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(54) Titre : COMPOSITIONS POUVANT ETRE UTILISEES COMME BIOCARBURANT
(54) Title: COMPOSITIONS WHICH CAN BE USED AS BIOFUEL

(57) Abrégé/Abstract:
The invention relates to a composition which contains an alkylester which is provided with an alkyl radical containing 1 - 8 carbon atoms and partial glycerides which have a glycerine content of free glycerine upto a maximum of 2 wt.% in relation to the total amount of the composition. The invention also relates to a first method for producing the inventive composition, wherein triglycerides are enzymatically reacted with an esterase in the presence of alcohols which are provided with a plurality of carbon atoms having 1 - 8 C atoms, said esterase being activated by adding alkaline salts. In an additional method, the esterases are immobilised and / or chemically modified. The invention further relates to a method, wherein the inventive composition is produced by chemical partial tranesterfication. The invention subsequently relates to a composition which can be obtained according to said method, in addition to the use of the inventive compositions as biodiesel or additives in fuel compositions.

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(54) Title: COMPOSITIONS WHICH CAN BE USED AS BIOFUEL

(54) Bezeichnung: ZUSAMMENSETZUNGEN VERWENDBAR ALS BIOTREIBSTOFF

(57) Abstract: The invention relates to a composition which contains an alkylester which is provided with an alkyl radical containing 1 - 8 carbon atoms and partial glycerides which have a glycerine content of free glycerine upto a maximum of 2 wt.% in relation to the total amount of the composition. The invention also relates to a first method for producing the inventive composition, wherein triglycerides are enzymatically reacted with an esterase in the presence of alcohols which are provided with a plurality of carbon atoms having 1 - 8 C atoms, said esterase being activated by adding alkaline salts. In an additional method, the esterases are immobilised and / or chemically modified. The invention further relates to a method, wherein the inventive composition is produced by chemical partial tranesterfication. The invention subsequently relates to a composition which can be obtained according to said method, in addition to the use of the inventive compositions as biodiesel or additives in fuel compositions.

(57) Zusammenfassung: Vorgeschlagen wird eine Zusammensetzung enthaltend Alkylester mit einem Alkylrest enthaltend 1 bis 8 Kohlenstoffatome und Partialglyceride die einen Glyceringehalt an freien Glycerin von maximal 2 Gew.-% bezogen auf die Gesamtmenge der Zusammensetzung hat. Weiterhin wird ein erstes Verfahren zur Herstellung der erfmdungsgemäßen Zusammensetzung vorgeschlagen, bei dem Triglyceride in Anwesenheit von Alkoholen mit einer Anzahl von Kohlenstoffatomen von 1 - 8 C- Atomen mit einer Esterase enzymatisch umgesetzt werden, welche durch Zugabe von alkalischen Salzen aktiviert wird. In einem weiteren Verfahren werden die Esterasen immobilisiert und / oder chemisch modifiziert eingesetzt. Weiterhin wird ein Verfahren vorgeschlagen, bei dem die erfmdungsgemäße Zusammensetzung durch chemische Partialumesterung hergestellt wird. Zusätzlich werden Zusammensetzung erhältlich nach dem erfindungsgemäßen Verfahren vor- geschlagen sowie die Verwendung der erfmdungsgemäßen Zusammensetzungen als Biodiesel oder als Additiv in Treibstoffzusammensetzungen.

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Compositions Which Can Be Used as Biofuel

Field of the Invention

This invention relates generally to glycerides and, more particularly, to compositions containing fatty acid esters and partial glycerides, to their production, for example by enzymatic catalysis, and to their use as a biofuel.

Prior Art

Hydrocarbon-based fuels, i.e. for example gas oils, heating oils, gasoline, diesel, kerosene, etc., contain numerous additives. Thus, besides corrosion inhibitors and lubricity additives, there are also flow improvers or compounds which improve the emission values of gases such as CO, CO_2 or NO_x.

Through Directive 2003/30/EC, Article 3.1 (b), the European Parliament decreed that, from 31.12.2005, all diesel fuels should contain 2% biofuel. This percentage is supposed to increase to 5.75% by 31.12.2010. In Article 2.2 of this Directive, biofuel is defined as follows: bioethanol, rapeseed oil methyl ester (RSME), biogas, biomethanol, biodimethylether, biohydrogen, synthetic biofuels and purely vegetable oils.

In general, rapeseed oil methyl ester (RSME) is used as biodiesel. It is already possible that engines are being powered by pure biofuel under the EU Directive. However, it is likely that up to 2% RSME is being added to normal diesel to comply with the EU Directive.

This RSME is produced by converting the natural triglyceride into a methyl ester or even an ethyl ester. The by-product of this process is crude glycerol. 100 kg free glycerol are formed in the production of 1 tonne of biodiesel as RSME. The availability of glycerol increases with the

increasing percentage of biodiesel. Since there is a limited market for glycerol, which is already covered by existing production, disposal problems arise. This situation potentially limits the normal production route for biodiesel because glycerol can no longer be factored in as additional profit, making this route economically unattractive.

A high glycerol level adversely affects the combustion performance of diesel and biodiesel, so that the glycerol has to be removed. One reason for this is the poor solubility of glycerol in rapeseed oil methyl ester. Excessive concentrations of glycerol in the methyl ester lead to the formation of a heavy glycerol phase which can settle, for example, in the fuel tank. If such a glycerol phase is injected into the engine, performance is reduced and the wear of individual engine components is potentially increased.

Now, one problem to be solved was to provide a biofuel which would comply with the guidelines of the European Parliament and in which glycerol would be present as a derivative so that very little free glycerol would be formed as a by-product in the production process. The production process would be environmentally friendly and economical.

Enzymes are being increasingly used as catalysts in chemical and biochemical syntheses. Thus, in many cases, hydrolases, more especially lipases (EC 3.1.1.3), are already being used for lipolysis or transesterification in industrial processes by virtue of the often relatively mild reaction conditions. These enzymes are produced by various microorganisms. To isolate the enzymes, fermentation of the microorganisms is followed by an expensive purification process. The effectiveness of these catalysts is often offset by the high costs of production and isolation, so that research groups are constantly striving to increase the yields of enzymes or the productivity of the enzymes. The standard chemical method for producing monoglycerides involves the base-catalyzed glycerolysis of triglycerides, a yield of 40 to 60%

monoglyceride, based on the total glycerides, typically being obtained. Further enrichment to a >90% monoglyceride content is achieved by physical separation techniques, such as molecular distillation or crystallization.

Various enzymatic routes suitable for the production of monoglycerides have been described in the literature: 1) enzymatic synthesis starting from fatty acid and glycerol; 2) enzymatic glycerolysis starting from triglyceride and glycerol which corresponds to the chemical process; 3) the 1,3-regioselective hydrolysis or alcoholysis of triglyceride. Summaries of these processes can be found, for example, in (a) **Recent Res. Devel. Oil Chem., 3 (1999), 93-106**; (b) **Hydrolases in Organic Synthesis, Wiley-VCH (1999), eds. Bornscheuer & Kazlaukas**.

WO 9013656 and **WO 9004033** (Enzytech. Inc.) and **US 5,939,828** and **US 5,316,927** (Opta Food Ingredients Inc.) describe the production of monoglycerides by enzymatic alcoholysis with various alcohols and a little water in the mixture. Lipases are used in powder form or immobilized. In the Examples, lipases are used in quantities of ca. 20% by weight, based on the triglyceride, and the alcohol component in a 20-fold excess.

WO 9116441, **WO 9116442** and **US 5,116,745** describe processes in which a mixed regioselective alcoholysis and hydrolysis to 1,2-diglycerides and 2-monoglycerides using lipases is carried out in the presence of a solvent, an alcohol and an aqueous buffer.

EP 407 959 describes a process for the production of monoester using a thermostable immobilized lipase in the presence of secondary or tertiary alcohols as solubilizers.

WO 0206505 (Nippon Suisan Kaisha Ltd.) describes regioselective alcoholysis using immobilized lipase, a large excess of alcohol and high concentrations of enzyme, followed by re-esterification of the monoglyceride.

JP 03108489 and **JP03187385** (Meito Sangyo Co. Ltd.) describe

the regioselective hydrolysis of triglycerides with alkaline lipase in the presence of alkaline salts. The lipase used is only active under alkaline conditions.

JP 03103499 (Meito Sangyo Co. Ltd.) describes the regioselective alcoholysis of PUFA triglycerides with isobutanol in the presence of an alkaline lipase.

Although the enzymatic production of partial glycerides has already been widely described, solvents are required in all the above-cited documents, the water of reaction has to be expensively removed or the lipases are very special and are not commercially available on an industrial scale.

Now, a first problem addressed by the present invention was to provide biofuel which would comply with the guidelines of the European Parliament and in which the glycerol would be present as a derivative, so that very little free glycerol would be formed as a by-product in the production process. The production process would be environmentally friendly and economical. A second problem resulting from this was to find an inexpensive enzymatic or chemical variant that would increase the yield of monoglycerides and diglycerides from polyol esters, such as triglycerides for example. In addition, the enzyme content in enzymatic alcoholyses would be kept minimal.

Description of the Invention

The present invention relates to a composition containing alkyl esters with a C_{1-8} alkyl group and partial glycerides with a free glycerol content of at most 2% by weight, based on the total quantity of the composition.

It has surprisingly been found that compositions which contain the above-mentioned components in the mixture and at most 2% by weight free glycerol solve the problem addressed by the invention in an

outstanding manner. A maximum glycerol content of 1.3% by weight is preferred and a maximum glycerol content of 1.0% by weight is particularly preferred, the evaluation being based on %-area in GC analysis and the values for glycerol having to be calibrated in view of the strong absorption.

In one particular embodiment, the composition contains methyl and/or ethyl esters as the alkyl esters.

In another particular embodiment, the composition has a partial glyceride content of at least 10% by weight and/or a triglyceride content of at most 5% by weight and/or an acid value of at most 5, based on the total quantity of the composition. A monoglyceride content of at least 25% by weight is preferred.

In another particular embodiment, the composition contains methyl and/or ethyl esters, monoglycerides and diglyceride in the following quantities:

methyl and/or ethyl esters: 30 to 70% by weight, preferably 55 to 60% by weight

monoglyceride: 10 to 35% by weight, preferably 25 to 33% by weight

diglyceride: 1 to 30% by weight, preferably 1 to 20% by weight.

The percentages by weight are evaluated via the %-areas in GC analysis.

Another particular embodiment are compositions in which the alkyl esters and partial glycerides represent fatty acid esters of saturated or unsaturated, linear or branched fatty acids with a C_{8-22} alkyl group. Fatty acid esters obtainable from vegetable oils, such as for example linoleate, oleate, palmitate, stearate and/or pelargonate, are particularly preferred for the purposes of the invention. Unsaturated representatives are, for example, lauroleic, myristoleic, palmitoleic, petroselaidic, oleic, elaidic, ricinoleic, linoleic, linolaidic, linolenic, gadoleic, arachidonic and erucic acid esters. Mixtures of the methyl and/or ethyl esters of these acids are also suitable.

Preferred oils for obtaining the fatty acid esters are sunflower oil,

rapeseed oil, thistle oil, soybean oil, linseed oil, peanut oil, tallows, olive oil, castor oil, palm oil, palm oil fractions, such as palm olein and palm stearin, yatropha oil, coconut oil and palm kernel oil.

Peanut oil contains on average (based on fatty acid) 54% by weight oleic acid, 24% by weight linoleic acid, 1% by weight linolenic acid, 1% by weight arachic acid, 10% by weight palmitic acid and 4% by weight stearic acid. Its melting point is 2 to 3°C.

Linseed oil typically contains 5% by weight palmitic acid, 4% by weight stearic acid, 22% by weight oleic acid, 17% by weight linoleic acid and 52% by weight linolenic acid. It has an iodine value of 155 to 205, a saponification value of 188 to 196 and a melting point of ca. -20°C.

Olive oil mainly contains oleic acid. Palm oil contains ca. 2% by weight myristic acid, 42% by weight palmitic acid, 5% by weight stearic acid, 41% by weight oleic acid, 10% by weight linoleic acid as fatty acid components.

Rapeseed oil typically contains ca. 48% by weight erucic acid, 15% by weight oleic acid, 14% by weight linoleic acid, 8% by weight linolenic acid, 5% by weight eicosenoic acid, 3% by weight palmitic acid, 2% by weight hexadecenoic acid and 1% by weight docosadienoic acid as fatty acid components. Rapeseed oil from new plants has higher levels of the unsaturated acids. Typical fatty acid levels here are erucic acid 0.5% by weight, oleic acid 63% by weight, linoleic acid 20% by weight, linolenic acid 9% by weight, eicosenoic acid 1% by weight, palmitic acid 4% by weight, hexadecenoic acid 2% by weight and docosadienoic acid 1% by weight.

80 to 85% by weight of castor oil consists of the glyceride of ricinoleic acid. Castor oil also contains ca. 7% by weight oleic acid glycerides, 3% by weight linoleic acid glycerides and ca. 2% by weight palmitic and stearic acid glycerides.

Soybean oil contains 55 to 65% by weight, based on total fatty acids, of polyunsaturated acids, more particularly linoleic and linolenic acid. The

situation is similar with sunflower oil of which the typical fatty acid spectrum, based on total fatty acids, is as follows: ca. 1% by weight myristic acid, 3 to 10% by weight palmitic acid, 14 to 65% by weight oleic acid and 20 to 75% by weight linoleic acid.

All the above-mentioned figures relating to the percentage fatty acid contents in the triglycerides are known to depend on the quantity of the raw materials and can vary accordingly.

The fatty acid composition in the mixture is made up of the particular native fatty acid composition of the vegetable oil used and the particular quality of the raw material from which the methyl and/or ethyl esters and the monoglycerides are produced.

The present invention also relates to a process for the production of biofuel in which triglycerides are enzymatically reacted with an esterase activated by the addition of alkaline salts, the reaction being carried out in the presence of alcohols containing 1 to 8 carbon atoms.

It has surprisingly been found that the addition of alkaline salts can activate esterases so that an increased yield of monoglycerides compared with known processes can be achieved in the alcoholysis of triglycerides.

In the process according to the invention, a triglyceride is split into a 2-monoglyceride and two fatty acid esters in the presence of an alcohol. In this process, more than 90% of the glycerol remains chemically bound in the product and the small concentrations of free glycerol remain dissolved in a single phase in the product. Accordingly, in contrast to conventional biodiesel production, no glycerol is formed as a by-product in the process according to the invention, so that the quantity of raw material (oil) required can be reduced accordingly. The composition according to the invention can be produced in an outstanding manner by this process.

The reaction can be carried out very economically through the use of small quantities of esterase, preferably lipase. The reaction is directly carried out with the enzyme concentrate in the presence of an added

alkaline inorganic salt which strongly activates the enzyme. In this way, a high conversion is achieved with a small quantity of enzyme, even without stabilization of the enzyme by immobilization. There is no need for the addition of solvents.

The alcoholysis is carried out at temperatures of 10°C to 40°C, preferably at 10°C to 30°C and more particularly – to maintain optimal regioselectivity and activity – at a temperature of 15°C to 25°C. The reaction is carried out with a water content of 0.1 to 10% by weight, preferably 0.1 to 5% by weight and more particularly 0.1 to 2% by weight, based on the quantity of triglyceride, the water content of the liquid enzyme preparation being included. Although the reaction can also be carried out with higher water contents, the content of free fatty acid formed is increased in that case. High levels of free fatty acid are undesirable because, when used in biodiesel, they can have a corrosive effect on engine parts at high temperatures.

The reaction time is preferably 12 to 48 hours, depending on the enzyme concentration used. In a preferred embodiment, all the reactants are mixed and the reaction is initiated by addition of the enzyme preparation.

The alcohol component containing 1 to 8 carbon atoms, preferably methanol and/or ethanol, preferably ethanol, is added either completely at the beginning of the reaction or over the duration of the reaction. The quantity of alcohol used is variable between a minimum of 2 mols alcohol to 1 mol oil and a maximum of 50% by weight alcohol and 50% by weight oil in the mixture.

In another step of the process according to the invention, the esterase can be deactivated by heat and the esterase precipitated may then optionally be filtered off, in which case not only the esterase precipitated, but also additives or formulation ingredients of the enzyme preparation used can be removed.

The following optional steps can be added onto the process according to the invention:

- addition of water-adsorbing agents during the enzymatic reaction to suppress the formation of free acids
- filtration of the reaction mixture through filter aids to remove additives or ingredients of the enzyme formulation
- refinement of the product mixture with water to remove free glycerol which is formed in small quantities as a by-product

Through the emulsifying character of the monoglycerides formed, any fatty acids formed, free glycerol and small amounts of water remain dissolved in a single phase in the product.

In one particular embodiment of the invention, alcohol and/or water is/are completely or partly removed, preferably by distillation. Any free glycerol still present - having been formed in small quantities as a by-product – may also be removed in the distillation step. Tests have shown that, even after blending of the biodiesel with diesel, these components remain dissolved in the diesel by virtue of the emulsifying effect of the monoglyceride.

Experimental data have shown that the addition of small quantities of alkaline inorganic salts greatly increases the enzyme activity of the esterases. In particular, non-immobilized lipases are activated by the alkaline salts.

The commercially obtainable liquid preparation is preferably used in a concentration of 0.05 to 2%, based on the quantity of triglyceride used. These commercially obtainable liquid enzyme preparations have an enzyme activity of on average 100,000 U/ml. One enzyme unit U is defined as the quantity of enzyme which reacts one micromol substrate per minute.

In the process according to the invention alkaline inorganic salts selected

from the group consisting of hydroxides, carbonates and phosphates of sodium, potassium, calcium, magnesium and ammonium predissolved in water are preferably used to activate the esterase. According to the invention, the quantity of alkaline inorganic salts for activating the esterase is between 0.00001 and 1% by weight and preferably between 0.0001 and 0.2% by weight, based on the quantity of triglyceride. The quantity of basic additive used depends on the quantity of buffered liquid enzyme preparation used and on the strength of the base. Where NaOH and <0.5% liquid enzyme preparation are used, the concentration is in the lower range; where Na_2CO_3 and 2% liquid enzyme preparation are used, the quantity of basic additive is in the upper concentration range.

Surprisingly, the strongest activation of the *Thermomyces lanugenosus* lipase was achieved when salts such as, for example, trisodium phosphate, sodium carbonate, sodium hydroxide or ammonium hydroxide were added to the commercially obtainable liquid enzyme preparation in quantities of 0.0001 to 0.2% by weight (based on the triglyceride content). Surprisingly, a faster monoglyceride synthesis rate was achieved than with *Thermomyces* lipase adsorbed onto polypropylene. The activation of the lipase is so strong that it cannot be explained by the pH shift in the reaction medium alone. If the *Thermomyces lanugenosus* lipase is used in immobilized form under the same conditions, there is no sign of equally strong activation by addition of salts. This strong activation is very surprising as it is generally accepted that a high activity level can only be achieved in the low-water medium with lipases fixed to a carrier. The strong activation eliminates the need for elaborate immobilization processes and leads to a simple plant concept. In addition, measurement of the pH value of the reacted product mixture shows that the pH is in the neutral to mildly acidic range which makes enzyme activation by pH shift alone improbable.

The present invention also relates to a process for the production of

monoglycerides in which triglycerides are enzymatically reacted with an immobilized and/or chemically modified esterase in the presence of alcohols containing 1 to 8 carbon atoms.

It has surprisingly been found that the composition according to the invention can also be produced in an outstanding manner by this enzymatic process. In this process, too, more than 90% of the glycerol remains chemically bound in the product and the small concentrations of free glycerol remain dissolved in a single phase in the product. Accordingly, in contrast to the conventional production of biodiesel, no glycerol is formed as a by-product in the process according to the invention, so that the quantity of raw material (oil) required can be distinctly reduced accordingly. By repeatedly using the immobilized and/or chemically modified esterase, preferably lipase, the reaction can be carried out very economically. There is no need for the addition of solvents.

The alcoholysis is carried out at temperatures of 10°C to 60°C, preferably at 10°C to 40°C and more particularly – to maintain optimal regioselectivity and activity – at a temperature of 15°C to 30°C. The reaction is carried out with a water content of 0.1 to 10% by weight, preferably 0 to 5% by weight and more particularly 0 to 2% by weight, based on the quantity of triglyceride. Although the reaction can also be carried out with higher water contents, the content of free fatty acid formed is increased in that case. High levels of free fatty acid are undesirable because, when used in biodiesel, they can have a corrosive effect on engine parts at high temperatures.

The reaction time is preferably 1 to 48 hours, depending on the enzyme concentration used. In a preferred embodiment, all the reactants are mixed and the reaction is initiated by addition of the enzyme preparation.

The alcohol component, preferably methanol and/or ethanol, preferably ethanol, is added either completely at the beginning of the

reaction or over the duration of the reaction. The quantity of alcohol used is variable between a minimum of 2 mols alcohol to 1 mol oil and a maximum of 50% by weight alcohol and 50% by weight oil in the mixture.

In another step of the process according to the invention, the esterase can be filtered off. The following optional steps can be added onto the process according to the invention:

- addition of water-adsorbing agents during the enzymatic reaction to suppress the formation of free acids
- filtration of the reaction mixture through filter aids to remove ingredients of the enzyme formulation or insoluble components of the oil used
- refinement of the product mixture with water to remove free glycerol which is formed in small quantities as a by-product.

Through the emulsifying character of the monoglycerides formed, any fatty acids formed, free glycerol and small amounts of water remain dissolved in a single phase in the product.

In one particular embodiment of the invention, alcohol and/or water is/are completely or partly removed, preferably by distillation. Any free glycerol still present - having been formed in small quantities as a by-product – may also be removed in the distillation step. Tests have shown that, even after blending of the biodiesel with diesel, these components remain dissolved in the diesel by virtue of the emulsifying effect of the monoglyceride.

Various carrier materials suitable for the formation of enzymes may be used for the process according to the invention. Plastics, mineral carriers or resins which bind the esterases through hydrophobic interactions, such as Amberlite 16 (Rohm & Haas), Celite or Accurel MP 1000 (Membrana) for example, may be used as carriers. Other suitable carriers are ion exchangers which bind the esterases through ionic and – in

part – hydrophobic interactions, such as Dowex Marathon WBA (Dow Chemicals) or Duolite A 568 (Rohm & Haas) for example. Other suitable carriers are those which are capable of binding the esterases through chemically reactive groups, such as Eupergit (Degussa) for example.

Chemical modifications are also suitable for adapting the esterases to the reaction system. Hydrophobic modifications, such as coating with surfactants for example, or chemical modification with fatty aldehydes may be used. Stabilization of the esterases through crosslinking, for example by glutaraldehyde, DMA or EDC, is also suitable.

A combination of chemical modification and immobilization is also suitable for adapting the esterases to the reaction system. In this case, either the esterases may first be immobilized and then modified on a carrier or esterases which have already been chemically modified are immobilized.

The esterases to be used in the enzymatic processes according to the invention are preferably those which emanate from an organism selected from the group consisting of *Thermomyces lanugenosus, Candida antarctica A, Candida antarctica B, Rhizomucor miehei, Candida cylindracea, Rhizopus javanicus, Porcine pancreas, Aspergillus niger, Candida rugosa, Mucor javanicus, Pseudomonas fluorescens, Rhizopus oryzae, Pseudompnas sp., Chromobacterium viscosum, Fusarium oxysporum and Penicilium camemberti.* Esterases from *Thermomyces lanugenosus* with the synonym *Humicola lanuginosa* are particularly preferred.

Esterases are enzymes which catalyze the formation and hydrolysis of esters; as hydrolases, they split their respective substrates with incorporation of the elements of water. The esterases include, for example, the fat-splitting lipases which represent preferred esterases for the process according to the invention. The use of 1,3-regiospecific lipases is particularly preferred for the process according to the invention, these lipases being distinguished by the fact that they preferentially split off the

fatty acids at the 1- and 3-positions of triglycerides. In principle, any 1,3-regioselective lipase or esterase in free or immobilized form may be used for the process according to the invention. The lipase of *Thermomyces lanugenosus* (manufacturer: Novozymes, name: Lipozyme TL 100 l or Lipoplase 100 EX) has proved to be particularly preferred for the process according to the invention.

The present invention also relates to a process for the production of monoglycerides in which triglycerides are chemically reacted in the presence of alcohols containing 1 to 8 carbon atoms. In this process, the alcohols are used in a molar concentration which is lower than the molar concentration of glyceride-bonded fatty acid. It has surprisingly been found that the composition according to the invention can be produced by this process. In this process according to the invention, at least a large part of the glycerol present in the triglyceride remains bound in the product, so that less glycerol is formed than in the conventional production of biodiesel.

In the process according to the invention, either alkaline catalysts are used in a low-pressure transesterification or strongly acidic catalysts are used in a low-pressure transesterification. High-pressure transesterifications in the presence of a chemical catalyst are also part of the process.

Preferred catalysts for the alkaline low-pressure transesterification in homogeneous catalysis are the salts of alcohols containing 1 to 8 carbon atoms with monovalent cations, the sodium and potassium salts of methanol and ethanol being particularly preferred. Preferred catalysts for the alkaline low-pressure transesterification in heterogeneous catalysis are carbonates and oxides such as, for example, sodium carbonate or calcium oxide. The catalysts are used in a concentration of 0.01% by weight to 5% by weight and preferably in a concentration of 0.1% by weight to 1% by weight. The alkaline catalysts may be prepared in situ from water-free NaOH or KOH and the corresponding alcohol. The transesterification is

carried out at a temperature of 40 to 120°C and under a pressure of at most 2 bar. The reaction is preferably carried out under a pressure of at most 1.2 bar. At the end of the reaction, the catalyst is neutralized by addition of an acid such as, for example, citric acid, phosphoric acid, hydrochloric acid or sulfuric acid and is removed by separation. The reaction time is preferably 0.1 to 10 h, depending on the catalyst concentration used and the reaction temperature.

Preferred catalysts for the acidic low-pressure transesterification in homogeneous catalysis are mineral acids, more especially sulfuric acid, or aliphatic and aromatic sulfonic acids. The catalysts are used in a concentration of 0.01% by weight to 5% by weight. The transesterification is carried out at a temperature of 40 to 160°C and under a pressure of at most 5 bar. At the end of the reaction, the catalyst is neutralized by addition of an alkali such as, for example, aqueous NaOH or KOH and removed by separation. The reaction time is preferably 0.5 to 25 h, depending on the catalyst concentration used and the reaction temperature.

Preferred catalysts for the high-pressure transesterification are metal salts or metal soaps, preferably salts or soaps of zinc such as, for example, zinc acetate or zinc stearate in a concentration of 0.01% by weight to 1% by weight. The transesterification is carried out at a temperature of 120 to 250°C and under a pressure of at most 20 to 200 bar. At the end of the reaction, the catalyst is removed by filtration. The reaction time is preferably 0.1 to 5 h, depending on the catalyst concentration used and the reaction temperature.

In the process according to the invention, the partial chemical transesterification may be carried out as a batch reaction or as a continuous reaction. In the continuous variant, the alcohol component may either be transported as a gas in countercurrent to the oil or, alternatively, may be transported in co-current as a single phase with the oil under high-

pressure conditions or low-pressure conditions. In a preferred embodiment, all the reactants are mixed and the reaction is initiated by addition of the catalyst. The alcohol component, preferably methanol and/or ethanol, preferably ethanol, is added either completely at the beginning of the reaction or over the duration of the reaction. The quantity of alcohol used is variable between a minimum of 10 mol-% alcohol and a maximum of 30 mol-% alcohol, based on the quantity of oil used in the mixture.

In another step of the process according to the invention, the catalyst can be filtered off or neutralized and washed out after the reaction. The following optional steps can be added onto the process according to the invention:

- addition of water-adsorbing agents during the reaction to suppress the formation of free acids
- filtration of the reaction mixture through filter aids to remove catalyst or insoluble components of the oil used
- refinement of the product mixture with water to remove free glycerol which is formed as a by-product.

Through the emulsifying character of the monoglycerides formed, any fatty acids formed, free glycerol and small amounts of water remain dissolved in a single phase in the product.

In one particular embodiment of the invention, alcohol and/or water is/are completely or partly removed, preferably by distillation. Any free glycerol still present - having been formed in small quantities as a by-product – may also be removed in the distillation step.

Acid-containing fats and oils may readily be used in the described acid-catalyzed low-pressure process and in the chemically catalyzed high-pressure process.

Triglycerides from fats and oils which have a high percentage

content of mono- and/or polyunsaturated fatty acids and which are selected from the group consisting of sunflower oil, rapeseed oil, thistle oil, soybean oil, linseed oil, peanut oil, tallows, olive oil, castor oil, palm oil, yatropha oil, coconut oil, palm kernel oil and old oils, for example used frying fat, are preferably used in the process according to the invention. The fats and oils may be used in refined or unrefined form in the process according to the invention. Acid-containing fats and oils may readily be used in the process according to the invention.

Alcohols containing 1 to 8 carbon atoms are preferably used as alcohol components for the process according to the invention. These alcohols may have linear or branched carbon chains and are preferably primary or secondary alcohols and are preferably selected from the group consisting of methanol, ethanol, 1-propanol, isopropanol; 1-butanol, sec. butanol. tert. butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 2-ethylhexanol. Particularly preferred alcohol components are methanol, ethanol or 1-propanol. Methanol and ethanol or mixtures thereof are particularly preferred, ethanol being most particularly preferred. The ethanol used is preferably obtainable from biological sources, for example from the fermentation of carbohydrates.

The alcohol content is preferably 10 to 50% by weight or 10 to 30 mol-% in the chemical process, based on the triglyceride used, 15 to 40% by weight or 15 to 25 mol-% preferably being used in the chemical process. The monoglyceride content is dependent on the quantity of alcohol used.

The present invention also relates to a composition obtainable by the process according to the invention. The composition thus obtained, which consists mainly of alcohol, alkyl ester, monoglyceride and diglyceride, may be directly added to diesel fuel. Through the emulsifying character of the monoglycerides formed, any fatty acids formed, free glycerol and small amounts of water remain dissolved in a single phase in the product. Traces of water are more effectively bound and no longer

adversely affect the combustion process. The monoglycerides formed enhance the lubricating properties. The effect of the components in their mixed form in the composition according to the invention can be that glycerol still present is more effectively burned in the combustion process. To reduce the flash point, alcohol can be completely or partly removed from the composition produced in accordance with the invention, for example by distillation, before it is added to the diesel.

Accordingly, the present invention also relates to a fuel composition containing 90 to 99.5% by weight gas oil and 0.5 to 10% by weight and preferably 2 to 6% by weight of a composition according to the invention or a composition obtainable by the process according to the invention.

In the context of the invention, gas oil is understood to encompass every possible fraction of petroleum in both the additive-containing and the additive-free state. Gas oil in the context of the present invention is preferably understood to be diesel. Additives which are present in the gas oil mentioned in its additive-containing state and which may be present in addition to the compositions according to the invention are additives selected from the group consisting of conductivity improvers, cetane number improvers, CFPP/CP improvers, defoamers, lubrication improvers, corrosion inhibitors and dehazers. These additives are used in the usual concentrations and are well-known in the oil industry.

The potential applications of this gas oil are included in the definition. This includes both uses in the traffic sector, for example as diesel for engines, and uses outside the traffic sector, for example as heating oil, tractor oil, diesel for mobile diesel engines, marine bunker oils or the like. The distillation range of the gas oil fractions extends from 140 to 400°C.

The use applies to all the gas oil fractions mentioned both in the additive-containing and in the additive-free state.

Diesel fuels are obtained from gas oil by cracking or from tars which

are obtained in the low-temperature carbonization of lignite or coal. Diesel fuels are poorly inflammable mixtures of liquid hydrocarbons which are used as fuels for constant-pressure or compression-ignition engines (diesel engines) and which consist mainly of paraffins with admixtures of olefins, naphthenes and aromatic hydrocarbons. Their composition is variable and depends in particular on the production method. Typical products have a density of 0.83 to 0.88 g/cm^3, a boiling point of 170 to 360°C and flash points of 70 to 100°C.

The present invention also relates to the use of the composition according to the invention containing alkyl esters with a C_{1-8} alkyl group and partial glycerides which has a free glycerol content of at most 2% by weight, based on the total quantity of the composition, or the preferred embodiments of this/these composition(s) obtainable by the process according to the invention as a biofuel.

The present invention provides a biofuel in which only small quantities of free glycerol are present as a by-product. More particularly, the enzymatic reaction of pure vegetable oil and bioalcohol gives a mixture of alkylester and partial glycerides, which may be used as a biofuel or as an additive in compliance with European Directive 2003/30/EC. It remains to be emphasized here that, besides the vegetable oil, the ethanol is also preferably produced from renewable raw materials, so that a biofuel retaining the advantages of raw materials from renewable sources is produced and made available.

Besides its production with few by-products, the advantage of the biofuel according to the invention lies in the introduction of additional oxygen into the combustion path which reduces emissions. In addition, the additional lubricating effect of the partial glycerides eliminates the need to use lubricity improvers. In contrast to the known production of rapeseed oil methyl ester, the first production process is energy-saving because it is purely enzymatic and does not involve major purification of the end

product. Tests have shows that the low-temperature behavior of commercially available diesels is not adversely affected by blending with the composition according to the invention. An important property, the cold filter plugging point (CFPP), is not adversely affected. Slight clouding of the mixture, but no precipitation or phase separation, was observed at temperatures around -20°C. The mixture remains thinly liquid and pumpable. No changes occurred during storage at 4°C.

The present invention also relates to the use of the composition according to the invention containing alkyl esters with a C_{1-8} alkyl group and partial glycerides and, more particularly, methyl and/or ethyl esters, monoglycerides and methanol and/or ethanol, which has a glycerol content of at most 2% by weight, based on the total quantity of the composition, or the preferred embodiments of this/these composition(s) obtainable by the process according to the invention as an additive in fuel compositions, preferably in quantities of 0.5 to 10% by weight and more particularly in quantities of 1 to 5% by weight. In a particularly preferred embodiment, the composition according to the invention is used as an additive for improving the lubricating performance of fuel compositions.

The use of various additives for fuels is known from the literature. Monoglycerides and other partially esterified or etherified polyols (for example even glycol monoesters) are added as a diesel additive because they have a good lubricating effect. Patent applications which describe such additives include, for example **EP 0 721 492** (Infineum USA L.P.), **WO 0119941** (Fina Research S.A.) and **WO 0063322** (Pure Fuels USA Inc.).

More particularly, glyceride mixtures with a high percentage of monoglyceride have good lubricating properties. Thus, it has been found that the monoglycerides produced by the process according to the invention can also be used as fuel additives in diesel fuel and show good lubricating properties.

The regiospecific fatty acid composition of the naturally occurring

oils can be utilized in the enzymatic processes according to the invention. The monoglyceride fraction mainly contains the fatty acid composition which is to be found in the 2-position of the oils. With most naturally occurring oils, the more highly unsaturated fatty acids are preferably bound in the 2-position. In this way, monoglycerides with a high linoleic acid content can be produced, for example, from sunflower or thistle oil. These monoglycerides have a reduced solidification point which is particularly important for the use of monoglycerides as a diesel additive. A monoglyceride with a high oleic acid content can be obtained, for example, from palm oil.

In the context of the present invention, fuel compositions are understood to be any energy-providing fuels of which the free combustion energy is converted into mechanical work. This includes all types of motor and aircraft fuels which are liquid at room temperature and normal pressure. Motor fuels, for example for automobile and truck engines, generally contain hydrocarbons, for example gasoline or higher-boiling petroleum fractions. The fuel compositions according to the invention are preferably diesel oil.

Examples

Example 1: Regioselective alcoholysis with various enzymes in free and immobilized form

16 mixtures consisting of 20 g rapeseed oil and 2.5 g ethanol were placed in glass beakers equipped with magnetic stirrers. 0.25 g water was added with stirring to mixtures 1 to 9, 15 and 16; 0.5 g water was added to mixtures 10 to 14. Lipases in free and immobilized form as listed in the following Table were then added. The mixtures were incubated with stirring for 24 h., another 2.5 g ethanol being added after 5 h. The alcoholysis of mixtures 1 to 14 was carried out at room temperature on a multistirrer plate.

Mixtures 15 and 16 were incubated at 45°C on a shaker. After 24 h, samples were taken and the content of glycerides and ethyl esters was analyzed by gas chromatography. The results were evaluated as percentage areas. Small amounts of fatty acid formed are contained in the ethyl ester area.

The immobilizates of mixtures 1 to 3, 15 and 16 were acquired in immobilized form direct from the manufacturer. The immobilizates of mixtures 4 to 8 were prepared by adsorption onto Accurel MP 1000 (Membrana). To this end, Accurel MP 1000 was incubated for 1 h in 10 ml ethanol. After the ethanol had been decanted off, 10 g water and 0.5 g of each lipase preparation were added. The mixture was incubated overnight at room temperature. The immobilizate was then separated by filtration and dried for 24 h at room temperature on sheets of paper.

Mixture	Enzyme	Manufacturer	Organism	Form
1	1 g Novozym 435	Novozymes	C.antarctica B	Immobilizate
2	1 g Lipozym RM IM	Novozymes	R.miehei	Immobilizate
3	1 g Lipozym TL IM	Novozymes	T.lanugenosus	Immobilizate
4	1 g Lipase FAP 15/MP 1000	Amano	R.oryzae	Immobilizate
5	1 g Lipase A/MP 1000	Amano	A.niger	Immobilizate
6	1 g Lipase M/MP 1000	Amano	M.javanicus	Immobilizate
7	1 g Lipase L115/MP 1000	Biocatalysts	Porcine pancreas	Immobilizate
8	1 g Lipomod 36/MP 1000	Biocatalysts	R.javanicus	Immobilizate
9	0.5 g Lipolase	Novozymes	T.lanugenosus	Free
10	0.5 g Lipase FAP 15/MP 1000	Amano	R.oryzae	Free
11	0.5 g Lipase A/MP 1000	Amano	A.niger	
12	0.5 g Lipase M/MP 1000	Amano	M.javanicus	
13	0.5 g Lipase L115/MP 1000	Biocatalysts	Porcine pancreas	
14	0.5 g Lipomod 36/MP 1000	Biocatalysts	R.javanicus	
15	1 g Novozym 435	Novozymes	C.antarctica B	Immobilizate
16	1 g Lipozym RM IM	Novozymes	R.miehei	Immobilizate

Mixture	% Ethyl ester	% Monoglyceride	% Diglyceride	% Triglyceride
1	18.2	1.4	5.0	75.4
2	39.3	16.2	14.5	29.5
3	62.7	23.5	10.9	0.5
4	58.5	29.6	9.6	0.0
5	5.2	1.6	4.6	88.6
6	41.7	16.5	27.7	14.1
7	82.4	6.8	7.0	2.9
8	57.7	32.7	8.3	0.0
9	15.9	4.1	14.8	65.2
10	0.0	0.0	2.1	96.2
11	2.0	0.4	1.6	96.0
12	3.4	0.0	2.4	94.2
13	2.2	0.4	2.3	95.1
14	3.3	0.0	2.8	93.9
15	41.0	0.0	2.2	55.8
16	3.7	0.0	2.3	94.0

Result:

It was found that all the immobilized lipases tested showed alcoholysis activity and are therefore suitable in principle for the production of the compositions according to the invention. Particularly good reactions were achieved with immobilized Thermomyces, Rhizopus and Porcine Pancreas; moderate conversion rates were observed with Rhizomucor and Mucor lipases. Under the test conditions, the free lipases showed distinctly poorer conversion rates. Only free lipase from Thermomyces showed significant product formation.

Example 2: Regioselective alcoholysis of sunflower oil with non-immobilized lipases

6 mixtures consisting of 40 g sunflower oil and 10 g ethanol were placed in glass beakers equipped with magnetic stirrers. 0.4 g water was

added with stirring. 40 mg solid Na_3PO_4 x 12 H_2O were added to mixtures 2, 4 and 6. 0.4 g lipolase (*Thermomyces lanugenosus* lipase, liquid preparation) was added to mixtures 1 and 2, 0.4 g Novozym 525 (*Candida antarctica B* lipase, liquid preparation) to mixtures 3 and 4 and 0.4 g Novozym 388 (*Rhizomucor miehei* lipase, liquid preparation) to mixtures 5 and 6. The alcoholysis was carried out at room temperature on a multistirrer plate. Samples were taken after 16 h and 44 h and the content of glycerides was analyzed by gas chromatography. The results were evaluated as percentage areas.

Mixture	Duration	% Ethyl ester	Monoglyceride content	Mono-:di-:triglyceride ratio
1	16	0	0 %	0 : 12 : 88
1	44	0.7	0 %	0 : 4 : 96
2	16	**55.1**	**26.5 %**	63 : 33 : 4
2	44	**61.1**	**23.3 %**	69 : 31 : 0
3	16	0.7	0 %	0 : 2 : 98
3	44	2.2	0 %	0 : 4 : 96
4	16	0.7	0 %	0 : 2 : 98
4	44	2.2	0 %	0 : 4 : 96
5	16	7.6	0 %	0 : 4 : 96
5	44	4.9	1.2 %	2 : 7 : 91
6	16	2.1	0 %	0 : 3 : 97
6	44	4.1	0.9 %	1 : 5 : 94

Result:

Lipolase in the presence of a basic salt showed significant activity (mixture 2). If, by contrast, no salt was added, only a very weak alcoholysis reaction could be detected. Weak activity was detected with Novozym 388, but was not dependent on the addition of salt.

Example 3: comparison of the activity of immobilized lipolase and lipolase liquid preparation

Mixtures containing 0.2 g lipolase liquid preparation or a corresponding amount of lipolase fixed to a carrier were compared.

Immobilization of lipolase on Accurel MP 1000 (Membrana): 5 g MP 1000 were placed in a 250 ml Erlenmeyer flask and 15 ml ethanol were added. The mixture was shaken for 1 hour, after which ethanol was decanted off. 50 g water were added to the MP 1000. After stirring for 1 h, the water was decanted off. 100 ml phosphate buffer, 20 mM, pH 6.0, were added and the immobilization was started by addition of 5 g lipolase liquid preparation. The mixtures were stirred overnight at 8°C, after which the enzyme immobilizate was filtered off. The immobilizate was dried overnight at room temperature between paper towels. The immobilizate was weighed out and a quantity of immobilizate corresponding to 0.2 g lipolase liquid preparation was used for the alcoholysis.

Immobilization of lipolase on Accurel MP 1000 (Membrana), alternative: Immobilization was carried out as described above. After the immobilizate had been filtered off, 5 ml of a 200 mM Na_3PO_4 solution were added. The complete mixture was dried in vacuo at room temperature. The object of this additional step was to prepare an already alkaline immobilizate. The immobilizate was weighed out and a quantity of immobilizate corresponding to 0.2 g lipolase liquid preparation was used for the alcoholysis.

Immobilization of lipolase on Dowex Marathon WBA (Dow Chemicals): 200 mg Dowex WBA were placed in a small glass beaker. 0.2 g lipolase liquid preparation were added by pipette and thoroughly mixed with the tip of a pipette. The mixture was incubated for 2 h at room temperature with occasional mixing. The complete mixture (Dowex + supernatant) was used for the transformation. Parallel tests where unbound lipolase was obtained

from the immobilizate by washing out showed that around 90% of the lipolase present was fixed to a carrier.

Immobilization of lipolase on Duolite A 568 (Rohm & Haas): 200 mg Duolite A 568 were placed in a small glass beaker. 0.2 g lipolase liquid preparation were added by pipette and thoroughly mixed with the tip of a pipette. The mixture was incubated for 2 h at room temperature with occasional mixing. The complete mixture (Duolite + supernatant) was used for the transformation. Parallel tests where unbound lipolase was obtained from the immobilizate by washing out showed that around 80% of the lipolase present was fixed to a carrier.

Test procedure:

10 mixtures consisting of 40 g sunflower oil and 10 g ethanol were placed in glass beakers equipped with magnetic stirrers. 0.4 g water was added with stirring. 50 mg solid Na_2CO_3 were added to mixtures 2, 4, 6, 8 and 10. 0.2 g lipolase (*Thermomyces lanugenosus* lipase, liquid preparation) was added to mixtures 1 and 2, the Dowex immobilizates to mixtures 3 and 4, the Duolite immobilizates to mixtures 5 and 6, the MP 1000 immobilizates to mixtures 7 and 8 and the MP 1000 immobilizates aftertreated with Na_3PO_4 to mixtures 9 and 10. The alcoholysis was carried out at room temperature on a multistirrer plate. Mixtures 3 to 10 were treated twice. Samples were taken after 16 h and the content of glycerides was analyzed by gas chromatography. The results were evaluated as percentages areas.

Mixture	% Ethyl ester	Monoglyceride content	Mono-:di-:triglyceride ratio
1	0	0 %	0 : 3 : 97
2	**56.1**	**28.5 %**	70 : 30 : 0
3 (1)	25.6	11.5 %	16 : 23 : 61

3 (2)	26.4	10.2 %	14 : 18 : 68
4 (1)	31.6	14.1 %	21 : 36 : 44
4(2)	37.9	15.7 %	26 : 30 : 45
5 (1)	17.6	7.4 %	9 : 13 : 78
5 (2)	22.6	9.3 %	12 : 15 : 73
6 (1)	35.5	17.1 %	27 : 34 : 39
6 (2)	28.5	12.8 %	18 : 19 : 63
7 (1)	15.5	5.5 %	7 : 20 : 73
7 (2)	24.8	8.5 %	11 : 27 : 61
8 (1)	26.1	10.5 %	14 : 37 : 49
8 (2)	44.1	20.0 %	36 : 40 : 24
9 (1)	24.4	9.1 %	12 : 43 : 45
9 (2)	14.2	3.5 %	4 : 13 : 83
10 (1)	8.4	2.4 %	3 : 18 : 79
10 (2)	15.9	4.3 %	5 : 14 : 81

Result:

All the immobilizates containing lipolase showed alcoholysis activity. With the exception of the immobilizate pretreated with Na_3PO_4, all the immobilizates showed additional activation by Na_2CO_3. However, the activation of the liquid lipolase by Na_2CO_3 is considerably stronger than the activation of the immobilizates. For the same weighed quantity of enzyme, alcoholysis with salt-activated lipolase (mixture 2) was much faster than with the immobilizates. By contrast, immobilization allowed repeated use of the enzyme and hence the use of a larger quantity of enzyme.

Example 4: reaction with various alcohols

Various mixtures consisting of 40 g sunflower oil and variable quantities of various alcohols were subjected to an alcoholysis reaction with lipolase at room temperature. The mixtures had the composition shown in the following Table:

Mixture	Alcohol	Water	Salt	Lipolase
1	10 g Ethanol	0.4 g	40 mg Na_3PO_4	0.4 g
2	13 g Propanol	0.4 g	40 mg Na_3PO_4	0.4 g
3	13 g Isopropanol	0 g	40 mg Na_3PO_4	1.2 g
4	16 g Butanol	0.4 g	40 mg Na_3PO_4	0.4 g
5	16 g Isobutanol	0 g	40 mg Na_3PO_4	1.2 g
6	19 g Isoamyl alcohol	0.4 g	40 mg Na_3PO_4	0.8 g
7	22 g Hexanol	0.4 g	40 mg Na_3PO_4	0.4 g
8	28 g 2-Ethylhexanol	0.4 g	40 mg Na_3PO_4	1.2 g
9	7 g Methanol	0 g	40 mg Na_3PO_4	1.2 g
10	16 g Butanol	0 g	25 mg Na_2CO_3	1.2 g
11	16 g Butanol	0 g	50 mg Na_2CO_3	0.6 g
12	16 g Butanol	0.8 g	50 mg Na_2CO_3	0.6 g
13	23 g Hexanol	0.8 g	25 mg Na_2CO_3	1.2 g
14	24 g Hexanol	2.8 g	25 mg Na_2CO_3	1.2 g
15	22 g Hexanol	2.8 g	50 mg Na_2CO_3	0.6 g

The content of glycerides and esters was analyzed by gas chromatography. The results were evaluated as percentage areas, the excess free alcohols not being included. Samples were taken at the times shown in the Table.

Mixture	Duration [h]	% Alkyl ester	Monoglyceride content	Mono-:di-:triglyceride ratio
1	16	59.3	26.4 %	72 : 28 : 0
2	16	58.8	28.3 %	74 : 26 : 0
3	16	30.6	8.7 %	13 : 55 : 32
4	44	42.1	17.1 %	30 : 44 : 26
5	44	41.4	17.9 %	31 : 41 : 28
6	44	43.5	17.1%	31 : 46 : 23
7	44	25.1	6.9 %	9 : 36 : 55
8	44	27.8	14.5 %	37 : 42 : 20
9	16	43.7	18.3 %	34 : 12 : 54

10	40	59.7	26.3 %	70 : 30 : 0
11	16	57.9	26.5 %	67 : 29 : 4
12	16	29.4	11.9 %	17 : 33 : 50
13	40	29.3	9.2 %	13 : 43 : 44
14	40	69.9	19.6 %	67 : 33 : 0
15	16	29.6	18.0 %	26 : 45 : 30

Result:

An alcoholysis reaction was observed with all the alcohols used. The enzyme accepts primary and secondary alcohols and linear and branched alcohols. The best reaction was observed with the alcohols ethanol and propanol in a reaction medium containing 2% water. For the other alcohols, the reaction conditions had to be slightly modified in part in order to achieve an optimal conversion. Detailed investigations with butanol (mixtures 10 to 12) and with hexanol (mixtures 13 to 15) showed that, even with these alcohols, the production of glycerides with a monoglyceride content of >60% is possible. The reaction with butanol takes place better in the medium containing relatively little water whereas the reaction with hexanol only takes place successfully in the presence of relatively large quantities of water. It may generally be concluded from this that the concentration of water has to be increased if the alcohol becomes more hydrophobic in order to achieve an optimal reaction rate.

Example 5: Influence of ethanol concentration on glycerol formation, acid formation and monoglyceride content

Various mixtures consisting of 40 g sunflower oil and variable quantities of ethanol were subjected to an alcoholysis reaction with 0.2 g lipolase at room temperature. Quantities of 25 mg Na_2CO_3 were added.

The mixtures had the composition shown in the following Table:

Mixture	Ethanol	Water

1	15 g	0.2 g
2	30 g	0.2 g
3	15 g	0.4 g
4	30 g	0.4 g
5	15 g	0.8 g
6	30 g	0.8 g

The content of glycerides was analyzed by gas chromatography. The results were expressed as percentage areas. The glycerol content was also analyzed by gas chromatography. The results are expressed as non-calibrated percentage areas. According to mass balance, the absolute glycerol contents are lower, although the key factor here is comparison of the relative values. GC samples were taken after a reaction time of 16 h for the glycerol determination and after a reaction time of 40 hours for the glyceride determination. Acid values were determined after 16 h.

Mixture	**Acid value**	**% Glycerol**	% Ethyl ester	% Monoglyceride	Mono-:di-:triglyceride ratio
1	2	1.5 %	62.2	29.2 %	86 : 14 : 0
2	1	0.3 %	34.5	11.4 %	18 : 35 : 47
3	3	**2.4 %**	64.3	26.2 %	86 : 14 : 0
4	1	**0.5 %**	58.9	30.6 %	77 : 23 : 0
5	5	2.8 %	64.7	25.8 %	87 : 13 : 0
6	2	1.1%	62.4	32.2 %	92 : 8 : 0

Since glycerol shows a comparatively stronger adsorption than the ethyl esters and glycerides in the GC method used, a calibration was carried out directly in a mixture of ethyl ester, free ethanol and glycerides. The adsorption over a concentration range of 0 to 1.0% by weight glycerol corresponds to the formula:

$y = 2.3x$ (y = adsorption, x = weighed amount)

The following pattern emerges from the above analysis:

Mixture	Glycerol measured	Glycerol (% by wt.) after calibration
1	1.5	0.65
2	0.3	0.13
3	2.4	1.04
4	0.5	0.22
5	2.8	1.22
6	1.1	0.48

Result:

The higher the concentration of alcohol used, the higher the monoglyceride contents obtained. Based on the total glycerides. monoglyceride contents of more than 90% can be achieved.

An increase in the alcohol content led to a reduction in the formation of by-products, such as free fatty acid or glycerol formed from the total hydrolysis of the oil.

The reaction rate was reduced when the alcohol content was increased. The reaction rate was improved by increasing the water content, so that good monoglyceride formation was achieved even with a large molar excess of ethanol (mixture 6).

Example 6: reaction with various oils

Hydrolysis was investigated in parallel tests using various oils. Quantities of 40 g of the oil were weighed into glass beakers with 10 g ethanol. Quantities of 0.4 g water were added with stirring, followed by the addition of 40 mg solid Na_3PO_4 x 12 H_2O. The reaction was started by addition of 0.4 g lipolase. After a reaction time of 16 h, a sample was taken for analysis by gas chromatography. The results are expressed as percentages areas.

Mixture	Oil	% Ethyl ester	% Monoglyceride	Mono-:di-:triglyceride ratio
1	Sunflower oil	59.3	26.4 %	72 : 28 : 0
2	Rapeseed oil	58.7	26.4 %	73 : 27 : 0
3	Thistle oil	60.9	26.0 %	76 : 24 : 0
4	Sunflower oil 2	60.0	26.7 %	76 : 24 : 0
5	Castor oil	57.5	30.0 %	73 : 27 : 0
6	Soybean oil	60.3	26.4 %	75 : 25 : 0
7	Fish oil	51.0	35.0 %	78 : 22 : 0
8	50% rapeseed oil + 50% palm oil	60.7	25.9 %	75 : 25 : 0
9	Lard	75.4	20.7 %	72 : 28 : 0

Result:

Good alcoholysis was observed with all the oils used. A monoglyceride content of > 70%, based on total glycerides, was achieved with all the oils.

Example 7: reaction with various alkaline salts

5 mixtures of 40 g sunflower oil and 10 g ethanol were weighed in. 0.4 g water was added with stirring to all 5 mixtures. 40 mg Na_3PO_4 x 12 H_2O was added to mixture 1, 11 mg Na_2CO_3 to mixture 2, 4 mg $Ca(OH)_2$ to mixture 3 and 31 mg trisodium citrate x 2 H_2O to mixture 4, No salt was added to mixture 5. The reactions were started by addition of 0.4 g lipolase. After a reaction time of 16 h, a sample was taken for analysis by gas chromatography. The results are expressed as percentage areas.

Mixture	% Ethyl ester	Monoglyceride content	Mono-:di-:triglyceride ratio
1	59.3	26.4 %	72 : 28 : 0
2	62.1	23.3 %	74 : 26 : 0
3	50.5	28.9 %	65 : 35 : 0
4	1.0	0 %	0 : 3 : 97

5	0.7	0 %	0 : 2 : 98

Result:

The alcoholysis reaction was successful with additions of phosphate salts, carbonate salts and hydroxides.

Example 8: optimization of the salt concentration used (for Na_2CO_3)

12 mixtures of 40 g sunflower oil and 10 g ethanol were weighed in. 0.2 g water was added with stirring to mixtures 1 to 6 and 0.4 g water to mixtures 7 to 12. Various quantities of salt as shown in the following Table were then added. The reactions were started by the addition of 0.2 g lipolase. After a reaction time of 16 h, a sample was taken for analysis by gas chromatography. The results are expressed as percentage areas.

Mixture	Na_2CO_3	% Ethyl ester	Monoglyceride content	Mono-:di-:triglyceride ratio
1	10 mg	30.0	14.7 %	21 : 32 47
2	25 mg	53.0	29.3 %	65 : 32 : 3
3	50 mg	54.5	30.2 %	70 : 30 : 0
4	100 mg	55.9	29.1 %	70 : 30 : 0
5	200 mg	43.4	22.4 %	41 : 41 : 19
6	500 mg	4.4	0.9 %	1 : 7 : 92
7	10 mg	44.2	23.5 %	43 : 38 : 19
8	25 mg	50.3	27.2 %	56 : 38 : 6
9	50 mg	55.4	30.2 %	72 : 28 : 0
10	100 mg	56.9	28.5 %	72 : 28 : 0
11	200 mg	57.2	27.5 %	70 : 30 : 0
12	500 mg	36.1	16.4 %	26 : 39 : 35

Result:

An increase in the water content in the mixture produces a slight shift in the optimal quantity of Na_2CO_3. With an addition of 0.2 g water, the

range for the optimal quantity of salt extends from 25 mg to 100 mg whereas, with an addition of 0.4 g water, the optimal range is between 50 mg and 200 mg.

It should be noted that the optimum of basic additive depends on the quantity of buffered enzyme solution used and on the strength of the base. The test series with Na_2CO_3 may be regarded as exemplary.

Example 9: influence of temperature on the transesterification rate

6 mixtures of 40 g sunflower oil and 10 g ethanol were weighed in. 0.4 g water and 50 mg Na_2CO_3 were added to the mixtures with stirring. The reactions were started by the addition of 0.2 g lipolase. The reactions were carried out at different temperatures as shown in the following Table. After a reaction time of 24 h, a sample was taken for analysis by gas chromatography. The results are expressed as percentage areas.

Mixture	Temperature °C	% Ethyl ester	Monoglyceride content	Mono-:di-:triglyceride ratio
1	20 °C	30.0	14.7 %	21 : 32 47
2	25°C	53.0	29.3 %	65 : 32 : 3
3	30°C	54.5	30.2 %	70 : 30 : 0
4	35C	55.9	29.1 %	70 : 30 : 0
5	40°C	43.4	22.4 %	41 : 41 : 19
6	45°C	4.4	0.9 %	1 : 7 : 92

Result:

The lipase is clearly deactivated even at temperatures as low as 30°C upwards. The optimal reaction temperature is in the range from 20 to 25°C

Example 10: synthesis of ethyl ester/partial glyceride mixtures with measured additions of ethanol

1200 g rapeseed oil, 75 g ethanol, 0.375 % water, based on the quantity of oil, and 0.025% NaOH with a concentration of 1 mol/l were introduced into a heatable 2-liter double-jacketed reactor. The mixture was cooled with stirring to 15°C, after which 0.25% lipolase, based on the quantity of oil, was added. The mixture was incubated while stirring for 48 h at 15°C. After 2.5 h, another 75 g ethanol and, after 5 h, 150 g ethanol were introduced into the reactor. After 48 h, the contents of the reactor were heated for 1 h to 80°C to deactivate the enzyme. The final product mixture was a single-phase mixture.

Analysis by gas chromatography produced the following composition (percentage areas, ethanol not included): 58.2% ethyl ester, 25.6% monoglyceride, 17.1% diglyceride, 0.7% triglyceride. Mathematically, the mixture still contains ca. 12% by weight free ethanol.

Example 11: synthesis with continuous addition of ethanol + working up of ethyl ester/partial glyceride mixtures

1000 g rapeseed oil, 50 g ethanol and 0.025% NaOH with a concentration of 1 mol/l were introduced into a heatable 2-liter double-jacketed reactor. The mixture was cooled with stirring to 17°C, after which 0.25% lipolase, based on the quantity of oil, was added. The mixture was incubated while stirring for 45 h at 17°C. After the reaction had been started, 200 g ethanol were pumped continuously into the reactor at a flow rate of 0.14 ml/min. After 45 h, 0.1% by weight Tonsil was introduced into the reactor and the contents of the reactor were heated. After incubation for 1 hour at 75°C, the contents of the reactor were filtered off. To remove residues of free glycerol, 500 g of the product were washed twice with 250 g water, the reaction system only being stirred slowly in order to avoid emulsion formation. The glycerol- and alkali-containing aqueous phase was separated from the oil. The final product mixture was a clear, single-phase mixture.

Analysis by gas chromatography produced the following composition (percentage area, ethanol not included):

A) Before removal of glycerol	B) After removal of glycerol
56.9% ethyl ester	59.9% ethyl ester
28.6% monoglyceride	29.6% monoglyceride
14.2% diglyceride	10.6% diglyceride
0.3% triglyceride	1.8% triglyceride

Mathematically, the mixture still contains ca. 12% free ethanol before washing with water. The free glycerol content of the washed end product is below 0.05% by weight. Before washing, the product had a glycerol content after calibration of 1.1% by weight.

Example 12: storage stability of the reaction products of Example 11

The products of Example 11 were placed in glass bottles and stored for 55 days in daylight at room temperature. Comparative GC analyses were carried out.

A) Before removal of glycerol		B) After removal of glycerol	
Day 1	Day 56	Day 1	Day 56
56.9 %	55.7 % Ethyl ester	59.9 %	
28.6 %	29.3 % Monoglyceride	29.6 %	28.6 % Monoglyceride
14.2 %	13.3 % Diglyceride	10.6 %	9.9 % Diglyceride
0.3 %	1.7 %	1.8 %	2.0 % Triglyceride

Result:

Within the accuracy limits of GC analysis, the samples were unchanged after 55 days. Accordingly, the biodiesel produced by the enzymatic process is stable in storage for at least 55 days.

Example 13: removal of glycerol from the reaction products of

Example 11

Quantities of 50 g of the unwashed product of Example 11 were washed twice with 2% by weight water and twice with 5% by weight water. After each washing step, the aqueous phase was separated. The following glycerol contents were obtained:

	Glycerol (% by weight)
Product before washing:	1.1
2x washing with 50% water (Example 10)	< 0.05
2x washing with 5% water	0.15
2x washing with 2% water	0.39

Result:

Glycerol can be removed from the product by washing with water over a broad concentration range and subsequent phase separation.

Example 14: Performance tests in diesel fuels

Two samples of enzymatically produced biofuel were tested as an additive to normal gas station diesel. The product of Example 10 was used for this purpose both without removal of glycerol (code: USC-CM-8327-131DS) and after removal of glycerol by washing with water (code: USC-CM-8327-131).

USC-CM-8327-131:

Mixture of ethyl ester + monoglyceride + ethanol, glycerol content <0.05% by weight

USC-CM-8327-131 DS:

Mixture of ethyl ester + monoglyceride + ethanol, glycerol-containing (glycerol content > 1% by weight)

The mixtures were tested for low-temperature behavior as 2.5, 3 and 5% by weight additions to gas station diesel. To this end, the CFPP values of the samples were determined.

Biofuel [%]	USC-CM-8327-131 CFPP value [°C]	USC-CM-8327-131 DS CFPP value [°C]
2.5	-15	-16
3	-15	-16
5	-14	-14

CFPP value, gas station diesel with no addition: -15°C

Result:

In relatively low concentrations, there were no significant deteriorations in the CFPP. Only relatively high concentrations produced an increase in the CFPP by 1°C.

Storage of the two mixtures at low temperatures led to slight clouding in the diesel/biofuel mixture at -20°C without any adverse effect on pumpability. At 4°C, the mixture remains unchanged, even after several weeks.

Example 15: production of monoglyceride-containing mixtures for testing lubricating properties

Mixture 1: 50 g Accurel MP 1000 were incubated for 1 h with 500 g ethanol. After removal of the ethanol, 500 g water and 50 g lipolase were added and the mixture was stirred for 24 h. After removal of the water, the immobilizate was dried. The immobilizate was placed in a 3-liter reactor and 1.6 kg sunflower oil, 0.4 kg ethanol and 8 g water were added. The reaction mixture was incubated with stirring for 24 h at room temperature. After the end of the reaction, the immobilizate was filtered off and the excess water/ethanol mixture was removed from the reactor. 16 g Tonsil and 2 g water were added to the sample, followed by incubation for 30 mins. at 80°C. The sample was then dried in vacuo and the Tonsil was removed by filtration. The ethyl ester/partial glyceride mixture thus obtained was used for the lubrication tests.

Mixture 2: 25 g lipolase was pipetted onto 25 g Dowex Marathon WBA. The mixture was mixed and stored for 2 h in a refrigerator for immobilization. 4 kg rapeseed oil and 1 kg ethanol were placed in a 6-liter reactor. The immobilizate was added to the reaction mixture with stirring, followed by incubation for 45 h with stirring. After the reaction, the immobilizate was filtered off and the excess water/ethanol mixture was removed in a rotary evaporator at 80°C/50 mbar. The ethyl ester/partial glyceride mixture was then subjected to short-path distillation. The ethyl esters were removed by distillation at 175°C under a vacuum of 0.3 mbar. The bottom product was used for the lubrication tests.

Mixture 3: 25 g lipolase was pipetted onto 25 g Dowex Marathon WBA. The mixture was mixed and stored for 2 h in a refrigerator for immobilization. 1.83 kg rapeseed oil and 0.7 kg butanol were placed in a 3-liter reactor. The immobilizate was added to the reaction mixture with stirring, followed by incubation for 60 h with stirring. After the reaction, the immobilizate was filtered off and the excess water/butanol mixture was removed in a rotary evaporator at 80°C/50 mbar. The butyl ester/partial glyceride mixture thus obtained was used for the lubrication tests.

The product compositions obtained are shown in Example 16.

Example 16: Testing of the lubricating properties in diesel fuel

The lubricating properties were subjected to an HFFR test (high-frequency reciprocating rig test) by CEC method F-06-T-94. Various diesel fuels and monoglyceride mixtures based on sunflower oil and rapeseed oil from Example 15, as shown in the following Table, were used.

Number	Sample	Raw material
Sample 1	Monoglyceride/ethyl ester mixture	Sunflower oil
Sample 2	Monoglyceride mixture distilled	Rapeseed oil

Sample 3	Monoglyceride/butyl ester mixture	Rapeseed oil

	Ester	Monoglyceride	Diglyceride	Triglyceride
Sample 1	56.0	27.8	12.8	< 1
Sample 2	3.5	61.0	32.0	2.5
Sample 3	66.0	21.5	9.0	< 1

Results:

Number	Concentration in diesel	HFFR value	Film
Diesel A	Blank	411 µm	19 µm
Sample 1	200 ppm	261 µm	67 µm
Diesel B	Blank	542 µm	20 µm
Sample 1	100 ppm	311 µm	65 µm
Sample 1	150 ppm	217 µm	70 µm
Sample 1	200 ppm	231 µm	68 µm
Diesel C	Blank	615 µm	
Sample 2	100 ppm	183 µm	
Sample 2	300 ppm	170 µm	
Sample 3	100 ppm	279 µm	
Sample 3	300 ppm	195 µm	

Result:

All samples significantly improve the lubricating properties of the diesel fuels used and reduce the HFFR values to below prescribed limits (for example currently 450 µm in Switzerland).

Example 17: Enzymatic synthesis of an ethanol-containing ethyl ester/partial glyceride mixture

A total of 1600 kg refined rapeseed oil, 640 kg ethanol, 600 ml 1 M NaOH, 7 l water and 250,000 U lipase (esterase from *Thermomyces*, units

according to the manufacturer) based on 1 kg rapeseed oil were placed in a 4000-liter reactor. The mixture was stirred for 40 h, heated with stirring to 80°C and then stirred for 2 h at 80°C, the reactor remaining closed so that no ethanol could escape. The mixture was then cooled to 50°C and filtered through a drum filter containing 10 kg Celatom FW 14. The product was poured into casks and stored at room temperature.

Result:

2200 kg product were obtained, corresponding to a yield of 98%.

Example 18: production of distilled ethyl ester/partial glyceride mixture

A total of 1600 kg refined rapeseed oil, 640 kg ethanol, 600 ml 1 M NaOH, 7 l water and 250,000 U lipase (esterase from Thermomyces, units according to the manufacturer), based on 1 kg rapeseed oil, were introduced into a 4000 l reactor. The mixture was stirred for 40 h and then heated with stirring to 120°C. Vacuum was applied to the reactor and the ethanol/water mixture was removed from the reactor. The vacuum was slowly reduced until no more ethanol escaped from the mixture. The mixture was then cooled to 50°C and filtered through a drum filter containing 10 kg Celatom FW 14. The product was poured into casks and stored at room temperature.

Result:

1742 kg product and 470 kg distillate were obtained, corresponding to a yield of 98%.

Example 19: analysis of the test products from Examples 17 and 18

The analyses shown in the following Table were carried out with the test products of Examples 17 and 18.

Values	**Product of Example 17**	**Product of Example 18**
Hydroxyl value	ca. 318 - 335	107
Iodine value	83	105
Peroxide value	9.1	9.6
Acid value	1.9	2.7
Saponification value	136	173
Density	0.875 g/ml	0.9 g/ml
Color values		
Lovibond 5-1/4	19 / 2.3	35 / 3.2
Lovibond 1	2.0 / 0.6	3.5 / 0.8
Gardner	2.6	3.9
Trace analysis		
Nitrogen	< 20 mg/kg	< 20 mg/kg
Sulfur	< 2 mg/kg	< 2 mg/kg
Sodium	8 mg/kg	10 mg/kg
Iron	0.4 mg/kg	0.7 mg/kg
Phosphorus	< 3 mg/kg	< 3 mg/kg
Composition		
Water content	0.3	0.01
Glycerol free	0.20%	0.30%
Glycerol bound	6.80%	9.00%
Ethanol	21.90%	0.20%
Ethyl ester	41.00%	55.50%
Monoglycerides	23.00%	32.20%
Diglycerides	13.00%	11.40%
Triglycerides	< 1 %	< 1 %
Fatty acid spectrum		
Palmitic acid	5.20%	4.80%
Stearic acid	1.30%	1.30%
Oleic acid	58.30%	60.20%
Linoleic acid	21.10%	20.80%
Linolenic acid	8.80%	8.30%

Result:

The test products are a mixture consisting mainly of ethyl esters and monoglycerides based on the fatty acid composition of rapeseed oil. Diglycerides are present in relatively small amounts; by-products are fatty acids and triglycerides. The non-distilled mixture additionally contains ethanol and a small amount of water. The test products have a good color corresponding to that of the oils used. The contents of organic and inorganic substances are low. Glycerol analysis shows that the glycerol of the triglyceride is almost completely bound in the form of the partial glycerides and less than 5% of the glycerol is present in free form.

Example 20: Stability of the test products of Examples 17 and 18

The products of Example 17 and Example 18 were placed in stoppered casks and stored for 3 months.

	Product of Example 17	**Product of Example 17**	**Product of Example 18**	**Product of Example 18**
Composition	After synthesis	After 3 months	After synthesis	After 3 months
Ethyl ester	41.00%	40.10%	55.50%	55.00%
Monoglycerides	23.00%	22.30%	32.20%	31.20%
Diglycerides	13.00%	13.30%	11.40%	12.00%
Triglycerides	< 1 %	1.90%	< 1 %	0.90%
Glycerol free (titr.)	0.20%	0.25%	0.30%	0.50%
Glycerol free (GC) (not calibrated / area%)	1.10%	0.70%	0.90%	0.90%
Values	After synthesis	After 3 months	After synthesis	After 3 months
Acid value	1.9	1.9	2.7	2.8
POV	9.1		9.6	
Lovibond	2.0 / 0.6	1.9 / 0.6	3.5 / 0.8	3.0 / 0.8
Gardner	2.6	2.6	3.9	3.6

Result:

The products are sufficiently stable in storage for use as a diesel additive or fuel additive for diesel.

Example 21: Comparison of the lubricating effect between FAME (fatty acid methyl ester) and the composition according to the invention

The HFFR values of different mixtures of diesel with FAME were determined in comparison with a 3% mixture of the composition of Example 18 according to the invention and the lubricating effect thus investigated. The test is described in ISO 12156. In the test, a metal pin is drawn over a metal plate and the size of the scar is determined. It follows that the smaller the scar, the better is the lubricating effect.

The composition according to the invention which was added to diesel to obtain a 3% mixture contained the following % by weight distribution: 55.5% ethyl ester, 32.3% monoglycerides, 11.4% diglycerides, <1% by-products

Results:

HFFR Test to ISO 12156	Wear scar [μm]
DIN EN 590	460
Diesel	600
Diesel + 0.5 % FAME	540
Diesel + 1 % FAME	370
Diesel + 2.5 % FAME	320
Diesel + 5 % FAME	310
Diesel + 3 % composition acc. to the invention	220

It was shown that the addition of the composition according to the invention to conventional diesel improves lubricity overproportionally by comparison with mixtures with fatty acid methyl ester in various concentrations. The specific EN limit for the wear scar is 460 µm.

Example 22: Suitability of the composition of Example 18 according to the invention as a fuel additive under EU guidelines

	Saybolt Institute	ISO/ASTM equivalent		2.97% composition acc. to invention	Diesel
Test	Test method	Test method	Unit	Analysis results	Specification
				Saybolt	EN 590
Cetane number	EN ISO 5165	ASTM D 613		51.8	min 51
Cetane index		ASTM D 976/ISO 4264		49.7/50.3	min 46
Density at 15°C	ASTM D 4052	ISO 12185	kg/l	0.8341	min 820/max 845
Flash point	ASTM D 93	ISO 2719	°C		min 55
Flash point (PM)		ISO 3679		67	
Viscosity at 40°C	ASTM D 445	ISO 3104	mm/2s	2.453	min 2.0/max 4.50
Carbon Residue Micro (on 10% dist res)	ASTM D 4530	ISO 10370	wt%	<0.1	max 0.30
Cloud point	ASTM D 2500	ISO 3015	°C	-8	only spec Arctic grades
Lubricity	ISO 12156	ASTM D 6079	µm	220	max 460
Distillation (atmospheric)	ASTM D 86	ISO 3405			
Distillation (vacuum)	ASTM D 1160				
IBP			°C	175.1	
5% v			°C	198.8	
10% v			°C	207.2	
15% v			°C	212.5	
20% v			°C	217.7	
30% v			°C	229.9	
40% v			°C	242.2	
50% v			°C	254.8	
60% v			°C	268.5	
70% v			°C	285.7	
78% v			°C		
80%			°C	307.5	
90% v			°C	333.3	
95% v			°C	349.7	max 360
FBP			°C	354.9	
% v recovered at 250°C			% vol	46.2	max 65
% v recovered at 350°C			% vol	95.3	min 85

Explanations:

Saybolt Institute: an institute in Rotterdam for independently determining the measure values.

Carbon residue micro (on 10% dist res): this test is carried out to determine the carbon residues in the diesel. To this end, a sample is evaporated in a stream of nitrogen and the residue is weighed. "Micro" stands for the method. For materials expected to produce a residue of less than 0.1%, a 10% distillation residue is first prepared and then measured.

Determination of distillation behavior:

IBP: initial boiling point

FBP: final boiling point. The percentage figure represents the percentage of diesel evaporated at the respective temperatures

The improved lubricity is apparent as a distinct advantage.

Example 23: Chemical partial transesterification

93 g rapeseed oil, 4 g methanol and 3 g sodium methylate in methanol (20%) were introduced into a flask. The reaction mixture was heated with stirring and incubated while stirring for 1 h under reflux through an attached reflux condenser. After the end of the reaction, the reaction mixture was neutralized with citric acid solution and washed with 50 g water. The product separated off was re-washed with 50 g water. The water phase was then removed. Samples were taken after the synthesis and after the two washing steps and analyzed by gas chromatography. The results are expressed as percentage areas. Small amounts of fatty acid formed are included in the ethyl ester area.

Step	Glycerol	Ethyl ester	Monoglyceride	Diglyceride	Triglyceride
After Synthesis	3.9 %	64.9 %	10.7 %	14.7 %	6.4 %
Wash 1	1.6 %	64.4 %	10.9 %	15.5 %	7.6 %
Wash 2	0.2 %	65.0 %	10.0 %	17.3 %	7.8 %

Result:

The chemical partial esterification gives a product mixture consisting of esters and partial glycerides from which large parts of glycerol can

readily be removed simply by washing. The mixture obtained is a single-phase mixture. Of the total of 10% glycerol present in the triglyceride, less than 50% is released in the partial transesterification. The remaining glycerol remains bound in the product. Accordingly, the stream of by-product glycerol is reduced by more than half in this process.

CLAIMS

1. Composition containing

a) alkyl esters with an alkyl group containing 1 to 8 carbon atoms,

b) partial glycerides,

characterized in that it has a free glycerol content of at most 2% by weight, based on the total quantity of the composition.

2. A composition as claimed in claim 1, characterized in that it contains methyl and/or ethyl esters as component (a).

3. A composition as claimed in at least one of claims 1 to 2, characterized in that it has a partial glyceride content of at least 10% by weight, based on the total quantity of the composition.

4. A composition as claimed in at least one of claims 1 to 3, characterized in that it has a triglyceride content of at most 5% by weight, based on the total quantity of the composition.

5. A composition as claimed in at least one of claims 1 to 4, characterized in that it has an acid value of at most 5.

6. A composition as claimed in at least one of claims 1 to 5, characterized in that alkyl esters, monoglycerides and diglycerides are present in the following quantities:

alkyl esters: 30 to 70% by weight
monoglyceride: 10 to 35% by weight
diglyceride: 1 to 30% by weight.

7. A composition as claimed in at least one of claims 1 to 6, characterized in that the alkyl esters and partial glycerides are derived from saturated or unsaturated, linear or branched fatty acids containing 8 to 22 carbon atoms.

8. A process for the production of biofuel, characterized in that triglycerides are enzymatically reacted with an esterase activated by addition of alkaline salts, the reaction being carried out in the presence of alcohols.

9. A process as claimed in claim 8, characterized in that the esterase is deactivated in another step.

10. A process as claimed in any of claims 8 to 9, characterized in that the alcoholysis is carried out at temperatures of 10°C to 40°C and with a water content of 0.1 to 10% by weight, based on the quantity of triglyceride.

11. A process as claimed in any of claims 8 to 10, characterized in that the esterases are used in quantities of 0.05 to 2% of the commercially obtainable liquid preparation, based on the quantity of triglyceride used.

12. A process as claimed in any of claims 8 to 11, characterized in that aqueous solutions of alkaline inorganic salts selected from the group consisting of hydroxides, carbonates and phosphates of sodium, potassium, calcium, magnesium and ammonium are used to activate the esterase.

13. A process as claimed in claim 12, characterized in that the salts are used in quantities of 0.00001 to 1% by weight, based on the triglyceride.

14. A process for the production of biofuel, characterized in that triglycerides are enzymatically reacted with an immobilized and/or chemically modified esterase in the presence of alcohols containing 1 to 8 carbon atoms.

15. A process as claimed in claim 14, characterized in that the esterase is separated from the product mixture in another step.

16. A process as claimed in any of claims 15 to 16, characterized in that the alcoholysis is carried out at temperatures of 10°C to 60°C and with a water content of 0 to 10% by weight, based on the quantity of triglyceride.

17. A process as claimed in any of claims 14 to 16, characterized in that the esterases are immobilized by hydrophobic interaction on plastics,

resins or mineral substrates or by ionic interactions on anion or cation exchangers or by chemical bonding to substrates containing activated chemical groups.

18. A process as claimed in any of claims 14 to 17, characterized in that the esterases are chemically modified by coating with surfactants, by hydrophobicizing the enzyme surface or by chemical crosslinking.

19. A process as claimed in any of claims 8 to 13 or 14 to 18, characterized in that the esterases emanate from organisms selected from the group consisting of *Thermomyces lanugenosus, Candida antarctica A, Candida antarctica B, Rhizomucor miehei, Candida cylindracea, Rhizopus javanicus, Porcine pancreas, Aspergillus niger, Candida rugosa, Mucor javanicus, Pseudomonas fluorescens, Rhizopus oryzae, Pseudompnas sp., Chromobacterium viscosum, Fusarium oxysporum and Penicilium camemberti*.

20. A process as claimed in claim 19, characterized in that the esterases used are lipases.

21. A process as claimed in any of claims 19 to 20, characterized in that 1,3-specific lipases are used.

22. A process as claimed in any of claims 19 to 21, characterized in that the lipase is lipase from *Thermomyces lanugenosus*.

23. A process as claimed in any of claims 8 to 13 or 14 to 18 and/or 19 to 22, characterized in that triglycerides from fats and oils which have a high percentage content of mono- and/or polyunsaturated fatty acids are used.

24. A process as claimed in claim 23, characterized in that triglycerides selected from the group consisting of sunflower oil, rapeseed oil, thistle oil, soybean oil, linseed oil, peanut oil, tallows, olive oil, castor oil, palm oil, yatropha oil, palm kernel oil, coconut oil and old oils are used.

25. A process as claimed in at least one of claims 8 to 13 or 14 to 18 and/or 19 to 22, characterized in that methanol or ethanol is used as the

alcohol component.

26. A process as claimed in at least one of claims 8 to 13 or 14 to 18 and/or 19 to 22, characterized in that the alcohol is used in quantities of 10 to 50% by weight, based on the triglyceride.

27. A process as claimed in at least one of claims 8 to 13 or 14 to 18 and/or 19 to 22, characterized in that the alcohol and/or water are partly or completely removed.

28. A process for the production of biofuel, characterized in that triglycerides are partly chemically reacted in the presence of alcohols containing 1 to 8 carbon atoms.

29. A process as claimed in claim 28, characterized in that the catalyst is separated from the product mixture in another step.

30. A process as claimed in any of claims 28 to 29, characterized in that the alcoholysis is carried out with alcohol concentrations of 10 mol-% to 30 mol-%, based on the oil used.

31. A process as claimed in any of claims 28 to 30, characterized in that the alcoholysis is preferably carried out with ethanol or methanol.

32. A process as claimed in any of claims 28 to 31, characterized in that the alcoholysis is carried out as a batch reaction or as a continuous reaction in co-current or countercurrent.

33. A process as claimed in any of claims 28 to 32, characterized in that the alcoholysis is carried out with an alkaline metal alcoholate in a concentration of 0.01% by weight to 5% by weight under pressures of up to 2 bar and at a temperature of 40°C to 120°C.

34. A process as claimed in any of claims 28 to 32, characterized in that the alcoholysis is carried out with sulfuric acid or a sulfonic acid in a concentration of 0.01% by weight to 5% by weight under pressures of up to 5 bar and at a temperature of 40°C to 120°C.

35. A process as claimed in any of claims 28 to 32, characterized in that the alcoholysis is carried out with metal salts or metal soaps in a

concentration of 0.01% by weight to 1% by weight under pressures of 20 to 200 bar and at a temperature of 120°C to 250°C.

36. A process as claimed in any of claims 28 to 35, characterized in that triglycerides from fats and oils which have a high percentage content of mono- and/or polyunsaturated fatty acids and which are selected from the group consisting of sunflower oil, rapeseed oil, thistle oil, soybean oil, linseed oil, peanut oil, tallows, olive oil, castor oil, palm oil, yatropha oil, coconut oil, palm kernel oil and old oils are used.

37. A process as claimed in any of claims 28 to 36, characterized in that alcohol and/or glycerol and/or water is partly or completely removed.

38. A composition obtainable by the processes claimed in claims 8 to

39. A fuel composition containing 90 to 99.5% by weight gas oil and 0.5 to 10% by weight (preferably 2 to 6% by weight) of the composition claimed in claims 1 to 7 or 38 as an additive.

40. The use of the composition claimed in claims 1 to 7 or claim 38 as a biofuel.

41. The use of the composition claimed in claims 1 to 7 or claim 38 as an additive in fuel compositions.

42. The use of the composition claimed in claims 1 to 7 or claim 38 as an additive for improving the lubricating performance of fuel compositions.

43. The use claimed in claim 41 and/or 42, characterized in that the composition claimed in claims 1 to 7 or claim 38 is present in quantities of 0.5 to 10% by weight.