

Description

The present invention relates to a method for the enzymatic interesterification of a lipid and an enzyme used therein.

As one of the methods for modifying a lipid, there has been employed an interesterification reaction, for example, between fatty acid residues in a lipid and other fatty acid residues in a reaction system. In the interesterification of a lipid, it has been known to use a metallic catalyst such as an alkaline metal, an alcoholate of an alkaline metal, an alkaline metal hydroxide or the like. However, when this interesterification is carried out in the presence of water, a free fatty acid, a peroxide or the like, the catalyst is deactivated. For example, it is reported that, when a fat or oil is treated by this interesterification, sodium methoxide used as the catalyst is deactivated at the rate of 0.3 part for each 0.1 part increase in water content and 0.1 part for each 0.1 increase in acid value (AV) [Journal of the American Oil Chemists' Society, 55, 796—804 (1978)]. Therefore, a fat or oil to be treated by this interesterification is thoroughly deacidified and dried before addition of such metallic catalyst and moisture content of the dried fat or oil to be treated is usually of the order of 0.01%.

It has also been proposed to use a lipolytic enzyme in the interesterification of a lipid (e.g. Japanese Kokai Nos. 15687/1976 and 104506/1977). Since a selective interesterification reaction which has not been expected in the interesterification using a metallic catalyst can be effected by utilizing specificity of a lipolytic enzyme, it is possible to modify a glyceride in the desired configuration and characteristics just as one wishes, that is, to obtain a so-called "tailor-made" glyceride by the interesterification using a lipolytic enzyme. In this enzymatic interesterification, unlike the above metallic catalyst, a lipid-degrading enzyme is not inactivated by water. On the contrary, it has generally been considered that the presence of water is essential for enzymatic reaction using various enzymes including the lipolytic enzyme, and an optimum pH thereof is also taken into consideration. Further, it is said that a lipolytic enzyme acts at interface in a heterogeneous system, one side of which is water. Therefore, almost conventional processes of the enzymatic interesterification are carried out with addition of water or without drying of a reaction system.

However, another problem which has not been encountered in the interesterification using a metallic catalyst arises in the enzymatic interesterification. That is, since hydrolysis is unavoidable in the enzymatic interesterification, a free fatty acid (FFA) and other hydrolyzates such as a monoglyceride (MG) or a diglyceride (DG) are formed and hence, yield of the desired esters such as a triglyceride (TG) is lowered and quality thereof is deteriorated because of difficulty of removing the hydrolyzates from a reaction mixture.

For example, in case of the preparation of TG, it is difficult to remove DG which is the most hardly removable among the hydrolyzates. Although, MG and FFA as well as DG form an eutectic mixture with TG and hinder the formation of crystal nuclei, MG and FFA contents in a reaction mixture can be considerably decreased by alkali refining and distillation. To the contrary, any effective industrial process for removing DG has not yet been established. K. G. Berger proves that, when palm oil contains 13% of DG, the ratio of the solid fat to the total oil at 21°C becomes as low as 80% of that containing no DG and when DG content becomes higher, it takes a longer time to transform the crystal form from α -form into β' -form and further into β -form [Oil Palm News, 22, 10—18 (1977)]. The presence of DG hinders the formation of crystal nuclei and hence, difficulties arise in a fractionation operation at a low temperature, a tempering step in chocolate production or the like. Generally, it is desirable to minimize DG content in a fat or oil. At most about 12%, particularly, in case of producing a cacao butter substitute, 6.5% or less of DG content is preferable.

Further, although a lipolytic enzyme has been employed in various uses such as digestives, enzymatic flavors, tanning agents, detergents, cosmetics, brewing, structural analysis of glycerides or the like, the worth of the enzyme is usually evaluated by the lipolytic activities (assay of the freed fatty acid) thereof, which is used as a measure in the preparation of an enzyme. In the conventional enzymatic interesterification, wherein lipolytic activities are also used as an important measure, as mentioned above, it has been considered that the enzymatic interesterification requires water, that is, the interesterification occurs as a result of a reversible reaction which involves both reactions of hydrolysis and synthesis and the hydrolysis is required for the enzymatic interesterification.

However, as a result of the present inventors' intensive study, it has been found that lipolytic activities of an enzyme is not necessarily corresponding to interesterification activities thereof, for example, some lipolytic enzymes show inferior interesterification activities in a system having a low water content, while they have good lipolytic activities, and further that when several enzyme preparations are prepared from enzymes in the same lot having interesterification activities, they sometimes show different interesterification activities from one another even if they have similar lipolytic activities.

Under these circumstances, it is required to carry out the enzymatic interesterification in a reaction mixture having low water content such as 0.18% or less in order to obtain a lipid in high quality and hence, it is required to study interesterification activities of a lipolytic enzyme in order to obtain an enzyme preparation being stable and having high interesterification activities even if in a reaction mixture having low water content.

The present inventors have surprisingly found that an enzyme preparation having interesterification activities increases its effectiveness by continuously or repeatedly using the preparation in a dried reaction system, that is, the formation of DG and other hydrolyzates is reduced and that an enzyme preparation having high interesterification activities which have not been attained by a conventional enzyme preparation can be obtained.

Accordingly, the present invention provides a method for the enzymatic interesterification of a lipid which comprises continuously or repeatedly contacting a dried enzyme or a dried enzyme preparation having interesterification activity with a fresh supply of a dried fatty ester substrate while maintaining the total amount of water in the reaction system at or below the solubility limit of water in the fatty acid ester used.

The present invention also provides a method for preparing an enzyme preparation which comprises dispersing, adsorbing or bonding an enzyme having lipolytic activity in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial rate of drying to activate or increase the interesterification activity of the enzyme.

Firstly, the concept and definition of the term "interesterification activity" are explained.

In general, when a degree of conversion and a reaction time are represented by the symbols "x" and "t (day)", respectively, and a reaction rate (dx/dt) is proportional to (1-x) wherein x=1 means a completely reacted state and x=0 means an unreacted state, the proportionality factor k is shown by the formula:

$$k = \frac{1}{t} \ln \frac{1}{1-x}$$

The degree of conversion of the interesterification is determined by selecting a suitable guide fatty acid and measuring the distributional change thereof which occurred in a dry reaction system. The term "completely reacted state" used herein means the state in which a constant distribution of a guide fatty acid has been substantially attained by reaction for an enough time. However, when the enzyme to be used apparently has a certain specificity, it is convenient and there is no hindrance to suppose a theoretical "completely reacted state". For example, when the interesterification is carried out by using a lipase which has specificity toward 1- and 3-positions of a glyceride (which does not substantially act on 2-position thereof), the state in which fatty acids at 1- and 3-position of the glyceride are completely distributed at random can be deemed as the "completely reacted state". The "interesterification activity" (absolute value) is expressed by the symbol "Ka" which is the product of the proportionality factor k and the value of "the amount of the substrate used/the amount of the enzyme used". The "interesterification activity" (relative value) is expressed by the symbol "Kr" which is determined by dividing Ka by the lipolytic activity of 1 g of the enzyme used.

The following illustrates the definition of "interesterification activity" of the present invention and the determination thereof in more detail.

A dry mixture of equivalent weight amounts of coconut oil (according to Japanese Pharmacopeia Standard) and methyl stearate (containing $C_{15}H_{31}COOCH_3$ and being substantially free from $C_{11}H_{23}COOCH_3$) (20 g, water content of the mixture being 0.02 wt. % or less) and an enzyme preparation, for example, a lipase preparation (1 g, moisture being removed as much as possible by vacuum drying) (total moisture content of the system being within the range of $0.08 \pm 0.02\%$) are placed in a 300 ml Erlenmyer flask with stopper. After replacing air in the flask with N_2 , the reaction is carried out with stirring at 300 to 500 r.p.m. at $40^\circ C$ for 24 hours (1 day). A sample (20 mg) of the resulting reaction mixture is collected and subjected to thin layer chromatography. The fraction containing fatty acid methyl esters is collected and subjected to gas chromatography to determine the fatty acid composition in the fraction. Lauric acid derived from coconut oil is regarded as the guide fatty acid and the ratios of the guide fatty acid to the total fatty acids in the fraction at completely reacted stage, $t=1$ (day) and $t=0$ are determined. When the ratios at completely reacted state, at $t=1$ and at $t=0$ are represented by the symbols "a", "b" and "c", respectively, x, k and Ka are as follows:

$$x = \frac{b}{a}, \quad k = \frac{1}{t} \ln \frac{a}{a-b}, \quad K_a = 20 \ln \frac{a}{a-b}$$

When the lipase used has specificity toward 1- and 3-positions of a glyceride, the symbol "a" can be determined by calculating the weight ratio of lauric acid residue bonded to 1- and 3-positions of the glyceride in coconut oil to the sum of the amounts of total fatty acid residues bonded to 1- and 3-positions of the glyceride and the fatty acid residues in methyl stearate. When an enzyme used does not show any substantial selectivity toward any certain position of a glyceride, "a" can be determined by calculating the weight ratio of total lauric acid residue bonded to the glyceride to the sum of the amount of total fatty acid residues bonded to the glyceride and the fatty acid residues in the methyl ester.

The lipolytic activity can be expressed by the amount (μ M) of fatty acids formed per 1 minute by 1 g of an enzyme used and is determined according to the method described in Fukumoto et al, J. Gen. Appl. Microbiol., 9, 353 (1963). The adequacy of the above definition is evaluated in Example 9 hereinafter.

Any enzyme or enzyme preparation which shows a certain interesterification activity in a dry reaction system and is capable of using repeatedly can be employed in the present invention. The method for preparing an enzyme preparation is not critical. Apparently, it is desirable that the K_a value of the enzyme or the enzyme preparation is higher and stability thereof is higher (i.e. activities are hardly lowered even being kept for a long period of time). Preferably, the enzyme or the enzyme preparation used in the present invention has K_a value of 5 or more.

However according to the present inventors' test of commercially available enzymes, it has been found that enzymes other than cell-bound enzymes (exocellular enzymes) hardly show the desired interesterification activity in a system having a low water content, and further that in case of enzymes having little interesterification activity, it is necessary to subject them to dispersion, adsorption or bonding in or to a carrier in an aqueous system, followed by drying the resulting mixture at an adequately slow initial drying rate. That is, even if an enzyme itself does not show interesterification activity or the activity thereof is insufficient, as far as the enzyme has lipolytic activity, the enzyme is modified and improved in terms of the interesterification activity in a dry reaction system by the above treatment.

Thus, in the method for preparing the enzyme preparation of the present invention, an enzyme or an enzyme-containing material such as microbial cells homogenates or extender containing material, having lipolytic activity is used as a raw material. As far as the raw material has lipolytic activity, interesterification activity thereof can be activated even if the raw material itself does not show the activity; but if it has no lipolytic activity, interesterification activity can not be activated by any treatment. Further, even if a drying rate is too fast to activate or increase interesterification activity as mentioned below, as far as a dried material keeps lipolytic activity, according to the remaining activity, the interesterification activity can be activated or increased by again hydrating the dried material and drying at a slow rate. Therefore, it is understood that the interesterification reaction requires a lipolytic active site. According to the present inventors' test of commercially available enzymes, a certain kind of cell-bound enzymes show a little interesterification activity, though they are not enough strong. However, the other lipolytic enzymes tested themselves hardly show interesterification activity. The origin, degree of purity and selectivity of the enzyme to be used are not critical and a wide variety of lipolytic enzymes such as those originated from microbe, molds, yeasts and, furthermore, tissues of higher animals and plants can be used. Examples of lipolytic enzymes used in the method for preparing the enzyme preparation of the present invention are those originated from microbes such as *Pseudomonas fluorescens*, *Thermomyces ibadanensis*, *Fumicorra ranuginosa* or the like; molds such as *Rhizopus delemar*, *Rhizopus japonicus* (cell-bound enzyme), *Rhizopus niveus*, *Aspergillus niger*, *Mucor javanicus*, *Rhizopus arrhizus* (cell-bound enzyme) or the like; and yeasts such as *Candida cylindracea*, *Geotrichum candidum* or the like; pancrease lipase; and rice bran lipase.

In order to utilize the advantages of the enzymatic interesterification and from practical viewpoint, it is preferable to use the enzyme having a certain selectivity such as that toward specific positions of a glyceride or toward a specific kind of fatty acids.

It is necessitated to disperse, adsorb or bond the enzyme or the enzyme-containing material having lipid-degrading activity in or to a carrier in an aqueous system. When the enzyme or the material is merely admixed with a carrier in a dry state, the interesterification activity can not be activated or increased. Although the term "in an aqueous system" means that the system contains enough water to hydrate protein of the enzyme, in order to shorten the time of the subsequent drying step, it is preferable to minimize the amount of water so as to be not much excess over the water retention capacity of the enzyme and the carrier. The suitable carrier to be used is that having high water retention capacity and low adsorptivity such as diatomaceous earth, kaolinite, pearlite, silicate gel, cellulose powder, polyvinyl alcohol, calcium carbonate or the like. When a carrier having high adsorptivity such as activated carbon, alumina or the like is used, the resulting interesterification activity is insufficient. This may be caused by blocking of an active site of the interesterification reaction. When a carrier having low or no water retention capacity such as glass powder or the like is used, it is difficult to activate or increase the interesterification activity since a large amount of carrier is needed so as to hydrate the carrier with enzyme solution and enzyme is insufficiently distributed on the carrier. The carrier may be in the form of powder, fibers or the like. However, it is preferable that the carrier is in the form of granules in view of continuous use of the resulting enzyme preparation. In order to disperse, adsorb or bond the enzyme or the enzyme-containing material in or onto the carrier, for example, the enzyme or the material is dissolved in water and then the resulting solution is admixed with the carrier, or the enzyme or the material is admixed with the carrier and then the resulting mixture is wetted by spraying water or contacting it with water vapor at a low temperature. The mixture ratio of the enzyme or the enzyme-containing material to the carrier varies according to water retention capacity of the carrier, but about 2:1 to 1:20 is preferable.

After the enzyme or the enzyme-containing material is dispersed, adsorbed or bonded in or to the

carrier in an aqueous system, the resulting mixture is dried. In this drying step, even if the mixture is merely dried as fast as possible at such a rate that the enzyme maintains its lipolytic activity, the inter-esterification activity can not be activated or increased. That is, in order to activate or increase the above-mentioned K_a or K_r value, it is necessitated to slow down the drying rate in the initial stage of the drying, that is, until water is removed to some extent from the starting hydrated mixture. The "slow initial drying rate" and the "period of the initial stage" being required to sufficiently activate or increase K_a or K_r value vary according to the components other than enzyme in the starting mixture, the kind and nature of the carrier used, the relation between the apparatus to be used in the treatment and the amount of the mixture to be treated or the like, and they can not be determined uniformly. However, they can be experimentally determined as follows:

Firstly, several runs are carried out with varying drying rate and the suitable "slow drying rate" is determined by measuring K_r value of each run. Secondly, several runs are again carried out by, initially, drying at the slow drying rate thus determined with varying periods of time and, in the course of drying at a faster drying rate. The suitable "initial stage" is the period after which the change of the drying rate no longer affects K_r value of the resulting enzyme preparation.

Usually, the initial stage is over before the starting mixture reaches 0.4 of degree of moisture (ratio of the amount of water in the mixture to the dry weight of the mixture by taking the latter as 1). The slow drying rate mostly depends on the nature of the carrier used. Generally, when using the carrier in the form of powder having high water retention capacity, the rate is such that the decrease in degree of moisture is at most 0.3 per hour, in case of using the carrier in the form of granules having about 2 mm in diameter and having high water retention capacity, the rate is such that the decrease in degree of moisture is at most 0.25 per hour, and in more generally, 0.1 or less per hour decrease is preferable.

The drying can be carried out by various methods in which the controlled slow drying rate can be effected including vacuum drying, pneumatical drying and high-frequency radiation vacuum drying. However, under the conditions of freezing water in the mixture such as in freeze-drying, interesterification activities can not be fully activated, while lipid-degrading activities are maintained. This may be caused by rapid solidification of water in the mixture. During the drying, if necessary, heating can be effected but it is preferable to maintain the temperature at 50°C or below at the initial stage of the drying. Dryness depends upon the particular use of the resulting enzyme preparation but, in order to use the preparation in a reaction system having a low water content such as 0.18% or less, it is preferable to dry the mixture of the enzyme or the enzyme-containing material until 2% of water content thereof is attained.

Thus, according to the method for preparing the enzyme preparation of the present invention, even if an enzyme does not show interesterification activity in a reaction system having a low water content, the activity can be activated or even if the activity thereof is weak, the intrinsic activity can be increased and hence, the enzyme preparation having high interesterification activity which has not been attained by a conventional enzyme preparation can be obtained.

The results obtained from the present inventors' test of commercial available enzymes are as follows:

K_a and K_r values of the commercially available lipases obtained were determined without any treatment of the enzymes. K_a and K_r values thereof are shown in Table 1.

TABLE 1

Origin of lipases	K_a	$K_r \times 10^3$
<i>Rhizopus niveus</i> (product of A Co.)	0.0	0.00
<i>Rhizopus japonicus</i>	12.0	7.5
<i>Aspergillus niger</i>	0.0	0.00
<i>Mucor javanicus</i>	0.9	0.09
Swine pancrease lipase	2.3	0.47
<i>Rhizopus niveus</i> (product of B Co.)	0.2	0.01
<i>Candida cylindracea</i>	0.0	0.00
<i>Rhizopus delemar</i> (product of C Co.)	0.0	0.00
<i>Rhizopus delemar</i> (product of D Co.)	0.0	0.00

Although it is preferable to use the enzyme or the enzyme preparation having K_a value of 5 or more in the present invention, as shown in Table 1, there is no lipase having K_r value of 0.01 or more in the commercially available lipases tested. Further, these lipases were treated by dispersing, adsorbing or bonding them in or to a carrier in an aqueous system and drying rapidly or freeze-drying without inactivation of their activity, but 0.005 increase or more in K_r value was not observed (see Reference Examples 2, 3 and 6 hereinafter).

In the enzymatic interesterification of the present invention, the cell-bound enzyme or the enzyme

preparation having interesterification activity can be used and it is preferable to dry it as dry as possible. However, the enzyme or the enzyme preparation may contain at most about 4 to 5% of water since, in general, it is difficult to completely remove water therein without inactivation of its activities. In the present invention, water content of the enzyme or the enzyme preparation is decreased by continuous or repeated use thereof. This decrease in water content of the enzyme or the enzyme preparation by continuous or repeated use together with use of a dried substrate containing a fatty ester controls formation of DG and the other hydrolyzates.

The fatty ester substrate used in the present invention should be dried in such dryness that at least water content of the enzyme or the enzyme preparation to be used is not increased by water in the substrate. The total amount of water including that in the enzyme or the enzyme preparation and that in the substrate continuously or repeatedly supplied to a reaction system is maintained at solubility limit of water in the fatty ester used or below. For example, when a fatty ester substrate is a fat or oil of glycerides, the fat or oil is preferably dried in such degree that the water content thereof becomes 0.18% or less. Even if the fat or oil contains no DG, though fats and oils generally contain a small amount of DG, hydrolyzates, particularly, about 6.5% of DG are formed in an interesterified product when the water content of the fat or oil is more than 0.18%. For example, in many cases, palm oil without fractionation contains 6 to 8% of DG. If DG content is increased as much as 6.5% by interesterification, total DG content of such the palm oil becomes more than 12% after the interesterification and the resulting oil is hardly used in such fats and oils processing that crystal characteristics are closely related to quality of products. It has been found that, when a fat or oil is a substrate to be continuously or repeatedly treated and moisture content (%) and DG content (%) thereof are represented by the symbols "w" and "y", respectively, it is desirable to decrease the value of $y+34w$, preferably, to less than 12, more preferably, to less than 6.5. However, since water content in a substrate to be treated becomes smaller, it becomes more difficult to dry, desired quality of products and economy of drying should be taken into consideration in order to determine to what extent below solubility limit of water in the substrate drying is effected. Generally, it is sufficient to dry the substrate used in the present invention to such degree as a stock oil used in a conventional interesterification using an alkaline metal catalyst which is elaborately dehydrated and dried (about 0.01% of water content).

As far as the substrate to be used is not deteriorated, any drying process including vacuum drying, vacuum heat drying and treatment with a dehydrating agent can be employed. In some cases, the interesterification may be carried out under a reduced pressure to remove water in the substrate or a dehydrating agent may be added to a reaction system during the interesterification. Examples of a dehydrating agent are zeolite, activated alumina, silica gel, anhydrous salts (e.g. anhydrous calcium carbonate, anhydrous sodium sulfate, etc.), ion exchange resins or the like.

Typical examples of the fatty ester substrate used in the present invention is a mixture of fats and oils of glycerides (e.g. animal fats and oils, vegetable fats and oils or microbial fats and oils, hydrogenated or fractionated fats and oils thereof) or a mixture of fats and oils of glycerides and C_1 — C_8 lower alcohol esters of fatty acids having C_{10} to C_{22} carbon atoms. Further the substrate include other fatty esters, on which a lipolytic enzyme can act, such as propylene glycol esters, phospholipids (e.g. phosphatidyl choline, phosphatidyl ethanolamine, lecithin etc.), cholesterol esters, terpene alcohol esters (e.g. geranyl esters, farnesol esters etc.) or the like. Fatty acids having C_3 to C_{22} carbon atoms may be present in the substrate together with these fatty esters. Besides the substrate should be in liquid at a reaction temperature and, when a material such as a saturated higher fatty acid having a melting point higher than the selected reaction temperature is used, the material may be dissolved in an inert organic solvent such as n-hexane. However, it is preferable to use the material as a lower alcohol ester thereof since a convenient apparatus and a convenient operation can be employed.

Particularly, in the production of a cacao butter substitute, it is desirable to obtain fats and oils of glycerides which comprise predominantly 1-palmityl-2-oleyl-3-stearyl glyceride, 1,3-distearyl-2-oleyl glyceride or mixtures thereof. In such case it is preferable to use a mixture of a fat or oil of glycerides containing a lot of oleic acid residue at 2-position, for example, olive oil, oleic safflower oil, palm oil, shear butter, sal fat (*Shorea Robusta*), mango fat, tea seed oil, camellia oil, a fractionated fat thereof, triolein or the like and a higher fatty acid or a lower alcohol esters thereof such as palmitic acid, stearic acid, or the like as the substrate and to act an enzyme preparation having specificity toward 1- and 3-positions thereon.

In the method of the present invention, the enzymatic interesterification is carried out by continuously or repeatedly contacting the enzyme or the enzyme preparation having interesterification activity with a fresh supply of the dried fatty ester substrate and, whereby the intermolecular or intramolecular interesterification between the fatty acid residues proceeds in the reaction system to give the fatty ester having desired configuration and characteristics.

The term "a fresh supply of the dried fatty ester substrate" used herein means that the interesterification of the substrate has not yet been completed to the desired extent.

In some cases, it is desirable to contact the substrate with a adsorbent before the interesterification. It is assumed that this may be due to the presence of an unknown interesterification inhibitor which may be a trace component resulted from deterioration of the substrate. Even if the substrate has been treated with an adsorbent, the substrate sometimes inhibits the interesterification when it is

allowed to stand for a long time. Therefore, it is preferable to subject the substrate to the inter-esterification reaction shortly after being treated with an adsorbent. Preferred examples of the adsorbent are activated clay, activated carbon, silica gel, used enzyme preparation, particularly, those containing carriers used in the present invention or the like. This treatment with an adsorbent can be carried out in a batchwise operation or can be continuously carried out by passing the substrate through a column packed with the above adsorbent before the interesterification.

The enzymatic interesterification of the present invention can be carried out in a batchwise operation or a continuous operation using, for example, a fluidized bed of the enzyme preparation or a column packed with the enzyme preparation. In view of continuous or repeated use of the enzyme preparation, as mentioned above, it is preferable to disperse, adsorb or bond the enzyme in or to the carrier so as to readily recover the enzyme preparation.

In the continuous or repeated use of the enzyme preparation, a small amount portions of the fresh enzyme preparation can be supplemented while a small amount portions of the enzyme preparation of which activities are mostly dropped are removed from the reaction system. By this operation, inter-esterification activities can be maintained for a long time and, in comparison with using the fresh enzyme preparation alone, there can be prevented undesirable increase in the formation of hydrolyzates and deterioration and variability of quality resulted therefrom. Further, loading can be increased and the amount of the hydrolyzates formed per a unit substrate or a unit time is decreased by this operation.

Generally, the interesterification reaction can be carried out at 20 to 75°C, preferably, at 20 to 60°C until the interesterification proceeds to a desired extent. When the enzyme preparation is thermal resistant, the reaction can be carried out at about 70°C with little inactivation of the enzyme preparation.

The following Examples and Reference Examples further illustrate the present invention in detail but are not to be construed to limit the scope thereof. In the Examples, all the "parts" and "%" are by weight unless otherwise stated.

Examples 1, 2 and 3 and Reference Examples 1, 2 and 3

A commercially available lipase originated from *Rhizopus niveus* (300 g) was dissolved in water (750 g) at about 5°C and the solution was slowly added to Celite 545 (diatomaceous earth produced by Johns Marville Sales Corp. U.S.A.) (750 g) with stirring to give a paste which turned into a wet powder. The powder was divided into 5 portions and one portion was freeze-dried (temperature: -23°C, degree of vacuum: 0.6 Torr) (Reference Example 2). Each of the other three portions was placed in a desiccator connected to a vacuum pump and vacuum-dried at 6 Torr for 4 hours (Reference Example 3), at 8 Torr for 20 hours (Example 1) or at 15 Torr for 4 days (Example 2) to attain the final water content of about 1.4%. During this vacuum drying, external heating and leakage of air were occasionally effected. The remaining one portion was also placed in a desiccator connected to a vacuum pump and vacuum-dried, firstly, under the same conditions as in Example 2 to attain 0.4 of degree of moisture, and then at 15 Torr for 1 day with increased external heating to attain the final water content of about 1.4% (Example 3). Lipid-degrading activities and interesterification activities of the above commercially available lipase (Reference Example 1) and the above-obtained enzyme preparation are shown in Table 2.

TABLE 2

Enzyme preparations	Lipolytic activities	Ka	Krx10 ³
Reference Example 1	4500	0	—
Reference Example 2	1250 (97.2%)*	3.0	2.4
Reference Example 3	1240 (97.3%)	3.2	2.6
Example 1	1210 (94.1%)	12.7	10.5
Example 2	1150 (89.4%)	28.5	24.8
Example 3	1200 (93.3%)	29.7	24.8

*) The percentages in the parentheses show the rate of remaining activities. The percentages are calculated based on the net amount of the lipase.

As is clear from Table 2, even if the lipase maintains its own activities, substantial Ka value is not activated unless it is dried at the adequately slow drying rate.

Example 4

A commercially available lipase originated from *Rhizopus niveus* (1 part) was dissolved in cold water (4 parts) and the solution was thoroughly stirred. Granular diatomaceous earth (2.5 parts) was added to the solution and the mixture was slowly dried under a reduced pressure to obtain an enzyme preparation having 1.5% of water content and 25.7 of Ka value.

The enzyme preparation (8 g) was packed in a glass column (16 mm in diameter) and a mixed oil (water content: 0.015%) which was prepared by previously mixing a middle melting point fraction of palm oil (iodine value: 34.5, DG content: 2.8%) and methyl stearate (purity: 90%) at the ratio of 1:1 and dried by vacuum heat drying was passed through the column at the superficial velocity in the column of 0.06/hr. The degree of conversion and DG content are shown in Table 3.

TABLE 3

Operating time (days)	1	2	5	8	10	12
Degree of conversion*	0.98	0.98	0.94	0.85	0.83	0.79
DG content (%)	9.3	6.7	4.5	4.1	4.3	4.0

*) Palmitic acid is regarded as the guide fatty acid and random distribution of fatty acid residues at 1- and 3-positions of the glyceride is regarded as "completely reacted state".

Example 5

Ten columns as in Example 4 were connected in series and a mixed oil as in Example 4 was passed through the column at the superficial velocity in the column of 0.5/hr. On the every seventh day after the operation has been started, a newly prepared column was connected to the outlet end of the columns and the column assembled to the inlet side of the columns was removed. From about 2 months after the operation has been started, the composition of the oil passed through the columns become constant. After removal of the methyl ester, the oil had the following composition:

Fatty acids composition: C₁₄ 0.4; C₁₆ 26.3; C₁₇ 6.2; C₁₈ 39.4; C_{18:1} 30.9; C_{18:2} 2.4; C₂₀ 0.3.
DG content: 4.1%.

When this oil was subjected to solvent fractionation at 6°C to remove solid therein, an oil quite similar to cacao butter was obtained. This oil was readily subjected to a tempering operation and could compare with cacao butter.

Reference Example 4

When the same procedure was repeated as described in Example 5 except that 0.3% of water was added to the oil to be passed through the columns, the resulting oil contained 13% of DG.

Example 6

Coconut oil (100 parts) and olive oil (100 parts) were mixed and dried by vacuum heat drying. The resulting mixed oil contained 2.1% of DG and 0.014% of moisture.

A commercially available lipase originated from *Rhizopus niveus* (1 part) was dissolved in cold water (4 parts) and the solution was thoroughly stirred. Perlite powder (2.5 parts) was admixed with the solution and the mixture was slowly dried at 25°C under a reduced pressure to give an enzyme preparation having 2.0% of water content and 20 of Ka valued.

The enzyme preparation (10 parts) was added the above-obtained mixed oil and the reaction was carried out with stirring at 40°C for 3 days under the conditions for preventing moisture absorption.

After completion of the reaction, the enzyme preparation was recovered from the reaction system and, according to the same procedure, the recovered enzyme preparation was again reacted with a fresh supply of the mixed oil for 3 days. This procedure was further repeated twice, but the third run was for 4 days and the fourth run was for 5 days. After completion of the reaction, DG content of each run was 4.5%, 3.1%, 2.9% or 2.8% in order.

Example 7 and Reference Examples 5 and 6

According to the same procedures as is described in Example 2 and Reference Example 2, enzyme preparation were prepared by using a commercially available lipase originated from *Rhizopus japonicus* and perlite as the carrier. Lipid-degrading activities and interesterification activities of the above commercially available lipase (Reference Example 5) and the above-obtained enzyme preparations (Reference Example 6 and Example 7) are shown in Table 4.

TABLE 4

Enzyme preparations	Lipolytic activities	Ka	Kr $\times 10^3$
Reference Example 5	1600	12.0	7.5
Reference Example 6 (freeze-dried)	455 (99.6%)*	4.4	9.7
Example 7	450 (98.5%)	7.1	15.8

*) See Table 2.

As is clear from Table 4, although the commercially available lipase has interesterification activities, Kr value is remarkably increased by the treatment of the present invention.

Equal amounts of a middle melting point fraction of palm oil (iodine value: 33.2) and methyl stearate (partially containing C₁₈ fatty ester) was mixed and dried by vacuum heat drying to give a substrate having 0.015% of water content.

The substrate (200 g) and the commercially available lipase (Reference Example 5, 5.62 g) or the enzyme preparation (Example 7, 20 g) were placed in a 500 ml flask and reacted at 40°C with stirring (200 r.p.m.) to attain 0.9 of degree of conversion (palmitic acid was regarded as the guide fatty acid and random distribution of fatty acid residues at 1- and 3-positions of the glyceride regarded as "completely reacted state"). After completion of the reaction, the enzyme or the enzyme preparation was separated from the reaction mixture and recovered. The same procedure was repeated by using the recovered enzyme or enzyme preparation and a fresh supply of the substrate to attain 0.9 of degree of conversion. The number of days necessary to attain 0.9 of degree of conversion in each run is shown in Table 5.

TABLE 5

Enzyme	1st run	2nd run	3rd run	7th run	9th run
Reference Example 5	6	7	9	—	—
Example 7	3	3	4	6	10

As is clear from Table 5, interesterification activities of the enzyme preparation of Example 7 is much higher than that of Reference Example 5 in each run and the enzyme preparation treated by the method of the present invention is sufficiently fit for repeated use.

Example 8

When the same procedure was repeated by using the lipase of Reference Example 5 and a middle melting point fraction of palm oil containing 4.2% of DG, DG content of 1st to 3rd runs were 7.2%, 5.5% and 5.6%, respectively.

Example 9

A commercially available lipase originated from *Rhizopus niveus* (300 g, Kr=0.0) was dissolved in cold water at about 5°C (1200 ml) and the solution was incorporated in granular diatomaceous earth (750 g). The mixture was slowly stirred so that the carrier was not destroyed and dried for 6 days under the reduced pressure of 15 Torr to obtain an enzyme preparation having 1.3% of water content (Ka=27.5, Kr=0.022).

According to the method of determination of interesterification activities as described hereinbefore, a substrate prepared by mixing equal amounts of coconut oil and methyl stearate was interesterified by using the above-obtained enzyme preparation (1 g). Change of the fatty acids composition of the resulting oil with time is shown in Table 6.

TABLE 6

Carbon number of fatty acids	t (day)						
	0/24	6/24	12/24	24/24	2	4	8
8	0	0.7	1.4	2.6	3.0	3.9	4.4
10	0.0	0.5	0.8	1.6	2.2	3.0	3.2
12	0	2.7	4.6	7.5	10.6	12.9	12.9
14	0.2	1.9	3.2	5.0	7.3	8.4	8.5
16	10.0	10.4	10.3	10.8	11.2	11.7	11.3
16-18	0.3	0.6	0.5	0.4	0.4	0.4	0.4
18:0	89.0	82.7	78.9	71.4	64.8	59.1	56.4
18:1							2.3
18:2	—	—	—	0.1	0.2	0.3	0.7
20:0	0.5	0.4	0.3	0.3	0.3	0.3	0.4
100 x	0	21.1	35.9	58.7	82.8	100	100
$\frac{1}{1-x}$	0	0.237	0.445	0.880	1.760	—	—

Since the amount of the target fatty acid (C_{12}) was not increased after $t=4$, x and

$$\ln \frac{1}{1-x}$$

were calculated by taking a and c as 0.128 and 0, respectively.

It is adequate to determine the activities by regarding the reaction as a first-order reaction since the relationship between t and

$$\ln \frac{1}{1-x}$$

is almost linear.

Example 10

Polyvinyl alcohol (2 g) was cyanogenated with cyanogen bromide and washed with water. 0.1 M phosphate buffer (pH 7.5) (50 ml) and a commercially available pancrease lipase (5 g) were added to the above cyanogenated polyvinyl alcohol and the mixture was stirred at 5°C overnight, filtered and dried under a reduced pressure to give an enzyme preparation (water content: 2.1%).

A mixture of equal amounts of olive oil and ethyl stearate was passed through a silica gel column and slowly dried by vacuum heat drying to give a substrate (water content: 0.01%).

The above-prepared enzyme preparation (1.5 parts) was added to the substrate (20 parts) and the mixture was reacted with 40°C. When the degree of conversion was reached to 0.8, the enzyme was separated from the reaction mixture and recovered. The degree of conversion was determined by regarding oleic acid as the target fatty acid. The same procedure was repeated by using the recovered enzyme preparation and a fresh supply of the substrate. DG content of the resulting oil in each run is shown in Table 7.

TABLE 7

	1st run	2nd run	3rd run	4th run
Reaction time (day)	4	5	6	6
DG content (%)	7.8	3.5	3.0	3.2

Example 11

A commercially available lipase originated from *Rhizopus delemar* (1 part) was dispersed in cold water (4 parts). Kaolinite (2.5 parts) was added to the dispersion and the mixture was dried at a slow initial drying rate in air flow at 20°C to give an enzyme preparation.

A mixed oil of equal amount of olive oil and coconut oil was divided into two portions. Clay (white earth) was added to the one portion of the mixed oil in the concentration of 2%. After stirring, the resulting mixture was dried by vacuum heat drying and clay was removed from the mixture. The other portion of the mixed oil was directly dried by vacuum heat drying. Both the resulting substrates contained 0.01% of water and 27% of triglyceride having 54 carbon atoms.

The above-prepared enzyme preparation was added to each substrate and the mixture was reacted with stirring at 40°C. When the content of the glyceride having 54 carbon atoms was decreased to 10% or less, the enzyme preparation was recovered from the reaction mixture and the same procedure was repeated by using the recovered enzyme preparation and a fresh supply of the substrate. DG content of the resulting oil in each run is shown in Table 8.

TABLE 8

		1st run	2nd run	3rd run	4th run
Enzyme without clay treatment	reaction time (day)	3	3	4	6
	DG content (%)	7.0	4.2	3.8	3.9
Enzyme with clay treatment	reaction time (day)	2	2	3	3
	DG content (%)	7.2	4.0	3.6	3.8

Example 12

A commercially available pancrease lipase (1.5 parts) was dissolved in cold water (5 parts) and granular perlite (4 parts) was added to the solution. The mixture was dried at 15°C under a reduced pressure to give an enzyme preparation (water content: 2%).

A mixture of equal amounts of propylene glycol dioleate and ethyl palmitate was dried by vacuum heat drying to give a substrate (water content: 0.01%).

The above prepared enzyme preparation was packed in a column and, according to the same procedure as in Example 5, the enzymatic interesterification was carried out by using the substrate. After removal of fatty esters from the resulting oil, the propylene glycol ester having lower content of mono-ester and higher content of palmitic acid was obtained.

Example 13

Activated clay (3 parts) was mixed with methyl laurate (30 parts) and egg yolk lecithin (50 parts) and the mixture was dried by vacuum heat drying to give a substrate (water content: 0.01%).

By using the substrate, the same procedure as is described in Example 12 was repeated to obtain a lauric acid-introduced lecithin.

Example 14

Ten columns as in Example 4 were arranged in a row and the same substrate as in Example 4 (1500 g) placed in a storage tank was uniformly circulated through the each column from the bottom to the top. When the oldest column of all ten columns was replaced with a newly prepared column, the substrate was also replaced with a fresh supply thereof on the every sixth day after the operation has been started, the fatty acids composition of the resulting oil was stable after removal of methyl esters.

Example 15

The same procedure as is described in Example 6 was repeated except that an enzyme preparation originated from *Candida cylindracea* ($K_a=17.8$) and a mixture prepared by mixing equal amounts of olive oil and oleic acid was substituted for the enzyme preparation and the substrate, DG content of each run was 5.9% (after reaction for 3 days), 3.8% (after reaction for 3 days), 3.5% (after reaction for 4 days) or 3.2% (after reaction for 5 days).

Example 16

The same procedure as is described in Example 6 was repeated except that the substrate was prepared by mixing a mixture of equal amount of safflower oil and stearic acid (DG content: 1.2%, water content: 0.009%) with hexane (300 parts) and 20 parts of the enzyme preparation was used. After removal of hexane and fatty acids from the reaction mixture, DG content of each run was 6.8% (after reaction for 3 days), 5.1% (after reaction for 3 days), 4.7% (after reaction for 4 days) or 4.3% (after reaction for 5 days).

Claims

1. A method for the enzymatic interesterification of a lipid which comprises continuously or repeatedly contacting a dried enzyme or a dried enzyme preparation having interesterification activity with a fresh supply of a dried fatty ester substrate while maintaining the total amount of water in the reaction system at or below the solubility limit of water in the fatty acid ester used.
2. A method as claimed in claim 1, wherein the enzyme is a cell-bound enzyme having lipolytic activity.
3. A method as claimed in claim 1, wherein the enzyme preparation is prepared by dispersing, adsorbing or bonding an enzyme having lipolytic activity in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial drying rate to activate or increase the interesterification activity of the enzyme.
4. A method as claimed in claim 4, wherein the initial water content of the enzyme or enzyme preparation is 5% or less.
5. A method as claimed in any one of the preceding claims, wherein the fatty acid ester is a glyceride mixture.
6. A method as claimed in any one of the preceding claims, wherein the substrate is a mixture of glycerides and a fatty acid.
7. A method as claimed in any one of claims 1 to 5, wherein the substrate is a mixture of glycerides and a lower alcohol ester of the fatty acid.
8. A method as claimed in claim 6 or claim 7, wherein the total amount of water in the substrate and the enzyme or enzyme preparation is 0.18% or less based on the weight of the substrate.
9. A method as claimed in any one of the preceding claims, wherein the substrate is contacted with an adsorbent prior to subjecting it to interesterification.
10. A method as claimed in claim 9, wherein the adsorbent is an activated clay, activated carbon, silica gel or a spent enzyme preparation containing the carrier.
11. A method as claimed in claim 1, wherein the enzyme preparation has a Ka value of 5.0 or more.
12. A method for preparing an enzyme preparation which comprises dispersing, adsorbing or bonding an enzyme having lipolytic activity in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial drying rate to activate or increase the interesterification activity (Kr value) of the enzyme.
13. A method as claimed in claim 12, wherein activation or increase in the Kr value is at least 0.005.
14. A method as claimed in claim 12 or claim 13, wherein the drying is effected by vacuum drying.
15. A method as claimed in claim 12 or claim 13, wherein the drying is effected by pneumatic drying.
16. A method as claimed in any one of claims 12 to 15, wherein the carrier is a diatomaceous earth, kaolinite, perlite, silica gel, cellulose powder, polyvinyl alcohol or calcium carbonate.
17. A lipase preparation having a Kr value of at least 0.01 which has been prepared by dispersing, absorbing or bonding lipase in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial drying rate to activate or increase the interesterification activity of the enzyme.

Revendications

1. Un procédé pour l'interestérification enzymatique d'un lipide, qui comprend le contact continu ou répété d'une enzyme séchée ou d'une préparation enzymatique séchée ayant une activité d'interestérification avec un apport frais d'un substrat constitué d'ester gras séché en maintenant la quantité totale d'eau dans le système réactionnel égale ou inférieure à la limite de solubilité de l'eau dans l'ester d'acide gras utilisé.
2. Un procédé comme revendiqué dans la revendication 1, dans lequel l'enzyme est une enzyme fixée aux cellules ayant une activité lipolytique.
3. Un procédé selon la revendication 1 dans lequel la préparation enzymatique est préparée par dispersion, adsorption ou liaison d'une enzyme ayant une activité lipolytique dans ou sur un support dans un système aqueux et séchage du mélange obtenu à une vitesse initiale de séchage suffisamment lente pour activer ou accroître l'activité d'interestérification de l'enzyme.

**Example 16**

The same procedure as is described in Example 6 was repeated except that the substrate was prepared by mixing a mixture of equal amount of safflower oil and stearic acid (DG content: 1.2%, water content: 0.009%) with hexane (300 parts) and 20 parts of the enzyme preparation was used. After removal of hexane and fatty acids from the reaction mixture, DG content of each run was 6.8% (after reaction for 3 days), 5.1% (after reaction for 3 days), 4.7% (after reaction for 4 days) or 4.3% (after reaction for 5 days).

Claims

1. A method for the enzymatic interesterification of a lipid which comprises continuously or repeatedly contacting a dried enzyme or a dried enzyme preparation having interesterification activity with a fresh supply of a dried fatty ester substrate while maintaining the total amount of water in the reaction system at or below the solubility limit of water in the fatty acid ester used.
2. A method as claimed in claim 1, wherein the enzyme is a cell-bound enzyme having lipolytic activity.
3. A method as claimed in claim 1, wherein the enzyme preparation is prepared by dispersing, adsorbing or bonding an enzyme having lipolytic activity in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial drying rate to activate or increase the interesterification activity of the enzyme.
4. A method as claimed in claim 4, wherein the initial water content of the enzyme or enzyme preparation is 5% or less.
5. A method as claimed in any one of the preceding claims, wherein the fatty acid ester is a glyceride mixture.
6. A method as claimed in any one of the preceding claims, wherein the substrate is a mixture of glycerides and a fatty acid.
7. A method as claimed in any one of claims 1 to 5, wherein the substrate is a mixture of glycerides and a lower alcohol ester of the fatty acid.
8. A method as claimed in claim 6 or claim 7, wherein the total amount of water in the substrate and the enzyme or enzyme preparation is 0.18% or less based on the weight of the substrate.
9. A method as claimed in any one of the preceding claims, wherein the substrate is contacted with an adsorbent prior to subjecting it to interesterification.
10. A method as claimed in claim 9, wherein the adsorbent is an activated clay, activated carbon, silica gel or a spent enzyme preparation containing the carrier.
11. A method as claimed in claim 1, wherein the enzyme preparation has a Ka value of 5.0 or more.
12. A method for preparing an enzyme preparation which comprises dispersing, adsorbing or bonding an enzyme having lipolytic activity in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial drying rate to activate or increase the interesterification activity (Kr value) of the enzyme.
13. A method as claimed in claim 12, wherein activation or increase in the Kr value is at least 0.005.
14. A method as claimed in claim 12 or claim 13, wherein the drying is effected by vacuum drying.
15. A method as claimed in claim 12 or claim 13, wherein the drying is effected by pneumatic drying.
16. A method as claimed in any one of claims 12 to 15, wherein the carrier is a diatomaceous earth, kaolinite, perlite, silica gel, cellulose powder, polyvinyl alcohol or calcium carbonate.
17. A lipase preparation having a Kr value of at least 0.01 which has been prepared by dispersing, absorbing or bonding lipase in or onto a carrier in an aqueous system and drying the resulting mixture at a sufficiently slow initial drying rate to activate or increase the interesterification activity of the enzyme.

Revendications

1. Un procédé pour l'intérestérification enzymatique d'un lipide, qui comprend le contact continu ou répété d'une enzyme séchée ou d'une préparation enzymatique séchée ayant une activité d'intérestérification avec un apport frais d'un substrat constitué d'ester gras séché en maintenant la quantité totale d'eau dans le système réactionnel égale ou inférieure à la limite de solubilité de l'eau dans l'ester d'acide gras utilisé.
2. Un procédé comme revendiqué dans la revendication 1, dans lequel l'enzyme est une enzyme fixée aux cellules ayant une activité lipolytique.
3. Un procédé selon la revendication 1 dans lequel la préparation enzymatique est préparée par dispersion, adsorption ou liaison d'une enzyme ayant une activité lipolytique dans ou sur un support dans un système aqueux et séchage du mélange obtenu à une vitesse initiale de séchage suffisamment lente pour activer ou accroître l'activité d'intérestérification de l'enzyme.



4. Un procédé comme revendiqué dans la revendication 4 dans lequel la teneur initiale en eau de l'enzyme ou de la préparation enzymatique est de 5% ou moins.
5. Un procédé comme revendiqué dans l'une quelconque des revendications précédentes dans lequel l'ester d'acide gras est un mélange de glycérides.
6. Un procédé comme revendiqué dans l'une quelconque des revendications précédentes dans lequel le substrat est un mélange de glycérides et d'un acide gras.
7. Un procédé comme revendiqué dans l'une quelconque des revendications 1 à 5 dans lequel le substrat est un mélange de glycérides et d'un ester d'alcool inférieur de l'acide gras.
8. Un procédé comme revendiqué dans la revendication 6 ou la revendication 7, dans lequel la quantité totale d'eau dans le substrat et l'enzyme ou la préparation enzymatique est de 0,18% ou moins par rapport au poids du substrat.
9. Un procédé revendiqué dans l'une quelconque des revendications précédentes dans lequel le substrat est mis en contact avec un adsorbant avant d'être soumis à une interestérification.
10. Un procédé comme revendiqué dans la revendication 9 dans lequel l'adsorbant est une argile activée, un charbon activé, un gel de silice ou une préparation enzymatique usée contenant le support.
11. Un procédé comme revendiqué dans la revendication 1 dans lequel la préparation enzymatique a une valeur K_a de 5,0 ou plus.
12. Un procédé pour la préparation d'une préparation enzymatique qui comprend la dispersion, l'adsorption ou la liaison d'une enzyme ayant une activité lipolytique dans ou sur un support dans un système aqueux et le séchage du mélange obtenu à une vitesse initiale de séchage suffisamment lente pour activer ou accroître l'activité d'interestérification (valeur K_r) de l'enzyme.
13. Un procédé comme revendiqué dans la revendication 12 dans lequel l'activation ou l'accroissement de la valeur K_r est d'au moins 0,005.
14. Un procédé comme revendiqué dans la revendication 12 ou la revendication 13 dans lequel le séchage est effectué par séchage sous vide.
15. Un procédé comme revendiqué dans la revendication 12 ou la revendication 13, dans lequel le séchage est effectué par séchage pneumatique.
16. Un procédé comme revendiqué dans l'une quelconque des revendications 12 à 15, dans lequel le support est la terre de diatomées, la kaolinite, la perlite, le gel de silice, la poudre de cellulose, l'alcool polyvinylique ou le carbonate de calcium.
17. Une préparation de lipase ayant une valeur K_r d'au moins 0,01 qu'on a préparée par dispersion, absorption ou liaison d'une lipase dans ou sur un support dans un système aqueux et séchage du mélange obtenu à une vitesse initiale de séchage suffisamment lente pour activer ou accroître l'activité d'interestérification de l'enzyme.

Patentansprüche

1. Verfahren zur enzymatischen Umesterung eines Lipids, dadurch gekennzeichnet, daß kontinuierlich oder wiederholt ein getrocknetes Enzym oder ein getrocknetes Enzym-Präparat mit Umesterungs-Aktivität mit einer frisch zugeführten Menge eines getrockneten Fettsäureester-Substrats in Berührung gebracht wird, wobei die Gesamtmenge des Wassers in dem Reaktionssystem an oder unterhalb der Löslichkeitsgrenze des Wassers in dem verwendeten Fettsäureester gehalten wird.
2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Enzyme ein zellgebundenes Enzym mit lipolytischer Aktivität ist.
3. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Enzym-Präparat hergestellt ist durch Dispergieren, Adsorbieren oder Binden eines Enzyms mit lipolytischer Aktivität in einem oder an einen Träger ein einem wäßrigen System und Trocknen der erhaltenen Mischung bei einer hinreichend langsamen anfänglichen Trocknungsrate, um das Enzym zu aktivieren oder die Umesterungs-Aktivität des Enzyms zu steigern.
4. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß der ursprüngliche Wasser-Gehalt des Enzyms oder Enzym-Präparats 5% oder weniger beträgt.
5. Verfahren nach irgendeinem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß der Fettsäureester ein Glycerid-Gemisch ist.
6. Verfahren nach irgendeinem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß das Substrat ein Gemisch aus Glyceriden und einer Fettsäure ist.
7. Verfahren nach irgendeinem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß das Substrat ein Gemisch aus Glyceriden und einem Ester der Fettsäure mit einem niederen Alkohol ist.
8. Verfahren nach Anspruch 6 oder Anspruch 7, dadurch gekennzeichnet, daß die Gesamtmenge des Wassers in dem Substrat und dem Enzym oder Enzym-Präparat 0,18% oder weniger, bezogen auf das Gewicht des Substrats, beträgt.
9. Verfahren nach irgendeinem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß das Substrat mit einem Adsorptionsmittel in Berührung gebracht wird, bevor es der Umesterung unterworfen wird.
10. Verfahren nach Anspruch 9, dadurch gekennzeichnet, daß das Adsorptionsmittel ein aktivierter Ton, Aktivkohle, Silicagel oder ein den Träger enthaltendes verbrauchtes Enzym-Präparat ist.



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11. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Enzym-Präparat einen Ka-Wert von 5,0 oder mehr besitzt.

12. Verfahren zur Herstellung eines Enzym-Präparats, dadurch gekennzeichnet, daß ein Enzym mit lipolytischer Aktivität in einem oder an einen Träger in einem wäßrigen System dispergiert, adsorbiert oder gebunden wird und die erhaltene Mischung bei einer hinreichend langsamen anfänglichen Trocknungsrate gerocknet wird, um das Enzym zu aktivieren oder die Umesterungs-Aktivität (Kr-Wert) des Enzyms zu steigern.

13. Verfahren nach Anspruch 12, dadurch gekennzeichnet, daß die Aktivierung oder Zunahme des Kr-Wertes wenigstens 0,005 beträgt.

14. Verfahren nach Anspruch 12 oder Anspruch 13, dadurch gekennzeichnet, daß das Trocknen durch Vakuumtrocknung bewirkt wird.

15. Verfahren nach Anspruch 12 oder Anspruch 13, dadurch gekennzeichnet, daß das Trocknen durch pneumatische Trocknung bewirkt wird.

16. Verfahren nach irgendeinem der Ansprüche 12 bis 15, dadurch gekennzeichnet, daß der Träger Diatomeenerde, Kaolinit, Perlit, Silicagel, Cellulose-Pulver, Polyvinylalkohol oder Calciumcarbonat ist.

17. Lipase-Präparat mit einem Kr-Wert von wenigstens 0,01, hergestellt durch Dispergieren, Adsorbieren oder Binden von Lipase in einem oder an einen Träger in einem wäßrigen System und Trocknen der erhaltenen Mischung bei einer hinreichend langsamen anfänglichen Trocknungsrate, um das Enzym zu aktivieren oder die Umesterungs-Aktivität des Enzymes zu steigern.