

that “[R]ejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.”
KSR. . . Exemplary rationales that may support a conclusion of obviousness include:

(A) Combining prior art elements according to known methods to yield predictable results;

(B) Simple substitution of one known element for another to obtain predictable results;

(C) Use of known technique to improve similar devices (methods, or products) in the same way;

(D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results;

(E) “Obvious to try”-choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;

(F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art;

(G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention. MPEP § 2141(II), (III) and 72 Federal Register 57527 (October 10, 2007).

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	14578075
	Filing Date	2014-12-14
	First Named Inventor	Jeff Addy
	Art Unit	1657
	Examiner Name	SRIVASTAVA, KAILASH C
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	1	Steinke et al., "High-yield Preparation of Wax Esters via Lipase-catalyzed Esterification Using Fatty Acids and Alcohols from Crambe and Camelina Oils", J. Agric Food Chem, February 2011, Vol. 49, No. 2, pp. 647-651.	

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- ☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.
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Signature	/Adam Stephenson/	Date (YYYY-MM-DD)	2019-03-13
Name/Print	Adam R. Stephenson	Registration Number	58349

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C11C 3/10, C07C 067/02 C07C 069/533	A1	(11) International Publication Number: WO 90/15127 (43) International Publication Date: 13 December 1990 (13.12.90)
(21) International Application Number: PCT/AU90/00253 (22) International Filing Date: 8 June 1990 (08.06.90) (30) Priority data: PJ 4660 9 June 1989 (09.06.89) AU PJ 6195 6 September 1989 (06.09.89) AU (71) Applicant (for all designated States except US): AG-SEED PTY LIMITED [AU/AU]; Carrington Road, Toowoomba, QLD 4350 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only) : RAE, Ian, David [AU/AU]; 52 Albert Street, Mount Waverley, VIC 3149 (AU). PHILPOTT, Arthur, Keith [AU/AU]; 129 Emerald Road, Upper Beaconsfield, VIC 3149 (AU).		(74) Agent: GRIFFITH HACK & CO.; GPO Box 4164, Sydney, NSW 2001 (AU). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), SU, US. Published <i>With international search report.</i>
(54) Title: ARTIFICIAL JOJOBA OIL (57) Abstract A compound, the properties of which in many respects are analogous to corresponding properties of naturally occurring jojoba oil, is synthesized by subjecting material containing a precursor of the compound to a transesterification reaction to yield the compound. Preferably, the compound is synthesized by transesterifying erucic acid with 2-octyldodecanol in the presence, as catalyst, of concentrated sulphuric acid.		

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ARTIFICIAL JOJOBA OILTechnical Field

This invention relates generally to an artificially synthesized compound, the properties of which are analogous to certain of the properties of naturally occurring jojoba oil, and to a method of producing the artificially synthesized compound.

Background of the Invention

Although the present invention will be described with particular reference to artificially synthesized jojoba oil and to a method of producing this material it is to be noted that the scope of the present invention is not so limited but may extend to include other similar materials and methods.

Natural jojoba oil has been known for centuries for its lubricant and moisturising properties, and is of a neutral pH. Previous attempts have been made to produce artificial jojoba oil due to the high cost and problems associated with the availability of the natural product. However, such attempts have not been entirely satisfactory.

The Wicken Chemical Company of U.S.A., produced a product under the name of WICKENOL 139 which is styled along the lines of a Synthetic jojoba oil. It is believed that WICKENOL 139 is an ester formed from unsaturated straight chain acids, predominantly C_{22} -containing acids and branched chain alcohols, predominantly C_{20} -containing acids and thus, has the empirical formula $C_{12}H_{20}O_2$. WICKENOL 139 is characterized by the infra-red spectrograph as shown in figure 3.

Scher Chemicals Inc describe the production of an oil (known as SCHEROBA OIL) in GB 2106507 which the applicants believe to be an ester comprising predominantly isostearylerycyl enucate. GB 2106507 discloses the use of alcohols with odd numbers of carbon atoms. SCHEROBA OIL is characterized by the infra-red spectrograph as shown in figure 4.

The aforementioned artificial oils are not entirely satisfactory. For example, the lubricity does not equal that of the naturally occurring jojoba oil.

Jojoba oil, both naturally occurring and artificially derived has many known commercial uses, which include such uses as high temperature lubricants, additives to mineral oil to improve their lubricity, additives and/or bases for cosmetic and skin care products such as moisturisers, use in electronic components, slip agents in plastic films, leather softening agents, anti-foam agents in preparations of penicillin and tetracyclin, non-corrosive permanent lubricants for scientific instruments and robotics and the like. Naturally occurring jojoba oil is composed of esters of monounsaturated straight-chain alcohols and acids.

Following intensive research, the present inventors have found a method for preparing a compound, the properties of which in many respects are analogous to the corresponding properties of a naturally occurring jojoba oil. One preferred method involves the use of mustard seeds having a high erucic acid content, from which mustard seed oil is extracted and transesterified. Mustard seed crops are readily grown in Australia and elsewhere in the world and yield good quantities of mustard seed oil (typically one tonne per hectare). Typically, the oil yield from mustard seed which is available for processing is in the order of 35% by weight.

Summary of the Invention

In a first aspect, the present invention provides a method of producing an artificially synthesized compound, the properties of which in many respects are analogous to corresponding properties of naturally occurring jojoba oil, the method comprising subjecting material containing a precursor of the said compound to a transesterification reaction and yielding the said compound.

Preferably, the precursor is erucic acid, an erucic acid containing material, or a mixture thereof.

It is further preferred that the alcohol is a branched-chain alcohol, more preferably, 2-octyldodecanol (C_{20}), which is marketed by Henckel Chemicals under the trade name Eutanol G, stearyl alcohol (C_{18}), cetyl alcohol (C_{16}), 1-eicosanol (C_{20}), 2-eicosanol (C_{20}), oleyl alcohol, isomers thereof, or a mixture of one or more thereof.

It is further preferred that the material containing the precursor comprises material obtained from vegetable oils containing at least 2-66% erucic acid residues, mustard (Brassica) species, rape (high-erucic) species, Crambe abyssinica, or naturally occurring jojoba. Typically, the erucic acid is combined with other acids. More typically the acid reactant of the transesterification reaction is a combination or collection of all of the acids present in the parent mustard seed oil or mustard oil. However, it is to be noted that any suitable alcohol, acid or catalyst may be used in the practice of the present invention. Furthermore, it is to be noted that sources other than mustard seed may be used to obtain artificially derived jojoba oil and such sources include seed of rapeseed varieties whose oil is high in erucic acid content.

Preferred additional acids include oleic acid ($C_{18:1}$), eicosenoic acid ($C_{20:1}$), erucic acid ($C_{22:1}$), nervonic acid ($C_{24:1}$), linoleic acid ($C_{18:2}$), or a mixture of one or more thereof.

Typically, the transesterification reaction mixture is heated for up to six hours at a temperature of up to 110°C and the resultant compound is extracted by a solvent. The solvent is preferably hexane or petroleum. The acid catalyst is typically a strong acid, such as sulphuric acid and is preferably concentrated sulphuric acid.

In a second aspect, the present invention provides an artificially synthesized compound, the properties of which in many respects are analogous to corresponding properties of naturally occurring jojoba oil.

Preferably, the artificially synthesized compound has the empirical formula $C_{40}H_{78}O_2$, and more preferably

the compound is characterized by having an infra-red spectrograph substantially as illustrated in figure 1.

Best Mode of Carrying out the Invention

Preferred embodiments of the present invention will now be described by way of Example only (which are not to limit the scope of the invention in any way) with reference to the accompanying drawings in which:

Figure 1 shows an infra-red spectrograph of one form of the compound of the present invention as described herein having the empirical formula $C_{40}H_{78}O_2$,

Figure 2 shows an infra-red spectrograph of refined natural jojoba oil,

Figure 3 shows an infra-red spectrograph of WICKENOL 139, and

Figure 4 shows an infra-red spectrograph of SHEROBA OIL.

Example 1

Mustard seed oil	25 grams
2-octyldodecanol	25 grams
concentrated H_2SO_4	1-2 grams

A reaction mixture is obtained by adding each of the above indicated reactants together in the proportion as indicated above. The reaction mixture is heated with stirring for approximately 2 hours at about $100^\circ C$ in a water

or oil bath. About 200 ml of water is added to the heated reaction mixture and the reaction mixture is then extracted from the water with hexane or petroleum ether in triplicate using 60 ml for each extraction (3 x 60 ml). The ether or hexane extracts are then combined into a single solution and are kept overnight over anhydrous Na_2SO_4 to remove any remaining traces of water. The solution is filtered and the solvent removed under reduced pressure to yield the ester. Typically, the amount of ester produced is about 41.4 grams which corresponds to about 82% yield. The yellow colour of the product is optionally removed by passing the product through activated silica.

The material made by Example I was subjected to column chromatography and a sample of the eluted material taken. The infra-red spectrograph was obtained using this sample of the eluted material. The infra-red spectra of naturally occurring jojoba oil exhibits peaks at the following locations,

- (i) a very strong triplet from 3000 to 2800,
 - (ii) a moderately strong peak at about 1740,
 - (iii) two weak single peaks at 1470 and 1170,
- respectively.

As can be readily seen from a comparison of Figures 1 and 2, the compound forming the subject matter of the present invention as evidenced by the infra-red spectra of Figure 1 has an infra-red spectra which mimics that obtained from naturally refined jojoba oil as is evidenced by the spectra shown in Figure 2, and thus may be regarded as an artificially derived synthetic substitute for naturally occurring jojoba oil.

The spectra of WICKENOL 139 shown in Figure 3 and that of SCHEROBA OIL shown in Figure 4 both contain additional peaks in the region 1400 to 1100 which illustrates that the compound produced by the present invention is more closely related to the naturally refined jojoba oil than is either WICKENOL 139 or SCHEROBA OIL.

Similar methods to those described for Example 1 were used to produce further synthetic substitutes for naturally occurring jojoba oil:

Example 2

A reaction mixture is obtained by adding each of the following reactants together:

High erucic acid rapeseed oil plus equal weight of oleyl alcohol (cis-9-octadecen-1-ol) with H_2SO_4 catalyst.

The product is an ester which is coloured, and has a detectable odour.

Example 3

5

A reaction mixture is obtained by adding each of the following reactants together:

High erucic acid rapeseed oil plus equal weight of
10 stearyl alcohol (octadecan-1-ol) with H_2SO_4 catalyst.

The product is an ester which is solid at room temperature.

15

Example 4

A reaction mixture is obtained by adding each of the following reactants together:

20 High erucic acid rapeseed oil plus equal weight cetyl alcohol (hexadecan-1-ol) gives ester product which is a solid at room temperature.

High erucic mustard seed oil was used in the above
25 trials with similar results.

30

35

Claims:-

1. A method of producing an artificially synthesized compound, the properties of which in many respects are analogous to corresponding properties of naturally occurring jojoba oil, the method comprising subjecting material containing a precursor of the compound to a transesterification reaction to yield the compound.
2. A method as claimed in claim 1 wherein the precursor is erucic acid, an erucic acid containing material, or a mixture thereof which is transesterified with an alcohol in the presence of an acid catalyst.
3. A method as claimed in claim 2 wherein the alcohol is a branched-chain alcohol, stearyl alcohol (C_{18}), cetyl alcohol (C_{14}), 1-eicosanol (C_{20}), 2-eicosanol (C_{20}), isomers thereof, or a mixture of one or more thereof.
4. A method as claimed in 3 wherein the alcohol is 2-octyl dodecanol or oleyl alcohol.
5. A method as claimed in any one of the preceding claims wherein the material containing the precursor comprises material obtained from one or more of the following sources:
- vegetable oils containing at least 2-66% erucic acid residues, mustard (Brassica) species, rape (high-erucic) species, Crambe abyssinica or naturally occurring jojoba.
6. A method as claimed in claim 5 wherein the material containing the precursor further comprises oleic acid ($C_{18:1}$), eicosenoic acid ($C_{20:1}$), erucic acid ($C_{22:1}$), nervonic acid ($C_{24:1}$), linoleic acid ($C_{18:2}$), or a mixture of one or more thereof.
7. A method as claimed in any one of claims 1-4 wherein the material containing the precursor is mustard seed oil or oil extracted from mustard seed.
8. A method as claimed in any one of the preceding claims wherein the transesterification reaction mixture is heated for up to six hours at a temperature of up to 110°C .

9. A method as claimed in any one of the preceding claims wherein the solvent for the transesterification reaction is water.

5 10. A method as claimed in any one of the preceding claims wherein the compound is extracted from the transesterification reaction mixture by a solvent.

11. A method as claimed in claim 10 wherein the solvent is hexane or petroleum.

10 12. A method as claimed in any one of the preceding claims wherein the acid catalyst is sulphuric acid.

13. A method as claimed in claim 12 wherein the sulphuric acid is concentrated sulphuric acid.

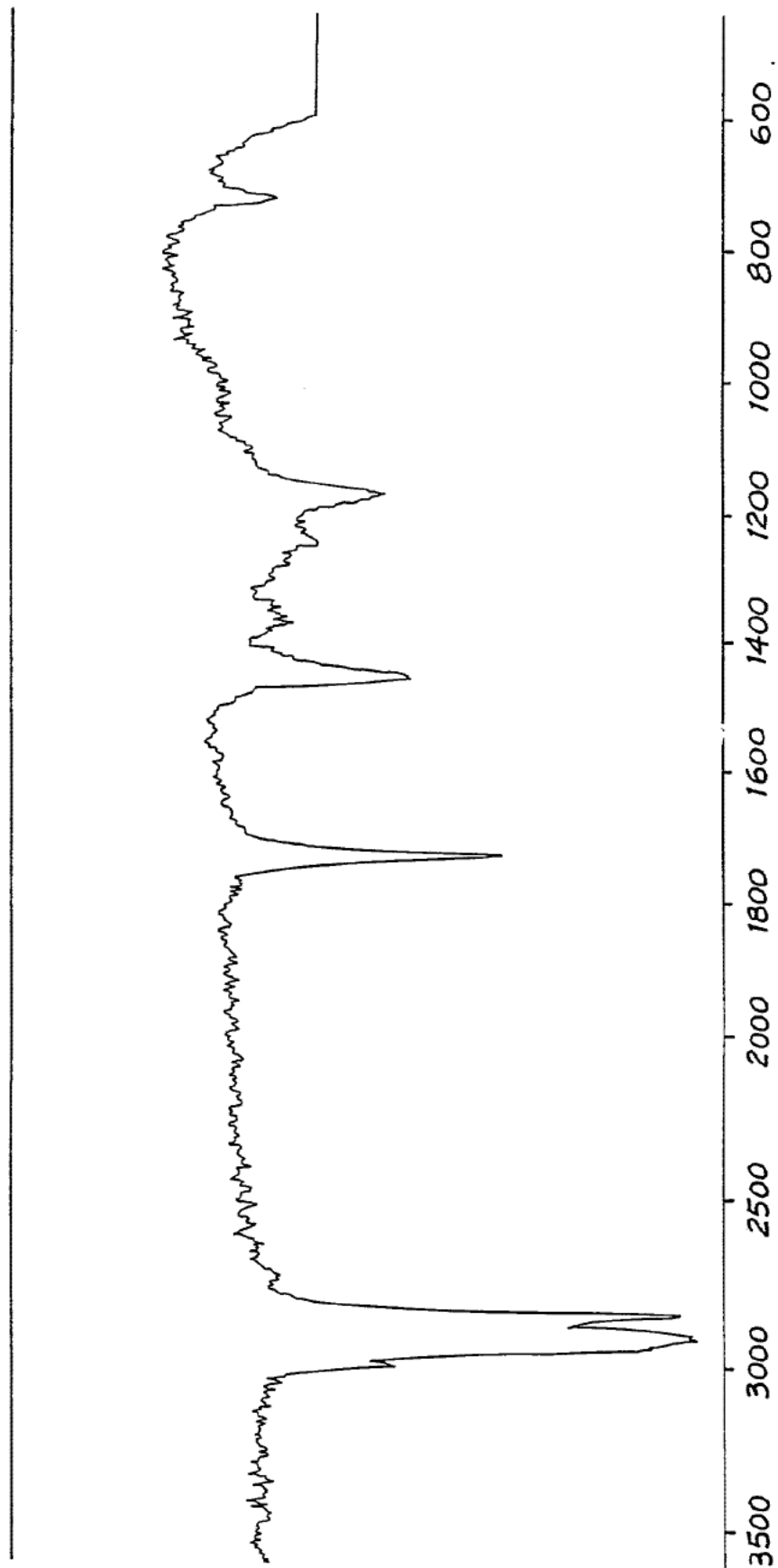
15 14. A method of producing an artificially synthesised compound, the properties of which in many respects are analogous to the corresponding properties of naturally occurring jojoba oil substantially as described herein with reference to any one of the Examples.

20 15. An artificially synthesized compound, the properties of which in many respects are analogous to the corresponding properties of naturally occurring jojoba oil, having substantially the infra-red spectrograph as shown in Figure 1.

25 16. An artificially synthesised compound, the properties of which in many respects are analogous to the corresponding properties of naturally occurring jojoba oil, prepared by a method as claimed in any one of claims 1-14.

30 17. An artificially synthesised compound, the properties of which in many respects are analogous to the corresponding properties of naturally occurring jojoba oil substantially as described herein with reference to any one of the Examples.

35 18. An artificially synthesised compound as claimed in any one of claims 15-17 having the empirical formula $C_{40}H_{78}O_2$.

*FIG. 1***SUBSTITUTE SHEET**

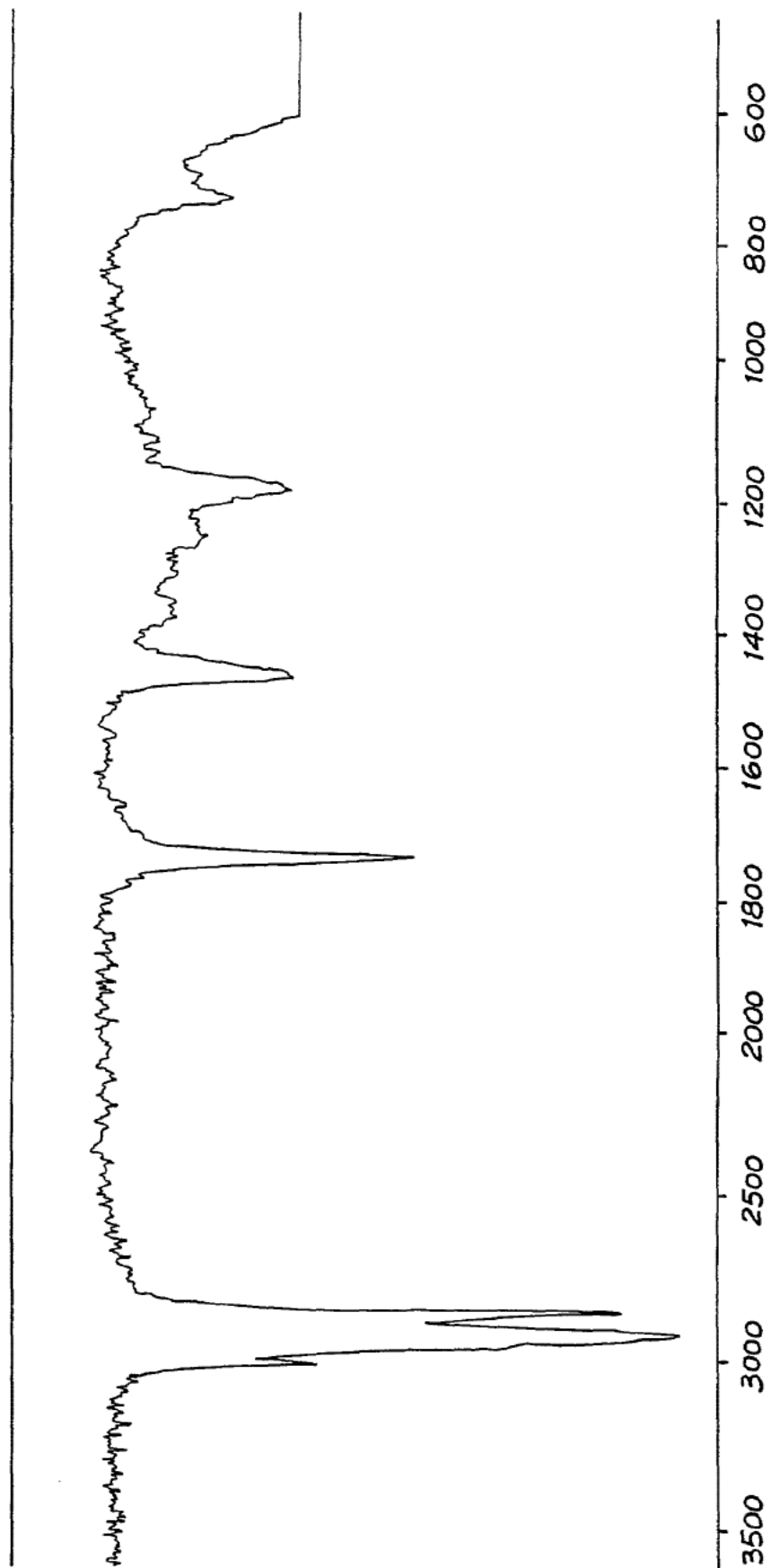


FIG. 2

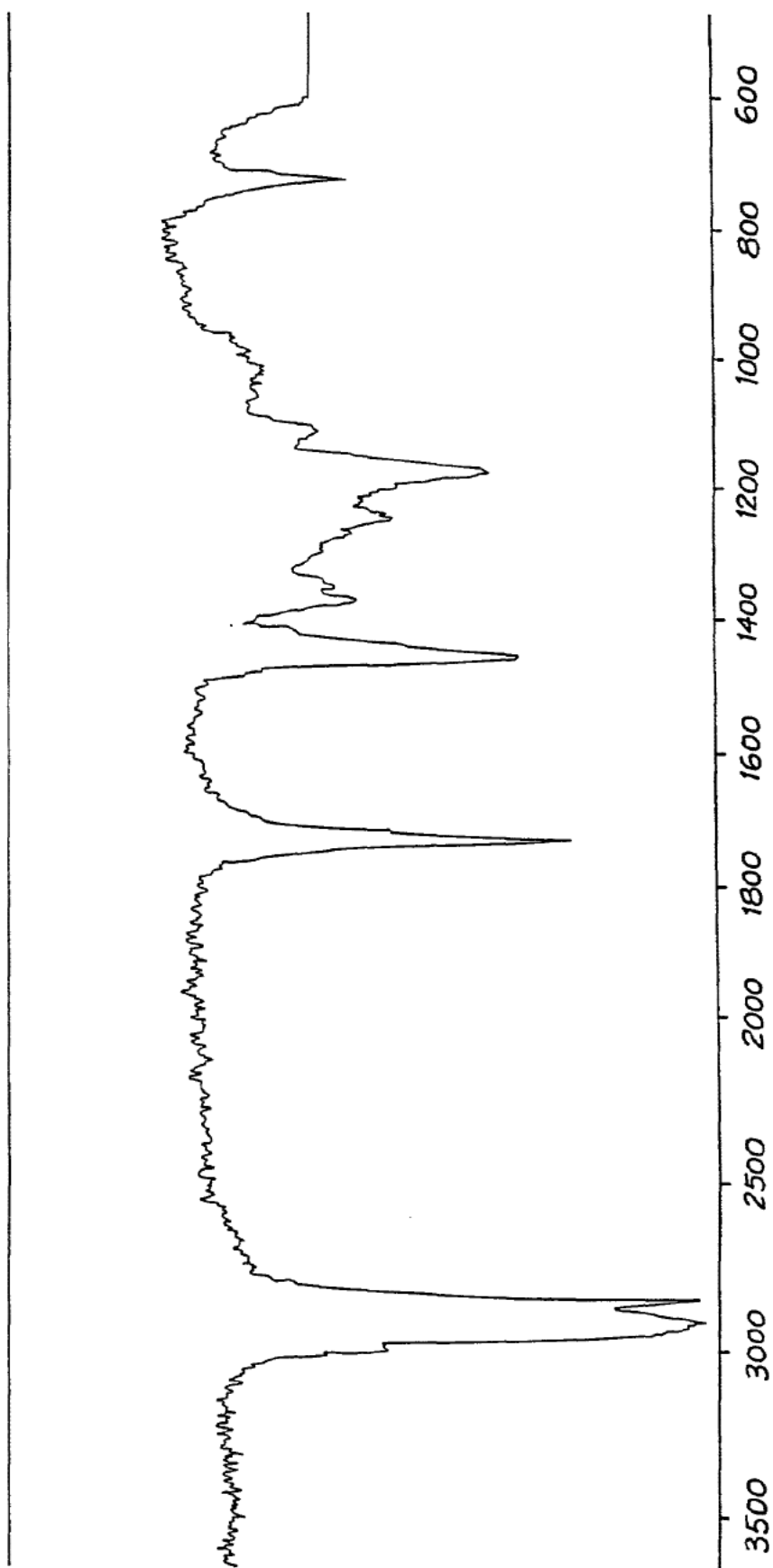
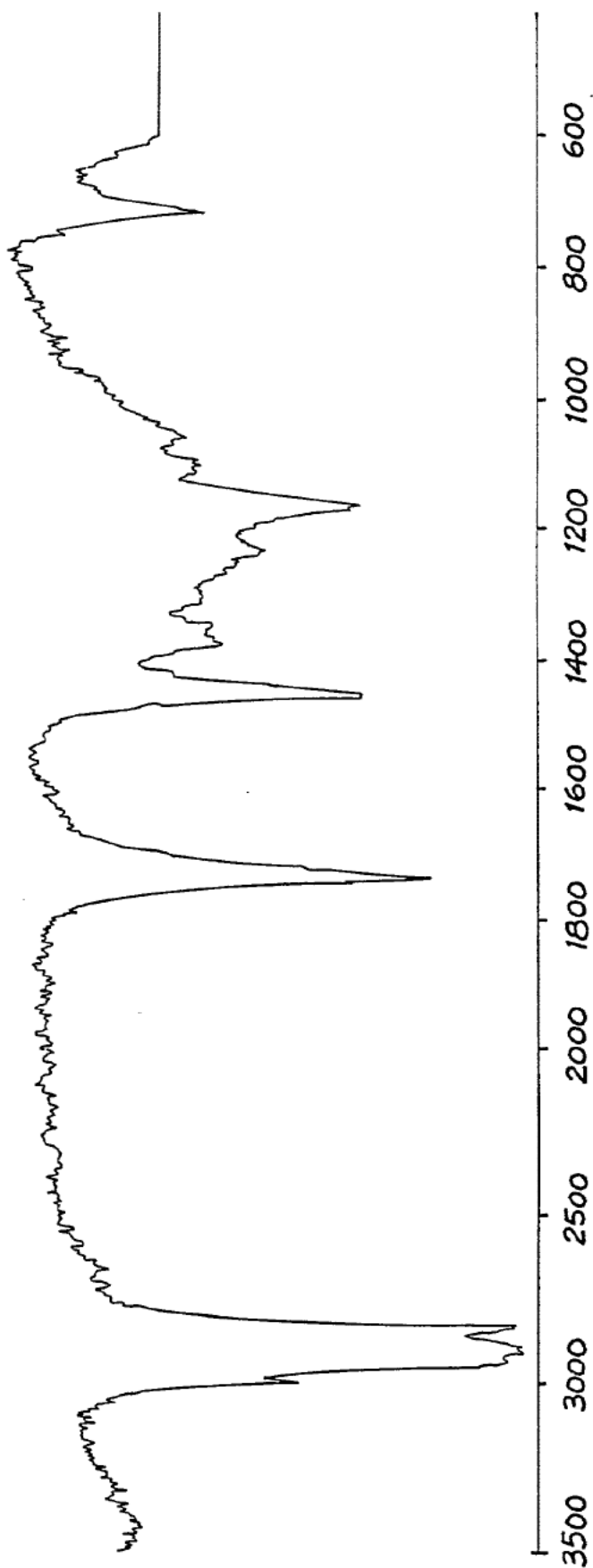


FIG. 3

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*FIG. 4***SUBSTITUTE SHEET**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int. Cl. ⁵ C11C 3/10, C07C 067/02, 069/533		
II. FIELDS SEARCHED		
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Classification System	Classification Symbols	
IPC ³⁻⁵ IPC ²	C11C 3/10, C07C 067/02, 069/533 C07C 069/52, 069/53	
Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched 8		
III. DOCUMENTS CONSIDERED TO BE RELEVANT 9		
Category*	Citation of Document, ¹¹ with indication, ¹² where appropriate, of the relevant passages	Relevant to Claim No 13
X	AU,B, 11275/83 (561896) (DYNAMIT NOBEL AG) 15 September 1983 (15.09.83) whole document	1, 2, 4, 10, 16-18
X	AU,A, 43950/72 (EMERY INDUSTRIES) 3 January 1974 (03.01.74) page 4 line 6-page 7 line 6	1-3, 5-6, 16
X	EP,A, 69946 (CIRTA) 19 January 1983 (19.01.83) whole document	1-7, 16-18
X	EP,A, 165457 (HENKEL KGaA) 27 December 1985 (27.12.85) whole document	1, 2, 16
X	EP,A, 195675 (KAO CORP) 24 September 1986, (24.09.86) page 4 line 5- page 6 line 14	1-4, 16, 17
X	Industrial and Engineering Chemistry Research, Volume 27, no. 11, 1988, pages 2179-2182, (Washington, USA) Martinez et al, "Synthesis of Esters of High Molecular Weight. An Analogue of Jojoba Oil. A Statistical Approach"	1, 16
X	Patent Abstracts of Japan, C-197, page 40, JP,A, 58-148836 (SHINEI KAGAKU K K) 5 September 1983 (05.09.83) (continued)	1-4, 16-17
* Special categories of cited documents: 10 "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention		
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X,Y	GB,A, 2106507 (SCHER CHEMICALS INC) 13 April 1983 (13.04.83) whole document	1-7, 16-18
X,Y	Journal of Agricultural Food Chemistry Volume 36, 1988, pages 1333-1336, (Washington, USA), Mukherjee et al, "Preparation of Esters Resembling Natural Waxes by Lipase-Catalyzed Reactions"	1-7, 16

V.[] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:
2. [] Claim numbers ..., because they relate to parts of the international application that do comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. [] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI.[] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1. [] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- [] The additional search fees were accompanied by applicant's protest.
 [] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 90/253

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Members			
AU 11275/83	BR 8301219 DK 1181/83 JP 58167543	CA 1215072 EP 88895 US 4510093	DE 3208930 ES 520491		
AU 43950/72	BE 785416 FR 2144334 JP 48056706	CA 1111017 GB 1379105 US 3740333	DE 2232323 IT 965846		
EP 69946	DE 3270747 JP 28065794	EP 69946	FR 2509322		
EP 165457	DE 3418887	JP 60260508			
EP 195675	CA 1258008 US 4675250	DE 3664969 JP 62001118	ES 553164 JP 62001119		
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(54) Title: BIODIESEL PRODUCTION

(57) Abstract: A process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant. The process includes treating a feedstock containing glycerides and/or free fatty acids with a lipase, a lower alkyl alcohol and an acid catalyst under conditions to produce said alkyl fatty acid esters.

WO 2008/092207 A1

BIODIESEL PRODUCTION

This application claims priority from Australian provisional patent application No. 2007900488 filed on 2 February 2007, the contents of which are to be taken as
5 incorporated herein by this reference.

Field of the Invention

The present invention relates to processes for the production of alkyl fatty acid ester
10 mixtures that are suitable for use as fuels, such as biodiesel, fuel additives and lubricants. The present invention also relates to fuels, such as biodiesel, produced using the processes, and to systems for producing the fuels.

Background of the Invention

15 Over 85% of the energy demands of modern society are met by the combustion of fossil fuels, such as coal, oil, and natural gas. However, fossil fuels are a non-renewable source of energy and our supply of these fuels has a finite end.

20 The environmental, health, political and social impacts of our reliance on fossil fuels are well known. The burning of fossil fuels by humans is the largest source of emissions of carbon dioxide, which is one of the greenhouse gases that contributes to global warming.

25 Alternative forms of energy are needed to help meet the increased global energy needs. Examples of alternative energy forms that are being investigated include nuclear energy, hydrogen fuels, and solar energy. However, each of these alternatives requires substantive redevelopment of infrastructure to enable their sustained and economical use.

30 Biofuels are an alternative to fossil fuels that do not have the same negative environmental impacts as fossil fuels because they are derived from atmospheric carbon dioxide, and thus do not increase the net amount of carbon dioxide in the atmosphere. Furthermore, the use of biofuels may not require substantive changes to
35 existing infrastructure and machinery as may be required from some other alternative energy sources.

Biodiesel is a biodegradable transportation fuel for use in diesel engines that is produced from plant- or animal-derived oils or fats. Biodiesel is used as a component of diesel fuel or as a replacement for diesel fuel. Biodiesel can be readily used in diesel-engine vehicles, which distinguishes biodiesel from the straight vegetable oils (SVO) or waste vegetable oils (WVO) used as fuels in some modified diesel vehicles. Biodiesel is biodegradable and non-toxic, and has significantly fewer emissions than petroleum-based diesel when burned and, therefore, its use can result in substantial environmental benefits.

Biodiesel manufactured from animal-derived fats show greater lubricity indexes than mineral- or plant-derived diesel/biodiesel fuels or mixtures, having a significant influence on engine maintenance by reducing engine component wear (for example see "Diesel Fuel Lubricity Reviewed", Paul Lacey, Sowthwest Research Institute, Steve Howell, MARC-IV Consulting, Inc., SAE paper 982567, International Fall Fuels and Lubricants Congress and Exposition, Oct. 19-22, 1998, San Francisco, California.)

Furthermore, biodiesel manufactured from animal-derived fats and high saturated plant fats have Cetane Index levels in excess of the ASTM D6751-07B Biodiesel Standard, which specifies a minimum requirement of a Cetane No. of 47. The average Cetane Index levels indicated by numerous studies by Universities and Research Institutes world wide indicate Cetane Numbers for biodiesel from animal fats ranging from approx 72 for biodiesel from lard to 61 for yellow grease biodiesel (for example see National Biodiesel Board "NBB, 2005a).

The use of biodiesel as an alternative to petroleum diesel is widely welcomed by environmental groups and the community. Biodiesel is Europe's dominant renewable fuel and, as part of a range of measures to reduce greenhouse gas emissions, the European Union is encouraging the use of biofuels. For example, the 2003 EU Biofuels Directive requires 5.75% of the energy for transport to come from renewable sources by the end of 2010, rising to 20% by 2020.

Biodiesel is comprised of a mix of mono-alkyl esters of long chain fatty acids, and is typically produced by transesterification of vegetable oil or animal fat with methanol using acid or basic catalysts. For example, International patent application

WO03/022961 describes a method for producing biofuel from waste oil or oil by-products by transesterification with methanol in the presence of sulfuric acid. In another example, International patent application WO2006/128881 describes a method for producing biodiesel from rapeseed oil or sunflower oil by transesterification with methanol in the presence of sodium hydroxide. There have also been suggestions that biodiesel can be produced from vegetable oils by an enzyme (*Candida cylindracea*) catalysed transesterification reaction (for example see published United States patent application 2005/0084941).

Whilst the integration of biodiesel as a transport fuel has generally been successful, there remain some problems with its use. For example, one of the physical properties of biodiesel that can lead to problems in use is the temperature at which the fuel starts to gel (the 'gel temperature'). The viscosity of biodiesel increases as it is cooled and the solution eventually crystallizes. Eventually, the crystals formed become large enough to block the pores of a fuel filter. The gel temperature of pure biodiesel varies depending on the glyceride composition from which it is formed and, therefore, the feedstock oil used to produce the biodiesel. Generally, the higher the saturated fatty acid content of the glyceride composition, the higher the gel temperature of the biodiesel formed from that material. For example, biodiesel produced from tallow, palm oil or coconut oil using the prior art methods tends to gel at around +15 to 16 °C. Many of these biodiesel fuels also have poor long term stability.

There is a need for processes for the production of new biodiesel fuels that do not have some of the disadvantages of prior art biodiesel fuels.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Summary of the Invention

The present invention arises out of studies into processes for producing biodiesel from beef tallow. We have found that beef tallow, and other suitable animal fats and plant oils, can be treated with lipases and transesterified with ethanol or other lower alkyl

alcohols to produce a biodiesel fuel that has superior properties to many currently available biodiesel fuels.

5 The present invention provides a process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant, the process including treating a feedstock containing glycerides and/or free fatty acids with a lipase, a lower alkyl alcohol and an acid catalyst under conditions to produce said alkyl fatty acid esters.

10 The feedstock containing glycerides and/or free fatty acids may be initially treated with the lipase in the presence of the lower alkyl alcohol to produce a first alkyl fatty acid ester mixture which is subsequently reacted with the lower alkyl alcohol in the presence of the acid catalyst to produce a second alkyl fatty acid ester mixture which is suitable for use as a fuel and/or lubricant.

15 The present invention also provides an alkyl fatty acid ester mixture prepared according to the process of the invention.

20 The present invention further provides a biodiesel fuel prepared according to the process of the invention.

The present invention also provides a system for use in the process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant according to the invention.

25 The feedstock containing glycerides and/or free fatty acids may be an animal fat or plant oil. Indeed, the process of the present invention is particularly suitable for producing biodiesel from beef tallow.

30 The lipase may be a latex lipase and/or a plant lipase from a high lipid content grain, legume or seed. The latex lipase from the papaya plant is suitable in combination with a plant lipase from rice bran. The active latex lipase may be a papain lipase, such as *Carica papaya* latex lipase, and the plant lipase may be rice bran lipase. However, it will also be appreciated that the latex lipase and/or the plant lipase may not be a single lipase and may indeed be a mixture of lipases. The active lipases may
35 be partially or wholly purified and used in the process of the present invention. Latex lipases and plant lipases from a number of other plant species may also be used in

the process of the present invention. The lipase may also be a suitable lipase of microbial origin.

Without intending to be bound by any specific theory, we believe that the lipase may display some selectivity for smaller chain length fatty acids in the feedstock. The lipase may also catalyse the transesterification of the naturally occurring glycerides in the plant oil or animal fat. Furthermore, the effect of the acid catalyst is to further assist in the transesterification reaction. We believe that the effect of the lipase and acid catalyst is to provide a biodiesel fuel product that is stable and which has a lower gel temperature than an equivalent product that is formed without the use of the lipase and acid catalyst combination.

The lipase may be immobilized on a substrate. For example, the latex lipase may be immobilised on a substrate formed from a saccharide and lignite. In an embodiment of the invention, the substrate is a pyrolised mixture of a saccharide and an acid precipitate of an alkaline lignite extract.

The lower alkyl alcohol may be selected from one or more of the group consisting of: methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol. In an embodiment of the invention, the lower alkyl alcohol is ethanol.

The acid catalyst may be a mineral acid, such as sulphuric acid.

The reaction of the first alkyl fatty acid ester mixture with the lower alkyl alcohol and the acid catalyst may be carried out under continuous reactive distillation conditions whereby any unreacted lower alkyl alcohol that is distilled from the reaction is returned to the reaction mixture and the second alkyl fatty acid ester mixture is distilled from the reaction mixture.

The distilled second alkyl fatty acid ester mixture may be treated, such as by gravity separation, to remove unreacted glycerine and soap. The purified second alkyl fatty acid ester mixture thus obtained may be used as a biofuel. However, we have also found that it is advantageous to mix the second alkyl fatty acid ester mixture with a rice bran oil ester mixture to form an alkyl fatty acid ester mixture suitable for use as a fuel. The rice bran oil ester mixture may be prepared by reacting rice bran oil with a lower alkyl alcohol in the presence of methane sulphonic acid. The lower alkyl alcohol

may be n-butanol. The n-butanol may be formed by enzymatic hydrolysis of the glycerine and soap mixture that is separated from the distilled second alkyl fatty acid mixture.

5 Brief Description of the Figures

The present invention will now be described in relation to various embodiments of which some aspects are illustrated in the accompanying figures. In the figures:

10 Figure 1 is a flow diagram showing details of a process for producing biodiesel in accordance with an embodiment of the present invention.

Figure 2(a) is a first part of a flow diagram showing details of an early part of a process for producing biodiesel in accordance with an embodiment of
15 the present invention.

Figure 2(b) is a second part of a flow diagram that is a continuation of Figure 2(a) showing details of a later part of the process shown in Figure 2(a).

Description of Embodiments of the Invention

20

Various embodiments of the present invention will now be described in more detail. However, it must be appreciated that the following description is not to limit the generality of the above description. Numerals corresponding to reference numerals used in Figures 1 to 2 are used in the following description.

25

The present invention provides a process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant. The process is particularly useful for the production of high quality biodiesel from relatively low grade feedstocks.

30 The process includes treating a feedstock 12 containing glycerides and/or free fatty acids with a lipase 14, a lower alkyl alcohol 16 and an acid catalyst 18 under conditions to produce alkyl fatty acid esters.

The feedstock 12 containing glycerides and/or free fatty acids is treated with the
35 lipase 14, the lower alkyl alcohol 16 and the acid catalyst 18 sequentially. In the embodiment that is illustrated in Figure 1, the feedstock 12 is reacted in a first step

with the lipase 14 in the presence of the lower alkyl alcohol 16 to produce a first alkyl fatty acid ester mixture 20 which is then reacted with the lower alkyl alcohol 16 in the presence of the acid catalyst 18 to produce a second alkyl fatty acid ester mixture 46.

5 Glyceride containing plant oils or animal fats are particularly suitable as the feedstock
12. As used herein the term "glyceride containing plant oil or animal fat" means any
oil or fat product of plant or animal origin that contains esters formed from glycerol and
fatty acids (i.e. glycerides). Glycerol has three hydroxyl functional groups which can
be esterified with one, two or three fatty acids to form monoglycerides, diglycerides,
10 and triglycerides, respectively. Plant oils and animal fats contain mostly triglycerides,
although they typically also contain some monoglycerides and diglycerides. The
glyceride containing plant oil or animal fat may be selected from the group consisting
of: animal tallow, vegetable oils, used cooking oils and fats, seeds, seed residue
feedstocks, and grease trap oils.

15 Animal fats are fats obtained from animal sources. Suitable animal fats that may be
used in the production of biodiesel include: tallow (beef fat), lard (pork fat), schmaltz
(chicken fat), blubber, cod liver oil, yellow grease, and the by-products of the
production of omega-3 fatty acids from fish oil. For example, we have found that the
20 process of the present invention is particularly suitable for converting tallow to
biodiesel fuel.

Suitable vegetable oils that may be used in the production of biodiesel include:
rapeseed oil, soybean oil, palm oil, mustard oil, castor oil, coconut oil (copra oil), corn
25 oil, cottonseed oil, false flax oil, hemp oil, peanut oil, radish oil, ramtil oil, rice bran oil,
safflower oil, sunflower oil, tung oil, algae oil, copaiba oil, honge oil, jatropha oil, jojoba
oil, milk bush oil, petroleum nut oil, walnut oil, sunflower oil, dammar oil, linseed oil,
poppyseed oil, stillingia oil, vernonia oil, amur cork tree fruit oil, apple seed oil,
balanos oil, bladderpod oil, brucea javanica oil, burdock oil (bur oil), candlenut oil
30 (kukui nut oil), carrot seed oil, chaulmoogra oil, crambe oil, cuphea oil, lemon oil,
orange oil, mango oil, mowrah butter, neem oil, rosehip seed oil, sea buckthorn oil,
shea butter, snowball seed oil (viburnum oil), tall oil, tamanu oil, and tonka bean oil
(cumaru oil).

35 The lower alkyl alcohol 16 may be a C₁-C₆ alcohol. In an embodiment of the invention
the lower alkyl alcohol 16 is selected from one or more of the group consisting of:

methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol. Methanol is an alcohol that is commonly used in biodiesel production but we have found ethanol to be particularly suitable for use in the process of the present invention. The ratio of lower alkyl alcohol 16 to glyceride containing plant oils or animal fats 12 in the combined stream 28 is about 1:1 mol ratio.

A feedstock supply stream 22 of the feedstock 12 containing glycerides and/or free fatty acids is fed through a flow control device 24 and is combined with a regulated stream 26 of the lower alkyl alcohol 16, which is fed from an injection pump. The combined stream 28 is mixed via a static mixer 30. The combined stream 28 containing lower alkyl alcohol 16 and plant oil or animal fat 12 is reacted in the presence of the lipase 14 in a suitable reaction vessel 32. The reaction vessel 32 may be temperature controlled.

The lipase 14 may be a latex lipase and/or a plant lipase from a high lipid content grain, legume or seed. In an embodiment of the invention the lipase 14 is a combination of a latex lipase and a plant lipase from a high lipid content grain, legume or seed. As used herein, the term "lipase" means an enzyme that catalyzes the hydrolysis of triglycerides. The term "latex lipase" means a lipase found naturally in the latex of a plant. Many plant species have a latex that contains a lipase. Such plant species include, without limitation, papaya, mango, sapote, mulberry, fig, kiwifruit, and blackberry. The latex lipase may be used as a crude mixture, a semi-purified mixture, or the lipase may be purified. The latex can be collected from plants using standard methods, such as tapping the fruits of the plant and collecting the exudate latex. The latex may be frozen for storage and/or it may be freeze dried to obtain a crude solid latex lipase preparation suitable for use. Alternatively, the latex lipase may be purified using known protein purification techniques, such as chromatography.

In an embodiment of the invention the latex lipase from the papaya plant is used. Latex from papaya is used in the food and beverage industries in the form of a protease preparation, papain. Papain (EC 3.4.22.2) from papaya latex (CAS Number: 9001-73-4) is commercially available in crude form or in a lyophilized form from Sigma-Aldrich (for example under Catalog No. P4762). The active latex lipase may be a papain lipase, such as *Carica papaya* latex lipase.

The term "plant lipase from a high lipid content grain, legume or seed" means a lipase found naturally in any grain, legume or seed having a relatively high lipid content. Such grains, legumes or seeds include, without limitation, castor beans, rice bran, wheat bran, oat bran, rye, oily legumes, and oily seeds (such as canola). The rice
5 bran lipase may be used in the form of ground rice bran, or as a semi-purified or purified preparation. Methods for the extraction of lipases from rice bran are known (for example see Prabhu, A.V. *et al.*, Biotechnol Prog. 1999;15(6):1083-9).

10 The lipase used may not be a single or homogenous lipase and may indeed be a mixture of different lipases. The lipase may also contain some microbial lipases. Furthermore, the active lipases may be partially or wholly purified and used in the process of the present invention.

15 The lipase 14 may be immobilised on a substrate. An important factor for the economic efficiency of an enzymatic process is a suitable method for the immobilisation of the enzyme in order to allow ready recovery and multiple use of the enzyme as well as to achieve, if possible, an increase of its stability under the reaction conditions. For a review on immobilisation of lipases see, for example F. X. Malcata
20 *et al.*, J. Am. Oil Chem. Soc. 1990, 67, 890-910.

In the illustrated embodiments of the present invention the lipase 14 is introduced into the combined stream 28 via a static mixer 34 and fed into the reaction vessel 32. The ratio of lipase to glycerol ester may be about 1:10 w/w to about 1:30 w/w. The reactants are continuously mixed and the reaction is carried out at a temperature of
25 from about 30°C to about 70°C. The reaction may be carried out for a time period of from about 30 minutes to about 90 minutes.

After the required time in the reaction vessel 32 the first alkyl fatty acid ester mixture 20 is pumped from the reaction vessel 32 through a flow control valve 38. The first
30 alkyl fatty acid ester mixture 20 may be separated from solid material, such as immobilised lipase 14. Separation of the first alkyl fatty acid ester mixture 20 from immobilised lipase 14 and/or other solid material may be accomplished using any suitable technique, including (but not limited to) mechanical separation, filtration, gravity separation, centrifugation, etc. In one embodiment of the invention the
35 separation is achieved by centrifugation. Thus, the first alkyl fatty acid ester mixture 20 may be pumped from the reaction vessel 32 through a flow control valve 38 to a

centrifuge 40. The purified first alkyl fatty acid ester mixture 20 may be drawn from the centrifuge in the usual manner. The centrifuge reject 41 may be pumped to a stripping tank 43, where the immobilized lipases may be extracted and re-cycled back to the reaction vessel 32.

5

The purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation is then reacted with the lower alkyl alcohol 16 in the presence of the acid 18 to form a second alkyl fatty acid ester mixture 46. The lower alkyl alcohol 16 may be any of the alcohols discussed previously herein and in one embodiment it is ethanol. The acid catalyst 18 may be any suitable mineral acid, such as hydrochloric acid, sulphuric acid, etc. In one embodiment of the invention the mineral acid is sulphuric acid.

The reaction of the purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation with the lower alkyl alcohol 16 in the presence of a mineral acid 18 may be carried out under suitable conditions. The lower alkyl alcohol and the mineral acid may be mixed in a ratio of about 1.5% mineral acid w/w of glycerol ester, in volume of lower alkyl alcohol that is calculated at a 12:1 molar ratio of the glycerol ester. Typically, the reaction will require heating. In one embodiment of the invention the reaction is carried out under reactive distillation conditions, such as continuous reactive distillation conditions. Thus, the purified alkyl fatty acid ester mixture 20, lower alkyl alcohol 16, and the mineral acid 18 are fed through a heated static mixer 48 into a reactive distillation tower 50. The heated static mixer 50 may be at a temperature of about 50°C to about 70°C. In one embodiment of the invention the heated static mixer is at a temperature of about 60°C.

25

Continuous reactive distillation is carried out in a tower and reboiler arrangement 50 with the reacting product flowing across a plate system via downcomers to the reboiler. We have found that a plate system comprising plates formed from expanded mesh is particularly effective. The expanded mesh maybe stainless steel mesh, zirconium coated steel mesh or nickel type steel mesh. Using this system we have been able to achieve 97% conversion. In the reboiler 50, the reaction is carried out a temperature of about 110°C to about 180°C. In one embodiment of the invention the reaction is carried out a temperature of about 125°C to about 165°C. Unreacted lower alkyl alcohol is vaporised off and flows up and is condensed and recycled back into a heated static mixer 48 inflow. In this way unreacted lower alkyl alcohol that is distilled

35

from the reaction is returned to the reaction mixture. The second alkyl fatty acid ester mixture 46 is distilled from the reaction mixture.

In an embodiment of the invention that is illustrated in Figures 2(a) and 2(b), the lipase 14 is a combination of a latex lipase 14a in combination with a plant lipase from a high lipid content grain, legume or seed 14b. We have found that a latex lipase 14a from the papaya plant in combination of a plant lipase 14b from rice bran is effective.

The latex lipase 14a may be immobilised on a substrate. We have found that a substrate formed from a saccharide and lignite is particularly suitable. The lignite may be an acid precipitate from an alkaline extract of lignite. We have found that a micronised, pyrolised mixture of a saccharide and the lignite extract is particularly suitable as a substrate for the papaya latex lipase 14a. The substrate may be formed as follows. Lignite is firstly micronised and the acid content of the lignite (e.g. humic acids, fulvic acids, etc) is then extracted under alkaline conditions. Suitable bases for the alkaline extraction include potassium hydroxide, magnesium hydroxide, and magnesium sulphate. The pH of the resultant extract is then adjusted to about 3 to about 5. We have found that a pH of about 4.0 to about 4.5 is particularly suitable. In this way the extracted acid content of the lignite precipitates and the precipitate is removed by filtration. The isolated precipitate is then mixed with a saccharide, such as (but not limited to) molasses, sucrose, etc, and the mixture is pyrolised at a temperature of between about 240°C and about 800°C. The pyrolised mixture of saccharide and lignite extract thus formed is then used as a substrate for the papaya latex lipase.

As best seen in Figure 2(a), the papaya latex lipase 14a and plant lipase from a high lipid content grain, legume or seed 14b (in the form of rice bran 19) are introduced into the combined stream 28 via a static mixer 30 and fed into the reaction vessel 32 containing the lower alkyl alcohol 16 and plant oil or animal fat 12. The rice bran 19 may be a rice bran residue that is from the in-situ esterification of the rice bran oil in a process that is described later.

The ratio of papaya latex lipase 14a to plant oil or animal fat 12 may be about 1:10 w/w to about 1:30 w/w. In one embodiment of the invention the ratio of papaya latex lipase 14a to plant oil or animal fat 12 is about 1:20 w/w. The reactants are continuously mixed and the reaction is carried out at a temperature of from about

30°C to about 70°C. In one embodiment of the invention the reaction is carried out at a temperature of about 50°C. The reaction may be carried out for a time period of from about 30 minutes to about 90 minutes. In one embodiment of the invention the reaction is carried out for a time period of about 60 minutes.

5

After the required time in the reaction vessel 32 the first alkyl fatty acid ester mixture 20 is separated from solid material, such as immobilised lipase 14a and rice bran 19, by centrifugation as described previously. The purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation is then reacted with the lower alkyl
10 alcohol 16 in the presence of the acid 18 to form a second alkyl fatty acid ester mixture 46 as described previously. The second alkyl fatty acid ester mixture 46 is distilled from the reaction mixture and is continuously drawn from the reboiler 50 by a pump 52 through a flow control valve 54.

15 The second alkyl fatty acid ester mixture 46 may be used as is or purified for use as a biofuel, or it may be further treated. For example, the distilled second alkyl fatty acid ester mixture may be treated to remove unreacted glycerine. The treatment may comprise gravity separation. Thus, the second alkyl fatty acid ester mixture 46 may be drawn from the reboiler 50 by a pump 52 through a flow control valve 54 to a static
20 glycerin/alkyl ester separator vessel 56. The separator vessel 56 is used to separate raw glycerin and un-reacted glycerol esters 58 from alkyl fatty acid ester mixture 46, which are drawn off continuously from the top layers of the separator vessel 56.

The raw glycerin and un-reacted glycerol esters 58 (i.e. the bottoms from the
25 separating vessel 56) may be recycled. For example, unreacted glycerol esters and unreacted ethanol may be separated from raw glycerin and soap based on the specific gravity of each component. Thus, the raw glycerin and un-reacted glycerol esters 58 may be pumped from the separating vessel 58 to a centrifuge 60 where they are separated based on specific gravity. The centrifuge extract 62 (i.e. primarily
30 unreacted glycerol esters and unreacted ethanol) may be pumped back to the reboiler 50.

The centrifuge reject 64 (i.e. primarily raw glycerin and soap) may be converted to butanol by fermentation. As illustrated in Figure 2(b), the centrifuge reject 64 may be
35 mixed with an appropriate enzyme and pumped via flow control valve 66 to a multi compartmented rotating biological disc bioreactor 68 with immobilized specific

bacteria and fermented to alcohols. Butanol is preferably produced using this method. Many microorganisms are known to produce butanol through fermentation. Several *Clostridium* spp (e.g. *Clostridium acetobutylicum*, *Clostridium beijerinckii* etc.) have been shown to catalyse the production of butanol may be used (see Jones, D.T. and Woods, D.R. Microbiol Rev. 1986 December; 50(4): 484–524). Other enzymes that could be used for this transformation include purified or crude mixtures containing microbial and/or fungal dehydrogenases. The alcohol produced may be distilled via a distillation apparatus 70 and used in the reactions discussed in more detail below.

10 We have found that a superior biodiesel product is formed if the alkyl fatty acid ester mixture 46 that has been separated from raw glycerin and un-reacted glycerol esters 58 is mixed with esters of rice bran oil.

15 Rice bran oil esters may be prepared using any suitable esterification or transesterification technique. However, we have found that it is advantageous to prepare rice bran oil esters using a sulphonic acid as the catalyst for the transesterification reaction. Suitable sulphonic acids include alkyl sulphonic acids, such as methane sulphonic acids. Thus, rice bran may be reacted with a lower alkyl alcohol in the presence of methane sulphonic acid to form a rice bran oil ester mixture. The lower alkyl alcohol may be selected from one or more of the group consisting of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol. Preferably, the lower alkyl alcohol is n-butanol. The n-butanol may be formed by enzymatic hydrolysis of a glycerine and soap mixture that is formed during the production of the second alkyl fatty acid mixture, as described previously.

25 Rice bran 19 with an oil content of about 19 to about 23% w/w is continuously charged into a reactor vessel 74 and mixed with a regulated flow of butanol 76 mixed with methane sulphonic acid 78. The butanol 76 and methane sulphonic acid 78 are pumped to a static mixer 80 prior to being injected into the reactor vessel 74. The ratio of methane sulphonic acid to n-butanol that is injected into the reactor vessel 74 may be about 1:40 w/w to about 1:50 w/w. In one embodiment the ratio of methane sulphonic acid to n-butanol is about 1:40 w/w to about 1:50 w/w.

110 Kg of rice bran per 1Kl of alkyl ester may be charged into the reactor vessel 74. The reactor vessel 74 may be a continuously stirred temperature controlled jacketed bio-reactor. The ratio of n-butanol to rice bran may be about 1:3 w/w to about 1:4

w/w. In one embodiment the ratio of n-butanol to rice bran in the starting reaction mixture is about 1:3.5 w/w.

5 The rice bran oil ester mixture 82 may be separated from solid material prior to mixing with the second alkyl fatty acid mixture 46. For example, the rice bran oil ester mixture 82 may be separated from the solid material by centrifugation. Thus, the rice bran oil ester mixture 82 may be drawn off and through a regulating flow control valve 84 to a centrifuge 86. The extract from the centrifuge (i.e. rice bran oil ester mixture 82) may be pumped to a mixing vessel 88 for mixing with the second alkyl fatty acid mixture 46.

Reject 90 from the centrifuge 86 (i.e. primarily rice bran) may be reused in the reaction to produce alkyl fatty acid ester mixture 20.

15 The alkyl fatty acid ester mixture 46, which is drawn off continuously from the top layers of the separator vessel 56, may be mixed with the rice bran oil ester mixture 82 in a static mixer 92 and pumped into a continuously pump mixed vessel 94. The pH of the mixture may be adjusted to about 6 to about 8. In one embodiment of the invention the pH of the mixture is adjusted to about 7.

20 The pH of the second alkyl fatty acid ester and rice bran oil ester mixture can be adjusted by adding an agent 95 selected from the group consisting of: potassium hydroxide, sodium hydroxide, magnesium hydroxide, ammonia, and calcium hydroxide. In one embodiment of the invention the pH of the mixture is adjusted by adding magnesium hydroxide.

The second alkyl fatty acid ester and rice bran oil ester mixture may be treated with a filtering agent 96. Suitable filtering agents 96 may be selected from the group consisting of diatomaceous earth, activated charcoal, and silicates. The silicate may be an aluminosilicate or a magnesium silicate. In one embodiment of the invention the filtering agent 96 is a magnesium silicate. Magnesium silicates are particularly beneficial as they not only act as filtering agent but they also extract residual water from the second alkyl fatty acid ester and rice bran oil ester mixture.

35 The filtering agent 96 may be separated from the second alkyl fatty acid ester and rice bran oil ester mixture by mechanical filtration. For example, the second alkyl fatty

acid ester and rice bran oil ester mixture, filtering agent and reacted pH adjusting agent may be pumped through a continuous belt filter press 97.

5 Filtered alkyl esters are the finished product 98 and may be retained for storage. We have found that the biodiesel produced has a gelling temperature somewhere between about 0°C and about 10°C depending on the starting feedstock material. For example, when the starting feedstock is beef tallow (which is a readily available, inexpensive starting material) we are able to produce biodiesel having a gelling temperature in this range. This is in contrast to prior art biodiesel fuels formed from
10 beef tallow, which we have found have a gelling temperature of about 16°C. Thus, using the processes of the present invention we are able to form a high grade biodiesel fuel from relatively low grade starting materials, such as tallow.

Furthermore, the biodiesel produced using the processes of the present invention has
15 excellent long term stability. For example, we have found that there is no evidence of oxidation of the sample after storage at room temperature in an open vessel for about 12 months. Without intending to be bound by theory, we believe that the oxidative stability of the biodiesel produced using the processes of the present invention may be due, at least in part, to the presence of alpha-tocopherol in the final product. Rice
20 bran is known to contain relatively high levels of alpha-tocopherol, which is a naturally occurring antioxidant. We believe, that at least some of the alpha-tocopherol present in the rice bran 19 used in the initial lipase catalysed reaction and/or the rice bran 74 used in the production of rice bran oil ester mixture 82 is carried through into the finished product 98. It is postulated that the use of methanesulphonic acid as the
25 catalyst in the preparation rice bran esters is advantageous because it does not appreciably degrade the alpha-tocopherol in the same way that some other acid catalysts might.

The process of the present invention may be operated in a continuous, zero-waste
30 manner.

The finished product is suitable for use as biodiesel and may be used as is or in admixture with mineral diesel. For example, prior art biodiesel fuels are typically mixed with mineral diesel in a ratio of 9:1 mineral to biodiesel. This is primarily done
35 because the high gelling point of the biodiesel limits the amount that can be used in

the mixture. However, the biodiesel produced according to the present invention may be mixed with mineral diesel in a ratio of about 7:3.

Advantageously, we have found that the cetane index of a biodiesel product can be increased by adding butyl esters of rice bran oil to the product.

Description of specific embodiment(s)

Example 1 – *Production of biodiesel*

10

Biodiesel is produced using the procedures and apparatus as described previously. Specifically the following steps are carried out.

15 A feedstock supply stream 22 of tallow 12 is fed through a flow control device 24 and is combined with a regulated stream 26 of ethanol 16, which is fed from an injection pump. The combined stream 28 is mixed via a static mixer 30. The combined stream 28 is reacted in the presence of *Carica papaya* latex lipase immobilised on a substrate formed from a saccharide and lignite and rice bran lipase 14 in a temperature controlled reaction vessel 32. The ratio of lipase to tallow is between 1:10 w/w and 20 1:30 w/w. The reactants are continuously mixed and the reaction is carried out at a temperature of between 30°C and 70°C for 30 to 90 minutes.

The reaction mixture (containing the first alkyl fatty acid ester mixture 20) is then pumped from the reaction vessel 32 through a flow control valve 38 to a centrifuge 40 25 where it is separated from solid material, such as immobilised lipase 14. The purified first alkyl fatty acid ester mixture 20 is drawn from the centrifuge in the usual manner. The centrifuge reject 41 is pumped to a stripping tank 43, where the immobilized lipases are extracted and re-cycled back to the reaction vessel 32.

30 The purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation is then reacted with methanol 16 in the presence of sulphuric acid 18 to form a second alkyl fatty acid ester mixture 46. The methanol 16 and sulphuric acid 18 are mixed in a ratio of about 1.5% sulphuric acid w/w, in a volume of methanol that is calculated at a 12:1 molar ratio. The reaction is carried out under continuous reactive distillation 35 conditions at a temperature of about 60°C. The second alkyl fatty acid ester mixture

46 is distilled from the reaction mixture and is continuously drawn from the reboiler 50 by a pump 52 through a flow control valve 54.

5 The second alkyl fatty acid ester mixture 46 is passed in to a static glycerin/alkyl ester separator vessel 56 where raw glycerin and un-reacted glycerol esters 58 are separated from the alkyl fatty acid ester mixture 46 by gravity filtration. The alkyl fatty acid ester mixture 46 is drawn off continuously from the top layers of the separator vessel 56.

10 The raw glycerin and un-reacted glycerol esters 58 (i.e. the bottoms from the separating vessel 56) are recycled by pumping from the separating vessel 58 to a centrifuge 60 where the unreacted glycerol esters and unreacted ethanol are separated from raw glycerin and soap based on the specific gravity of each component. The centrifuge extract 62 (i.e. primarily unreacted glycerol esters and
15 unreacted ethanol) is pumped back to the reboiler 50.

The centrifuge reject 64 (i.e. primarily raw glycerin and soap) is pumped via flow control valve 66 to a multi compartmented rotating biological disc bioreactor 68 containing immobilized *Clostridium* spp bacteria which ferment the material to butanol.
20 The butanol is distilled via a distillation apparatus 70 and used in the production of rice bran oil esters 82.

To produce rice bran oil esters 82, rice bran 74 with an oil content of about 19 to about 23% w/w is continuously charged into a reactor vessel 74 and mixed with a
25 regulated flow of butanol 76 mixed with methanesulphonic acid. The butanol 76 and methane sulphonic acid 78 are pumped to a static mixer 80 prior to being injected into the reactor vessel 74. The ratio of methane sulphonic acid to n-butanol that is injected into the reactor vessel 74 is about 1:40 w/w to about 1:50 w/w. The ratio of n-butanol to rice bran is 1:3 w/w to 1:4 w/w. 110 Kg of rice bran per 1Kl of alkyl ester is
30 charged into the reactor vessel 74. The reactor vessel 74 is continuously stirred and temperature controlled.

The rice bran oil ester mixture 82 is drawn off from the vessel 74 and through a regulating flow control valve 84 to a centrifuge 86. The extract from the centrifuge (i.e.
35 rice bran oil ester mixture 82) is pumped to a mixing vessel 88 for mixing with the

second alkyl fatty acid mixture 46. Reject 90 from the centrifuge 86 (i.e. primarily rice bran) is reused in the reaction to produce alkyl fatty acid ester mixture 20.

5 The alkyl fatty acid ester mixture 46 is mixed with the rice bran oil ester mixture 82 in a static mixer 92 and pumped into a continuously pump mixed vessel 94. The pH of the mixture is adjusted to about to about 7 by adding magnesium hydroxide. Magnesium silicate is then added as a filtering agent and a drying agent and it is then separated from the second alkyl fatty acid ester and rice bran oil ester mixture pumping it through a continuous belt filter press 96.

10

The filtered alkyl esters are the finished product 98 and are retained for storage.

15 Example 2 – *Characterisation of biodiesel*

The alkyl fatty acid ester composition of the finished product 98 obtained from the reaction sequence described were analysed by gas chromatography (GC) essentially as described in British Standard Institution (BSI) reference method BS EN
20 14103:2003; " Fat and oil derivatives-Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl ester contents".

The present invention however relates to the formation of mixtures which are produced by way of transesterification reactions of animal fat and rice bran oil
25 performed in the presence of ethanol and n-butanol, respectively, to produce fatty acyl ethyl esters and fatty alkyl butyl esters, respectively. Therefore in the spirit of the EN 14103:2003 Standard, the present invention reports all information necessary for the complete identification of the sample; the sampling method used; the test method used with reference to the European Standard; and the test results obtained.

30

Both fatty alkyl ethyl esters (FAEE) and fatty alkyl butyl esters (FABE) were analysed using an Agilent 7890A GC system (Agilent Technologies Wilmington, DE, USA), equipped with flame ionization detector (FID) and an HP-Innowax capillary column (1909N-113; J & W Scientific; 30m x 320um x 0.25um) using high-purity Helium as
35 carrier gas. FAEE and FABE were analysed using identical temperature

programming conditions of 140°C for 0min, then 5°C / min to 220°C for 3min, then 20°C / min to 260°C for 8 mins.

FAEEs were identified from their retention times against mixes of commercially
5 obtained FAEE standards (Nu-Chek Prep, Inc., Elysian, MN, USA) which were
aliquoted in heptane containing 0.005% (w/v) butylated hydroxy anisole as
antioxidant. FABEs were identified from their retention times against FABE mixes
prepared from commercially available triacylglycerides (TAG) (Nu-Chek). To prepare
FABEs, 10mg of homogeneous acyl fatty acid (with respect to the sn-1, sn-2 and sn-3
10 positions) TAGs dissolved in chloroform / methanol (9:1; v/v, containing 0.005% (w/v)
butylated hydroxy anisole as antioxidant), were dried under nitrogen and reacted with
2ml of 2.5% (v/v) methanesulfonic acid in n-butanol (final ratio methanesulfonic acid to
n-butanol (1:40; v/v)) for 3 hours at 80°C. After cooling, FABEs were extracted in the
heptane phase by separation of the phases using the ratio of (approximately), n-
15 butanol : water : methanol : heptane of 1:1:1.2:1.3 (by volume) or by selectively
breaking the phase by changing the proportions of each solvent as required on a
sample by sample basis. The FABEs in the heptane phase were dried overnight
using anhydrous sodium sulphate and then each FABE made up in 2ml heptane,
capped under nitrogen. For both the FAEEs and the FABEs, appropriate mixes of
20 individual esters were prepared to enable identification of all major peaks from
chromatograms of all biodiesel samples.

Fatty acids are designated by their carbon length followed by the number of
unsaturated double bonds and expressed as wt (%) of the total fatty acids. FA(ee);
25 fatty acid ethyl esters; be; FA(be): fatty acid butyl esters. Other fatty acids represent
fatty acids with retention times greater than C22:6 n-3ee or C22:6 n-3be.

Table 1 shows the fatty acid composition of the mixture derived from the esterification
of animal fat in stage 1 of the biodiesel process in which this material is treated with
30 an immobilised plant lipase in the presence of ethanol and later sulphuric acid. The
fatty alkyl esters identified in this mixture were only of the ethyl ester type as opposed
to methyl esters described for other biodiesel mixtures. Ethyl esters were primarily
C16:0 (palmitate) 23.8%, C18:0 (stearate) 22.2%, and C18:1cis 9 (oleate) 37.9%, with
an overall degree of saturation of 48.2% consistent with the fatty acids of the starting
35 material being of animal origin. Minor fatty acid ethyl esters detected comprised
isomeric forms of C18:1 likely to be elaidic and/or vaccenic ethyl esters, as well as

- 20 -

ethyl esters with retention times longer than C22:6 likely to be of the C24 ethyl ester type.

Table 1 - Fatty acid analysis of biodiesel mixtures produced by esterification of animal fat (Biodiesel sample # 2).

Fatty acid	% (w/w)
14:0 ee	2.2
16:0 ee	23.8
16:1 ee	2.8
18:0 ee	22.2
18:1 ee (cis 9)	37.9
18:1 ee (other isomers)	3.7
18:2 ee	2.3
Other fatty acids (ee)	5.0
Σ Saturated fatty acids	48.2

Table 2 shows the fatty acid composition of the mixture derived from the esterification of rice bran oil in stage 2 of the biodiesel process in which this material is treated in the presence of n-butanol and methanesulphonic acid. The fatty alkyl esters identified in this mixture were only of the butyl ester type as opposed to methyl esters described for other biodiesel mixtures and the ethyl esters described for stage 1 of the present invention. Butyl esters were primarily C16:0 (palmitate) 19.1%, C18:1 cis 9 (oleate) 32.7% and C18:2 (linoleate) 28.7%, with an overall degree of saturation of 23.6% consistent with the fatty acids of the starting material being of plant origin. Several other fatty alkyl ethyl esters were detected but these were difficult to unequivocally identify with the butyl ester standards available, and together comprised about 14.5% of the total fatty acid esters in this sample.

20

Table 2 - Fatty acid analyses of biodiesel mixtures produced by esterification of rice bran oil (Biodiesel sample # 3).

5	Fatty acid	% (w/w)
	16:0 be	19.1
	18:0 be	2.2
10	18:1 be (cis 9)	32.7
	18:2 be	28.7
	18:3 be	0.8
	22:0 be	2.3
15	Other fatty acids (be)	14.5
	Σ Saturated fatty acids	23.6

20 Table 3 shows the fatty acid composition of the combined mixture derived from both the esterification of animal fat in stage 1 of the biodiesel process in which this material is treated with an immobilised plant lipase in the presence of ethanol and later sulphuric acid, and the esterification of rice bran oil in stage 2 of the biodiesel process in which this material is treated with in the presence of n-butanol and methane

25 sulphonic acid. Both ethyl esters and butyl esters are identified in this mixture with the ethyl esters comprising approximately 91% and the butyl esters 9% of the total fatty alkyl esters. The complete fatty alkyl ester composition of this biodiesel mixture is shown as both total % FAEE plus FABE (left hand column) and % FAEE or % FABE (right hand column). The FAEE profile compares well with that shown in Table 1, while

30 for the butyl esters, the major species present, C 16:1 (18.0%), C 18:1 (30.1%) and C 18:2 (30.0%), are also similar in proportion to that shown in Table 2 for the rice bran derived fatty alkyl butyl esters. The other fatty alkyl butyl esters present in Biodiesel sample #3, for which identification was difficult due to the relatively lower proportion of butyl to ethyl esters in this mixture, comprised approximately 2.0% of the total ester

35 content or 21.9% of the total butyl esters.

Table 3 - Fatty acid analysis of biodiesel produced by esterification of animal fat and rice bran oil. (Biodiesel sample # 4).

Fatty acid (ethyl esters)	% (w/w) (total FAEE plus FABE)	% (w/w) (% of FAEE or FABE)
Fatty acid ethyl esters (FAEE)		
14:0 ee	2.1	2.3
16:0 ee	22.5	24.8
16:1 ee	2.7	2.9
18:0 ee	20.5	22.6
18:1 ee (cis 9)	35.1	38.6
18:1 ee (other isomers)	3.4	3.7
Other fatty acids (ee)	4.7	5.1
Σ Fatty acids	91.0	100.0
Σ Saturated fatty acids (FAEE)		49.6
Fatty acid butyl esters (FABE)		
16:0 be	1.6	18.0
18:1 be (cis 9)	2.7	30.1
18:2 be	2.7	30.0
Other fatty acids (be)	2.0	21.9
Σ Fatty acids	9.0	100.0
Σ Saturated fatty acids (FABE)		18.0
Σ Saturated fatty acids (FAEE + FABE)		46.7

The embodiments have been described by way of example only and modifications within the spirit and scope of the invention are envisaged. Thus, it must be appreciated that there may be other various and modifications to the configurations described herein which are also within the scope of the present invention.

Claims

1. A process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant, the process including treating a feedstock containing glycerides and/or free fatty acids with a lipase, a lower alkyl alcohol and an acid catalyst under conditions to produce said alkyl fatty acid esters.
2. A process according to claim 1, wherein the feedstock containing glycerides and/or free fatty acids is treated with the lipase in the presence of the lower alkyl alcohol to produce a first alkyl fatty acid ester mixture which is then reacted with the lower alkyl alcohol in the presence of the acid catalyst to produce a second alkyl fatty acid ester mixture which is suitable for use as a fuel and/or lubricant.
3. A process according to either claim 1 or claim 2, wherein the lipase is selected from one or more of the group consisting of: a latex lipase and a plant lipase from a high lipid content grain, legume or seed.
4. A process according to claim 3, wherein the lipase is a combination of a latex lipase and a plant lipase.
5. A process according to either claim 3 or claim 4, wherein the latex lipase is a papaya latex lipase.
6. A process according to claim 5, wherein the papaya latex lipase is immobilized on a substrate formed from sucrose and lignite.
7. A process according to claim 6, wherein the substrate is a pyrolised mixture of a saccharide and an acid precipitate from an alkaline extract of lignite.
8. A process according to any one of claims 3 to 7, wherein the plant lipase from a high lipid content grain, legume or seed is a rice bran lipase.
9. A process according to any one of the preceding claims, wherein the feedstock containing glycerides and/or free fatty acids is a plant oil or animal fat.

10. A process according to claim 9, wherein the feedstock containing glycerides and/or free fatty acids is tallow.
11. A process according to any one of the preceding claims, wherein the lower alkyl alcohol is selected from one or more of the group consisting of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol.
12. A process according to claim 11, wherein the lower alkyl alcohol is ethanol.
13. A process according to any one of claims 5 to 12, wherein the ratio of papaya latex lipase to feedstock containing glycerides and/or free fatty acids is about 1:10 w/w to about 1:30 w/w.
14. A process according to claim 13, wherein the ratio is about 1:20 w/w.
15. A process according to any one of claims 2 to 14, wherein the reaction to produce the first alkyl fatty acid ester mixture is carried out at a temperature of from about 30°C to about 70°C.
16. A process according to claim 15, wherein the reaction is carried out at a temperature of about 50°C.
17. A process according to any one of claims 2 to 16, wherein the reaction to produce the first alkyl fatty acid ester mixture is carried out for a time period of from about 30 minutes to about 90 minutes.
18. A process according to claim 17, wherein the reaction is carried out for a time period of about 60 minutes.
19. A process according to any one of claims 2 to 18, further including separating the first alkyl fatty acid ester mixture from solid material.
20. A process according to claim 19, wherein the first alkyl fatty acid ester mixture is separated from solid material by centrifugation.

21. A process according to any one of the preceding claims, wherein the acid catalyst is a mineral acid.
22. A process according to claim 21, wherein the mineral acid is sulphuric acid.
- 5 23. A process according to any one of claims 2 to 22, wherein the reaction to produce the second alkyl fatty acid mixture is carried out under reactive distillation conditions.
- 10 24. A process according to claim 23, wherein the reaction is carried out under continuous reactive distillation conditions.
25. A process according to either claim 23 or claim 24, wherein the reaction is carried out a temperature of about 110°C to about 180°C.
- 15 26. A process according to claim 25, wherein the reaction is carried out a temperature of about 125°C to about 165°C.
27. A process according to any one of claims 23 to 26, wherein unreacted lower alkyl alcohol that is distilled from the reaction is returned to the reaction mixture.
- 20 28. A process according to any one of claims 23 to 27, wherein the second alkyl fatty acid ester mixture is distilled from the reaction mixture.
- 25 29. A process according to claim 28, wherein the distilled second alkyl fatty acid ester mixture is treated to remove unreacted glycerine.
30. A process according to claim 29, wherein said treatment comprises gravity separation.
- 30 31. A process according to any one of claims 2 to 30, wherein the pH of the second alkyl fatty acid ester mixture is adjusted to between about 6 and about 8.
32. A process according to claim 31, wherein the pH of the second alkyl fatty acid ester mixture is adjusted to about 7.
- 35

33. A process according to either claim 31 or claim 32, wherein the pH of the second alkyl fatty acid ester mixture is adjusted by adding an agent selected from the group consisting of: potassium hydroxide, sodium hydroxide, magnesium hydroxide, ammonia, and calcium hydroxide.

5

34. A process according to claim 33, wherein the pH of the second alkyl fatty acid ester mixture is adjusted by adding magnesium hydroxide.

35. A process according to any one of claims 2 to 34, wherein the second alkyl fatty acid ester mixture is filtered to remove suspended solids.

10

36. A process according to any one of claims 2 to 35, wherein the second alkyl fatty acid ester mixture is treated with a filtering agent.

37. A process according to claim 36, wherein the filtering agent is selected from the group consisting of diatomaceous earth, activated charcoal, and silicates.

15

38. A process according to claim 37, wherein the silicate is selected from the group consisting of aluminosilicates and magnesium silicates.

20

39. A process according to claim 38, wherein the silicate is a magnesium silicate.

40. A process according to any one of claims 36 to 39, wherein the filtering agent is separated from the second alkyl fatty acid ester mixture by mechanical filtration.

25

41. A process according to any one of claims 2 to 40, further comprising:
- reacting rice bran oil with a lower alkyl alcohol in the presence of methane sulphonic acid to form a rice bran oil ester mixture; and
- mixing the rice bran oil ester mixture with the second alkyl fatty acid ester mixture to form an alkyl fatty acid ester mixture suitable for use as a fuel.

30

42. A process according to claim 41, wherein the lower alkyl alcohol is selected from one or more of the group consisting of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol.

35

43. A process according to claim 42, wherein the lower alkyl alcohol is n-butanol.

44. A process according to claim 43, wherein the n-butanol is formed by enzymatic hydrolysis of a glycerine and soap mixture that is formed during the production of the second alkyl fatty acid mixture.

5

45. A process according to any one of claims 36 to 39, wherein the rice bran oil ester mixture is separated from solid material prior to mixing with the second alkyl fatty acid mixture.

10 46. A process according to claim 45, wherein the rice bran oil ester mixture is separated from the solid material by centrifugation.

47. A process according to any one of claims 41 to 46, wherein the ratio of n-butanol to rice bran oil is about 1:3.5 w/w.

15

48. A process according to any one of claims 41 to 42, wherein the ratio of n-butanol to rice bran oil in the starting reaction mixture is about 1:3 w/w to about 1:4 w/w.

20 49. A process according to claim 48, wherein the ratio of n-butanol to rice bran oil is about 1:3.5 w/w.

50. A process according to any one of claims 41 to 49, wherein the ratio of methane sulphonic acid to n-butanol in the starting reaction mixture is about 1:40 w/w to about 1:50 w/w.

25

51. A process according to claim 50, wherein the ratio of methane sulphonic acid to n-butanol is about 1:40 w/w to about 1:50 w/w.

30 52. An alkyl fatty acid ester mixture prepared according to the process of any one of claims 1 to 52.

53. A biodiesel fuel prepared according to the process of any one of claims 1 to 52.

35

54. A biodiesel fuel according to claim 53, wherein the gelling temperature of the fuel is between about 0°C and about 10°C.
55. A biodiesel fuel that is substantially stable to oxidation under ambient
5 conditions for a period of at least twelve months.
56. A system for carrying out the process of any one of claims 1 to 51.
57. A process according to claim 1 and substantially as hereinbefore described
10 with respect to the accompanying example(s).

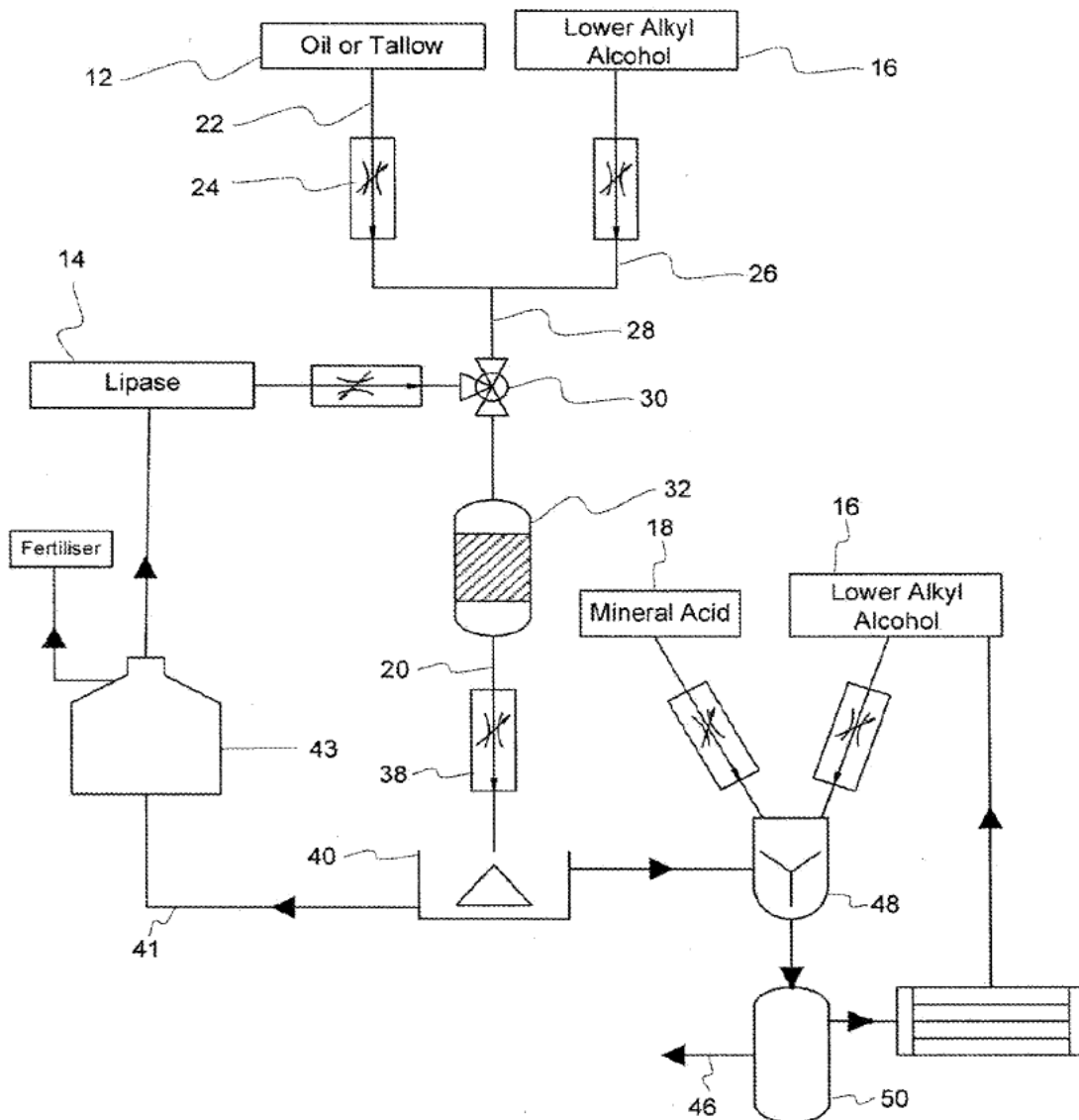


FIGURE 1

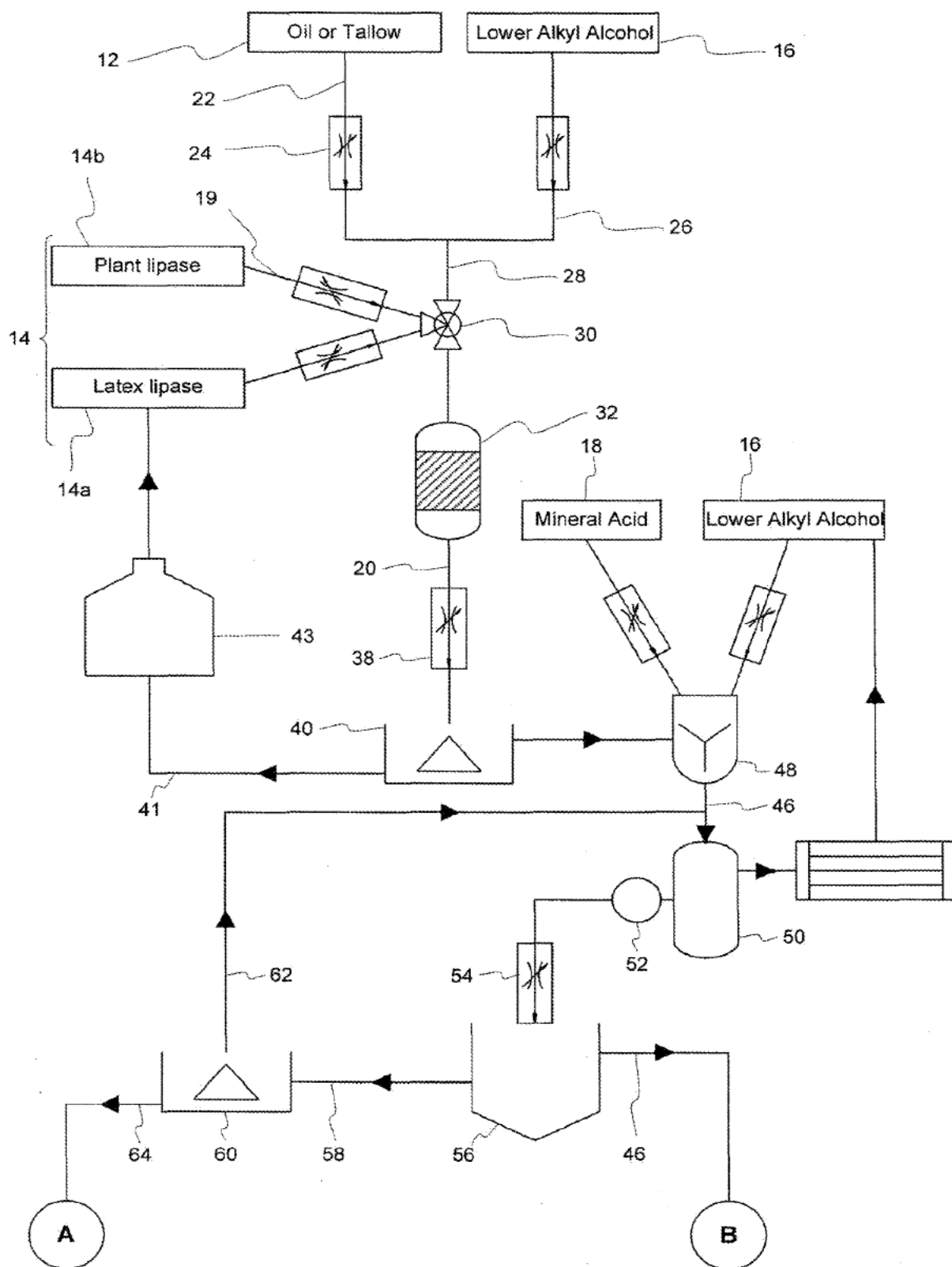


FIGURE 2(a)

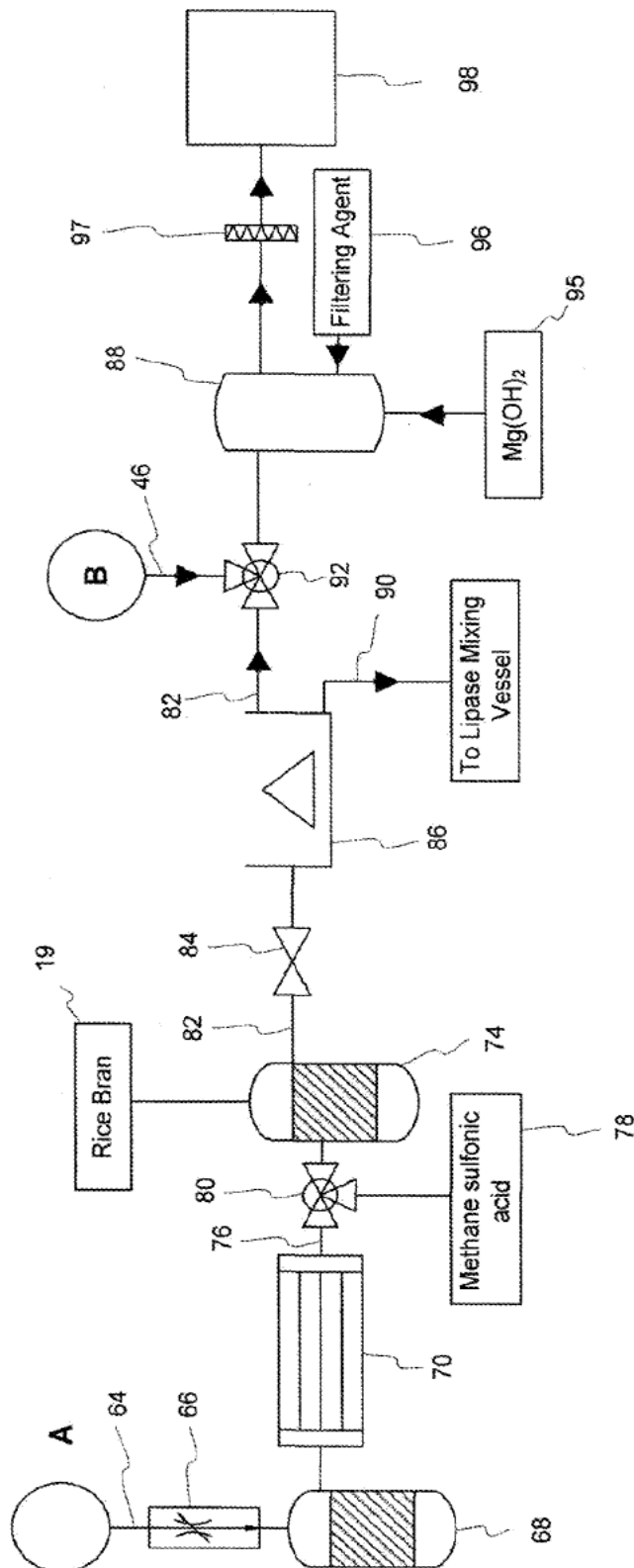


FIGURE 2(b)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/000112

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. *C11C 3/00* (2006.01) *C10M 107/26* (2006.01) *C11C 3/04* (2006.01) *C10L 1/02* (2006.01)
C10M 107/32 (2006.01) *C11C 3/08* (2006.01) *C10M 101/04* (2006.01) *C10M 129/95* (2006.01)
C11C 3/10 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPAT, JAPIO and Espacenet: c11c-3/00/ic, c10l-001/02/ic, c10m-101/04/ic, c10m-107/26/ic, c10m-107/32/ic, c10m-129/95/ic, c11c-3/04/ic, c11c-3/10/ic, C11C-3/08/IC, biodiesel+ or biofuel+ or fuel+ or lubric+, lipase+, alcohol+, (acid catalyst+); biodiesel fuel and stability and oxidation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/050589 A1 (COGNIS IP MANAGEMENT GMBH) 18 May 2006 See Abstract; claim 9; page 3, line 27 to page 4, line 20; page 5, lines 1-2	1-4, 9, 11-12, 19-22, 36-37, 40, 52-53, 55-56

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
26 February 2008

Date of mailing of the international search report
18 MAR 2008

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DEBASHIS ROY
 AUSTRALIAN PATENT OFFICE
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 Telephone No : (02) 6225 6125

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/000112

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>Patent Abstracts of Japan, JP 2006-272326 A (NATIONAL UNIV CORP SHIZUOKA UNIV et al.) 12 October 2006 (machine translation, retrieved 8 May 2007 from Internet) <URL: http://www4.ipdl.inpit.go.jp/Tokujitu/PAJdetail.ipdl?N0000=60&N0120=01&N2001=2&N3001=2006-272326 See Abstract and Paragraph [0018] of machine-based translation</p>	1-4, 9, 11-12, 19-22, 31-34, 52-53, 55-56
X	<p>Patent Abstracts of Japan, JP 2005-171099 A (MIZUSAWA IND CHEM LTD) 30 June 2005 (machine translation, retrieved 8 May 2007 from Internet) <URL: http://www4.ipdl.inpit.go.jp/Tokujitu/PAJdetail.ipdl?N0000=60&N0120=01&N2001=2&N3001=2005-171099 See Abstract and Paragraph [0023] of machine-based translation</p>	1-4, 9, 11-12, 19-22, 31-34, 52-53, 55-56
X	<p>US 2005/0084941 A1 (ABE et al.) 21 April 2005 See whole document especially Paragraph [0039]</p>	1-4, 8-12, 15-22, 31-34, 52-53, 55-56
X	<p>US 2006/0219979 A1 (ASBAHR et al.) 05 October 2006 See Abstract and page 6</p>	55
A	<p>US 2003/0032826 A1 (HANNA) 13 February 2003 See whole document especially Paragraph [0019]</p>	1-57
A	<p>US 6982155 B1 (FUKUDA et al.) 03 January 2006 See whole document</p>	1-57

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2008/000112

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	2006050589	BR	PI0419166	CA	2586889	CN	101056985
		EP	1809755				
JP	2006272326						
JP	2005171099						
US	2005084941	AU	2003211577	BR	0303369	CN	1522293
		EP	1484384	JP	2003336082	WO	03076553
US	2003032826						
US	6982155	AU	14179/01	WO	0138553		
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							

Electronic Patent Application Fee Transmittal

Application Number:	14578075			
Filing Date:	19-Dec-2014			
Title of Invention:	Processes and Systems for Catalytic Manufacture of Wax Ester Derivatives			
First Named Inventor/Applicant Name:	Jeff Addy			
Filer:	Adam Reid Stephenson/Dee Wilson			
Attorney Docket Number:	FloraTech002			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	2806	1	120	120
Total in USD (\$)				120

Electronic Acknowledgement Receipt

EFS ID:	35413404
Application Number:	14578075
International Application Number:	
Confirmation Number:	2053
Title of Invention:	Processes and Systems for Catalytic Manufacture of Wax Ester Derivatives
First Named Inventor/Applicant Name:	Jeff Addy
Customer Number:	93756
Filer:	Adam Reid Stephenson/Dee Wilson
Filer Authorized By:	Adam Reid Stephenson
Attorney Docket Number:	FloraTech002
Receipt Date:	13-MAR-2019
Filing Date:	19-DEC-2014
Time Stamp:	16:11:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$ 120
RAM confirmation Number	031419INTEFSW16120800
Deposit Account	
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Vantage Exhibit 1002

Page 547 of 1215
CARGILL 00000572

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	SIDS002_2.pdf	1035240	no	4
			2ecf73ece7215f251db33058f988e431a9d763a5		
Warnings:					
Information:					
2	Foreign Reference	WO9015127A1.pdf	617689	no	17
			bf32fc56b7d163492a68701a501f16ca898ade2b		
Warnings:					
Information:					
3	Foreign Reference	WO2008092207A1.pdf	1429268	no	36
			990c65f470a01f2426ae34d7c82967d656035641		
Warnings:					
Information:					
4	Non Patent Literature	Steinke_etal.pdf	421809	no	5
			821aeda4b3fe53d638c85003f594c38eaf332057		
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30487	no	2
			893f23b9ca80a3d271816abbe7731e25c11721c9		
Warnings:					
Information:					
Total Files Size (in bytes):			3534493		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/578,075	12/19/2014	Jeff Addy	FloraTech002	2053
93756	7590	06/06/2019		
IPTechLaw 8350 E Raintree Dr., Ste 245 Scottsdale, AZ 85260			EXAMINER PATURY, SRIKANTH	
			ART UNIT	PAPER NUMBER
			1657	
			NOTIFICATION DATE	DELIVERY MODE
			06/06/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

adam@iptech.law
ipdocket@iptech.law

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Status of the Claims

The amendments filed on 12/06/2018 are acknowledged and have been fully considered. Claims 1-7 are pending. Claim 1 has been amended. Claims 8-20 were previously canceled. Claims 1-7 are now under consideration and the subject of this office action.

It is noted the application has been transferred to Examiner Srikanth Patury from Examiner Srivastava.

Information Disclosure Statement

The references provided in the information disclosure statement(s) were considered and have been made of record to the extent that each was provided.

Declaration Under 37 CFR 1.132

The declaration under 37 CFR 1.132 by applicant Jeff Addy filed on 12/06/2018 has been fully considered and entered.

Objections/Rejections Withdrawn

The previous rejections of claims 1-7 are withdrawn in light of the claim amendments and declaration under 37 CFR 1.132 filed on 12/06/2018 and new

rejections are presented below.

Claim Objections

Claims 3 and 4 are objected to because of the following informalities: the phrase “without one of removing and degrading...” in claims 3 and 4 is awkward. It is suggested to amend to “without removing or degrading...”.

Appropriate correction is needed.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Claim Rejections - 35 USC § 103

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the

claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-5 are rejected under 35 U.S.C. 103 as being unpatentable over **Gunawan** (Journal of Oleo Science, 2004; Of Record) **in view of Austic** (WO 2012/106701; Pub. Aug. 9, 2012) **and as evidenced by Busson-Breysse** (JAOCS, 1994) **and Fucosterol** (Cayman Chemical, CAS Entry, 2019).

Gunawan teaches transesterifying palm oil using oleyl alcohol and lipase (abstract, pg. 472 col. 1, Exp. Procedures). Gunawan teaches the palm oil comprises wax esters (pg. 472 col. 1 para. 2 Exp. Procedures). Gunawan teaches adding a lipase (Lipozyme) to catalytically transesterify the oils to produce transesterified wax esters (Fig.1, Table 1). Gunawan is silent regarding the oxidative stability index (OSI) of the transesterified product being at least 2.5 times greater than the OSI of the feedstock. Gunawan teaches that wax esters can be extracted from plant materials such as jojoba oil but the process is too expensive (pg. 471 col. 2 para 1). It was also known in the art that jojoba oil can be esterified using a lipase to produce wax esters. Austic teaches that feedstocks comprising esters including jojoba oil, palm oil etc., can be esterified with an alcohol and a lipase (pg. 4 lines 25- pg. 5 lines 3, pg. 8 lines 19 – pg. 9 lines 20, Ex. 4;

Re. claim 5). Austic teaches lipase including Lipozyme TL IM (Thermomyces lanuginosus) from Novozyme can be used in its methods (pg. 9 Enzymes). In light of these teachings, it would have been obvious to one of ordinary skill before the effective filing date of the claimed invention to use jojoba oil instead of palm oil to produce transesterified wax esters in the methods of Gunawan and the artisan would be motivated to do so because it was known in the art that jojoba oil can also be used in transesterification processes with lipases and the artisan is substituting jojoba oil instead of palm oil in the methods of Gunawan. Gunawan also teaches it is expensive to extract wax esters from jojoba oil, so therefore it would be obvious to produce wax esters by the lipase-catalyzed synthesis method from oils as taught by Gunawan and substitute jojoba oil instead of palm oil in the methods of Gunawan.

Gunawan in view of Austic are also silent regarding the oxidative stability index (OSI) of the transesterified product being at least 2.5 times greater than the OSI of the feedstock. However, the combined teachings of Gunawan and Austic, teach a process in which jojoba oil is catalyzed by contacting with a lipase (Lipozyme TL IM), the same process taught in the instant specification (par. [0057]). Therefore it is reasonably expected that the process of Gunawan in view of Austic would also result in the OSI of the transesterified product being at 2.5 times greater than an OSI of the feedstock.

Regarding claims 3 and 4; Gunawan and Austic do not teach the feedstock to further comprise an antioxidant (claim 3), a skin conditioning agent and a volatile compound (claim 4). Jojoba oil comprises various sterols such as fucosterol (see Busson-Breyse Table 4), which is an antioxidant (see Fucosterol pg. 1 Description). The instant specification discloses that jojoba oil comprises various sterols, antioxidants

(tocopherols etc.,) function as skin conditioning agents and are volatile (see par. [0050]). Gunawan in view of Austic teach using jojoba oil in their methods, and teach a process in which jojoba oil is catalyzed by contacting with a lipase (Lipozyme TL IM), the same process taught in the instant specification (par. [0057]). Therefore it is reasonably expected that the process of Gunawan in view of Austic is teaching a method in which the feedstock (jojoba oil) comprises an antioxidant, skin conditioning agent and a volatile compound and the transesterification process would occur without removing or degrading the antioxidant, skin conditioning agent and a volatile compound.

Regarding claim 2, Gunawan teaches using oleyl alcohol and does not teach using methanol in its methods to catalyze the feedstock, therefore the transesterified product would not comprise methyl esters.

The teachings of Gunawan and Austic would render claims 1-5 obvious.

Claims 1-7 are rejected under 35 U.S.C. 103 as being unpatentable over **Gunawan** (Journal of Oleo Science, 2004; Of Record) in view of Austic (WO 2012/106701; Pub. Aug. 9, 2012) **and as evidenced by Busson-Breysse** (JAOCS, 1994) **and Fucosterol** (Cayman Chemical, CAS Entry, 2019) **and further in view of Arquette** (US 5,968,530; Iss. Oct. 19, 1999; Applicant IDS).

Regarding claims 6 and 7, Gunawan and Austic do not teach the jojoba oil comprises hydrogenated wax esters. Arquette teaches preparing esterified products for use in cosmetic products and teaches using jojoba oil in the esterification methods (col. 4 lines 40-65). Arquette teachings jojoba oil is also called jojoba wax esters (col. 5 lines 1-15) and teaches jojoba wax esters, hydrogenated jojoba wax esters or a mixture of

the two are used in the esterification reaction to produce the emollients (lubricants) for use cosmetic compositions (col. 8 lines 1-30,49-60).

In light of these teachings, it would have been obvious to one of ordinary skill before the effective filing date of the claimed invention to use jojoba oil comprising hydrogenated jojoba esters instead of jojoba oil to produce transesterified wax esters in the methods of Gunawan in view of Austic as taught by Arquette. The artisan would be motivated to do so because it was known in the art that jojoba oil, hydrogenated jojoba oil and mixtures thereof can also be used in transesterification processes to produce emollient compositions and the artisan is substituting hydrogenated jojoba oil instead of jojoba oil in the methods of Gunawan/Austic. Gunawan, Austic and Arquette teach a method using hydrogenated jojoba oil in their methods, and teach a process in which jojoba oil is catalyzed by contacting with a lipase (Lipozyme TL IM), the same process taught in the instant specification (par. [0057]). Therefore it is reasonably expected that the process of Gunawan, Austic and Arquette would also produce a transesterified product whose dropping point is greater than the dropping point of the feedstock and the dropping point increases as a percentage of hydrogenated jojoba wax esters in the feedstock increases. Since Arquette teaches using mixtures of jojoba oil and hydrogenated jojoba oil, the percentage of hydrogenated wax esters can be varied, and the dropping point would change based on the hydrogenation since the same method steps (providing a feedstock, contacting with a lipase and catalytically transesterifying the wax esters) are taught by Gunawan, Austic and Arquette.

The teachings of Gunawan, Austic and Arquette would render claims 1-7 obvious.

Response to Arguments

Applicant's arguments filed 12/06/2018 have been fully considered but are moot because the previous rejections are withdrawn in light of the claim amendments filed on 12/06/2018 and new grounds of rejections have been presented above.

Applicants have submitted a declaration under 37 CFR 1.132 disclosing experiments performed by the inventor that the prior art methods of catalyzing feedstocks comprising wax esters with a lipase does not necessarily produce a transesterified product with an OSI of at least 2.5 times greater the OSI of the feedstock. The declaration discloses experiments when crambe oil was catalyzed with lipase, the OSI only increased by 1.75 times as compared to when jojoba esters were used the OSI increased by 3.8 times (sec. 8 and 10). Applicants argue that Gunawan teaches palm oil which is similar to crambe oil and therefore similar results of OSI increase would be expected in the methods of Gunawan and therefore Gunawan would not teach the limitation of atleast 2.5 increase in the OSI of the product (remarks pg. 6,7).

However, this is not persuasive, because as discussed above, the combined teachings of Gunawan and Austic, teach a process in which jojoba oil is catalyzed by contacting with a lipase (Lipozyme TL IM), the same process taught in the instant specification (par. [0057]). Therefore it is reasonably expected that the process of Gunawan in view of Austic would also result in the OSI of the transesterified product being at 2.5 times greater than an OSI of the feedstock.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

U.S. Patent Application No. 14/841,242

Claims 1-7 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 8-14 of co-pending Application No. 14/841,242. Although the conflicting claims are not identical, they are not patentably distinct from each other because the scope of the claims 8-14 of

the '242 application renders obvious that of the instant claims. The co-pending claims also recite process to transesterify feedstocks comprising wax esters, contacting with a lipase and catalytically transesterifying the feedstock to produce a transesterified product. The co-pending claims also teach feedstock to comprise jojoba wax esters (claim 11), hydrogenated jojoba wax esters (claim 12), the feedstock to comprise antioxidants and catalyzing without removing or degrading the antioxidant (claim 10). The co-pending claims are silent regarding the OSI and dropping point of the product, feedstock comprising skin conditioning agent and volatile compounds. However, the co-pending claims teach the same feedstock, jojoba oil, which comprises skin conditioning agents and volatile compounds. Regarding OSI, dropping point of the product and performing the transesterification without removing/degrading the skin conditioning agent, the co-pending claims teach the same process as the instant claims, catalyzing jojoba oil with lipase, therefore it is expected the product of the co-pending claims would also have the same functional characteristics.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

Claims 1-7 fail to be patentably distinguishable over the state of the art discussed above and cited on the enclosed PTO-892 and/or PTO-1449. Therefore, the claims are properly rejected.

The remaining references listed on the enclosed PTO-892 and/or PTO-1449 are

cited to further show the state of the art.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SRIKANTH PATURY whose telephone number is (571)270-1020. The examiner can normally be reached on Monday-Friday, 9:30 - 6:30 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Louise W Humphrey can be reached on 571-272-5543. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Srikanth Patury/
Examiner, Art Unit 1657

/LOUISE W HUMPHREY/
Supervisory Patent Examiner, Art Unit 1657

<i>Notice of References Cited</i>	Application/Control No. 14/578,075		Applicant(s)/Patent Under Reexamination Addy et al.	
	Examiner SRIKANTH PATURY		Art Unit 1657	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-4826767-A	05-1989	Hansen; Tomas T.	C12P7/6436	435/134
*	B	US-8742148-B1	06-2014	Liu; Zengshe	C09F7/06	508/496
*	C	US-20140120589-A1	05-2014	Austic; Greg	C12M21/18	435/134
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	WO 2012/106701	08-2012	WIPO	Austic, Greg	C12P 7/64
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Busson-Breyse, Jojoba Wax: Its Esters and Some of Its Minor Components, JAOCS, Vol. 71, no. 9 (September 1994)
	V	Fucosterol, Fucosterol, CAS Entry, Cayman Chemical, 2019
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes 	Application/Control No. 14/578,075	Applicant(s)/Patent Under Reexamination Addy et al.
	Examiner SRIKANTH PATURY	Art Unit 1657

US Classification - Searched*			
Class	Subclass	Date	Examiner
Partial text search in WEST	Partial text search in WEST	3/20/2017	KCS
CPC	C12P7/6436 C11C3/003	3/20/2017	KCS
IPC	C12P7/64)	3/20/2017	KCS
USPC	435/134	3/20/2017	KCS
Updated Partial text search in WEST	Updated Partial text search in WEST	10/19/2017	KCS
CPC	C12P7/6436, C11C3/003	10/19/2017	KCS
IPC	C12P7/64	10/19/2017	KCS
USPC	435/134	10/19/2017	KCS
Re-updated Partial text search in WEST	Re-updated Partial text search in WEST	06/23/2018	KCS
CPC	C12P7/6436 C11C3/003 C11C3/10	06/23/2018	KCS
IPC	C12P7/64	06/23/2018	KCS
USPC	435/134	06/23/2018	KCS

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

	/SRIKANTH PATURY/ Examiner, Art Unit 1657
--	--

Search Notes 	Application/Control No. 14/578,075	Applicant(s)/Patent Under Reexamination Addy et al.
	Examiner SRIKANTH PATURY	Art Unit 1657

Search Notes		
Search Notes	Date	Examiner
Inventor name search in PE2E & PALMsearch in	3/1/2017	KCS
Assignee name search in PE2E & PALM	3/1/2017	KCS
Verified Application priority data in PE2E & PALM	3/1/2017	KCS
Partial Text Search in WEST:DWPI, EPAB, FPRS, JPAB, USOC, USPT according to search stratgy and terms in WEST search history appende hereto	3/20/2017	KCS
STN Search	09/20/2016	KCS
Updated Inventor name search in PE2E, WEST & PALM	10/19/2017	KCS
Updated Assignee name search in PE2E & PALM	10/19/2017	KCS
Reverified Application priority data in PE2E & PALM	10/19/2017	KCS
Updated Partial Text Search in WEST:DWPI, EPAB, FPRS, JPAB, USOC, USPT according to search stratgy and terms in WEST search history appende hereto	10/19/2017	KCS
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(54) Title: FATTY ACID ESTERIFICATION PROCESS

(57) Abstract: The invention relates to the utilisation of fatty acid feedstocks with substantial free fatty acid content in the production of biodiesel by the use of microbial enzymes.

Vantage Exhibit 1002

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FATTY ACID ESTERIFICATION PROCESS**REFERENCE TO A SEQUENCE LISTING**

This application contains a Sequence Listing in computer readable form, which is
5 incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a process for preparing carboxylic acid derivatives. In
particular the invention relates to the utilization of fatty acid feedstocks with substantial free fatty
10 acid content in the production of biodiesel.

BACKGROUND OF THE INVENTION

As a result of the increasing interest in renewable resources in general and biofuels in
particular, a number of processes has been developed for the production of esters of fatty acids
15 and lower alcohols, which esters are also referred to as 'biodiesel'. Early 'biodiesel' processes
prescribed the use of neutral raw materials and thereby competed with food applications.
Accordingly, there is an incentive to exploit cheaper alternative sources of fatty acid moieties as
raw material for biodiesel production. This often means that such fatty acid feedstocks may
contain free fatty acids and that their FFA contents can vary over a wide range.

Before such fatty acid feedstock is suitable for use in a transesterification process for
20 biodiesel production the FFA content needs to be reduced to $\leq 0.25\%$. This may be performed
by esterifying the FFA with a lower alcohol. However, the esterification reaction is reversible and
leads to an equilibrium where one of the products formed by the esterification is water. Shifting
the reaction equilibrium to the ester side therefore requires either the use of a large excess of
25 lower alcohol or the removal of the formed water.

Accordingly, U.S. Patent No. 4,164,506 discloses a process comprising the esterification
of free fatty acids of unrefined fats with a lower alcohol in an amount larger than its solubility in
the fats in the presence of an acid catalyst. However, several lower alcohols have a boiling point
that is lower than the boiling point of water which implies that it is impossible to remove the
30 water formed by the esterification while retaining the lower alcohol in the reaction mixture.
Shifting the esterification equilibrium to the ester side therefore requires the use of a large
excess of lower alcohol.

This disadvantage can be overcome by using a high boiling alcohol such as glycerol as
disclosed in U.S. Patent No. 2,588,435. Using such high boiling alcohols has the additional
35 advantage that the reaction can be carried out at a higher temperature, which increases the rate
constant of the esterification reaction, without having to operate under superatmospheric
pressure. In fact, as disclosed in U.S. Patent No. 6,822,105, the esterification can now be

carried out under vacuum, which promotes the evaporation of the water formed by the esterification reaction which is thereby shifted towards the ester side. The use of nitrogen during a vacuum stripping operation further facilitates the water evaporation.

However, as demonstrated by the examples in U.S. Patent Nos. 6,822,105 and 7,087,771, the esterification reaction is quite slow and it can take some 7 to 11 hours before the acid value of the reaction mixture, which is indicative of the residual free fatty acid content, has decreased to a value below 0.4 (mg KOH per g oil), which in industrial practice is the maximum value for a starting material for a transesterification process leading to biodiesel. The example in U.S. Patent Application Publication No. 2004/0186307 employing a solid esterification catalyst, which is present in a packed bed inside the esterification reactor, also mentions a reaction time of 5 hours at a temperature of 200°C. Holding fatty materials at such a high temperature for long periods of time can lead to the formation of unwanted side-products.

Accordingly, there is a strong preference for an esterification reaction at lower temperatures and for a catalyst that does not cause side-products to be formed. Operating at a lower temperature can also lead to energy savings. In this context, the use of enzymes in general and of lipases in particular merits consideration. However, the use of enzymes is far from straightforward. Their activity depends on the water concentration but water also affects the position of the esterification equilibrium. Moreover, the reagents should be well mixed, which is why the literature often mentions the use of solvents, e.g., Pastor et al., 1994, *Applied Biochemistry and Biotechnology* 50: 251-263: Synthesis of Mono-and Diolelyglycerols Using an Immobilized Lipase. For industrial processes the use of solvents raises the cost of operation and is therefore preferably avoided.

WO 2008/125574 discloses an esterification process wherein the formed water is removed from the reaction mixture by stripping with an inert gas. However, there is further need for enzymatic processes that allows fatty raw materials with variable free fatty acid contents to be utilized as raw material for biodiesel production.

OBJECTS OF THE INVENTION

Accordingly, it is an object of the invention to overcome the various disadvantages of the prior art processes for utilizing fatty raw materials with a high free fatty acid content for the production of fatty acid esters of lower alcohols by the use of lipolytic enzymes.

It is another object of the invention to avoid the use of solvents.

It is also an object of the invention to use the lipolytic enzyme in such a way that its productivity is maximized.

It is a further object of the invention to enable the maximization of the yield of lower alkyl esters of fatty acids based on the fatty acid moiety content of the raw material.

It is a further object of the invention to enable the maximization of the yield of lower alkyl esters of fatty acids based on the lower alcohol consumption of the process.

It is a further object of the invention to enable the maximization of the yield of lower alkyl esters of fatty acids while minimizing the energy consumption of the process.

5 It is yet another object of the invention to provide a process that can accommodate a wide range of raw materials with varying free fatty acid contents.

These and further objects of the invention will become apparent from the description and the examples hereinafter.

10 SUMMARY OF THE INVENTION

It has surprisingly been found that in an esterification process where water is removed from the reaction mixture by stripping with an inert gas, the stripping gas, following drying by passage through a volume of a lower alcohol, can be reused.

15 It has furthermore surprisingly been found that in the above esterification process, the volume of a lower alcohol, following use for drying of the stripping gas, may be reused in a transesterification process, such as disclosed in WO 2006/072256, wherein some water is needed. The process of the invention may be applied in a biodiesel production process, a) to esterify the free fatty acids (FFA) in a glyceride feedstock to lower the FFA content sufficiently for allowing the feedstock with fatty acid esters to be used in a chemical catalyzed biodiesel
20 process, b) to esterify the FFA in a glyceride feedstock originating from an enzymatic transesterification process to achieve the biodiesel standard of $\leq 0.25\%$ FFA.

The advantage is a simplified total biodiesel production process wherein the "wet" lower alcohol from the esterification process of the invention does not have to be dewatered but can be reused at the biodiesel refinery in a transesterification process. This results in reduced
25 energy consumption.

Accordingly, the invention provides a process for the production of fatty acid alkyl esters from a fatty acid feedstock comprising free fatty acids, comprising the steps of: a) providing a reaction mixture (R) that comprises the fatty acid feedstock, a lower alcohol, and a lipolytic enzyme; b) allowing the reaction mixture (R) to react under formation of fatty acids alkyl esters;
30 wherein a gas flow (G) is passed through the reaction mixture (R), and thereafter passed through a volume of lower alcohol (A), before being recirculated through the reaction mixture (R). Furthermore, the invention provides a production system for processing a reaction mixture (R), said production system comprising:

(a) one or more enclosed reaction vessels(s) (RV) suitable for comprising said reaction
35 mixture (R), each reaction vessels (RV) comprising at least one first gas inlet (GI1) and at least one first gas outlet (GO1),

(b) one or more gas wash tank(s) (GWT) suitable for comprising a gas wash liquid (W), each gas wash tank (GWT) comprising at least one second gas inlet (GI2) and at least one second gas outlet (GO2),

(c) comprising one or more gas pump(s) (P) capable of delivering a gas flow (G), which can be passed by the first gas inlet (GI1) through the reaction vessels (RV), discharged by the first gas outlet (GO1) and thereafter passed by the second gas inlet (GI2) through the gas wash liquid (GWL), and discharged by the second gas outlet (GO2), before being recirculated to the first gas inlet (GI1) into the reaction vessels (RV).

10 DETAILED DESCRIPTION OF THE INVENTION

Biodiesel

Fatty acid alkyl esters (FAAE) of short-chain alcohols, such as fatty acid methyl esters (FAME) and fatty acid ethyl esters (FAEE) are also called biodiesel, because they are used as an additive to or as a replacement of fossil diesel.

15

Alcohol

The alcohol used as gas wash liquid in the method of the invention is preferably a short-chain, branched or linear, alcohol having 1 to 5 carbon atoms (C₁, C₂, C₃, C₄, or C₅, "a lower alcohol") and mixtures thereof. Preferred lower alcohols are methanol, ethanol and propanol.

20 The alcohol content is preferably less than 4, 3, 2, 1.5 or 1.0 molar equivalents to the amount of fatty acids in the reaction mixture (free and glyceride bound fatty acids). The alcohol may be added stepwise (such as in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more steps) and/or continuously to the reaction mixture.

25 Fatty acid feedstock

The term "fatty acid feedstock" is defined herein as a substrate comprising fatty acid derivatives. The substrate may comprise fatty acid alkyl esters, triglyceride, diglyceride, monoglyceride, free fatty acid or any combination thereof. Any oils and fats of vegetable or animal origin comprising fatty acids may be used as substrate for producing fatty acid alkyl esters in the process of the invention. Also fatty acid feedstock consisting substantially of fatty acid alkyl esters is suitable as feedstock (biodiesel feedstock) for the present invention. Preferably, the free fatty acid content of the fatty acid feedstock is above 0.25%, above 0.30%, above 0.35%, above 0.50%, above 0.75%, above 1.0%, above 5.0%, above 10.0%, above 15.0%, above 20.0%, above 25.0%, above 30.0%, above 40%, or even above 50.0%.

35 The fatty acid feedstock may be oil selected from the group consisting of: algae oil, canola oil, coconut oil, castor oil, coconut oil (copra oil), corn oil, cottonseed oil, flax oil, fish oil, grape seed oil, hemp oil, jatropha oil, jojoba oil, mustard oil, canola oil, palm oil, palm stearin,

palm olein, palm kernel oil, peanut oil, rapeseed oil, rice bran oil, safflower oil, soybean oil, sunflower oil, tall oil, and oil from halophytes, pennycress oil, camelina oil, jojoba oil, coriander seed oil, meadowfoam oil, seashore mallow oil, microbial oils or any combination thereof.

The fatty acid feedstock may be fat selected from the group consisting of: animal fat, including tallow from pigs, beef and sheep, lard, chicken fat, fish oil, or any combination thereof.

The fatty acid feedstock may be crude, refined, bleached, deodorized, degummed, or any combination thereof.

Food quality oils and fats are expensive, and therefore, waste and by-products from their processing as well as non-food grade oils and fats have become increasingly attractive feedstock for producing fatty acid alkyl ester. Soap stock is the fraction of oil obtained in an oil refinery by treating the oil with a base to convert free fatty acids to soaps (e.g., sodium soaps). The soap stock usually contains a fraction of glycerides beside the soaps. Acid oil is the by-product from the oil refinery produced by acidification of soap stock to solubilize the soaps. It mainly contains free fatty acids (FFA) and acylglycerols. Distillates like Palm Fatty Acid Distillate (PFAD) is the by-product from oil refining coming from a distillation process used to eliminate free fatty acid from the oil.

The feedstock may be an intermediate product, a waste product or a by-product of oil or fat refining selected from the group consisting of: soap stock; acid oil; fatty acid distillates such as PFAD, soy fatty acid distillate, rapeseed fatty acid distillate, rice bran fatty acid distillate, poultry fat fatty acid distillate, beef tallow fatty acid distillate, etc.; gums from degumming; by-products from the production of omega-3 fatty acids derivatives from fish oil; fat trap grease; yellow grease, and brown grease, free fatty acids like oleic acid; or fractions of oil obtained by physical separations; or any combinations thereof.

The process of the invention provides an economic and environmentally friendly alternative to the use of the acid catalyzed esterification processes that are currently used as it can accommodate raw materials with widely different FFA contents. These can be crude or degummed oils and fats of vegetable or animal origin and preferably those that have such a high FFA content that their neutralization by conventional means is uneconomic. High acidity rice bran oil is a prime example of such oils, but palm oil may occasionally also exhibit high FFA contents. In general, oils and fats that are used for the production of soap because their FFA content is too high for economic use as food are suitable.

The FFA contents of the raw materials mentioned above can vary widely. Crude rice bran oil for example can have an FFA content of more than 10%, even more than 20% or even more than 30%. Fatty acid distillates originating from the physical refining process can contain in excess of 90% FFA; it is an advantage of the process of the invention that it can effectively handle all these raw materials.

Reactor design

The process of the invention is preferably performed in a reactor (*i.e.*, a production system) in which the catalyst is freely distributed in the reaction mixture. The process may apply a heterogeneous catalyst or a homogeneous catalyst, *e.g.*, an immobilized enzyme composition, or a liquid enzyme composition.

A gas flow serves to remove volatile reaction products, for example water in esterification reactions from the reaction mixture, and hence to shift the equilibrium to the product side. The gas flow further serves to mix the reaction mixture. In a preferred embodiment wherein a homogeneous catalyst, *e.g.*, an immobilized enzyme composition, is applied, the gas flow serves to keep the heterogeneous catalyst suspended and in contact with the reaction mixture. In another preferred embodiment using a homogeneous liquid catalyst, such as a liquid enzyme, effective mixing increases contact between the polar and non-polar phases, decreasing total reaction time.

A rotary jet head (EP 1324818) may be used to mix the gas flow into the reaction mixture as well as providing efficient mixing of the reaction mixture.

As the gas flow (G) thereafter is passed through a volume of a lower alcohol (A), *e.g.*, comprised in a column, the gas releases the volatile reaction product (*e.g.*, water) to the lower alcohol (A), and the so dried gas can be recirculated through the reaction mixture (R).

The reactor may further be equipped with a stirrer in order to increase the mixing of the reaction mixture.

During step (b) the temperature of the reaction mixture (R) is preferably kept between 25°C and 80°C, more preferably between 30°C and 50°C, and most preferably between 35°C and 45°C.

The volume of lower alcohol (A) may comprises the same lower alcohol as comprised in the reaction mixture (R). However, for biodiesel production more than one lower alcohol as well as a mixture of two or more lower alcohols may be applied.

The volume of lower alcohol (A) will over time accumulate the volatile reaction product (*e.g.*, water), and the process efficiency will decrease. Preferably the amount of water in the volume of lower alcohol (A) is 70% or less, 65% or less, 60% or less, 55% or less, 50% or less, 45% or less, 40% or less, 35% or less, 30% or less, more preferably 25% or less, even more preferably 20%, 15%, 10% or less.

The temperature control on the volume of the lower alcohol determines the moisture equilibrium in the production system. Lower temperatures will result in a lower moisture equilibrium and higher temperatures will result in a higher moisture equilibrium in the production system. In a preferred embodiment wherein the lower alcohol is methanol the temperature of the volume of the lower alcohol (A) is between 25°C and 35°C, preferably around 30°C.

The lower alcohol may undergo distillation, pervaporation or other suitable separation technique where after it can be reused in the process of the invention. The water-containing lower alcohol without any separation may also be reused as a reactant in the transesterification of triglycerides, where some water is needed and excess water will be leaving the product stream in the glycerol phase.

A production system suitable for performing the process of the present invention may comprise

(a) one or more enclosed reaction vessels(s) (RV) suitable for comprising said reaction mixture (R), each reaction vessels (RV) comprising at least one first gas inlet (GI1) and at least one first gas outlet (GO1),

(b) one or more gas wash tank(s) (GWT) suitable for comprising a gas wash liquid (W), each gas wash tank (GWT) comprising at least one second gas inlet (GI2) and at least one second gas outlet (GO2),

(c) comprising one or more gas pump(s) (P) capable of delivering a gas flow (G), which can be passed by the first gas inlet (GI1) through the reaction vessels (RV), discharged by the first gas outlet (GO1) and thereafter passed by the second gas inlet (GI2) through the gas wash liquid (GWL), and discharged by the second gas outlet (GO2), before being recirculated to the first gas inlet (GI1) into the reaction vessels (RV). The gas pump applied in the above production system may be any device suitable for creating a gas flow, e.g., by physical or mechanical action.

In an embodiment of the process of the invention wherein a homogeneous catalyst is applied in a continuous reactor, the polar phase (in which the catalyst is dissolved) may be isolated using gravity separation or mechanical separation after exiting the reactor vessel. After separation, the polar phase may be recycled back to the reactor. To replace any catalyst loss through deactivation or imperfect separation an amount of catalyst may be added continuously or stepwise to the recycled stream.

In a preferred embodiment of the process of the present invention, the process is performed in the above production system and the reaction mixture comprises fatty acid feedstock, a lower alcohol, and a lipolytic enzyme, and the gas wash liquid is a lower alcohol, e.g., methanol or ethanol.

The gas

According to the invention, the gas or gasses used may be any inert gas, i.e., a gas that does not react with the reactants, the catalyst or the reactor materials. Preferably the gas is atmospheric air, oxygen, nitrogen, noble gases or carbon dioxide, or any mixture thereof. The gas used can be supplied from suitable pressure vessels or pumps, for example gas bottles, or by means of compressors.

Catalyst

The use of heterogeneous catalysts has the advantage that they can be removed in a simple manner after the reaction and if appropriate reused. Examples of such heterogeneous catalysts are metal salts, ion exchange resins or catalysts immobilized on suitable supports. Particularly suitable catalysts are those with particle sizes which allow simple and rapid removal from the reaction medium, e.g., enzymes, which are immobilized on supports. The supports used for enzyme immobilization are frequently ion exchange resins or polymer particles which possess suitable particle size distributions.

The catalysts used in accordance with the invention may be those whose particle size is such that they can be retained in the reaction vessel without any great pressure drop using the customary available filter systems, i.e., larger than 0.5 μm , preferably larger than 5 μm , more preferably larger than 10 μm , especially larger than 25 μm . They may be polymeric catalysts of appropriate particle size, or catalysts immobilized on suitable supports. Examples of the polymeric catalysts are ion exchangers, for example sulphonated polystyrenes or zeolites. According to the invention, the catalysts immobilized on suitable supports may be chemical catalysts or immobilized enzymes.

In embodiments where a homogeneous catalyst is applied the catalyst is preferably in solution with a high boiling point liquid, preferably glycerol. The reaction takes place at the interface layer between the polar and non-polar phases. The byproduct water is stripped from the solution by the gas flow (G), though at a slightly reduced rate as compared to a heterogeneous system due to the presence of a high boiling point polar phase (e.g., glycerol). The polar phase may be recovered from the non-polar phase downstream of the reactor by simple gravity or mechanical separation methods and returned to the reactor vessel.

The homogeneous catalysts used in the process of the invention are preferably an enzymatic catalyst, e.g., a lipase from *Thermomyces lanuginosus* (TL) or *Candida antarctica* Lipase B (CALB) in glycerol solutions, using glycerol as the high boiling point polar phase.

Enzyme immobilization

The use of immobilized enzymes in processing of oils experience significant growth due to new technology developments that have enabled cost effective methods. A fundamental advantage of immobilized enzymes is that they can be recovered and re-used from a batch process by simple filtration.

Various ways of immobilizing lipolytic enzymes are well known in the art. A review of lipase immobilization is found in "Immobilized lipase reactors for modification of fats and oils – a review" Malcata et al., 1990, *J. Am. Oil Chem. Soc.* 67: 890-910, where examples of representative lipase immobilizing carriers are illustrated, including inorganic carriers such as

diatomaceous earth, silica, porous glass, etc.; various synthetic resins and synthetic resin ion exchangers; and natural polysaccharide carriers such as cellulose and cross-linked dextrin introduced with ion exchange groups.

In some embodiments, the invention relates to a method, wherein the lipolytic enzyme is immobilized either on a carrier; by entrapment in natural or synthetic matrices, such as sol-gels, alginate, and carrageenan; by cross-linking methods such as in cross-linked enzyme crystals (CLEC) and cross-linked enzyme aggregates (CLEA); or by precipitation on salt crystals such as protein-coated micro-crystals (PCMC).

In some embodiments, the invention relates to a method, wherein the carrier is a hydrophilic carrier selected from the group containing: porous inorganic particles composed of alumina, silica or silicates such as porous glass, zeolites, diatomaceous earth, bentonite, vermiculite, hydrotalcite; and porous organic particles composed of carbohydrate polymers such as agarose or cellulose.

In some embodiments, the invention relates to a method, wherein the carrier is a hydrophobic carrier selected from the group containing: synthetic polymers such as nylon, polyethylene, polypropylene, polymethacrylate, or polystyrene; and activated carbon.

Enzyme

The lipolytic enzyme may be selected from lipases, cutinases or esterases, for example a lipase selected from the *Candida antarctica* lipase A (CALA) as disclosed in WO 88/02775, the *C. antarctica* lipase B (CALB) as disclosed in WO 88/02775 and shown in SEQ ID NO:1 herein, the *Thermomyces lanuginosus* (previously *Humicola lanuginosus*) lipase disclosed in EP 258068), the *Thermomyces lanuginosus* variants disclosed in WO 2000/60063 or WO 95/22615, in particular the lipase shown in positions 1-269 of SEQ ID NO: 2 of WO 95/22615, the *Hyphozyma* sp. lipase (WO 98/18912), and the *Rhizomucor miehei* lipase (SEQ ID NO: 5 in WO 2004/099400), a lipase from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218272), *P. cepacia* (EP 331376), *P. glumae*, *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012); a *Bacillus* lipase, e.g., from *B. subtilis* (Dartois et al., 1993, *Biochemica et Biophysica Acta* 1131: 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422). Also preferred is a lipase from any of the following organisms: *Absidia corymbifera*, *Absidia reflexa*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus tubingensis*, *Fusarium heterosporum*, *Fusarium oxysporum*, *Penicillium camembertii*, *Rhizopus delemar* (*oryzae*), *Aspergillus foetidus*, *Rhizomucor miehei*, and *Thermomyces lanuginosus*, such as a lipase selected from any of SEQ ID NOs: 1 to 15 in WO 2004/099400.

Preferably, the lipolytic enzyme is an enzyme having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or even at least 99% identity to any of the aforementioned lipases.

More preferred, the lipolytic enzyme has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least or even at least 99% identity to the amino acid sequence shown as positions 1-342 of SEQ ID NO: 1 herein.

Liquid (non-immobilized or free) lipolytic enzyme compositions suitable for use in the process of the invention and examples of commercially available immobilized lipolytic enzyme include the ones sold under the trade names LIPOZYME TL 100L, LIPOZYME CaLB L, CALLERA ULTRA L, and CALLERA TRANS from Novozymes A/S, Bagsvaerd, Denmark.

Immobilized lipolytic enzyme compositions suitable for use in the process of the invention and examples of commercially available immobilized lipolytic enzyme include the ones sold under the trade names NOVOZYM 435, LIPOZYME RM IM or LIPOZYME TL IM from Novozymes A/S, Bagsvaerd, Denmark, or Amano PS, from Amano, Japan.

Typically, the enzyme is used in a concentration corresponding to 1 LU/g fatty acid feedstock to 1000 LU/g fatty acid feedstock. Preferably the enzyme is used in a concentration of between 5 LU/g fatty acid feedstock to 500 LU/g fatty acid feedstock, more preferably between 10 LU/g fatty acid feedstock to 100 LU/g fatty acid feedstock.

Typically, the enzyme is used in a concentration corresponding to 1 PLU/g fatty acid feedstock to 1000 PLU/g fatty acid feedstock. Preferably the enzyme is used in a concentration of between 5 PLU/g fatty acid feedstock to 500 PLU/g fatty acid feedstock, more preferably between 10 PLU/g fatty acid feedstock to 100 PLU/g fatty acid feedstock.

Enzyme sources and formulation: The lipolytic enzyme used in the process of the invention may be derived or obtainable from any of the sources mentioned herein. The term "derived" means in this context that the enzyme may have been isolated from an organism where it is present natively, *i.e.*, the identity of the amino acid sequence of the enzyme are identical to a native enzyme. The term "derived" also means that the enzymes may have been produced recombinantly in a host organism, the recombinant produced enzyme having either an identity identical to a native enzyme or having a modified amino acid sequence, *e.g.*, having one or more amino acids which are deleted, inserted and/or substituted, *i.e.*, a recombinantly produced enzyme which is a mutant and/or a fragment of a native amino acid sequence. Within the meaning of a native enzyme are included natural variants. Furthermore, the term "derived" includes enzymes produced synthetically by, *e.g.*, peptide synthesis. The term "derived" also encompasses enzymes which have been modified, *e.g.*, by glycosylation, phosphorylation etc., whether in vivo or in vitro. The term "obtainable" in this context means that the enzyme has an amino acid sequence identical to a native enzyme. The term encompasses an enzyme that has

been isolated from an organism where it is present natively, or one in which it has been expressed recombinantly in the same type of organism or another, or enzymes produced synthetically by, e.g., peptide synthesis. With respect to recombinantly produced enzyme the terms "obtainable" and "derived" refers to the identity of the enzyme and not the identity of the host organism in which it is produced recombinantly.

Accordingly, the lipolytic enzyme may be obtained from a microorganism by use of any suitable technique. For instance, an enzyme preparation may be obtained by fermentation of a suitable microorganism and subsequent isolation of an enzyme preparation from the resulting fermented broth or microorganism by methods known in the art. The enzyme may also be obtained by use of recombinant DNA techniques. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector comprising a DNA sequence encoding the enzyme in question and the DNA sequence being operationally linked with an appropriate expression signal such that it is capable of expressing the enzyme in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may also be incorporated into the genome of the host cell. The DNA sequence may be of genomic, cDNA or synthetic origin or any combinations of these, and may be isolated or synthesized in accordance with methods known in the art.

The optimum parameters for enzymatic activity will vary depending upon the enzyme used. The rate of enzyme degradation depends upon factors known in the art, including the enzyme concentration, substrate concentration, temperature, the presence or absence of inhibitors and presence of water. These parameters may be adjusted to optimise the esterification reaction.

During the enzymatic treatment step, the temperature of the suspension should be adjusted to provide effective enzyme activity. In general, a temperature of about 30°C to about 90°C is used, particularly from about 35°C to about 60°C.

MATERIALS AND METHODS

Degree of identity

Sequence identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity". For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution

matrix. The output of Needle labeled "longest identity" (obtained using the `-nobrief` option) is used as the percent identity and is calculated as follows:

$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the `-nobrief` option) is used as the percent identity and is calculated as follows:

$$(\text{Identical Deoxyribonucleotides} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment}).$$

15 **Lipolytic Activity**

Lipase Unit (LU): The lipolytic activity may be determined using tributyrine as substrate. This method is based on the hydrolysis of tributyrin by the enzyme, and the alkali consumption to keep pH constant during hydrolysis is registered as a function of time.

One Lipase Unit (LU) is defined as the amount of enzyme which, under standard conditions (*i.e.*, at 30°C; pH 7.0; with 0.1% w/v Gum Arabic as emulsifier and 0.16 M tributyrine as substrate) liberates 1 micromol titrable butyric acid per minute. One KLU is 1000 LU.

Propyl Laurate Units: The ester synthesis activity of immobilized lipases may be determined as Propyl Laurate Units per gram product: PLU/g.

The immobilized lipase esterifies lauric acid with 1-propanol, forming propyl laurate. The activity ($\mu\text{mol/g/min}$) is determined by quantification of formed propyl laurate and consumed lauric acid by GC. Reaction temperature is 60°C and reaction time 20 min.

One PLU unit corresponds to 1 $\mu\text{mol/g/min}$, *e.g.*, 1 μmol propyl laurate formed per g of enzyme product per minute.

30 **Enzyme**

NOVOZYM 435 is a commercial enzyme product from Novozymes A/S comprising an immobilized lipase B from *Candida antarctica* (SEQ ID NO:1 herein). The product has an activity of 10000 PLU/g. NOVOZYM 435 was used in Examples 1-5.

CALLERA ULTRA L is a commercial enzyme product from Novozymes A/S comprising an liquid lipase B from *Candida antarctica* (SEQ ID NO: 1 herein). The product has an activity of 50 KLU/g. CALLERA ULTRA L was used in Example 6.

Example 1

Esterification of free fatty acids from high FFA feedstock was performed using three different methods of eliminating water from the reaction mixture:

1. A process of the invention with continuous water removal
2. Vacuum/evaporation and refill with alcohol after the vacuum step
3. Heat/evaporation and refill with alcohol after the heat step

The feedstock was yellow grease with 14.1% of FFA. Batch size was 40 g oil. Methanol was used as the alcohol. The amount of water in the reaction mixture at time = 0 was 891 ppm. The enzyme was Novozym 435 in a dosage of 4% w/w of oil. The reaction was performed at a temperature of 45°C with shaking at 300 rpm.

In the process of the invention, an air flow rate of 617 ml/min was applied and re-circulated in a 20 ml methanol column.

The vacuum/evaporation treatment was carried out in a rotary evaporator at 65°C at 50 mbar for 3 hours after 30 and 60 minutes reaction time, respectively.

The heat/evaporation treatment used warm storage at 50°C overnight in an open vessel to evaporate water and alcohol. In the two latter methods methanol was added to make up for the amount lost during evaporation. The results are presented in table 1.

Table 1				
Method	Reaction time, minutes	Vol % MeOH added	Water ppm (w/w)	% FFA
1	30	1.69	n.a.	2.85
2	30	2.87	4632	3.08
3	30	2.87	4988	3.27
1	60	1.69	n.a.	0.88
2	60	4.66	1328	0.63
3	60	6.74	2325	0.89
1	100	1.69	577	0.25
2	105	6.50	309	0.38

Only with the process of the invention was it possible to achieve $\leq 0.25\%$ FFA as required for biodiesel. Also, the amount of alcohol used in methods 2 and 3 are higher than method 1 which will cost more in a production setting. Lastly, the process of the invention is the only method of the three that are operating continuously and therefore having a shorter total operating time. In method 2 two interruptions for vacuum treatment were used and in method 3 one (long) heating/settling period was used.

Example 2

Esterification of free fatty acids in a biodiesel feedstock with 4.0% unconverted FFA feedstock was performed using two different methods for eliminating water from the reaction mixture:

- 5 1. A process of the invention with continuous water removal
2. Vacuum treatment and refill with alcohol

The setup was as described in example 1. Four vacuum steps were used after 15, 30, 45, and 60 minutes, respectively. The water content in the reaction mixture at time = 0 was 500 ppm. The results are presented in table 2.

10

Table 2				
Method	Reaction time, minutes	Vol % MeOH added	Water ppm	% FFA
1	30	0.49	n.a.	0.83
2	30	0.80	1225	1.43
1	50	0.49	n.a.	0.30
2	60	7.88	260	0.54
1	65	0.49	129	0.17
2	90	7.88	348	0.49

Only with the process of the invention was it possible to achieve $\leq 0.25\%$ FFA. The result was achieved with a low consumption of methanol.

15 **Example 3**

In this example we tested the efficiency of the invention to supply alcohol from the alcohol column to the reactor by the re-circulation of air. The feedstock was yellow grease with 15.44% FFA. All other conditions were as in example 1, treatment 1. The results are presented in table 3.

20

Table 3			
Reaction time, minutes	Vol % MeOH added	Water ppm (w/w)	% FFA
15	0	n.a.	6.18
30	0	680	1.34
40	0	n.a.	0.52
60	0	n.a.	0.20
80	0	n.a.	0.18

The amount of methanol transferred from the methanol column by the air re-circulation was sufficient to obtain $\leq 0.25\%$ FFA.

5 Example 4

The conditions are as in example 3 except that methanol was substituted with ethanol. The results are presented in table 4.

Table 4			
Reaction time, minutes	Vol % EtOH added	Water ppm (w/w)	% FFA
15	0	n.a.	8.85
30	0	610	3.00
40	0	n.a.	1.57
60	0	n.a.	0.38
80	0	n.a.	0.21

10 Example 5

The conditions are as in examples 3 and 4 except that the feedstock is a biodiesel feedstock consisting of yellow grease FAME with 15% FFA added. The results are presented in table 5.

Table 5					
	Methanol			Ethanol	
Reaction time, minutes	Water (w/w)	ppm	% FFA	Water (w/w)	% FFA
15	n.a.		4.41	n.a.	8.39
30	530		0.82	569	3.05
40	n.a.		0.44	n.a.	1.32
60	n.a.		0.11	n.a.	0.30
80	n.a.		0.04	n.a.	0.20
100	n.a.		0.12	n.a.	0.18
120	52		0.09	76	0.17

Example 6

In this example a liquid enzyme composition, commercially sold as Lipozyme CALB-L. 40 grams of neat biodiesel doped with oleic acid to 2.1% FFA, 1 wt% (0.4 g) Lipozyme CALB-L, 2.57% (1.026 g) methanol, and 9–10% ACS grade glycerol were combined in a 120 ml bottle. The mixtures were reacted in a heated shaker at 300 rpm and 35°C for 220 minutes. Samples were tested for % FFA at 50, 105, 165, and 220 minutes. Before sampling, the mixture was allowed to settle for 2 minutes to ensure no enzyme or glycerol was removed in the sample. The results are presented in Table 6. Three methods were tested:

Method 1: A process of the invention for continuous water removal as described in example 1 with 10% (4 g) glycerol;

Method 2: Addition of 10% glycerol (no continuous water removal);

Method 3: Addition of 9% (3.6 g) glycerol and 1% (0.4 g) water (no continuous water removal).

Table 6			
Time (min.)	Method 1 (% FFA)	Method 2 (% FFA)	Method 3 (% FFA)
0	2.10	2.10	2.10
50	1.40	1.90	1.96
105	0.71	1.60	1.81
165	0.38	1.37	1.68
220	0.23	1.58	1.17

CLAIMS

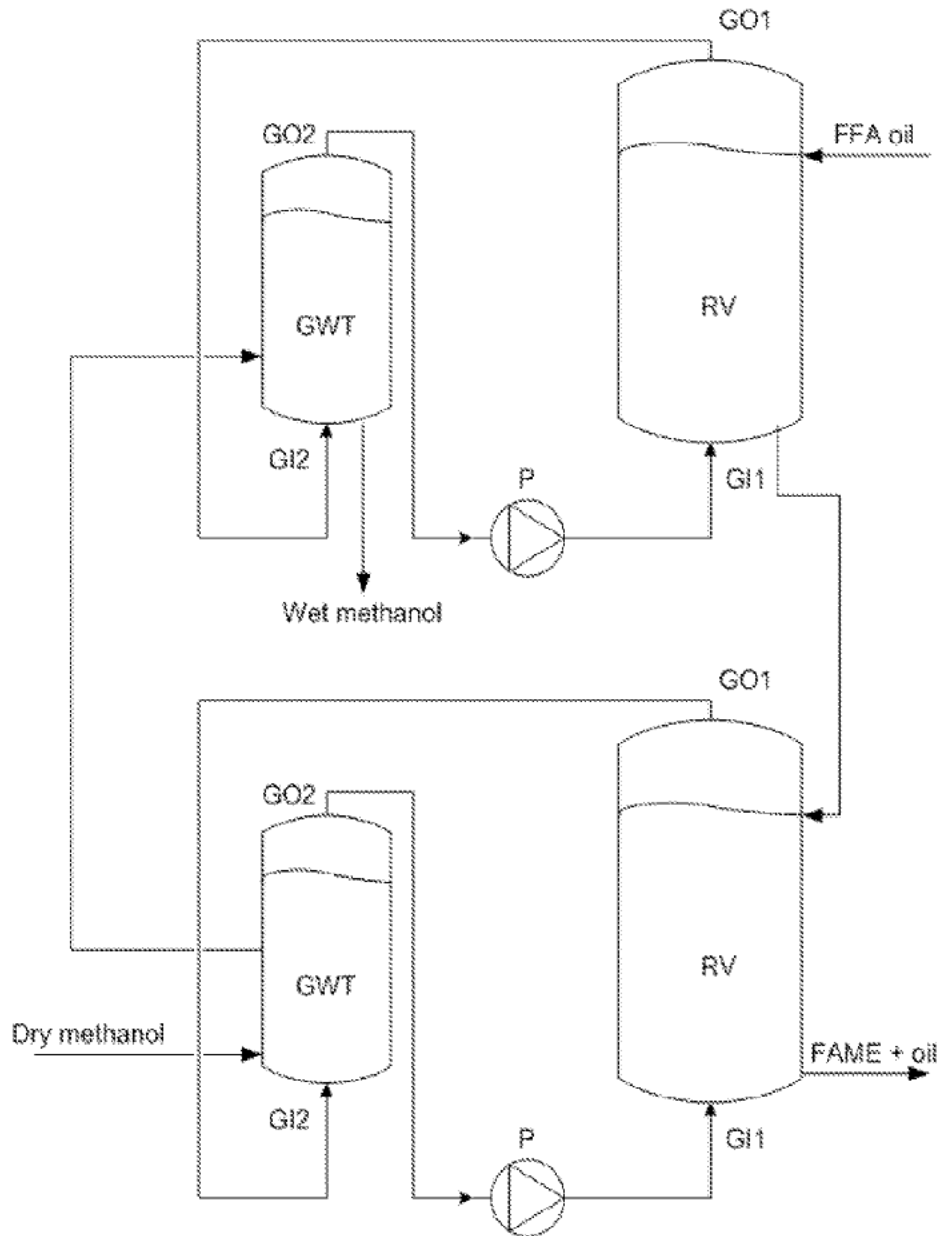
1. A process for the production of fatty acid alkyl esters from a fatty acid feedstock containing free fatty acids, comprising the steps of:
 - 5 (a) providing a reaction mixture (R) that comprises the fatty acid feedstock, a lower alcohol, and a lipolytic enzyme;
 - (b) allowing the reaction mixture (R) to react under formation of fatty acids alkyl esters;wherein a gas flow (G) is passed through the reaction mixture (R) and thereafter passed
10 through a volume of lower alcohol (A), before being recirculated through the reaction mixture (R).
2. The process according to claim 1 in which the lower alcohol is selected from methanol, ethanol, and propanol, including mixtures thereof.
15
3. The process according to claim 1 or 2 in which the gas is selected from atmospheric air, oxygen, nitrogen, noble gases, carbon dioxide, or any mixture thereof.
4. The process according to any of the preceding claims in which the lipolytic enzyme is a
20 lipase, cutinase and/or esterase.
5. The process according to any of the preceding claims in which the lipase is the *Candida antarctica* lipase B, such as an enzyme having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least or even at least 99%
25 identity to the amino acid sequence shown as SEQ ID NO: 1.
6. The process according to any of the preceding claims in which the lipolytic enzyme is applied in a liquid composition.
- 30 7. The process according to any of the preceding claims in which the lipolytic enzyme is immobilized on a support, such as a hydrophilic carrier selected from the group containing: porous inorganic particles composed of alumina, silica or silicates such as porous glas, zeolites, diatomaceous earth, bentonite, vermiculite, hydrotalcite; and porous organic particles composed of carbohydrate polymers such as agarose or cellulose, or such as a hydrophobic carrier
35 selected from the group containing: synthetic polymers such as nylon, polyethylene, polypropylene, polymethacrylate, polystyrene; and activated carbon.

8. The process according to any of the preceding claims in which the process is performed as a batch, or a continuous process.
9. The process according to any of the preceding claims in which the volume of lower alcohol (A) following being passed through by the gas flow (G) is dewatered, e.g., by distillation, pervaporation or use of a molecular sieve.
10. The process according to any of the preceding claims in which the volume of lower alcohol (A) comprises the same lower alcohol as comprised in the reaction mixture (R).
11. The process according to any of the preceding claims in which the lower alcohol comprised in the reaction mixture (R) is supplied from the volume of lower alcohol (A) by the gas flow (G).
12. The process according to any of the preceding claims in which the volume of lower alcohol (A) is reused in a transesterification process, e.g. a transesterification process for production of biodiesel.
13. The process according to any of the preceding claims in which the temperature of the reaction mixture (R) in step (b) is kept between 25°C and 80°C and preferably between 30°C and 50°C, and most preferably between 35°C and 45°C.
14. The process according to any of the preceding claims in which the reaction mixture (R) is stirred.
15. The process according to any of the preceding claims in which the gas flow (G) is dispersed in the reaction mixture (R) by means of a rotary jet head.
16. A production system for processing a reaction mixture (R), said production system comprising:
- (a) one or more enclosed reaction vessels(s) (RV) suitable for comprising said reaction mixture (R), each reaction vessels (RV) comprising at least one first gas inlet (GI1) and at least one first gas outlet (GO1),
 - (b) one or more gas wash tank(s) (GWT) suitable for comprising a gas wash liquid (W), each gas wash tank (GWT) comprising at least one second gas inlet (GI2) and at least one second gas outlet (GO2),

(c) comprising one or more gas pump(s) (P) capable of delivering a gas flow (G), which can be passed by the first gas inlet (GI1) through the reaction vessels (RV), discharged by the first gas outlet (GO1) and thereafter passed by the second gas inlet (GI2) through the gas wash liquid (GWL), and discharged by the second gas outlet (GO2), before being
5 recirculated to the gas inlet (GI1) into the reaction vessels (RV).

1/1

Fig. 1



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/023927

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P7/64
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/125574 A1 (NOVOZYMES AS [DK]; DESMET BALLESTRA GROUP S A NV [BE]; DE GREYT WIM [B] 23 October 2008 (2008-10-23)	16
A	the whole document	1-15
X	----- JEONG J C ET AL: "Enzymatic esterification reaction in organic media with continuous water stripping: Effect of water content on reactor performance and enzyme agglomeration", BIOTECHNOLOGY TECHNIQUES, vol. 11, no. 12, December 1997 (1997-12), pages 853-858, XP002674156, ISSN: 0951-208X	16
A	the whole document ----- -/--	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

18 April 2012

Date of mailing of the international search report

04/05/2012

Name and mailing address of the ISA/

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Vantage Exhibit 1002
Roscoe, Richard Page 587 of 1215

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/023927

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3 064 952 A (BROWN RONALD W) 20 November 1962 (1962-11-20)	16
A	the whole document -----	1-15
X	WO 93/12241 A1 (KARLSHAMNS OILS & FATS AB [SE]) 24 June 1993 (1993-06-24)	16
A	the whole document -----	1-15
A	WO 88/02775 A1 (NOVO INDUSTRI AS [DK]) 21 April 1988 (1988-04-21)	1-16
	the whole document -----	

Vantage Exhibit 1002
Page 588 of 1215

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/023927

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
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3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/023927

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008125574	A1	23-10-2008	AR 066015 A1 15-07-2009
			AT 516362 T 15-07-2011
			CN 101657543 A 24-02-2010
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WO 8802775	A1	21-04-1988	AT 117018 T 15-01-1995
			CA 1339844 C 28-04-1998
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			DE 3750982 T2 14-06-1995
			EP 0287634 A1 26-10-1988
			JP 2628667 B2 09-07-1997
			JP H01501120 A 20-04-1989
			WO 8802775 A1 21-04-1988

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

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Application Number	14578075
Filing Date	2014-12-14
First Named Inventor	Jeff Addy
Art Unit	1657
Examiner Name	SRIVASTAVA, KAILASH C
Attorney Docket Number	FLORATECH002

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Filing Date	2014-12-14
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Art Unit	1657
Examiner Name	SRIVASTAVA, KAILASH C
Attorney Docket Number	FLORATECH002

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	1	Steinke et al., "High-yield Preparation of Wax Esters via Lipase-catalyzed Esterification Using Fatty Acids and Alcohols from Crambe and Camelina Oils", J. Agric Food Chem, February 2011, Vol. 49, No. 2, pp. 647-651.	

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Application Number	14578075
Filing Date	2014-12-14
First Named Inventor	Jeff Addy
Art Unit	1657
Examiner Name	SRIVASTAVA, KAILASH C
Attorney Docket Number	FLORATECH002

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See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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Signature	/Adam Stephenson/	Date (YYYY-MM-DD)	2019-03-13
Name/Print	Adam R. Stephenson	Registration Number	58349

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Vantage Exhibit 1002

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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	2874	((("ADDY") near3 ("Jeff")) OR (("DALY" near3 ("Clay")) OR (("BROWN") near3 ("James"))).INV.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:41
S2	32	S1 and "wax ester"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:43
S3	4842	(C12P7/6436 or C11C3/003 or C11C3/10).cpc.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:54
S4	262	S3 and jojoba	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:54
S5	132	S4 and lipase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:54
S6	128	jojoba same lipase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:54
S7	69	"jojoba oil" same lipase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:55
S8	19	S7 and "wax ester"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 00:55
S9	11	("20130052701" "5968530" "20130149414" "20140017741" "4329298" "4360387" "6123979" "8349594" "20050176597" "20130185991" "20130253211").PN.	US-PGPUB; USPAT	OR	ON	2019/05/29 01:05
S10	777	jojoba same (transesterif\$4 or transesterif\$4 or esterif\$4)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:06

S11	124	S10 and lipase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:06
S12	4	"20140017741"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:06
S13	3	"20140120589"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:31
S14	5	S9 and "jojoba oil"	US-PGPUB; USPAT	OR	ON	2019/05/29 01:37
S15	336	("jojoba oil") same (transesterif\$4 or trans-esterif\$4 or esterif\$4)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:42
S16	24	S15 and (lipase or lipolytic)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:43
S17	871	("jojoba oil") same (transesterif\$7 or trans-esterif\$7 or esterif\$7)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:44
S18	60	S17 and (lipase or lipolytic)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2019/05/29 01:44

EAST Search History (Interference)

< This search history is empty >

5/ 29/ 2019 9:33:43 AM

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 14/578,075		Filing Date 12/19/2014		☐ To be Mailed		
ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO										
APPLICATION AS FILED - PART I										
		(Column 1)	(Column 2)							
FOR		NUMBER FILED	NUMBER EXTRA	RATE (\$)		FEE (\$)				
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A	N/A	N/A						
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))		N/A	N/A	N/A						
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A	N/A	N/A						
TOTAL CLAIMS (37 CFR 1.16(i))		minus 20 =	*	x \$40 =						
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =	*	x \$210 =						
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).								
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))										
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL						
APPLICATION AS AMENDED - PART II										
		(Column 1)	(Column 2)	(Column 3)						
AMENDMENT	09/19/2019	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	* 7	Minus	** 20	= 0	x \$50 =		0		
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0	x \$230 =		0		
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE		0		
		(Column 1)	(Column 2)	(Column 3)						
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	*	Minus	**	=	x \$0 =				
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x \$0 =				
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE				
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.						LIE				
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".						/GLORIA J TRAMMELL/				
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".										
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.										

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Electronic Patent Application Fee Transmittal

Application Number:	14578075			
Filing Date:	19-Dec-2014			
Title of Invention:	Processes and Systems for Catalytic Manufacture of Wax Ester Derivatives			
First Named Inventor/Applicant Name:	Jeff Addy			
Filer:	Adam Reid Stephenson			
Attorney Docket Number:	FloraTech002			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension - 1 month with \$0 paid	2251	1	100	100
Miscellaneous:				
Total in USD (\$)				100

Electronic Acknowledgement Receipt

EFS ID:	37220695
Application Number:	14578075
International Application Number:	
Confirmation Number:	2053
Title of Invention:	Processes and Systems for Catalytic Manufacture of Wax Ester Derivatives
First Named Inventor/Applicant Name:	Jeff Addy
Customer Number:	93756
Filer:	Adam Reid Stephenson
Filer Authorized By:	
Attorney Docket Number:	FloraTech002
Receipt Date:	19-SEP-2019
Filing Date:	19-DEC-2014
Time Stamp:	20:35:43
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$ 100
RAM confirmation Number	E20199IK36085733
Deposit Account	
Authorized User	

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Vantage Exhibit 1002

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CARGILL 00000625

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Req. Reconsideration-After Non-Final Reject	OAR5.pdf	171548	no	13
			849b0a71892cd13d2af3d7e96857785dbb450f96		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30159	no	2
			a6f77fe77044924115f00df31c76a3bedb4b27fa		
Warnings:					
Information:					
Total Files Size (in bytes):			201707		
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FLORATECH002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

AMENDMENT/REPLY

Applicant: Jeff Addy

Docket No: FLORATECH002

Examiner: PATURY, SRIKANTH

Ser. No: 14578075

Filed: December 19, 2014

Group Art Unit: 1657

Conf. No.: 2053

Title: Processes and Systems for Catalytic Manufacture of Wax Ester Derivatives

**RESPONSE TO OFFICE ACTION
DATED JUNE 6, 2019**

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir/Madam:

The following is responsive to the Office Action mailed June 6, 2019. Applicant respectfully requests a 1 month extension of time the fee for which is submitted herewith and reconsideration of the application in view of the following:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

Please amend claims 3-4 as indicated among the following complete set of pending claims:

1. (Previously presented) A process for transesterifying wax esters, the process comprising:
 - providing a feedstock comprising wax esters;
 - contacting the feedstock with a lipase;
 - catalytically transesterifying the wax esters in the feedstock with the lipase to form a transesterified product;
 - wherein an oxidative stability index (OSI) of the transesterified product is at least 2.5 times greater than an OSI of the feedstock.
2. (Original) The process of claim 1, wherein the transesterified product comprises no methyl esters.
3. (Currently amended) The process of claim 1, wherein the feedstock further comprises an antioxidant and catalytically transesterifying the wax esters in the feedstock with the lipase further comprises catalytically transesterifying without ~~one of removing and or~~ degrading the antioxidant in the feedstock.
4. (Currently amended) The process of claim 3, wherein the feedstock further comprises a skin conditioning agent and a volatile compound and catalytically transesterifying the wax

esters in the feedstock with the lipase further comprises catalytically transesterifying without ~~one of removing and or~~ degrading the skin conditioning agent and the volatile compound in the feedstock.

5. (Original) The process of claim 1, wherein the wax esters of the feedstock are jojoba wax esters.

6. (Original) The process of claim 5, wherein the jojoba wax esters further comprise hydrogenated jojoba wax esters.

7. (Original) The process of claim 6, wherein a dropping point of the transesterified product is greater than a dropping point of the feedstock and the dropping point of the transesterified product increases as a percentage of hydrogenated jojoba wax esters in the feedstock increases.

8-20. (Canceled).

REMARKS

Claims 1 – 7 are currently pending in the application. Applicant has amended claims 3 and 4. Applicant requests reconsideration of the application in light of the following remarks.

Objections to Claims

The Examiner has objected to claims 3 and 4 for informalities. Applicant has amended claims 3 and 4 as suggested by the Examiner, to make the claims “less awkward.” Applicant respectfully requests that the objections to claims 3 and 4 be withdrawn.

Double Patenting Rejection

Claims 1-7 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 8-14 of co-pending Application No. 14/841,242 to Addy. The Applicant notes that this rejection was made in the 11/30/2017 OA and subsequently withdrawn in the 08/06/2018 OA. Similar arguments will now be made against this obviousness type double patenting rejection.

The Examiner’s analysis of why claims 1-7 of the present application are rejected based on the claims of the ‘242 patent application is error. Specifically, the Examiner states on p.9 of the 6/6/19 OA that “Although the conflicting claims are not identical, they are not patentably distinct from each other because the scope of the claims 8-14 of the ‘242 application renders obvious that of the instant claims. The co-pending claims also [a] recite process to transesterify feedstocks comprising wax esters, contacting with a lipase and catalytically transesterifying the feedstock to produce a transesterified product. The co-pending claims also teach feedstock to comprise jojoba wax esters (claim 11), hydrogenated jojoba wax esters (claim 12), the feedstock to comprise antioxidants and catalyzing without removing or degrading the antioxidant (claim 10). The co-pending claims are silent regarding the OSI and dropping point of the product, feedstock comprising skin conditioning agent and volatile compounds. However, the copending claims teach the same feedstock, jojoba oil, which comprises skin conditioning agents and volatile compounds. Regarding OSI, dropping point of the product and performing the transesterification without removing/degrading the

skin conditioning agent, the co-pending claims teach the same process as the instant claims, catalyzing jojoba oil with lipase, therefore it is expected the product of the co-pending claims would also have the same functional characteristics.” These statements are error and wholly fail to conduct the analysis required by the MPEP in making out an obviousness type double patenting rejection—particularly in view of the fact that the ‘242 patent is a continuation-in-part of the present application.

Specifically in MPEP § 804 (II)(B) Requirements of a Double Patenting Rejection – Nonstatutory Double Patenting, the Examiner is required to perform an Anticipation Analysis followed by an Obviousness Analysis if the previously patented claims are not found to anticipate the claims. In performing the anticipation analysis, the Examiner must determine whether the entire scope of the reference claim falls within the scope of the examined claim so that the claim under examination is anticipated by the reference claim. MPEP § 804 (II)(B)(1).

In this case, independent claim 1 of the present application fails to recite major elements of the method of independent claim 8 of the ‘242 application. Independent claim 8 of the ‘242 application states that the method includes “introducing into the feedstock an alcohol with a carbon number ranging from 1 to 34 carbons, the alcohol selected from the group consisting of a straight chain alcohol, a branched chain alcohol, any combination of straight chain alcohols, any combination of branched chain alcohols, and any combination thereof.... wherein the enzymatically transesterified product is adapted to produce a finished product having a certain formula that has a viscosity that substantially matches a viscosity of a finished product having the same certain formula comprising chemically catalyzed transesterified wax esters.” Clearly, the claim 1 of the present application does not anticipate the claim under examination, as the claim 1 in this application does not include the element of “introducing into the feedstock an alcohol with a carbon number ranging from 1 to 34 carbons, the alcohol selected from the group consisting of a straight chain alcohol, a branched chain alcohol, any combination of straight chain alcohols, any combination of branched chain alcohols, and any combination thereof...” as disclosed in Claim 8. This is not a trivial part of the claim, as **this element is**

not disclosed in the specification of the present application, which is why the ‘242 application is a continuation-in-part of the present application.

Since the independent claims are not anticipated by the claims of the ‘242 application, the next step in the analysis is to determine whether the independent claims in the present application would be “an obvious variation of the invention defined in the claim of a patent...” MPEP § 804 (II)(B)(2)(a). The disclosure of the present application in this analysis cannot be used as prior art.

It would not be obvious to one skilled in the art to introduce an alcohol into the feedstock before contacting the feedstock with the lipase because it is not fundamentally obvious to conduct a reaction while adding an additional step to that reaction. No person of ordinary skill would be led to add the step of adding an alcohol to the feedstock of claim 1 in view of the claims of ‘242 application. There is no teaching pointed out by the examiner’s analysis that would teach one of ordinary skill to add an additional step suggested in Claims 1-7. Furthermore, the addition of the alcohol in Claim 8 of the ‘242 application produced unexpected results as described at least in para. [0075]-[0076] of that application because the addition of alcohol to the feedstock prior to transesterification unexpectedly results in a change in viscosity of finished products made using the transesterified products formed using the claimed process of the ‘242 patent. Because adding alcohol to the feedstock yields unexpected results, the claims of the present application, which lack the element of the claims of the ‘242 patent are clearly not an obvious variation of what is claimed in the present application. Furthermore, the ‘242 application is a continuation-in-part application and claim 8 is drawn to the newly added subject matter, making an obviousness type double patenting rejection even more unusual, given that the Applicant could not claim the subject matter of claim 8 in the present application in the first place (this alone obviates any issues of unjustifiable extension of patent term). The Examiner’s provisional rejection of claim 8 for obviousness-type nonstatutory double patenting is clearly erroneous, and the Applicant respectfully requests that it be withdrawn.

Rejections under 35 U.S.C. 103

Claims 1 – 5 were rejected under 35 U.S.C. 103 as being unpatentable over Gunawan (Journal of Oleo Science, 2004 “Gunawan”), in light of Austic et al. (WO Patent Publication No. WO 2012/106701A1, hereinafter “Austic”), and as evidenced by Busson-Breysse (JAOCS, 1994, hereinafter “Busson-Breysse”) and Fucosterol (Cayman Chemical, CAS Entery, 2019, hereinafter “Fucosterol”). Claims 1 – 7 were rejected under 35 U.S.C. 103 as being unpatentable over Gunawan, in light of Austic, and as evidenced by Busson-Breysse and Fucosterol, in further light of Arquette (U.S. Patent No. 5,968,530, hereinafter “Arquette”). Applicant respectfully traverses this rejection and requests reconsideration of the claims.

Referring to the legal standards set forth in Appendix A, Applicant observes that the Examiner has not explicitly stated which of the obviousness rationales endorsed in *KSR International Co. v. Teleflex Inc.* 550 U.S. 398, 127 S.Ct. 1727, 167 L.Ed.2d 705 (2007) are being used. The Applicant believes the rationale being used is A/E:

“(A) Combining prior art elements according to known methods to yield predictable results;

(E) “Obvious to try”-choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;”

MPEP § 2141(II), (III) and 72 Federal Register 57527 (October 10, 2007).

Independent claim 1

Claim 1 was rejected under 35 U.S.C. 103 as being unpatentable over Gunawan in view of Austic. In making the 103 rejection of claim 1 the Examiner argues, on p. 5 of the 6/6/2019 OA, that “it is reasonably expected that the process of Gunawan in view of Austic would also result in the OSI of the transesterified product being at 2.5 times greater than an OSI of the feedstock.” The Examiner’s argument, while not being fully stated, is that the OSI effect recited in claim 1 is inherently disclosed by the combination of the teachings of the disclosures of the methods of Gunawan and Austic.

As the Office well knows, M.P.E.P. § 2112, under the heading “Examiner Must Provide Rationale or Evidence Tending to Show Inherency”, states that “[t]he fact that a

certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993).” M.P.E.P. § 2112 further states that “[i]n relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art.” *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original).

The Examiner’s rejection of claims 1-7 over Gunawan and Austic hang entirely on their alleged teaching of the same method steps as those claimed in independent claim 1. This is because neither Gunawan nor Austic actually include any OSI data of either the feedstock materials or the reaction products—**there is no data in either reference to support the Examiner’s assertion**. There is simply no way one of ordinary skill would have, in view of the disclosures of Gunawan and Austic alone, have been able to predict this result **necessarily would be present** if the teachings were combined. Because of this, neither Gunawan or Austic disclose, expressly or inherently, the subject matter of claim 1.

Further, while the Examiner acknowledges that Gunawan teaches jojoba oils contain wax esters, he completely ignores that the entire statement about jojoba oils teaches away from using jojoba oil because the reference states jojoba oils are expensive. The entirety of Gunawan teaches a method to make further use of palm oil because so much palm oil is produced in Malaysia and Indonesia and it is accordingly less expensive than jojoba oil. This reference would not lead someone skilled in the art to use a reaction mixture including jojoba oil which is both more expensive and produced in different regions of the world than was focused on in Gunawan.

The Examiner argues that the combination of Gunawan and Austic teach the same process as the current application. This is simply not true. The reaction in Gunawan differs from the method of the present application in a number of ways. Gunawan teaches using a solvent, hexane, in containers in small batch reactors. See pg. 472, col. 1, paragraph 2. The method in the present application is solventless and includes continuous flow through packed column reactors. It would not be obvious to take a reaction including a solvent, remove the

solvent and change the reactors entirely. Because the chemical arts are unpredictable this significant change in the method would not clearly lead to the same results. The reactants have been changed which will most likely lead to different results.

Gunawan also teaches increasing the temperature of the reaction from 60 degrees Celsius to 70 degrees Celsius reduces the yield of the reaction. This statement in Gunawan again teaches away from the method in claim 1 because one skilled in the art would not increase the temperature of a reaction if they were trying to increase the yield of the reaction after reading Gunawan. However, the disclosed process in the present application found that increasing the reactant temperature to 70-72 C unexpectedly produces higher yields than disclosed in Gunawan. Gunawan reports yields of 75% using Lipozymes while the present method produced yields greater than 99%. Clearly, the present method produces unexpected results in view of the teachings of Gunawan when the temperature of the reactants in the present disclosure was raised higher than taught in Gunawan and produced better results.

Referring to FIG. 4 of Gunawan, yields are illustrated having a high of 35% with higher levels of enzymes than that disclosed in the present application. In spite of this, the disclosed reaction in the present application produced yields at greater than 99%. Not only does Gunawan not teach the method of claim 1 it does not teach the unexpected results observed when the claimed methods were carried out. Therefore, any rejection of claim 1 using Gunawan is in error and should be withdrawn.

The teachings of Austic do not make up for the deficiencies in Gunawan and therefore the combination does not make claim 1 obvious. For example, Austic teaches using large vessels for reacting gases with the feedstock. See Para. [0021]-[0024] of Austic. As previously described, the present application discloses use of packed columns and liquid reactants. Austic further teaches performing the reactions preferably under lower temperatures between 35 degrees Celsius to 65 degrees Celsius than the temperatures disclosed in the present application. Para. [0040] and [0085]-[0087] of Austic. Even if one skilled in the art

tried jojoba oil in the method of Gunawan based on a teaching of Austic in large vessels the steps would not be the same as the elements of claim 1 and would therefore not result in the unexpectedly high yields experienced in the present application as explained in both the originally filed application in para. [0073] and the Addy Declaration submitted with the Applicant's response on 12/6/2018. The failure of Gunawan to provide teachings that would clearly predict the results in the disclosed application is a classic example of the unpredictability of the chemical arts. Even a minor change in temperature or concentrations can change the product or characteristics of a given chemical reaction. The 103 rejection of claim 1 in view of Gunawan and Austic alone or in combination is therefore inappropriate and the Applicant respectfully requests that the rejection be withdrawn.

Claims 2-7 are allowable for, among other reasons, depending from independent claim 1.

Applicant respectfully requests that the obviousness rejections of claims 1-7 be withdrawn.

Regarding Doctrine of Equivalents

Applicant hereby declares that any amendments herein that are not specifically made for the purpose of patentability are made for other purposes, such as clarification, and that no such changes shall be construed as limiting the scope of the claims or the application of the Doctrine of Equivalents.

CONCLUSION

Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

If any fees, including extension of time fees or additional claims fees, are due as a result of this response, please charge Deposit Account No. 60-1853. This authorization is intended to act as a constructive petition for an extension of time, should an extension of time be needed as a result of this response. The examiner is invited to telephone the undersigned if this would in any way advance the prosecution of this case.

Respectfully submitted,

Date: 9/19/2019

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Appendix A:
Legal Standard for Finding Obviousness under 35 U.S.C. 103

The most recent guidelines published by the USPTO regarding the implementation of the Supreme Court's decision in *KSR International Co. v. Teleflex Inc.* 550 U.S. 398, 127 S.Ct. 1727, 167 L.Ed.2d 705 (2007) state that:

An invention that would have been obvious to a person of ordinary skill at the time of the invention is not patentable. See 35 U.S.C. 103(a). As reiterated by the Supreme Court in *KSR*, the framework for the objective analysis for determining obviousness under 35 U.S.C. 103 is stated in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). Obviousness is a question of law based on underlying factual inquiries. The factual inquiries enunciated by the Court are as follows:

- (A) Determining the scope and content of the prior art;
- (B) Ascertaining the differences between the claimed invention and the prior art; and
- (C) Resolving the level of ordinary skill in the pertinent art...

Office personnel fulfill the critical role of fact-finder when resolving the *Graham* inquiries... When making an obviousness rejection, Office personnel must therefore ensure that the written record includes findings of fact concerning the state of the art and the teachings of the references applied. In certain circumstances, it may also be important to include explicit findings as to how a person of ordinary skill would have understood prior art teachings, or what a person of ordinary skill would have known or could have done. Factual findings made by Office personnel are the necessary underpinnings to establish obviousness...

In short, the focus when making a determination of obviousness should be on what a person of ordinary skill in the pertinent art would have known at the time of the invention, and on what such a person should reasonably have been able to do in view of that knowledge. This is so regardless of whether the source of that knowledge and ability was documentary prior art, general knowledge in the art, or common sense...

The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. The Supreme Court in *KSR* noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit. The Court quoting *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006) stated

that “[R]ejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.”
KSR. . . Exemplary rationales that may support a conclusion of obviousness include:

(A) Combining prior art elements according to known methods to yield predictable results;

(B) Simple substitution of one known element for another to obtain predictable results;

(C) Use of known technique to improve similar devices (methods, or products) in the same way;

(D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results;

(E) “Obvious to try”-choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;

(F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art;

(G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention. MPEP § 2141(II), (III) and 72 Federal Register 57527 (October 10, 2007).

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	14578075
	Filing Date	2014-12-19
	First Named Inventor	Jeff Addy
	Art Unit	1657
	Examiner Name	PATURY, SRIKANTH
	Attorney Docket Number	FLORATECH002

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	2	H07-079786	JP	A	1995-03-28	The Nisshin Oil Mills Ltd		☒
	3	2003-532705	JP	A	2003-11-05	Fan Tech Ltd.		☒

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¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	14578075
Filing Date	2014-12-19
First Named Inventor	Jeff Addy
Art Unit	1657
Examiner Name	PATURY, SRIKANTH
Attorney Docket Number	FLORATECH002

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Adam Stephenson/	Date (YYYY-MM-DD)	2019-11-05
Name/Print	Adam R. Stephenson	Registration Number	58349

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Bibliography

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- (19) [Publication country/region] Japan Patent Office (JP)
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- (54) [Title of the invention] 3-improved lipid composition containing 4-methoxybenzylthio

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CARGILL 00000644

urea derivatives and said derivatives

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C09K 15/28

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// A61K 7/00

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(31) [Application number of the priority] 60/202,562

(32) [Priority date] Heisei 12(2000) May 10 (2000.5.10)

(33) [Priority claim country/region] U.S. (US)

(81) [Designated country/region] EP (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OA (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG), AP (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), EA (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

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Page 620 of 1215

CARGILL 00000645

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4C083
4H006
4H025
4H059
[F-term (reference)]
4C076 DD55S EE53 EE54 EE55 FF51
4C083 AA081 AA121 AC761 BB47 EE01
4H006 AA01 AA03 AB83
4H025 AA45 AC07
4H059 BA73 BB14 BB45 BC03 BC13 EA03

Overview

Close

(57) [Overview]

To provide a 1 - (4-methoxybenzyl) - 3 - substituted thio Urea antioxidant compound and an improved lipid composition supplemented with an effective amount of the antioxidant compound to enhance the oxidative stability of the base. 3. Further, there is provided a method for improving the oxidative stability of lipids by adding at least one 1 - (3 - methoxybenzyl) - 3 - substituted thio Urea compound of the present invention to a lipid which is a base for which oxidation stability needs to be improved.

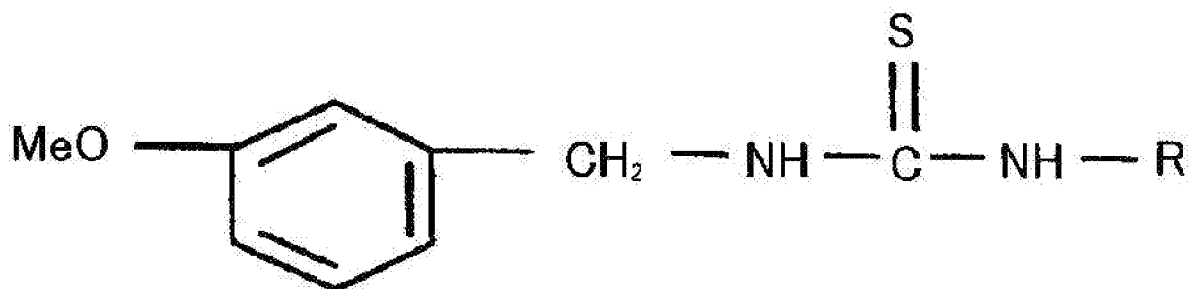
Scope of Claims

Close

[Patent Claims]

[Claim 1]The compound of a following formula.

[Chemical formula 1]



In the formula, R is a straight or branched alkyl group having 1 to 20 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, and

An alkoxy substituted cycloalkyl group having 5 to 7 carbon atoms, a hydroxy substituted cycloalkyl group having 5 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms, an aryl group having 6 to 7 hydroxy substituted carbon atoms, or an alkoxy substituted aryl group having 6 to 7 carbon atoms.

[Claim 2]A compound according to claim 1, wherein R is a straight or branched alkyl group of 1 to 20 carbon atoms.

[Claim 3]A compound according to claim 1, wherein R is a cycloalkyl group having 5 to 7 carbon atoms, an alkoxy substituted cycloalkyl group having 5 to 7 carbon atoms, or a hydroxy substituted cycloalkyl group having 5 to 7 carbon atoms.

[Claim 4]The compound according to claim 1, wherein R is an aryl group having 6 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms substituted with hydroxy, or an aryl group having 6 to 7 carbon atoms substituted with alkoxy.

[Claim 5]R A methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, The compound according to claim 2 which is an isobutyl group, a tertiarybutyl group, a pentyl group, a 2-methylpentyl ester group, a 3-methylpentyl ester group, a hexyl group, an octyl group, a decyl group, a nonyl group, or the dodecyl.

[Claim 6]A compound according to claim 3, wherein R is a cyclopentyl, cyclohexyl or cycloheptyl group.

[Claim 7]R is a phenyl group, a benzyl group, a hydroxyphenyl group, a hydroxybenzyl group, a methoxyphenyl group, an ethoxyphenyl group, a methoxybenzyl group, and

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A compound according to claim 4, which is a group of the formula :.

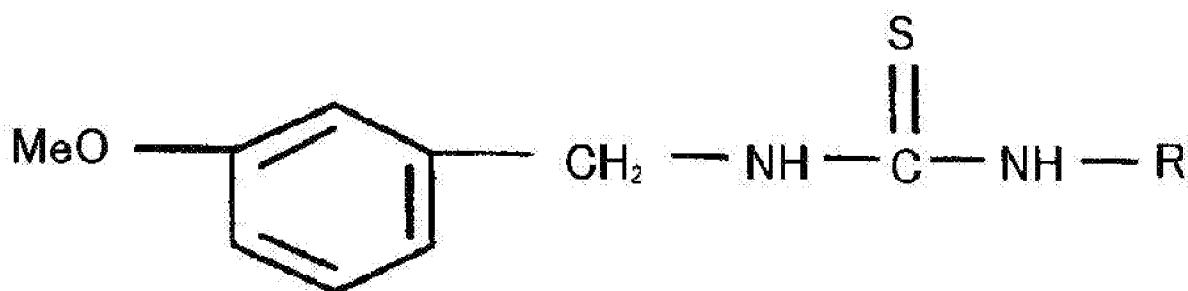
[Claim 8]A compound according to claim 4, wherein R is a 3-hydroxy substituted or 3 alkoxy substituted aryl group.

[Claim 9]The compound of claim 4, wherein the compound is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-ethyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-propyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-hexyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-dodecyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-(4-hydroxyphenyl)-2-thioic Urea, or a 1-(3-methoxybenzyl methoxybenzyl)-3-(3-methoxyphenyl)-2-thioic Urea.

[Claim 10]The compound of claim 9, wherein the compound is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea.

[Claim 11]A composition comprising a base lipid or oil and an amount of a compound of the formula :.

[Chemical formula 2]



In the formula, R is a straight or branched alkyl group having 1 to 20 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, a hydroxy substituted cycloalkyl group having 5 to 7 carbon atoms, and the like.

This composition is excellent in oxidation stability of a lipid or oil or an oil which is an aryl group having 6 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms substituted with hydroxy, or an aryl group having 6 to 7 carbon atoms substituted with alkoxy, and the oxidation stability of the composition is a base before the addition of the compound.

[Claim 12]A composition according to claim 11, wherein R is a straight or branched alkyl group of 1 to 20 carbon atoms.

[Claim 13]R is a cycloalkyl group of the carbon numbers 5-7, and alkoxy substitution.

A composition according to claim 11, which is a cycloalkyl group of 5 to 7 carbon atoms or a hydroxy substituted cycloalkyl group of 5 to 7 carbon atoms.

[Claim 14]The composition according to claim 11, wherein R is an aryl group having 6 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms substituted with hydroxy, or an

aryl group having 6 to 7 carbon atoms substituted with alkoxy.

[Claim 15]A composition according to claim 11, wherein from about 0.01% to about 5.0% by weight of said compound is added to the base lipid or oil, based on the total weight of said lipid or oil.

[Claim 16]The composition of claim 15, wherein from about 0.05% to about 2.0% by weight of the compound is added to the base lipid or oil.

[Claim 17]The composition of claim 16, wherein from about 0.1% to about 1.0% by weight of the compound is added to the base lipid or oil.

[Claim 18]The composition of claim 14, wherein the compound is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-ethyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-propyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-hexyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-dodecyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-(4-hydroxyphenyl)-2-thioic Urea, or a 1-(3-methoxybenzyl methoxybenzyl)-3-(3-methoxyphenyl)-2-thioic Urea.

[Claim 19]The composition of claim 18, wherein the compound is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea.

[Claim 20]A composition according to claim 11, wherein the base lipid or oil is a seed oil or a vegetable oil.

[Claim 21]A composition according to claim 11 wherein the base lipid or oil is meadowfoam oil, peanut oil, corn oil, cottonseed oil, safflower oil, soybean oil, high oleic sunflower oil, castor seed oil, rapeseed oil, palm oil, olive oil, jojoba wax ester, jojoba oil, lecithin or other vegetable oils.

[Claim 22]The base lipid or oil is jojoba oil, meadow-foam oil, high-oleic sunflower oil, soybean oil or castor seed oil and is a base.

The composition of claim 21, wherein said compound is added to said lipid or oil in an amount of from about 0.1% to about 1.0% by weight.

[Claim 23]The composition of claim 11, wherein the base lipid or oil comprises one or more benzylamine or N-substituted benzylamine compounds.

[Claim 24]The composition of claim 23, wherein the base lipid or oil is meadow-foam seed oil.

[Claim 25]A composition according to claim 11, wherein the base lipid or oil further comprises an amount of one or more benzylamines or N-substituted benzylamine compounds which enhance oxidative stability.

[Claim 26]The composition of claim 11, wherein when the oxidation stability index test is conducted at a temperature of from about 110 ° C. to about 130 ° C., the oxidation stability index of the composition is at least about 10% greater than the oxidation stability index of the base lipid or oil prior to the addition of the compound.

[Claim 27]The composition of claim 26, wherein the oxidative stability index of the composition is at least about 100% greater than the oxidative stability index of the base lipid or oil.

[Claim 28]The composition of claim 27, wherein the oxidative stability index of the composition is at least about 200% greater than the oxidative stability index of the base lipid or oil.

[Claim 29]The composition of claim 28, wherein the oxidative stability index of the composition is at least about 500% greater than the oxidative stability index of the base lipid or oil.

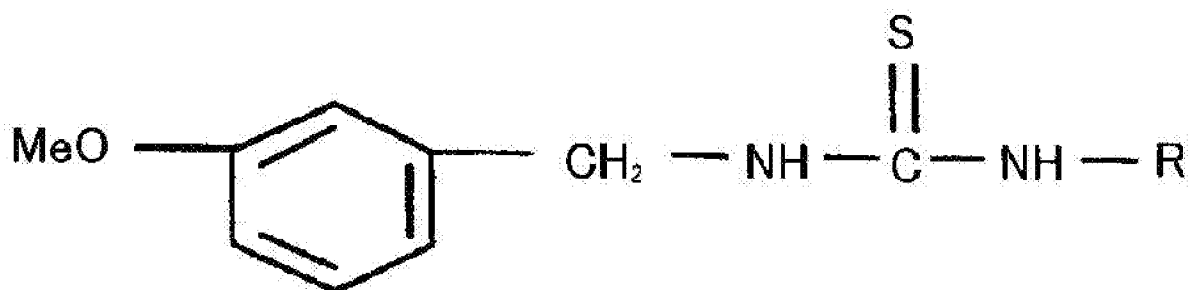
[Claim 30]The composition of claim 29, wherein the oxidative stability index of the composition is at least about 800% greater than the oxidative stability index of the base lipid or oil.

[Claim 31]The composition of claim 30, wherein the oxidative stability index of the composition is at least about 1000% greater than the oxidative stability index of the base lipid or oil.

[Claim 32]The composition of claim 31, wherein the oxidative stability index of the composition is at least about 1500% greater than the oxidative stability index of the base lipid or oil.

[Claim 33]A method for improving the oxidative stability of a lipid or oil as a base, comprising the step of combining a base lipid or oil with an amount of a compound of the formula which enhances oxidative stability.

[Chemical formula 3]



In the formula, R is a straight or branched alkyl group having 1 to 20 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, and

An alkoxy substituted cycloalkyl group having 5 to 7 carbon atoms, a hydroxy substituted cycloalkyl group having 5 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms, an aryl group having 6 to 7 hydroxy substituted carbon atoms, or an alkoxy substituted aryl group having 6 to 7 carbon atoms.

[Claim 34]The method of claim 33, wherein from about 0.05% to about 2.0% by weight of the compound is combined with the base lipid or oil.

[Claim 35]The method of claim 34, wherein said compound is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-ethyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-propyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-hexyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-dodecyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-(4-hydrxyphenyl)-2-thioic Urea, or a 1-(3-methoxybenzyl methoxybenzyl)-3-(3-methoxyphenyl)-2-thioic Urea.

[Claim 36]The method of claim 35, wherein said compound is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea.

Detailed Description

Close

[Detailed description of the invention]

[Technical field to which the invention belongs]

[0001]

This invention relates to novel 1 - (3 - methoxybenzyl) - 3 - substituted thio Urea compounds, and more particularly to improved oxidative and oil compositions of the present invention.

[Background of the Invention]

[0002]

Natural lipids and oils have been used in a variety of industrial articles such as pharmaceuticals, foods, cosmetics, and lubricants, paints, inks, paints, and the like. Such lipids are susceptible to oxidative degradation which may affect color, odor, viscosity and lubricity, and adversely affect the quality of the lipid containing product. In the food, cosmetic, and pharmaceutical industries, it is important to avoid the degree of unsaturation of the lipid or oil and the amount of natural or synthetic antioxidants contained therein, as well as to avoid acidification by oxidation that is affected by factors such as oxygen concentration, light and heat, and to keep the high quality color and odor of the oil or other lipid intact. Biodegradable lipids, oils and their derivatives used as cutting lubricants have been found to be adversely affected by thermal oxidation.

It has been demonstrated that seed oil of meadow-foam (Rimnan's Limnantes alba) is very stable to oxidation. While it is now not clear what compounds contribute to the comparable oxidative stability of meadow-foam oil, mixing meadow-foam oil with other oils improves the oxidative stability of the mixture

(Isbell,T.A.,Abbott,T.A.and Carlson,K.D.1999.Ind.Corps Prod.9 (2) : 115 - 123). However, a number of minor components of the meadow-foam oil are known

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(Abbott,T.P.and Isbell,T.A.1998., U.S. Pat. No. 89, Meeting Abstracts, Chicago,IL,1998 May 10-13, 66). It is found from refined meadowfoam oil (and other refined seed oils and vegetable oils) that the purification process reduces oxidative stability. In a meadowfoam,

It is known that 3-methoxyphenylacetonitrile, 3 - *****-* and 3-methoxybenzaldehyde are contained. Even if these compounds are added in an amount ratio of from about 0.1% to about 1.0% relative to the meadow-foam oil after purification, the antioxidant effect exhibited by these compounds is at most low to moderate.

[0003]

In oils in which Urea has been shown to have an antioxidant action (tongue book, Murakami, Japan Nutritional Food Society 51 (4) : 207 - 212, 1998 ; Chemical Abstract 129:188538), Urea is hardly soluble in oils. When an ester-based synthetic lubricating oil (I. e., not a vegetable oil) was stabilized with an amine-based antioxidant, the oxidative stability of the lubricating oil was found to be improved by a specific thio Urea (Chao and Kjonaas Amer.Chem.Soc.Preprints,Div.Pet.Chem.27 (2) : 362 - 379,1982). European Patent Application EP 91 810474 to Camenzind and Rolf, Chemical Abstracts 117:30273, has shown that certain acylated thiophenols enhance the oxidative stability of a lubricant and a working liquid. Urea.

Also, mono - and di-substituted thio Urea compounds are described in US Patent 2154341,2662096,3852348 and 3991008. Phytochem.16 (11) : 1719 - 1721, (1977)Migirab et al.,' - bis[(4-methoxyphenyl)methyl]-thioic Urea (CAS #22313 - 70-8), which is isolated from West African Strawberry (P.Brazzeana), discloses such a methoxy substituted aromatic thio Urea.

[0004]

[Problem to be solved by the invention]

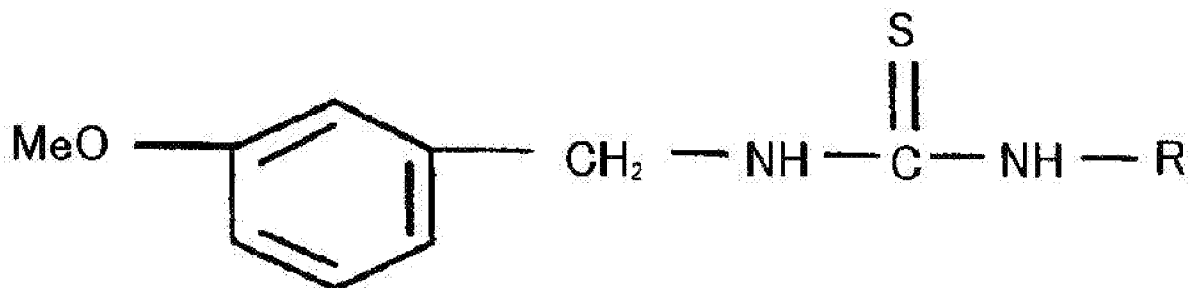
There is a need for antioxidant compounds and compositions which, inter alia, are natural antioxidants or derivatives thereof, which can be dissolved in lipids or oils to enhance the oxidative stability of lipids and oils even at low concentrations of addition.

[Means for solving the problem]

We have unexpectedly discovered that compounds of Formula I provide excellent oxidative stability for lipids and oils.

[0005]

[Chemical formula 4]



(The inside of a formula and R are a straight chain of the carbon numbers 1-20, such as a methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a tertiarybutyl group, a pentyl group, a hexyl group, an octyl group, a decyl group, a nonyl group, and dodecyl, or an alkyl-group; cyclopentyl group of branching.) A cycloalkyl group having 5 to 7 carbon atoms, such as a cyclohexyl group or a cycloheptyl group ; an aryl group having 6 to 7 carbon atoms, such as a phenyl group or a benzyl group ; or a hydroxyphenyl group ; It is an aryl group of the carbon numbers 6-7 of hydroxy or alkoxy substitution like a methoxyphenyl group, an ethoxyphenyl group, a hydroxybenzyl group, a methoxybenzyl group, and an ethoxybenzyl group. In the inside of the compound of the formula I, While 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea is presently preferred, this is a compound wherein R is a 3-methoxybenzyl methoxybenzyl group in Formula I. An amount of a compound of Formula I sufficient to provide oxidative stability to a lipid or oil (or a composition containing a lipid or oil) is from about 0.01% to about 5.0% by weight based on the total weight of the lipid or oil.

The present invention also provides an oxidatively stable lipid composition comprising from about 95% to about 99.99% by weight of a base lipid or oil and from about 0.01% to about 5.0%, preferably from about 0.05% to about 2.0%, more preferably from about 0.1% to about 1.0% by weight of a compound of Formula I. The lipids or oils of the present invention comprising from about 3% to about 5% or more by weight of the total weight of the lipids or oil composition based on the substituted thio Urea compound of Formula I are used as "concentrates".

To provide a lipid or oil composition of the present invention which is suitably added to processed seed oils or other lipids which require improved oxidative stability.

[0006]

In addition, the present invention provides a method of providing oxidative stability to a lipid or oil composition which is a base requiring enhanced oxidative stability, the method comprising adding a lipid or oil based on a compound of Formula I in an amount sufficient to enhance the oxidative stability of the lipid or oil.

That it is supposed with the compound of the formula I that it is preferable now, and 1,3-

di(3-methoxybenzyl methoxybenzyl)thioicurea, A 1-(3-methoxybenzyl methoxybenzyl)-3-ethyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-propyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-hexyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-dodecyl-2-thioic Urea, a 1-(3-methoxybenzyl methoxybenzyl)-3-(4-hydroxyphenyl)-2-thioic Urea, and a 1-(3-methoxybenzyl methoxybenzyl)-3-(3-methoxyphenyl)-2-thioic Urea. For a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea, we have confirmed that they are important natural antioxidants contained in meadow-foam seed oils, which is particularly preferred among the compounds of the present invention. It has also been surprisingly found that a compound of formula I, when combined with a benzylamine compound such as an N-substituted benzylamine, synergistically exhibits an oxidative stability enhancing effect in lipids and oils. Various naturally occurring lipids and oils, such as seed oils and vegetable oils, include benzylamine compounds. In such a case, a synergistic effect would be obtained by supplementing the base lipid or oil with a compound of Formula I and optionally supplementing an amount of benzylamine in an amount sufficient to further enhance oxidative stability. Thus, as another aspect of the invention, there is necessarily provided a lipid composition comprising (i) a compound of Formula I and (ii) a benzylamine or an N-substituted benzylamine compound for the purpose of enhancing oxidative stability.

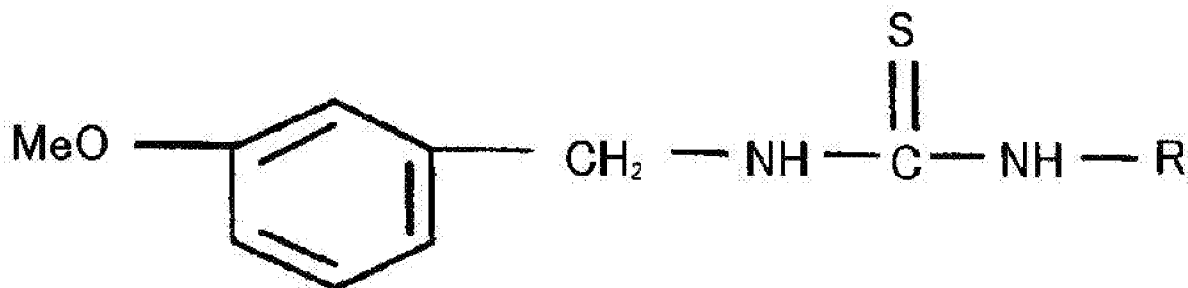
[0007]

[Embodiment of the invention]

As an aspect of the invention, a 1-(3-methoxybenzyl methoxybenzyl) - 3 substitution of the formula :

To provide a thio Urea compound.

[Chemical formula 5]



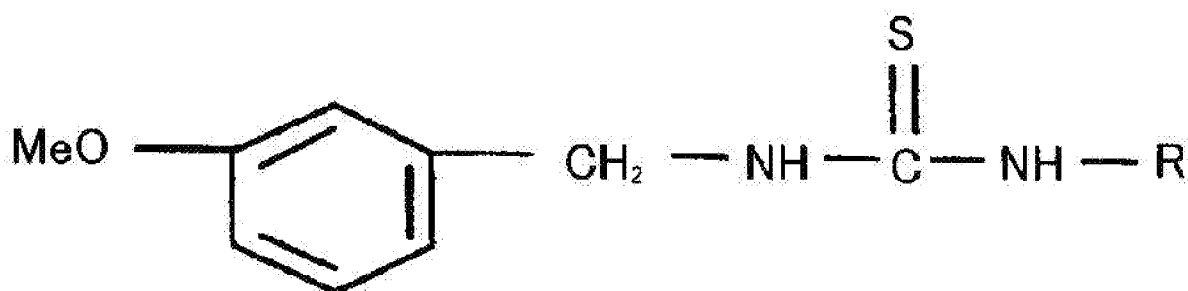
In the formula, R is a straight or branched alkyl group having 1 to 20 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, a hydroxy substituted cycloalkyl group having 5 to 7 carbon atoms, and the like. In particular preferred embodiments, the aryl group having 6 to 7 carbon atoms is selected from the group consisting of an aryl group having from 6 to 7 carbon atoms, an aryl group

having from 6 to 7 carbon atoms having a hydroxy substituent, and an aryl group having from 3 to carbon atoms, and the aryl group having a substituent is a 2-hydroxy-substituted aryl compound or a 2-alkoxy-substituted aryl compound. 3.

According to another aspect of the invention, from about 95% to about 99.99% by weight of a base lipid is provided. To provide a lipid composition having improved oxidative stability containing from about 0.01 wt.% to about 5.0 wt.%, preferably from about 0.05 wt.% to about 2.0 wt.%, and more preferably from about 0.1 wt.% to about 1.0 wt.% of a 1 - (3 - methoxybenzyl) - 3 - substituted thio Urea compound of the formula :

[0008]

[Chemical formula 6]



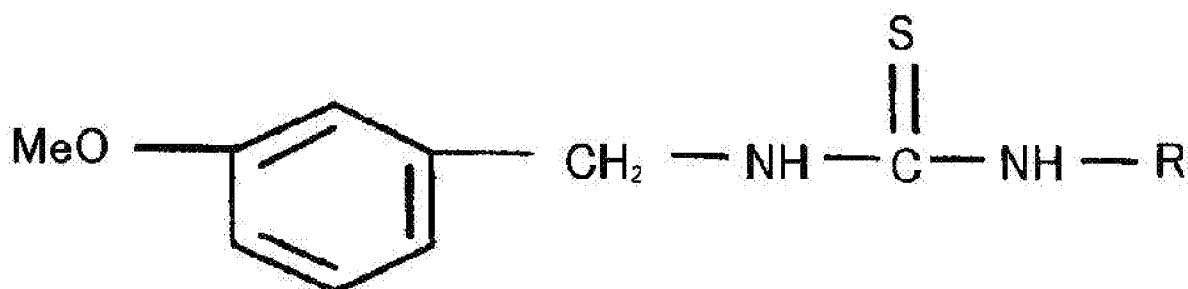
Wherein R is a straight or branched alkyl group having 1 to 20 carbon atoms, a cycloalkyl group having 5 to 7 carbon atoms, a hydroxy or alkoxy substituted cycloalkyl group having 5 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms, and a hydroxy or alkoxy substituted carbon atom 6.

It is chosen out of the aryl group which is -7.)

According to yet another aspect, the present invention provides a method of enhancing oxidative stability of a lipid comprising mixing a lipid with an amount of a compound of Formula I that has improved oxidative stability.

[0009]

[Chemical formula 7]



In the formula, R is selected from a linear or branched alkyl group having 1 to 20 carbon

atoms, a cycloalkyl group having 5 to 7 carbon atoms, a hydroxy or alkoxy substituted cycloalkyl group having 5 to 7 carbon atoms, an aryl group having 6 to 7 carbon atoms, and an aryl group having 6 to 7 carbon atoms substituted with an alkoxy group.

[0010]

The compounds of the present invention may be added to any lipid essentially provided that the compound is capable of dissolving and enhancing the oxidative stability of the lipid. As used herein, the term "lipid" includes vegetable oils, seed oils, triglycerides, waxes of triglycerides and phospholipids. Lipids which may be added to the compounds of the present invention to provide excellent oxidative stability include vegetable oils, peanut oil, corn oil, cottonseed oil, safflower oil, soybean oil, rapeseed oil (canola oil), palm oil, olive oil, jojoba wax esters, and lecithin. As used herein, a "base lipid", "base oil" or equivalent term means a lipid or oil before the compound of Formula I is added externally.

As used herein, "a straight or branched alkyl group having 1 to 20 carbon atoms" is a methyl group. An ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a tertiarybutyl group, a pentyl group, a 2-methyl-pentylgroup, a 3-methyl-pentylgroup, a hexyl group, an octyl group, a decyl group, dodecyl, etc. are contained. "Cycloalkyl group having 5 to 7 carbon atoms" is a cyclopentyl group, a cyclohexyl group.

And cycloheptyl groups. "Hydroxy-substituted or alkoxy-substituted cycloalkyl group of 5 to 7 carbon atoms" includes a cyclopentyl group, a cyclohexyl group, and a cycloheptyl group substituted with a hydroxyl group, a methoxy group, an ethoxy group, a propoxy group, or the like. The "aryl group having 6 to 7 carbon atoms" includes a phenyl group and a benzyl group. "Hydroxy-substituted or alkoxy-substituted aryl group of 6 to 7 carbon atoms" includes a phenyl group substituted with a hydroxyl group, a methoxy group, an ethoxy group or a propoxy group, and a benzyl group.

[0011]

The use of "excellent oxidative stability", "improved oxidative stability" or equivalent term means that the ability to inhibit oxidation by measurement of the oxidative stability index (OSI) disclosed in the specification is increased when compared to the lipid or oil (I. e., the compound of Formula I is not externally added) based on the lipid composition of the present invention. When the OSI test is conducted at a temperature of from about 110 ° C. to about 130 ° C., the OSI value of the lipid or oil composition of the present invention is determined. It is preferred that the base lipid or oil be at least 10% greater than the OSI value of the base lipid or oil to be compared, more preferably at least 100% or more, and most preferably at least about 200% or more.

[0012]

A compound of formula I is synthesized by reacting a 3-methoxybenzylamine with an isothiocyanate compound of formula II wherein $S = C = R$. Wherein R is as defined for

the compound of formula I. This reaction may be carried out by gradually adding an isothiocyanate to an aqueous 3-methoxybenzylamine under a nitrogen atmosphere. While the thio Urea product of reaction is a compound of Formula I, the product can be purified and recovered by mixing solvents such as methylene chloride, chloroform, toluene or diethyl ether which is not miscible with water but becomes a solvent for Urea. The aqueous layer may or may not be acidified to enhance separation and recovery of the thio Urea compound of the present invention. While Urea is dissolved in the solvent layer, it is separated from the aqueous layer and dried, and the crude Urea obtained is purified by recrystallization from a suitable solvent such as ethanol. For Example 1 and Summary, a Moore and Crossley is provided.

Reference can be made to the procedure of "Organic Synthesis" 2, Vol. 617 - 618 (Note 4). [0013]

The 3-methoxybenzylamine as defined above and the appropriate isothiocyanate compound as reactants can be obtained commercially available or can be synthesized by conventional methods known in the art. The resulting compound of Formula I can be readily isolated by conventional methods well known to those skilled in the art. Suitable isothiocyanate reactants for synthesizing the compounds of the present invention are obtained by degrading glucosinolates contained in seed oils or other lipids, as is well known in the art. The glucosinolate compound is degraded in aqueous solution containing thioglucosidase enzyme to obtain isothiocyanate and other degradation products. J.Chem.Ecol.22,1939 49 (1996) of Vaughn et al. ; M.Rechcigl (1983) of Naturally Occurring Food Toxicants CRC Press,Boca Raton,FL. of glass (knitted). C.VanEtten and H.Tooley Reference can be made to Glucosinolates 15 30. Isothiocyanates which are part of the degradation products of glucosinolates can thus be isolated and reacted with the above 3 - methoxybenzylamine to give the compounds of the invention. Approximately 100 glucosinolate compounds have been discovered from 11 different families of plants, including mustard, rapeseed, cabbage, ariaria, and comics (S.F.Vaughn,1999,Glucosinolates as Natural Pesticides in Biologically Active Natural Products:Agrochemicals, H.G.Cutler and S.J.Cutler,Eds,CRC Press,Boac Raton,FL.). Oils isolated from glucosinolate containing plants usually remove odors by vapor spraying to remove volatile compounds containing isothiocyanate and amine. Various isothiocyanate and benzylamine compounds can thus be obtained from the distillation waste produced in the process of refining oil and serve as reactants in synthesizing the compounds of the present invention.

[0014]

3-methoxybenzylamine can be purchased on the market. Alternatively, it may be isolated

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by extracting from the meadow-foam oil into an acidified aqueous layer which is immiscible, separating the aqueous layer from the oil, washing with a non-polar solvent, lowering the pH value with a base and extracting the amine into an immiscible solvent. This 3-methoxybenzylamine compound is purified by recrystallization from ethanol or similar solvents and / or by high performance liquid chromatography (HPLC) using a reverse phase column (C 18), with a concentration gradient initially starting from 100% methanol and about 80% methanol. It may be purified by separating and eluting up to 20% chloroform. A peak containing 3-methoxybenzylamine can be identified by comparing the retention time on an HPLC column with the retention time of a known 3-methoxybenzylamine standard. Other naturally occurring amines may be purchased commercially or may be extracted from natural sources from natural sources as described above and identified by standard chemical methods of amine identification (e.g., chromatography, infrared spectroscopy, mass spectrometry, elemental analysis, nuclear magnetic resonance analysis, etc.).

[0015]

For the oxidative stability of the lipid, it is sufficient to determine whether or not the compound of the present invention is added, whether or not the compound of the present invention is added, by the procedures described in the literature. See, e.g., K.Tian and P.Dasgupta, Anal.Chem.71,1692 98 (1999). A preferred oxidative stability measure of lipids and oils employs an oxidative stability index (OSI) which determines the oxidative stability of an oil by passing air through a sample under stringent temperature control.

(Firestone, Oxidative Stability Index(OSI): Official Methods of Recommended Practices of the American Oil Chemists' Society, 4th version, U.S. oil recovery society, and Champaign IL Cd 126-92) In this gentleman method, if air is poured into an oil sample, triglyceride will decompose quickly and will become volatile organic acid. The acid evaporated from the oil by the force of the air stream is fed into the conductivity cell filled with water, and the acid dissolves in the cell. These acids will dissolve in aqueous solution once, but will then dissociate into ions, which will change the conductivity of water.

It changes. Thus, if the conductivity of a cell is measured continuously by a computer, it can be seen that the conductivity rises rapidly, which is a point of induction and corresponds to the oxidative degradation of the sample. The time to the point of induction is the OSI time. The AOC S standard method has recently been developed and coextensive research has also been published (Jebe et al., 61 (1993)), and it has been confirmed by the OSI method that reproducibility is reproducible between samples and beyond the laboratory (, et al.). J.Am.Oil Chem.Soc.70,1055. Saturated fatty acid methyl esters (FAME) standards of Alltech Associates (Deerfield, IL) may be used in calibrating the OSI measurements. The OSI measurement may be performed according to the AOCS method described in the

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Firestone reference disclosed above in an oxidation stability meter manufactured by Omniion (Rockland,MA). A sample of lipid or oil is operated at 110 ° C. and FAME may be tested at 90 ° C., with a final speed of 35 kPa and a final speed of about 140 ml / min. A preferred method of determining an OSI value is described in T.A.Isbell 123 (1999), such as Industrial Crops and Products 9,115.

[0016]

The compound of formula I may be mixed in a conventional manner with a suitable lipid or oil and dissolved in a concentration of 3.0 to 5.0% or more. At present, a concentration of about 0.1 to 1.0% of the compound of Formula I is considered to be 10 minutes to increase the oxidative stability of the base lipid or oil requiring an increase in oxidative stability by about 2 to 10 times. However, the lipid or oil of the present invention containing from 3.0 to 5.0% of a compound of Formula I is useful as a "concentrate" which can be suitably diluted to 30 to 50 times or more with a lipid which is a base requiring improved oxidative stability. The basic composition of such concentrates may itself be a lipid or oil such as seed oil, vegetable oil or an edible solvent. Furthermore, the compound of formula I may optionally be formulated as an oil-in-water or water-in-oil emulsion with an amine compound such as 3-methoxybenzylamine. To enhance the oxidative stability of biodegradable cutting lubricants, such as canola oil, soybean oil, vegetable oil eslite (estolyte), or other cutting lubricants, it is believed to be particularly useful to add to the above emulsions.

In a preferred embodiment of the lipid or oil composition of the invention, an effective amount of a compound of Formula I is added to the base lipid or oil, I. e., a sufficient amount of a benzylamine compound is added to enhance the oxidative stability conferred by the compound of Formula I, as well as a concentration of about 0.1% to 1.0%. The amount of benzylamine compound added to the lipid or oil composition of the present invention may be determined while observing an increase in the OSI value as a function of the amount of benzylamine compound added. While the base lipid or oil may inherently contain an amine compound, an amine compound, preferably a 3-methoxybenzylamine, may be further added to the lipid or oil to which the compound of Formula I has been added for the purpose of improving the oxidative stability of the lipid or oil composition of the present invention. In order to achieve a synergistic antioxidant effect, the amount of the amine compound added to the lipid or oil composition of the present invention may be determined experimentally by adding a predetermined amount of an amine compound to an aliquot of a lipid or oil composition containing a compound of Formula I, and measuring an increase in the OSI value.

The following examples do not limit the invention and describe the preparation of the compounds of the invention. Unless otherwise indicated, all percentages mean weight percent (wt%).

[0017]

Example 1

This example demonstrates the synthesis of 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea. To a 100 ml three necked flask equipped with a condenser, a rubber ***** (syringe septum) and a nitrogen-introducing tube, 20 ml of water and 3.6 g (25.8 mmol) of 3-methoxybenzylamine were added. The reactor was purged with nitrogen and stirred with a Teflon-coated magnetic stirrer. 2.59 ml (3.0g,16.7mmol) of 3 - *****-* was dropped from a glass syringe (a drop of 5 to 10 seconds). A separation layer was formed and the mixture was stirred at room temperature for one hour. The aqueous layer was acidified to pH 5.5 using 1 M hydrochloric acid (about 10 ml). Methylene chloride (15 ml) was added and the 2 layers transferred to a separatory funnel. The lower layer (methylene chloride) was removed. The aqueous layer was washed with methylene chloride and washed 2 more times with 10 ml methylene chloride and the resulting CHCl₃ sub. 2 solution was washed successively with 0.1 m hydrochloric acid and water. C

The HCl 2 solution was dried over molecular sieves (3 A) and evaporated to dryness on a rotary solvent evaporator. The resulting viscous liquid was placed in 20 ml of ethanol heated to 35. degree. C. and recrystallized 2 times from ethanol while cooling in a condenser, and the white crystals were dried in vacuo at room temperature and weighed. Recrystallization was also carried out from the mother liquor to recover the additional product, with a yield of 79.8% in the first batch of crystals and a yield of 83.2% when the batches of crystals were combined. NMR, mass spectrometry and elemental analysis showed that this product is a 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea.

[0018]

Example 2

Jojoba oil (which is extracted from jojoba seeds with hexane) is a wax ester in which the monohydric unsaturated acid having 20 and 22 carbon atoms and the alcohol together are esterified. Jojoba oil (20 g) was mixed with 20 mg (0.1%) of the 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea prepared in Example 1. A sample of 5 g of this mixture was subjected to repeated oxidative stability tests at 110 ° C. (3 times) and found to have an OSI time of 64.2 hours, which is an improvement of 30% compared to 49.2 hours which is an OSI time of only jojoba oil. The OSI time refers to the time from which a bubble of oxygen generated in an oil at a constant rate will decompose the oil and produce a detectable oxidation product. When the 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea product of Example 1 was added at a concentration of 1%, the OSI time extended to 168 hours and increased by 241% compared to the jojoba oil alone.

[0019]

Example 3

Purified meadowfoam oil is a highly monounsaturated vegetable oil and its oxidative stability is reduced in the refining process. 20 g of meadow-foam oil (Lot #C-9773, The Fanning) was mixed with 20 mg (0.1%) of the 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea prepared in Example 1. A sample of 5 g of this mixture is subjected to an oxidative stability test at 110 ° C. (3 times) and found to have an OSI time of 76.4 hours, which is an improvement of 15% compared to 66.3 hours, which is the OSI time of the meadowfoam oil alone. Example 1 1,3 - di (3)

When methoxybenzyl) Urea product was added at a concentration of 1%, the OSI time increased to 218% by 211 hours.

[0020]

Example 4

The 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea prepared in Example 1 was mixed with a purified meadowfoam oil (Lot #CW- 4551, The Fanning) at a rate of 0.1%, 0.5% or 1.0%, and the oxidation stability of the resulting mixture and the oxidative stability of the purified meadowfoam oil were compared at 130 ° C. The OSI time of purified meadow-foam oil without additive of lot was 14.9 hours at 130 ° C., while the OSI time of the mixture containing thio Urea was 49.8 hours, 159 hours, 172 hours. Thus, the addition of 1,3-di-3-methoxybenzylthio Urea to purified meadowfoam oil at a rate of 0.1%, 0.5% and 1.0% resulted in an OSI time of 234%, 967% and 1054%, respectively.

[0021]

Example 5

High oleic sunflower oil is a highly monounsaturated vegetable oil. 20 g of high oleic sunflower oil (Florasun Brand, Floratech, Gilbert, AZ) was mixed with 200 mg (1.0%) of the 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea prepared in Example 1. A sample of 5 g of this mixture was subjected to repeated oxidative stability tests at 130 ° C. (3 times) and found to have an OSI time of 157 hours, which is an improvement of 1720% compared to 9.13 hours, which is an OSI time of only sunflower oil.

[0022]

Example 6

A 2 group substituted thio Urea without a commercially available 3-methoxybenzyl methoxybenzyl group has a low antioxidant effect on a monounsaturated oil. Thus, when a 1,3-bis(2-methoxyphenyl)-2-thioic Urea (manufactured by Aldrich Chem Co., Ltd.) is added to purified meadowfoam oil at a rate of 0.1%, an OSI time of 37.1 hours at 130 ° C. is obtained.

This is 34% lower than that of the thio Urea produced in Example 1. Similarly, when a 1,3-bis(3-methoxyphenyl)-2-thioic Urea is added to purified meadowfoam oil at a rate of 0.1%,

the OSI time is 27.0 hours, which is 84% lower than that of the thio Urea produced in Example 1.

[0023]

Example 7

Soybean oil is a highly polyunsaturated vegetable oil. Soybean oil (20 g) was mixed with 200 mg (1.0%) of the 1,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea prepared in Example 1. A sample of 5 g of this mixture is subjected to an oxidative stability test at 130 ° C. (3 times) and found to have an OSI time of 6.4 hours, which is an improvement of 831% compared to 0.77 hours, which is an OSI time of only soybean oil.

[0024]

Example 8

The castor seed oil is a highly polyunsaturated vegetable oil. 20 g of castor seed oil (extracted from seeds with hexane) was mixed with 200 mg (1.0%) of the thio Urea prepared in Example 1. A sample of 5 g of this mixture was subjected to repeated oxidation stability tests at 130 ° C. (3 times) and found to have an OSI time of 2.78 hours, which is an improvement of 654% compared to 0.42 hours, which is the OSI time of only spruce seed oil.

[0025]

Example 9

The crude meadowfoam oil and refined meadowfoam oil were analyzed by high performance liquid chromatography (hplc), and the crude product showed a peak at a retention time of 9.5 minutes but did not reveal a refined oil. The retention time of the compound synthesized in Example 1 was 9.575 to 9.613 when the concentration was changed. Further, when the crude meadow-foam oil is extracted with an acetonitrile solvent and subjected to high performance liquid chromatography, each component is separated, and one of the components is identified as a 1,3-di(3-methoxybenzyl methoxybenzyl) Urea by mass spectrometry, NMR and elemental analysis, which is an oxidized form of the synthesized thio Urea of claim 1. Therefore, 1

It can be seen that the ,3-di(3-methoxybenzyl methoxybenzyl)thioic Urea is a natural ingredient contained in crude meadow-foam oil when extracted from seeds rather than refined meadow-foam oil.

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C07C 69/30 (2006.01)

C07C 69/58 (2006.01)

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A23D 9/007 (2006.01)

C12R 1/645 (2006.01)

C12R 1/845 (2006.01)

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4C206DB48
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4C206MA04
4C206MA72
4C206NA09
4C206NA11
4C206ZC01
4H006AA01
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Overview	Close
<p>(57) [Overview]</p> <p>[Problem] To obtain a highly unsaturated fatty acid-containing fat or oil or triglyceride which can be expected to be applied in the fields of infant formula, food and health food, has high digestive absorbability and hardly causes oxidative deterioration.</p> <p>[Means for solving] An ester exchange of fatty acid containing high unsaturated fatty acid or triglyceride with vegetable oil or fat or triglyceride is performed using a 1,3 position specific lipase.</p>	

Scope of Claims	Close
<p>[Patent Claims]</p> <p>[Claim 1]</p> <p>An oil or triglyceride containing ≥ 50 to 100 pts. mass of fat or oil or triglyceride containing $\geq 20\%$ highly unsaturated fatty acids containing ≥ 20 and ≥ 2 carbon atoms and ≥ 2 carbon atoms is transesterified by using a 1,3 position specific lipase of 0 - to 50 pts. mass of a vegetable oil or triglyceride. To provide a method for producing an ester exchange fat or triglyceride.</p> <p>[Claim 2]</p> <p>The method according to claim 1, wherein the transesterification is performed in a deoxygenated state.</p> <p>[Claim 3]</p> <p>The method according to claim 1 or 2, wherein the fatty acid produced by the fungus is a highly unsaturated fatty acid containing 2 or more of 6 or more carbon atoms and 20 or more carbon atoms of 2 carbon atoms.</p> <p>[Claim 4]</p> <p>The method according to claim 1 or 2, wherein the fatty acid produced by the fungus is a highly unsaturated fatty acid containing 2 or more of 9 or more carbon atoms and 20 or more carbon atoms of 2 carbon atoms.</p> <p>[Claim 5]</p>	

A process according to claim 1 or 2, wherein the fatty acid produced by fungi is arachidonic acid.

[Claim 6]

The method according to claim 1 or 2, wherein the fatty acid is arachidonic acid produced by microorganisms of the genus *Mortierella* (*Mortierella*).

[Claim 7]

A process according to claim 1 or 2, wherein the fatty acids produced by the fungi are dihomogamma-linolenic acid.

[Claim 8]

A process according to claim 1 or 2, wherein the fatty acid produced by the fungus is mead acid.

[Claim 9]

Lipase is *Rhizopus delemar* (*Rhizopus delemar*), A process according to any one of claims 1 to 8, which is a lipase produced by *Rhizopus neberus* (*Rhizopus niveus*), *Rhizomucor miehe* (*Rhizomucor miehei*) or *Rhizopus oryzae* (*Rhizopus oryzae*).

[Claim 10]

An interesterified fat or triglyceride obtainable by a process according to any one of claims 1 to 9.

[Claim 11]

Fat or oil or triglyceride containing $\geq 20\%$ highly unsaturated fatty acid containing ≥ 2 carbon atoms and ≥ 2 double bonds obtained by the manufacturing method according to any one of claims 1 to 9, and ≥ 2 carbon atoms and ≥ 20 double bonds in 1 molecule; and ≥ 2 carbon atoms and ≥ 20 carbon atoms. The transesterified fat or triglyceride contains a triglyceride containing ≥ 1 residue triglyceride containing 40 residues and / or containing 3 residue of the same high unsaturated fatty acid containing ≥ 20 and ≥ 2 double bonds at a ratio of $\leq 4.0\%$, and / or triglycerides containing $\geq 2\%$ of triglycerides.

[Claim 12]

A fat or oil or triglyceride containing $\geq 20\%$ highly unsaturated fatty acids containing ≥ 2 carbon atoms and ≥ 2 double bonds of 20 to 6 carbon atoms of fungal production obtained by the production method according to any one of claims 1 to 9, wherein ≥ 2 carbon atoms and $\geq$ double bonds of $\omega 6$ system are contained in 1 molecule. 20 2The transesterified fat or triglyceride contains a triglyceride containing 1 residue of triglyceride containing 40% or more of a triglyceride and / or containing 3 residues of the same polyunsaturated fatty acid containing 2 or more of 20 or more and 2 or more carbon atoms of the $\omega 6$ system of fungal production at a ratio of 4.0% or less.

[Claim 13]

Fat or oil containing $\geq 15\%$ highly unsaturated fatty acids containing ≥ 2 and ≥ 2 double bonds of the fungal production $\omega 9$ system obtained by the manufacturing method

according to any one of claims 1 to 9, or a trime containing $\geq$ %. 20

A highly unsaturated fatty acid which is a chloride and contains ≥ 1 carbon atoms and ≥ 2 double bonds of $\omega 9$ - series in the 20 molecules ; and 2The transesterified fat or triglyceride contains a triglyceride containing 1 residues of triglyceride containing 40% or more of a triglyceride and / or containing 3 residues of the same polyunsaturated fatty acid containing 2 or more of 20 or more and 2 or more carbon atoms of the $\omega 9$ system of fungal production at a ratio of 4.0% or less.

[Claim 14]

An oil or a triglyceride or a triglyceride containing 20% or more of arachidonic acid obtained by the production method according to any one of claims 1 to 9. This transesterified fat or triglyceride contains $\geq 40\%$ triglyceride containing 1 residues of arachidonic acid in 1 molecules and / or $\leq 4.0\%$ of AAA (triglycerides containing 3 residues of arachidonic acid in 1 molecules).

[Claim 15]

A fat or oil or triglyceride containing at least 20% of a dihomogamma-linolenic acid obtained by the method of any one of claims 1 to 9. An interesterified fat or triglyceride contains at least 4.0% of a triglyceride containing 1 residues of dihomogamma-linolenic acid in 1 molecules and / or contains 40% or more of DDD (triglycerides containing 1 residues of dihomogamma-linolenic acid in 3 molecules).

[Claim 16]

A fat or oil or triglyceride containing at least 20% of mead acid obtained by the method of any one of claims 1 to 9. An ester exchange fat or triglyceride contains at least 4.0% by weight of triglycerides containing 1 residues of mead acid in 1 molecules and / or containing 40% or more of MMM (triglycerides containing 1% of mead acid for 3 molecules).

[Claim 17]

Fat or oil or triglyceride containing $\geq 20\%$ highly unsaturated fatty acids containing ≥ 20 and ≥ 2 double bonds and containing ≥ 2 carbon atoms, and ≥ 20 carbon atoms and ≥ 2 double bonds in 1 molecules contains ≥ 2 high unsaturated fatty acids. The transesterified fat or triglyceride contains a triglyceride containing ≥ 1 residue triglyceride containing 40 residues and / or containing 3 residue of the same high unsaturated fatty acid containing ≥ 20 and ≥ 2 double bonds at a ratio of $\leq 4.0\%$, and / or triglycerides containing $\geq 2\%$ of triglycerides.

[Claim 18]

This oil or triglyceride is an oil or fat or triglyceride containing 20% or more of arachidonic acid, and contains 40% or more of a triglyceride containing 1 residues of arachidonic acid in 1 molecules and / or contains 4.0% or less of AAA.

[Claim 19]

An oil or fat or triglyceride is fat or triglyceride containing 20% or more of dihomogamma-linolenic acid, containing 40% or more of triglycerides containing 1 residues of dihomogamma-linolenic acid in 1 molecules, and / or containing 4.0% or less of DDD.

[Claim 20]

An oil or fat or triglyceride is fat or triglyceride containing 20% or more of mead acid and containing 40% or more of triglycerides containing 1 residues of mead acid in 1 molecules and / or containing 4.0% or less of M.

[Claim 21]

The method for producing the transesterified fat or triglyceride comprises a highly unsaturated fatty acid containing 20 or more carbon atoms and 2 or more double bonds and 1 or more medium chain fatty acids as constituent fatty acids. This method for transesterifying 1 or more kinds of fats or oils or triglycerides produced by fungi containing at least 20% highly unsaturated fatty acids containing 20 or more carbon atoms and 2 or more double bonds and 50 to 100 pts. wt. of 1 or more medium chain fatty acid triglycerides (ZZ) with 0 to 50 pts. wt. of a 1,3 - position specific lipase.

[Claim 22]

The method according to claim 21, wherein the highly unsaturated fatty acid containing 20 or more carbon atoms and 2 or more double bonds is arachidonic acid.

[Claim 23]

The method according to claim 21 or 22, wherein the medium chain fatty acid triglyceride is a triglyceride of trioctanoate.

[Claim 24]

An interesterified fat or triglyceride obtainable by the process of any one of claims 21 to 23.

[Claim 25]

Triglyceride comprising arachidonic acid (A) and medium chain fatty acid (Z) as a constituent fatty acid, ZAZ (triglyceride having 2 residues of medium chain fatty acid and 1 residues of arachidonic acid) ; A transesterified fat or triglyceride comprising at least 1% by weight of individual at least 2% by weight of a ZZa (triglycerides having 2 residues of medium chain fatty acids and 1 residues of arachidonic acid, wherein arachidonic acid is bound at 1 or 3).

[Claim 26]

The transesterified fat of claim 25, wherein said medium chain fatty acid is octanoic acid.

[Claim 27]

A human nutritional composition comprising an oil or fat or triglyceride according to any one of claims 10 to 20 and 24 to 26.

[Claim 28]

A food composition comprising an oil or fat or triglyceride according to any one of claims 1

to 20 and 24 to 26.

[Claim 29]

The food composition of claim 28, wherein the food composition is a functional food, a dietary supplement, a pre-infant infant formula, an infant formula, an infant food, a food for a pregnant woman or an elderly food.

[Claim 30]

An animal feed comprising an oil or a triglyceride according to any one of claims 10 to 20 and 24 to 26.

Detailed Description

Close

[Detailed description of the invention]

[Technical field]

[0001]

The present invention relates to a method for improving fat or oil or triglyceride comprising highly unsaturated fatty acids produced by microorganisms as constituent fatty acids by transesterification of a lipase, and to a human nutritional composition comprising an improved fat or oil or triglyceride and an improved fat and oil.

[Background of the Invention]

[0002]

Eicosapentaenoic acid (hereinafter referred to as "EPA") of highly unsaturated fatty acids, and docosahexaenoic acid (hereinafter referred to as "DHA") Having many physiological functions especially by the preventive effect of adult diseases, such as arteriosclerosis and thrombosis, an anticancer working effect, the potentiation of study ability, etc. is known, and various trials are made by use to drugs and food for specified health use. However, recently, attention has been paid to the physiological function of these other highly unsaturated fatty acids.

[0003]

Arachidonic acid, which is 1 kinds of highly unsaturated fatty acids, occupies about 10% of fatty acids constituting important organs such as blood and liver (for example, the ratio of fatty acid in phospholipids of human blood is in the ratio of fatty acids). Arachidonic acid is 11%, eicosapentaenoic acid is 1%, docosahexaenoic acid is 3%, and is involved in regulating the fluidity of the membrane as a main constituent of the cell membrane, and exhibits various functions in metabolism in the body, and plays an important role as a direct precursor of prostaglandins. In particular, recently, attention has been paid to the role of

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arachidonic acid as an infant nutrition, and as a constituent fatty acid of an endogenous cannabinoid (2 - arachidonoyl monoglycerol, anandamide) exhibiting nerve activity. Usually, when food products rich in linoleic acid are ingested, arachidonic acid is converted into arachidonic acid, but in adult disease patients and their preworkers, infants, and elderly people, the action of enzymes involved in biosynthesis is reduced, and these arachidonic acid is insufficient.

As a result, it is desired to be taken directly as a triglyceride type oil and fat.

[0004]

There is a rich source of fish oil for EPA and DHA, but dihomo-gamma-linolenic acid, arachidonic acid and 4,7,10,13,16 - docosapentaenoic acid (22:5 ω 6). Fats and oils or triglycerides which are hardly obtainable from conventional oil and fat sources and which are currently obtained by fermenting microorganisms as constituent fatty acids have been generally used. For example, a method has been proposed in which various microorganisms capable of producing fats or oils or triglycerides containing arachidonic acid as a constituent fatty acid are cultured to obtain fats or oils or triglycerides containing arachidonic acid as a constituent fatty acid. Among them, it is known that, especially by using microorganisms of the genus *Mortierella*, fats or oils containing arachidonic acid or triglycerides can be obtained (see, for example, Patent Documents 1 and 2). Arachidonic acid has begun to be used in an essential application, for example, in the field of infant nutrition, e.g., in the field of infant nutrition, and in particular, triglycerides in which arachidonic acid obtained by fermentation is used as a constituent fatty acid.

[0005]

In the field of nutrition of infants, a method of adding and using an arachidonic acid-containing fat and oil to a formula is disclosed (see Patent Documents 3 and 4). An arachidonic acid-containing oil and fat used as the additive raw material is produced by fungi and is molecularly considered. It is known that 6% to 24% of AAA (a triglyceride containing 3 residues of arachidonic acid in 1 molecules) is contained, and as the content of arachidonic acid becomes higher, AAA becomes higher (see Non-Patent Document 1).

[0006]

Since fats and oils containing a high concentration of AAA are not subject to the action of digestive enzymes (pancreatic lipase) of humans under physiological conditions unlike vegetable oils and fats, infants and elderly persons having low pancreatic lipase activity become fats and oils which are hardly absorbed and absorbed (for example, see Non-Patent Document 1).

Since the content of arachidonic acid contained in human milk is 0.5% in total fatty acids (see, for example, Non-Patent Document 2), it is estimated that there is a high possibility that 1 residues of arachidonic acid are present in 1 molecules of triglyceride rather than in

form in which arachidonic acid is condensed in 1 molecules of AAA as described above.

Thus, it is not possible to formulate the arachidonic acid-containing fats and oils obtained by fermentation of fungi into adjusted milk only by considering the ratio of the fatty acid content.

[0007]

Many attempts have been made to enzymatically modify fats and oils to improve physical properties (meltability in mouth, crystallinity, and heat resistance). Many of them have been enzymatic modification of vegetable fats and oils represented by the enzymatic synthesis of cacao substitute fat (see, for example, Patent Documents 5 and 6). In the production technique presented here, the reactivity of lipase is good, and in the case of fats and oils containing many highly reactive unsaturated fatty acids, it is difficult to adapt them from the viewpoint of lipase reactivity.

[0008]

Since fats or oils or triglycerides containing highly unsaturated fatty acids have poor reactivity enzymatically, enzymatic modification has not been made much. Attempts have been made to make structural lipids of highly unsaturated fatty acids using medium-chain fatty acids and highly unsaturated fatty acid containing fats and oils by devising an immobilized enzyme (see Patent Document 7, International Patent Application No. PCT / JP 02 06702). In this method, a free fatty acid in the free form is released in order to replace the fatty acid bound to the 1,3 position of the triglyceride with octanoic acid. Therefore, fats and oils containing high unsaturated fatty acids cannot be expected. Furthermore, there is a problem that high and complex purification techniques such as precision distillation are required for removal of free fatty acids.

[0009]

Fujimoto et al. have studied in detail an influence that an oil and fat structure has on the oxidation stability of highly unsaturated fatty acids since a fish oil (particularly an oil and fat containing EPA and DHA) is extremely oxidized and a sufficient oxidation stability is not obtained by the addition of an antioxidant (for example, see Non-Patent Document 3). A specific fat (C. sub. 8 of medium chain fatty acid and C. sub. 14 triglyceride) is added to sardine oil (high polyunsaturated fatty acid).

To provide a method for oxidizing a highly unsaturated fatty acid by using an EEE (tri-EPA) of a chemically synthesized highly unsaturated fatty acid, and to oxidize the highly unsaturated fatty acid in the presence form scattered between molecules and a state condensed in the same molecule.

It is also confirmed that the dispersibility is good, compared with the ease of the dispersion.

[0010]

In order to prevent the oxidation of highly unsaturated fatty acids contained in fish oil or

the like, a method of stabilizing fats and oils by an ester exchange reaction is also disclosed (see Patent Document 8). However, highly unsaturated fatty acids contained in fish oil or the like have low reactivity of lipase used in the transesterification reaction. Therefore, in the present method, a vegetable oil or fat having high lipase reactivity is used, and a large amount of fat and oil containing high unsaturated fatty acids purified from fish oil or the like is diluted to ensure the reactivity of lipase. Thus, it is not possible to increase the content of highly unsaturated fatty acids in the stabilized fat and oil.

[0011]

[Patent document 1]JP S 63 - 44891A

[Patent document 2]JP S 63 - 12290A

[Patent document 3]JP H 11 - 151075A

[Patent document 4]The extraction-of-a-square-root No. 191886 [ten to] gazette

[Non-patent document 1]Jim-Wen Liu et al., "Hydrolysis of fungal oils in a test tube reaction : The molecular species of arachidonic acid-containing triglycerides (In Vitro Hydrolysis of Fungal Oils: Distribution of Arachidonic Acid - Containing Triacylglycerol molecular Species)", Journal of the American Petrochemical Society (J. Am. Oil Chem. Soc.), 75, p. 507 - 510 (1998).

[Non-patent document 2]Christie, W.W. work and Hamilton, R.J. -- and Volume "connecting position (The Positional Distribution of Fatty Acids in Triglycerides) of fatty acid in triglyceride" on Russell, J.B.,

Analysis of fat and oil (Analysis of Oil and Fats), p. 313 - 339, Elsevier Applied Science (London), (1986).

[Patent document 5]JP S 55 - 71797A

[Patent document 6]Japanese Patent laid-open No. 58 - 42697

[Patent document 7]JP H 08 - 214891A

[Non-patent document 3]4 ** "Influence of Fat and oil structure on oxidative stability of highly unsaturated fatty acids", Science and Industrial Science, 75,, p. 53-60. 2001

[Patent document 8]JP H 6 - 287593A

[Patent document 9]JP H 5 - 91887A

[Patent document 10]JP H 5 - 91888A

[Patent document 11]WO 98/39468.

[Non-patent document 4]Higashiyama et al., "Enhancement of arachidonic acid productivity according to strain 1 S - 4 of Mortierella" (Enhancement of Arachidonic Acid Production by Mortierella Alpina 1S - 4, J. Am. Oil Chem. Soc.), 75, p. 1501 - 1505 (1998)).

[Disclosure of invention]

[0012]

To provide fat or oil containing high unsaturated fatty acid and triglyceride which can be expected to be applied in the fields of infant formula, food and health food, and has

characteristics such as high digestive absorbability and hardly oxidative deterioration. To achieve this, there is provided a method for producing fats and oils containing highly unsaturated fatty acids, such as arachidonic acid, and triglycerides, and a composition containing them, and a food and health food containing them, using an ester interchange reaction of lipase to improve the fat or oil containing high unsaturated fatty acids represented by arachidonic acid.

[0013]

High unsaturated fatty acid containing high unsaturated fatty acid content, high in the content of high unsaturated fatty acids, high in digestive absorbability, and hardly deteriorated in oxidative deterioration. 50-100 parts by mass, vegetable fat and oil, or 0-50 parts by mass of triglyceride is changed into a deoxidization state for the fats and oils or triglyceride which contains the higher unsaturated fatty acid which true fungi produce and includes 20 or more carbon numbers and two or more double bonds 20% or more using specific lipase the 1 or 3rd place, It could be obtained by increasing the reaction temperature and transesterifying.

[0014]

Namely, the present invention,

A method for removing oxygen contained in an immobilized enzyme itself is found by using an immobilized enzyme to enhance heat stability, enabling use of an enzyme at a high reaction temperature, and removing oxygen contained in the immobilized enzyme itself, thereby completing an enzyme reaction suitable for practical use by preventing oxidative deterioration.

Since the transesterification reaction is a reversible reaction between a hydrolysis reaction and an ester synthesis reaction, a method for suppressing the amount of free fatty acid / monoglyceride / diglyceride secondarily generated by efficiently removing moisture for activating an enzyme after activation of an enzyme has completed an enzyme reaction suitable for practical use.

Thus, it is an object of the present invention to provide a method suitable for commercial production of fats and oils converted by transesterification.

Accordingly, the present invention provides a method for producing an edible transesterified fat and oil utilizing the transesterification reaction of lipase using a highly unsaturated fatty acid-containing fat or oil produced by fungi or triglycerides as a raw material, and a composition containing these fats and oils.

[Best mode for carrying out the invention]

[0015]

Since the arachidonic acid content in the milk fat is less than 1% (% by weight), the arachidonic acid residue contained in the triglyceride 1 molecules is estimated to be at mo

1 residues. The molecular species of high - acid-containing fats and oils (Mortierella smut, trade name, manufactured by SUNTGA40S: Sangyo Co., Ltd.) prepared by the fermentation process of the genus Mortierella are shown in Table 1 below.

[0016]

[Table 1]

表1 SUNTGA40Sのアラキドン酸の存在形態

分子種	存在量 (質量%)
AAA	10.57
XAA	37.56
XXA	26.52
XXX	25.35

[0017]

In this case, A is arachidonic acid bound to triglycerides, X is a fatty acid bonded to triglycerides other than arachidonic acid, and specifically, "AAA" is triglyceride containing 3 residues of arachidonic acid in 1 molecules. "XAA" refers to triglycerides having 2 residues of arachidonic acid residues and 1 residues of fatty acids other than arachidonic acid bonded thereto, and "XXA" refers to triglycerides bonded to 1 residues of arachidonic acid and 2 residues of fatty acids other than arachidonic acid. While triglycerides containing arachidonic acid are 74.7% of all triglycerides, XXA, which is presumed to be present in breast milk, is 26.52%, and AAA and XAA, which are considered non-existent, are also present in 48%. It is believed that by converting this 48% AAA and XAA into XXA form, it is in the form of arachidonic acid close to breast milk.

[0018]

For the purpose of improving the high content of fat containing arachidonic acid and triglyceride derived from fermented fungi as described above, the present inventors have experimentally made a transesterified fat and oil with a relatively small amount of vegetable fat and oil using a 1,3 position specific lipase in a deoxygenated state. The arachidonic acid which was contained in fungus oil arachidonic acid fats and oils so much, and combined with the 1 or 3rd place AAA where digestion is not carried out at the raw material is distributed, and it is the structure of people's mother's milk.

It was also successful to approach Form (XXA).

Fats and oils improved in the present transesterification method can be used in foods, health foods and the like as well as in the field of pharmacy and in the field of nutrition of infants.

[0019]

Method for the activation and removal of enzymes

Methods for enzymatically transesterifying and improving fats have been carried out for a long time, as described in the prior art. To solve the problem that a large number of arachidonic acid residues are contained in an oil and fat containing high contents of arachidonic acid, which hardly acts on physiological reaction conditions of lipase, and there is a problem in the reactivity of an enzyme in an ester exchange reaction. In order to increase efficiency of the transesterification reaction, a lipase is immobilized and heat resistance is imparted to increase the reaction temperature, thereby improving an ester exchange ratio (see, for example, Patent document 7 and International Patent Application PCT / JP 02 06702). However, when the reaction is brought to a high temperature in order to improve the reaction efficiency, the oil and fat containing high content of arachidonic acid as a raw material tends to cause oxidative deterioration. In particular, when an immobilized enzyme is used, removal of oxygen permeated into an immobilized carrier becomes a problem. Thus, we found an efficient method for removing oxygen contained in this immobilized enzyme, and found a method of transesterifying under this hypoxic condition.

[0020]

The POV value (peroxide value) is widely used as one parameter indicating the degree of oxidation of fats and oils. The amount of oxygen entering the reaction system was changed to perform the transesterification reaction, and the degree of oxidation of the oil and fat was examined by measuring the POV value. The amount of oxygen was performed at the following 4 levels :. In other words, "degassing & nitrogen blowing", in which the most deoxygenation level is high, "nitrogen blowing", "nitrogen blowing", in which nitrogen is replaced by nitrogen, "nitrogen", which is a level "nitrogen-free" at which no nitrogen substitution is performed at all, and "control", which is controlled under a nitrogen non-substitution condition, using only a carrier of an immobilized enzyme. The POV value was measured after carrying out a prescribed period reaction at 30 and the temperature of 45 or 55 degrees C. As shown in Table 2, it was found that the POV value increased in proportion to the amount of oxygen remaining, and in the case of a degassing state "nitrogen blowing" at a level at which nitrogen substitution was performed in a sense of common sense in particular, it was considered that the removal of oxygen was insufficient because the POV value increased as the reaction temperature increased. It was found from this result that, if the reaction is performed under this condition, deterioration of fat and oil is prevented, an

increase in the POV value is suppressed by increasing the deoxidation level up to the next "degassing and nitrogen blowing", and thus, a high-quality oil and fat can be obtained.

[0021]

[Table 2]

表2 各種条件下で反応した時のPOV値			
	30℃、1日	45℃、3日	55℃、1日
脱気&窒素ブロー	0.87	0.88	0.87
窒素ブロー	1.07	1.23	2.75
窒素無置換	1.28	1.86	3.15
コントロール	2.69	4.50	4.05

(POV : meq/kg油脂)

[0022]

In the case of lipases of the *Rhizopus* (*Rhizopus delemar*, current *Rhizopus oryzae*), it is necessary to add a small amount of water beforehand (about 2%) and activate the enzymes. Thus, manipulation is required to remove excess moisture in the activation and immobilization enzymes. It is necessary to perform deoxidation by adding this operation.

However, the operation of removing oxygen can be performed simultaneously in the activation of the enzyme and the moisture removal step. Upon activation of an enzyme, a raw oil and a small amount of water are added to an immobilized enzyme, and the enzyme is reacted at 30 to 45 ° C for about 1 days to activate an enzyme. As long as the activation of the immobilized enzyme is only activated, inexpensive vegetable oil may be used instead of the high unsaturated fatty acid-containing fat and oil. The removal of oxygen may be carried out at a stage where a raw oil containing a small amount of water is added to an immobilized enzyme or before the addition of an oil or fat, or may be carried out by discharging a nitrogen gas from the raw oil fat and the immobilized enzyme by using a vacuum pump or the like, and then leaking the nitrogen gas, instead of being leaked by air, when returning to normal pressure. By repeating the operation at the reduced pressure and the normal pressure, oxygen in the immobilized enzyme can be removed. After completion of the activation reaction, fat and oil are removed, and the same pressure-reduced nitrogen gas leak as above is repeated for the immobilized enzyme which has absorbed the fat and

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oil to remove oxygen, and the oxygen is removed (nitrogen-substituted raw material fat and oil), and thus the oil and fat can be prevented from oxidative deterioration even under high-temperature conditions.

[0023]

A method for removing water used for activating an enzyme and a method for removing water contained in an enzyme are provided.

It is necessary to remove excess water from the immobilized enzyme activated in the above operation. The presence of excess water leads to a hydrolysis reaction by the lipase, and during the transesterification reaction, a diglyceride, monoglyceride, and free fatty acid are generated. To prevent this, it is necessary to remove water from the reaction system. The fats and oils used for activation from immobilized enzyme activated for removing excessive moisture are removed, and it may react to the washing back with the fats and oils (fats and oils deoxidized and dried preliminarily) of reaction raw materials, and may react to petroleum stock fat at 45 degrees C once for one day. From this reaction, an esterified fat and oil in which the amount of diglycerides is suppressed is obtained. For the purpose of reducing the production cost, inexpensive vegetable oil such as palm oil or the like may be used instead of the highly unsaturated fatty acid containing high unsaturated fatty acid for the activation and the water removal reaction.

[0024]

In the case of the immobilized enzymes of *R. delemar*, the activation of enzymes by water is necessary, but immobilized enzymes (*Rhizomucor miehei* lipases) are commercially available. In the case of a Boezyme L - 9, C. - f., C2, lyo manufactured by Boehringer Mannheim, the activation of enzymes by water is not necessary, and is activated by a trace amount of water contained in the immobilized enzymes. However, this immobilized enzyme also contains extra water, and it is necessary to remove moisture as in the case of the *Rhizopus decamer* (*R. delemar*). This method of removing water may be used, and no special operation is required. In addition, in the case of this enzyme, removal of oxygen may be performed in this dehydration step.

[0025]

About the reaction vessel of an enzyme reaction

Heretofore, a case has been described in which an enzyme reaction is carried out in batch form and transesterification.

As a method of performing the transesterification reaction of fats and oils using an immobilized enzyme, there is a column type reaction method in addition to a batch type reaction method. That is, it is the method of introducing petroleum stock fat into a column and taking out ester interchange fats and oils from an exit, filling immobilized enzyme in column-type a container with a jacket, keeping warm and heating it, and carrying out temperature control of the column. It is also possible, if appropriate, to pass the column

through the column while circulating the fat and oil to increase the conversion rate. In this case, the raw oil fat and oil are passed through a column at the time of activation and at the time of water removal, and oil is made to be compatible with the immobilized enzyme inside, so that deaeration under reduced pressure and forcible blow of nitrogen gas are combined to remove oxygen. It is preferable to perform the transesterification reaction of the fat and oil after the water is removed by being activated.

[0026]

Raw oil of transesterification reaction

An oil or triglyceride containing high unsaturated fatty acid as a constituent fatty acid is mixed with 50 to 100 pts. mass of a triglyceride or triglyceride and 0 to 50 pts. mass of a vegetable oil or triglyceride. The fat and oil containing high unsaturated fatty acids can be improved by performing a transesterification reaction by an immobilized enzyme which becomes a liquid oil and fat in a uniform state and has been removed from oxygen by the method described above.

[0027]

<Vegetable oil and fat>

As the vegetable oil and fat of the reaction, those which are generally used as an edible raw material can be used. The vegetable fats and oils used may also be converted fats and oils. This vegetable oil is used as one of the sources of the saturated fatty acids of transesterification. For example, a linoleic acid system such as soybean oil, safflower oil, olive oil, rice bran oil, sesame oil, and the like, a oleic-linoleic acid oil and fat, palm oil, palm kernel oil, cacao oil, and a cacao oil. Mention may be made of oils and fats of saturated fatty acids such as coconut oil, lard oil and the like and oils obtained by the conversion of triglyceride vegetable oils such as MCT (including saturated medium chain fatty acids of 8 carbon atoms and saturated medium chain fatty acids of 10 carbon atoms and triglycerides composed of the same or different fatty acids). More preferred are fats and oils of saturated fatty acids rich in saturated fatty acids from the viewpoint of imparting resistance to oxidation and from the standpoint of approximating the milk component.

[0028]

If the palm oil of a saturated fatty acid system and the soybean oil of a linoleic acid series with comparatively much unsaturated fatty acid compare the vegetable fat and oil used for the supply source of the saturated fatty acid of an ester interchange, both the fats and oils of the reduction speed of AAA in an ester exchange reaction are substantially equivalent, It is considered that there is no difference in the content of AaA aaAA which is a problem in the digestibility of fats and oils, and the effect of improving the fat and oil having good digestive absorbability is exhibited. However, due to the difference in the fatty acids introduced in the transesterification reaction, there is a difference in the strength against

oxidative deterioration.

[0029]

MCT, particularly triglycerides containing octanoic acid as constituent fatty acids, and microbial arachidonic acid triglycerides, are transesterified according to the invention in order to produce triglycerides comprising arachidonic acid as a constituent fatty acid, for example "8 a 8", "88 a". This method is useful for economically producing oils and fats containing arachidonic acid without waste arachidonic acid and the arachidonic acid-containing fats obtained therefrom are useful as new oils and fats having improved digestibility and absorbency.

[0030]

Further, fats and oils which have been fractionated from fats and oils and have increased saturated fatty acid content by partial hydrogenation can be raised, but the fats and oils which can be used are not limited thereto.

[0031]

<Fats and oils which become considering a higher unsaturated fatty acid as constituent fatty acids>

It is essential to culture a microorganism capable of producing fats or oils or triglycerides comprising highly unsaturated fatty acids as constituent fatty acids. As the microorganism described here, a microorganism which mainly produces at least 1 kinds of highly unsaturated fatty acids having 20 or more carbon atoms and 2 or more double bonds, ω 6 series or ω 9 series of unsaturated fatty acids, and a high unsaturated fatty acid of ω 3 series or ω 2 series of unsaturated fatty acids is desirable.

[0032]

As a highly unsaturated fatty acid having 20 or more carbon atoms and having 2 or more of double bonds, ω 6 system, ω 9 system or ω 3 system, dihomo- γ -linolenic acid (8,11,14 - eicosatrienoic acid), arachidonic acid(5,8,11,14-eicosatetraenoic acid), 7,10,13,16 - docosatetraenoic acid (22:4 ω 6), or the like. DPA ω 6 (4,7,10,13,16 - Docosapentaenoic acid), 8,11-eicosadienoic acid, mead acid(5,8,11-eicosatrienoic acid), 8,11,14,17 - eicosatetraenoic acid (20:4 ω 3) Mention may be made of EPA (5,8,11,14,17 - eicosapentaenoic acid), DPA ω 3 (7,10,13,16,19 - docosapentaenoic acid) and DHA (4,7,10,13,16,19 - docosahexaenoic acid).

[0033]

Accordingly, in the present invention, any microorganisms capable of producing fats or oils or triglycerides containing highly unsaturated fatty acids as constituent fatty acids can be used. For example, as a microorganism which has the productivity of the fats and oils or triglyceride which changes considering arachidonic acid as constituent fatty acids, they are the *Mortierella* (*Mortierella*) group and a **** *obolus* (*Conidiobolus*) group, A ***** (*Pythium*) group, the *Phytophthora* (*Phytophthora*) group, A *****-* (*Penicillium*) group, :

clo *** Ruhm (*Cladosporium*) group, the *Mucor* (*Mucor*) group, a ****-* (*Fusarium*) group, *Aspergillus* (*Aspergillus*) genera of the genus *Rhodotorula* (*Rhodotorula*). Mention may be made of microorganisms belonging to the genera *entomostrobin* (*Entomophthora*), *echinosporangium* (*Echinosporangium*), and *peprolegunia* (*Saprolegnia*). Microorganisms belonging to the genus *Mortierella* (*Mortierella*) genus *Mortierella* (*Mortierella*)

Mention may be made of (*Mortierella elongata*), *titierella* (*Mortierella exigua*), *mortierella phyllaphylla* (*Mortierella hygrophila*), *mortierella alpina* (*Mortierella alpina*), etc. Specifically, *titierella elongant* (*Mortierella elongata*) IFO 8570, *Mortierella eruga* (*Mortierella exigua*) IFO 8571, *Mortierella phygrophila* (*Mortierella hygrophila*) IFO 5941, *mortierella alapide* Mention may be made of strains such as (*Mortierella alpina*) IFO8568, ATCC16266, ATCC32221, ATCC42430, CBS219.35, CBS224.37, CBS250.53, CBS343.66, CBS527.72, CBS529.72, CBS608.70, CBS754.68. For example, as microorganisms capable of producing DHA, *Crypthecodinium* (*Crypthecodenium*), *Thraustolium* (*Thrautochytrium*), *Schizochytrium* (*Schizochytrium*), and the like. Mention may also be made of microorganisms belonging to the genus *Ulkenia*, of the genus *Japanica* (*Japonochytrium*) or of the genus *Horofatris* (*Haliphthoros*).

[0034]

Any of these strains can be obtained without any limitation from the National Institute of Fermentation (IFO) of Osaka, Japan and the American Type Culture Collection (American Type Culture Collection, ATCC) and Centrralbureau voor Schimmelcultures (CBS). It is also possible to use the strain *Mortierella elongata* SAM02 19 (FERM No. 8703) which is separated from soil by the research group of the present invention (FERM No. 1239).

[0035]

In order to culture the strain used in the present invention, a spore of the strain, a mycelial, or a seed culture obtained by previously culturing the strain or a bacterial cell recovered from a seed culture is inoculated into a liquid medium or a solid medium and is cultured in a main culture. In the case of a liquid medium, any commonly used carbon source such as glucose, fructose, xylose, saccharose, maltose, soluble starch, molasses, glycerol, mannitol, and glycated starch may be used, but is not limited thereto. As the nitrogen source, there can be used a natural nitrogen source such as peptone, a yeast extract, a malt extract, a meat extract, a casamino acid, a corn steep liquor, a soybean protein, a defatted soybean, and a cotton seed meal ; an organic nitrogen source such as Urea ; and an inorganic nitrogen source such as sodium nitrate, ammonium nitrate, or sulfuric. Particularly, there may be mentioned a nitrogen source obtained from soybean, specifically, soybean, defatted soybean, soybean flakes, edible soy protein, okay, soybean milk, cedar powder, etc. In particular, it is possible to use a material obtained by subjecting defatted soybeans to heat treatment, more preferably, a material obtained by subjecting defatted soybeans to heat

treatment at about 70 to 90 ° C., and further removing an ethanol-soluble component, singly or in combination with the nitrogen source. Other than this, metal ions such as iron, copper, zinc, manganese, nickel, cobalt, and the like, and vitamins can be used as trace nutrients in addition to phosphate ions, potassium ions, sodium ions, magnesium ions, and calcium ions.

[0036]

No particular limitation is imposed on these medium components so long as they do not impair the growth of microorganisms. In practice, in general, the total amount of carbon source is 0.1 to 40% by mass, preferably 1 to 25% by mass. The total amount of the nitrogen source added is preferably 2 to 15% by weight, more preferably 2 to 10% by weight, more preferably 1 to 5% by weight of the initial carbon source added, 3 to 8% by weight of the initial nitrogen source, and even more preferably only the carbon source, and still more preferably only the carbon source is fed. In order to increase the yield of unsaturated fatty acids, for example, hexadecane or * is a hydrocarbon such as octadecane or, and oleic acid or a salt thereof is oleic acid or *. It is possible to use fatty acid esters such as ethyl esters, Glycerin fatty esters, sorbitan fatty esters ; or olive oil, soybean oil, rapeseed oil, cotton seed oil or * alone or in combination with fats and oils such as Coconut Oil. The amount of the substrate to be added is 0.001 to 10% by weight, preferably 0.5 to 10% by weight, based on the medium. These substrates may also be cultured as a sole carbon source.

[0037]

The culture temperature of the microorganism producing high unsaturated fatty acid depends on the microorganism used, but is 5.

It is also possible to produce an unsaturated fatty acid by culturing at 40 ° C., preferably 20 to 30 ° C., and culturing at 20 to 30 ° C., followed by culturing at 5 to 20 ° C. Such temperature control also makes it possible to increase the proportion of highly unsaturated fatty acids in the fatty acid produced. The pH of the culture medium is from 4 to 10, preferably from 5 to 9, and is subjected to aeration agitation culture, shaking culture, solid culture, or static liquid culture. This culture is usually carried out for 2 to 30 days, preferably 5 to 20 days, more preferably 5 to 15 days.

[0038]

Microorganisms belonging to the genus *Mortierella* subgenus are known as microorganisms capable of producing fats or oils or triglycerides comprising arachidonic acid as the main constituent fatty acid. A microorganism capable of producing fat or oil or triglyceride or triglyceride having dihomo-gamma-linolenic acid as a main constituent fatty acid by subjecting a strain to a mutation treatment (see Patent Document 9) ;Microorganisms capable of producing fats or oils or triglycerides having ω 9 highly unsaturated fatty acids

main constituent fatty acids have been obtained (see Patent Document 10). Furthermore, microorganisms resistant to high concentrations of carbon source (see Patent Document 11) have been obtained, and these microorganisms are microorganisms belonging to the genus *Mortierella*. However, the present invention is not limited to microorganisms belonging to the genus *Mortierella* genus *Mortierella*, and microorganisms capable of producing fats or oils or triglycerides having high unsaturated fatty acids as constituent fatty acids can be used.

[0039]

As a method for obtaining crude oil from microorganisms cultured as described above, after completion of the culture, a culture liquid is directly or after being subjected to a treatment such as sterilization, concentration, acidification or the like, and a cultured bacterial cell is obtained by conventional solid-liquid separation means such as natural sedimentation, centrifugation and / or filtration. To aid in solid-liquid separation, a flocculant or a filter aid may be added. Examples of the aggregating agent include aluminum chloride, calcium chloride, algin, chitosan, and the like. As a filter aid, for example, diatomaceous earth can be used. The cultured cells are preferably washed with water, crushed and dried. Drying can be performed by freeze drying, air drying, fluidized bed drying, freeze drying, or the like. As a means for obtaining a crude oil from a dry cell, an extraction method using an organic solvent or a compression method can be used, and preferably, extraction is performed with an organic solvent under a nitrogen stream. As the organic solvent, ethanol, hexane, methanol, ethanol, chloroform, dichloromethane, petroleum Ether, acetone, and the like can be used, and alternately extraction of methanol and petroleum Ether and a solvent of a single system of chloroform-methanol and water can be used. However, the extraction method used for obtaining crude oil is not limited to the above method, and any method for efficiently extracting fat and oil within a bacterial cell can be used. For example, supercritical extraction methods and the like can be used as effective means.

[0040]

By removing an organic solvent or a supercritical fluid component from an extract extracted with an organic solvent or a supercritical fluid under a condition such as a reduced pressure, a desired crude oil can be obtained. In addition, extraction can be performed using a wet cell instead of the above method. In this case, a solvent compatible with water such as methanol, ethanol and acetone or a mixed solvent compatible with water containing water and / or other solvent is used. Other procedures are the same as described above.

[0041]

In addition, not only crude oil but also refined fats can be used as substrates for transesterification. As the oil and fat refining step, a conventional method such as degumming, deacidification, deodorization, decolorization, column treatment, molecular distillation, and winterization can be used.

[0042]

A lipase used in the transesterification reaction.

Examples of lipases that can be used in the present invention include microorganisms produced by microorganisms such as *Rhizopus*, *Rhizomucis* (*Rhizomucor*), and *Aspergillus* (*Aspergillus*), and porcine pancreatic lipase. For such a lipase, a commercially available lipase can be used. For example, lipases of the genus *Rhizopus* (*Rhizopus deleamar*) (manufactured by Kabushiki Kaisha).

Lipase (*Rhizopus deleamar* is currently classified as a taxonomic *Rhizopus oryzae*), *Rhizopus Neberus* (*Rhizopus niveus*) lipases (Amano Mailer)

(A) Neuase F3G) (a *Rhizopus niveus* is the same as a *Rhizopus deleamar* and is unified with a *Rhizopus oryzae*) ;*Rhizomucan mehigh* (manufactured by Novo Nordisk Co., Ltd.) lipases (manufactured by Novo Nordisk Co., Ltd.) *Rhizomucor miehei* Examples thereof include a ribozyme IM, a Boehringer Mannheim L - 9, c. - f., C2, lyo manufactured by Boehringer Mannheim Co., Ltd., and a lipase of *Aspergillus niger* (*Aspergillus niger*). Without being limited to these enzymes, any 1,3 - position specific lipase can be used.

[0043]

It is desirable to use a lipase immobilized on an immobilized carrier for the purpose of increasing the reaction efficiency of the lipase to the high unsaturated fatty acid or for the purpose of imparting stability for repeated use. As an immobilizing carrier, there is used Celite, an ion exchange resin, a ceramic, an adsorbing resin capable of adsorbing a protein, and the like. For example, powdery diatomaceous earth, granular diatomaceous earth, Dowex MARATHON WBA (Dow Chemical), Diaion WA 30 (Mitsubishi Chemical Co., Ltd.), and SM - 10 (Japanese Industrial Chemical Co., Ltd.) are listed in Celite, and Diaion HP 20 (Mitsubishi Chemical Co., Ltd.) is used in an adsorption resin.

The above ion exchange resins and the like are merely an example, and a resin is evolved every day and better is marketed. It is believed that these resins can be used even if they have emerged.

[0044]

The reaction temperature and time of the transesterification reaction.

In view of the reactivity of the enzyme, the transesterification reaction is preferably from 30 to 60 ° C. In view of the stability of the immobilized enzyme, it is preferable to carry out the reaction at 30 to 50 ° C. in view of the stability of the immobilized enzyme. Although the shorter the time required for the reaction is, the more preferable is the reaction for 1 to 7 days in view of the content of XXA and AAA contained in the transesterification fat and oil after the reaction. In the case of the reaction, since the raw fat and oil (fat and oil rich in high unsaturated fatty acid) is easily oxidized, it is desirable to take measures to remove oxygen as described above and to react under no oxygen.

[0045]

Purification after transesterification.

Thus, fats and oils improved in an ester exchange reaction can be easily separated from an immobilized enzyme from an enzyme reaction tank (either a column reaction or a batch reaction). Most of the components of the resulting fat and oil are triglyceride type fats and oils and can be purified by a method used for purification of ordinary fats and oils such as deoxidation, degumming, decoloring and deodorization.

[0046]

The fat and oil of the present invention is fat or oil or triglyceride which is an oil or fat or triglyceride having a high unsaturated fatty acid produced by microorganisms as a constituent fatty acid, and contains $\geq 20\%$ of a highly unsaturated fatty acid containing ≥ 20 and ≥ 2 triple bonds and ≥ 2 carbon atoms. The higher unsaturated fatty acid which includes 20 or more carbon numbers and two or more double bonds in one molecule. Examples thereof include transesterified fats or oils or triglycerides containing 40% or more of triglycerides containing 1 residues and / or containing 3 residues of the same polyunsaturated fatty acids containing 2 or more of 20 or more and 2 or more bonds at a ratio of 4.0% or less.

[0047]

In the present invention, the fat and oil contain at least 90% of monoglyceride, preferably triglyceride. Fats and oils are present in any form, but in the present invention they comprise diglycerides, monoglycerides and possibly minor amounts of free fatty acids and triglycerides as the products obtained by transesterification.

[0048]

The oil or fat of the present invention is an oil or fat containing 20% or more of arachidonic acid, for example.

Use of an interesterified fat or triglyceride containing 40% or more of triglycerides containing 1 residues of arachidonic acid in 1 molecules and / or containing AAA in a ratio of 4.0% or less, and a raw material of a food, a beverage, a cosmetic, and a pharmaceutical, can be used as an infinite potential.

It can be used as an additive. In addition, there is no limitation on the purpose of use and the amount of use.

[0049]

Examples of the food composition include, in addition to general foods, functional foods, nutraceuticals, pre-infant preparations, infant formulas, baby foods, pregnant women, and geriatric foods. The natural food which contains original fats and oils, such as meat, a fish, or nuts, as an example of foodstuffs containing fats and oils, The foodstuffs etc. which spray or apply fats and oils at the time of processing finishing of the processed food which adds

fats and oils at the time of processing of fats-and-oils foodstuffs, such as foodstuffs, and butter, etc. using fats and oils as heat transfer media, such as foodstuffs, and a doughnut, etc. which add fats and oils at the time of cooking of soup etc., Cookie, etc., or a hard biscuit are mentioned. In addition, it can be added to an agricultural food, a fermented food, a livestock food, a water food or a beverage, which do not contain fats and oils. It may be a form of functional food and drugs, for example, may be a processing form of an enteral hyperalimentation drug, powder, granulation, troches, liquid for oral administration, suspension, an emulsion, syrup, etc.

Next, the present invention will be described in more detail with reference to Examples. However, the present invention is not limited to the examples.

[Examples]

[0050]

Preparation of immobilized enzyme (addition and deoxygenation of stability by immobilization of a 1,3 - position specific lipase) 1.

100 g of an ion-exchange resin carrier (Dowex MARATHON WBA : Dow Chemical) was suspended in 80 ml of an aqueous solution of Rhizopus delesmer (Rhizopus deleamar) lipase (manufactured by Taisase, 12.5%, manufactured by Kakaki Seiyaku Co., Ltd.) and dried under reduced pressure to obtain immobilized enzymes. Next, triglycerides containing 40% arachidonic acid (SUNTGA 40 s, trade name, trade name, trade name) (25 g), palm oil (15 g), the above immobilized lipase (4 g), and water (800 μ l) were weighed into a bottle capable of being sealed. The bottle was placed in a dessicator in a semi-open state and a vacuum pump was connected to the dessicator to reduce the volume of the contents (the air containing oxygen is released from the immobilized enzyme carrier containing the fat and oil into the bottle in the desiccator into the oil). Next, in order to return the temperature of the desiccator to normal pressure, nitrogen gas was leaked into the desiccator. This pressure reduction and atmospheric pressure operation was repeated and the oxygen present in the matrix of the immobilized carrier was replaced with nitrogen and the bottle was sealed. Next, to activate the immobilized enzyme, shaking (100 rpm) was performed at 30 ° C. for 24 hours. After completion of the activation, the fat and oil used for activation were removed, and a deoxidation operation was performed once again to obtain an activated immobilized enzyme. When an ester exchange reaction with other vegetable oil fats and oils is performed, vegetable oil and fat used for the transesterification reaction are used instead of palm oil when the immobilized enzyme is activated. Similarly to palm oil described above, SUNTGA 40 s (25 g), plant fat (15 g), immobilized lipase (2 g), and water (800 μ l) were mixed and subjected to the above-mentioned deoxygenation operation and activation of the immobilized enzyme, and used for the transesterification reaction.

[0051]

Method for determining fatty acid content in oil and fat 2

The fat and oil were collected into a 10 to 20 mg screw-open tube, and 2 mL of methanol hydrochloride and 1 mL of dichloromethane were added thereto, and the mouth of the test tube was sealed and allowed to react at 50 ° C. for 3 hours. After completion of the reaction, the mixture was cooled to room temperature, hexane was added to extract the fatty acid methyl ester, and the recovered hexane layer was concentrated under reduced pressure. The resulting fatty acid methyl ester was dissolved in hexane so that the total fatty acid methyl ester concentration was about 1% and subjected to gas chromatography analysis. Gas chromatographic analysis conditions and component identification were as follows : "Official Methods and Recommended Practices of the AOCS 5th Edition (American Oil Chemists' Society, 1998) "Fatty Acid Composition by GLC (Method Ce 1b" 89) and "Supelcowax - 10". The ratio of the peak area of arachidonic acid methyl ester to the detected peak area sum was defined as the arachidonic acid content in the fat and oil. For fatty acids other than arachidonic acid, each fatty acid content was determined in a similar manner.

[0052]

Example 3. Analytical method of triglyceride

The present invention relates to a method for the production of triglyceride in fats and oils which have been analyzed using HPLC under the following conditions :

Measurements were taken.

Column : reverse phase column (Cosmosil 4.6 x 250 mm 5C18 - MS).

Solvent : Acetone / Acetonitrile (1:1) 1 ml / min.

Analytical time: 55 minutes

Column oven temperature : 40 ° C.

Detector : Differential refractometer detector (cell temperature 40 ° C.)

Sample : 5 µ l of a 10% solution of fat or triglyceride in chloroform is injected.

[0053]

Various triglycerides were analyzed under the above measurement conditions.

Determination of the molecular species of each triglyceride was performed according to the method disclosed in Table 2000 to 513575. That is, the peaks of each triglyceride were separated under the above conditions, and after hydrolysis, they were converted into methyl esters. Gas chromatography was used to determine its molecular species.

[0054]

By this method, each triglyceride of SUNTGA 40 s and fat and oil obtained in the transesterification reaction was analyzed and calculated, and it was possible to indicate the presence form of arachidonic acid in SUNTGA 40 s and the ratio and content of triglycerides in each of XXA. XXA is the sum of percent by weight of the

LLA,PGA,OLA,PLA,OOA,POA,PPA,SOA,PSA,SSA,LC22A. Abbreviations used herein include L : linoleic acid, P : palmitic acid, G : γ - linolenic acid, and the like. O : oleic acid, S : Stearic Acid, C 22 : straight chain saturated fatty acids having 22 carbon atoms, X : fatty acids other than arachidonic acid, A : arachidonic acid (the position of the respective fatty acid residues bonded to the Glycerin is not defined).

[0055]

Modification of arachidonic acid-containing fat and oil (SUNTGA 40 s ; containing about 40% as arachidonic acid) using immobilized lipase of the activated type obtained in Example 1 ; and 4

The reaction was carried out by shaking (100 rpm) at 30 ° C. with the raw material composition shown in Table 3 below, and the reaction was continued while sampling for the purpose of confirming the progress of the transesterification reaction. The amount of change in AAA was measured by performing triglyceride analysis of the sampled fat and oil.

[0056]

[Table 3]

表3 反応の原料組成

SUNTGA40S	25g
パーム油 または 大豆油	15g
固定化リパーゼ	2g

[0057]

FIG. 1 shows changes in AAA content obtained when an oil or fat containing high unsaturated fatty acid and an arachidonic acid-containing fat or oil as a kind of triglyceride are mixed with palm oil or soybean oil of vegetable oil or fat and transesterified. In addition, the rate of decrease of the AAA was the same as that of the soybean oil of the linoleic acid oil having a low saturated fatty acid content and a high unsaturated fatty acid content.

This is evidence that the disappearance rate of the AAA according to the present invention is the same if the mixing ratio is the same regardless of the type of vegetable oil and fat.

[0058]

EXAMPLE 5 CDM TEST

Enzyme conversion oil for the purpose of confirming oxidation stabilization of enzymatically converted arachidonic acid highly rich fat and oil

Fat was prototyped and oxidation stability was measured by CDM test. In the CDM test, while the sample fat and oil are heated (fixed temperature) in a reaction vessel, clean air is fed, and the volatile decomposition product generated by oxidation is collected in water, and the stability of the sample fat and oil is expressed in a time up to a bending point where the conductivity of water abruptly changes. As this time becomes shorter, the oil and fat easily undergoes oxidative deterioration.

[0059]

Based on Example 3, an immobilized enzyme (activated) was added to raw oil fat (40 g) in an amount of 2 times (4 g), and reacted at 30 ° C. at 100 rpm. After the reaction, the fat and oil and the immobilized enzyme were separated by decantation to obtain a reaction fat and oil. The AAA content of the resulting fat was analyzed by HPLC and analyzed for stability by CDM testing. Varying reaction times gave modified oil fats 1 and modified oils 2 with different AAA contents.

[0060]

[Table 4]

表4 修飾油脂（エステル交換油脂）のAAA含量と安定性

試料	CDM (Hr)	AAA含量 (%)
パーム油/SUNTGA40S		
修飾油脂 1	6.4	3.90
修飾油脂 2	5.7	4.44
未修飾油脂	4.6	6.46
大豆油/SUNTGA40S		
修飾油脂 1	4.1	3.69
修飾油脂 2	3.6	4.28
未修飾油脂	3.5	7.01

[0061]

As shown in Table 4, it was shown that, by using a saturated fatty acid-based oil such as palm oil, the vegetable oil and fat of the reaction raw material also decreased the AAA

content in question and increased the stability against the oxidation of the fat and oil. On the other hand, when soybean oil of linoleic acid type oil is used as a reaction raw material, it has been shown that AAA is reduced as in the case of palm oil, but as for the stability shown by CDM, a higher effect is obtained as compared with palm oil.

[0062]

FIG. 6 : Time OF EACH COMPONENT IN THE TRANSESTERIFICATION REACTION OF HIGH ARACHIDONIC ACID-CONTAINING FATS AND OILS (SUNTGA 40 s) diluted with palm oil Since the high-arachidonic acid-containing fat and oil produced by fungi contains many AAA, the present inventors have found that, in order to approach the presence form (XXA) of arachidonic acid of breast milk, it is possible to convert the AAA from AAA to XXA by the transesterification reaction of the lipase and experimentally measure the change in the time course and the ratio of each component.

[0063]

[Table 5]

表5 エステル交換反応における各トリグリセリド成分の時間変化

トリグリセリド成分 (%)	経過時間 (hr)			
	0 hr	48 hr	96 hr	176hr
AAA	6.37	5.16	4.33	3.41
XAA	23.19	19.09	17.00	14.86
XXA	16.11	29.33	35.41	40.55

[0064]

The immobilized enzyme was activated by the method performed in Example 1, and 25 g of SUNTGA 40 s and 15 g of palm oil were added to 2 g of activated immobilized enzyme, and the mixture was deoxygenated, followed by reaction at 30 ° C. Samples were sampled at each time and each triglyceride was analyzed by the method described in Example 2 (HPLC analysis) and each component was calculated. As shown in Table 5, AAA decreased over time, and XXA considered as the conversion body increased. In order for the AAA to be about 1/2 after fat mixing and 2 times or more for XXA, a time of 1 weeks was required under this condition. In the following examples, the reaction temperature and the amount of immobilized enzyme added are examined and optimized.

[0065]

Effect of reaction temperature in the transesterification reaction of Example 7.

The influence of the reaction temperature in the transesterification reaction was investigated using the immobilized enzyme prepared in Example 1. The immobilized enzyme was activated by the method performed in Example 1, and 25 g of SUNTGA 40 s and 15 g of palm oil were added to 2 g of activated immobilized enzyme, and the reaction was carried out at each temperature after deoxidation. After 1 days of reaction, the fat and oil reacted were sampled and each triglyceride was analyzed by the method described in Example 2 (HPLC analysis) to calculate the remaining amount of AAA (Table 6).

[0066]

[Table 6]

表6 エステル交換反応の温度の影響

反応温度	AAA残存量 (%)	AAA減少率 (%、24時間当たり)
反応前	6.37	
30°C	5.86	9%
45°C	4.50	29%
55°C	2.43	62%

[0067]

Since it is considered that the transesterification reaction is performed at 60 ° C. or less, the reaction is carried out at 30 ° C., 45 ° C., and 55 ° C. to confirm the reactivity. As shown in the table, the higher the reaction temperature, the higher the reaction temperature.

It was confirmed that it can be efficiently performed. For this reason, the deoxidation step is important in terms of preventing oxidative deterioration by reaction at high temperature.

[0068]

EXAMPLE 8 Investigation of Rhizomucis Mehig (Rhizomucor miehei) with immobilized enzyme agents

As in Example 5, the amount of enzyme to be added is 10%, and SUNTGA 40 s diluted with palm oil. Lipase of the Rhizopus delemar (R. delemar) activated [fixed] in Example 1 using the trade name, and fixed lipase of a *** call I high (R. miehei) in which it is available as :

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CARGILL 00000693

commercial item and activation by water is unnecessary (made by Boehringer Mannheim)
The transesterification activity of the Quillazyme L - 9, c. - f., C2, Iyo was compared.
[0069]

[Table 7]

表7 R. delemar リパーゼと R. miehei リパーゼによるエステル交換反応
の比較

分子種	<u>R. delemar</u>	<u>R. miehei</u>
AAA	2. 86	2. 53
XAA	14. 95	14. 05
XXA	43. 33	44. 20

[0070]

Each triglyceride was analyzed by the method described in Example 3 (HPLC analysis) and each component was calculated. As shown in Table 7, both reactions were reacted for 48 hours at 45 ° C., but the decrease in AAA and the rate of increase in XXA were almost equal to each other, indicating that they can be used in the present transesterification reaction.

[0071]

Example 9. Improvement by the ester exchange reaction which high-contains arachidonic acid

The present inventors have disclosed a method for obtaining a purified fat and oil by culturing a microorganism belonging to the genus *Mortierella*, obtaining a microorganism capable of producing an oil or a triglyceride having arachidonic acid as a main constituent fatty acid, and fermenting and producing it on an industrial scale (see Non-Patent Document 4). An oil or a triglyceride containing arachidonic acid obtained by this method as a main constituent fatty acid is used as a raw material fat and oil, and is deoxidized and activated by the method of Example 1, and is transesterified with palm oil of vegetable oil and fat by using an immobilized enzyme (*R.delemar* lipase) activated by the method of Example 1. Fat (250 g) containing 40% by weight of arachidonic acid, palm oil (150 g), and immobilized enzyme (40 g) were reacted at 45 ° C. for 48 hours. 97% of the raw material fats and oils were in the form of triglycerides, 25% arachidonic acid as a constituent fatty acid, and 6.37% tri-arachidonic acid (AAA) in triglycerides. The fat and oil obtained by the transesterification reaction were separated from the immobilized enzyme by filtration to

prepare 385 g of an oil or fat containing arachidonic acid containing 95.1% of triglyceride, 2.83% of AAA, and 42.3% of XXA (monoarachidonic acid having 1 arachidonic acid residues bound thereto) or triglyceride.

[0072]

To provide an improved ester interchange reaction of fats and oils containing a high content of dihomo-gamma-linolenic acid (DGLA) in Example 10.

The present invention relates to a method for treating a microorganism belonging to the genus *Mortierella* genus *Mortierella*.

Thus, there is disclosed a method for obtaining a refined fat and oil by obtaining a microorganism capable of producing fats or oils or triglycerides having dihomo-gamma-linolenic acid (DGLA) as main constituent fatty acids and fermenting and producing them on an industrial scale (see Patent Document 9). The oil or triglyceride obtained by using DGLA obtained by this method as the main constituent fatty acid was subjected to an exchange reaction with palm oil of vegetable fat and oil using an immobilized enzyme (*R. delemar* lipase) which was deoxygenated by the method of Example 1 and activated. Oil (25 g) containing 40% by weight of DGLA as the main constituent fatty acid, palm oil (15 g), and immobilized enzyme (4 g) were reacted at 45 ° C. for 48 hours. At the beginning of the reaction, 97.2% of triglyceride and 25% of DGLA as a constituent fatty acid were present, and 6.09% of tri- dihomo-gamma-linolenic acid (DDD) in triglyceride was present. In this reaction, the triglyceride was 95.3% and DDD can be reduced to 3.03%, and the DDD contained in the fat and oil containing DGLA as the main constituent fatty acid as well as arachidonic acid-containing fat and oil or triglyceride can be reduced.

[0073]

Preparation of arachidonic acid-containing oils and fats (crude oil) 11

As arachidonic acid-producing bacteria, a *Mortierella alpina* (*Mortierella alpina*) CBS754.68 was used. The stock strain was inoculated into a medium containing 1% yeast extract, 2% glucose, and 6.3 pH, and the seed culture (first step) was started under the conditions of 100 rpm and 28 ° C. and cultured for 3 days. Next, 1% of yeast extract, 2% of glucose, and 30 L of soybean oil 0.1%, pH6.3 were prepared in a 50 L ** stirring culture tank, and seed culture (first stage) was inoculated with seed culture (first stage), and seed culture (2 stage) was started under the conditions of a stirring speed of 200 rpm, a temperature of 28 ° C., and an internal pressure of 150 kPa, and cultured for 2 days.

[0074]

Next, 4500 L of medium (Medium A : soybean powder 270kg, KH₂PO₄ 16.2kg, MgCl₂ 6H₂O 2.7kg, CaCl₂ 2H₂O 2.7kg, soybean oil 54 kg) was sterilized at 121 ° C. for 20 minutes. As a separate medium, 800 L of medium (Medium B : 108 kg of hydrous glucose) was sterilized at 140 ° C. for 40 seconds and added to the medium A to prepare a medium C. After

adjusting the medium C to pH 6.1, a seed culture solution (2 stage) was inoculated and adjusted to a total of 5400 L of initial culture medium volume (culture tank volume 10 kL). Cultivation was started at a temperature of 26 ° C., an aeration amount of 49 Nm³ / hr, and an internal pressure of 200 kPa. Further, as shown in Table 8, a medium feed was performed, and a main culture was performed for 210 hours. At the end of the culture, a culture volume of 7100 L was obtained due to an increase in culture volume and a decrease in decrease due to evaporation.

[0075]

[Table 8]

表8：実施例1の培地流加

本培養時間	流加培地
19時間後	(含水グルコース243kg +大豆油54kg) / 400L
43時間後	含水グルコース243kg / 400L
67時間後	含水グルコース216kg / 380L
91時間後	含水グルコース162kg / 300L
120時間後	含水グルコース108kg / 200L

[0076]

After completion of the culture, sterilization is performed at 120 ° C. for 20 minutes, and then the wet cells are collected by a filter press.

The dried cells were dried to a moisture content of 2% by weight in a fluidized bed drier, and dried cells were transported to a filling place using an air transporter. The obtained dried cells were filled into an aluminum pouch bag having a volume of about 100 L together with nitrogen gas, and the bag mouth portion was heat-sealed and sealed, and then stored in a refrigerating chamber of 10 ° C. or lower.

[0077]

The dried cell taken out of the container bag was subjected to hexane extraction, a hexane solution was filtered to remove the solid content, and then heated under reduced pressure to remove hexane, thereby obtaining a crude oil containing arachidonic acid as a

constituent fatty acid.

Analysis of the crude oil revealed a triglyceride content of 94% and an arachidonic acid content of 29.6% in total fatty acids.

[0078]

Transesterification of fats and oils prepared in Example 12 11

An attempt was made to improve the crude oil using only the transesterification reaction of the lipase without adding the vegetable oil and fat to a crude oil (triglyceride 94%) containing 29.6% of arachidonic acid prepared in Example 11. Using an immobilized lipase of *R. delemar*, an immobilized enzyme (4 g) was added to a crude oil (40 g) containing 29.6% by deoxygenation and containing arachidonic acid as described in Example 1, followed by shaking for 48 hours at 45. degree. C. At the start of the reaction, fat and oil having a composition of 6.08% AAA and 24.15% XXA had a composition of 3.21% AAA and 40.37% XXA at the end of the reaction. When such crude oil is used, it has been shown that the fat and oil are improved in the same manner as in the transesterification reaction of fats and oils which mainly constitute the microorganism producing highly unsaturated fatty acids as the main constituent fatty acids, as shown in Examples 6 and 8, even if the vegetable oil and fat are not added.

[0079]

Addition of milk to milk 13

To 100 g of powdered milk, 0.44 g of modified oil fat 1 obtained in Example 9 (new transesterified fat and oil) was added to prepare adjusted milk close to human milk. The ratio of arachidonic acid to total fatty acids of the prepared milk was 0.5%.

[0080]

Example 14. Addition to butter

To 100 g of butter fat from which butter milk had been removed in a stirring operation (churning) of a butter manufacturing process, 1.97 g of the modified oil fat 1 (new transesterification fat and oil) obtained in Example 9 was added and subjected to a kneading operation (working) to obtain a butter to which arachidonic acid was added as an equivalent composition.

[0081]

Example 15. Addition to juice

2 g of β - dextrin was added to 20 ml of 20% ethanol aqueous solution, and 0.05% by weight of Vitamin E was added to the modified oil fat 1 obtained from Example 9 (new transesterification fat oil) while stirring with a stirrer, and 100 mg was added thereto and incubated at 50 ° C. for 2 hours. After room temperature cooling (about 1 hours), incubation was continued for 10 hours at 4 ° C. with continued stirring. The resulting precipitate was collected by centrifugation, washed with n-hexane, and lyophilized to obtain 1.8 g of a cyclodextrin inclusion compound containing arachidonic acid-containing

triglyceride. 1 g of this powder was uniformly mixed with 10 L of sucrose to prepare a fat containing an arachidonic acid-containing transesterification fat.

[0082]

Preparation of triglycerides containing medium chain fatty acids and arachidonic acid in the same molecule by transesterification reaction and purification of fats and oils thereof 16 The immobilized lipase prepared according to the method of Example 1 was subjected to deoxygenation and activation according to Example 1. Namely, a triglyceride containing 40% of arachidonic acid (SUNTGA 40 s, trade name, trade name) (200 g), MCT (Cocoard RT, Trade Name of Kao Co., Ltd.) (200 g), the immobilized lipase (40 g) and water (8 ml) were put into a bottle which can be sealed, and the deoxygenation and the activation of the enzyme of Example 1 were carried out.

The fat and oil used in the activation procedure was removed and fresh SUNTGA 40 s (200 g) and MCT (200 g) were placed in a bottle containing the immobilized enzyme and shaken at 30 ° C. for 48 hours (100 rpm).

[0083]

After completion of the reaction, fat and oil were taken out of the bottle and analyzed for triglyceride of the fat and oil transesterified by analysis by HPLC shown in Example 3. Analysis results are shown in Table 9. In the method of this HPLC, triglyceride containing medium chain fatty acids and arachidonic acid obtained by transesterification (e.g., triglyceride (1,2 a) in which octanoic acid is bound at position 3, arachidonic acid at position 88, and arachidonic acid at position 1,3, and triglyceride (8 A8) in which arachidonic acid is bound at position 2) cannot be analyzed. Then, 88 A and 8 A 8 are analyzed for separation by adding GC (analytical conditions below). 88 a appeared chromatographically at 17.01 min and 8 A 8 at 17.15 min.

Accordingly, in the present reaction, various triglyceride contents were as shown in Table 10.

[0084]

[GC analytical method]

Column : Frontier Ultra ALLOY UA - 17-15 M - 0.1 F (15 m × 0.25 × 0.1 μ m).

Column temperature: 260 degree-C-(1 degree C/(minute))-290 degree-C-(10 degrees C/(minute))-390 degree C (5 minutes)

Analytical time: 45 minutes

Inlet temperature: 310 degrees C

Detector temperature : 370 ° C. (hydrogen ionization detector).

Carrier gas : helium

Linear velocity : 40 cm / min.

Sample : 1 μ l of a 1% solution of fat (triglyceride) in hexane is injected.

Further, by molecular distillation of the transesterified fat and oil remaining after the analysis, it was possible to remove almost 85% of the MCT remaining in the reaction.
[0085]

[Table 9]

表 9 : トリグリセリドの割合

HPLC分析の結果

トリグリセリド	反応前 (重量%)	反応後 (重量%)
8A8, など	0.00	15.87
8P8	0.00	6.96
8L8	0.00	6.58
808	0.00	15.23
AAA	4.84	1.67
8AA	0.00	6.25
GAA	0.79	0.00
LAA	3.06	2.86
PAA	5.55	4.71
SAA	3.97	1.45
G22AA	1.30	0.28
G24AA	3.72	0.52
LLA	0.55	1.45
PGA	1.88	0.91
OLA	0.67	0.28
PLA	3.04	1.01
OOA	0.60	0.00
POA	2.97	0.84
PPA	1.32	0.41
SOA	0.90	0.70
PSA	1.30	0.23
SSA	0.93	0.44
LAC22	0.57	0.27
888	50.00	23.27
その他のトリグリセリド	12.04	7.81

[0086]

8 A8 etc. : a mixture of 8A8 (88A, 8A8), 8G8, 8D8.

Each fatty acid is represented by an alphanumeric character, wherein the attachment position to the Glycerin skeleton is not defined, provided that the triglyceride other than 88 A and 8 A 8 does not define the attachment position to the Glycerin skeleton in this table.

The symbols and names of fatty acids are as follows :.

8 : Octanoic acid, A : arachidonic acid, D : DGLA, P : palmitic acid, S : Stearic Acid, O : oleic acid, G : γ - linolenic acid, L : linoleic acid, C 22 : saturated straight chain fatty acids having 22 carbon atoms, C 24 : saturated straight chain fatty acids having 24 carbon atoms.

[0087]

[Table 10]

表10：トリグリセリドの割合

GCとHPLCの組合せ分析結果

トリグリセリド	反応前（重量%）	反応後（重量%）
88A	0.00	8.36
8A8	0.00	5.37
88G + 8G8	0.00	0.35
88D + 8D8	0.00	1.79
88L + 8L8	0.00	6.58
88P + 8P8	0.00	6.96
88O + 8O8	0.00	15.23
8AA + A8A	0.00	6.25
AAA	4.84	1.67
XAA + AXA	18.39	9.82
XXA + XAX	14.73	6.54
888	50.00	23.27
その他のトリグリセリド	12.04	7.81

[0088]

The various triglycerides described above are indicated by the type of fatty acid constituting, in which the binding position of each fatty acid is shown in the 1,2,3 or 3,2,1 position. In the case of triglycerides, position 2 of Glycerin is an asymmetric carbon, and strictly, positions 1 and 3 are sterically different. However, since the 1 and 3 positions are substantially identical, as can be seen from the recognition of the lipase physiologically, the fatty acid shown in the middle in this notation is at the 2 position, and the same triglyceride is displayed as the same triglyceride even if both ends are interchanged.

[0089]

Description of the Invention

Each fatty acid is represented by an alphanumeric character.

The symbols and names of fatty acids are as follows :.

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8 : Octanoic acid, A : arachidonic acid, D : DGLA, P : palmitic acid, S : Stearic Acid, O : oleic acid, G : γ - linolenic acid, L : linoleic acid, C 22 : saturated straight chain fatty acids having 22 carbon atoms, C 24 : saturated straight chain fatty acids having 24 carbon atoms.

The total of XAA+AXA:GAA, LAA, PAA, SAA, C22AA, C24AA shows.

The total of XXA+XAX:LLA, PGA, OLA, PLA, OOA, POA, PPA, SOA, PSA, SSA, LAC22 shows.

Specific triglycerides (e.g., PGA) designated as XAA or XXA do not specify the position of the fatty acid. Specific contents are shown in Table 9.

[Brief Description of the Drawings]

[0090]

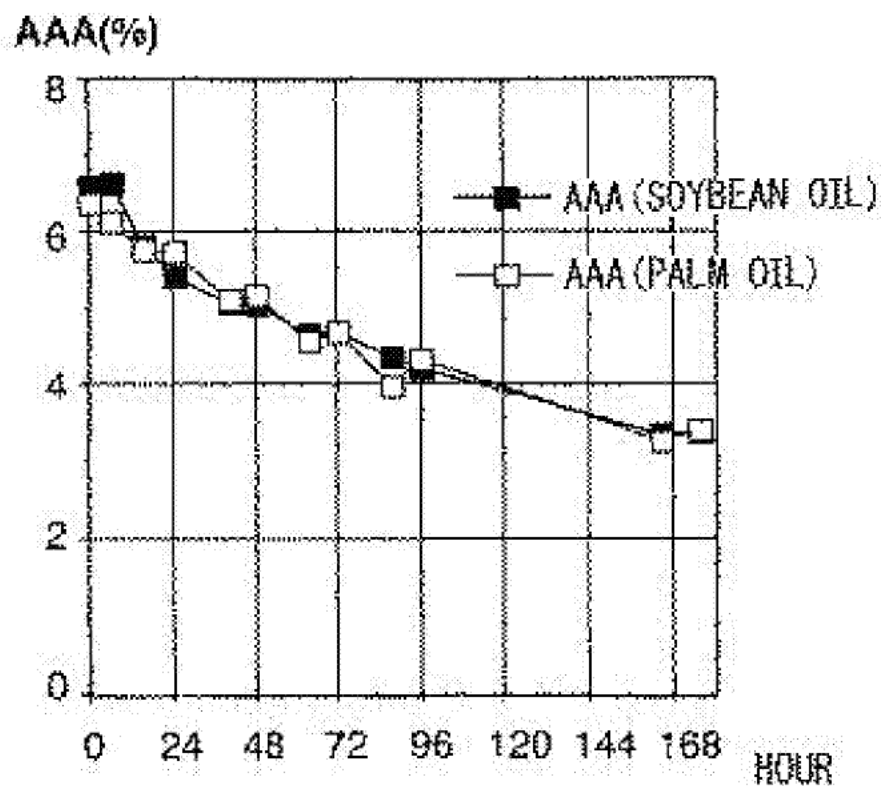
[Fig. 1]FIG. 1 shows the effect of palm oil and soybean oil on reduction of AAA in the transesterification reaction.

Drawings

Close

[FIG.1]

Fig.1



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Overview

Close

(57) [Overview]

PURPOSE: To obtain a white and soft bread with thin crust, irrespective of stock formulation and also without altering its baking process from conventional ones.

CONSTITUTION: Such a fat composition as to contain 0.001-5wt.% of at least one kind of compound selected from phytic acid and alkali or alkaline earth metal salts thereof and 1-5 in the pH value of the aqueous phase, with the pH level of the bread dough on finishing its final hot drying adjusted within the range of 4.5 to 5.2, is incorporated with 0.001-5wt.% of at least one kind selected from among curdlan, carboxymethylcellulose, carrageenan, gelatin and tamarind feed gum. Using the resultant fat composition, the bread can be obtained.

Scope of Claims

Close

[Patent Claims]

[Claim 1] This oil and fat composition contains 1 to 5 wt.% of one or 2 or more selected from alkali salts or alkaline earth metal salts of phytic acid and phytic acid, and is prepared by adjusting pH of an aqueous phase part to 1 to 5 0.001.

[Claim 2] The fat and oil composition according to claim 1, wherein 1 or 2 or more of curdlan, carboxy methylcellulose, carrageenan, gelatine or tamarind seed are contained in

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an amount of 0.001 to 5% by weight.

[Claim 3]A method for producing bread comprising using 0.5 to 15 parts by weight of an oil / fat composition according to claim 1 or 2 per 100 parts by weight of wheat flour.

[Claim 4]A method according to claim 3, wherein the pH of the dough at the end of the stream is adjusted to 4.5 to 5.2.

Detailed Description

Close

[Detailed description of the invention]

[0001]

[Industrial Application]The present invention relates to an oil composition used for producing bread and a method for producing bread using the fat composition.

[0002]

[Prior art] Bread is manufactured by baking a dough made of wheat flour or the like to be changed into a porous structure of a porous structure, and the like, and each component in flour undergoes a change in a physical, chemical and biochemical reaction during firing. That is, dough is thermally expanded, undergoes thermal denaturation of proteins, swelling and gelatinization of starch granules, and the like, and creates a crust. In crusts, browning reactions proceed, resulting in unique color and aroma.

[0003]In order to produce high-quality bread, it is necessary to strictly control the baking process. For example, when a large amount of sugar and a large amount of protein are blended, coloring of a crust is fast, so that when baking is performed under normal conditions, coloring of a crust is too advanced and a texture is hard, and there is a problem that a large amount of debris is generated when sliced. In addition, there is a case in which baking is performed for a long time or at a lower temperature, but in this case, a phenomenon called *-* occurs in the bread, and a problem arises in that productivity is greatly reduced.

[0004]In view of the above problems, Japanese Patent Laid-Open No. 2 (1989) - 117340 proposes a method of producing bread by suppressing the amount of liquid sugar (isomerized sugar, sucrose) or white sugar during bread blending to a minimum amount necessary for fermentation of yeast and adding a sugar alcohol.

[0005]

[Problem to be solved by the invention] However, although this method has an effect in which the amount of sugar and protein is small originally, it is inferior in flavor, and in addition, in the case where a large amount of auxiliary raw material such as powdered milk

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or egg is used, it is impossible to exert sufficient effect only by adjusting the sugar content. [0006]Then, the present invention takes an example by the above-mentioned point, and is * of a bread.

To provide an oil-and-fat composition capable of producing a white and soft bread having a thin crust thickness and a white and soft bread irrespective of the baking process, regardless of whether the composition is used or not, and to provide a method for producing a bread using the composition.

[0007]

[Means for solving the problem] To namely, the oil and fat composition which the present invention contains 1 type chosen from the alkali salt or alkaline earth metal salt of phytic acid and phytic acid, or 2 type or more 0.001 to 5% by weight, and adjusts PH of a water phase part to 1-5 and also the aforementioned oil and fat composition, An oil / fat composition contains 1 to 5 wt.% of one or 2 or more of curdlan, carboxy methylcellulose, carageenan, gelatine or tamarind seed gum from 0.001 to wt.%. It is therefore an object of the present invention to provide a method for producing bread which is capable of producing a bread having a low crust thickness and a white and soft bread thickness.

[0008]Although the pH of the aqueous phase portion of the oil and fat composition can be arbitrarily adjusted within the range of 1 to 5, the objective of the present invention can be achieved by adjusting the pH of the bread dough at the end of the final line to within the range of 4.5 to 5.2. When the pH of the bread dough is lower than 4.5, the dough is sticky, the mechanical resistance is deteriorated, and the gas holding force is lowered, and the bread is not swollen, and the merchantability of the bread itself is eliminated. In addition, when the pH of the bread dough is higher than 5.2, normal bread (ordinary bread dough PH 5.2 to 5.7) and the thickness, color, hardness, and the like of the crust become unchanged. In this case, various acids can be used as means for adjusting pH, but it is more preferable to use an acid and various alkali or base in combination because the pH tends to fluctuate due to the influence of the auxiliary raw material only by the acid.

[0009]The edible oil and fat used in the oil and fat composition of the present invention is not particularly limited, and any of animal and vegetable oils such as soybean oil, cotton seed oil, Rape Seed Oil, palm oil, Coconut Oil, peanut oil, corn oil, jojoba oil, *** oil, fish oil, Beef Tallow, Lard, and milk fat can be used. In addition, although the limit of the solid fat index of the oil and fat which can be used in the present invention is not particularly limited, it may be a range in which the oil and fat are usually kneaded.

[0010]In addition, a Glycerin fatty acid ester, a Propylene Glycol fatty acid ester and a fatty acid ester which are commonly used in the food field are used in preparing the oil and fat composition of the present invention. Emulsifiers such as sorbitan fatty acid esters, polyglycerol fatty acid esters, polyglycerol condensed lysinol acid esters, sucrose fatty acid

esters, and lecithin can be used alone or in combination of 2 or more.

[0011]The method for producing the oil composition of the present invention may be exemplified by the following methods, but is not particularly limited thereto. In other words, a previously adjusted aqueous phase portion (optionally, a taste agent such as a salt, a protein, a sugar, or the like) is added to an oil phase portion, and stirred and mixed.

Combine to form an emulsion. Next, a liquid containing curdlan, carboxy methylcellulose, carrageenan, gelatine or tamarind seed gum is mixed with a liquid oil or fat represented by a Rape Seed Oil or the like to obtain a uniform composition. In preparing the oil and fat composition of the present invention, the ratio (weight ratio) of the oil phase to the oil phase is suitably 50:50~90:10.

[0012]Next, examples of a method for producing bread using the above oil composition include a method of adding yeast, yeast food, fat (shortening, lard, margarine, butter, liquid fat and the like), water, a milk product, a salt, a sugar and the like to wheat flour as a main raw material and baking through a kneading fermentation step.

[0013]

[Examples]

(Example 1) The heating and dissolving of 0.1 % by weight of lecithin and 0.3 % by weight of the glycerine fatty acid esters were added and carried out to 70 % by weight of hardened fish oil with a melting point of 35 degrees C, and 70 % by weight of mixed oils which consists of 30 % by weight of rapeseed oil, and it was considered as the oil phase part. Next, 6% by weight of 50% lactic acid and 2% by weight of 50% phytic acid were added and dispersed in 21.6% by weight of water, followed by heating to obtain an aqueous phase portion. Incidentally, pH of the aqueous phase portion was 3.8. The oil phase portion was gradually added with the aqueous phase portion while stirring, followed by emulsification and mixing with a rapid cooling plasticizing machine (combinator) to obtain an oil and fat composition.

[0014]To 70% by weight of the oil mixture used in Example 1, 0.1% by weight of lecithin and 0.3% by weight of Glycerin fatty acid ester were added and dissolved by heating to prepare an oil phase portion (Example 2). Next, 6% by weight of 50% lactic acid and 2% by weight of Ca 2 - phytate were added to 21.6% by weight of water, and the mixture was heated to obtain an aqueous phase portion. Incidentally, pH of the aqueous phase portion was 3.9. Emulsification, mixing, and rapid cooling plasticization were carried out under the same conditions as in Example 1 to obtain an oil composition.

[0015]To 60% by weight of the oil mixture used in Example 1, 0.1% by weight of lecithin and 0.3% by weight of Glycerin fatty acid ester were added and dissolved by heating to prepare an oil phase portion (Example 3). Next, in 19.6 % by weight of water, the distributed dissolution was carried out moreover, 8 % by weight of citrate, 4 % by weight of

trisodium citrate, and 2 % by weight of 50% phytic acid were warmed, and it was considered as the water phase part. Incidentally, pH of the aqueous phase portion was 3.7. Emulsification, mixing, and rapid cooling plasticization were performed under the same conditions as in Example 1 to obtain a kneaded product. To 94 parts of this kneaded product, 6 parts of a dispersion prepared by dispersing 2 parts of curdlan in 4 parts of Rape Seed Oil was mixed to obtain an oil and fat composition.

[0016]To 60% by weight of the oil mixture used in Example 1, 0.1% by weight of lecithin and 0.3% by weight of Glycerin fatty acid ester were added and dissolved by heating to prepare an oil phase portion (Example 4). Next, in 19.6 % by weight of water, the distributed dissolution was carried out moreover, 8 % by weight of citrate, 4 % by weight of trisodium citrate, and 2 % by weight of 50% phytic acid were warmed, and it was considered as the water phase part. Incidentally, pH of the aqueous phase portion was 3.8. Emulsification, mixing, and rapid cooling plasticization were performed under the same conditions as in Example 1 to obtain a kneaded product. This kneading article 94

To the part, 6 parts of a dispersion prepared by dispersing 2 parts of carboxy methylcellulose in 4 parts of Rape Seed Oil was uniformly mixed to obtain an oil and fat composition.

[0017]50% by weight of the mixed oil used in Example 1 was dissolved by heating, and 0.5% by weight of polyglycerol fatty acid ester was added to and mixed with the mixed oil to obtain an oil phase portion (Comparative Example 1). Next, 6% by weight of 50% lactic acid was added and dispersed in 43.5% by weight of water, followed by heating to obtain an aqueous phase portion. Incidentally, pH of the aqueous phase portion was 3.9.

Emulsification, mixing, and rapid cooling plasticization were carried out under the same conditions as in Example 1 to obtain an oil composition.

[0018]To 85% by weight of the oil mixture used in Example 1, 0.1% by weight of lecithin and 0.3% by weight of Glycerin fatty acid ester were added and dissolved under heating to obtain an oil phase portion (Comparative Example 2). Next, 4% by weight of citric acid was added to 10.6% by weight of water and dissolved, and heated to obtain an aqueous phase portion. Incidentally, pH of the aqueous phase portion was 4.1. Emulsification, mixing, and rapid cooling plasticization were carried out under the same conditions as in Example 1 to obtain an oil composition.

[0019]To 85% by weight of the oil mixture used in Example 1, 0.1% by weight of lecithin and 0.3% by weight of Glycerin fatty acid ester were added, and the mixture was heated and dissolved to obtain an oil phase portion (Comparative Example 3). Next, 14.6% by weight of water was heated to obtain an aqueous phase portion. Incidentally, pH of the aqueous phase portion was 5.7. Emulsification, mixing, and rapid cooling plasticization were carried out under the same conditions as in Example 1 to obtain an oil composition.

[0020]Using the fat composition obtained in Examples 1 to 4 and Comparative Examples 1 to 3, bread was produced using the formulations shown in Table 1 below and evaluated.

[0021]

[Table 1]

食パンの配合（重量部）

	中 種	本 捏
強力粉	7 0	3 0
上白糖	—	6
食塩	—	2
油脂組成物	—	5
イースト	2.2	—
イーストフード	0.1	—
脱脂粉乳	—	2
加水	4 0	2 3

[0022]A vertical mixer (20 coat of Kanto Mixer), a hook and a medium mix material are put into a ball, and kneaded at a low speed of 2 minutes and a medium speed of 1 minutes, and a ** temperature is 24 ° C.

A medium dough was prepared. Then, this was fermented (medium fermentation).

Conditions at this time are as follows.

Medium species fermentation temperature 28.

Medium seed fermentation time 4

[0023]Next, the present ** material was added to the seed fermented dough, mixed and kneaded at a low speed of 2 minutes, a medium speed of 2 minutes and a high speed of 2 minutes, and then the oil and fat were added and kneaded at a low speed of 2 minutes, a medium speed of 2 minutes and a high speed of 3 minutes to obtain a kneaded dough. At this time, the dough temperature was about 27.5 ° C.

[0024]Next, a floor time was taken for 15 minutes to recover the damaged dough by kneading and then divided into 225 g of dough. In order to recover the damaged dough by dividing, a bench time was taken for 20 minutes, and the dough was shaped in a blender. The shaped article was put into a Panman's pan form and fermented (coated). At the end of the run, the dough was measured on a PH meter (Horiba PH Meter, D - 14). Incidentally, the conditions of the roller are as follows.

*** temperature 37°C

*** relative humidity 80%

48 time segment

[0025]The dough thus prepared was calcined in an oven at an inlet temperature of 220 ° C. and an outlet temperature of 200 ° C. for 35 minutes. After firing, the mixture was stored at 20 ° C. for 2 days to obtain a bread sample.

[0026](Comparative Example 4) In the bread formulation shown in Table 1, the fat and oil composition of Comparative Example 3 is used using 2 parts by weight of glucose and 10 parts by weight of sugar alcohol instead of 6 parts by weight of white sugar.

In the same way as above, bread was prepared and evaluated as described above.

[0027]The upper surface of the bread was measured at 10 locations using a color difference meter (manufactured by Minolta, CHROMA METER CR 200), and the average value of the lightness index L was determined. It is to be noted that, as L is larger, color is white and brighter.

[0028]Bread was sliced by 2 cm in a slicer, and the upper section of the 6, 10, and 14 sheets of the bread was observed using a stereomicroscope (manufactured by Mitsubishi Chemical Co., Ltd.), and the average value of the crust thickness (colored portion) was determined by image processing.

[0029]Bread was sliced by 4 cm in a slicer, and the upper part of the bread pan was subjected to a rupture test using a plunger having a diameter of 0.3 cm at a speed of the 0.5 mm/sec of 5 times at a speed of the, and the average value of the breaking strength was determined. It is to be noted that the smaller the value of the breaking strength, the

softer the crust.

[0030]After baking, the bread after baking was left at room temperature for 2 hours, and then the weight of the dust which emerged when 3 of 3 bars were sliced with a slicer of 2 cm thickness was measured and evaluated.

[0031]The above test results are summarized in Table 2 below.

[0032]

[Table 2]

食パンの評価結果

	ホイロ終了時の生地PH	クラストの色	クラスト厚(mm)	破断強度(dyne/cm ²)	スライスくず量
実施例 1	4.7	57.5	2.3	6.0X10 ⁶	
実施例 2	4.7	57.0	2.3	6.2X10 ⁶	
実施例 3	4.9	59.0	2.2	5.7X10 ⁶	
実施例 4	5.0	59.0	2.2	5.8X10 ⁶	7.5g
比較例 1	4.7	56.5	2.4	6.5X10 ⁶	
比較例 2	5.3	51.0	3.0	7.6X10 ⁶	
比較例 3	5.5	50.0	3.0	7.5X10 ⁶	12.9g
比較例 4	5.6	52.0	3.0	7.5X10 ⁶	

[0033]

[Effect of the Invention] As is apparent from the results shown in Table 2, according to the present invention, the crust thickness is significantly thinner than that of the conventional method, and a white and soft bread can be obtained.

Because of the thick and hard crust thickness in conventional bread making, it is also possible to eliminate the problem of large debris when the bread is sliced.

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Overview

Close

(57) [Overview]

PURPOSE: To provide a process for the efficient transesterification of high- melting oil and fat, etc., in the adsence of solvent by effectively utilizing the advantageous properties of a lipase having site specificity, etc.

CONSTITUTION: The transesterification process comprises reacting a molten organic raw material for transesterification with a lipase obtained from microorganism of the genus Alcaligeous under an substantially solvent-free anhydrous condition at 81-130°C.

Scope of Claims

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[Patent Claims]

[Claim 1]This transesterification method is characterized by allowing a lipase obtained from a microorganism belonging to the genus Alcaligenes to act at 81 to 130 ° C on a molten organic raw material for transesterification under substantially solvent-free and anhydrous conditions.

[Claim 2]The method according to claim 1, wherein the temperature of the lipase is 91 to 120 ° C.

Detailed Description

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[Detailed description of the invention]

[0001]

[Industrial Application]The present invention relates to a transesterification method using a lipase, which performs transesterification without using a solvent.

[0002]

[Prior art] The transesterification reaction is an important technique for the preparation of esters of various fatty acids, sugar esters and steroids, as well as the modification of animal and vegetable oils and fats. As a catalyst for the transesterification reaction, a lipase which is an oil and fat hydrolase is used, whereby the side reaction can be suppressed and energy cost can be reduced compared with a chemical reaction, and further, since lipase as a catalyst is a natural substance, safety is obtained. In addition, there is an advantage that the target substance can be efficiently produced due to its substrate specificity and position specificity. However, when an ester exchange reaction is performed using a lipase, the reaction proceeds only under mild conditions from room temperature to about 70 ° C., and thus it has been practically impossible to transesterify, for example, fats and oils having a high melting point exceeding 90 ° C. in the absence of an organic solvent, or the like, using a lipase. The same applies to the *Alcaligenes* lipase, and also in the case of the ester synthesis using lipase PL manufactured by the name sugar industry (Japanese Patent Laid-Open No. 62 104589), a reaction is usually carried out in a temperature range of 20 to 60 ° C., and an ester synthesis reaction is carried out at 40 ° C. in the examples. As described above, conventionally, a means by a chemical reaction has been used when an oil or fat having a high melting point or a wax or the like is transesterified, so that it has been difficult to obtain a desired target substance, e.g., an ester exchange reaction product requiring substrate specificity and position specificity. On the other hand, in the transesterification reaction using lipase, a high temperature reaction of about 100 ° C. is substantially impossible.

[0003]

[Problem to be solved by the invention] It is an object of the present invention to provide a method for efficiently transesterifying an oil or fat having a high melting point without solvent, by effectively utilizing the advantage of a lipase having position specificity or the

like.

[0004]

[Means for solving the problem] The present invention has been made on the basis of the finding that the above problems can be solved when the transesterification reaction is carried out at 81. degree. to 130. degree. C. under substantially solvent-free and anhydrous conditions using a specific lipase. That is, the present invention is a fruit to the organic raw material for ester interchanges which carried out melting.

To provide an ester exchange method which is characterized in that a lipase obtained from a microorganism belonging to the genus *Alcaligenes* is allowed to act at 81 to 130 ° C under qualitatively anhydrous and anhydrous conditions. Here, the term "ester interchange" means that, for example, 1 or 2 or more esters selected from esters of a carboxylic acid and an alcohol or a carboxylic acid or an alcohol is allowed to coexist therewith, and a carboxylic acid residue or * of the ester is exchanged for an alcohol residue.

[0005] Suitable examples of the carboxylic acid include a saturated or unsaturated, linear or branched aliphatic monobasic acid having 2 to 50 carbon atoms, a fatty acid, an aliphatic dibasic acid, and a tri- or tri-phosphoric acid as a preferable example of the carboxylic acid.

2. As such aliphatic monobasic acids, acetic acid, butyric acid, caprylic acid, isooctyl acid, isononanoic acid, capric acid, lauric acid, palmitic acid, palmitoleic acid, heptadecanoic acid, Stearic Acid, isothiazole, oleic, elaidic acid, and elaidic acid are used. Stearic Acid 10-hydroxystearic acid, 12-hydroxystearic acid, lysinol acid, linoleic acid, linolenic acid, erucic acid, behenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, cerotic acid, montanic acid, nonacosanoic acid, melissic acid, melissic acid, montanic acid, and melicolic acid ; Examples thereof include pentatriacontanoic acid, hexatriacontanoic acid, and ***** acid. As aliphatic dibasic acid, succinic acid, malic acid, fumaric acid, maleic acid, tartaric acid, glutaric acid, azelaic acid, sebacic acid, 1,12-dodecylcarboxylic acid, etc. can be illustrated, and citrate etc. can be illustrated as aliphatic series tribasic acid.

[0006] As the alcohol, for example, a saturated or unsaturated, linear or branched aliphatic monohydric alcohol having 1 to 50 carbon atoms, and a 2 to 6 valent alcohol are suitable. As an example of these alcohols, methanol, ethanol, isopropyl alcohol, hexanol, isooctanol, isononanol, lauryl alcohol, Cetanol, Stearyl Alcohol, iso Stearyl Alcohol, and oleylalcohol are used. A monohydric alcohol such as behenyl alcohol, octacosanol, a monoproline alcohol 425 (average molecular weight : 510) manufactured by Petrolite Co., Ltd., a homophosphorus alcohol 550 (average molecular weight : 660), an allilin alcohol 700 (average molecular weight : 850), and the like. Dihydric alcohol, such as ethylene glycol, a diethylene glycol, propylene glycol, dipropylene glycol, 1,8-octanediol, and 1,10-decanediol, Examples thereof include alcohols having 3 or more valences such as Glycerin, diglycerin, triethanolamine, polyglycerin, pentaerythritols, neopentyl glycol, trimethylolethane,

trimethylolpropane, Xylitol, sorbitol, and. Glycerin. Further, as an alcohol, xylose, fructose, galactose, glucosamine, glucose, β - ***** , and β - are used.

Saccharides such as maltose and raffinose, cholesterol, lanosterol and lanosterol ;Mention may also be made of sterols such as Testosterone, androstototriol, nandrolone, ergophyphenol, cholestaphenol, 2,2-dihydroergosterol, dimosterol, and the like, and sphingosine, *****-***** , and the like.

[0007]Such esters of carboxylic acid and alcohol can be obtained by extracting from natural products such as animals, plants, fish, microorganisms, and minerals, or by synthesizing esters by a conventional method. Preferred examples of esters in the present invention are fats and oils and waxes. In the present invention, it is preferable to use those having a melting point of 81 to 130 ° C., but when 2 or more kinds of organic raw materials for transesterification are used, the mixture of the organic raw materials for transesterification may be melted at 81 to 130 ° C. even if the melting point is not within the range of 81 to 130 ° C. Particularly preferred is 91 to 120 ° C. In the present invention, the aforementioned ester is used as a raw material substrate, the lipase from microorganism which belongs to this at Alcaligenes can be added, an ester exchange reaction can be carried out at 81-130 degrees C, preferably 91-120 degrees C, and ester can be used either alone or as mixtures. If necessary, 1 or 2 or more of the aforementioned carboxylic acids, or 1 or 2 or more of the alcohols may be co-present as a raw material substrate in the above ester.

[0008]The lipase used in the present invention is a lipase produced by microorganisms belonging to the genus Alcaligenes, and any lipase can be used as long as it has an ester exchange activity under substantially anhydrous conditions and at 81 to 130 ° C. Among these lipases, a lipase obtained from an Alcaligenes sp. (Alcaligenes sp.) strain is used. Specifically, it is a lipase QL (produced by the name of the name of the name sugar industry) produced by the lipase-producing bacterial name saccharide PL - 266 which is disclosed in Japanese Patent Publication No. 58 36953, and deposited as FERM P. 2985 (FERM P - 3187:FERM BP - 2985). This lipase is a bile activating lipase having a position specificity for the 1 and 3 positions of the glyceride and an optimum pH for the alkaline side, and has an enzymatic property described in Japanese Patent publication No. 58 36953. In accordance with the present invention, it is also possible to use a separate lipase PL (manufactured by Alcaligenes sp.). In order to carry out the transesterification reaction according to the method of the present invention, it may be carried out as follows. That is, without adding water to the raw material substrate, the lipase is added, and the mixture is heated to 81 to 130 ° C., preferably 91 to 120 ° C., more preferably 91 to 110 ° C., most preferably 95 to 100 ° C. in the absence of a solvent, and is subjected to a reaction for a predetermined time, for example, 10 minutes to 100 hours by standing, stirring or shaking.

A reactant is collected and refined after ending reaction and with a conventional method, and it is ester.

An exchange reaction can be obtained.

[0009]In the present invention, it is important to carry out the reaction in a substantially anhydrous state. If, for example, water content in the reaction is about 0.1% or more, such as in a transesterification reaction using a conventional lipase, the method of the present invention lowers the reactivity and is undesirable because of the side reactions that produce diglycerides from triglycerides. In other words, in the present invention, it is preferable to reduce the moisture content to less than 0.1%, including water in a discontinuous state which is originally dissolved in a substrate, or small free water or bound water contained in an enzyme or an immobilized enzyme. In the case where water in the system may exceed this condition, it is preferable to use the organic material in advance by removing the water content of the organic material or the enzyme. Although the reaction can be carried out in either a closed system or an open system, the initial water content in the system is consumed by evaporation or hydrolysis of some of the esters, and is gradually reduced in the range of 1 ppm as long as the transesterification reaction proceeds in a range of 81 ° C. to 130 ° C. as long as the transesterification reaction proceeds.

[0010]As the form of the enzyme used in the present invention, an enzyme powder or an enzyme immobilized on diatomaceous earth or an ion exchange resin is exemplified. As a method of performing the transesterification reaction, a continuous reaction method using an enzyme packed in a column, a batch method of dispersing an enzyme in a reaction solution, or the like can be used. The lipase particles which are dispersed in the reaction solution can be recovered by a method such as fine membrane filtration, centrifugation, or washing with an inert organic solvent, and can be subjected to a new transesterification reaction again.

[0011]

[Effect of the Invention] According to the present invention, it is possible to efficiently transesterify high melting point fats and oils and the like by effectively utilizing the advantage of lipase. In addition, since no solvent is used in the method of the present invention, it is possible to obtain a product having a good final product and a high purity. Next, the present invention will be described by way of examples.

[0012]

[Examples]

Example 1

50 g of ethyl ester of split fat acid (the Hoechst A.G. make, trade name:S wax) of a montan wax, medium-chain-fatty-acid triglyceride of caprylic acid/capric acid =75/25 (made by the Nisshin Oil Mills, Ltd.) 50 g of ODO and 1 g of lipases derived from *****-

(Alcaligenes sp.) (trade name : Lipase QL, manufactured by the name of the sugar Industry) were stirred at a stirring speed of 300 rpm using a magnetic stirrer to carry out a transesterification reaction. Reaction temperature was 95 degrees C which is more than the melting point of a montan wax, and reacted for 7 hours. Gas chromatographic analysis of the reaction confirmed that 83% of the montan wax fatty acid was introduced into the triglyceride, ODO. This *

From this, it is found that a wax which is a kind of high melting point lipid can be transesterified in a substantially anhydrous state without solvent using lipase at a high temperature of 95 ° C.

[0013]Example 2

20 g of dibasic acid (the NIKKO KYODO [CO., LTD.] make, a trade name: tetradecane ***** acid) of the carbon number 14, 80 g of tributyrin (manufactured by Wako Pure Chemical Industries, Ltd., trade name : tributyrin), which is a triester of Glycerin and butanoic acid, and 1 g of lipase QL used in Example 1 were stirred using a magnetic stirrer at a stirring speed of 350 rpm to carry out a transesterification reaction. The reaction temperature was set at 120 ° C. for dissolving the above-mentioned dibasic acid, and the reaction was carried out for 6.5 hours at 2 ° C. From the results of gas chromatographic analysis of the reaction product, it was confirmed that 93% of the long-chain dibasic acid was introduced into the tributyrin 2. From this, it can be seen that a lipase is used at a high temperature as high as 120 ° C. and a long chain 2 fatty acid which is a kind of high melting point lipid can be transesterified in a substantially anhydrous state without a solvent.

[0014]Example 3

120 g of Carnauba Wax, 10 g of Glycerin and 1 g of the lipase QL used in Example 1 were subjected to an exchange reaction using a magnetic stirrer at a stirring speed of 300 rpm. Reaction temperature reacted for 36 hours at 90 degrees C which is more than the melting point of a carnauba wax. From the results of gas chromatographic analysis, the production of 1-monoglyceride and 1,3-diglyceride was confirmed. For this purpose, a high melting point wax is used at a high temperature of 90 ° C.

It has been shown to be able to transesterify in a substantially anhydrous state, while maintaining the position specificity of the lipase.

[0015]Example 4

A glass tube having an inner diameter of 2 cm and a length of 15 cm was filled with 80 g of the lipase QL used in Example 1 immobilized on granular diatomaceous earth by a conventional method, to prepare an immobilized lipase packed column. A mixture of 20 g of Carnauba Wax and 80 g of tritriazan cetin (manufactured by Wako Pure Chemical

Industries, Ltd., trade name : Thoria cetin), which is a triester of Glycerin and acetic, was flowed through a column of 2.0 ml per hour, and the transesterification reaction was continuously carried out at 100 ° C. Gas chromatographic analysis of the reaction confirmed the introduction of Carnuba Wax fatty acids into the triglyceride triamide cetin. From this fact, it is found that a wax which is a kind of high melting point lipid can be continuously transesterified in a substantially anhydrous state without solvent using lipase at a high temperature of 100 ° C.

[0016]Comparative example 1

Water was added to the raw material of Example 1 to obtain a water content of 1.0%. The reaction was carried out in the same manner as in Example 1, and the reaction product was analyzed. As a result, a side reaction product accompanied by hydrolysis was generated, and hardly the formation of a glyceride having a montan wax decomposed fatty acid residue was observed, and the thermal stability of the enzyme was reduced.

Comparative example 2

Example 2 was repeated under the same conditions except that the reaction temperature was 50 ° C. in Example 2. As a result, only 15% of the long-chain 2 acids were introduced into the tributyrin.

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(54)【発明の名称】 油脂組成物およびそれを用いたパンの製造方法

(57)【要約】

【目的】 原料配合の如何にかかわらず、しかも焼成工程を変えることなく、クラスト厚が薄く、白くて柔らかいパンを製造可能とする。

【構成】 フィチン酸、フィチン酸のアルカリ塩またはアルカリ土類金属塩から選んだ1種または2種以上を0.001~5重量%含有し、水相部のPHを1~5として最終的にホイロ終了時のパン生地のPHを4.5~5.2の範囲内に調整してなる油脂組成物に、カードラン、カルボキシメチルセルロース、カラギーナン、ゼラチンまたはタマリンド種子ガムから選んだ1種または2種以上を0.001~5重量%含有する油脂組成物を用いてパンを製造する。

【特許請求の範囲】

【請求項1】 フィチン酸、フィチン酸のアルカリ塩またはアルカリ土類金属塩から選んだ1種または2種以上を0.001～5重量%含有し、かつ水相部のPHを1～5に調整してなることを特徴とする油脂組成物。

【請求項2】 カードラン、カルボキシメチルセルロース、カラギナン、ゼラチンまたはタマリンド種子から選んだ1種または2種以上を0.001～5重量%含有することを特徴とする請求項1記載の油脂組成物。

【請求項3】 小麦粉100重量部に対し、請求項1または請求項2に記載された油脂組成物を0.5～1.5重量部用いてなることを特徴とするパンの製造方法。

【請求項4】 ホイロ終了時のパン生地を4.5～5.2に調整することを特徴とする請求項3記載のパンの製造方法。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、パンの製造に用いられる油脂組成物、および、この油脂組成物を用いたパンの製造方法に関するものである。

【0002】

【従来の技術】パンは、小麦粉等からなるパン生地を焼成することによって多孔質の構造に変化させて製造されるものであり、この焼成中に小麦粉中の各成分は物理的、化学的、生化学的変化を受ける。即ち、パン生地は、熱により膨張し、タンパク質の熱変性、デンプン粒の膨潤糊化などを受け、更にクラストを作る。クラストでは褐変反応が進み、独特の色、香りが生ずる。

【0003】そこで、品質のよいパンを製造するためには、まず、焼成工程を厳密に管理する必要があるが、例えば、糖、タンパク質が多い配合だとクラストの着色が進みすぎ、食感も硬いものになるだけでなく、スライスした時にくずが多く出る、ということが問題となっている。また、短時間、またはより低温で長時間焼成したりする場合もあるが、この場合には、パンにケーキングという現象が発生したり、また、生産性が大幅に低下するということが問題となっている。

【0004】上記のような問題に対して、特開平2-117340号には、パン配合中の液糖（異性化糖、ショ糖）もしくは上白糖の量を酵母の醗酵に必要な最低量に抑え、糖アルコールを加えてパンを製造する方法が提案されている。

【0005】

【発明が解決しようとする課題】ところが、この方法は、もともと糖、タンパク質量の少ない配合では効果はあるものの、風味の点で通常より劣り、更に、粉乳、卵等の副原料の多い配合では、このような糖含量の調整だけでは充分な効果は発揮できなくなる。

【0006】そこで本発明は上記の点に鑑み、パンの製

造において、原料配合の如何にかかわらず、かつ、焼成工程を変えることなく、クラスト厚が薄く、白くて柔らかいパンを製造する油脂組成物、およびこの油脂組成物を用いたパンの製造方法を提供せんとするものである。

【0007】

【課題を解決するための手段】即ち、本発明は、フィチン酸、フィチン酸のアルカリ塩またはアルカリ土類金属塩から選んだ1種または2種以上を0.001～5重量%含有し、水相部のPHを1～5に調整してなる油脂組成物、更に、前記油脂組成物に、カードラン、カルボキシメチルセルロース、カラギナン、ゼラチンまたはタマリンド種子ガムから選んだ1種または2種以上を0.001～5重量%含有する油脂組成物、およびこれを用いたパンの製造方法を提供せんとするものであり、これにより、クラスト厚が薄く、白くて柔らかいパンを製造することができる。

【0008】前記油脂組成物の水相部のPHは1～5の範囲に任意に調整できるが、最終的にホイロ終了時のパン生地を4.5～5.2の範囲内に調整すれば、本発明の目的を達成することができる。このパン生地のPHが4.5より低い場合は、生地がベタついて機械耐性が悪くなり、また、ガス保持力が低下し、膨らまなくなりパン自体の商品性がなくなる。また、パン生地のPHが5.2より高い場合には、通常のパン（通常のパン生地PH5.2～5.7）と、クラストの厚さ、色、硬さ等が変わらなくなってしまう。この場合、PHを調整する手段として種々の酸を用いることができるが、酸のみでは副原料の影響でPHが変動しやすいために酸と種々のアルカリまたは塩基を併用する方がより好ましい。

【0009】本発明の油脂組成物に使用される食用油脂については特に制限はなく、大豆油、綿実油、ナタネ油、パーム油、ヤシ油、落花生油、コーン油、ホホバ油、クヘア油、魚油、牛脂、豚脂、乳脂といった、動植物油のいずれも使用することができる。また、本発明に使用し得る油脂の固体脂指数の制限は特にないが、通常練り込まれる範囲であればよい。

【0010】また、本発明の油脂組成物を製造する際には乳化剤を使用するが、食品分野で一般的に用いられるグリセリン脂肪酸エステル、プロピレングリコール脂肪酸エステル、ソルビタン脂肪酸エステル、ポリグリセロール脂肪酸エステル、ポリグリセロール縮合リノール酸エステル、ショ糖脂肪酸エステル、レシチン等の乳化剤を単独で、または2種以上を混合して使用することができる。

【0011】本発明の油脂組成物を製造する方法は、下記に示す方法等を挙げることができるが、特にこれらの製造方法に限定されるものではない。即ち、予め調整された水相部（必要に応じて食塩、タンパク質、糖類等の呈味剤を添加してもよい）を油相部に添加し、攪拌、混

合し、乳化物を作る。次いで、常法により急冷可塑性し、得られた捏和品にナタネ油等に代表される液状油脂にカードラン、カルボキシメチルセルロース、カラギーナン、ゼラチンまたはタマリンド種子ガムを分散させた液を混合し、均一な組成物を得る。また、本発明の油脂組成物を製造するに際しては、油相と水相の比率（重量比）は50：50～90：10とするのが適当である。

【0012】次に、前記の油脂組成物を用いてパンを製造する方法としては、主原料として小麦粉にイースト、イーストフード、油脂類（ショートニング、ラード、マーガリン、バター、液状油脂等）、水、乳製品、食塩、糖類等を添加し、混捏醗酵工程を経て焼成する方法等が挙げられる。

【0013】

【実施例】

（実施例1）融点35℃の硬化魚油70重量%、ナタネ油30重量%からなる混合油70重量%に、レシチン0.1重量%、グリセリン脂肪酸エステル0.3重量%を添加して加熱溶解し、油相部とした。次に、水21.6重量%に、50%乳酸6重量%、50%フィチン酸2重量%を添加分散し、加温し、水相部とした。尚、水相部のPHは3.8であった。油相部を攪拌しながら水相部を徐々に加えて乳化、混合後、急冷可塑性機（コンビネーター）を通し、油脂組成物を得た。

【0014】（実施例2）実施例1で用いた混合油70重量%に、レシチン0.1重量%、グリセリン脂肪酸エステル0.3重量%を添加して加熱溶解し、油相部とした。次に、水21.6重量%に50%乳酸6重量%、Ca2-フィテート2重量%を添加分散し、加温し、水相部とした。尚、水相部のPHは3.9であった。乳化、混合、急冷可塑性は実施例1と同じ条件で行い、油脂組成物を得た。

【0015】（実施例3）実施例1で用いた混合油60重量%に、レシチン0.1重量%、グリセリン脂肪酸エステル0.3重量%を添加して加熱溶解し、油相部とした。次に、水19.6重量%にクエン酸8重量%、クエン酸三ナトリウム4重量%、50%フィチン酸2重量%を加えて分散溶解し、加温し、水相部とした。尚、水相部のPHは3.7であった。乳化、混合、急冷可塑性は実施例1と同じ条件で行い、捏和品を得た。本捏和品94部に対し、ナタネ油4部にカードラン2部を分散させた分散液6部を混合し、油脂組成物を得た。

【0016】（実施例4）実施例1で用いた混合油60重量%に、レシチン0.1重量%、グリセリン脂肪酸エステル0.3重量%を添加して加熱溶解し、油相部とした。次に、水19.6重量%にクエン酸8重量%、クエン酸三ナトリウム4重量%、50%フィチン酸2重量%を加えて分散溶解し、加温し水相部とした。尚、水相部のPHは3.8であった。乳化、混合、急冷可塑性は実施例1と同じ条件で行い、捏和品を得た。本捏和品94

部に対し、ナタネ油4部にカルボキシメチルセルロース2部を分散させた分散液6部を均一に混合し、油脂組成物を得た。

【0017】（比較例1）実施例1で用いた混合油50重量%を加熱溶解し、これにポリグリセロール脂肪酸エステル0.5重量%を添加混合し、油相部とした。次に、水43.5重量%に、50%乳酸6重量%を添加分散し、加温し、水相部とした。尚、水相部のPHは3.9であった。乳化、混合、急冷可塑性は実施例1と同じ条件で行い油脂組成物を得た。

【0018】（比較例2）実施例1で用いた混合油85重量%に、レシチン0.1重量%、グリセリン脂肪酸エステル0.3重量%を添加して加熱溶解し、油相部とした。次に、水10.6重量%にクエン酸4重量%を添加溶解して加温し、水相部とした。尚、水相部のPHは4.1であった。乳化、混合、急冷可塑性は実施例1と同じ条件で行い油脂組成物を得た。

【0019】（比較例3）実施例1で用いた混合油85重量%に、レシチン0.1重量%、グリセリン脂肪酸エステル0.3重量%を添加し、加熱溶解して油相部とした。次に、水14.6重量%を加温し、水相部とした。尚、水相部のPHは5.7であった。乳化、混合、急冷可塑性は実施例1と同じ条件で行い、油脂組成物を得た。

【0020】（食パン生地および食パンの製造条件、製法並びに評価方法）実施例1～4、および比較例1～3で得られた油脂組成物を用い、下記表1に記載した配合で食パンを製造し、評価を行った。

【0021】

【表1】

食パンの配合（重量部）

	中 種	本 捏
強力粉	70	30
上白糖	—	6
食塩	—	2
油脂組成物	—	5
イースト	2.2	—
イーストフード	0.1	—
脱脂粉乳	—	2
加水	40	23

【0022】（生地作成）縦型ミキサー（関東ミキサー20コート）、フックを用いて中種配合材料をボールに入れ、低速2分、中速1分で混捏し、捏上温度を24℃

として中種生地を調製した。次に、これを醗酵（中種醗酵）させた。この時の条件は下記のとおりである。

中種醗酵温度 28℃
中種醗酵時間 4時間

【0023】次に、この中種醗酵生地に本捏配合材料を添加し、低速2分、中速2分、高速2分で混捏した後、油脂を添加し、更に低速2分、中速2分、高速3分で混捏し、本捏生地とした。この時の生地温度は約27.5℃であった。

【0024】次に、混捏でダメージを受けた生地を回復させるためにフロアタイムを15分とり、その後225gの生地に分割した。分割でダメージを受けた生地を回復させるために、ベンチタイムを20分とり、モルダーで整形した。整形物をブルマンのパン型にいれ醗酵（ホイロ）を行った。ホイロ終了時、生地をPHメーター（堀場PHメーター、D-14）で測定した。尚、ホイロの条件は下記のとおりである。

ホイロ温度 37℃
ホイロ相対湿度 80%
ホイロ時間 48分

【0025】このようにして調製したパン生地を、入口温度220℃、出口温度260℃のオープンで3.5分間焼成した。焼成後、20℃で2日間保存し、食パンのサンプルとした。

【0026】（比較例4）表1に示す食パン配合において、上白糖6重量部のかわりにブドウ糖2重量部、糖アルコール10重量部を用い、比較例3の油脂組成物を使用
食パンの評価結果

	ホイロ終了時の生地PH	クラストの色	クラスト厚(mm)	破断強度 (dyne/cm ²)	スライスくず量
実施例1	4.7	57.5	2.3	6.0X10 ⁶	
実施例2	4.7	57.0	2.3	6.2X10 ⁶	
実施例3	4.9	59.0	2.2	5.7X10 ⁶	
実施例4	5.0	59.0	2.2	5.8X10 ⁶	7.5g
比較例1	4.7	56.5	2.4	6.5X10 ⁶	
比較例2	5.3	51.0	3.0	7.6X10 ⁶	
比較例3	5.5	50.0	3.0	7.5X10 ⁶	12.9g
比較例4	5.6	52.0	3.0	7.5X10 ⁶	

【0033】

【発明の効果】表2の結果から明らかなように、本発明によれば、従来の方法に比べて著しくクラスト厚が薄く、また白くて軟らかいパンを得ることができ、また、

い、前記と同様にパンを製造し、前記と同様に評価した。

【0027】（クラスト色の測定）食パンの上部表面を色差計（ミノルタ社製、CHROMA METER CR-200）にて10箇所測定し、明度指数Lの平均値を求めた。尚、Lは大きいほど色が白く、明るいことを表す。

【0028】（クラスト厚の測定）食パンをスライサーにて2cmずつスライスし、オープン入口より6枚目、10枚目、14枚目の上部断面を実体顕微鏡（三菱化成（株）社製、マイクロウォッチャー）で観察し、画像処理によりクラスト厚（焼き色の付いた部分）の平均値を求めた。

【0029】（クラストの硬さ）食パンをスライサーにて4cmずつスライスし、食パン上部を直径0.3cmのプランジャーで0.5 mm/sec の速度でクリープメーター（山電（株）社製）にて5回ずつ破断試験し、破断強度の平均値を求めた。尚、破断強度の値が小さいものほど、クラストが軟らかいことを表す。

【0030】（スライスくずの量）焼成後のパンを室温で2時間放置した後、スライサーにて2cm厚で3斤棒を3本スライスしたときに出るくずの重量を測定し、評価した。

【0031】以上の各試験結果を、まとめて下記表2に示す。

【0032】

【表2】

従来のパン製造における、クラスト厚が厚くて硬いために、パンをスライスしたときのくずが多い、といった問題も解消しうるのである。

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(54)【発明の名称】 リパーゼによるエステル交換法

(57)【要約】

【目的】 位置特異性等を有するリパーゼの利点を有効に活用し、かつ無溶媒下で、高融点の油脂類などを効率的にエステル交換する方法を提供すること。

【構成】 溶融したエステル交換用有機原料に、実質的に無溶媒かつ無水条件下で、アルカリゲネス属に属する微生物から得られるリパーゼを、81〜130℃で作用させることを特徴とするエステル交換法。

【特許請求の範囲】

【請求項1】 溶融したエステル交換用有機原料に、実質的に無溶媒かつ無水条件下で、アルカリゲネス属に属する微生物から得られるリパーゼを、81～130℃で作用させることを特徴とするエステル交換法。

【請求項2】 リパーゼに作用させる温度が91～120℃である請求項1記載のエステル交換法。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、溶媒を使用しないでエステル交換を行う、リパーゼを用いたエステル交換法に関するものである。

【0002】

【従来の技術】エステル交換反応は、動植物油脂類の改質をはじめ、各種脂肪酸のエステル、糖エステルやステロイドの製造法として重要な技術である。このエステル交換反応の触媒として、油脂加水分解酵素であるリパーゼを用いることにより、化学反応に比べ副反応の抑制やエネルギーコストが低減でき、さらには触媒としてのリパーゼが天然物であることから安全性も高いという利点が得られている。また、その基質特異性や位置特異性により目的物を効率よく生産することができるという利点もある。ところが、リパーゼを用いてエステル交換反応を行う場合、室温ないし約70℃程度までの温和な条件下でしか反応が進行しないため、有機溶媒等の非存在下に例えば90℃を超えるような高融点の油脂類やワックス等をリパーゼを用いてエステル交換することは事実上不可能であった。アルカリゲネス属リパーゼについても同様であり、名糖産業社製リパーゼPLを用いたエステル合成に関する特許（特開昭62-104589号）においても、通常は20～60℃の温度範囲で反応を行うとしており、実施例では40℃でエステル合成反応を行っている。このように、従来は、高融点の油脂類やワックス等をエステル交換する際には化学反応による手段が用いられてきたため、反応がランダムに進み、所望の目的物、たとえば基質特異性、位置特異性を必要とするエステル交換反応物を高純度で得ることは困難であった。一方、リパーゼを用いるエステル交換反応では、100℃前後の高温反応は実質的に不可能であった。

【0003】

【発明が解決しようとする課題】本発明は、位置特異性等を有するリパーゼの利点を有効に活用し、かつ無溶媒下で、高融点の油脂類などを効率的にエステル交換する方法を提供することを目的とする。

【0004】

【課題を解決するための手段】本発明は、特定のリパーゼを用い、実質的に無溶媒かつ無水条件下で、エステル交換反応を81～130℃で行うと、上記課題を解決できるとの知見に基づいてなされたものである。すなわち、本発明は、溶融したエステル交換用有機原料に、実

質的に無溶媒かつ無水条件下で、アルカリゲネス属に属する微生物から得られるリパーゼを、81～130℃で作用させることを特徴とするエステル交換法を提供する。ここで、エステル交換とは、例えば、カルボン酸とアルコールとのエステルから選ばれる1種もしくは2種以上のエステル、あるいはこれにカルボン酸またはアルコールを共存させ、前記エステルのカルボン酸残基若しくはアルコール残基を交換することである。

【0005】上記カルボン酸としては、例えば、炭素数2～50の飽和または不飽和、直鎖状または側鎖状の脂肪酸一塩基酸いわゆる脂肪酸、脂肪酸二塩基酸および三塩基酸が好適なものとしてあげられる。かかる脂肪酸一塩基酸としては、酢酸、酪酸、カプリル酸、イソオクタリン酸、イソノナン酸、カプリン酸、ラウリン酸、パルミチン酸、パルミトオレイン酸、ヘプタデカン酸、ステアリン酸、イソステアリン酸、オレイン酸、エライジン酸、10-ヒドロキシステアリン酸、12-ヒドロキシステアリン酸、リシノール酸、リノール酸、リノレン酸、エルジン酸、ベヘン酸、アラキドン酸、エイコサペンタエン酸、ドコサヘキサエン酸、セロチン酸、モンタン酸、ノナコサン酸、メリシン酸、ペンタトリアコンタン酸、ヘキサトリアコンタン酸、ヘキサテトラコンタン酸等を例示でき、脂肪酸二塩基酸としてはコハク酸、リンゴ酸、フマル酸、マレイン酸、酒石酸、グルタル酸、アゼライン酸、セバシン酸、1,12-ドデカジカルボン酸等を例示でき、また脂肪酸三塩基酸としてはクエン酸等を例示できる。

【0006】また、アルコールとしては、例えば、炭素数1～50の飽和または不飽和、直鎖状または側鎖状の脂肪酸一価アルコール、及び二価～六価アルコールが好適なものとしてあげられる。これらのアルコール類の例として、メタノール、エタノール、イソプロピルアルコール、ヘキサノール、イソオクタノール、イソノナノール、ラウリルアルコール、セタノール、ステアリルアルコール、イソステアリルアルコール、オレイルアルコール、ベヘニルアルコール、オクタコサノール、米国パトロライト社製のユニリンアルコール425（平均分子量：510）、同ユニリンアルコール550（平均分子量：660）、同ユニリンアルコール700（平均分子量：850）等の一価アルコール、エチレングリコール、ジエチレングリコール、プロピレングリコール、ジプロピレングリコール、1,8-オクタンジオール、1,10-デカンジオール等の二価アルコール、グリセリン、ジグリセリン、トリグリセリン、ポリグリセリン、ペンタエリスリトール、ネオペンチルグリコール、トリメチロールエタン、トリメチロールプロパン、キシリトール、ソルビトール、マンニトール等の三価以上のアルコールを挙げることができる。さらに、アルコール類として、キシロース、フラクトース、ガラクトース、グルコサミン、グルコース、 β -メチルグルコサイド、

マルトース、ラフィノースなどの糖類、コレステロール、ラノステロール、テストステロン、アントドロスタンジオール、ナンドロロン、エルゴカルシフェノール、コレカルシフェノール、2, 2-ジヒドロエルゴステロール、ジモステロールなどのステロール類やスフィンゴシン、リグノセリールスフィンゴシンなどもあげられる。

【0007】かかるカルボン酸とアルコールのエステルは、動物、植物、魚介類、微生物、鉱物等の天然物から抽出し、あるいは常法によりエステル合成して得ることができる。本発明におけるエステルの好適例は油脂およびワックスである。本発明では、このうち、融点が81～130℃にあるものを使用するのが好ましいが、2種以上のエステル交換用有機原料を使用する場合には、それらの融点が81～130℃の範囲内でなくとも、該エステル交換用有機原料の混合物が81～130℃で溶融すればよい。特に好ましくは91～120℃である。本発明では前記のエステルを原料基質とし、これにアルカリゲネス属に属する微生物由来のリパーゼを添加して81～130℃、好ましくは91～120℃にてエステル交換反応させるものであり、エステルは単独あるいは混合して用いることができる。さらに必要に応じて前記したカルボン酸の1種あるいは2種以上、またはアルコールの1種あるいは2種以上を原料基質として前記エステルに共存させることもできる。

【0008】本発明において使用するリパーゼは、アルカリゲネス属に属する微生物の生産するリパーゼであって、実質的に無水条件下でかつ81～130℃でエステル交換活性を有するものであればいずれのものでも使用できる。このうち好ましいリパーゼは、アルカリゲネス *Esper* (*Alcaligenes* sp.) 菌株から得られるのリパーゼである。具体的には、特公昭58-36953号公報に記載され、微工研菌寄第2985号 (FERM P-3187: FERM BP-2985) として寄託されているリパーゼ生産菌名糖PL-266号が生産するリパーゼQ1 (名糖産業 (株) 製) である。このリパーゼはグリセリドの1位および3位に対して位置特異性を有し、アルカリ側に至適pHを有する胆汁賦活性リパーゼであり、特公昭58-36953号公報に記載の酵素的性質を有する。本発明では、アルカリゲネス *Esper* (*Alcaligenes* sp.) 菌株から得られる上記とは別のリパーゼPL (名糖産業 (株) 製) も使用することができる。本発明の方法により、エステル交換反応を行うには、次のようにすればよい。すなわち前記原料基質に水分を添加することなく、上記リパーゼを加え、溶媒の不存在下、81～130℃、好ましくは91～120℃、より好ましくは91～110℃、最も好ましくは95～100℃に加熱し、静置、攪拌または振とうして所定の時間、例えば、10分～100時間反応を行う。反応終了後、常法により反応物を回収、精製してエステル

交換反応物を得ることができる。

【0009】本発明では、エステル交換反応は実質的に無水の状態で反応を行うことが重要である。従来のリパーゼを用いるエステル交換反応におけるような、例えば反応中の含水率が0.1%程度もしくはそれ以上になると、本発明の方法では反応性が低下し、またトリグリセリドからジグリセリド、モノグリセリドを生成するような副反応を伴うので好ましくない。つまり、本発明では、もともと基質に溶解している不連続状態の水、酵素もしくは固定化酵素に含まれる僅かな自由水もしくは結合水を含めて水分含量を0.1%未満とするのが好ましい。系内水分がこの条件を越える恐れのある場合は、事前に有機原料や酵素の水分を除いて使用するのがよい。反応は、閉鎖系、開放系のいづれでも行えるが、系内初発水分は反応と共に蒸発もしくは一部のエステルの加水分解により消費され、序々に減少していくが、81℃～130℃の範囲でエステル交換反応が進行する限りにおいて1ppmであっても、本発明の実質的に無水条件の範囲に含まれる。

【0010】なお本発明において用いる酵素の形態としては、酵素粉末、又は珪藻土やイオン交換樹脂に固定した酵素があげられる。エステル交換反応を行う方法は、カラムに充填した酵素を使用する連続反応法や反応液中に酵素を分散させるバッチ法などを使用することができる。反応液中で分散状態となっているリパーゼ粒子を、精密膜濾過、遠心分離、不活性有機溶媒による洗浄等の方法で回収することができ、これを再び新たなエステル交換反応に供することができる。

【0011】

【発明の効果】本発明によれば、リパーゼの利点を有効に活用して高融点の油脂類などを効率的にエステル交換することができる。そして、本発明の方法では、溶媒を使用しないので、最終生成物の分離が良好で、簡易に高純度の生成物を得ることができる。次に実施例により本発明を説明する。

【0012】

【実施例】

実施例1

モンタンワックスの分解脂肪酸 (ヘキスト社製、商品名: Sワックス) のエチルエステル50g、カプリル酸/カプリン酸=75/25の中鎖脂肪酸トリグリセリド (目清製油 (株) 製、商品名: ODO) 50g、およびアルカリゲネス *Esper* (*Alcaligenes* sp.) 由来のリパーゼ (名糖産業 (株) 製、商品名: リパーゼQ1) 1gを、マグネチックスターラーを用い攪拌速度300rpmで攪拌してエステル交換反応を行った。反応温度はモンタンワックスの融点以上である95℃とし、7時間反応した。反応物のガスクロマトグラフィー分析の結果から、モンタンワックス脂肪酸の83%がトリグリセリドであるODO中へ導入されたことが確認された。このこ

とから、95℃という高温でリパーゼを用い、高融点脂質の一種であるワックスを無溶媒下に、実質的に無水状態でエステル交換反応できることがわかる。

【0013】実施例2

炭素数14の二塩基酸（日鉱共石（株）製、商品名：テトラデカンジオイックアシッド）20g、グリセリンとブタン酸のトリエステルであるトリブチリン（和光純薬（株）製、商品名：トリブチリン）80g、および実施例1で使用したリパーゼQ L 1gをマグネチックスターラーを用い、攪拌速度350rpmで攪拌してエステル交換反応を行った。反応温度は、前記二塩基酸を溶解させるため120℃とし、6、5時間反応した。反応物のガスクロマトグラフィー分析による結果から、前記長鎖二塩基酸の93%がトリブチリン中へ導入されていることが確認された。このことから、120℃という高温でリパーゼを用い、高融点脂質の一種である長鎖二塩基酸を無溶媒下に、実質的に無水状態でエステル交換できることがわかる。

【0014】実施例3

カルナウバロウ120g、グリセリン10gおよび実施例1で使用したリパーゼQ L 1gをマグネチックスターラーを用い攪拌速度300rpmでエステル交換反応を行った。反応温度はカルナウバロウの融点以上である90℃にて36時間反応した。反応物のガスクロマトグラフィー分析結果から、1-モノグリセリドおよび1,3-ジグリセリドの生成を確認した。このことから、90℃という高温で、リパーゼを用い、高融点ワックスを無溶

媒下に、実質的に無水状態で、しかも該リパーゼの位置特異性を維持してエステル交換できることが示された。

【0015】実施例4

実施例1で使用したリパーゼQ Lを常法により粒状ケイソウ土に固定化したもの（凍結乾燥品）80gを内径2cm、長さ15cmのガラス管に充填し、固定化リパーゼ充填カラムを調製した。カルナウバロウ20g、グリセリンと酢酸のトリエステルであるトリアセチン（和光純薬（株）製、商品名：トリアセチン）80gの混合物を毎時2、0mlカラムに流し、100℃にて連続的にエステル交換反応を行った。反応物のガスクロマトグラフィー分析の結果から、トリグリセリドであるトリアセチン中へのカルナウバロウ脂肪酸の導入が確認された。このことから、100℃という高温でリパーゼを用い、高融点脂質の一種であるワックスを無溶媒下に、実質的に無水状態で連続エステル交換反応できることがわかる。

【0016】比較例1

実施例1の原料に加水し、水分を1、0%とした。これを実施例1と同様の操作で反応を行い、反応物を分析した。その結果、加水分解に伴う副反応物を生成し、モンタンワックス分解脂肪酸残基を有するグリセリドの生成はほとんど認められず、酵素の熱安定性も低下した。

比較例2

実施例2において反応温度を50℃とした以外は同じ条件で実施例2を繰り返した。その結果、長鎖二塩基酸の15%しかトリブチリン中へ導入されなかった。

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(54) 【発明の名称】 3-メトキシベンジルチオ尿素誘導体及び該誘導体を含有する改善された脂質組成物

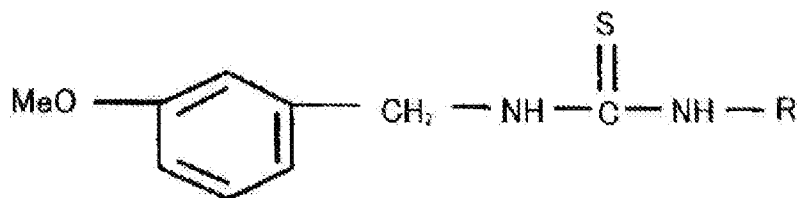
(57) 【要約】

1-(3-メトキシベンジル)-3-置換チオ尿素酸化防止化合物、及び基剤となる脂質の酸化安定度を向上させるのに有効量の前記の酸化防止化合物を補って改善した脂質組成物を提供する。さらに、酸化安定度を向上させる必要のある基剤となる脂質に、少なくとも一種の本発明の1-(3-メトキシベンジル)-3-置換チオ尿素化合物を添加することによって脂質の酸化安定度を向上させる方法を提供するものである。

【特許請求の範囲】

【請求項1】 次式の化合物。

【化1】



(式中、Rは炭素数1～20の直鎖又は分岐のアルキル基、炭素数5～7のシクロアルキル基、アルコキシ置換の炭素数5～7のシクロアルキル基、ヒドロキシ置換の炭素数5～7のシクロアルキル基、炭素数6～7のアリール基、ヒドロキシ置換の炭素数6～7のアリール基、又はアルコキシ置換の炭素数6～7のアリール基である。)

【請求項2】 Rが炭素数1～20の直鎖又は分岐のアルキル基である請求項1記載の化合物。

【請求項3】 Rが炭素数5～7のシクロアルキル基、アルコキシ置換の炭素数5～7のシクロアルキル基、又はヒドロキシ置換の炭素数5～7のシクロアルキル基である請求項1記載の化合物。

【請求項4】 Rが炭素数6～7のアリール基、ヒドロキシ置換の炭素数6～7のアリール基、又はアルコキシ置換の炭素数6～7のアリール基である請求項1記載の化合物。

【請求項5】 Rがメチル基、エチル基、プロピル基、イソプロピル基、ブチル基、イソブチル基、ターシャリブチル基、ペンチル基、2-メチルペンチル基、3-メチルペンチル基、ヘキシル基、オクチル基、デシル基、ノニル基又はドデシル基である請求項2記載の化合物。

【請求項6】 Rがシクロペンチル基、シクロヘキシル基又はシクロヘプチル基である請求項3記載の化合物。

【請求項7】 Rがフェニル基、ベンジル基、ヒドロキシフェニル基、ヒドロキシベンジル基、メトキシフェニル基、エトキシフェニル基、メトキシベン

ジル基又はエトキシベンジル基である請求項4記載の化合物。

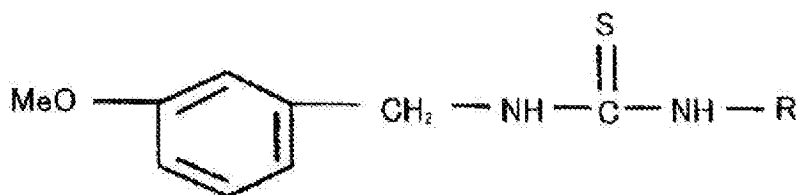
【請求項8】 Rが3-ヒドロキシ置換又は3-アルコキシ置換アリール基である請求項4記載の化合物。

【請求項9】 化合物が1, 3-ジ(3-メトキシベンジル)チオ尿素、1-(3-メトキシベンジル)-3-エチル-2-チオ尿素、1-(3-メトキシベンジル)-3-プロピル-2-チオ尿素、1-(3-メトキシベンジル)-3-ヘキシル-2-チオ尿素、1-(3-メトキシベンジル)-3-トデシル-2-チオ尿素、1-(3-メトキシベンジル)-3-(4-ヒドロキシフェニル)-2-チオ尿素、又は1-(3-メトキシベンジル)-3-(3-メトキシフェニル)-2-チオ尿素である請求項4記載の化合物。

【請求項10】 化合物が1, 3-ジ(3-メトキシベンジル)チオ尿素である請求項9記載の化合物。

【請求項11】 基剤となる脂質又はオイルに、酸化安定度が向上する量の次式の化合物が添加されている組成物。

【化2】



(式中、Rは炭素数1～20の直鎖又は分岐のアルキル基、炭素数5～7のシクロアルキル基、アルコキシ置換の炭素数5～7のシクロアルキル基、ヒドロキシ置換の炭素数5～7のシクロアルキル基、炭素数6～7のアリール基、ヒドロキシ置換の炭素数6～7のアリール基、又はアルコキシ置換の炭素数6～7のアリール基であり、組成物の酸化安定度が前記化合物を添加する前の基剤となる脂質又はオイルの酸化安定度よりも優れている。)

【請求項12】 Rが炭素数1～20の直鎖又は分岐のアルキル基である請求項11記載の組成物。

【請求項13】 Rが炭素数5～7のシクロアルキル基、アルコキシ置換

の炭素数5～7のシクロアルキル基、又はヒドロキシ置換の炭素数5～7のシクロアルキル基である請求項1記載の組成物。

【請求項14】 Rが炭素数6～7のアリール基、ヒドロキシ置換の炭素数6～7のアリール基、又はアルコキシ置換の炭素数6～7のアリール基である請求項1記載の組成物。

【請求項15】 基剤となる脂質又はオイルに、該脂質又はオイルの全重量を基準として約0.01重量%から約5.0重量%の前記化合物が添加されている請求項1記載の組成物。

【請求項16】 基剤となる脂質又はオイルに、約0.05重量%から約2.0重量%の化合物が添加されている請求項15記載の組成物。

【請求項17】 基剤となる脂質又はオイルに、約0.1重量%から約1.0重量%の化合物が添加されている請求項16記載の組成物。

【請求項18】 化合物が1, 3-ジ(3-メトキシベンジル)チオ尿素、1-((3-メトキシベンジル)-3-エチル-2-チオ尿素、1-((3-メトキシベンジル)-3-プロピル-2-チオ尿素、1-((3-メトキシベンジル)-3-ヘキシル-2-チオ尿素、1-((3-メトキシベンジル)-3-ドデシル-2-チオ尿素、1-((3-メトキシベンジル)-3-(4-ヒドロキシフェニル)-2-チオ尿素、又は1-((3-メトキシベンジル)-3-(3-メトキシフェニル)-2-チオ尿素である請求項14記載の組成物。

【請求項19】 化合物が1, 3-ジ(3-メトキシベンジル)チオ尿素である請求項18記載の組成物。

【請求項20】 基剤となる脂質又はオイルが種子油又は植物油である請求項1記載の組成物。

【請求項21】 基剤となる脂質又はオイルがメドウフォーム油、ピーナツ油、コーン油、綿実油、ペニバナ油、大豆油、高オレイン酸ヒマワリ油、トウワタ種子油、菜種油、パーム油、オリーブ油、ホホバワックスエステル、ホホバ油、レシチン又は他の植物油である請求項1記載の組成物。

【請求項22】 基剤となる脂質又はオイルがホホバ油、メドウフォーム油、高オレイン酸ヒマワリ油、大豆油又はトウワタ種子油であって、基剤となる

脂質又はオイルに前記化合物が約0.1重量%から約1.0重量%添加されている請求項2.1記載の組成物。

【請求項2.3】 基剤となる脂質又はオイルが一種以上のベンジルアミン又はN-置換ベンジルアミン化合物を含有する請求項1.1記載の組成物。

【請求項2.4】 基剤となる脂質又はオイルがメドウフォーム種子油である請求項2.3記載の組成物。

【請求項2.5】 基剤となる脂質又はオイルに、酸化安定度を向上させる量の一種以上のベンジルアミン又はN-置換ベンジルアミン化合物がさらに添加されている請求項1.1記載の組成物。

【請求項2.6】 酸化安定度指数テストを約110℃から約130℃の温度で行った際、組成物の酸化安定度指数が、前記化合物を添加する前の基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約10%高い請求項1.1記載の組成物。

【請求項2.7】 組成物の酸化安定度指数が、基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約100%高い請求項2.6記載の組成物。

【請求項2.8】 組成物の酸化安定度指数が、基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約200%高い請求項2.7記載の組成物。

【請求項2.9】 組成物の酸化安定度指数が、基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約500%高い請求項2.8記載の組成物。

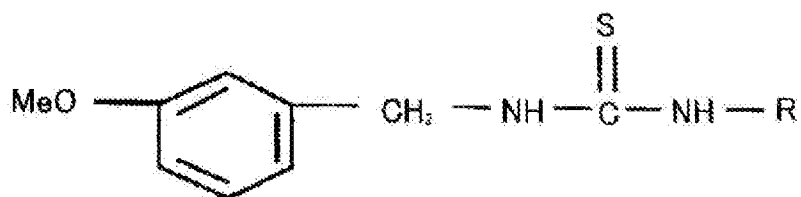
【請求項3.0】 組成物の酸化安定度指数が、基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約800%高い請求項2.9記載の組成物。

【請求項3.1】 組成物の酸化安定度指数が、基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約1000%高い請求項3.0記載の組成物。

【請求項3.2】 組成物の酸化安定度指数が、基剤となる脂質又はオイルの酸化安定度指数よりも少なくとも約1500%高い請求項3.1記載の組成物。

【請求項3.3】 基剤となる脂質又はオイルに酸化安定度を向上させる量の次式の化合物を組合せる工程を有することを特徴とする基剤となる脂質又はオイルの酸化安定度を向上させる方法。

【化3】



(式中、Rは炭素数1～20の直鎖又は分岐のアルキル基、炭素数5～7のシクロアルキル基、アルコキシ置換の炭素数5～7のシクロアルキル基、ヒドロキシ置換の炭素数5～7のシクロアルキル基、炭素数5～7のアリール基、ヒドロキシ置換の炭素数6～7のアリール基、又はアルコキシ置換の炭素数6～7のアリール基である。)

【請求項34】 基剤となる脂質又はオイルに、約0.05重量%から約2.0重量%の前記化合物が組合されている請求項33記載の方法。

【請求項35】 前記化合物が1, 3-ジ(3-メトキシベンジル)チオ尿素、1-(3-メトキシベンジル)-3-エチル-2-チオ尿素、1-(3-メトキシベンジル)-3-プロピル-2-チオ尿素、1-(3-メトキシベンジル)-3-ヘキシル-2-チオ尿素、1-(3-メトキシベンジル)-3-ドデシル-2-チオ尿素、1-(3-メトキシベンジル)-3-(4-ヒドロキシフェニル)-2-チオ尿素、又は1-(3-メトキシベンジル)-3-(3-メトキシフェニル)-2-チオ尿素である請求項34記載の方法。

【請求項36】 前記化合物が1, 3-ジ(3-メトキシベンジル)チオ尿素である請求項35記載の方法。

【発明の詳細な説明】

【発明の属する技術分野】

【0001】

本発明は新規な1-（3-メトキシベンジル）-3-置換チオ尿素化合物に関するものであり、又、この化合物を添加した酸化安定度の向上した脂質及びオイル組成物に関するものである。

【発明の背景】

【0002】

天然の脂質及びオイル類は、医薬品、食品、化粧品、及び潤滑剤、塗料、インキ、ペイント、プラスチック等の様々な工業用品に用いられている。このような脂質類は色、臭い、粘度及び潤滑性に作用しうる酸化崩壊を受けやすく、脂質を含有している商品の品質に悪影響を及ぼすことになる。食品、化粧品及び医薬業界においては、脂質又はオイルの不飽和度や含有されている天然又は合成の酸化防止剤の量だけでなく、酸素濃度、光及び熱といった要因の影響を受ける酸化による酸敗を避け、オイルや他の脂質の高品質な色や臭いをそのまま保つことが重要となる。切削用潤滑剤として使用される生分解性の脂質、オイル及びそれらの誘導体は熱による酸化の悪影響を受けることがわかっている。

メドウフォーム（リムナンテス *Limnanthus alba*）の種子油が酸化に対して非常に安定していることが立証されてきた。メドウフォーム油の類まれな酸化安定度に含有している化合物が何であるかは今のところまだわからないが、メドウフォーム油と他の油類とを混合することによって、その混合物の酸化安定度は改善される（Isbell, T. A., Abbott, T. A. and Carlson, K. D. 1999, Ind. Corps Prod. 9 (2) : 115-123）。しかしながら、メドウフォーム油中の酸化安定度を漸減させるか、わずかに上昇させるいくつかの微量成分については既知である（Abbott, T. P. and Isbell, T. A. 1998, 米国油化学会第80回大会アブストラクト; Chicago, IL, 1998年5月10-13日, 66頁）。精製されたメドウフォーム油（及び他の精製された種子油や植物油）から、精製プロセスによって酸化安定度が低下することがわかる。メドウフォームには、

3-メトキシフェニルアセトニトリル、3-メトキシベンジルイソチオシアネート及び3-メトキシベンズアルデヒドが含有されていることは知られている。これらの化合物が精製後のメドウフォーム油に対して約0.1%から1.0%の量比で添加されても、これらの化合物が示す酸化防止効果は、せいぜい低から中程度である。

【0003】

油類においてチオ尿素が酸化防止作用を有することが明らかにされたが（堀本、村上、日本栄養食糧学会誌51（4）：207-212、1998年；Chemical Abstract 129：188538）、チオ尿素は油にほとんど溶解しない。エステル系合成潤滑油（即ち、植物油ではないもの）をアミン系酸化防止剤で安定化させた場合、その潤滑油の酸化安定度は特定のチオ尿素によって向上していることがわかった（Chao and Kjønaas Amer. Chem. Soc. Preprints, Div. Pet. Chem. 27（2）：362-379、1982）。Camenzind及びRolfの欧州特許出願EP91-810474、ケミカルアブストラクト117：30273には、特定のアルキル化されたチオ尿素が潤滑剤や作動液の酸化安定度を向上させることが示されている。

また、モノー及びジー置換チオ尿素化合物も米国特許2154341、2662096、3852348及び3991008に記載されている。Phytochem. 16（11）：1719-1721、（1977）には、Migirah等がN、N'-ビス〔（4-メトキシフェニル）メチル〕-チオ尿素（CAS #22313-70-8）、これは西アフリカイチゴ（P.Brazzeana）から単離されるものであるが、このようなメトキシ置換の芳香族チオ尿素類を開示している。

【0004】

【発明が解決しようとする課題】

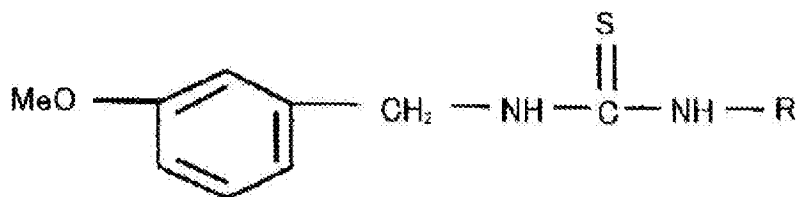
酸化防止化合物及び組成物が必要とされているが、とりわけ天然の酸化防止剤又はそれらの誘導体であって、脂質やオイルに溶解し低濃度の添加でも脂質やオイルの酸化安定度を向上させることができるものが必要とされている。

【課題を解決するための手段】

本発明者らは、式1の化合物が脂質やオイル類に優れた酸化安定度をもたらすことを予断せずして発見した。

【0005】

【化4】



(式中、Rはメチル基、エチル基、プロピル基、イソプロピル基、ブチル基、イソブチル基、ターシャリブチル基、ペンチル基、ヘキシル基、オクチル基、デシル基、ノニル基、ドデシル基等の炭素数1～20の直鎖又は分岐のアルキル基；シクロペンチル基、シクロヘキシル基、又はシクロヘプチル基等の炭素数5～7のシクロアルキル基；フェニル基又はベンジル基等の炭素数6～7のアリール基；或いはヒドロキシフェニル基、メトキシフェニル基、エトキシフェニル基、ヒドロキシベンジル基、メトキシベンジル基、エトキシベンジル基のようなヒドロキシ又はアルコキシ置換の炭素数6～7のアリール基である。)式1の化合物のなかで、1, 3-ジ(3-メトキシベンジル)チオ尿素が目下のところ好ましいが、これは上記式1においてRが3-メトキシベンジル基となる化合物である。脂質又はオイル(或いは脂質又はオイルを含有した組成物)に酸化安定度を与えるのに十分な式1の化合物の量は、脂質又はオイルの全重量基準で約0.01重量%から約5.0重量%である。

また本発明は、約9.5重量%から約99.99重量%の基剤となる脂質又はオイルと、約0.01重量%から約5.0重量%、好ましくは約0.05重量%から2.0重量%、さらに好ましくは約0.1重量%から1.0重量%の式1の化合物とを含有する酸化安定性のある脂質組成物を提供する。式1の置換チオ尿素化合物を基剤となる脂質又はオイル組成物の全重量の約3重量%から約5重量%又はそれ以上含有する本発明の脂質又はオイル類は「濃縮物」として用いること

ができ、酸化安定度の向上を必要とする加工種子油又は他の脂質に適宜添加され、本発明の脂質又はオイル組成物を提供する。

【0006】

さらに本発明は、酸化安定度の向上を必要とする基剤となる脂質又はオイル組成物に酸化安定度をもたらす方法を提供するもので、その方法とは、式Iの化合物を基剤となる脂質又はオイルの酸化安定度が向上するのに十分な量添加する工程からなる。

式Iの化合物で現在好ましいとされるのは、1, 3-ジ(3-メトキシベンジル)チオ尿素、1-(3-メトキシベンジル)-3-エチル-2-チオ尿素、1-(3-メトキシベンジル)-3-プロピル-2-チオ尿素、1-(3-メトキシベンジル)-3-ヘキシル-2-チオ尿素、1-(3-メトキシベンジル)-3-ドデシル-2-チオ尿素、1-(3-メトキシベンジル)-3-(4-ヒドロキシフェニル)-2-チオ尿素、及び1-(3-メトキシベンジル)-3-(3-メトキシフェニル)-2-チオ尿素である。1, 3-ジ(3-メトキシベンジル)チオ尿素については、本発明者らはメドウフォーム種子油に含まれる重要な天然の酸化防止剤であると確認しており、これは本発明の化合物のなかでもとりわけ好ましい。

また驚いたことに、式Iの化合物は、N-置換ベンジルアミンのようなベンジルアミン化合物と一緒にすると脂質やオイル類中で相乗的に酸化安定度向上効果を示すこともわかった。種子油や植物油のような様々な天然に存在する脂質やオイル類はベンジルアミン化合物を含む。このような場合、基剤となる脂質又はオイルに式Iの化合物を補い、任意で酸化安定度をさらに向上させるに足る量のベンジルアミンを外から補うことによってその相乗効果が得られるであろう。従って、本発明のもう一つの態様として、酸化安定度を高めることを目的に、(1)式Iの化合物と(11)ベンジルアミン又はN-置換ベンジルアミン化合物とを含む脂質組成物を必然的に提供することになる。

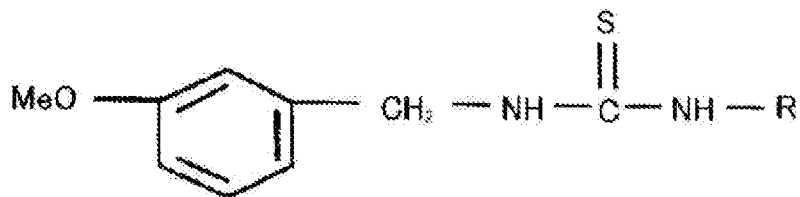
【0007】

【発明の実施の形態】

本発明の態様の一つとして、次式の1-(3-メトキシベンジル)-3-置換

チオ尿素化合物を提供する。

【化5】

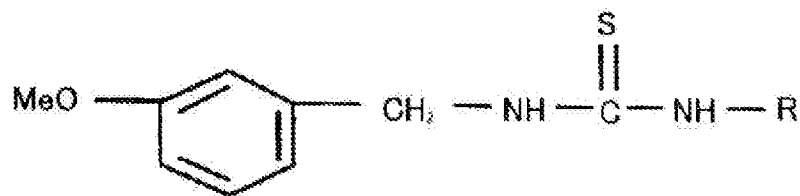


(式中、Rは炭素数1～20の直鎖又は分岐のアルキル基、炭素数5～7のシクロアルキル基、アルコキシ置換の炭素数5～7のシクロアルキル基、ヒドロキシ置換の炭素数5～7のシクロアルキル基、炭素数6～7のアリール基、ヒドロキシ置換の炭素数6～7のアリール基、及びアルコキシ置換の炭素数6～7のアリール基から選ばれる。) とりわけ好ましい実施態様としては、置換基を有するアリール基が、3-ヒドロキシ置換アリール化合物又は3-アルコキシ置換アリール化合物である。

本発明の別の態様によると、約9.5重量%から約99.99重量%の基剤となる脂質と、約0.01重量%から約5.0重量%、好ましくは約0.05重量%から2.0重量%、さらに好ましくは約0.1重量%から約1.0重量%の次式の1-(3-メトキシベンジル)-3-置換チオ尿素化合物とを含有する酸化安定度の改善された脂質組成物を提供する。

【0008】

【化6】



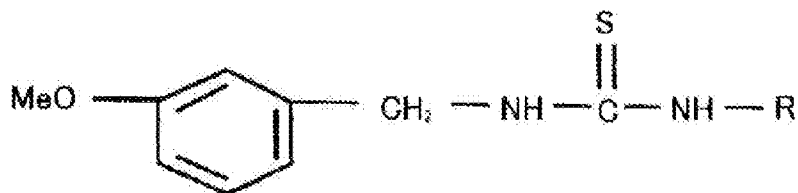
(式中、Rは炭素数1～20の直鎖又は分岐のアルキル基、炭素数5～7のシクロアルキル基、ヒドロキシ又はアルコキシ置換の炭素数5～7のシクロアルキル基、炭素数6～7のアリール基、及びヒドロキシ又はアルコキシ置換の炭素数6

～7のアリール基から選ばれる。)

さらに別の態様によると、本発明は脂質に酸化安定度が向上する量の式Iの化合物を混合するステップを有する脂質の酸化安定度を増強する方法を提供する。

【0009】

【化7】



(式中、Rは炭素数1～20の直鎖又は分岐のアルキル基、炭素数5～7のシクロアルキル基、ヒドロキシ又はアルコキシ置換の炭素数5～7のシクロアルキル基、炭素数6～7のアリール基、及びヒドロキシ又はアルコキシ置換の炭素数6～7のアリール基から選ばれる。)

【0010】

本発明の化合物は、基本的に、その化合物が溶解し脂質の酸化安定度を向上させることができるならば、いかなる脂質に添加してもよい。ここで使われている「脂質」ということばは、植物油、種子油、トリグリセリド、トリグリセリドのワックス類及びリン脂質を含む。優れた酸化安定度を与えるために本発明の化合物を添加できる脂質には、植物油、ピーナッツ油、コーン油、綿実油、パーム油、大豆油、菜種油（カノーラ油）、バーム油、オリーブ油、ホホバワックスエステル、及びレシチンがある。ここで使われている「基剤となる脂質」、「基剤となるオイル」又はそれに相当する用語は、式Iの化合物が外的に添加される前の脂質又はオイルを意味する。

ここで使用されているように、「炭素数1～20直鎖又は分岐のアルキル基」にはメチル基、エチル基、プロピル基、イソプロピル基、ブチル基、イソブチル基、ターシャリブチル基、ペンチル基、2-メチルペンチル基、3-メチルペンチル基、ヘキシル基、オクチル基、デシル基、ドデシル基等が含まれる。「炭素数5～7のシクロアルキル基」には、シクロペンチル基、シクロヘキシル基

及びシクロヘプチル基が含まれる。「ヒドロキシ置換又はアルコキシ置換の炭素数5〜7のシクロアルキル基」には、ヒドロキシル基、メトキシ基、エトキシ基又はプロポキシ基等で置換されたシクロペンチル基、シクロヘキシル基、及びシクロヘプチル基が含まれる。「炭素数6〜7のアリール基」にはフェニル基とベンジル基が含まれる。「ヒドロキシ置換又はアルコキシ置換の炭素数6〜7のアリール基」には、ヒドロキシル基、メトキシ基、エトキシ基又はプロポキシ基等で置換されたフェニル基及びベンジル基が含まれる。

【0011】

「すぐれた酸化安定度」、「向上した酸化安定度」又はこれに相当する用語の使用は、本発明の脂質組成物が基剤となる脂質又はオイル（即ち、式Iの化合物を外から加えていないもの）と比較した際、明細書に開示した酸化安定度指数（OSI）の測定による酸化を抑える能力が高まったことを意味している。OSIテストを約110℃から約130℃の温度で行った場合、本発明の脂質又はオイル組成物のOSI値は、比較すべき基剤となる脂質又はオイルのOSI値よりも少なくとも10%以上大きいことが好ましく、さらに好ましくは少なくとも100%以上、また少なくとも約200%以上大きいことがもっとも好ましい。

【0012】

式Iの化合物は、3-メトキシベンジルアミンと式IIの $S=C=N-R$ で表されるイソチオシアネート化合物から適宜選ばれたものを反応させることによって合成される。式中、Rは式Iの化合物で定義したものと同一である。この反応は、好ましくは窒素雰囲気下でイソチオシアネートを3-メトキシベンジルアミン水溶液に徐々に添加しておこなえばよい。反応によるチオ尿素生成物が式Iの化合物であるが、水とは混和しないがチオ尿素の溶媒となるような溶媒、例えば、塩化メチレン、クロロフォルム、トルエン又はジエチルエーテルを混合することによって生成物を精製し回収することができる。本発明のチオ尿素化合物の分離、回収を向上させるために水層を酸性化してもよいし、しなくてもよい。チオ尿素は溶媒層の方に溶解しているが、水層から分離させ乾燥し、それによって得た粗製物であるチオ尿素をエタノールのような適当な溶媒で再結晶させて精製する。実施例1、及び、概要についてはMoore and Crossleyの

「Organic Synthesis」2巻617-618（注4）の手順を参照できる。

【0013】

反応物である上記に定義した3-メトキシベンジルアミンと適切なイソチオシアネート化合物は市販のものから入手することもできるし、この分野では既知の通常の方法によって合成することもできる。結果として生成された式1の化合物は、この分野の当業者には周知の通常の方法によって容易に単離できる。本発明の化合物を合成するのに適したイソチオシアネート反応物は、この分野ではよく知られているように、種子油や他の脂質に含まれているグルコシノレート分解することによって得られる。チオグルコシダーゼ酵素含有の水溶液中でグルコシノレート化合物は分解され、イソチオシアネート及び他の分解物が得られる。Vaughn等のJ. Chem. Ecol. 22, 1939-49 (1996)；M. Rechcigl (編) の「Naturally Occurring Food Toxicants」CRC Press, Boca Raton, FL のC. VanEtten and H. Tooke (1983) Glucosinolates 15-30を参照できる。グルコシノレートの分解物の一部であるイソチオシアネートをこうして単離し、上記3-メトキシベンジルアミンと反応させて本発明の化合物を得ることができる。およそ100種のグルコシノレート化合物が、カラシ、ナタネ、キャベツ、アリアリア、及びハマナを含む11種にわたる異なる科の植物から発見されている（S. F. Vaughn, 1999, Glucosinolates as Natural Pesticides in Biologically Active Natural Products: Agrochemicals, H. G. Cutler and S. J. Cutler, Eds. CRC Press, Boca Raton, FL.）。グルコシノレート含有植物から単離されたオイル類は、通常、蒸気噴霧によって臭気を取り除きイソチオシアネート及びアミンを含んだ揮発性化合物が取り除かれる。様々なイソチオシアネート化合物及びベンジルアミン化合物は、このように油を精製するプロセスで生じた蒸留廃棄物から得ることができ、本発明の化合物を合成する際の反応物として供される。

【0014】

3-メトキシベンジルアミンは市場で購入することができる。又は、メドウフ
 ォーム油から不混和性の酸性化させた水性層に抽出して単離し、水性層を油から
 分離し非極性溶媒で洗浄し、塩基でpH値を下げ、アミンを不混和性溶媒に抽出
 させればよい。この3-メトキシベンジルアミン化合物は、エタノール又は同様
 な溶媒からの再結晶による精製及び／又は逆相カラム（C18）を使った高速液
 体クロマトグラフィー（HPLC）によって濃度勾配を最初は100%メタノー
 ルから始めメタノール約80%、クロロフォルム20%まですすめて分離溶出し
 て精製してもよい。3-メトキシベンジルアミンを含有するピークは、HPLC
 カラムでの保持時間を既知の3-メトキシベンジルアミン標準試料の保持時間と
 比較することで同定できる。他の天然アミン類についても、市場で購入してもよ
 いし、或いは天然原料から上記と同様に抽出し既知の標準試料を参照して精製、
 及び／又はアミン同定の標準的な化学的方法（例えば、クロマトグラフィー、赤
 外分光、質量分析、元素分析、核磁気共鳴分析等）によって同定すればよい。

【0015】

脂質の酸化安定度については、本発明の化合物が添加されている場合も添加さ
 れていない場合も文献記載の手順によって求めればよい。例えば、K. Tian
 and P. Dasgupta, Anal. Chem. 71, 1692-98
 (1999)を参照する。脂質及びオイル類の好ましい酸化安定度測定法として
 は酸化安定度指数(OSI)を採用するが、これは厳しい温度管理のもとサンプ
 ルに空気を通過させることによってオイルの酸化安定度を求めるものである。（
 Firestone, Oxidative Stability Index (OSI): Official Methods of Recommended
 Practices of the American Oil Chemi
 sts' Society, 4版、米国油化学会、Champaign IL, C
 d 128-92）。この方法では、オイルサンプルに空気を流しこむとトリグリ
 セライドがすばやく分解し揮発性の有機酸になる。気流の勢いでオイルから酸化
 した酸は水を満たした伝導度セルに送り込まれ、酸がセル中で溶解する。これら
 の酸は一旦は水溶液に溶解するが、その後イオンに解離するので水の伝導度が変

化する。そのため、セルの伝導度をコンピュータで連続的に測定すれば、伝導度が急速に上昇する時がわかり、これが誘導点でありサンプルの酸化劣化に相当する。誘導点にいたる時間がOSI時間である。AOC S標準方法が近年開発され共同研究も発表され(Jebs等、J. Am. Oil Chem. Soc. 70, 1055-61 (1993))、OSI方法によるとサンプル間や実験室をこえて再現性がよいことが確認されている。Alltech Associate社(Derfield, IL)の飽和脂肪酸メチルエステル(FAME)標準品をOSI測定値を校正する際に使用するとよい。

OSI測定は、Omnion社(Rockland, MA)製の酸化安定度測定器で上記に開示したFirestoneの参考文献に記載されているAOC S法に従って行えばよい。気流を35 kPaに調整し最終的な速度を約140 ml/分として、脂質又はオイルのサンプルを110℃で動作させ、FAMEは90℃で試験に供すればよい。OSI値を求める好ましい方法については、T. A. Isbell等、Industrial Crops and Products 9, 115-123 (1999)に記載されている。

【0016】

式Iの化合物は適当な脂質又はオイルと一般的方法で混合し、3.0～5.0%又はそれ以上の濃度で溶解させるとよい。現在のところ、式Iの化合物の濃度が約0.1～1.0%であれば、酸化安定度の向上を必要とする基剤となる脂質又はオイルの酸化安定度を約2倍から10倍ひきあげるのに十分であると考察されている。しかしながら、式Iの化合物を3.0～5.0%まで含有している本発明の脂質又はオイルは、酸化安定度の向上を必要とする基剤となる脂質で30～50倍以上に適宜希釈することができる「濃縮物」として有用なのである。このような濃縮物の基本組成はそれ自体が種子油、植物油又は食用溶剤のような脂質又はオイルであってよい。さらには、式Iの化合物は、任意に3-メトキシベンジルアミンのようなアミン化合物と一緒に水中油形又は油中水形乳濁液等にしてもよい。カノーラ油、大豆油、植物油エストライト(castolene)のような生分解性の切削用潤滑剤、又はその他の切削用潤滑剤の酸化安定度を向上させるためには、上記の乳濁液にして添加することがとりわけ有用であると考えられる。

本発明の脂質又はオイル組成物の好ましい実施態様としては、基剤となる脂質又はオイルに式1の化合物を有効量、即ち、約0.1%～1.0%の濃度で添加するだけでなく、式1の化合物によって与えられる酸化安定度を増強させるに十分な量のベンジルアミン化合物も添加する。本発明の脂質又はオイル組成物に添加されるベンジルアミン化合物の量は、OSI値の増加を添加されたベンジルアミン化合物の量の関数として観察しながら求めればよい。基剤となる脂質又はオイルは生得的にアミン化合物を含有していてもよいが、本発明の脂質又はオイル組成物の酸化安定度の向上を目的に式1の化合物がすでに添加されている脂質又はオイルに、アミン化合物、好ましくは3-メトキシベンジルアミンをさらに加えてもよい。相乗的な酸化防止効果を達成するために本発明の脂質又はオイル組成物に添加する上記のアミン化合物の量は、式1の化合物を含有する脂質又はオイル組成物を等分に分けたものにアミン化合物をそれぞれ所定の量添加し得られるOSI値の増加を測定しながら実験的に求めればよい。

以下の実施例は本発明を限定するものではなく、本発明の化合物の調製を記載したものである。特に記されていなければ、パーセントはすべて重量パーセント（wt%）を意味する。

【0017】

実施例1

本実施例は1,3-ジ（3-メトキシベンジル）チオ尿素の合成を明示する。冷却器、ゴム製シリンジセプタム（syringe septum）及び窒素導入管を装着した100mLの三口フラスコに20mLの水と3.6g（2.5,8mmol）の3-メトキシベンジルアミンを加えた。反応器の中を窒素でバージし、テフロン（登録商標）被覆の電磁攪拌機で攪拌した。2.59mL（3.0g、16.7mmol）の3-メトキシベンジルイソチオシアネートをガラス製のシリンジから滴下（一滴を5～10秒）した。分離層が形成され混合物を室温で一時間攪拌した。水層を1Mの塩酸（約10mL）を使ってpH3.5の酸性にした。塩化メチレン（15mL）を加え、二相を分液漏斗に移した。下の層（塩化メチレン）を取り除いた。水層を塩化メチレンで洗浄し、10mLの塩化メチレンでさらに二度洗浄し、できたCHCl₃溶液を0.1Mの塩酸及び水で順次洗浄した。C

HCl溶液をモレキュラシーブ(3A)で乾燥し、回転式溶媒蒸発装置で蒸発乾固した。得られた粘ちょう液体を35℃に加熱した20mlのエタノール中に入れ、冷却器のなかで冷却しながらエタノールから三回再結晶させ、白い結晶として得たものを室温で真空乾燥し重さを量った。母液からも再結晶を行い追加生成物を回収し、最初の結晶バッチでは79.8%の収率で、また結晶バッチを合わせると83.2%の収率だった。NMR、質量分析及び元素分析によりこの生成物が1,3-ジ(3-メトキシベンジル)チオ尿素であることがわかった。

【0018】

実施例2

ホホバ油(ホホバの種子からヘキサンで抽出したもの)は、炭素数20及び22の一価不飽和酸とアルコールが一緒になってエステル化したワックスエステルである。ホホバ油(20g)を実施例1で調製した1,3-ジ(3-メトキシベンジル)チオ尿素20mg(0.1%)と混合した。この混合物の5gのサンプルに対して110℃で酸化安定度試験を反復し(3回)、OSI時間が64.2時間であることが判明したが、これはホホバ油のみのOSI時間である49.2時間に比べて30%の向上である。OSI時間とは、一定の速度でオイル内に発生する酸素の泡がオイルを分解し検出可能な酸化生成物を生成するまでの時間をさす。実施例1の1,3-ジ(3-メトキシベンジル)チオ尿素生成物を1%の濃度で添加した際、OSI時間は168時間まで伸びホホバ油のみの場合と比べて241%向上した。

【0019】

実施例3

精製されたメドウフォーム油は高度単不飽和の植物油であり、その酸化安定度は精製プロセスにおいて減退する。メドウフォーム油(Lot #C-9773, The Panning社製)20gを実施例1で調製した1,3-ジ(3-メトキシベンジル)チオ尿素20mg(0.1%)と混合した。この混合物の5gのサンプルに対して110℃で酸化安定度試験を反復し(3回)、OSI時間が76.4時間であることが判明したが、これはメドウフォーム油のみのOSI時間である66.3時間に比べて15%の向上である。実施例1の1,3-ジ(3-

ーメトキシベンジル)チオ尿素生成物を1%の濃度で添加した際、OSI時間は211時間まで伸び218%向上した。

【0020】

実施例4

実施例1で調製した1,3-ジ(3-メトキシベンジル)チオ尿素を0.1%、0.5%又は1.0%の割合で精製されたメドウフォーム油(Lot #CW-4551, The Fanning社製)と混合し、できた混合物の酸化安定度と精製メドウフォーム油の酸化安定度を130℃で比較した。上記ロットの添加物を含まない精製メドウフォーム油のOSI時間は130℃で14.9時間であるのに対し、チオ尿素含有の混合物のOSI時間はそれぞれの40.8時間、159時間、172時間であった。従って、精製メドウフォーム油に1,3-ジ-3-メトキシベンジルチオ尿素を0.1%、0.5%及び1.0%の割合で添加することによって、OSI時間がそれぞれ234%、987%及び1054%のびた。

【0021】

実施例5

高オレイン酸ヒマワリ油は高度単不飽和植物油である。高オレイン酸ヒマワリ油(Florasun Brand, Floratech, Gilbert, AZ)20gを実施例1で調製した1,3-ジ(3-メトキシベンジル)チオ尿素200mg(1.0%)と混合した。この混合物の5gのサンプルに対して130℃で酸化安定度試験を反復し(3回)、OSI時間が15.7時間であることが判明したが、これはヒマワリ油のみのOSI時間である9.13時間に比べて72.0%の向上である。

【0022】

実施例6

市販の3-メトキシベンジル基をもたない2基置換のチオ尿素は、単不飽和油に対して酸化防止効果が低い。そこで、精製メドウフォーム油に1,3-ビス(2-メトキシフェニル)-2-チオ尿素(Aldrich Chem社製)を0.1%の割合で添加したところ、130℃で37.1時間というOSI時間であ

り、これは実施例1で生成されたチオ尿素の場合より3.4%低い値である。同様に、精製メドウフォーム油に1, 3-ビス(3-メトキシフェニル)-2-チオ尿素を0.1%の割合で添加したところ、OSI時間は27.0時間であり、これは実施例1で生成されたチオ尿素の場合より8.4%低い値である。

【0023】

実施例7

大豆油は高度多価不飽和植物油である。大豆油(20g)を実施例1で調製した1, 3-ジ(3-メトキシベンジル)チオ尿素200mg(1.0%)と混合した。この混合物の5gのサンプルに対して130℃で酸化安定度試験を反復し(3回)、OSI時間が6.4時間であることが判明したが、これは大豆油のみのOSI時間である0.77時間に比べて83.1%の向上である。

【0024】

実施例8

トウワタ種子油は高度多価不飽和植物油である。トウワタ種子油(ヘキサンで種子から抽出したもの)20gを実施例1で調製したチオ尿素200mg(1.0%)と混合した。この混合物の5gのサンプルに対して130℃で酸化安定度試験を反復し(3回)、OSI時間が2.78時間であることが判明したが、これはトウワタ種子油のみのOSI時間である0.42時間に比べて65.4%の向上である。

【0025】

実施例9

粗製のメドウフォーム油と精製メドウフォーム油を高速液体クロマトグラフィー(hplc)で分析したところ、粗製物のほうは保持時間9.5分でピークがあらわれたが、精製油のほうはあらわれなかった。上記実施例1で合成された化合物の保持時間は、濃度をかえると9.575から9.613であった。また、粗製のメドウフォーム油をアセトニトリル溶媒で抽出したものを高速液体クロマトグラフィーにかけると各成分に分離され、その中の一つの成分が質量分析、NMR及び元素分析によって1, 3-ジ(3-メトキシベンジル)尿素と同定されたが、これは請求項1の合成されたチオ尿素が酸化された形である。従って、1

、3-ジ（3-メトキシベンジル）チオ尿素は、精製メドウフォーム油ではなく、種子から抽出した時の粗製のメドウフォーム油に含まれる天然成分であることがわかる。

【國際調查報告】

INTERNATIONAL SEARCH REPORT

Int. Appl. No.
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A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7	A61K7/06	A61K7/48 C11B5/00 C07C335/12 C07C335/14
According to International Patent Classification (IPC) or to both national classification and IPC		
B. RELEVANT SEARCHES		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 7 A61K C11B C07C		
Documentation searched other than minimum documentation to the extent that each document is included in the lists referred to		
Electronic data base consulted during the international search (name of data base used, where practical, source terms used)		
CHEM ABS Data, BEILSTEIN Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 28008 A (GUERLAIN S.A.) 19 September 1996 (1996-09-19) claims 1,2	11
X	M. ETTLINGER ET AL.: "the mustard oil of limnanthes douglasii seed, m-methoxybenzyl isothiocyanate" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 78, no. 9, 1956, pages 1952-1954, XPCQ1031424 page 1954, column 2, paragraphs 2,3	1
A	EP 0 903 349 A (F. HOFFMANN-LA ROCHE AG) 24 March 1999 (1999-03-24) page 25, line 29	11
-/-		
☒ Further documents are listed in the continuation of box C. ☒ Potentially relevant documents are listed in box C.		
* Special categories of cited documents "A" document claiming the general state of the art which is not considered to be of particular relevance "E" earlier document but published in or after the international filing date "I" document which may throw doubt on priority, clarity or which is cited to establish the publication date of another document or other special reasons (see searched) "O" document informing to no one document, i.e., citation of other means "P" document published prior to the (re-)filing date but after the priority date claimed "T" later document submitted after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention "X" document of substantive relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken into account "Y" document of substantive relevance: the claimed invention cannot be considered to involve an inventive step when the document is considered with one or more other such docu- ments such combination being obvious to a person skilled in the art "Z" document: none of the above categories		
Date of this search completion of the international search		Date of mailing of the international search report
13 November 2001		23/11/2001
Name and mailing address of the ISA European Patent Office, P.O. Box 1, Patentstr. 2 8001 Zurich, Switzerland Tel. (+41-70) 300-2000, Te. (+41-70) 300-2000 Fax (+41-70) 300-2000		SEARCHED BY Glickman, J-F

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