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DESCRIPTION

[0001] This invention relates to a process for producing a glyceride composition.

[0002] The invention is defined by the claims. Triglyceride fats and oils are important commercial products and are used extensively in, for example, the food industry. Some triglycerides are nutritionally important and the triglyceride 1,3-dioleoyl-2-palmitoyl glyceride is known to be an important component of human milk fat.

[0003] Fat compositions containing the principal fatty acids found in human milk fat, in similar amounts to those found in human milk fat, may be derived from oils and fats of vegetable origin. However, a significant difference in composition arises because most glycerides of vegetable origin are unsaturated in the 2-position. In contrast, a substantial amount of palmitic acid occupies the 2-position of glycerides in human milk fat.

[0004] The difference in the distribution of acids along the glyceride positions is believed to have important dietary consequences. The distribution of fatty acids in the triglycerides of some milk fats of nutritional importance was studied by Freeman et al, (J. Dairy Sci., 1965, p.853), who reported that human milk fat contains a greater proportion of palmitic acid in the 2-position, and a greater proportion of stearic acid and oleic acid in the 1,3-positions than the milk fat of ruminants. The greater absorption of palmitic acid in the 2-position of triglycerides by infants was reported by Filer et al (J. Nutrition, 99, pp. 293-298), who suggest that the relatively poor absorption of butter fat by infants compared with human milk fat is attributable to its substantially uniform distribution of palmitic acid between the glyceride positions of the fat.

[0005] In order to most closely match the chemical and/or physical properties of triglyceride fats or oils obtained from natural sources, to that of human milk fat, therefore, it is necessary to control the distribution of the fatty acid residues on the glyceride positions. Processes are thus known for selectively producing the triglyceride 1,3-dioleoyl-2-palmitoyl glyceride (OPO) for use in replacements for human milk fat.

[0006] EP-A-0209327 discloses milk replacement fat compositions comprising the triglyceride 1,3-dioleoyl-2-palmitoyl glyceride (OPO). The fat compositions can be obtained by subjecting fatty mixtures of 2-palmitoyl glycerides to reaction with oleic acid in the presence of a catalyst, such as a lipase, which is regiospecific in activity in the 1- and 3- positions of the glycerides. Enzymatic processes of this kind are also described in GB-A-1577933. Under the influence of the catalyst, unsaturated fatty acid residues may be introduced into the 1- and 3- positions of the 2-palmitoyl glycerides by exchange with unsaturated free fatty acids or their alkyl esters.

[0007] WO 2005/036987 discloses a process for producing a fat base by reacting a palmitic rich oil with unsaturated fatty acids such as oleic acid. The total palmitic acid residue content of the fat base is at most 38% and at least 60% of the fatty acid moieties are in the 2- position of the glyceride backbone. A related disclosure can be found in WO 2005/037373, filed on the

same day.

[0008] WO 2006/114791 discloses human milk fat substitutes. The only palm oil stearins that are specifically mentioned have iodine values (IVs) of 34 and 15 and these are interesterified with triglyceride oils or chemically randomised before further use.

[0009] EP-A-1477070 and US 2004/126475 describe fat compositions comprising crystals of β form having a double chain length structure. The compositions are for use in baking.

[0010] EP-A-0417823 describes a process that involves the transesterification of triglycerides.

[0011] JP-H06-70786 discloses a method for producing triglycerides having a high palmitic acid content at the 2- position.

[0012] Commercial processes for producing triglycerides generally employ starting materials that are readily available at reasonable cost in large quantities. One suitable starting material that is relatively rich in triglycerides having palmitic acid in the 2- position is palm oil. Typically, palm oil is fractionated before being employed in this type of process.

[0013] Our copending international application no PCT/GB2006/003343 describes a process for the production of a composition comprising 1,3-dioleoyl-2-palmitoyl glyceride (OPO) in which a palm oil stearin, with an iodine value (IV) between about 2 and about 12 is subjected to enzymic transesterification, with oleic acid or a non-glyceride ester of oleic acid.

[0014] There remains a need to provide a more efficient process for the production of 1,3-dioleoyl-2-palmitoyl glyceride (OPO). For example, there is a need to increase the yield of the process and/or make the process more efficient.

[0015] According to the invention, there is provided a process for the production of a composition comprising 1,3-dioleoyl-2-palmitoyl glyceride (OPO), wherein the process comprises:

1. (i) providing one or more palm oil stearin fractions comprising tripalmitoyl glyceride and having an iodine value between 18 and 40;
2. (ii) enzymatically interesterifying the one or more palm oil stearin fractions to form a randomly interesterified palm oil stearin;
3. (iii) subjecting the randomly interesterified palm oil stearin to enzymic transesterification with oleic acid or a non-glyceride ester thereof using an enzyme having selectivity for the 1- and 3- positions of a glyceride; and
4. (iv) separating palmitic acid or palmitic non-glyceride esters from the product obtained in (iii) to form a composition comprising OPO glyceride.

[0016] In another aspect, the invention provides the use of a randomly interesterified palm oil

product (such as palm oil stearin) in a process for the production of a composition comprising 1,3-dioleoyl-2-palmitoyl glyceride (OPO).

[0017] The invention allows the production of OPO glyceride in a more cost effective manner from commercially available starting materials. The invention is at least partly based on the finding that it is possible to increase the yield of the process by carrying out a step of interesterifying the one or more palm oil stearin fractions to form a randomly interesterified palm oil stearin prior to the 1-, 3- selective reaction with the oleic acid (or an oleoyl ester) in the presence of an enzyme. It has been found that this formation of a randomly interesterified palm oil stearin increases the amount of the 2-palmitoyl glyceride that is present in the final product and thus increases the yield of the process.

[0018] The process of the invention employs one or more palm oil stearin fractions in part (i). The palm oil stearin fractions may be a single palm oil stearin or a mixture of palm oil stearins, for example obtained in different fractionation processes and/or having different physical and/or chemical properties, such as different melting temperatures or different iodine values (IVs). Preferably, the one or more palm oil stearin fractions is or are bleached and deodorised before step (ii). Bleaching and deodorising can be carried out using techniques that are well known in the art.

[0019] The term "stearin", as used in this specification, includes a triglyceride mixture or fat blend from which at least 10 % by weight of the lower melting constituents have been removed by some kind of fractionation, e.g., dry fractionation or solvent fractionation.

[0020] The optional bleaching of the palm oil stearin is typically performed above 95 °C, more preferably above 100 °C (such as at from 105 °C to 120 °C). In the deodorising step, volatile impurities are removed from the palm oil stearin to yield deodorised palm oil stearin, typically at temperatures above 200 °C. The impurities removed in the deodorising step commonly include free fatty acids, aldehydes, ketones, alcohols and other hydrocarbon impurities. The bleaching and deodorising may be carried out in a single process step or two or more process steps. For example, the steps may be carried out at reduced pressures (e.g., 10 mm Hg or below), wherein the palm oil stearin is contacted with steam to help vaporise the impurities. Bleaching and deodorising the palm oil stearin may help to improve the yield of the process.

[0021] Preferably, the one or more palm oil stearins is or are provided by fractionating palm oil or a derivative thereof. Although fractionation may be carried out with or without a solvent, it is preferred that the fractionation of the palm oil comprises dry fractionation. Thus, the process of fractionation is preferably carried out in the absence of a solvent.

[0022] The palm oil stearin has an iodine value from 18 to 40, preferably from 18 to 35. It will be appreciated that when a mixture of two or more palm oil stearins is employed, the iodine value refers to the iodine value of the mixture.

[0023] It has surprisingly been found, according to the invention, that it is possible to use as

starting materials palm oil stearins having relatively high iodine values and yet still achieve high levels of C16:0 at the Sn-2 position in the composition comprising OPO i.e., after (iv).

[0024] The process of the invention may, in a preferred embodiment, use a palm oil stearin which comprises a mixture of at least two palm oil stearins having different iodine values. For example, the palm oil stearin may comprise a mixture of a first palm oil stearin having an iodine value of from about 10 to about 20 and a second palm oil stearin having an iodine value of from about 25 to 50. This permits the use of palm oil stearins having even higher iodine values in the process for making OPO.

[0025] Step (ii) in the process of the invention is carried out enzymatically to effect random interesterification of the triglycerides in the one or more palm oil stearins. The enzyme that is used is typically a lipase. The lipase will generally be substantially non-selective for the positions on the glyceride backbone, under the reaction conditions, in order to achieve the optimum random interesterification. Selective lipases may be used, provided that the reaction conditions are such that no significant selectivity is observed, for example by running the reaction for extended periods of time. Suitable lipases include the lipases from *Thermomyces lanuginosa*, *Rhizomucor miehei*, *Rhizopus delemar* and *Candida rugosa*. Preferably, the lipase is suitable for use with food products. It will be appreciated that the randomly interesterified palm oil stearin produced in (ii) is derived from the palm oil stearin only and not from other added triglycerides such as vegetable oils.

[0026] In step (ii), the palm oil triglycerides, which typically have more palmitic acid residues in the 1- and 3- positions than in the 2- position, are converted into glyceride compositions having a greater amount of palmitic acid at the 2- position of the glyceride. The interesterification may not be fully random but is preferably random to a large extent, for example greater than 75 %, more preferably greater than 85 %, such as greater than 95 % of the fatty acid residues may change during the reaction i.e., less than 25 %, more preferably less than 15 %, such as less than 5 % of the fatty acids retain their original position in the starting triglyceride. The percentages are based on the number of fatty acyl groups present. The degree of randomisation can be determined by measuring the Sn-2 value of the C16:0 fatty acids in the product. The Sn-2 value is the number of moles (or weight) of palmitoyl residues present at the 2- position of the glyceride divided by the total number of moles (or weight) of palmitoyl residues present in the glyceride. Thus, for example, pure OPO will have an Sn-2 value for C16:0 of 1 and pure POO will have an Sn-2 value of 0, whereas a fully randomised form of OPO (i.e., containing POO and OPO) will have an Sn-2 value of 0.33. Preferably, in the present invention, the randomisation in step (ii) is carried out to give an Sn-2 value for C16:0 of from 0.300 to 0.333, more preferably from 0.310 to 0.333, even more preferably from 0.315 to 0.333, such as 0.320 to 0.333 or 0.325 to 0.333.

[0027] Preferably in step (ii), the tripalmitoyl glyceride content (C48) decreases to about 60 to 20 %, such as 60 to 40 %, or 60 to 50 % (e.g., 58 to 54 %) and the dioleoyl-monopalmitoyl glyceride content (C50) increases to about 28 to 40%, or 28 to 38% (e.g., 30 to 36 %).

[0028] In step (iii) of the process, the randomly interesterified palm oil stearin is selectively transesterified with oleic acid or an oleoyl ester. This reaction preferentially replaces residues at the 1- and 3- positions of the glyceride relative to those at the 2- position. Thus, the product has greater amounts of the oleoyl residue at the 1- and 3- positions than at the 2- position. In the enzymic transesterification of step (iii), the fatty acids on the 2-position of the triglycerides typically do not change (for example, less than 40 % by moles (or weight) of fatty acyl groups in the 2-position, more preferably less than 20 %, such as less than 5 % or less than 1 %, change during the process). The conditions of the process are selected so as to provide the desired degree of selectivity from the enzyme. Preferred enzymes for use in step (iii) are lipases from *Rhizopus delemar* and *Rhizomucor miehei*. The transesterification reaction is typically performed to reach or approach equilibrium at a conversion ratio of a minimum of at least 50 %, preferably at least 60 %, most preferably at least 70 %.

[0029] Preferably, in the transesterification reaction of step (iii), randomly interesterified palm oil stearin is mixed with an oleic acid concentrate (comprising free oleic acid at a concentration of greater than 65 % by weight, preferably greater than 70 % by weight, most preferably greater than 75 % by weight). Alternatively, the oleic acid may be provided as a mixture comprising oleic acid (preferably in an amount of greater than 65 % by weight), linoleic acid and, optionally, one or more other fatty acids. The ratio of the randomly interesterified palm oil stearin to oleic acid concentrate is preferably from 0.1:1 to 2:1, more preferably from 0.4:1 to 1.2:1, even more preferably from 0.4:1 to 1:1, most preferably from 1:1.1 to 1:2 on a weight basis. The reaction is preferably carried out at a temperature of from 30 °C to 90 °C, preferably from 50 °C to 80 °C, such as about 60 °C to 70 °C, and may be conducted batchwise or in continuous fashion, with or without a water-immiscible organic solvent.

[0030] Before the enzyme transesterification reaction of step (iii), the humidity is preferably controlled to a water activity between 0.05 and 0.55, preferably between 0.1 and 0.5, depending on the type of biocatalyst enzyme system used. The reaction may be performed, for example, at 60 °C in a stirred tank or in a packed bed reactor over biocatalysts, based on concentrates of Lipase D (*Rhizopus oryzae*, previously classified as *Rhizopus delemar*, from Amano Enzyme Inc., Japan) or immobilised concentrates of *Rhizomucor miehei* (Lipozyme RM IM from Novozymes A/S, Denmark).

[0031] The non-glyceride esters of oleic acid that are optionally used in the invention, in addition to or as an alternative to, oleic acid, are preferably alkyl esters. The term "alkyl", as used herein, includes straight chain or branched saturated hydrocarbons having from 1 to 12, more preferably 1 to 6, carbon atoms.

[0032] In step (iv) of the process, palmitic acid or palmitic non-glyceride esters are separated from the desired OPO glyceride product. It will be appreciated that the separation is not usually complete and that both the materials separated and the product that remains will be mixtures. Also, the separation of the palmitic acid or palmitic non-glyceride esters will normally also separate other fatty acids or fatty acid non-glyceride esters from the product. The term "fatty acid", as used herein, refers to straight chain, saturated or unsaturated, carboxylic acids

having from 12 to 24 carbon atoms.

[0033] In order to separate palmitic acid and other fatty acids or palmitic non-glyceride esters and other glycerides from OPO in step (iv), the transesterified mixture (optionally after further treatment, such as isolation of the fat phase) is preferably distilled. Distillation is preferably carried out at low pressure (e.g., lower than 10 mbar) and elevated temperatures (e.g., greater than 200 °C) to remove the fatty acids from the product triglyceride fraction.

[0034] The process of the invention may further comprise the step (v) of dry fractionating the product obtained in (iv) to form a fraction comprising an increased amount of OPO. However, this step may not be required depending on the purity of the final product and the desired end use of the product.

[0035] The composition or fraction produced according to the invention preferably comprises not more than 45 wt%, more preferably not more than 42 wt% of palmitic acid, based on total fatty acid content, and/or at least 53 wt%, more preferably at least 55 wt%, such as at least 58 wt%, of the palmitic acid residues are present in the 2-position of the glyceride.

[0036] A preferred composition or fraction of the invention preferably comprises not more than 42 wt% of palmitic acid, based on total fatty acid content, and at least 56 wt%, more preferably at least 58 wt %, of the palmitic acid residues are present in the 2-position of the glyceride.

[0037] Preferably, the tripalmitoylglyceride (PPP) content of the composition or fraction produced according to the invention is less than 9 % (more preferably less than 8 %) by weight of the composition.

[0038] Preferably, the composition of (iv) or the fraction of (v) is blended with at least one vegetable oil to form a fat composition for use as the fat in a human milk fat replacement product. Typically, the amount of vegetable oil varies from 1 % to 75 %, more preferably from 5 to 60 %, such as from 10 to 50 %, by weight of the fat blend. Examples of suitable vegetable oils include sunflower oil, high oleic sunflower oil, palm kernel oil, rapeseed oil and soybean oil and mixtures thereof. The resulting fat blends preferably have a Solid Content Index measured by NMR-pulse on non-stabilised fats within the following ranges: NO = 35-55; N10 = 25-50 and N30 \leq 10. These values were preferably obtained by melting the fat blend at 80 °C, holding at 60 °C or higher for at least 10 minutes, cooling to 0 °C and holding at 0 °C for 16 hours, heating to the measurement temperature N and holding at that temperature for 30 minutes before measuring the N value.

[0039] Alternatively, the composition of (iv) or the fraction of (v) may be blended with a source of docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), such as fish oil or a microbial oil, more preferably in a weight ratio of from 10:1 to 1:10.

[0040] The fat compositions or fat blends produced by the process of the invention may be suitable for replacing at least a part of the fat in infant food formulations. Infant food

formulations may comprise the fat composition or fat blend of the invention together with one or more of protein, carbohydrate, minerals and vitamins. The present invention also therefore contemplates a method for the production of infant food compositions comprising fat, protein and carbohydrate components, for example in the approximate relative weight proportions 2.5:1:5, wherein at least a part of the fat normally used in such formulations is replaced by the fat composition or fat blend made in accordance with the present invention. Dry formulations containing this mixture, together with additional components customary in such formulations such as protein, carbohydrate, minerals and vitamins, may be dispersed for use in sufficient water to produce an emulsion of approximately 2 to 5 grams of fat per 100 ml of dispersion. Therefore, an infant food formulation may be prepared by packaging and labelling a composition comprising OPO triglyceride.

[0041] The following non-limiting examples illustrate the invention and do not limit its scope in any way. In the examples and throughout this specification, all percentages, parts and ratios are by weight unless indicated otherwise.

Examples

Example 1 (Referential)

Randomisation of palm oil stearin fraction by chemical means

[0042] Chemical randomisation was carried out to have a reference for the enzyme-catalysed randomisation. 1 kg of palm oil stearin fraction with iodine value of 14 (POs14) was heated under vacuum to 110 °C for 15 minutes to remove all water. The vacuum was interrupted to add 0.15 % sodium-ethanolate and the reaction was continued under vacuum during 30 minutes at 110°C. The reaction was stopped by washing the oil several times with demineralised water at 95°C.

[0043] Finally the fat was dried by vacuum. The analysis results are given below in Example 2.

Example 2 (Referential)

Randomisation of palm oil stearin fraction enzymatically in batch reaction

[0044] The following three parameters apply to the randomisation of palm oil stearin fraction and to the manufacturing of a structured lipid comprising OPO (1,3-dioleoyl-2-palmitoyl

glyceride) in general.

- Overall fatty acid composition: FAME
- triglyceride carbon number:
 - C48 tripalmitoyl glyceride,
 - C50 mono-oleoyl-dipalmitoyl glyceride,
 - C52 dioleoyl-monopalmitoyl glyceride,
 - C54 trioleoyl glyceride
- relative position of palmitic acid: Sn-2 C16:0.

[0045] Sn-2 is as defined above and refers to the number of moles (or weight) of palmitoyl residues present at the 2- position of the glyceride divided by the total number of moles (or weight) of palmitoyl residues present in the glyceride.

[0046] POs14 (palm oil stearin fraction with iodine value of 14) was randomised in batch reaction using immobilised lipase TL-IM (*Thermomyces lanuginosus*, Novozymes) as catalyst.

[0047] 170 g POs14 was melted in a 250 ml roundbottom flask at 70 °C under nitrogen. 0.5 % g/g or 0.85 g enzyme was added and the flask was closed. The reaction time was six days, while stirring with a magnetic stirrer. At intervals samples were taken for analysis.

[0048] There is a prominent decrease in the tripalmitin concentration (C48) and increase in the mono-olein-dipalmitin concentration (C50).

[0049] The results of the analyses are listed in Table 1. It appears that enzymatic randomisation indeed increases the Sn-2 C16:0 value to about the maximal random value of 0.33.

[0050] The tripalmitoyl glyceride content (C48) decreases from 62% to 56% and the dioleoyl-monopalmitoyl glyceride content (C50) increases from 24.5% to 33-34%.

[0051] Comparison with the analyses obtained from the chemical randomisation shows that the enzymatic reaction was almost complete.

Table 1. Randomisation of POs14 with lipase TL-IM. analysis data

	regular POs14	randomised Pos14	
		chemical	enzymatic
C14:0	1.2	1.2	-
C16:0	80.3	79.8	-

	regular POs14	randomised Pos14	
		chemical	enzymatic
C18:0	5.2	5.4	-
C18:1	10.4	10.6	-
C18:2	2.1	2.3	-
C46 (PPM)	3	2.8	3.2
C48 (PPP)	62.3	55.8	55.8
C50 (PPO&POP)	24.5	33.4	33.6
C52 (OPO&OOP)	8.5	6.7	6.8
C54 (OOO)	1.7	0.6	0.8

Example 3 (Referential)

Manufacture of structural lipid containing 1,3-dioleoyl-2-palmitoyl glyceride (OPO) with regular and with randomised palm oil stearin fraction (POs14)

[0052] The structural lipid comprising OPO was manufactured by enzymatic 1,3- specific acidolysis between oleic fatty acid and POs14. A continuous packed bed reactor containing enzyme was used. This enzyme was lipase D (*Rhizopus delemar*, Amano) immobilised on microporous polypropylene beads (Accurel).

[0053] The source oil consisted of a mixture of POs14 and oleic fatty acid with ratio 1:1.4 g/g. The mixture was pumped slowly through a column containing the immobilised enzyme. During the passage of the oil/fatty acid mixture, the acidolysis reaction takes place, whereby the conversion level is proportional to the residence time in the column. The residence time is the void volume in the enzyme bed divided by the flow rate. The residence time used in this example was 53 minutes.

[0054] Water was added in order to keep the enzyme active. The water content in the POs14/oleic acid mixture was maintained at 0.15% g/g. The reaction temperature was 60°C. The duration of each experiment was three days.

[0055] The product emerging from the column was at intervals analysed for its Sn-2 value and glyceride content by carbon number.

[0056] In Table 2, the average results of four experiments, two with regular and two with randomised POs14, are listed. The composition of each POs14 source is included in columns 2

and 4.

[0057] From these data the following can be deduced.

1. 1. The difference between the regular and randomized POs14, which were from another randomisation reaction is essentially the same as shown in Example 2.
2. 2. The large difference in C-number composition between both POs14 sources does not have much effect on the corresponding C-number composition of the resulting fats, which are surprisingly alike.
3. 3. There is a significant increase of the Sn-2 value in the fat originating from the randomised POs14.

Table 2. Average analysis of fat product from regular POs14 and from random POs14 (2x2 experiments)

	regular POs 14	structured fat product	randomized POs14	structured fat product
C48	60.5	8.8	53.7	8.6
C50	24.3	34.3	32.5	34.7
C52	8.8	43.5	7.2	44.6
C54	2.4	11.6	3.0	10.4
Sn-2	0.297	0.555 ± 0.005	0.319	0.584 ± 0.002

Example 4

Enzymatically randomized POsIV35 (ER(POsIV35)) as feedstock

[0058] About 9.0 kg of POsIV35 was enzymatically randomized by stirring the oil at 70°C for 48 hours in the presence of 3%(wt) lipase Lipozyme TL IM (*Thermomyces lanuginosa*, Novozymes). After this, the enzyme was filtered off and the oil was physically refined. Physically refining includes bleaching the randomized oil with 0.5%(wt) bleaching earth at 90°C and deodorizing after filtration at 215°C for 3 hours. A sample of the refined oil was submitted for analysis. The results are shown in Table 3.

[0059] The randomized POsIV35 was interesterified with oleic acid in a continuous process. This reaction was performed at 60°C and catalyzed by Lipase D (from *Rhizopus delemar*). After the acidolysis reaction, the excess of fatty acids was removed by mean of short path distillation. A sample was submitted for analysis. The results are shown in Table 3.

Example 5

POsIV35 as feedstock

[0060] POsIV35 was directly interesterified with oleic acid in a continuous process. This reaction was performed at 60°C and catalyzed by Lipase D (from *Rhizopus delemar*). After the acidolysis reaction, the excess of fatty acids was removed by mean of short path distillation. A sample was submitted for analysis. The results are shown in Table 3.

Table 3. Analysis of samples removed during the production of Fat base from ER(POsIV35) and POsIV35

	POsIV35	Refined ER(POsIV35)	Fat base ER(POsIV35)	Fat base POsIV35
sn-2 C16	19.3	34	56.5	37.2
Carbon numbers				
C46	2	1.9	0.7	0.1
C48	24.7	24.1	4.8	4.9
C50	41.9	41.8	24.4	21.3
C52	25.2	25.8	46.4	42.2
C54	5.9	6.2	22.9	30.6
C56	0.3	0.3	0.8	0.9
Fatty acid composition				
C12:0	0.14	0.19	0.21	0.17
C15:0	0.1	0.1	0	0
C14:0	1.2	1.3	0.7	0.6
C16:0	58.3	57.2	31.6	29.4
C18:0	4.7	4.9	3.8	3.1
C18:1T	0.1	0	0.1	0.2
C18:1C	28.1	28.8	56.6	58.5
C18:2T	0.2	0.2	0.2	0.2
C18:2C	6.2	6.4	5.5	6.6
Total Trans	0.3	0.2	0.4	0.4
C20:0	0.4	0.4	0.3	0.3
C20:1C	0.1	0.1	0.2	0.2
C22:0	0.1	0.1	0.3	0.3
C24:0	0.1	0	0.1	0.1
SAFA	65.1	64.2	37.1	34
MUFA	28.4	29.1	57	59

Fatty acid composition				
PUFA	6.5	6.7	5.9	7

Example 6**Randomisation of palm oil stearine fraction POs20 in an enzyme packed bed reactor.**

[0061] Palm oil stearine fraction POs20 -having an iodine value IV of 20- was made by mixing POs14 and POs35 in a 2:1 ratio. This mixture was pumped in plug-flow mode through a packed bed reactor containing immobilized lipase TL-IM (*Thermomyces lanuginosus*, Novozymes). The pump rate was about 2 bedmasses of oil *per* hour, at a temperature of 70°C. At various intervals samples were collected to determine the glyceride composition with carbon number analysis and the relative amount of palmitic acid on the 2-position of the glycerol, Sn-2 C16:0.

[0062] The analyses of regular and randomized POs20 are shown in Table 4, columns 2 and 4. The Sn-2 C16:0 increases from 0.267 to 0.316.

Example 7**Manufacture of structural lipid containing 1,3-dioleoyl-2-palmitoyl glyceride (OPO) with regular and with randomised palm oil stearine fraction POs IV20**

[0063] The structural lipid comprising OPO was manufactured by enzymatic 1,3- specific acidolysis between oleic fatty acid and regular or randomized POs20. As in Example 4, a continuous packed bed reactor containing immobilized lipase D (*Rhizopus delemar*, Amano) was used, and the ratio of POs20 and oleic acid was 1:1.4 g/g, alike with regular or randomized POs20

[0064] At various intervals samples were collected to determine the glyceride composition with carbon number analysis and the relative amount of palmitic acid on the 2-position of the glycerol, Sn-2 C16:0.

[0065] In Table 4, the average results of two experiments, one with regular and one with randomised POs20, are listed. The composition of each POs20 source and the resulting structured fat product are given.

Table 4. Analysis of non-randomized POs20 and of randomized POs20, and of the structured

fat products thereof.

	POs20 non-randomized	structured fat product	POs20 randomized	structured fat product
C48	50.0	7.7	43.6	7.1
C50	29.2	31.4	39.0	32.2
C52	14.0	43.1	12.4	45.9
C54	3.4	16.5	1.6	13.5
Sn-2 C16:0	0.267	0.489	0.316	0.551

REFERENCES CITED IN THE DESCRIPTION

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P a t e n t k r a v

1. Fremgangsmåde til fremstilling af en sammensætning omfattende 1,3-dioleoyl-2-palmitoylglycerid (OPO), hvor fremgangsmåden omfatter:
- 5 (i) at tilvejebringe en eller flere palmeoliestearin-fraktioner omfattende tripalmitoylglycerid med et iodtal på mellem 18 og 40;
- (ii) enzymatisk at interesterificere den ene eller de flere palmeoliestearin-fraktioner til dannelse af en tilfældigt interesterificeret palmeoliestearin;
- 10 (iii) at udsætte den tilfældigt interesterificerede palmeoliestearin for enzymatisk transesterificering med oliesyre eller en ikke-glycerid-ester deraf under anvendelse af et enzym med selektivitet for 1- og 3-positionerne af et glycerid; og
- (iv) at separere palmitinsyre eller palmitin-ikke-glycerid-estere fra produktet opnået i (iii) til dannelse af en sammensætning omfattende OPO-glycerid.
- 15 2. Fremgangsmåde ifølge krav 1, hvor den ene eller de flere palmeoliestearin-fraktioner bleges og deodoriseres før trin (ii).
3. Fremgangsmåde ifølge krav 1 eller krav 2, yderligere omfattende trinnet (v) med at tørfraktionere produktet opnået i (iv) til dannelse af en fraktion omfattende en øget mængde af OPO.
- 20 4. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 3, hvor den ene eller de flere palmeoliesteariner tilvejebringes ved fraktionering af palmeolie eller et derivat deraf.
- 25 5. Fremgangsmåde ifølge krav 4, hvor fraktionering af palmeolie omfatter tørfraktionering.
6. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 5, hvor palmeoliestearinen har et iodtal på mellem 18 and 35.
- 30 7. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 6, hvor palmeoliestearinen omfatter en blanding af mindst to palmeoliesteariner med forskellige iodtal.
- 35

8. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 7, hvor palmeoliestearinen omfatter en blanding af en første palmeoliestearin med et iodtal på fra 10 til 20 og en anden palmeoliestearin med et iodtal på fra 25 til 50.

5 **9.** Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 8, yderligere omfattende trinnet at blande sammensætningen fra (iv) eller fraktionen fra (v) med mindst en vegetabilsk olie, fortrinsvis udvalgt blandt solsikkeolie, solsikkeolie med højt oliesyreindhold, palmekerneolie, rapsolie og sojaolie og blandinger deraf.

10 **10.** Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 8, yderligere omfattende trinnet at blande sammensætningen fra (iv) eller fraktionen fra (v) med en kilde til docosahexaensyre (DHA) eller eicosapentaensyre (EPA).

15 **11.** Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 10, hvor sammensætningen eller fraktionen omfatter ikke mere end 45 vægt-% palmitinsyre, baseret på det samlede fedtsyreindhold, og hvor mindst 53 vægt-% af palmitinsyreresterne er til stede i 2-positionen af glyceridet, fortrinsvis er mindst 55 vægt-% af palmitinsyreresterne til stede i 2-positionen af glyceridet.

20 **12.** Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 10, hvor sammensætningen eller fraktionen omfatter ikke mere end 42 vægt-% palmitinsyre, baseret på det samlede fedtsyreindhold, og hvor mindst 56 vægt-% af palmitinsyreresterne er til stede i 2-positionen af glyceridet.

25 **13.** Fremgangsmåde ifølge krav 12, hvor mindst 58 vægt-% af palmitinsyreresterne er til stede i 2-positionen af glyceridet.

30 **14.** Fremgangsmåde ifølge et hvilket som helst af kravene 11 til 13, hvor tripalmitoylglycerid (PPP)-indholdet af sammensætningen eller fraktionen er mindre end 9 vægt-% af sammensætningen.