(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 349 606 B1**

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **06.12.95**

(51) Int. Cl.[6]: **C11C 1/04**, C12P 7/64

(21) Application number: **88909701.0**

(22) Date of filing: **07.10.88**

(86) International application number:
PCT/US88/03480

(87) International publication number:
WO 89/03419 (20.04.89 89/09)

The file contains technical information submitted after the application was filed and not included in this specification

(54) **PROCESS FOR PRODUCING HIGHLY PURE OLEIC ACID BY HYDROLYSIS OF SUNFLOWER OIL.**

(30) Priority: **13.10.87 US 108241**

(43) Date of publication of application:
10.01.90 Bulletin 90/02

(45) Publication of the grant of the patent:
06.12.95 Bulletin 95/49

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(56) References cited:
EP-A- 0 232 933
EP-A- 0 239 470
EP-A- 0 245 076
GB-A- 2 176 480
GB-A- 2 188 057
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EP 0 349 606 B1

Description

This invention relates to the field of methods for producing cis-9 octadecenoic acid, i.e. oleic acid. More particularly, the invention relates to the enzymatic hydrolysis of high oleic sunflower seed oil to produce a highly pure form of oleic acid as well as highly pure oleic acid compositions derived from such hydrolysis.

Each year about 500 000 tons of fatty acids are produced in the United States. About 80% of the fatty acids produced are derived from the industrial hydrolysis of tallow. (See M.W. Formo, in Bailey's Industrial Oil and Fat Products, 4th Edition, Volume 2, D. Swern, Ed., J. Wiley, New York, 1982, Page 379). Most fatty acids are produced by industrial fat splitting methods, however, there has been a recent increase in interest with respect to the use of enzymes in connection with the hydrolysis of fats in order to produce fatty acids. The main advantages of using enzymes as compared to conventional high-pressure steam for fat splitting are (1) a cleaner purer product due to a more specific reaction; (2) a lower energy requirement; and (3) the resulting sweet water is clearer, i.e. the glycerin water mixture resulting from the hydrolysis is clearer. Oleic acid is a monounsaturated fatty acid of the formula $CH_3(CH_2)_7(CH{:}CH)(CH_2)_7COOH$ present within natural fats and oils or biological lipids. Oleic acid is a very important substance in both industry and biology. Cleaner, purer products are inherently safer when used in connection with products such as pharmaceuticals; and purer starting materials allow for the production of purer fine chemical derivatives.

Oleic acid is most generally obtained from high-pressure steam fat splitting processes using tallow as the starting material. When produced by such fat splitting processes, oleic acid is not generally obtained in a pure form. Highly purified oleic acid is both colorless and odorless and has excellent stability with respect to oxidative degradation. These properties make it extremely useful in connection with a large number of food and pharmaceutical products. Pure oleic acid can be used safely due to its excellent physical, chemical and physiological properties. Due to such properties oleic acid is actively and widely utilized in the fine chemical or specialty chemical fields. For example, oleic acid is extensively used in pharmaceuticals, cosmetics and foods and has found application in biochemical areas in connection with biosensors and biosurfactants. Oleic acid has also found application in connection with electronics for the stimulation of biological function as well as a number of other quickly developing high technology fields.

Many uses for oleic acid require that the oleic acid be very pure, and commercially available oleic acid generally includes fatty acid homologs having different carbon numbers and double bond numbers. In addition, commercially available oleic acid often contains various minor impurities. Oleic acid compositions which are impure have properties and characteristics which make them unsatisfactory with respect to color, odor, stability, safety and the like making such compositions incapable of performing adequately in a number of high technology applications.

Chemical and physical processing steps in a method of producing highly purified oleic acid have been described in U.S. Patent 4,601,856. However, a number of more conventional oleic acid purification processes are described in Bailey's Industrial Oil and Fat Products, Vol 2, 4th Ed. John Wiley & Sons, N.Y. 1982 (p.379-387). The processes disclosed in Bailey's are those most likely to be presently used commercially.

EP-A-0 232 933 describes the hydrolysis of fats in an aqueous medium using lipase immobilized on certain porous polymeric supports in order to prevent inactivation of the lipase. The only lipase used as described in the examples is Candida rugosa (Candida cylindracea) which hydrolyzes olive oil in a yield of only 10-20% (Example 5, page 14, line 37). Lipase from Candida rugosa shows no substrate specificity; see page 2, lines 44 - 48.

GB-A-2 176 480 also describes the hydrolysis of fat or oil in an aqueous medium using a lipase. The particular technical problem to be solved seems to be to prevent inactivation of the lipase in the reaction system by maintaining the glycerol concentration in the aqueous phase in the reaction system constant within a range of 10 to 40% by weight; see abstract.

The lipase derived from Candida rugosa (Candida cylindracea) is used for the hydrolysis of soybean oil (Ex. 1), beef tallow (Ex. 8) and olive oil (Ex. 9).

Furthermore, in example 9, results for the hydrolysis of olive oil are reported. Starting with a mixture of 3 liter hydrolyzed fatty acids, 2 liter 15% glycerol in water and 1.5 g enzyme, an amount of 0.15 liter olive oil and 0.1 liter water were added every hour. The mixture was stirred at a temperature of 30 °C. Once an hour about 0.5 liter of emulsion was withdrawn from the reaction vessel, centrifuged, and the hydrolysis ratio of the oil and the glycerol concentration was determined. The results are shown in Table 12, page 12. A hydrolysis ratio of 92 % was found after 20 hours reaction time under the particular reaction conditions of this example.

In Kirk-Othmer, Encyclopedia of Chemical Technology, 3rd. Edition, Vol. 9 (1980), pages 804-805, Table 3, the properties and composition of commercially significant fats are summarized. The composition

of conventional sunflower seed oil and olive oil is shown.

The two conventional oils comprise fatty acids of different types in different amounts.

In US-A-4 627 192 sunflower seed oil is described which has a high content of oleic acid moieties and a low content of linoleic acid moieties; see column 8, Table 3. The sunflower seed oils were hydrolyzed with aqueous sodium hydroxide solution; see column 8, lines 3-7.

It has been indicated above that enzymes can be utilized in order to produce fatty acids from triglycerides and that the hydrolytic reactions resulting from the application of such enzymes to triglycerides can find application within fields of high technology. For example, the quantitative determination of mono-, di- and particularly triglycerides in the body fluids of man has been used in the clinical diagnosis of many diseases or disorders. Clinical analysis generally requires that the glycerol esters first be hydrolyzed to liberate glycerol and the corresponding fatty acids. In connection with such techniques an enzyme composition found to be useful for glycerol ester determination is described in U.S. Patent 4,056,442. The patent discloses a composition useful for hydrolysis in an aqueous medium comprising a mixture of from 15 to 95 units of Rhizopus arrhizus lipase and from 5 to 85 units of Candida cylindracea lipase per 100 units of total lipase present.

A specific method and composition for the hydrolysis of triglycerides is described in U.S. Patent 4,259,440. The method includes the steps of adding lipase and cholesterol esterase to a triglyceride in combination with a glycerol assay system and determining the amount of triglycerides present based on the amount of glycerol produced. Other patents which refer generally to the enzymatic hydrolysis of triglycerides are referred to within U.S. patent 4,259,440.

Since one of the potential disadvantages of carrying out hydrolysis with the use of enzymes is cost, enzyme techniques have been developed which involve the immobilization of the enzyme on a substrate. U.S. Patent 4,275,011 describes a process for the interesterification of oils and fats comprising treating such oils and fats with a water-soluble microbial enzyme. The microbial enzyme is adsorbed on an inert, powdered, water insoluble dispersing agent. Thereafter, the enzyme which is adsorbed onto the inert substrate is recovered from the reaction medium.

The technical problem underlying the present invention is to provide an efficient and low energy process for producing highly pure oleic acid.

This problem is solved by a process comprising

a) subjecting sunflower seed oil wherein the triglycerides contain oleic acid moieties in an amount of about 80% or more and the triglycerides have a ratio of oleic acid moieties to linoleic acid moieties of 1 : (less than 0.09) to enzymatic hydrolysis in an aqueous medium in the presence of a combination of hydrolase enzymes under conditions permitting hydrolysis of the sunflower seed oil triglycerides,

b) allowing a layer of an oleic acid containing composition to form and separate from the aqueous glycerol containing medium obtained, and

c) separating the oleic acid containing composition from the aqueous glycerol containing layer.

The high oleic sunflower seed oils such as used in connection with the present invention have an oleic content of, preferably 88% or more, and most preferably about 95%. Such high oleic oils are subjected to enzymatic hydrolysis by contacting the triglycerides with a combination of hydrolase enzymes to provide a reaction product which includes a high purity oleic acid. The reaction medium resulting from the enzymatic hydrolysis contains the oleic acid, glycerol, and a number of contaminant acids. By carrying out the reaction in an aqueous medium the glycerol and other water soluble compounds can be easily separated from the water insoluble oleic acid.

In connection with the invention the terms "high oleic sunflower seed oil," "high oleic sunflower oil" and "high oleic oil" will be used synonymously to mean an oil extracted from the seed of a sunflower plant which oil contains triglycerides which have fatty acid moieties and wherein 80% or more of such moieties are oleic acid moieties (preferably 88% or more, most preferably about 95%) and further wherein the ratio of oleic acid moieties : linoleic moieties is 1:(less than 0.09), preferably in the range of from about 1:0.09 to about 1:0.01 and most preferably in the range from about 1:0.09 to about 1:0.01.

Further, the term "high purity oleic acid" refers to oleic acid compositions obtained by using a "high oleic sunflower oil" starting material and carrying out the process of the present invention. A typical high purity oleic acid obtained according to the present invention would have approximately the following physical characteristics:

	Typical Sample
Specific Gravity (at 15.6 °C)	0.899
Color (ASTM)	L2.0
Color (Gardner)	5-6
% H_20	0.13
Acid value	201
Iodine Value	87.8
Titer	18 °C

These physical parameters will vary somewhat based on the oleic content of the starting oil.

An advantage of the process of the present invention is that the hydrolysis of the triglycerides within the sunflower oils can be carried out in an energy efficient manner.

A feature of the process of the present invention is that the reaction product resulting from the hydrolysis of the high oleic sunflower seed oil is a high purity oleic acid having a variety of uses within high technology fields.

Another feature of the process of the present invention is that it combines technological advancements from the unrelated fields of (1) agricultural plant development; (2) biochemical enzymatic hydrolysis and (3) chemical engineering purification procedures respectively.

These features and, advantages of the present invention will become apparent.

It must be noted that as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a triglyceride" includes mixtures of triglycerides, reference to "an enzyme" includes reference to mixtures of enzymes and reference to "the hydrolysis" includes a plurality of hydrolysis reactions and so forth.

The sunflower (genus Helianthus) is second only to the soybean as a source worldwide for vegetable oil. In the United States alone, there are approximately four million acres planted annually in sunflower, primarily in the Dakotas and in Minnesota. Average sunflower yields in the United States range from about 1200 to about 1400 kilograms per hectare, with the oil content from harvested seed averaging about 40 to 45% on a dry weight basis. Increasing both the oil content (as a percentage of total plant weight) and the yield of these sunflower plants are major objectives of plant breeding projects which the present invention utilizes as source material.

Sunflower seed oil is comprised primarily of palmitic, stearic, oleic and linoleic acids, with oleic acid and linoleic acid accounting for about 90% of the total fatty acid content of the conventional sunflower seed oils. However, sunflower seed oil is known to contain 13 varieties of fatty acids including linoleic, oleic, palmitic, stearic, linolenic, palmitoleic, arachidic, margaric and behenic acid; see T. Cuprina et.al., "The Relative Amount of Fatty Acids in Sunflower Oil of Certain Inbred Lines and in Hybrids of Sunflowers", Institute for Agriculture and Horticulture, Yugoslavia, 1983 The unsaturated acids have one, two or three double bonds, e.g. respectively oleic, linoleic and linolenic. Other acids such as stearic and palmitic are saturated.

It has been recognized that there was an inverse relationship between oleic and linoleic acid which was highly influenced by environmental factors, especially temperature during the growing season. The totals of the oleic and linoleic acid contents is generally about 90% of the acid content of the oil. Therefore, as the linoleic content increases to about 10% the oleic content decreases to about 80%. This relationship is merely a rule of thumb and should not be strictly construed. Cool northern climates generally yield higher linoleic acid-content sunflower seed, whereas high oleic acid values are more characteristic of seeds grown in warmer southern areas. A high linoleic acid concentration is desirable in sunflower oil used in soft margarines and salad dressings, high oleic acid content is preferred for many other applications. For example, a high oleic sunflower seed oil is desirable with respect to the present invention which involves the production of a high purity oleic acid. The purity of the oleic acid, with respect to its lack of linoleic acid, increases the oxidative stability of the product obtained. As a consequence, the oxidative stability of conventional crude sunflower oil derived from seed grown in southern climates is nearly twice that of the crude oil extracted from northern-grown sunflower seeds.

As indicated above, the various fatty acids, such as stearic acid, oleic acid and linoleic acid, are characteristic of the oil of a given variety of seed. Such acid contents may be expressed as a percentage of the total fatty acid content of the trigylceride making up the oil. This method of describing the oils obtained from sunflower seeds used in connection with the present invention is utilized herein. For example, the dimensionless ratios of oleic acid to linoleic acid mentioned below are calculated by dividing the linoleic

acid percentage of total fatty acid moieties on the triglyceride by the like percentage of oleic acid moieties. Thus, smaller numbers represent a larger percentage of oleic acid relative to linoleic acid.

As will be indicated below, it is possible to utilize enzymes which selectively remove particular fatty acids from the triglyceride of the sunflower seed oil. However, the selectivity of the enzyme is often not sufficiently specific to differentiate between linoleic and oleic acids containing the same number of carbons and an overlapping unsaturated position. Accordingly, it is particularly important to use a sunflower seed oil which has a dramatically lower linoleic content coupled with a high oleic content of 80% or greater by weight, and more preferably 88% oleic or greater and most preferably about 95% oleic content. It should be noted that in connection with the present invention, the sunflower seed oil is obtained from a substantially homogeneous assemblage of sunflower seeds. Any particular sunflower seed within the assemblage may well contain higher or lower amounts of oleic acid and different ratios of linoleic to oleic acid. However, the resulting statistical mixture of triglycerides obtained from the substantially homogeneous assemblage of sunflower seeds provides an oil which on average contains 80% or more, more preferably 88% or more oleic most preferably 95% or more oleic with the ratio of the oleic to linoleic of 1:(less than 0.09). A typical sunflower seed oil used in connection with the present invention would include the following acid moieties in the given percent amounts:

Acid Moiety	% present
Oleic (18 carbons, one double bond)	80.0
Linoleic (18 carbons, 2 double bonds)	8.1
Stearic (18 carbons, no double bond)	5.5
Palmitic (16 carbons, no double bond)	4.2
Behenic (22 carbons, no double bond)	0.7
Linolenic (18 carbons, 3 double bonds)	0.2

In general, a variation ± 10% would be within the scope of the present invention.

It is possible to obtain such high oleic sunflower seed oil by breeding techniques as described in U. S. Patents 4,627,192 and 4 743 402.

As indicated above, there are a number of different methods for the hydrolysis of fats and oils which involve decomposition by saponification or acid hydrolysis. Such methods include the decomposition of the triglycerides by the application of high temperature and steam pressure and the Twitchell decomposition method. In general, the fatty acid compositions obtained utilizing such decomposition methods are not particularly pure as indicated by their darker colors. Their impurity contributes to their oxidative instability and unsuitability for use in connection with many high tech applications. In order to purify the hydrolyzed products obtained from these techniques, it is necessary to carry out distillation steps which increase the amount of energy necessary to produce the final product.

The distillation steps required vary depending on the chain length of the fatty acid being isolated. As the chain length increases, the amount of temperature and vacuum required to carry out distillation also increases which further increases the expense due to the additional energy requirements. Further, as the temperature of distillation is increased, reactions can occur among the fatty acids themselves resulting in polymerization and the oxidative degradation. This decreases the yield of the fatty acid obtained from such techniques. In addition to the occurrence of polymerization reactions, some fatty acids isomerize at their double bonds creating large amounts of isomerized fatty acids which decrease the yield of the fatty acid product obtained.

Having noted the above problems, the process of the present invention does not make use of any high pressure, high temperature techniques in order to separate the fatty acids from the trigylcerides within the sunflower seed oil. The process of the present invention utilizes enzymatic hydrolysis to carry out decomposition of the sunflower seed oil. The enzymatic hydrolysis reactions carried out in accordance with the present invention are very selective and have a very low energy requirement. The selectivity of the reaction increases the amount of a particular fatty acid removed from the triglyceride, thus, increasing the purity of the resulting oleic acid. Further, since high temperatures are not required during the hydrolysis and are actually undesirable, fatty acids are not lost by polymerization or isomerization reactions which occur under high temperature.

The ability of enzymes derived from specific microbes to hydrolyze a material is often specific to the material. Accordingly, the enzymatic hydrolysis reaction used in connection with the present invention are carried out only on high oleic sunflower seed oils which have been described above. To develop the process of the present invention, particular reaction conditions necessary to enzymatically hydrolyze such

high oleic sunflower seed oil have been carefully studied with regard to the type and amount of enzyme, the pH of the reaction mixture, the type and amount of additives, the temperature and the amount of water necessary to obtain both a high purity oleic acid and a high yield. Adjustment of these parameters can increase the percent of hydrolysis and/or selectivity of the reaction.

In connection with the hydrolase enzymes, the enzymes used in connection with the present invention can be divided into different categories as follows:

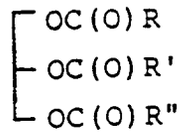
(1) non-site-specific enzymes;

(2) site-specific enzymes; and,

(3) fatty acid selective enzymes.

Various enzymes and particular combinations of enzymes have been found to be particularly useful in connection with the present invention to obtain both high purity oleic acid in a high yield based on the amount of high oleic sunflower oil starting material.

High oleic sunflower seed oil used in connection with the present invention is comprised of trigylcerides having the following general structural formula (I):



wherein R, R' and R'' are hydrocarbon moieties of the acid moieties, 80% or more of which are oleic acid moieties. As indicated above, preferably 88% or more of the acid moieties are oleic and most preferably about 95% are oleic moieties.

When a non-site-specific enzyme is brought into contact with a triglyceride of general structural formula (I), the enzyme will separate all of the fatty acid moieties at all three positions and leave a mixture of glycerol and the separated fatty acids. When a site-specific enzyme is utilized in connection with the triglyceride, the site-specific enzyme generally removes the fatty acid moiety from the two primary positions of the triglyceride. Thus, a 100% efficient reaction of such a site-specific enzyme with such a triglyceride would remove two-thirds of the fatty acid moieties. When a fatty acid selective enzyme is reacted with the triglyceride, the enzyme will react with fatty acid positions wherein particular fatty acids are located (the acid generally being recognized by a particular unsaturated position). For example, the enzyme could react with only oleic fatty acid moieties which have an unsaturated position at the delta nine carbon. However, such fatty acid selective enzymes might also react with other non-oleic moieties which also have an unsaturated position at the ninth carbon.

In connection with the present invention, it was first discovered that the yield and purity of the oleic acid product obtained could be increased by utilizing high oleic sunflower seed oil wherein the triglycerides had a particularly high amount of oleic moieties. Thereafter, differences in various enzymes and reaction conditions were studied to make the best possible use of the best possible starting material. This was done by varying the enzymes and combining different enzymes in different manners at various pH's, additive amounts and amounts of water. Accordingly, one embodiment of the invention involves the use of the high oleic sunflower seed oil starting material in combination with enzymatic hydrolysis techniques to obtain a high yield of oleic acid in a relatively high purity. By carrying out the enzymatic hydrolysis at a water/oil interface, the resulting hydrolyzed product is comprised of fatty acids and glycerol with the glycerol being soluble within the aqueous phase. The aqueous phase is then separated away, leaving a relatively high yield of a high purity oleic acid composition.

It is possible to use different types of enzymes in connection with the present invention, by combining the enzymes or using them step-wise to obtain yields in step-wise reactions. In terms of biochemical nomenclature enzymes are divided into six groups. The present invention involves the use of a combination of hydrolase enzymes, and more specifically water soluble lipases.

The following is a listing of microbes from which are derived non site-specific lipases used in connection with the present invention:

Candida rugosa (cylindracea)

Chromobacterium viscosum

Humicola lanuginosa

Candida lipolytica

The following is a listing of microbes from which are derived site-specific enzymes used in connection

with the present invention:

Aspergillus niger, Mucor miehei, Mucor pusillus,
Rhizopus sp., Pseudomonas sp.,
Penicillium cyclopium.

Geotrichum candidum microbes are the source of enzymes which are selective for fatty acids with a delta nine carbon atom.

Some particular combinations of enzymes are found to be particularly useful in connection with the present invention are derived from the following combinations of microbes.

1) Candida rugosa/Penicillium cyclopium
2) Aspergillus niger/Penicillium cyclopium
3) Mucor miehei/Candida rugosa/Penicillium cyclopium
4) Mucor pusillus/Penicillium cyclopium
5) Chromobacterium viscosum/Penicillium cyclopium
6) Mucor miehei/Penicillium cyclopium
7) Pseudomonas sp./Penicillium cyclopium

In addition to microbial hydrolase enzyme sources, animal sources may be used in connection with this invention such as Porcine pancreatic lipase, Bovine pancreatic lipase and Porcine liver esterase.

In connection with the enzymatic hydrolysis reactions carried out in connection with the present invention, the amount of the hydrolase enzymes utilized depends on the amount of the triglyceride to be hydrolyzed. The amount of the enzymes is expressed in units (U) of activity in connection with the hydrolytic decomposition of the triglyceride. The amount of enzymes utilized varies depending on the particular enzyme used. Since the present invention only applies enzymes to high oleic sunflower seed oils, the amount and type of enzymes does not vary substantially depending upon the oil being hydrolyzed. However, other parameters do affect the amount of the enzymes utilized such as the reaction time, temperature and pH of the reaction medium. In connection with the present invention, it is useful to utilize 10 to 5,000 preferably 10 to 100 more preferably 20 to 40 units per gram of high oleic sunflower seed oil.

In order to demonstrate how the amount of enzyme used varies with enzyme source, some ranges on the amounts are suggested below:

1) Candida rugosa: 7-10 U/m.eq. 23.8-34.0 U/g.
2) Porcine pancreas: 100-1000 U/m.eq. 340-3,400 U/g.
3) Geotrichum candidum: 25 U/m.eq. 85.0 U/g.
4) Pseudomonas: 34 U/g.
5) Penicillium: 1 U/m.eq. 3.4 U/g.
6) Aspergillus niger: 10-100 U/m.eq. 340 U/g.

When used in commercially acceptable processes less enzyme is generally used. Some preferred ranges for some specific enzyme sources are as follows:

1) Candida rugosa: 23.8-34.0 U/g.
2) Porcine pancreas: 340 U/g.
3) Geotrichum candidum: 85.0 U/g or less.
4) Pseudomonas: 34 U/g.
5) Mucor: 85.0 U/g or less.
6) Aspergillus niger: 340 U/g or less.

The enzymatic hydrolysis reaction carried out in accordance with the process of the present invention is preferably carried out in the presence of water in an amount in the range of about 0.5 to 1.5 times the amount of the high oleic sunflower seed oil. The water must be present in a sufficient amount to allow for the hydrolysis to efficiently proceed. However, the inclusion of too much water can also decrease the efficiency of hydrolysis and make it difficult to effectively remove and dispose of the aqueous phase of the reaction medium. The enzymatic hydrolysis reactions of the present invention take place at the sunflower seed oil/water interface.

The relative amounts of sunflower seed oil (oil) and water vary with each system and must be adjusted in order to obtain the best results. In general the oil/water ratio is (1-$\frac{1}{2}$):(1-1$\frac{1}{2}$) preferably 1:(1-1$\frac{1}{2}$) at a temperature which is most preferably about 35 °C ± 2 °C. The temperature is generally about 38 °-40 °C with a combination of hydrolase enzymes specific for the 1,3 position of the triglyceride. more generally the temperature can range from 20 °C to 60 °C.

The pH of the enzymatic reaction mixture affects the hydrolysis. The hydrolytic reaction yields a carboxylic acid which is not water soluble. However, it may be desirable to include a sufficient amount of a buffer to control the pH within the range of about 4.5 to about 10.0, more preferably, 5.5 to 9. Further, the reaction can be carried out in the presence of other additives although other additives are not generally

useful in connection with the present invention.

The enzymatic hydrolysis of the present invention is preferably carried out in a temperature range of about 20 °C to 60 °C, more preferably, about 30 °C to 50 °C. The temperature must generally be kept above 20 °C in order to allow for the reaction to proceed quickly enough to economically carry out the procedure and must be carried out below 60 °C in order to avoid deactivation of the enzyme prior to its interaction with the trigylceride. It is also desirable to continually agitate the reaction mixture in order to promote the enzymatic hydrolysis of the triglycerides.

The relative amounts of oil/water vary with other factors such as the amount of agitation, temperature and the enzyme source. Clearly, the amount of oil/water interface is affected by agitation and to some extent by temperature. The oil to water ratio is preferably 1:(1½), (more preferably 1: 1.2) the temperature is preferably 30 °C to 50 °C and agitation is generally carried out at sufficient speed in order to keep the oil and water phases in a homogeneous dispersion.

The following examples illustrate the invention. Unless indicated otherwise, parts are parts by weight, temperature is in degrees C, and pressure is at or near atmospheric.

EXAMPLE 1

In 35 ml of water is dissolved 1000 U of Candida rugosa and 100 units of Penicillium cyclopium. Then add 100 m. eq. of high oleic sunflower seed oil containing 80% or more oleic acid moieties. Allow the mixture to react at a temperature of about 35 °C to 40 °C for about 6 hours with stirring. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. The hydrolysis rate will rise to above 90% in 6 hours and if continued the hydrolysis rate will rise above 98% at 24 hours.

EXAMPLE 2

In 35 ml of water is dissolved 1000 U of Mucor miehei and 100 units of Penicillium cyclopium. Then add 100 m. eq. of high oleic sunflower seed oil. Allow the mixture to react at a temperature of about 35 °C to 40 °C for about 22-24 hours with stirring. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis rate should be above 96%.

EXAMPLE 3

Add about 70ml of distilled water to 200 units of the lipase obtained from Penicillium cyclopium and 2,000 units of the lipase obtained from the Pseudomonas (10 U/m.eq. oil). Then add 200 m. eq. of high oleic sunflower seed oil containing 80% or more oleic acid moieties. Allow the mixture to react at a temperature of about 35 °C to 40 °C for about 22-24 hours. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis rate should be above 93%.

EXAMPLE 4

Add about 105 ml of distilled water to 1,000 units of the lipase obtained from Candida rugosa; 1,000 units of the lipase obtained from Mucor miehei and 300 units of the lipase obtained from Penicillium cyclopium. Then add 300 m. eq. of high oleic sunflower seed oil. Allow the mixture to react at a temperature of about 35 °C for about 22-24 hours at a pH in the range of about 5.5. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis rate should be in the range of about 80 to 95%.

The specific purity of the oleic acid varies somewhat depending on the starting high oleic sunflower oil used. The oleic acid obtained from utilizing the high oleic sunflower seed oil and subjecting the oil to hydrolysis in accordance with the process of the present invention is considered to be the broadest aspect of the present invention. However, this oleic acid obtained from the high oleic sunflower seed oil can be further purified by physical and chemical procedures which will now be described in detail.

Firstly, after carrying out enzymatic hydrolysis the reaction product separates into two phases with the upper phase being comprised of the fatty acids and the lower aqueous phase being comprised largely of water having dissolved therein glycerol and certain contaminants from the high oleic sunflower seed oil.

Accordingly, the first step in purification of the enzymatic hydrolysis product is to separate away the lower aqueous phase containing the water soluble compounds. The remaining upper phase will contain oleic acid in a very high concentration.

The characteristics of the oleic acid will vary depending on the starting sunflower seeds used. The following is typical of such a high oleic acid:

Physical characteristics

Specific gravity (at 15.6 °C)	0.899
Color (ASTM)	L2.0
Color (Gardner)	5-6
% H_2O	0.13
Acid value	201
Iodine Value	87.8
Titer	18 °C

Chemical characteristics

Oleic acid 80%, linoleic acid 8.1%, stearic acid 5.5%, palmitic acid 4.2%, behenic acid 0.7% and linolenic acid 0.2%. The oil might also include some metals such as Ca, Zn, and Fe in small amounts, e.g. 1-100 ppm. Each of the above physical and chemical characteristics might vary to different degrees, but in general might vary ± 10%.

From the above it is clear that the upper phase will include contaminant fatty acids such as linoleic acid as well as other fatty acids which having longer and/or shorter chains than oleic acid as well as greater and lesser degrees of unsaturation. These contaminant fatty acids can then be separated away by utilizing one or more chemical or physical separation techniques. For example, it is possible to use chemical separation techniques such as those described in U.S. Patent 4,601,856.

The highly pure oleic acid obtained in the process of the invention is obtained as the upper phase resulting from the enzymatic hydrolysis process described above. This highly pure oleic acid includes several different types of fatty acids and other contaminants as indicated above. It is possible to separate away fatty acids having different chain lengths and fatty acids having different degrees of unsaturation in order to further purify the oleic acid composition. More specifically, those fatty acids having more or less than 18 carbon atoms or more or less than one unsaturated bond can be separated away from the oleic acid which contains 18 carbon atoms and a single unsaturated bond.

A highly pure oleic acid obtained in the process of the invention can be winterized by subjecting the oleic acid containing reaction mixture to a low temperature treatment. By reducing the temperature gradually until crystallization begins, it is possible to separate away those fatty acids which contain higher degrees of saturation than oleic acid. Accordingly, by reducing the temperature gradually a point will be reached wherein fatty acids such as stearic and palmitic acid will crystallize and precipitate within the composition. These fatty acids can then be removed to provide a further purified high oleic acid composition.

Polar solvents such as acetone and methanol allow saturated acids such as stearic acid and palmitic acid to crystallize almost quantitatively while the unsaturated acids such as oleic acid remain dissolved within a solvent. Accordingly, separation can be accomplished by including acetone and/or methanol in the oleic acid containing reaction mixture in an amount sufficient to bring about crystallization of the contaminant palmitic and stearic acids. After the crystallization occurs filtration can be carried out in order to remove the crystallized contaminant palmitic and stearic acids. In general the acetone or methanol solvents are added to the reaction mixture in a ratio of 3-4 liters of solvent per liter of fatty acid. After adding the solvent the temperature is reduced to bring about crystallization. In general the temperature is reduced to -10 to -15 °C and filtration is carried out utilizing a vacuum rotary filter after crystallization occurs. The filter can then be sprayed with cold acetone to remove any free oleic acid. Solvents are removed from the oleic acid by flash evaporation and steam stripping.

In addition to the techniques referred to above it is possible to purify oleic acid by chilling the fatty acids containing reaction mixture in water which contains a detergent such as sodium decyl sulfate. Crystals formed within the aqueous dispersion are coated with a film of detergent. These crystals remain in the

water phase when the mixture is centrifugally separated. The oil phase is free of crystals and moisture.

It is also possible to purify the oleic acid containing utilizing fractional distillation. Such reaction mixture methods are described in U.S. Patents 2,054,096; 2,224,984; 2,322,056; and 2,674,570.

Example 5

First the enzymatic hydrolysis procedure is carried out. Thereafter the lower aqueous phase is separated. The upper phase includes the oleic acid. Approximately 1 liter of the oleic acid containing phase is mixed with 3 liters of methanol and the temperature is reduced to a range between -10 and -15 °C. The mixture is kept at the reduced temperature until crystallization appears to be complete. The crystallized material is filtered off and a highly purified oleic acid is obtained.

Claims

1. A process for producing highly pure oleic acid by hydrolysis of sunflower seed oil, comprising
a) subjecting sunflower seed oil wherein the triglycerides contain oleic acid moieties in an amount of about 80% or more and the triglycerides have a ratio of oleic acid moieties to linoleic acid moieties of 1 : (less than 0.09) to enzymatic hydrolysis in an aqueous medium in the presence of a combination of hydrolase enzymes under conditions permitting hydrolysis of the sunflower seed oil triglycerides,
b) allowing a layer of an oleic acid containing composition to form and separate from the aqueous glycerol containing medium obtained, and
c) separating the oleic acid containing composition from the aqueous glycerol containing layer.

2. The process as claimed in claim 1, wherein the oleic acid moieties are present on the triglycerides in an amount of about 88% or more.

3. The process as claimed in claim 1 or 2, wherein the triglycerides have a ratio of oleic acid moieties to linoleic acid moieties in the range of from about 1 : 0.09 to about 1 : 0.01.

4. The process as claimed in any one of claims 1 to 3, wherein the oleic acid moieties are present in an amount of about 95% and the triglycerides have a ratio of oleic acid moieties to linoleic acid moieties in the range of from about 1 : 0.09 to about 1 : 0.01.

5. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzymes are selected from the group consisting of an enzyme derived from Candida rugosa, Chromobacterium viscosum, Humicola languinosa, Candida lipolytica, Aspergillus niger, Mucor miehei, Mucor pusillus, Geotrichum candidum, Rhizopus sp., Pseudomonas sp. and Penicillium cyclopium.

6. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Candida rugosa and Penicillium cyclopium.

7. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Aspergillus niger and Penicillium cyclopium.

8. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Mucor miehei, Candida rugosa and Penicillium cyclopium.

9. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Mucor pusillus and Penicillium cyclopium.

10. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Chromobacterium viscosum and Penicillium cyclopium.

11. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Mucor miehei and Penicillium cyclopium.

12. The process as claimed in any one of claims 1 to 4, wherein the hydrolase enzyme is derived from a combination of Pseudomonas sp. and Penicillium cyclopium.

13. The process as claimed in any one of claims 1 to 12, wherein the aqueous medium contains a buffer selected from the group consisting of acetate and phosphate buffers capable of maintaining the pH in the range of from 4.5 to about 10 during the enzymatic hydrolysis.

14. The process as claimed in any one of claims 1 to 13, wherein the enzymatic hydrolysis is carried out at a temperature in the range of from about 20 °C to about 60 °C at a pH in the range of from about 4.5 to about 10.0.

15. The process as claimed in any one of claims 1 to 14, wherein the ratio of sunflower seed oil to water is 1 : 0.5 to 1 : 1.5, the temperature is in the range of from 30 °C to 50 °C and the pH is in the range of from about 5.5 to about 9.0, and the mixture of sunflower seed oil and aqueous medium is agitated at sufficient speed in order to keep the oil and water phases in a homogeneous dispersion.

16. The process as claimed in claim 15, wherein the aqueous medium contains a buffer capable of maintaining the pH in the range of from 5.5 to 9.0 during the enzymatic hydrolysis.

17. The process as claimed in claim 1(c), further comprising: adding a polar solvent to the oleic acid containing composition obtained in step 1(c);
reducing the temperature of the liquid mixture gradually to about 0 °C to -20 °C;
maintaining the temperature in the range of from 0 °C to -20 °C until crystallization of the saturated fatty acids occurs; and
removing the crystallized saturated fatty acids from the oleic acid.

18. The process as claimed in claim 17, wherein acetone or methanol is used as polar solvent.

19. The process as claimed in claim 17, comprising the steps of:
adding acetone or methanol to the oleic acid containing composition in a ratio of 3 to 4 liters per liter oleic acid containing composition;
reducing the temperature of the liquid mixture to a temperature in the range of from -10 °C to -15 °C;
maintaining the temperature in the range of from -10 °C to -15 °C until crystallization of the saturated fatty acids occurs; and
removing the crystallized saturated fatty acids from the oleic acid.

Patentansprüche

1. Verfahren zur Herstellung hochreiner Ölsäure durch Hydrolyse von Sonnenblumenkernöl, umfassend:
a) Unterwerfen von Sonnenblumenkernöl, in dem die Triglyeride Ölsäurereste in einer Menge von ungefähr 80% oder mehr enthalten und die Triglyceride ein Verhältnis von Ölsäureresten zu Linolsäureresten von 1: (weniger als 0,09) aufweisen, einer enzymatischen Hydrolyse in einem wäßrigen Medium in Anwesenheit einer Kombination von Hydrolaseenzymen unter Bedingungen, die die Hydrolyse der Sonnenblumenkernöl-triglyceride erlaubt,
b) Bildenlassen einer Schicht von Ölsäure-enthaltender Zusammensetzung und Absitzenlassen vom erhaltenen wäßrigen Glycerin-enthaltenden Medium, und
c) Abtrennen der Ölsäure-enthaltenden Zusammensetzung von der wäßrigen Glycerin-enthaltenden Schicht.

2. Verfahren nach Anspruch 1, wobei die Ölsäurereste in einer Menge von ungefähr 88% oder mehr in den Triglyceriden vorhanden sind.

3. Verfahren nach Anspruch 1 oder 2, wobei das Verhälnis von Ölsäureresten zu Linolsäureresten im Bereich von ungefähr 1:0,09 bis ungefähr 1:0,01 in den Triglyceriden liegt.

4. Verfahren nach einem der Ansprüche 1 bis 3, wobei die Ölsäurereste in einer Menge von ungefähr 95% vorhanden sind und die Triglyceride ein Verhältnis von Ölsäureresten zu Linolsäureresten

aufweisen, das im Bereich von ungefähr 1:0,09 bis ungefähr 1:0,01 liegt.

5. Verfahren nach einem der Ansprüche 1 bis 4, wobei die Hydrolaseenzyme aus einer Gruppe ausgewählt werden, die aus einem Enzym aus Candida rugosa, Chromobacterium viscosum, Humicola languinosa, Candida lipolytica, Aspergillus niger, Mucor miehei, Mucor pusillus, Geotrichum candidum, Rhizopus sp., Pseudomonas sp. und Penicillium cyclopium besteht.

6. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus einer Kombination von Candida rugosa und Penicillium cyclopium stammt.

7. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus einer Kombination von Aspergillus niger und Penicillium cyclopium stammt.

8. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus einer Kombination von Mucor miehei, Candida rugosa und Penicillium cyclopium stammt.

9. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus der Kombination von Mucor pusillus und Penicillium cyclopium stammt.

10. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus einer Kombination von Chromobacterium viscosum und Penicillium cyclopium stammt.

11. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus einer Kombination von Mucor miehei und Penicillium cyclopium stammt.

12. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Hydrolaseenzym aus einer Kombination von Pseudomonas sp. und Penicillium cylopium stammt.

13. Verfahren nach einem der Ansprüche 1 bis 12, wobei das wässrige Medium einen Puffer enthält, der ausgewählt ist aus der Gruppe bestehend aus Acetat- und Phosphatpuffern, die den pH-Wert während der enzymatischen Hydrolyse im Bereich von 4,5 bis etwa 10 halten können.

14. Verfahren nach einem der Ansprüche 1 bis 13, wobei die enzymatische Hydrolyse bei einer Temperatur im Bereich von etwa 20 °C bis etwa 60 °C und bei einem pH-Wert im Bereich von etwa 4,5 bis etwa 10,0 durchgeführt wird.

15. Verfahren nach einem der Ansprüche 1 bis 14, wobei das Verhältnis von Sonnenblumenkernöl zu Wasser 1:0,5 bis 1:1,5 beträgt, die Temperatur im Bereich von 30 °C bis 50 °C liegt und der pH-Wert im Bereich von etwa 5,5 bis etwa 9,0 liegt, und das Gemisch von Sonnenblumenkernöl und wässrigem Medium in einer ausreichenden Geschwindigkeit gerührt wird, um die Ölphase und die wässrige Phase in einer homogenen Dispersion zu halten.

16. Verfahren nach Anspruch 15, wobei das wässrige Medium einen Pfuffer enthält, der den pH-Wert während der enzymatischen Hydrolyse in einem Bereich von 5,5 bis 9,0 halten kann.

17. Verfahren nach Anspruch 1(c), weiterhin umfassend:

Zusetzen eines polaren Lösungsmittels zu der Ölsäure-enthaltenden Zusammensetzung, die in Schritt 1(c) erhalten wird;

Allmähliches Vermindern der Temperatur des flüssigen Gemischs auf etwa 0 °C bis -20 °C;

Halten der Temperatur im Bereich von 0 °C bis -20 °C bis die Kristallisation der gesättigten Fettsäuren eintritt; und

Entfernen der kristallisierten gesättigten Fettsäuren von der Ölsäure.

18. Verfahren nach Anspruch 17, wobei Aceton oder Methanol als polares Lösungsmittel verwendet wird.

19. Verfahren nach Anspruch 17, folgende Schritte umfassend:

Zusetzen von Aceton oder Methanol zu einer Ölsäure-enthaltenden Zusammensetzung in einem Verhältnis von 3 bis 4 Litern pro Liter Ölsäureenthaltender Zusammensetzung;

Verminderung der Temperatur des flüssigen Gemischs auf eine Temperatur im Bereich von -10 bis -15 °C;

Halten der Temperatur in einem Bereich von -10 bis -15 °C, bis die Kristallisation der gesättigten Fettsäuren eintritt; und

Abtrennen der kristallisierten gesättigten Fettsäuren von der Ölsäure.

Revendications

1. Procédé de préparation d'acide oléique très pur par hydrolyse d'une huile de graines de tournesol, dans lequel:
   a) on soumet de l'huile de graines de tournesol dans laquelle les triglycérides qui contiennent des parties d'acides oléiques en une quantité d'environ 80% ou plus et les triglycérides ont un rapport des parties à l'acide oléique aux parties acide linoléique de 1: (moins de 0,09) à une hydrolyse enzymatique dans un milieu aqueux en présence d'une combinaison d'enzymes hydrolase dans des conditions permettant l'hydrolyse des triglycérides d'huile de graines de tournesol,
   b) on laisse se former une couche d'une composition contenant de l'acide oléique et on la laisse séparer du milieu aqueux contenant du glycérol obtenu et,
   c) on sépare la composition contenant l'acide oléique d'avec la couche aqueuse contenant du glycérol.

2. Procédé selon la revendication 1, dans lequel les parties acide oléique sont présentes sur les triglycérides en une quantité d'environ 88% ou plus.

3. Procédé tel que revendiqué dans la revendication 1 ou 2, dans lequel les triglycérides ont un rapport des parties acide oléique aux parties acide linoléique compris entre environ 1:0,09 à environ 1:0,01.

4. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 3, dans lequel les parties acide oléique sont présentes en une quantité d'environ 95% et les triglycérides ont un rapport des parties acide oléique aux parties acide linoléique compris dans un intervalle d'environ 1:0,09 à environ 1:0,01.

5. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel les enzymes hydrolase sont choisies dans le groupe constitué par une enzyme dérivée de Candida rugosa, Chromobacterium viscosum, Humicola languinose, Candida lipolytica, Aspergillus niger, Mucor miehei, Mucor pusillus, Geotrichum candidum, Rhizopus sp., Pseudomonas sp. et Penicillum cyclopium.

6. Procéde tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Candida rugosa et Penicillium cyclopium.

7. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Aspergillus niger et Penicillium cyclopium.

8. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Mucor miehei, Candida rugosa et Penicillium cyclopium.

9. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Mucor pusillus et Penicillium cyclopium.

10. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Chromobacterium viscosum et Penicillium cyclopium.

11. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Mucor miehei et Penicillium cyclopium.

12. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 4, dans lequel l'enzyme hydrolase est dérivée d'une combinaison de Pseudomonas sp. et Penicillium cyclopium.

13. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 12, dans lequel le milieu aqueux contient un tampon choisi dans le groupe constitué par les tampons d'acétate et de phosphate capables de maintenir le pH dans un intervalle de 4,5 à environ 10 au cours de l'hydrolyse enzymatique.

14. Procéde tel que revendiqué dans l'une quelconque des revendications 1 à 13, dans lequel l'hydrolyse enzymatique est réalisée à une température comprise entre environ 20 °C et environ 60 °C à un pH compris entre environ 4,5 et environ 10,0.

15. Procédé tel que revendiqué dans l'une quelconque des revendications 1 à 14, dans lequel le rapport de l'huile de graines de tournesol à l'eau est de 1:0,5 à 1:1,5, la température est comprise entre 30 °C et 50 °C et le pH est compris entre environ 5,5 et environ 9,0, et le mélange d'huile de graines de tournesol et le milieu aqueux est agité à une vitesse suffisante pour maintenir les phases huile et aqueuse en une dispersion homogène.

16. Procédé tel que revendiqué dans la revendication 15, dans lequel le milieu aqueux contient un tampon capable de maintenir le pH dans un intervalle de 5,5 à 9,0 au cours de l'hydrolyse enzymatique.

17. Procédé tel que revendiqué dans la revendication 1(c), dans lequel en outre:
on ajoute un solvant polaire à la composition contenant de l'acide oléique obtenu dans l'étape 1(c);
on réduite graduellement la température du mélange liquide à environ 0 °C à -20 °C;
on maintient la température dans un intervalle de 0 °C à -20 °C jusqu'à ce que se produise une saturation des acides gras saturés, et
on enlève les acides gras saturés cristallisés de l'acide oléique.

18. Procédé tel que revendiqué dans la revendication 17, dans lequel on utilise de l'acétone ou du méthanol comme solvant polaire.

19. Procédé tel que revendiqué dans la revendication 17, comprenant les étapes de :
addition d'acétone ou de méthanol à la composition contenant de l'acide oléique dans un rapport de 3 à 4 litres par litre de composition contenant de l'acide oléique;
réduction de la température du mélange liquide à une température comprise entre -10 °C et -15 °C;
maintien de la température dans un intervalle de - 10 °C à -15 °C jusqu'à ce que se produise une cristallisation des acides gras saturés; et
enlèvement des acides gras saturés cristallisés de l'acide oléique.