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(54) Title: ENZYMATIC MODIFICATION OF TRIGLYCERIDE FATS

(57) Abstract: Process wherein the fatty acid residues on a glyceride moiety are randomised over the terminal and middle positions, wherein the process proceeds to a conversion degree on the terminal positions, Re, ranging from 0.3 to 0.95, and wherein a conversion degree on the middle position, Ra, ranges from 0.06 to 0.75, and wherein Ra is greater than 0.32Re - 0.08, the process comprises the exposure of a triglyceride fat to a catalyst comprising a lipase wherein the lipase is a *Thermomyces lanuginosa* lipase which has an activity of at least 250 IUN corresponding to 22 g/(g\*h) at the onset of the process.



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## Enzymatic modification of triglyceride fats

### Field of the invention

The present invention relates to an interesterification process  
5 of triglyceride fats. More particularly the process concerns an  
enzymatic interesterification process which is further denoted  
as an enzymatic rearrangement process.

### Background

10 Chemical interesterification of a triglyceride fat aims at an  
exchange of the fatty acid residues of the glyceride moiety of  
the fat. After interesterification, on the resulting  
triglycerides the fatty acid residues have been exchanged by  
other residues. The fatty acid residues may originate from the  
15 same or from a different triglyceride molecules or they may  
come from free fatty acids which were present in the reaction  
mixture.

The exchange of fatty acid residues eventually results in a  
20 statistically random distribution of the fatty acid residues  
over the terminal and middle positions of the glyceride  
molecule. The obtained fat is said to have become fully  
randomised.

25 The chemical interesterification process needs a catalyst,  
which usually is an alkali metal hydroxide or an alkali metal  
alkanolate, such as sodium methanolate.

However, consumers increasingly prefer food and food  
30 ingredients which have not been exposed to chemicals during  
their preparation. Therefore a general need has arisen for non-  
chemical modification processes of triglyceride fats.  
Intesterification may also occur via enzymatic rearrangement.

Such enzymatic process does not affect the naturalness of the fat.

Contrary to chemical interesterification which proceeds  
5 instantaneously, enzymatic rearrangement proceeds gradually,  
and therefore takes more time.

For enzymatic rearrangement (ER) a lipase enzyme is used as  
catalyst. Lipases used for ER comprise the microbial *Mucor*  
10 *miehei* lipase, *Thermomyces lanuginosa* lipase and *Rhizopus*  
*oryzae* (formerly *Rhizopus delemar*).

Generally, the lipases used in an ER process are sn-1 and sn-3  
specific meaning that only the terminal fatty acid residues are  
15 effected.

In the course of the enzymatic reaction, some randomisation at  
the middle position may occur. However when this happens it is  
due to acylmigration (see Torres et al., JAOCS vol 79, no.8  
20 (2002) p775-781, Torres et al JOACS, vol 79 no 7 (2002) p655-  
661, and Zhang et al JAOCS vol 78, no. 1 (2001) p 57-64) which  
is a chemical side reaction which take place at long reaction  
times. The acylmigration is due to the presence of  
diacylglycerides which arise abundantly at long reactions times  
25 and in the presence of water.

This difference in randomisation results in triglyceride  
products with a triglyceride composition and with properties  
that are quite different from the fully randomised triglyceride  
30 fat resulting from chemical interesterification. Unfortunately,  
the extensive knowledge and experience acquired by using fully  
randomised chemically interesterified fats for manufacturing

food products can not be used for enzymatically interesterified fats (Zhang et al JAOCS, Vol.78, no 1 (2001) pp. 57-64).

Furthermore the middle position in a natural feedstock fat is usually is an unsaturated fatty acid, often oleic acid. Triglycerides of the type palmitic-oleic-palmitic may cause graininess in the fat blend. Because the middle position of the triglycerides in an enzymatic rearrangement reaction is hardly affected, triglycerides with a saturated middle position are barely present in enzymatically rearranged fats, unless already present in the starting material. This typical distribution of natural fatty acids over the triacylglycerides has some consequences. In the first place it is nutritionally beneficial to have an unsaturated fatty acid at the middle position, since the lipase activity in our digestive system delivers a 2-monoacylglyceride and 2 free fatty acids which are derived from the terminal triacylglycerides positions. This digestive effect is confirmed by Nielsen (Oils and fats international (vol 18, no 4 (2002)). He established that immobilised Lipozyme TL IM action is restricted to the 1 and 3 position on the triglyceride, leaving the middle position unaltered. However, this configuration of fatty acids over the glycerol backbone of the triacylglycerides also has a downside. With respect to food structuring functionality these triacylglycerides, with a unsaturated middle position, are less functional. This is due to their lower melting point compared to fully saturated triacylglycerides and their complicated crystallisation behaviour.

As explained before, rearrangement on the middle position may occur during enzymatic rearrangement, however in order to occur at a appreciable amount the enzymatic rearrangement has to proceed at equilibrium (100% conversion of sn1 and sn-3

position) or beyond. Chemical rearrangement proceeds instantly, meaning that instantly a complete randomisation is obtained. It is the nature of the enzymatic reaction that in the beginning of the reaction the conversion of sn-1 and sn3 position runs  
5 quickly, but that towards equilibrium the conversion rate proceeds more and more slowly (see figure 1). Consequently, to attain 100% conversion long enzyme contact times are needed.

The enzymatic re-arrangement process, even though strictly sn-1  
10 and sn-3 specific, is always accompanied by a some change of the fatty acid distribution on the sn-2 position. This is due to the unavoidable chemical process of acyl-migration that occurs in partial fatty acid glycerides. Xu et al (Enzymatic Production of Structured lipids: Process reactions and Acyl  
15 migration, inform 11 (2000) p1121-1131) reported that acylmigration can be attributed primarily to longer residence times. However, the related low flow through the packed bed reactor makes the process expensive for use on industrial scale. (Xu et al JAOCS, Vol.79, no 6 (2002) pp. 561-565).  
20 Indeed, Torres et al. recommend short reaction times to reduce randomisation of the fatty acids residues (JOACS, vol 79, no 8 (2002) p 775-781).

Without wishing to be bound by theory the process of  
25 acylmigration is independent on the enzyme used, however it is due to the relatively slow rate of the process. Significant effect on the middle position only occur at very high conversion rates, often 100%, which relates to very long contact times. This is illustrated by Fig. 3.

30

The processes reported in the prior art typically refer to time and enzyme concentration combinations that relate to 100% conversion of the sn-1 and sn-3 position (equilibrium) and often

in excess of the time needed to obtain 100% conversion on the terminal positions. As a logical consequence these reactions yield also a certain amount of randomisation of the middle position. However, these processes are economically not  
5 attractive, because of the long contact times needed to obtain a reasonable amount of sn-2 randomisation.

For example Berben et al in society of chemical industry (online 16 february 2001) describe a process of enzymatic  
10 rearrangement wherein they have the reaction proceed until equilibrium and obtain a randomisation on the middle position of 18%.

WO96/14756 describes ER of fat blends using sn-1 and sn-3-  
15 specific SP392 as lipase catalyst. The process is characterised in that the rearrangement does not proceed beyond a conversion degree of the the sn-1 and sn-3 position of 90% (but being at least 20%), which results in shorter reaction times. However, no randomisation at the sn-2 position is observed.

20

Some rare lipases including *Candida cylindracea* and *Arthrobacter* lipases are non-specific. An ER process using those lipases delivers a fat rearranged at all glyceride positions. However, those lipases either have been found to be  
25 not suited for use at an industrial scale and/or have not been approved for food manufacture.

The process described in EP 652289 uses a common sn-1 and sn-3-  
specific lipase. The rearrangement requires the presence of a  
30 substantial amount of at least 4 wt.% of diacylglycerides (also denoted as diglycerides) in the reaction mixture. The fat becomes rearranged at all three positions, but at the end it

contains much diglycerides and other byproducts, all of which need to be removed by a subsequent purification process.

A cost effective ER process is needed which results in substantial rearrangement also at the middle position. Such a  
5 process would make a new range of naturally modified triglyceride fats economically available.

It is therefore an object of the present invention to provide an enzymatic rearrangement process wherein an appreciable  
10 amount of rearrangement on the middle position occurs. A further object of the invention is a process with a short reaction time. Another object of the invention is to provide an enzymatic rearrangement process without the deliberate use of diacylglycerides.

15

Surprisingly one or more of the above mentioned objects is obtained by using a catalyst with an activity exceeding 250 IUN (22 g/(g\*h)) as measured at the onset of the process.

#### 20 **Description of figures:**

Fig. 1: plot of conversion degree  $Re$  vs time.

Fig. 2: plot of randomisation on 2-position ( $Ra$ ) versus contact time.

Fig. 3: Plot of randomisation on 2-position ( $Ra$ ) versus time  
25  $Re > 1$  indicates that the reaction time has exceeded the time needed to reach equilibrium with respect to the randomisation of the sn-1 and sn-3 positions.

#### **Detailed description of the invention**

30 The present invention relates to a rearrangement process wherein the fatty acid residues on a glyceride moiety are randomised over the terminal and middle positions,

wherein the process proceeds to a conversion degree on the terminal positions,  $R_e$ , ranging from 0.3-0.95, and wherein a conversion degree on the middle position,  $R_a$ , ranges from 0.06-0.75, and wherein  $R_a$  is greater than  $0.32R_e - 0.08$ ,

5

the process comprises the exposure of a triglyceride fat to a catalyst comprising a lipase characterised in that the lipase is a *Thermomyces lanuginosa* lipase which has an activity of at least 250 IUN corresponding to 22 g/(g\*h) at the onset of the  
10 process.

The IUN is a measure of the activity of the enzyme and is determined according to the procedure as described below in the experimental section.

15

The activity of the enzyme can also be measured with an other, more convenient method. The method measure the amount of oil converted per amount of catalyst in one hour (g/(g\*h)). An activity of 250 IUN corresponds to 22 g/(g\*h). The method is  
20 described in the experimental section.

The invented process has the benefit over prior art enzymatic rearrangement processes that it provides an appreciable amount of rearrangement at the middle position of the triglycerides,  
25 it does not need an excess of diacylglycerides and therefore does not need a cumbersome purification step at the end. In addition, the process of the invention has short contact times.

Conversion degree  $R_e$  is the actual conversion over the sn-1 and  
30 sn-3 position. It is the conversion degree at a certain time divided by the equilibrium state (100% conversion of sn-1 and sn-3 position). Conversion degree  $R_a$  is the actual conversion on the sn-2-position only. It is the conversion of the sn-2-

position at a certain time divided by the equilibrium state at the sn-2-position which is identical to the sn-2 fatty acid distribution of chemically interesterified mixture.

Determination of the conversion degree  $R_e$  and  $R_a$  of a

5 rearranged fat sample is based either on the change of its carbon number profile or on the change in molar fractions of specific types of triglycerides. The measurements are explained in the experimental section.

10 The process of the present invention is such that even before attaining equilibrium with respect to sn-1 and sn-3 randomisation ( $R_e=1$ ), a substantial randomisation of the middle position ( $R_a$ ) is achieved, ranging from 0.06-0.75. The process of the present invention does therefore not proceed further  
15 than a conversion degree  $R_e$  of 0.95. This allows short residence time such as described in WO96/14756.

The processes of the prior art proceed until equilibrium or beyond see e.g. Berben et al in society of chemical industry (online 16 february 2001), Torres et al., JAOCS vol 79, no.8  
20 (2002) p775-781, Torres et al JOACS, vol 79 no 7 (2002) p655-661, and Zhang et al JAOCS vol 78, no. 1 (2001) p 57-64.

With the process of the present invention long contact time and long reaction times which belong to the processes of the prior  
25 art, are avoided.

In addition, even at short contact times, i.e. low  $R_e$  conversion degree, already a minimum randomisation at the sn-2-position occurs. For  $R_e$  between 0.3 and 0.95, the rearrangement on the middle position ( $R_a$ ) is greater than  
30  $0.32R_e - 0.08$ . Preferably  $R_a$  is at least  $0.32R_e - 0.06$  and even more preferably  $R_a$  is at least  $0.32R_e - 0.04$ .

Preferably the conversion degree  $R_e$  is less than 0.9, more preferably less than 0.85.

The conversion degree  $R_e$  is preferably at least 0.35, and even more preferably at least 0.4.

5

The supplier of Lipozyme® TL IM recommends a flow of 1500 kg oil per hour per 400 kg catalyst in a packed bed reactor. This results in residence time of 32 minutes. A general description of this process can also be found in Nielsen, Oils and Fat  
10 international, vol 18, no 4 (2002).

In contrast the present invention where the enzyme has an activity of at least 250 IUN corresponding to 22 g/(g\*h), allows processing of 4400 kg oil per hour employing the same  
15 amount of enzyme resulting in a residence time of only 11 minutes. However, still a substantial amount of randomisation at the middle position occurs.

Suitably during the first hour of conducting oil through a  
20 packed bed reactor the residence time of the oil in the Lipozyme® TL IM catalyst bed of the present invention preferably is less than 25 min, more preferably less than 20 min and still more preferably less than 15 min. These residence times are the residence times in the beginning of the  
25 rearrangement when fresh catalyst is present. In the course of the process the catalyst gradually loses activity and longer residence times are needed for the maintenance of the degree of conversion  $R_e$ . Longer residence times may be accomplished by reducing the flow of the oil through the catalyst bed reactor.  
30 For the present invention, even when the activity of the catalyst in the course of the reaction is reduced, and the flow is adjusted, still the randomisation of the sn-2-position is at an appreciable rate (see fig 2).

The present invention may also be used in a batch process. However instead of short residence time or high oil flow, low concentrations of a catalyst may be used. In comparison with prior art processes where the concentration of the catalyst is 5 10 wt.%, the catalyst concentration of the present invention in a batch process may range from 0.05 - 9 wt.%, more preferably from 0.05 - 5 wt.% and still more preferably 0.05 - 3 wt.% calculated on the reaction mixture.

10 The present process preferably uses the *Thermomyces lanuginosa* lipase containing ER catalyst Lipozyme® TL IM which is commercially available from NOVOZYMES, Denmark, as an aggregate of enzyme and silica. The IUN catalyst activity is defined by its method for measuring by NOVOZYMES, as described below in 15 the experimental section. Alternatively the activity can be measured by another more convenient activity measurement which is also described in the experimental section.

In the art of enzymatic rearrangement the presence of silica is 20 reported to catalyse randomisation of the middle position. Without wishing to be bound to theory, it is thought that silica retains water and that water hydrolyses the triglycerides to diacylglycerides which catalyse rearrangement at the sn-2-position. So, in the processes of the prior art, 25 more silica leads to more diacylglycerides, which leads to more sn-2-randomisation. However, when using the highly active lipase according to the present invention, it was surprisingly found that less silica leads to a increase of randomisation at the middle position.

An economy related benefit of the present process is that it does not need the cumbersome final purification from diglycerides as is necessary for the process of EP 652289.

5 From the prior art the Lipozyme® TL IM catalyst is dispensed with water and then added to the reaction mixture. The present process however, is preferably carried out with relatively low content of water. Preferably the amount of water is in the range 0.001 - 0.1 wt.%, more preferably in the range of 0.001 -  
10 0.05 wt.%.

Water content measurement is determined by means of standard Karl Fischer titration, but not earlier than 30 minutes after contacting the catalyst with the feedstock in a batch reactor.  
15 When processing in a packed bed reactor, water measurement is done in an oil sample taken downstream of the reactor but not earlier than 30 minutes after the oil has started to flow through the catalyst bed in order to allow for reliable measurements.

20

Suitably the temperature of the reaction mixture is from 40 to 85°C, preferably from 45 to 80°C, more preferably from 50 to 75°C.

25 The process of the invention may be applied to a variety of triglyceride fat blends, but is most appropriate for triglyceride fat blends in which there is a difference in the distribution of fatty acids over the sn-1, sn-3 and sn-2 position of the glyceride molecule.

30

The process of the present invention is particularly suitable for a feedstock comprising

- any mixture comprising of a liquid oil and a hydrogenated oil, preferably the hydrogenated oil is fully hydrogenated as that leads to no trans fatty acids, or

- any triglyceride fat which has not been subjected to  
5 hydrogenation, or,

- a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.

The mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction is preferred.

10

The invention also comprises the use of an aggregate of *Thermomyces lanuginosa* lipase and silica as catalyst for partially rearranging fatty acid residues of a triglyceride fat to a conversion degree of the terminal positions  $R_e$  of 0.3 to  
15 0.95, comprising a rearrangement on the middle position to a conversion degree  $R_a$  of 0.06 to 0.75, wherein the lipase/silica aggregate has an activity of at least 250 IUN (22 g/(g\*h)), preferably at least 300 IUN (25.5 g/(g\*h)), more preferably at least 350 IUN (29 g/(g\*h)).

20

The invention also comprises a triglyceride fat obtainable by enzymatic rearrangement of which the conversion degree of the terminal positions  $R_e$  is from 0.3 to 0.95 and of which the conversion degree of the middle position of the triglyceride  $R_a$   
25 is from 0.06 to 0.75, while  $R_a$  is greater than  $0.32R_e - 0.08$ , preferably greater than  $0.32R_e - 0.06$ , more preferably greater than  $0.32R_e - 0.04$ .

Because the enzymatic rearrangement process of the present  
30 invention is different from the often used chemical interesterification and the enzymatic conversion process proceeding to 100% conversion at the sn-1 and sn-3 position, the fats resulting from the enzymatic rearrangement process of

the present invention have different properties than those obtained by the conventional chemical interesterification and the enzymatic conversion process proceeding to 100% conversion on the sn-1 and sn-3 position (like Berben et al in society of  
5 chemical industry (online 16 february 2001), Nielsen, Oils and Fat international, vol 18, no 4 (2002), Torres et al., JAOCS vol 79, no.8 (2002) p775-781, Torres et al JOACS, vol 79 no 7 (2002) p655-661, and Zhang et al JAOCS vol 78, no. 1 (2001) p 57-64).

10

Preferably the triglyceride fat is obtained by enzymatic rearrangement of which the conversion degree of the terminal positions  $R_e$  is less than 0.9, preferably less than 0.85.

Preferably the conversion degree  $R_e$  is at least 0.35,

15 preferably at least 0.4.

Fats according to the invention are suitable for the preparation of food compositions, particularly for the preparation of a constituting fat phase which comprises a  
20 liquid oil and a structuring fat. Such fat phases are widely used for the preparation of fat continuous emulsions used in the manufacture of e.g. spreads.

Because an enzymatic rearrangement process qualifies as  
25 natural, those fats too may be qualified as natural.

The process according to the invention allows the production of fats enriched with triglycerides which have a saturated fatty acid residue on the middle position. Such fats are used in food  
30 applications where the crystallisation behaviour of the lipid phase is critical for end product quality. Said triacylglycerides strongly influence both stability of the end products against adverse temperature conditions and

crystallisation related process parameters based on the intrinsic crystallisation properties of the fat.

So the process according to the invention allows the production  
5 of fats like the ones in EP 831711 with low graininess despite having a high palmitic acid content. Although the feedstock is a blend with palm oil or a palm oil fraction, the rearranged fat phase does not cause graininess in fat continuous emulsion spreads prepared with such fat phase, or at least the  
10 graininess is substantially reduced.

The invention also comprises the food products in which a fat is incorporated which is obtained by the process of the present invention.

15

The present invention has the benefit over the prior art enzymatic rearrangement processes that it has much shorter reaction time, thus allowing an economically feasible process. Furthermore a substantial amount of randomisation on the sn-2-  
20 position is possible with the process of the invention, while still keeping the naturalness of the fat. In addition, because there is already a substantial amount of randomisation of the middle position at short reaction time and low conversion degree on the sn-1 and sn-3 position, the rearrangement process  
25 can be stopped at various times, resulting in fats with different properties. One may choose the conversion degree,  $R_e$  and  $R_a$ , by stopping the reaction at a certain time and thereby fine-tuning the property of the obtained fat.

**Experimental section**Determination of residence time.

Residence time in hours is determined by taking the volume of  
5 the catalyst bed inclusive oil, subtracting the volume of the  
catalyst and dividing the difference by the volume of oil  
passing the bed in one hour.

10 Establishing the IUN activity of lipase catalyst**1. PRINCIPLE**

The method is provided by the catalyst supplier and is based on  
15 interesterification of triglycerides by an immobilised lipase.  
The conversion of tristearin in a substrate composed of 27 w/w  
% fully hydrogenated soy bean oil and 73 w/w % refined,  
bleached and deodorized soy bean oil at 70 °C and 200 rpm  
stirring is used to quantify the activity of the catalyst. The  
20 concentration of tristearin is determined using an HPLC based  
analytical method.

**2. SPECIFICITY AND SENSITIVITY**

Components having the same retention time as tristearin in the  
25 chromatographic method used will cause an overestimation of the  
tristearin concentration.

**3. DEFINITION OF UNITS**

The interesterification activity is defined as the initial  
30 conversion rate for tristearin at standard conditions. 1 IUN  
corresponds to a conversion rate of 0.01 g tristearin/l  
/minute/ gram catalyst.

#### 4. APPARATUS

Analytical balance

Shaker water bath

Pipettes                    Positive displacement pipette for taking oil  
5                                samples (due to high viscosity of the liquid).  
                                  Standard pipettes (e.g. Finnpiquette) for the  
                                  other steps in the analysis.

HPLC system including the following modules: HPLC pump,  
10 injection system and column heater.

Light scattering detector, e.g. Sedex 55.

HPLC Column RP 18e (5 $\mu$ m), LiChroCart 250-4.

Data acquisition        Data can be acquired and processed using  
chromatography software.

15

#### ANALYTICAL CONDITIONS

Column temp.:                    40°C  
20

Flow:                                1.5 mL/min.

Mobile phase:                    The mobile phase consists of 50 v/v %  
25                                        acetonitrile, HPLC grade and 50 v/v %  
    dichloromethane, HPLC grade.

Injection volume:                20  $\mu$ L.

Run time:                            At least 11 minutes.  
30

Column temperature:            40°C ( $\pm$ 2°C)

Detector:                            40°C ( $\pm$ 2°C), nitrogen pressure 2.3 bar.

35 Detector sensitivity: Gain 6

**5. REAGENTS AND SUBSTRATES**

## CHEMICALS

Tristearin. SIGMA grade, Approx. 99%

## 5 SOLUTIONS

Substrate (20 grams):

27 w/w % fully hydrogenated soy bean oil (delivered by ADM, Illinois, USA)

73 w/w % refined, bleached and deodorised soy bean oil

10 (delivered by ADM, Illinois, USA)

Catalyst (2 gram): The samples are conditioned at  $a_w = 0.3$  for at least 24 hours.

15 **6. SAMPLES AND STANDARDS**

## STANDARDS

A standard curve of tristearin is made in the concentration range from 0.25 to 2.0 mg/ml.

## 20 LEVEL CONTROL

Reference sample: Lipozyme® TL IM reference PPW6503-3, particle size fraction 425-500µm, is used (single determination).

25 **7. PROCEDURE**

As substrate a blend of 27 w/w % fully hydrogenated soy bean oil (FH SBO) and 73 w/w % refined, bleached and deodorised soy bean oil (RBD SBO) is made. The oil mixture is heated to 80 °C in a water bath, and is well mixed.

30

The oil mixture is weighed out in 100 ml conical flask with a screw top wail, about 20 grams in each. The precise weight is

notated with 2 decimals. The flasks (batches) are placed in the shaker water bath at 70°C and 200 rpm.

A catalyst amount corresponding to approx. 10% of the amount of oil is weighted out (approx. 2 grams). The precise weight of the catalyst is determined with 2 decimals.

When the oil mixture is homogeneous, a time zero-sample is taken. 100µl of the oil is taken with a positive displacement pipette (Gilson microman). The pipette tip is wiped off by a Kleenex to remove outside oil mixture and the sample is deposited in a HPLC vial (type BROWN 12x32mm with Silicone/PTFE Septa).

The weighed amount of catalyst is added to the flasks containing the oil mixture and samples are taken to the times: 15, 30, 45 and 60 minutes. All samples are 100 µl. The samples are stored in a freezer until HPLC analysis.

The samples in the HPLC vials are diluted with 900 µl dichloromethane. This solution is mixed on a whirl-mixer, before a further dilution of 100 µl to 900 µl dichloromethane is made (total dilution of 100x). After a further mixing this sample is being analysed by HPLC.

25

The diluted samples are analysed by HPLC-ELSD.

The response versus concentration of the tristearin standards are fitted to an exponential model.

30

**8. CALCULATION**

The concentration of tristearin in the samples is calculated by use of the standard curve.

5 The conversion of (the decrease in) tristearin concentration versus time is fitted to a exponential model, by non linear parameter estimation. The model is:

$$C_{Tristearin}(t) = C_{0,est} \cdot \exp(-k_{est} \cdot t)$$

10

Where

$C_{tristearin}(t)$  = is the concentration of tristearin in the reaction  
15 mixture at time, t.

$C_{0,est}$  = start concentration of tristearin (estimated  
parameter)

$k_{est}$  = rate constant (estimated parameter)

t = reaction time

20

From the estimated rate constant,  $k_{est}$ , the activity at standard conditions, the IUN activity, can be calculated according to the following formula:

$$IUN/g = k_{est} \cdot C_{0,std} \cdot 100 \cdot \frac{W_{std}}{W} \cdot \frac{M_{oil}}{M_{oil,std}}$$

25

$C_{0,std}$  = start concentration of tristearin at standard condition.

$W_{std}$  = weight of catalyst under standard conditions (2g)

W = actual weight of catalyst

$M_{oil}$  = actual weight of oil

30  $M_{oil,std}$  = weight of oil under standard conditions (20g)

Determination of activity in g/(g\*h)**Calculating activity**5 *Substrates:*

50g refined bleached and deodorised quality dry fractionated palm stearin fraction mp 53

50g refined bleached and deodorised quality palm kernel oil an amount of immobilised enzyme catalyst such that a conversion degree Re between 0.2 and 0.4 is obtained at approximately one hour. Some experiments with different amounts of enzyme is usually sufficient to find the right amount of enzyme. For an enzyme with an activity of 455 IUN/38 g/(g\*h) a conversion degree Re between 0.2 and 0.4 is obtained at approximately 15 hour with an amount of 1 wt%.

*Procedure:*

The two oils are mixed together well in a closed 100ml reaction vessel and brought to 70°C under agitation. The enzyme catalyst is added to the oil substrate and the mixture agitated at 70°C. A sample of oil is taken after 1 hour, ensuring that no enzyme catalyst is present, and then every hour until the reaction is at equilibrium.

25 A plot is made of conversion degree Re against time as shown in figure 1.

The activity is then determined according to the following equation:

30

$$\text{Activity} = -\ln(1-x) * \frac{\text{amount of oil (g)}}{(\text{amount cat (g)} * t \text{ (h)})}$$

where,

x is the conversion degree, Re (determined as described) and is a point after approximately 1 hour when Re is between 0.2 and Re=0.4. However Re should never be above 0.4.

5 t is the time

the amount of oil and catalyst is in grams, and are known parameters.

Care should be taken that the point x is taken on the linear part of the curve and not too close to the starting point.

10

#### Determination of Re and Ra

Determination of the conversion degree Re of a rearranged fat sample is based either on the change of its carbon number  
15 profile or on the change in molar fractions of specific types of triglycerides. The determination proceeds as follows:

For a to be rearranged fat sample the overall fatty acids composition, the composition of fatty acids on the middle  
20 position and the triglyceride composition is analysed. Common analysis methods are used comprising FAME-analysis, the GLC/carbon number method and the HPLC/silver phase method as are described in for example EP 78568, EP 652289, JAOCS, (1991), 68(5), 289-293 and Hammond E.W.J., Chromatography, 203,  
25 397, 1981.

The molar fraction ( $p_{A_{sn1,3}}$ ) of any specific fatty acid residue (A) on a terminal position (either the sn-1 or the sn-3 position, which positions are assumed to be identical) is  
30 calculated according to the following formula:

$$p_{A_{sn1,3}} = (3 * p_{A_{total}} - 1 * p_{A_{sn2}}) / 2$$

where  $pA_{total}$  denotes the total occurrence of a particular fatty acid residue A in the fat blend and  $pA_{sn2}$  the occurrence at the 2-position of fatty acid residue A situated. For all occurring fatty acid residues having a molar fraction larger than 0.002  
5 the value  $pA_{sn1,3}$  is established. The distribution of these molar fractions should be normalized to 1.0.

The triacylglyceride profile of a fully randomised triglyceride fat is calculated by simple statistics known to the man skilled  
10 in the art. The mole fraction  $p(ABB)$  of the triacylglyceride ABB, for example, is calculated using the formula:  $p(ABB) = 2$   
\*  $pA_{sn1,3}$  \*  $pB_{sn2}$  \*  $pB_{sn1,3}$

Generally, the carbon number of a specific triglyceride  
15 molecule is the total number of carbon atoms on its three fatty acid residues. The carbon number profile of a particular fat blend consists of the occurrence percentages of all carbon numbers of that fat blend.

20 The carbon number profile of a fat blend is derived from its triacylglyceride composition (the mole fraction collection of all triacylglycerides).

From the triacylglycerol profile of the fat blend the carbon  
25 number profile can be derived easily.

By analysis the carbon number profile is established for the starting fat blend and for a specific partially rearranged sample taken from a reaction mixture. The carbon number profile  
30 of the theoretically sn-1 and sn-3 fully randomised fat blend is found by a statistical calculation of the triglycerides profile as described above.

The degree of conversion of the partially rearranged fat sample is calculated as follows:

For each carbon number in the range 30 - 60 the differences are  
5 calculated between the mole fraction at the beginning of the reaction and the mole fraction at full sn-1 and sn-3 randomisation (equilibrium). The sum of the (absolute) values of these differences (100% absolute change of carbon numbers) defines 100% rearrangement of the fat blend.

10

In the same way for a specific sample taken from a reaction mixture for each carbon number between 30 to 60 the differences are calculated of the mole fraction at the begin of the reaction and the actual mole fraction when sampling the  
15 reaction product. Again the absolute values of these differences are accumulated. The sum is the actual absolute change of carbon numbers until the moment of sampling.

For the specific sample the conversion degree  $Re = (\text{actual absolute change of carbon numbers}) / (\text{100\% absolute change of carbon numbers})$ .

This equation applies for triglyceride products where the abovementioned 100% absolute change of carbon numbers is at  
25 least 0.15. If not, the degree of conversion  $Re$  should be determined in an alternative way:

For each triacylglyceride of the type H3, H2O and H2L (where H indicates fatty acid residues of palmitic or stearic acid, O of  
30 oleic acid and L of linoleic acid) the mole fraction is established by analysis. For each of these triglycerides the absolute change between the molar fraction at the beginning of the reaction and at full sn-1 and sn-3 randomisation is

calculated. The sum of the absolute values of these changes belongs to the state of 100% conversion.

For a specific sample taken during the proceeding reaction the difference between the mole fraction at the begin of the reaction and at the actual state of the reaction product is calculated for the same selected triglycerides. The absolute values of the differences in mole fraction are accumulated. The sum defines 100% rearrangement of the fat blend.

10

The degree of conversion  $R_e$  of the specific sample follows from the equation:

$$R_e = (\text{actual change of triacylglycerides}) / (100\% \text{ absolute change of triacylglycerides}).$$

Determination of  $R_a$ , the degree of rearrangement at the middle position, proceeds as follows:

For the initial feedstock and for a sample taken from the reaction mixture first the total occurrence of fatty acid residues and the occurrence of fatty acid residues situated at the 2-position of the triacylglyceride is established using GLC-FAME and 2-position analysis. For methods see the references described above.

For each fatty acid occurring in the triacylglyceride fat at a molar level of at least 0.002 the absolute difference of its Sn-2 molar fraction at the beginning of the reaction and its molar fraction in the reaction mixture is calculated, wherein the latter mixture is equal to chemically randomised fat. The sum of the absolute values of these changes ( $F_a - S_{n2} - 100\%$ ) for

all selected fatty acids defines the status of 100% degree of Sn-2 randomness.

For a sample taken during the proceeding reaction in the same way the absolute change between the Sn-2 molar fraction at the begin of the reaction and at the actual state of the reaction product is calculated for each of the selected fatty acids. The sum of the absolute values of these changes (Fa-Sn2-actual) is the actual absolute change of fatty acids on the Sn-2 position.

10

For the specific sample the Ra value follows from the equation:

$$Ra = (Fa-Sn2-actual) / (Fa-Sn2-100\%)$$

#### Example 1

15

60 g of palm oil and 40 g of palm kernel oil were mixed together in a 100 ml reaction vessel and heated to 70°C. 1 wt.% of Lipozyme® TL IM (activity 455 IUN, 38 g/(g\*h)) was added to the mixture and this was stirred at 70°C. Samples were taken at intervals and the degree of conversion Re and the degree of conversion Ra were determined according to the methods described elsewhere in this specification by means of establishing changes in carbon number distribution and changes in composition of fatty acids on the middle position.

25

At the conversion degrees Re of 0.85 and 0.5, the following degrees of rearrangement of the middle position have been found:

TABLE I

Degree of conversion Re	Degree of conversion Ra
0.85	0.28
0.5	0.1

30

**Example 2**

Example 1 has been repeated but using a low activity Lipozyme® TL IM lipase catalyst and the 10% catalyst concentration as used in the prior art.

**Example 3**

Example 1 has been repeated using the same high activity Lipozyme® TL IM catalyst, but with the same high catalyst concentration as used in comparison example 2, allowing a contact time which is much shorter than necessary in examples 1 and 2.

TABLE II

ER using Lipozyme® TL IM catalyst	Example 1	Example 2	Example 3
Catalyst conc. (wt.%)	1	10	10
Water (wt.%)	0.013	0.025	0.024
Reaction time (h)	5.25	2.75	0.60
Activity (IUN)	455	160	455
Activity g/(g*h)	38	18	38
Conversion Re	0.85	0.85	0.85
Randomness Ra	0.28	0.08	0.21
$Ra > 0.32Re - 0.08$	yes	no	yes
$Ra > 0.32Re - 0.06$	yes	no	equal
$Ra > 0.32Re - 0.04$	yes	no	no

The high activity Lipozyme® TL IM catalyst (example 1) causes at a low concentration the same conversion Re as the prior art low activity catalyst of example 2 but accompanied by a high rearrangement of the middle composition. When increasing the catalyst concentration according to example 3 the same effect as example 1 is obtained with a shorter contact time, which enables a higher throughput.

**Examples 4 - 6**

Examples 1 - 3 have been repeated under the same conditions, respectively, but using shorter contact times. The shorter  
5 contact times result in a lower degree of conversion  $Re$ , but even at these lower conversion degrees the same relatively high rearrangement on the middle position is observed.

TABLE III

ER using Lipozyme® TL IM catalyst	Example 4	Example 5	Example 6
Catalyst conc. (wt.%)	1	10	10
Water (wt.%)	0.013	0.025	0.024
Activity (IUN)	455	160	455
Activity g/(g*h)	38	18	38
Conversion $Re$	0.4	0.4	0.4
Randomness $Ra$	0.08	0.02	0.08
$Ra > 0.32Re - 0.08$	yes	no	yes
$Ra > 0.32Re - 0.06$	yes	no	yes
$Ra > 0.32Re - 0.04$	no	no	no

10

**Example 7 Graininess in spreads**

Hardstock A was prepared by the following method:

50wt % of palm oil and 50wt % of palm kernel oil were mixed  
15 together in a reaction vessel and 0.53 wt% of Lipozyme® TL IM (activity 38 g/(g\*h)) was added to the mixture. This was stirred at 70 °C until a conversion  $Re$  was reached of 0.63. Rearrangement of the sn-2 position  $Ra$  was determined as being 0.18.

20

A fatblend was prepared from the following:

- 42 wt% hardstock A
- 5 wt% of interesterified dry fractionated palm oil stearin
- 5 (62 wt%) with palm kernel oil (38wt%)
- 53 wt% rapeseed oil

A fat phase was prepared by mixing 99.7 parts of fatblend, 0.2 parts of lecithin and 0.1 parts of monoglyceride (Hymono 8903).

10 An aqueous phase was prepared from 96.3 parts water, 2.2 parts sour whey powder and 1.5 parts salt. The pH was adjusted to 4.6 by means of addition of citric acid.

80 parts fat phase and 20 parts aqueous phase were combined and processed in a conventional manner using a votator, to obtain a  
15 spread which is packed in tubs. The spreads were produced using an AAC sequence. The temperature after the second A-unit was 8°C and after the C-unit was 16°C. The A-units were operated at 600rpm and the C-unit at 230rpm. The residence time in the C-unit was approximately 90 sec. The product was stored  
20 at 5°C for 5 weeks.

For comparison, margarines were made using a fatblend in which:

- i. Hardstock B (Re 0.62, Ra 0.10) was used, which was prepared with Lipozyme® TM IM of activity 18 g/(g\*h)
- 25 ii. Hardstock C (Re 0.60, Ra 0.05) was used which was prepared with Lipase D (Rhizopus oryzae on Accurel carrier).

Everything else was kept the same.

30 An expert panel judged the products for the presence of tropical grains.

In margarines which had been made with hardstock A, grains were scarce and barely noticeable.

In margarines which had been made with hardstock B, grains were apparent at unacceptable levels creating an intolerable product  
5 defect.

In margarines which had been made with hardstock C, grains were clearly abundant throughout the product and thus a clear unacceptable defect.

Table IV

	Enzyme catalyst		
	Lipozyme® TL IM (hardstock A)	Lipozyme® TL IM (hardstock B)	Lipase D (hardstock C)
Activity (g/(g*h))	38	18	53
IUN	455	160	-
Concentration wt%	0.53	1.03	0.34
Re	0.63	0.62	0.60
Ra	0.18	0.10	0.05
Presence of grains in final product	Scarce, acceptable	Numerous, unacceptable	Abundant, unacceptable

10

In the product made with the process of the present invention hardstock A, where Ra is highest, no or hardly any tropical grains were detected. On the contrary, products made with either a low activity (hardstock B) or a sn-1 and sn-3 specific  
15 lipase (hardstock C) give rise to unacceptable levels of tropical grains.

#### Example 8: Graininess

60 wt% of palm oil and 40 wt% of palm kernel oil were mixed  
20 together in a reaction vessel and 0.9 wt% of Lipozyme® TL IM (activity 455 IUN, 38 g/(g\*h)) was added to the mixture. This

was stirred at 70 °C to give hardstock A with Re 0.68. Ra was determined to be 0.22. Harstock B was made with Re 0.26 and Ra 0.08 and 0.6 wt% Lipozyme® TL IM (activity 455 IUN, 38 g/(g\*h)).

5

A fatblend was prepared from the following:

42 wt% hardstock A

5 wt% interesterified palm stearin with palm kernel oil

53 wt% rapeseed oil

10

A fat phase was prepared by mixing 0.9971 parts of fatblend, 0.0020 parts of lecithin and 0.0009 parts of monoglyceride.

An aqueous phase was prepared from 0.9630 parts water, 0.0220 parts sour whey powder and 0.0150 parts salt and citric acid to

15 a pH of 3.8.

80 parts fat phase and 20 parts aqueous phase were combined and processed in a conventional manner using a votator, to obtain a spread which is packed in tubs. The spreads were produced

using an ACAA sequence. The temperature after the first A-unit

20 was 15°C, after the C-unit was 18°C and after the last A unit

was 10°C. The A-units were operated at 600rpm and the C-unit at 200rpm. The residence time in the C-unit was approximately 90

sec. The product was stored at 5°C for 5 weeks. No tropical grains developed in the product.

25

For comparison, margarines were made using a fatblend in which Hardstock B was used.

In products in which hardstock A was used, no tropical grains

30 had developed.

In products in which hardstocks B were used, tropical grains were identified after 5 weeks of storage at 5°C.

Table V

	Hardstock	
	A	B
Activity IUN	455	455
g/(g*h)	38	38
Re	0.68	0.26
Ra	0.22	0.08
Tropical grains	No	Yes

**Example 9: Ra vs reaction time**

5 Following the same procedure as outlined in example 1, four experiments in a batch reactor were performed. In table IV the type of enzyme, its activity, and its concentration in the reactor are given.

Table VI

experiment	Lipase	Concentration (w/w) %	Activity g/g/h
1	Lipozyme® TL IM	2.4	38
2	Lipozyme® TL IM	10	38
3 comparative	Lipozyme® TL IM	10	18
4 comparative	Lipase D	10	53

10

The reaction mixture again consists of 60 parts palm oil and 40 parts palm kernel fat. The four reactions were monitored closely over time. A number of samples were taken and analysed. The last sample was taken after 10 hours reaction time. From  
 15 the analytical data the degree of conversion (Re) and the randomisation of the sn-2 position (Ra) were derived as outlined in the text. Figure 3 illustrates the evolution of Re and Ra over time for the different reactions.

The graph clearly shows, that for the Lipase D (experiment 4) and the low activity Lipozyme® TL IM (experiment 3) the small changes in the composition of the sn-2 position are progressing linear with time. This is in line with the prior art stating  
5 the effect of acyl migration in partial fatty acid glycerides (see Torres et al., JAOCS vol 79, no.8 (2002) p775-781, Torres et al JOACS, vol 79 no 7 (2002) p655-661, and Zhang et al JAOCS vol 78, no. 1 (2001) p 57-64).

10 In contrast to this, Lipozyme® TL IM with a high activity and a concentration level of 10 (w/w) % (experiment 2) yields a dramatically increased randomisation of the sn-2 position at the same reaction time.

It should be pointed out that in comparison to the experiment 4  
15 (lipase D) these timings are actually corresponding to practically identical degrees of conversion with respect to the randomisation of the sn-1 and sn-3 positions (Re). The different shapes of the curves also illustrate this.

20 Also experiment 1, with a lower Lipozyme® TL IM concentration shows a clearly different behaviour than the the comparative experiments (3 and 4). When comparing the experiments 1 and 2 one finds that for a given degree of conversion (the randomisation of the sn-1 and sn-3 positions (Re)) the  
25 experiment employing a low concentration of the enzyme catalyst actually yields higher degrees of randomisation of the sn-2 position (Ra). This increase in Ra is coinciding with the presence of less silica in the reaction vessel.

30 As this example clearly demonstrates, the process we have found is substantially different from the processes reported in the prior art.

**Example 10: RA vs contact time**

60 parts of palm oil is mixed well with 40 parts of palm kernel oil at 70°C and added to the feed tank of a packed bed reactor (PBR) system, of which the temperature is also 70°C.

5 A PBR of 20ml volume is filled with 3g of Lipozyme® TL IM (Activity IUN 455, 38 g/(g\*h)) to give a catalyst bed volume of 7ml.

The flow meter, controlling the oil flow through the reactor is set to 35g/h, which corresponds to a residence time of around 8  
10 minutes, in order to achieve an Re of approximately 0.6.

Samples are taken at regular intervals and the flow adjusted accordingly in order to compensate for the deactivation of the enzyme and thus retain an Re of 0.6.

Table VII

Relative activity (%)	Re	Ra	Flow (g/h)	Residence time (min)
100	0.58	0.15	35	8
42	0.63	0.15	13	22
32	0.62	0.14	10	28
9	0.64	0.12	3	105

15

In the same way, a second experiment was carried out whereby the flow was set to 20 g/h, which corresponds to a residence time of 14 minutes, in order to achieve a Re of approximately  
20 0.8.

Table VIII

Relative activity (%)	Re	Ra	Flow (g/h)	Residence time (min)
100	0.84	0.30	20	14
77	0.83	0.29	16	17
47	0.83	0.27	10	28
8	0.84	0.25	2	165

The results documented in the tables VII and VIII above,  
25 illustrate that in order to maintain a constant degree of

conversion over time in a packed bed flow reactor, it is necessary to adjust the flow rate in a reciprocal way to the deactivation of the enzyme catalyst (reduction of activity). To our surprise, we have found that the resulting randomisation of the sn-2 position (Ra) is practically not affected by these dramatically increased residence times, from 14 to 165 minutes. If the found randomisation would be the result of the often-cited acyl migration in partial fatty acid glycerides, the sn-2 randomisation (Ra) should increase dramatically with increasing residence time. The process according to the invention, however, yields that the sn-2 randomisation (Ra) is practically constant when the residence time is increased.

The data also show that the sn-2 randomisation (Ra) is not changing when an enzyme catalyst with high starting activity, according to the invention, is as a consequence of the ongoing process functioning at a reduced activity. This proves that the high starting activity of the enzyme catalyst, as defined in the text, is the key element to the establishment of the sn-2 randomisation (Ra).

**Claims**

1. Process wherein the fatty acid residues on a glyceride moiety are randomised over the terminal and middle positions, wherein the process proceeds to a conversion degree on the terminal positions,  $R_e$ , ranging from 0.3-0.95, and wherein a conversion degree on the middle position,  $R_a$ , ranges from 0.06-0.75, and wherein  $R_a$  is greater than  $0.32R_e - 0.08$ , the process comprises the exposure of a triglyceride fat to a catalyst comprising a lipase characterised in that the lipase is a *Thermomyces lanuginosa* lipase which has an activity of at least 250 IUN at the onset of the process.
2. Process according to claim 1, characterised in that the catalyst has an activity of at least 300 IUN, more preferably at least 350 IUN.
3. Process according to claims 1 or 2, characterised in that  $R_a$  is greater than  $0.32R_e - 0.06$ , preferably greater than  $0.32R_e - 0.04$ .
4. Process according to any one of claims 1 - 3, characterised in that the amount of catalyst when used in a batch reactor is 0.05 - 9 wt.%, preferably 0.05 - 5 wt.%, more preferably 0.05 - 3 wt.% calculated on the reaction mixture.
5. Process according to any one of claims 1 - 3, characterised in that in the first hour of conducting oil through a packed bed reactor the residence time of the oil in the catalyst bed is less than 25 min, preferably less than 20 min, more preferably less than 15 min.

6. Process according to any one of claims 1 - 5, characterised in that the triglyceride fat is selected from the list comprising  
any mixture comprising a liquid oil and a hydrogenated oil,  
any triglyceride fat which has not been subjected to hydrogenation, and  
a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.
7. Process according to any one of claims 1 - 6, characterised in that the conversion degree  $Re$  is less than 0.9, preferably less than 0.85.
8. Process according to any one of claims 1 - 7, characterised in that the conversion degree  $Re$  is at least 0.35, preferably at least 0.4.
9. Process according to any one of claims 1 - 8, characterised in that the content of water in the reaction mixture is from 0.001 to 0.1 wt.%, preferably from 0.001 to 0.05 wt.%.
10. Process according any one of claims 1 - 9, characterised in that the temperature of the reaction mixture is from 40 to 85°C, preferably from 45 to 80°C, more preferably from 50 to 75°C.
11. A triglyceride fat obtainable by enzymatic rearrangement of which the conversion degree of the terminal positions  $Re$  is from 0.3 to 0.95 and of which the conversion degree of the middle position of the triglyceride  $Ra$  is from 0.06 to 0.75, while  $Ra$  is greater than  $0.32Re - 0.08$ , preferably greater

than  $0.32Re - 0.06$ , more preferably greater than  $0.32Re - 0.04$ .

12. A triglyceride fat according to claim 11 which is obtainable by enzymatic rearrangement of a fat selected from the list comprising
  - any mixture comprising a liquid oil and a fully hydrogenated oil,
  - any triglyceride fat which has not been subjected to hydrogenation, and
  - a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.
13. A triglyceride fat according to claim 11 or 12, characterised in that the conversion degree  $Re$  is less than 0.9, preferably less than 0.85.
14. A triglyceride fat according to any one of claims 11 - 13, characterized in that the conversion degree  $Re$  is at least 0.35, preferably at least 0.4.
15. Use of an aggregate of *Thermomyces lanuginosa* lipase and silica as catalyst for partially rearranging fatty acid residues of a triglyceride fat to a conversion degree of the terminal positions  $Re$  of 0.3 to 0.95, comprising a rearrangement on the middle position to a conversion degree  $Ra$  of 0.06 to 0.75, wherein the lipase/silica aggregate has an activity of at least 250 IUN, preferably at least 300 IUN, more preferably at least 350 IUN.
16. A food products which comprises a fat according to any one of claims 11 - 14.

Fig. 1

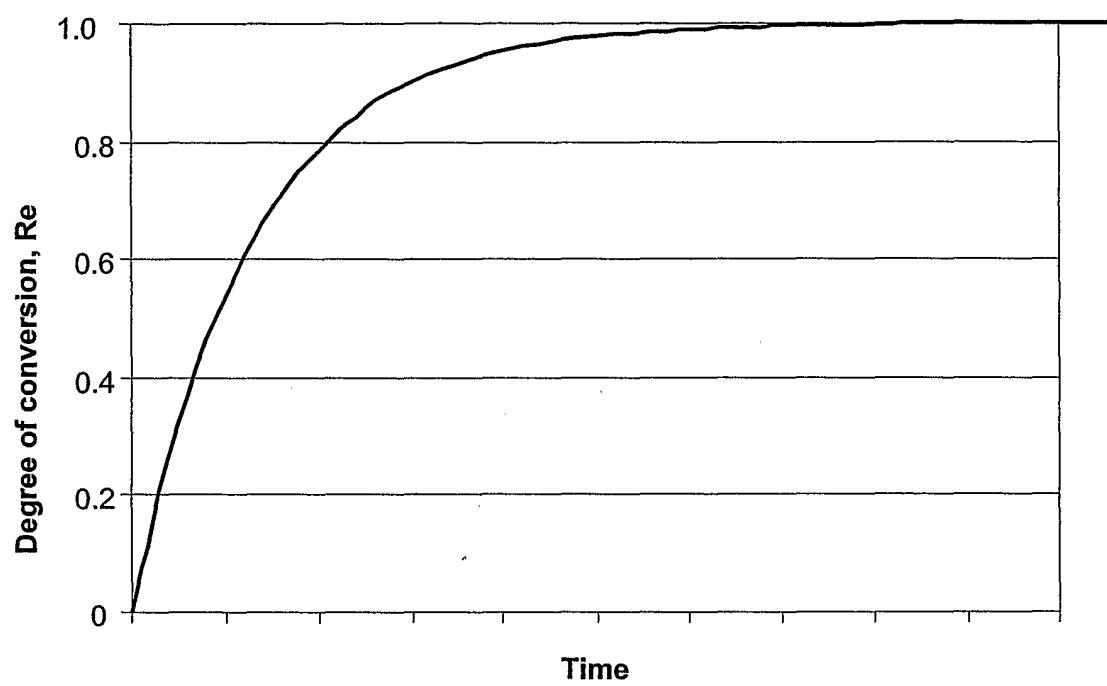


Fig. 2

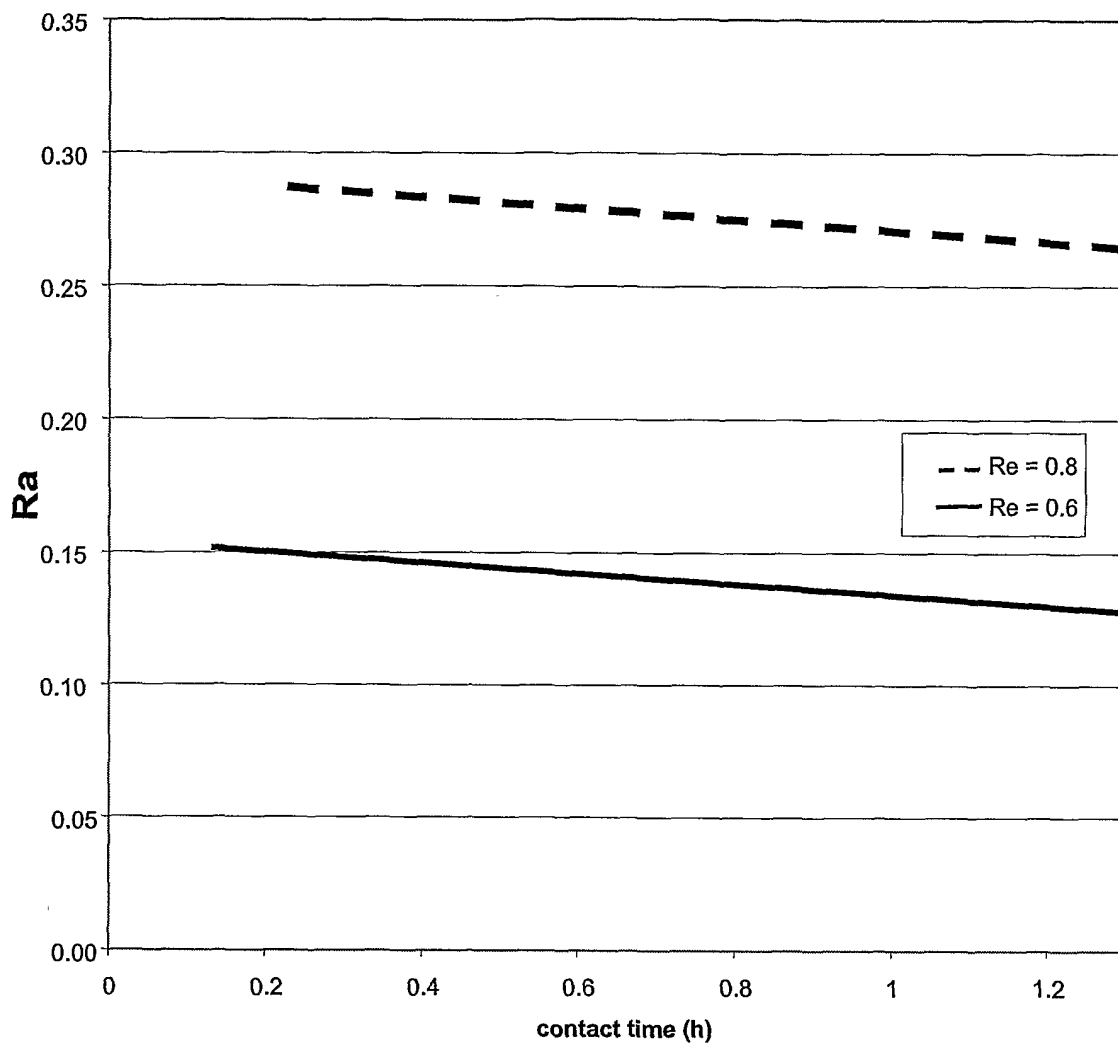
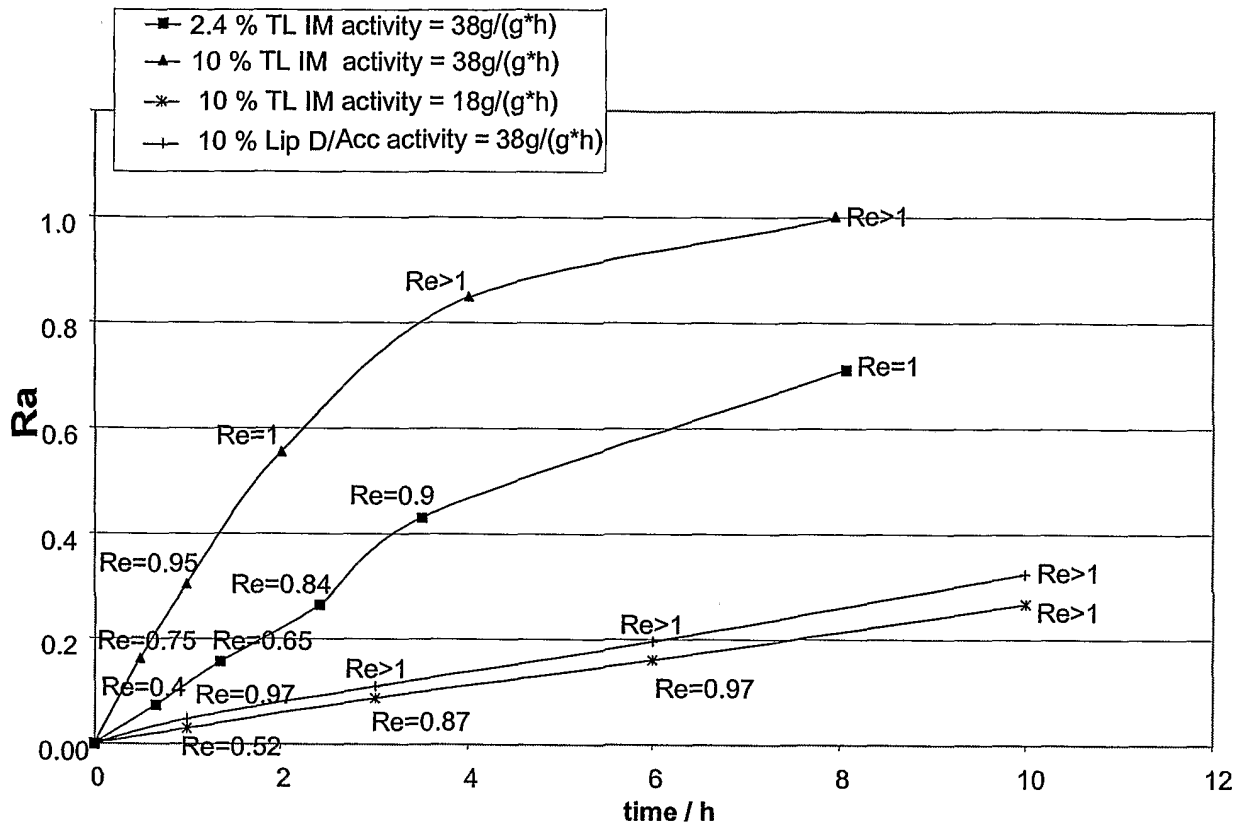


Fig. 3



**INTERNATIONAL SEARCH REPORT**

national Application No  
PCT/EP2004/014772

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C11C3/10 A23D9/00 A23D7/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**  
Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C11C A23D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  
EPO-Internal, WPI Data, PAJ, BIOSIS, FSTA, MEDLINE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PH BERBEN, C GROEN, MW CHRISTENSEN, HC HOLM: "Interesterification with immobilized enzymes" SOCIETY OF CHEMICAL INDUSTRY, 'Online! 16 February 2001 (2001-02-16), XP002286715 Retrieved from the Internet: URL:www.soci.org/SCI/publications/2001/pdf/pb88.pdf> 'retrieved on 2004-06-24! the whole document	1-16
X	NIELSEN P M: "A NATURAL VEGETABLE FAT" OILS AND FATS INTERNATIONAL, INTERNATIONAL TRADE PUBLICATIONS, REDHILL,, GB, vol. 18, no. 4, July 2002 (2002-07), pages 18-19, XP008025554 ISSN: 0267-8853 the whole document	1-16

Further documents are listed in the continuation of box C.  Patent family members are listed in annex.

° Special categories of cited documents :

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- \* & \* document member of the same patent family

Date of the actual completion of the international search <b>25 February 2005</b>	Date of mailing of the international search report <b>09/03/2005</b>
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer <b>Muller, I</b>
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TORRES C F ET AL: "CATALYTIC TRANSESTERIFICATION OF CORN OIL AND TRISTEARIN USING IMMOBILIZED LIPASES FROM THERMOMYCES LANUGINOSA" JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, AMERICAN OIL CHEMISTS' SOCIETY. CHAMPAIGN, US, vol. 79, no. 8, August 2002 (2002-08), pages 775-781, XP001124617 ISSN: 0003-021X page 775, right-hand column, paragraph 2 - paragraph 3; table 3 page 779, right-hand column, line 9 - line 14; figures 1,5 page 780 - page 781</p>	1-16
X	<p>H. ZHANG, X. XU, J. NILSSON, H. MU, J. ADLER-NISSEN, C.-E. HOY: "Production of Margarine Fats by Enzymatic Interesterification with Silica-Granulated Thermomyces lanuginosa Lipase in a Large-Scale Study" JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 78, no. 1, 2001, pages 57-64, XP001182133 US page 57, right-hand column, line 8 - page 58, right-hand column, line 19 page 60, right-hand column, last paragraph - left-hand column, paragraph 3 page 62</p>	1-16
X	<p>C. R. TORRES, F. MUNIR, L. P. LESSARD, C. G. HIL JR.: "Lipase-Mediated Acidolysis of Tristearin with CLA in a Packed-Bed Reactor: A Kinetic Study" JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 79, no. 7, 2002, pages 655-661, XP001182205 US page 655, left-hand column, paragraph 2 - right-hand column, paragraph 1 page 660, right-hand column</p>	1-16