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(54) Titre : COMPOSITION A BASE D'HUILES DE POISSON

(54) Title: COMPOSITION BASED ON FISH OIL

(57) **Abrégé/Abstract:**

Fish oil concentrates, having: ≥ 40 % of long chain poly unsaturated fatty acids, < 20 % of saturated fatty acids (C_{14} - C_{18}), < 15 % oleic acid, < 12 % $C_{16:1}$ - fatty acid, and with a weight-ratio: DHA = 0.5 - 3.0, EPA, can be obtained by a process involving the following steps: subjecting a fish oil to an enzymic conversion, removing free fatty acids or esters from conversion product, subjecting product of above to enzymic hydrolysis, using 1.3-specific lipase or partial glyceride-specific lipase, wash and dry product, re-esterify dried product.

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(54) Title: COMPOSITION BASED ON FISH OIL (57) Abstract Fish oil concentrates, having: ≥ 40 % of long chain poly unsaturated fatty acids, < 20 % of saturated fatty acids (C_{14}-C_{18}), < 15 % oleic acid, < 12 % $C_{16:1}$ - fatty acid, and with a weight-ratio: <u>DHA</u> = 0.5 - 3.0, EPA, can be obtained by a process involving the following steps: subjecting a fish oil to an enzymic conversion, removing free fatty acids or esters from conversion product, subjecting product of above to enzymic hydrolysis, using 1.3-specific lipase or partial glyceride-specific lipase, wash and dry product, re-esterify dried product.		

Composition based on fish oil

Fat-compositions, based on fish oils are well-known in the prior art. Many of these compositions were made and applied, because of the health benefits. E.g. Wo 90/04012 discloses triglycerides, containing saturated C₈-C₁₀ fatty acid residues in 1.3 and polyunsaturated fatty acid residues in the 2-position. It is stated, that these triglycerides have benificial nutritional properties. According to Wo 94/00044 unhardened fish oils have significant health benefits. Above known oils however have one main, common disadvantage: i.e. either the total level of polyunsaturated fatty acids (such as: EPA, DHA, DPA, etc.) is rather low (i.e.: below 35 wt % in total), or if this level is above 35 wt % its oxidative stability is low, while it also displayed high off-taste. Although it is known in the prior art that the oxidative stability of a triglyceride, containing polyunsaturated fatty acids can be increased by incorporation of saturated fatty residues into the triglycerides, the levels of saturated fatty acids were rather high in order to achieve acceptable stability.

We found new triglycerides, which overcome the drawbacks of the compositions of the prior art. These new compositions combine high levels of polyunsaturated long chain fatty acids, with relatively low levels of saturated fatty acid residues, relatively low off-taste and relatively high oxidative stability. Oxidative stability and off-taste can be evaluated by establishing mean smell scores upon evaluation by a taste-panel. Simultaneously a preference of the panellists can be measured for oils according to the invention, compared with comparative oils. Another method is to measure peroxide values of the different oils. Above triglyceride-compositions can be characterized as fish-oil concentrates, comprising glycerides with:

- (i) at least 40 wt %, preferably 40-55 wt %, more preferably 42-52 wt % of w-3 long chain poly-

- 2 -

- unsaturated fatty acids, preferably consisting of two or more of the group consisting of DHA, EPA and DPA (ie: $C_{22:6}$: $C_{20:5}$ and $C_{22:5}$ respectively).
- 5 (ii) less than 20 wt %, preferably 2-18 wt %, most preferably 5 - 15 wt % of total saturated fatty acid with 14 - 18 C - atoms.
- (iii) less than 15 wt %, preferably < 12 wt % of $C_{18:1}$ - fatty acid.
- 10 (iv) less than 12 wt %, preferably < 7 wt % of $C_{16:1}$ - fatty acid.
- (v) while DHA and EPA are present in a weight-ratio of 0.5 - 3.0, preferably 0.7 - 2.0.
- 15 Preferred fish oil concentrates comprise triglycerides and diglycerides in a weight ratio of tri:di > 3, preferably 3-50, more preferable 10-35. These concentrates are rich in long chain polyunsaturated fatty acids, whereas its oxidative stability, off-taste and peroxide values are the
- 20 same or even improved compared with comparative compositions. The SAFA-content of these concentrates is lower than could be expected for above properties.
- Although above concentrates could be added perse to
- 25 foodproducts, it is often better or easier to use a blend with other triglycerides. Therefore our invention also concerns blends of triglycerides comprising:
- 0.3 - 95 wt% of the concentrate according to claim 1 or 2, and
- 30 99.7 - 5 wt% of a complementary fat, having a solid fat index at 10 ° C (N_{10}) that is either at least 5 % more, or at least 5 % less than the N_{10} of the concentrate according to claim 1 or
- 35 2.

- 3 -

The complementary fat often provides structuring properties to the fatblend. The amounts of complementary fat applied can vary from 98-20 wt %, preferably from 95-60 %. In order to provide structuring characteristics it was found, that
5 the solid fat content (NMR-pulse, not stabilized) of the complementary fat should be >15 at 20°C, preferably >20. Very suitable blends are obtained, if the complementary fat is selected from cocoa butter equivalents, cocoa butter, palm oil or fractions thereof, palmkernel oil or
10 fractions thereof, interesterified mixtures of above fats or fractions or hardened components thereof, or from liquid oil, such as sunflower oil, high oleic sunflower oil, fish oil, soybean oil, rapeseed oil, cottonseed oil, safflower oil, high oleic safflower oil, maize oil or MCT oils,
15 hardened liquid oils or fractions thereof or mixtures of one or more of the fats or oils mentioned.

The composition of the blend should preferably be selected in such a way, that the blend displays a solid fat content
20 (NMR-pulse; not stabilized) of 0 - 85, preferably 10 - 70, most preferably 20 - 60 at 5 ° C and < 30, preferably < 20, most preferably < 5 at 35 ° C.

In order to improve the oxygen stability of our
25 triglycerides or blends, containing them, we prefer to add an effective amount of an oxidation stabilizer, selected from the group consisting of: natural or synthetic tocopherols, BHT, BHA, TBHQ, propylgallate, ascorbylester of fatty acids, free radical scavengers, enzymes with anti-
30 oxidant properties.

Our invention further concerns food-products, comprising a fat phase, such as spreads, margarine, cream alternative, infant food, chocolate, confectionery, bakery products,
35 sauces, ice-creams, ice-cream coatings, cheese, soups, mayonnaise, dressings, enteral or parental products,

- 4 -

wherein the fat phase contains a concentrate or a blend according to claims 1 - 8.

A very efficient way to dose our new triglycerides, is to
5 make capsules from them. These capsules comprise a filling, encapsulated in an edible coating, wherein the filling consists of the concentrate according to claim 1 or 2 or the blends according to claims 3-8. In this way our triglycerides can also be eaten, without noticing the
10 disadvantageous off-taste of triglycerides, based on fish-oils.

Our new composition can be made by blending of the individual triglycerides. However, this is not a very
15 economical way. We found a new, more sophisticated process to prepare them, comprising the following steps:

- (i) a refined fish oil is subjected to an enzymic hydrolysis or alcoholysis, preferably, using Cand. Rugosa or Geotrichum Candidum
- 20 (ii) the product of (i) is subjected to a treatment for the removal of free fatty acids or its alkylesters
- (iii) the product of (ii) is subjected to an enzymic hydrolysis, in particular using a 1,3-selective
25 lipase or a lipase with a specificity for mono- and diglycerides, such as Amono G-lipase
- (iv) the product of (iii) is washed for the removal of glycerol and dried
- (v) whereupon the product of (iv) is re-esterified.

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In a preferred embodiment the hydrolysis process according to step (i) is performed to a hydrolysis rate of 50 - 80 %. The enzymic treatment according to step (iii) is preferably performed till such a level of free fatty acids, which is
35 at least enough to re-esterify all remaining partial glycerides in the reaction mixture.

- 5 -

Step (v) of above process can be performed as a directed or non-directed enzymic esterification, or as a directed or non-directed chemical esterification, using a base (in particular Na-methanolate) as a catalyst. Directed chemical
5 esterification is performed by removing the triglycerides formed during the conversion, which are insoluble in the reaction solution.

In the most preferred embodiment above process is performed
10 on a fish-oil, having following characteristics:

- (a) 10 - 35 wt % of w-3 long chain, polyunsaturated fatty acids, preferably being DHA, EPA and DPA.
- (b) 15 - 35 wt % of saturated fatty acids with 14 - 18 C-atoms.
- 15 (c) 10 - 15 wt % of $C_{18:1}$ - fatty acid.
- (d) 7 - 15 wt % of $C_{16:1}$ - fatty acid.
- (e) DHA : EPA - ratio of 0.5 - 1.5.

- 6 -

EXAMPLES

Example 1:

20kg of refined fish oil, with a fatty acid composition
5 shown in table 1.1, was mixed with 200ppm of TBHQ
antioxidant and 10kg of 50mM phosphate buffer pH 7. A
suspension of 8g of *Candida rugosa* lipase in a small
quantity of buffer was then added and the mixture stirred
vigorously under a nitrogen atmosphere at 25°C. After 26
10 hours the free fatty acid (FFA) level in the reaction
mixture reached 60%. The oil was heated to 90°C for 10
minutes and the aqueous phase was allowed to settle and
drained off. The oil was washed twice with 10kg
degassed/demineralised water and dried at 100°C under
15 vacuum. The FFA was separated from the partial glycerides
by molecular distillation and the fatty acid composition of
each fraction is shown in table 1.1. The corresponding
glyceride and FFA compositions are shown in table 1.2.
7.7kg of the partial glyceride fraction obtained as
20 described above was bleached by mixing at 105°C, under
vacuum, with 4% of bleaching earth and 0.08% citric acid
for 30 minutes followed by filtration. To this oil was
added 200ppm TBHQ antioxidant and an equal amount of
degassed/demineralised water followed by 3% immobilised
25 *Rhizomucor miehei* lipase based on the weight of oil.
Hydrolysis was carried out by stirring for 3.5 hours at
35°C. The lipase was inactivated by heating the reaction
mixture to 90°C for 15 minutes and the aqueous phase was
allowed to settle and drained off with inactivated lipase.
30 The partially hydrolysed oil was washed twice with
distilled water and after draining the aqueous phases was
dried under vacuum at 100°C for 0.5 hours. The composition
of the resulting oil is shown in table 1.2.
After cooling to 55°C, 5% immobilised *Rhizomucor miehei*
35 lipase based on the weight of oil was added and a vacuum of
ca. 50mbar was applied. After 24 hours the diglyceride
level had dropped to 2.9% and the reaction was stopped by

- 7 -

removing the lipase by filtration. The oil was refined and the fatty acid and glyceride composition of the product is given in table 1.1 and table 1.2 respectively.

- 8 -

Table 1.1 Fatty acid compositions (wt%).

	C14:0	C16:0	C16:1	C16:u	C18:0	C18:1	C18:2	C18:3	C18:4
Fish oil	6.9	18.3	7.9	5.9	3.8	13.6	1.5	0.8	2.1
Glyceride fraction	4.1	5.9	4.5	6.4	2.5	10.1	1.1	0.6	2.9
FFA fraction	8.9	26.1	10.1	6.0	4.6	16.8	2.0	1.0	1.6
Product	5.3	6.1	5.0	9.3	2.3	9.2	1.5	0.6	3.6

	C20:0	C20:1	C20:5	C20:u	C22:0	C22:1	C22:5	C22:6	C22:u
Fish oil	0.2	1.6	15.9	2.3	0	1.3	2.4	12.3	1.4
Glyceride fraction	0.3	1.8	20.2	3.4	0	2.0	4.5	25.7	2.5
FFA fraction	0.2	1.3	12.3	2.0	0	0.9	0.9	2.4	1.1
Product	0.2	1.4	21.9	2.6	0.2	1.7	3.9	21.9	1.6

Table 1.2 Glyceride and FFA content (wt%).

	Triglyceride	Diglyceride	Monoglyceride	FFA
Glyceride fraction	72.6	24.6	1.3	1.5
Partially hydrolysed glyceride fraction	43.6	25.1	4.8	26.5
Product	96.8	2.9	0.1	0.1

- 9 -

Example 2:

A refined fish oil concentrate was made from refined fish oil according to the process described in example 1. The fatty acid composition of the original fish oil and the concentrate is shown in table 2.1. A 10g sample of each was placed in glass bottles with free exposure to air. The bottles were stored at 50°C for 1 week, 40°C for 2 weeks, 20°C for 3 weeks, 5°C for 4 weeks. A group of 6 trained panellists evaluated the quality of the samples by smelling the oil at time 0 and after the storage period. Each panellist assigned a score to the sample on a scale of 0 to 6. A score of 0 corresponds to no detectable odour while a score of 6 is an extremely strong odour. The quality of the samples was determined at time 0 and at the end of the storage period. The mean value of the scores of all the panellists is shown in table 2.2. Table 2.3 shows the preferences of the individual panellists for each sample i.e. the number of people who exhibited a preference for one sample over the other, or who found the samples to have the same odour. The samples were also analyzed for peroxide value (PV) as a measure of oxidative deterioration, a higher PV indicating a greater degree of oxidation. The measured PVs at time 0 and after the storage period are shown in table 2.4.

Table 2.2: Mean smell scores for fish oil and concentrate at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	Mean smell score	
		Fish oil	Concentrate
	0	0.9	1
50	1	2.9	2.9
40	2	2.8	3.4
20	3	2.7	2.7
5	4	2.3	2.1

Table 2.3: Preferences of individual panellists for fish oil, concentrate or no preference at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	No. of panellists who exhibited a preference		
		for fish oil	none	for concentrate
	0	1	4	1
50	1	2	2	2
40	2	4	1	0
20	3	0	4	1
5	4	1	3	1

Table 2.4: Peroxide values (PV) for fish oil and concentrate at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	Peroxide Value (PV)	
		Fish oil	Concentrate
	0	1.8	0.8
50	1	13.3	8.2
40	2	12.2	7.6
20	3	8.5	2.4
5	4	3	1.2

- 13 -

Example 3:

A partial glyceride concentrate was prepared from refined fish oil by treatment with *C. rugosa* lipase according to the process described in example 1. This partial glyceride material was partially hydrolysed using SP392 lipase also as described in example 1. A portion of this material was then reesterified according to the process in example 1. The fatty acid composition and FFA content of the partially hydrolysed material is shown in table 3.1. A 10g sample of each was placed in glass bottles with free exposure to air. The bottles were stored at 50°C for 1 week, 40°C for 2 weeks, 20°C for 3 weeks, 5°C for 4 weeks. A group of 6 trained panellists evaluated the quality of the samples by smelling the oil at time 0 and after the storage period. Each panellist assigned a score to the sample on a scale of 0 to 6. A score of 0 corresponds to no detectable odour while a score of 6 is an extremely strong odour. The quality of the samples was determined at time 0 and at the end of the storage period. The mean value of the scores of all the panellists is shown in table 3.2. Table 3.3 shows the preferences of the individual panellists for each sample i.e. the number of people who exhibited a preference for one sample over the other, or who found the samples to have the same odour. The samples were also analyzed for peroxide value (PV) as a measure of oxidative deterioration, a higher PV indicating a greater degree of oxidation. The measured PVs at time 0 and after the storage period are shown in table 3.4.

Table 3.2: mean smell scores for partially hydrolysed concentrate and reesterified concentrate at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	Mean smell score	
		Hydrolysed	Reesterified
	0	4.4	4.5
50	1	4.7	4.9
40	2	4.7	4.8
20	3	5.8	4.9
5	4	5.0	4.6

Table 3.3: preferences of individual panellists for partially hydrolysed concentrate, reesterified concentrate or no preference at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	No. of panellists who exhibited a preference		
		for hydrolysed	none	for reesterified
	0	2	3	1
50	1	1	4	1
40	2	2	3	0
20	3	0	1	5
5	4	0	1	4

- 17 -

Table 3.4: Peroxide values (PV) for partially hydrolysed concentrate and reesterified concentrate at time 0 and after storage at different temperatures for different time periods

		Peroxide Value (PV)	
Temperature (°C)	Storage time (weeks)	Hydrolysed	Reesterified
	0	2.9	2.1
50	1	1.5	4.5
40	2	1.4	5.1
20	3	5.6	5.6
5	4	5.9	3.7

Example 4:

A refined fish oil concentrate was prepared from refined fish oil according to the process described in example 1. A high saturates concentrate was prepared by dissolving 2 parts of Dynasan (fully hardened soybean oil) in 9 parts of the fish oil concentrate at 80°C for 15 minutes. The concentrate without Dynasan was also heated at 80°C for 15 minutes. Upon cooling to room temperature, the concentrate was liquid and completely clear but the high saturates concentrate formed a opaque solid. The fatty acid composition of the concentrate and the high saturates concentrate is shown in table 4.1. A 10g sample of each was placed in glass bottles with free exposure to air. The bottles were stored at 50°C for 1 week, 40°C for 2 weeks, 20°C for 3 weeks, 5°C for 4 weeks. A group of 6 trained panellists evaluated the quality of the samples by smelling the oil at time 0 and after the storage period. Each panellist assigned a score to the sample on a scale of 0 to 6. A score of 0 corresponds to no detectable odour while a score of 6 is an extremely strong odour. The quality of the samples was determined at time 0 and at the end of the storage period. The mean value of the scores of all the panellists is shown in table 4.2. Table 4.3 shows the preferences of the individual panellists for each sample i.e. the number of people who exhibited a preference for one sample over the other, or who found the samples to have the same odour. The samples were also analyzed for peroxide value (PV) as a measure of oxidative deterioration, a

- 19 -

higher PV indicating a greater degree of oxidation. The measured PVs at time 0 and after the storage period are shown in table 4.4.

Table 4.2: Mean smell scores for concentrate and high saturates concentrate at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	Mean smell score	
		Concentrate	High saturates concentrate
	0	1.6	1.6
50	1	2.9	2.5
40	2	2.9	2.9
20	3	2.7	2.9
5	4	2.3	2.3

- 22 -

Table 4.3: Preferences of individual panellists for concentrate and high saturated concentrate at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	No. of panellists who exhibited a preference		
		for concentrate	none	for high saturated concentrate
	0	1	2	3
50	1	1	1	4
40	2	1	3	1
20	3	1	4	0
5	4	1	3	1

- 23 -

Table 4.4: Peroxide values (PV) for concentrate and high saturates concentrate at time 0 and after storage at different temperatures for different time periods

Temperature (°C)	Storage time (weeks)	Peroxide Value (PV)	
		Concentrate	High saturates concentrate
	0	1.1	1.2
50	1	8.6	3.9
40	2	9.9	4.3
20	3	3	3.2
5	4	1.5	1.9

Claims:

1. Fish-oil concentrate, comprising glycerides with:
 - (i) at least 40 wt % of w-3 long chain poly-unsaturated fatty acids, comprising at least DHA (c22:6 = dodecahexaenoic acid) and EPA (c20:5 = eicosapentaenoic acid).
 - (ii) less than 20 wt % of total saturated fatty acid with 14 - 18 C - atoms
 - (iii) less than 15 wt % of C_{18:1} - fatty acid
 - (iv) less than 12 wt % of C_{16:1} - fatty acid
 - (v) while DHA and EPA are present in a weight-ratio of 0.5 - 3.0.

2. Fish oil concentrate, according to claim 1, wherein the concentrate comprises triglycerides and diglycerides in a weight-ratio of >3.

3. Blends of triglycerides comprising:

0.3 - 95 wt %	of the concentrate according to claim 1 or 2, and
99.7 - 5 wt %	of a complementary fat, having a solid fat index at
	10 ° C (N ₁₀) that is either at least 5 % more, or at
	least 5 % less than the N ₁₀ of the concentrate
	according to claim 1 or 2.

4. Blends of triglycerides, according to claim 3, comprising 2 - 80 wt % of the concentrate according to claim 1 or 2, and 98 - 20 wt % of the complementary fat.

5. Blends according to claim 3 or 4, wherein the complementary fat has a solid fat content (NMR-pulse; not stabilized) of more than 15 to 20 ° C.
6. Blends according to any one of claims 3 to 5, wherein the complementary fat is selected from cocoa butter equivalents, cocoa butter, palm oil or fractions thereof, palmkernel oil or fractions thereof, interesterified mixtures of above fats or fractions or hardened components thereof, or from liquid oil or MCT oils (Medium Chain Triglycerides), hardened liquid oils or fractions thereof or mixtures of one or more of the fats or oils mentioned.
7. Blends according to any one of claims 3 to 6, wherein the blend displays a solid fat content (NMR-pulse; not stabilized) of 0 - 85 at 5 ° C and < 30 at 35 ° C.
8. Triglyceride compositions or blends containing them, according to any one of claims 1 to 7, wherein the compositions or the blends contain an effective amount of an oxidation stabilizer, selected from the group consisting of:
natural or synthetic tocopherols, propylgallate, TBHQ (tert Butyl Hydroquinon), free radical scavengers, enzymes with anti-oxidant properties, and ascorbylesters of fatty acids.
9. Food products, comprising a fat phase, wherein the fat phase contains a concentrate or a blend according to any one of claims 1 to 8.

10. Capsules comprise a filling, encapsulated in an edible coating, wherein the filling consists of the concentrate according to claim 1 or 2 or the blends according to any one of claims 3 to 8.

11. Process for the preparation of a fish oil concentrate with the composition of claims 1 or 2, wherein

- (i) a refined fish oil is subjected to an enzymatic hydrolysis or alcoholysis
- (ii) the product of (i) is subjected to a treatment for the removal of free fatty acids or its alkylesters
- (iii) the product of (ii) is subjected to an enzymic hydrolysis
- (iv) the produce of (iii) is washed for the removal of glycerol and dried
- (v) whereupon the product of (iv) is re-esterified.

12. Process according to claim 11, wherein the hydrolysis according to step (i) is performed to a hydrolysis rate of 50 - 80 %.

13. Process according to claim 11, wherein the enzymic hydrolysis according to step (iii) is performed till such level of free fatty acids, which is at least enough to re-esterify all remaining partial glycerides in the reaction-mixture.

14. Process according to claim 11, wherein step (v) is performed as a directed or non-directed enzymic esterification.

15. Process according to claim 11, wherein step (v) is performed as a directed or as a non-directed chemical esterification.

16. Process according to any one of claims 11 to 15, wherein the fish oil of step (i) of claim 11 comprises:

- (a) 10 - 35 wt % of w-3 long chain, polyunsaturated fatty acids
- (b) 15 - 35 wt % of saturated fatty acids with 14 - 18 C-atoms
- (c) 10 - 15 wt % of C_{18:1} - fatty acid
- (d) 7 - 15 wt % of C_{16:1} - fatty acid
- (e) DHA (c22:6 = dodecahexaenoic acid) : EPA (c20:5 = eicosapentaenoic acid) - ratio of 0.5 - 1.5.

17. Fish-oil concentrate as claimed in claim 1, comprising 40 - 55 wt % of w-3 long chain polyunsaturated fatty acids.

18. Fish-oil concentrate as claimed in claim 1, comprising 2 - 18 wt % of total saturated fatty acid with 14 - 18 C atoms.

19. Fish-oil concentrate as claimed in claim 1, comprising < 12 wt % of C_{18:1} - fatty acid.

20. Fish-oil concentrate as claimed in claim 1 wherein DHA (c22:6 = dodecahexaenoic acid) and EPA (c20:5 = eicosapentaenoic acid) are present in a weight-ratio of 0.7 - 2.0 %.

21. A food product as claimed in claim 9, selected from the group consisting of spreads, margarine, cream alternative, infant food, chocolate, confectionery, bakery products, sauces, ice-creams, ice-cream coatings, cheese, soups, mayonnaise, dressings, enteral or parental products.

22. The process as claimed in claim 11, wherein the enzymic hydrolysis or alcoholysis of step (i) is performed using *Cand. Rugosa* or *Geotrichum Candidum*.

23. The process as claimed in claim 11, wherein the enzymic hydrolysis of step (iii) is performed using a 1.3-selective lipase or a lipase with a specificity for mono- and diglycerides.

24. The process according to claim 16 wherein the w-3 long chain, polyunsaturated fatty acids comprise DHA (c22:6 = dodecahexaenoic acid), EPA (c20:5 = eicosapentaenoic acid) and DPA (c22:5 = dodecapentaenoic acid).