistance of the PUFAs to enzymatic cleavage, compared to saturated and monounsaturated acyls- it is possible to isolate intermediate compositions of glycerols still partially acylated and enriched in PUFAs, to be submitted to hydrolysis reactions to acids or of alcoholisis to esters in separate steps. Further, they can also be submitted to subsequent optional processes of fractionation of the components and/or enrichment to give compositions of acids or their salts, esters and even of "structured" glycerides, all enriched and concentrated in certain polyunsaturated components.

[0083] Also these methods by enzymatic catalysis are well described by "Breivik 2007", Chapter "Enzymatic processing of omega-3 specialty oils", p. 141-164, and are clearly adoptable also with the purified compositions of the invention.

[0084] It follows therefore that, starting from the glycerides purified according to the method, it will be possible to obtain all the compositions comprising the corresponding acids in free form, or their salts, or even their esters. In addition, it will still be possible to obtain all the respective compositions enriched in polyunsaturated components, exactly as feasible e.g. starting from "fish oils natural" or other oils, with the only difference that one can obtain in this case compositions that are essentially free from the various pollutants defined POPs and from the consequent risks to the health of the user.

[0085] While the acids are obtained by hydrolysis of the glycerides by chemical or enzymatic route as reported above, the salts of the polyunsaturated acids are obtainable from these e.g. by direct salification and are represented by the salts with alkali metals, e.g. sodium and potassium, alkaline-earth metals, e.g. calcium, with basic amino acids such as lysine and arginine, meglumine, with choline and mono-, di-, and triethanolamine, and the like, if pharmacologically acceptable.

[0086] The alkyl esters are obtainable e.g. by chemical or enzymatic alcoholysis and are represented by esters with aliphatic alcohols, even very long chain alcohols as found in "natural waxes", but are preferably represented by esters with lower alcohols C1-C3, in particular the ethyl ester.

[0087] The glycerides reconstituted and concentrated in PUFAs can be prepared by esterification e.g. of concentrated and purified omega-3 acids with glycerol, or by transesterification of esters with glycerol.

[0088] Similarly, by chemical or enzyme assisted esterification/transesterification e.g. of concentrated and purified omega-3 acids with the mono- and/or di- glycerides purified from POPs according to the present process, it will be possible to obtain reconstituted tri-glycerides which are totally purified and concentrated in PUFAs

[0089] Coming to a greater detail, one can thus obtain, by way of example, a generic composition of lipids and some compositions of acids or esters which can derive therefrom as described below.

--a composition consisting of mono-glycerides and/or di-glycerides of any origin, wherein the glycerol is at least partially esterified with long-chain fatty acids having 18-22 carbon atoms, preferably 20-22 carbon atoms, and of polyunsaturated type (PUFAs), i.e. containing 2-6 double bonds, preferably 5-6 double bonds, belonging to the class of omega-6 and/or omega-3 acids, preferably to the class of the omega-3 acids, more preferably represented by eicosapentaenoic acid (EPA, C20:5 n-3, all cis) and/or docosahexaenoic acid (DHA, C22:6 n-3, all cis), in which the composition further contains the following environmental persistent organic pollutants (POPs) :

- polychloro-dibenzo-para-dioxins (PCDDs) and polychloro-dibenzo-furans (PCDFs) in an overall concentration of less than or equal to 1.0 pg/g, value determined in accordance with the Toxic Equivalent Factors(TEFs) of WHO and expressed as Toxic Equivalents (TEQs);
- PCDDs and PCDFs and polychloro-biphenyls (PCBs) dioxin-like (DL-PCBs) in overall concentration of less than or equal to 5.0 pg/g, value determined in accordance with the values of TEF of WHO and expressed as TEQs;
- PCBs markers in overall concentration of less than or equal to 5.0 ng/g;
- polybromo- diphenyl ethers (PBDEs) in overall concentration of less than or equal to 5.0 ng/g; and
- a sum of polycyclic aromatic hydrocarbons (PAHs), expressed as marker substance benzo[a]pyrene, of less than or equal to 1.0 ng/g;

each of said POPs being preferably contained in said composition in a concentration below its limit of quantification LOQ with standard methodology GC-MS.

-- a composition that comprises EPA and/or DHA in the form of free acids or in the form of ethyl esters, wherein the total concentration is comprised between 15 and 100%, preferably between 50 and 100%, of the weight of the composition, and a content of pollutants as described above and in accordance with the purified compositions of glycerides from which they derive.

-- a composition wherein the concentration by weight of EPA ethyl ester or DHA-ethyl ester, is greater than or equal to 80%, preferably greater than or equal to 90%, or their sum is greater than or equal to 80%, preferably greater than or equal to 84%, being the concentration of EPA ethyl ester greater than or equal to 40%, the concentration of DHA ethyl ester greater than or equal to 34% and the sum of the concentrations of all the omega-3 ethyl esters greater than or equal to 90% in accordance with the specifications of the European Pharmacopoeia (EP), and a content of pollutants as described above.

-- a composition in which the sum of the weight concentrations of EPA ethyl ester and DHA ethyl ester is between 80.0 and 88.0%, being the concentration of EPA ethyl ester between 43.0 and 49.5%, the concentration of DHA ethyl ester between 34.7 and 40.3%, and the sum of the concentrations of all the omega-3 ethyl esters greater than or equal to 90% in accordance with the specifications of the United States Pharmacopoeia (USP), and a content of pollutants as described above.

[0090] Having defined some of the compositions obtainable by chemical or enzymatic route starting from the purified glycerides, a fourth aspect of the invention comprises the addition to the purified glycerides and derivatives thereof of suitable diluents, excipients, suspending agents, etc. and/or preservatives, antioxidants, etc., according to all the technologies known in the art, to give all the formulations known in the art to enable their use in all the proposed uses. Said formulations comprise, in addition to their direct inclusion e.g. in various foods, or in the form of micro-encapsulated or nano-encapsulated or encochleates obtained according to the prior art, also the formulations for the oral use - as drops, soft gelatin capsules, auto-sealants capsules of hard gelatin, tablets, if necessary after adsorption on a solid substrate or as an inclusion complex, if necessary even in enteric formulation, etc. - as known in the art; or for the topical use - such as creams, ointments, etc. - as well as known in the art; or even for the injection use - such as vials for intramuscular use, bottles for slow intravenous drip infusion, etc., after sterilization and/or if necessary after modification by chemical and/or physical methods, e.g. as emulsified glycerides, as well as known in the art.

[0091] With reference to their use, said purified compositions and their formulations will be addressed to the preparation and use as a food or food ingredient, of every kind and purpose, as food and diet supplement, food for special medical purposes (functional food), both new and deductible from the pharmaceutical use, feed for animal consumption and for aquaculture, infant food formulas, cosmetic and pharmaceutical preparation, all containing or enriched with long chain fatty acids or derivatives thereof, having polyunsaturated character or specifically of the omega-3 series.

[0092] For all uses, but in particular the preparation for pharmaceutical use will preferably be enriched and concentrated in polyunsaturated components, in particular in EPA and/or DHA, or salt thereof, or their ethyl ester, or their glyceride.

[0093] Of great and growing importance are foods for special medical purposes, or functional foods - including drinks and supplements - which include specific ingredients, such as in particular the omega-3 acids in their every form, capable of imparting certain specific benefits for health (fortified foods). These functional foods are currently known under the English term FOSHU (Foods for Specified Health Use) or with the Japanese term Tokuho.

[0094] Going on to the particular, the use of the compositions will be directed to the prevention and treatment of risk factors for heart diseases, cardiovascular and cardio-circulatory illnesses, such as hypertension, severe and moderate hypertriglyceridemia (resp. >500mg/dl and >200mg/dl) and hypercholesterolemia, in particular the familial and genetic forms, also in combination with other drugs and in particular with statins, and as the defects of coagulation and platelet aggregation.

[0095] A use of great importance is the one for the prevention and treatment of heart diseases, and cardiovascular and cardio-circulatory illnesses, such as coronary- atherosclerotic illnesses and cardiac and cerebral ischemic states, including myocardial and cerebral infarction, and for reducing the risk of sudden cardiac death consequent to myocardial infarction; those of electrical origin and involving the onset and the propagation of the heart rhythm, including arrhythmia and atrial and/or ventricular fibrillation; and those due to mechanical defects of the heart pump, such as heart insufficiency and decompensation and/or the "congestive heart failure".

[0096] In other pharmaceutical uses the compositions are used for the prevention and treatment of disorders of the central nervous system (CNS), including epilepsy, the various depressive forms, the bipolar disorders, the pediatric pathologies by defects of the attention and hyperactivity disorders (ADHD), defects of learning and memory, the various forms of schizophrenia, Alzheimer's disease and various forms of dementia. The memory enhancements, particularly in pediatric age, are of extreme interest.

[0097] Still other pharmaceutical uses include the prevention and the treatment of retinopathy and symptoms of dry eye, of the metabolic syndrome, defects of metabolism and related to obesity, type 2 diabetes, liver disorders, diseases of connective tissue and joints, of inflammatory states, autoimmune diseases, ulcerative colitis, psoriasis and tumor disease.

[0098] The final aspect of the present invention finally concerns the purification of a composition consisting of a monoglyceride and/or diglyceride of any origin, esterified with long-chain fatty acids consisting of 16-20, preferably 18-20 carbon atoms, of type saturated and/or monounsaturated, and the purified composition which is obtained.

EXPERIMENTAL EXAMPLES

[0099] The invention will now be described by means of some examples which have not however any limitative character.

EXAMPLE 1

[0100] The purification from POPs was carried out on a composition containing a mixture of monoglycerides (52.4%) and diglycerides (46.0%) of polyunsaturated fatty acids (PUFAs).

[0101] The composition has been obtained by direct esterification with glycerol of a concentrated composition at more than 80% of n-3 PUFAs derived at their turn from their corresponding ethyl esters (batch 201308, origin from fish oil; GC/MS analysis: EPA 51% b.w., DHA 39% b.w.) by standard alkaline hydrolysis (KOH in aqueous ethanol and reflux for 2 hours).

[0102] The synthesis of glycerides was carried out in the presence of a preparation of immobilized lipase from Candida antarctica (NV-435) and of molecular sieves, and the ratios of components, the temperatures and the reaction times adopted were those reported for the experiment 2 of Noriega-Rodriguez J.A. et al, J. Food Res. 2(6),97, 2013.

[0103] The product obtained and analyzed by HPLC, as indicated by the same author, has finally been submitted to a quick distillation under high vacuum in order to eliminate a small distillation residue mainly constituted by triglycerides. After final control for presence of pollutants, the product has been subjected to purification as described here below.

Procedure - Step 1

[0104] 200 mL of methanol and 100 g of urea are charged into a flask, and then the mixture is heated to reflux under stirring obtaining a substantially limpid solution.

[0105] 25 g of the above oily composition (ratio by weight oil: urea 1 : 4) are then added, and the mixture is then left under stirring and boiling for 10 min, obtaining a slightly yellowish mixture with some amount of oil still in suspension. Then 20 mL of n-butanol are added if necessary to complete the dissolution, and reflux is continued for 30 minutes.

[0106] The mixture is left to cool with stirring and at about 45°C quickly starts the precipitation of a precipitate, which is then cooled for a few minutes with running water and then left to rest for 1 hour in a refrigerator at about 5°C to complete the precipitation.

[0107] At the end the precipitate is collected by filtration on a buchner and squeezed well to separate to the maximum extent the methanol mother liquors, which are kept aside for a second treatment. No separation of oily substances from methanol is noticed.

[0108] The collected precipitate is washed thoroughly on the buchner with two 50 mL portions of a urea solution in methanol, obtained by dissolving 16 g of urea in 100 mL of methanol under heating and then cooling to 5°C, thus obtaining at the end colorless washing waters.

[0109] 95 g still wet of precipitate are so obtained, from which one proceeds directly to the recovery of the purified glycerides by dissolution in 200 mL of 5% solution of NaCl in water and subsequent extraction with 2 portions each of 50 mL of n-hexane.

[0110] The two organic phases are separated from the aqueous phase and pooled, then the n-hexane is evaporated to dryness at about 35°C and under reduced pressure up to constant weight, obtaining 20.8 g of residue consisting of refined mixture of mono- and diglycerides that is kept around 5°C in a nitrogen atmosphere.

[0111] The product is usually used as such, any eventual traces of solvents can be eliminated, if desired, with known methods and quantitative yields.

[0112] The analysis GCMS of the corresponding methyl esters FAME demonstrates that the composition is similar to that of the starting product.

[0113] The analysis of POPs is reported here only as the ratio of the number of congeners that cannot be quantified, to the number of controls carried out, as indeed their content is always lower than their LOQ.

[0114] For comparison, the corresponding values of the original composition are also reported.

	Purified product	Raw material
Dioxins	7 / 7	7 / 7
Furans	10 / 10	8 / 10
Dioxin-like PCBs	12 / 12	1 / 12
PCB markers	6 / 6	3 / 6
PBDEs	9 / 9	2 / 9
PAHs	4 / 4	0 / 4

[0115] In the purified product, all congeners of toxic and carcinogenic environmental pollutants (POPs: persistent organic pollutants) were less than the limit of quantification and essentially absent, in 48 cases out of 48. This leads us to believe that the complexation with urea acts as a sort of "size exclusion" chromatography and that this purification can be extended to all classes of environmental pollutants of similar structure and steric hindrance, without risk of exceptions.

[0116] In relation to the reference starting product, it can be noted that the content of dioxins and furans is acceptable, since the control rules are more rigorous and applicable for longer times. For other pollutants, equally toxic, the situation is much worse and shows some concentration peaks at high risk for the user and poor adherence to the ethical standards and to legislation.

Step 2

[0117] The methanol mother liquors isolated by filtration of the urea inclusion complex of step 1, containing eventual butanol and combined with the methanol washing as described above, are added with a further 50 g of urea (total ratio by weight glycerides : urea of 1 : 6) and brought to the boiling for 15 minutes under stirring, obtaining almost complete solution.

[0118] It is allowed to cool under stirring, obtaining at about 45°C a quick precipitation, and then to rest for 1 hour in a refrigerator at about 5°C.

[0119] At the end the precipitate is filtered on a buchner, the mother liquors are thoroughly removed and the precipitate is washed carefully with 2 portions of 50 mL of a urea solution in methanol obtained as described above and pre-cooled to 5°C.

[0120] There is thus obtained an abundant precipitate, from which still wet further purified product is recovered as described in Step 1, by dissolving in 200 mL of 5% aqueous solution of NaCl and extracting with two 50 mL portions of n-hexane.

[0121] Evaporation to dryness of the extracts in n-hexane as described above leads to a further oily residue of 3.3 g, constituted also by purified glycerides, which is stored at 5°C under nitrogen.

[0122] The analysis GCMS confirms the purity of the product and demonstrates that its composition is essentially equal to that obtained in Step 1, therefore the products are combined, thus leading to a total yield 24.1 g.

[0123] The analysis of POPs demonstrates that their content is still not measurable and "essentially zero", in 48 cases out of 48.

EXAMPLE 2

[0124] The purification from POPs was performed on a composition containing substantially only monoglycerides (98.5%) of PUFAs, obtained as above by direct esterification with glycerol of the same above concentrated composition of n-3 PUFAs.

[0125] The preparation of monoglycerides was done in this case according to Experiment 5 of the Author above cited, and at the end the product was submitted twice to molecular distillation to eliminate limited amounts of more substituted glycerides.

[0126] The content of PUFAs in the monoglyceride, determined by GC/MS after analytical transesterification to fatty acid methyl esters (FAME), was EPA 53.5% b.w. and DHA 38%.

[0127] The presence of pollutants was still confirmed.

[0128] The purification of this product was carried out by following the procedure of Example 1, in 2 steps, giving an overall purification yield of 95%.

[0129] The analysis of the 6 classes of POPs by us routinely checked, confirmed that the 48 congeners of various families, as above, were all below their limit of quantification LOQ and then essentially absent.

[0130] Also absent resulted to be some pesticides of the family of 2,2 bis-(p-dichlorophenyl)-ethane variously chlorinated, like DDT, as well as the polybrominated biphenyls and hexacloro-derivatives of benzene and cyclohexane, present instead in a concentration around 1-2 ng/g in the starting product.

Claims

1. Method for the purification from Persistent Organic Pollutants (POPs) of a composition consisting of mono-glycerides and/or di-glycerides of any origin, wherein glycerol is at least partially esterified with saturated or unsaturated long chain fatty acids having 16-22 carbon atoms, said composition containing said POPs, wherein said method comprises the phases of:

   a) forming an inclusion urea complex that contains essentially all of said composition by treating one part by weight of said composition with at least 3 parts by weight of urea in a polar solvent, such as a lower alcohol or ketone, optionally containing up to 5% of water, at a temperature comprised between 20°C and the boiling temperature of the solvent;
   b) cooling to about 4-5°C until precipitation of said urea complex, isolating it by filtration or by centrifugation, from the mother waters containing said POPs, and washing it with said polar solvent, previously saturated with urea and cooled to a temperature of about 4-5 °C ;
   c) obtaining a purified composition having a reduced content of POPs, by dissolution in water of said inclusion urea complex and separation of the oily phase formed following said dissolution, or by extraction of said oily phase with an organic solvent unmixable with water, typically hexane or the like, followed by evaporation of said solvent to dryness, or by direct extraction from said inclusion urea complex by means of supercritical fluids, particularly carbon dioxide.

2. Method according to claim 1, wherein said mother waters and washing waters of said isolated urea complex are pooled and treated with further 2-3 parts by weight of urea (referred to the starting composition), repeating the phases from a) to c) of forming the inclusion urea complex, cooling, isolating and obtaining the purified composition.

3. Method according to any of claims 1 and 2, wherein said long chain fatty acids have 18-22 carbon atoms, preferably 20-22 carbon atoms, and are polyunsaturated acids (PUFA), that is containing 2-6 double bonds, preferably 5-6 double bonds, belonging to the class of omega-6 and/or omega-3 acids, preferably to the class of omega-3 acids, more preferably represented by eicosapentaenoic acid (EPA, C20:5 n-3, *all cis*) and/or docosahexaenoic acid (DHA, C22:6 n-3, *all cis*).

4. Method according to any of claims 1-3, wherein the phase a) of treatment of said composition with urea is performed by using as polar solvent at least one C_1-C_4-alcohol or at least one C_1-C_6 alkyl ketone or a mixture thereof.

5. Method according to claim 4, wherein said polar solvent is selected from the group consisting of methanol, ethanol, n-butanol, i-butanol, acetone, methyl ethyl ketone and methyl isobutyl ketone.

6. Method according to claim 5, wherein the phase a) of treatment of said composition with urea is performed by using as polar solvent methanol in an amount of 4.5-7 parts by weight, or ethanol in an amount of 45-65 parts by weight.

7. Method according to each of claims 1-6, wherein said environmental persistent organic pollutants (POPs) mainly comprise polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs), and/or dioxin-like (DL-) and marker polychlorinated biphenyls (-PCBs), and/or polybrominated diphenyl-ethers (PBDEs), and/or polycyclic aromatic hydrocarbons (PAHs), and their presence in the purified composition obtained in the phase c) results to be at least 2 times reduced, preferably at least 5 times reduced, most preferably at least 10 times reduced, and in any case lower - for each component - than the respective limit of quantification (LOQ) determined with standard GC-MS method.

8. Composition obtained with the method according to any of claims 1-7, consisting of mono-glycerides and/or diglycerides of any origin, wherein glycerol is partially esterified with long chain fatty acids having 18-22 carbon atoms, preferably 20-22 carbon atoms, and are of polyunsaturated structure (PUFA), that is containing 2-6 double bonds, preferably 5-6 double bonds, belonging to the class of omega-6 and/or omega-3 acids, preferably to the class of omega-3 acids, more preferably represented by eicosapentaenoic acid (EPA, C20:5 n-3, *all cis*) and/or docosahexaenoic acid (DHA, C22:6 n-3, *all cis*), said composition further containing the following environmental persistent organic pollutants (POPs):

    - polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs) in total concentration of less than or equal to 1.0 pg/g, value determined in agreement with the Toxicity Equivalency Factors (TEFs) of WHO and expressed as Toxic Equivalents (TEQs);
    - PCDDs, PCDFs, and dioxin-like polychlorinated biphenyls (DL-PCBs) in total concentration of less than or equal to 5.0 pg/g, value determined in agreement with the TEF values of WHO and expressed as TEQs;
    - PCBs marker in total concentration of less than or equal to 5.0 ng/g;
    - polybrominated diphenyl-ethers (PBDEs) in total concentration of less than or equal to 5.0 ng/g;
    - a sum of polycyclic aromatic hydrocarbons (PAHs), expressed as benzo[a]pirene marker substance, in total concentration of less than or equal to 1.0 ng/g;

    each of said POPs being preferably contained in said composition in concentration lower than its limit of quantification (LOQ) determined with standard GC-MS method.

9. Composition according to claim 8, wherein the total content of said polyunsaturated fatty acids is comprised between 15 and 90% by weight of the composition.

10. Use of a composition according to any of claims 8 and 9 for the preparation from said monoglycerides and/or diglycerides of the corresponding triglycerides, by means of chemical or enzymatic esterification or transesterification, in presence of said PUFAs as defined in claim 3 or of C_1-C_3 alkyl esters thereof, preferably ethyl esters thereof.

11. Use of a composition according to any of claims 8 and 9 for the preparation from said mono-glycerides and/or diglycerides of the corresponding polyunsaturated fatty acids, or their salts, by means of chemical or enzymatic hydrolysis, or for the preparation of the relevant alkyl esters C1-C3, preferably ethyl esters, by means of transesterification by chemical or enzyme catalyzed route, in the presence of aliphatic alcohols C1-C3, preferably of ethanol.

12. Use according to claim 11, wherein the obtained acids or esters are submitted to a procedure of concentration of the polyunsaturated components by fractioned distillation, or molecular or "short path" distillation, or by fractioning

by means of progressive complexation with urea of saturated components and possibly of other components with a lower degree of unsaturation, followed by elimination of the formed complexes and final distillation of the concentrate, or even by extracting and/or fractioning with supercritical fluids, or by using combined technologies.

13. Composition comprising said polyunsaturated fatty acids or their salts or alkyl esters C1-C3 obtained by chemical or enzymatic hydrolysis according to any of claims 11 and 12, comprising EPA and/or DHA in the form of free acids or ethyl esters, in total concentration comprised between 15 and 100%, preferably between 50 and 100% by weight of the composition, and a content of pollutants POPs as defined in any of claims 7 and 8.

14. Composition according to claim 13, comprising EPA ethyl ester, or DHA ethyl ester, wherein the concentration of EPA ethyl ester, or of DHA ethyl ester, is higher than or equal to 80%, preferably higher than or equal to 90% by weight of the composition, or the sum of the concentrations of EPA ethyl ester and of DHA ethyl ester is higher than or equal to 80%, preferably higher than or equal to 84%, being the concentration of EPA ethyl ester higher than or equal to 40%, the concentration of DHA ethyl ester higher than or equal to 34% and the sum of the concentrations of all omega-3 ethyl esters higher than or equal to 90% by weight of the composition, in agreement with the specifications of monograph 1250 of the EP 8.0.

15. Composition according to claim 13, comprising EPA ethyl ester and DHA ethyl ester in total concentration comprised between 80.0% and 88.0%, being the concentration of EPA ethyl ester comprised between 43.0% and 49.5%, the concentration of DHA ethyl ester comprised between 34.7% and 40.3%, and the sum of the concentrations of all omega-3 ethyl esters higher than or equal to 90% by weight of the composition, in agreement with the specifications of the USP 37, pages 4059-61.

16. Use of a composition according to each of claims 8, 9, 13, 14 and 15 for the preparation of formulations useful as food ingredients, food and diet supplements, foods for special medical purposes (functional foods), foods for animal and aquaculture use, infant food formulas, cosmetic and pharmaceutical preparations, due to their high content of long chain fatty acids or their derivatives, having polyunsaturated character or specifically belonging to the omega-3 series.

17. Pharmaceutical formulation comprising a composition according to any of claims 8, 9, 13, 14 and 15 and a pharmaceutically acceptable vehicle.

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EUROPEAN SEARCH REPORT

Application Number
EP 18 16 2570

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The present search report has been drawn up for all claims

1

Place of search	Date of completion of the search	Examiner
Munich	12 April 2018	Adechy, Miriam

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X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons
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