
Ozonolysis of unsaturated fatty acids and derivatives thereof

The invention relates to a method for the ozonolysis of unsaturated fatty acids and derivatives thereof, comprising the steps a) ozonolysis of an unsaturated fatty acid or of a derivative thereof in an organic phase comprising an organic solvent, and b) contacting the organic phase with an aqueous phase comprising catalase and preferably a buffer, where the unsaturated fatty acid or the derivative thereof has a linear chain having at least eight carbon atoms, and the use of catalase for removing reactive oxygen species from a reaction mixture comprising ozonolysis products of an unsaturated fatty acid or of a derivative thereof.

Ozonolysis is a method established in synthetic organic chemistry for cleaving a C=C double bond of an unsaturated hydrocarbon to form two carbonyl compounds. The latter may in particular be carboxylic acid, aldehydes and alcohols. In mechanistic terms, ozonolysis involves a [3+2]-cycloaddition of the ozone onto the double bond, which gives a primary ozonide, an unstable intermediate, which decomposes to give an aldehyde and a carbonyl oxide. The latter can either polymerize and/or dimerize to give a 1,2,4,5-tetraoxolane or, in a further cycloaddition, form a secondary ozonide. The secondary ozonide can then be worked-up oxidatively to give a carboxylic acid or reductively to give an aldehyde. The aldehyde can be reduced further as far as the alcohol (Vollhardt/Scholer, 1995).

A disadvantage of the method is that from the carbonyl oxide is formed not only the secondary ozonide, which can be worked-up to give the desired products, but also a number of by-products, including the tetraoxolane dimer, an adduct, referred to as hydroperoxide, of ozonide and water and also hydrogen peroxide produced therefrom. Moreover, the formation and accumulation of secondary ozonide is unacceptable with regard to the safety of the reaction implementation since compounds of this type are known to be explosive.

The formation of the other by-products lowers the yield of the reaction, especially since the hydrogen peroxide produced is also able to oxidize products of subsequent steps and, in so doing, to form additional by-products. Undesired carbonyl compounds that are formed can lead to the formation of numerous undesired condensation products.

Against this background, the problem addressed by the invention consists in providing an improved method in which the appearance of harmful secondary ozonides and troublesome by-products is reduced and the yield of the reaction is accordingly improved.

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Furthermore, the object of the present invention is to develop a method in which the reactive oxygen species in a reaction mixture comprising ozonolysis products of unsaturated fatty acids and derivatives thereof are broken down without consuming reduction equivalents.

10 Furthermore, the object of the present invention is to develop a method in which the degradation of the reactive oxygen species in a reaction mixture comprising ozonolysis products of unsaturated fatty acids and derivatives thereof is achieved by an alternative catalyst which has improved properties compared to catalysts used hitherto.

15 Furthermore, the object of the present invention is to develop a method in which the treatment of a reaction mixture comprising ozonolysis products of unsaturated fatty acids and derivatives thereof for the purposes of reducing the concentration of secondary ozonides, takes place under neutral conditions in order to avoid secondary reactions of aldehydic ozonolysis products favoured by extreme pH values.

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A further object of the invention is to improve the method such that it results in an accelerated degradation of reactive oxygen species without requiring a change of solvent.

25 These and other objects are achieved by the subject matter of the present invention and in particular also by the subject matter of the attached independent claims, with embodiments arising from the subclaims.

According to the invention, the object is achieved in a first aspect by a method comprising the steps:

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- a) ozonolysis of an unsaturated fatty acid or a derivative thereof in an organic phase comprising an organic solvent, and
- b) contacting the organic phase with an aqueous phase comprising catalase and preferably a buffer,

where the unsaturated fatty acid or the derivative thereof has a linear chain with at least eight carbon atoms.

- 5 In a first embodiment of the first aspect, the organic solvent is an organic solvent which, in the presence of an aqueous phase at room temperature, forms an organic phase separate from the aqueous phase.

10 In a second embodiment, which is one embodiment of the first embodiment, step a) takes place in the presence of an aqueous phase.

In a third embodiment, which is one embodiment of the second embodiment, before step b), the aqueous phase is removed from step a).

- 15 In a fourth embodiment of the first aspect, which is also one embodiment of the first to third embodiment, the unsaturated fatty acid or the derivative thereof is a compound of the formula (I)



- 20 where R^1 is selected from the group which comprises hydrogen, HO-, HS-, CHO-, R^2OC -, unsubstituted or substituted, preferably halogenated, alkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl,

25 where R^2 is selected from the group which comprises HO-, substituted or unsubstituted alkoxy, alkenoxy, alkynoxy and cycloalkoxy having one to seven carbon atoms, $-NR^3R^4$, aryloxy and benzyloxy,

where R^3 and R^4 in each case, and independently of one another, are selected from the group which comprises hydrogen, alkyl, preferably methyl or ethyl, alkenyl, alkynyl, aryl and benzyl,

30 and where x and y, in each case and independently of one another are 0 or a larger integer.

In a fifth embodiment which is one embodiment of the fourth embodiment, R^1 is hydrogen, R^2 is HO-, methoxy or ethoxy and the sum of x and y is nine or more.

In a sixth embodiment of the first aspect, which is also one embodiment of the first to fifth embodiment, the fatty acid or the derivative is selected from the group which comprises oleic acid and erucic acid and esters thereof, preferably methyl or ethyl esters.

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In a seventh embodiment of the first aspect, which is also one embodiment of the first to sixth embodiment, the organic solvent is selected from the group which comprises $R^5R^6R^7C-O-R^8$ or intramolecular ethers thereof, where R^5 , R^6 and R^7 in each case, and independently of one another, are selected from the group which comprises hydrogen, an alkyl or cycloalkyl having one to seven carbon atoms, and also unsubstituted and substituted aryl, where R^8 is selected from the group which comprises hydrogen, an alkyl, cycloalkyl, hydroxyalkyl and alkoxyalkyl having one to seven carbon atoms, and unsubstituted or substituted aryl and benzyl, and where preferably at least one of the radicals R^5 , R^6 and R^7 is methyl.

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In an eighth embodiment, which is one embodiment of the seventh embodiment, the organic solvent is a compound of the formula $R^5R^6R^7C-OH$, and R^5 , R^6 and R^7 are, in each case and independently of one another, selected from the group which comprises methyl, ethyl, propyl, butyl and pentyl.

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In a ninth embodiment, which is one embodiment of the eighth embodiment, the organic solvent is 2-methyl-2-butanol.

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In a tenth embodiment of the first aspect, which is also one embodiment of the first to ninth embodiment, the volume fraction of the aqueous phase upon contacting the organic phase, relative to the total volume of both phases, is more than 0 to 50, more preferably 5 to 30, even more preferably 15 to 25 percent.

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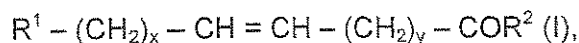
In an eleventh embodiment of the first aspect, which is also one embodiment of the first to tenth embodiment, catalase is present in step a).

In a twelfth embodiment of the first aspect, which is also one embodiment of the first to eleventh embodiment, the catalase is beef liver catalase or a variant thereof.

In a thirteenth embodiment of the first aspect, which is also one embodiment of the first to twelfth embodiment, the pH of the aqueous phase in step b), before contacting with the organic phase, is 3 to 11, preferably 5 to 8, yet more preferably 6.5 to 7.5.

5 According to the invention, the object is achieved in a second aspect through the use of catalase for removing reactive oxygen species, preferably organic reactive oxygen species, from a reaction mixture comprising ozonolysis products of an unsaturated fatty acid or a derivative thereof.

10 In a first embodiment of the second aspect the unsaturated fatty acid or the ester thereof is a compound of the formula



15 where R^1 is selected from the group which comprises hydrogen, HO-, HS-, CHO-, R^2OC -, unsubstituted or substituted, preferably halogenated, alkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl,

where R^2 is selected from the group which comprises HO-, substituted or unsubstituted alkoxy, 20 alkenoxy, alkynoxy and cycloalkoxy having one to seven carbon atoms, $-NR^3R^4$, aryloxy and benzyloxy,

where R^3 and R^4 in each case, and independently of one another, are selected from the group which comprises hydrogen, alkyl, preferably methyl or ethyl, alkenyl, alkynyl, aryl and benzyl,

25 and where x and y, in each case and independently of one another are 0 or a larger integer.

In a second embodiment of the second aspect, which is also one embodiment of the first embodiment, the reaction mixture further comprises an organic solvent, preferably one which, 30 in the presence of an aqueous phase at room temperature, forms an organic phase separate from the aqueous phase,

where the organic solvent is selected from the group which comprises $R^5R^6R^7C-O-R^8$ or intramolecular ethers thereof, where R^5 , R^6 and R^7 in each case, and independently of one

another, are selected from the group which comprises hydrogen, an alkyl or cycloalkyl having one to seven carbon atoms, and also unsubstituted and substituted aryl, where R^8 is selected from the group which comprises hydrogen, an alkyl, cycloalkyl, hydroxyalkyl and alkoxyalkyl having one to seven carbon atoms, and unsubstituted or substituted aryl and benzyl, and where preferably at least one of the radicals R^5 , R^6 and R^7 is methyl.

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and where the organic solvent is yet more preferably 2-methyl-2-butanol.

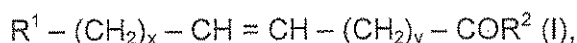
In a third aspect, the object of the invention is achieved by a reaction mixture comprising on the one hand an organic phase comprising ozonolysis products of an unsaturated fatty acid or of a derivative thereof, and an organic solvent and on the other hand, an aqueous phase comprising catalase and preferably a buffer, where the fatty acid or the derivative thereof has a linear chain having at least eight carbon atoms.

In a first embodiment of the third aspect, the organic solvent, in the presence of an aqueous phase at room temperature, forms an organic phase separate from the aqueous phase.

In a second embodiment of the third aspect, which is one embodiment of the first embodiment, step a) takes place in the presence of an aqueous phase.

In a third embodiment of the third aspect, which is one embodiment of the second embodiment, before step b) the aqueous phase is removed from step a).

In a fourth embodiment of the third aspect, which is also one embodiment of the first to second embodiment, the unsaturated fatty acid or the derivative thereof is a compound of the formula (I)



where R^1 is selected from the group which comprises hydrogen, HO-, HS-, CHO-, R^2OC -, unsubstituted or substituted, preferably halogenated, alkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl,

where R^2 is selected from the group which comprises HO-, substituted or unsubstituted alkoxy, alkenoxy, alkynoxy and cycloalkoxy having one to seven carbon atoms, $-NR^3R^4$, aryloxy and benzyloxy,

- 5 where R^3 and R^4 in each case, and independently of one another, are selected from the group which comprises hydrogen, alkyl, preferably methyl or ethyl, alkenyl, alkynyl, aryl and benzyl,

and where x and y, in each case and independently of one another are 0 or a larger integer.

- 10 In a fifth embodiment of the third aspect, which is one embodiment of the fourth embodiment, R^1 is hydrogen, R^2 is hydrogen or methyl or ethyl and the sum of x and y is nine or more.

- In a sixth embodiment of the third aspect, which is also one embodiment of the first to fifth embodiment, the fatty acid or derivative is selected from the group which comprises oleic acid
15 and erucic acid and esters thereof, preferably methyl or ethyl esters.

- In a seventh embodiment of the third aspect, which is also one embodiment of the first to sixth embodiment, the organic solvent is selected from the group which comprises $R^5R^6R^7C-O-R^8$ or intramolecular ethers thereof, where R^5 , R^6 and R^7 in each case, and independently of one
20 another, are selected from the group which comprises hydrogen, an alkyl or cycloalkyl having one to seven carbon atoms, and also unsubstituted and substituted aryl, where R^8 is selected from the group which comprises hydrogen, an alkyl, cycloalkyl, hydroxyalkyl and alkoxyalkyl having one to seven carbon atoms, and unsubstituted or substituted aryl and benzyl, and where preferably at least one of the radicals R^5 , R^6 and R^7 is methyl.

- 25 In an eighth embodiment of the third aspect, which is one embodiment of the seventh embodiment, the organic solvent is a compound of the formula $R^5R^6R^7C-OH$, and R^5 , R^6 and R^7 in each case, and independently of one another, are selected from the group which comprises methyl, ethyl, propyl, butyl and pentyl.

- 30 In a ninth embodiment of the third aspect, which is one embodiment of the eighth embodiment, the organic solvent is 2-methyl-2-butanol.

In a tenth embodiment of the third aspect, which is also one embodiment of the first to ninth embodiment, the volume fraction of the aqueous phase upon contacting the organic phase, relative to the total volume of both phases, is more than 0 to 50, more preferably 5 to 30, even more preferably 15 to 25 percent.

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In an eleventh embodiment of the third aspect, which is also one embodiment of the first to tenth embodiment, catalase is present in step a).

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In a twelfth embodiment of the third aspect, which is also one embodiment of the first to eleventh embodiment, the catalase is beef liver catalase or a variant thereof.

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In a thirteenth embodiment of the third aspect, which is also one embodiment of the first to twelfth embodiment, the pH of the aqueous phase in step b), before contacting with the organic phase, is 3 to 11, preferably 5 to 8, more preferably 6.5 to 7.5.

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The inventors of the present invention have surprisingly found that the efficiency and yield of the ozonolysis of unsaturated fatty acids and derivatives thereof, and also the purity of the reaction products can be increased by the presence of catalase in the reaction mixture after carrying out the ozonolysis. The inventors have also surprisingly found that the activity of catalase is compatible with the reaction mixture that is formed during the ozonolysis. Finally the inventors have surprisingly found that the presence of catalase in the ozonolysis reaction mixture reduces the concentration of reactive oxygen species such as secondary ozonide as well as the formation of further by-products, and increases the rate of formation of the carbonyl products.

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In a preferred embodiment, the term "ozonolysis" as used herein, means a method in which a C=C double bond of a hydrocarbon, more preferably an unsaturated fatty acid or a derivative thereof, is oxidatively cleaved as a result of the action of ozone on the molecule to form carbonyl products. The methods, agents and instruments suitable for carrying out the ozonolysis are known to the person skilled in the art from the prior art (Bally, 1978). It is usually carried out in alcohols as solvents, the reaction mixture further comprising at least 0.5 percent by weight of water, based on the total amount of solvent. Usually, the unsaturated fatty acid or its derivative is present in a concentration of 0.1 to 1 mol/l. The ozonolysis is carried out preferably at temperatures from 0 to 40°C, more preferably at temperatures from 10 to 35°C, and particularly preferably at temperatures from 20 to 30°C. Usually, to produce the ozone, an

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ozone generator is used which uses technical-grade air or a mixture of carbon dioxide and oxygen as feed gas. The ozone is produced from the oxygen by means of non-luminous electric discharge. In the process, oxygen radicals are formed which form ozone molecules with further oxygen molecules.

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A particular strength of the method according to the invention is that an improvement in the method can be achieved by adding an enzymatic activity, not by using aggressive or hazardous compounds and/or reaction conditions. The use of active enzymes in organic synthesis has hitherto primarily been limited to methods on a small scale. Enzymes are not only high-cost
10 agents with low substrate specificity, but are incompatible with numerous solvents customary in organic synthesis on account of various effects, and their use is in many cases still a prerequisite for economical work-up of the desired reaction products. As polypeptides, enzymes generally have a more or less globular structure which is in particular stabilized by the fact that the aliphatic amino acid side chains form a hydrophobic core on the inside, whereas hydrophilic,
15 polar amino acid side chains predominate at the surface of the enzyme. Accordingly, for its stabilization, the enzyme requires a hydrate sheath made of water molecules and has a tendency towards denaturation when it is in contact with a solvent which competes with it for a few available water molecules. In the case of biphasic systems comprising an aqueous phase and an organic phase, interfacial effects often result which can include an absorption and
20 inactivation of the enzyme molecules at the phase boundary. The presence of organic solvents can also influence the kinetic properties of the enzyme, i.e. its activity and its selectivity, for example as a result of reversible or irreversible denaturation of the enzyme or as a result of changing its selectivity or as a result of influencing the reaction equilibrium. Also, the reliable establishment of a certain pH range, which is essential for the activity of enzymes, is difficult in
25 the presence of organic solvents (Wong, 1994).

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In a preferred embodiment, the term "catalase" as used herein, is understood to mean an enzymatically active polypeptide which catalyzes the decomposition of hydrogen peroxide to give water and oxygen. In a preferred embodiment a catalysis is present if the decomposition of
hydrogen peroxide proceeds more rapidly in the presence of detectable amounts of the catalytically effective polypeptide than its absence. In one preferred embodiment, the enzymatically active polypeptide is a multifunctional enzyme which, besides the catalase activity has one or more than one further activity. Examples of polypeptides which can be used according to the invention include beef liver catalase (database code NP_001030463),

horseradish peroxidase, for example database code, CAA00083.1, all other catalases described in the prior art (Abad-Zapatero, 1992; Abe *et al.*, 1973; Altomare *et al.*, 1974; Baird *et al.*, 1987), Balasubramaniam *et al.*, 1987; Barlow & Margoliash, 1969; Beers & Sizer, 1952; Beyer & Fridovich, 1985; Boismenu *et al.*, 1989; Browett & Stillman, 1980; Chance, 1951; Chance, 1955, 5 Chance, 1973, Chance *et al.*, 1973; Chatterjee *et al.*, 1989; Ch *et al.*, 1975; Corral *et al.*, 1974; Darr & Fridovich, 1985; Davison *et al.*, 1986; Deisseroth & Dounce, 1969; Diesseroth *et al.*, 1967; Eglinton *et al.*, 1983; Escobar *et al.*, 1990; Evans & Recheigl, 1967; Feinstein *et al.*, 1967; Feinstein *et al.*, 1967; Fita & Rossman, 1985; Furuta *et al.*, 1974; Ghadermarzi & Moosavi-Movahedi, 1996; Goldacre & Galston, 1953; Goldstein, 1968; Goth, 1989; Goth, 1991; Gregory 10 & Fridovich, 1974; Gadermarzi & Moosavi-Movahedi, 1996; Goldacre & Galston, 1953; Goldstein, 1968; Goth, 1989; Goth, 1991; Gregory & Fridovich, 1974; Haining & Legan, 1972; Halliwell, 1973; Heidrich, 1968; Higashi *et al.*, 1974; Holmes, 1972; Holmes & Masters, 1972; Johansson & Borg, 1988; Jones & Masters, 1975; Jones, 1973; Jones & Middlemiss, 1972; Kirkman & Gaetani, 1984; Kiselev *et al.*, 1967; Kremer, 1970; Kroll *et al.*, 1989; Kyle *et al.*, 15 1987; Lanir & Schejter, 1975; Lardinois, 1995; Lee *et al.*, 1987; Liebermann & Ove, 1958; Loewen & Seitala, 1987; Longley, 1967; Maehly & Chance, 1954; Maimoni *et al.*, 1999; Margoliash & Novogorodsky, 1958; Marklund, 1972; Mastersc, 1986; McPherson & Rich, 1973; Mitchel & Anderson, 1965; Miyahara *et al.*, 1978; Murphy *et al.*, 1967; Madler *et al.*, 1986; Nelson & Niesow, 1972M Nicholls *et al.*, 1963; Orr, 1967a; Orr, 1967b; Oshino *et al.*, 1973; 20 Percy *et al.*, 1990; Perrin *et al.*, 1990; Pitts, 1992; Poznansky *et al.*, 1974; Pritchard & Hudson, 1967; Roth & Jensen, 1967; Rossman & Labaw, 1967; Sakamoto & Highashi, 1974; Samejima; Kita, 1969; Samejima *et al.*, 1962; Schroeder *et al.*, 1969; Scibior & Czczot, 2006; Scott *et al.*, 1991; Seah & Kaplan, 1973; Seah *et al.*, 1973; Sharma *et al.*, 1989; Shimiza *et al.*, 1984; Spreti *et al.*, 1995; Sumner & Dounce, 1937; Sumner *et al.*, 1940; Sund *et al.*, 1967; Tanaka *et al.*, 25 1991; Tanford & Lovrien, 1962; Tauber & Petit, 1952; Tudhope, 1967; Vuilaum *et al.*, 1988; Wheeler *et al.*, 1990; Whiteside *et al.*, 1988; Yasukochi *et al.*, 1972; Zidoni & Kremer, 1974) and variants thereof.

If, for the activity of the catalase, as well as hydrogen peroxide, a further substrate and/or one or 30 more than one co-factor is also required, the catalase comprises this. E.g. in the case of using horseradish peroxidase, the presence of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) is required.

The catalase used can be added to the reaction mixture in different forms and in different purification stages, from an unprocessed solution or a pellet of cells having catalase activity ranging to a completely purified enzyme. In a preferred embodiment, the catalase is a polypeptide associated with a whole, either viable or lysed cell, for example one which is expressed by a prokaryotic whole-cell catalyst, preferably bacterial in nature. In this connection, a cell, if it is used as a whole-cell catalyst does not have to express the catalase on its inside, but a catalase localized on the outer membrane is also possible, for example a fusion protein with an endogenous outer membrane protein. In a further preferred embodiment, the lysate is a cell, preferably bacterial in nature, which expresses the catalase in the inside of the cell. In a further embodiment, it is a solution or a lyophilizate or a lyophilizate, preferably at least 60, 70, 80, 90 or 95, particularly preferably 100% pure, polypeptide. Preparations of these types of cells or polypeptides are commercially available or can be produced for the person skilled in the art by reference to standard biochemical methods.

The concentration of the catalase in one preferred embodiment is 0.001 to 1, more preferably 0.1 to 0.5, most preferably 0.025 to 0.1 percent by weight of the aqueous phase in step b).

The sequence and implementation of steps a) and b) can take place in all conceivable variations provided the catalase comes into contact with the products of the ozonolysis of the fatty acid or of the derivative thereof. Whereas the presence of an aqueous phase comprising catalase is essential in step b), it is optional in step a). Thus, the same aqueous phase can be present in step a) and b), although it is also possible to carry out step a) in the presence of one aqueous phase, but to remove this and to use a different aqueous phase for step b). Likewise it is possible to add an aqueous phase with or without catalase to the organic phase during or before step a) and to add the catalase to the aqueous phase when the ozonolysis in step a) is complete. In one particularly preferred embodiment, the catalase is only added to the organic phase after the ozonolysis.

Numerous methods and protocols according to which catalytically active catalases can be purified from wild-type cells or recombinant cells are known to the person skilled in the art, see for example Yumoto *et al.*, 2000.

Suitable starting materials for the ozonolysis are all conceivable fatty acids and derivatives thereof, both naturally occurring ones and also artificially synthesized ones, those with an even

or uneven number of carbon atoms and branched and also unbranched ones, in particular undecylenic acid, myristoleic acid, palmitoleic acid, petroselinic acid, oleic acid, elaidic acid, vaccenic acid, gadoleic acid, icosenic acid, cetoleic acid, erucic acid, nervonic acid and derivatives thereof, preferably their methyl esters. Furthermore, it is also possible to use polyunsaturated fatty acids, for example linoleic acid, linolenic acid, calendic acid, punicic acid, elaeostearic acid, arachidonic acid, timnodonic acid, clupanodonic acid and cervonic acid and derivatives thereof, preferably their methyl esters. In one preferred embodiment, the fact that "the fatty acid or the derivative thereof has a linear chain with at least eight carbon atoms", as used herein, means that the carbon atom of the carboxyl group is part, preferably at the end, of a linear chain having at least eight carbon atoms which must have branches if the length of eight carbon atoms is given over the longest possible linear chain in the carbon backbone.

In one preferred embodiment, the substituents referred to as "alkoxy", "alkenoxo" or "alkynoxo" as used herein, are substituents of the formula $R-O-$, where R is an alkyl, alkenyl or alkynyl.

A particular advantage of the method according to the invention is that ozonolysis and removal of the reactive oxygen species can be carried out in solvents or solvent mixtures compatible with both reactions, i.e. in a one-pot reaction, meaning that a complex change of solvent can be omitted. In a preferred embodiment, step b) is accordingly carried out in the same reaction vessel without changing the solvent or without separating off constituents of the reaction mixture from step a).

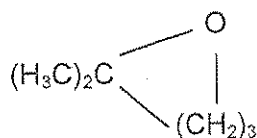
In one particular embodiment, the volume fraction of the aqueous phase upon contacting the organic phase, based on the total volume of both phases, is more than 0.5, 1, 5, 10, 15, 20, 25 or more than 0 to 50, more than 1 to 40, more than 2.5 to 25, more preferably 5 to 30, even more preferably 15 to 25 percent.

In principle, a large number of solvents can be used for step a), for example aliphatics such as hexane or cyclohexane, aromatics alkylated aromatics such as benzene, toluene or xylene, halogenated aliphatics or aromatics such as dichloromethane or chlorobenzene, ketones, for example acetone or cyclohexanone, alcohols and ethers thereof such as 2-butanol, 2-methyl-2-propanol or methyl tert-butyl ether, carboxylic acids and esters thereof, such as acetic acid or ethyl acetate, sulfoxides such as dimethyl sulfoxide, and heterocycles such as tetrahydrofuran or dioxane. Particularly preferred solvents include secondary and tertiary

alcohols, including in particular 2-propanol and tert-butanol. If the solvent is a solvent of the formula $R^5R^6R^7C-O-R^8$, then the radicals R^5 , R^6 , R^7 and R^8 can, in each case in pairs and independently of one another, form alkylene bridges of the formula $-(CH_2)_z-$, where z is selected such that the corresponding ring has at least 3, preferably at least 5, members.

- 5 Furthermore, the radicals can also form intramolecular ethers, which are formally the result of the condensation of two hydroxy-containing radicals. For example, for the case that $R^5 = R^6 =$ methyl, R^7 is hydroxypropyl and R^8 is hydrogen, a solvent according to the invention is the compound with the formula

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Separating off the organic solvents, particularly if these form a phase separate from the aqueous phase without assistance, is simple for the person skilled in the art and takes place for example by means of decanting, use of a separating funnel and the like.

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In one preferred embodiment, the term "an organic phase separate from the aqueous phase", as used herein, is understood as meaning a homogeneous liquid phase consisting, at room temperature, predominantly preferably to at least 90 volume percent, of organic solvents which, after contacting with an aqueous phase, settles from this without further assistance, as can preferably be established by purely visible means, and resulting, after the equilibrium has been established, in the formation of two separate phases.

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Steps a) and b) are generally followed by a further processing of the ozonolysis product freed from reactive oxygen species. For example, the reductive amination is possible, in which an aldehyde or ketone is aminated, i.e. in the case of an ω -oxocarboxylic acid ozonolysis product the result is the formation of an ω -aminocarboxylic acid. Such a reductive amination is preferably carried out with the aid of a Raney nickel catalyst and hydrogen, as described in the prior art, for example in Chan *et al.*, 1994. Alternatively, a further oxidation to the α,ω -dicarboxylic acid can be useful.

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The present invention is illustrated further by the following figures and non-limiting examples, which reveal further features, embodiments, aspects and advantages of the present invention.

Fig. 1 shows the reduction in the concentration of reactive oxygen species following the treatment according to the invention of a solution of ozonolysis products with various amounts of lyophilized beef liver catalase, as described in example 1. The y-axis shows the amount of reactive oxygen species in weight percent.

Example 1: Ozonolysis of methyl *cis*-13-docosenoate (methyl erucate) with subsequent catalase treatment

All chemicals and solvents were acquired in the highest possible grade (at least p.a.) on the free market and used without further purification and/or treatment. Water only in demineralized grade. Phosphate buffer solution (pH = 7.0) was acquired from Bernd Kraft GmbH (Duisburg, Germany), freeze-dried beef liver catalase from Sigma-Aldrich (St. Louis, USA). The fatty acid esters methyl *cis*-9-octadecenoate (methyl oleate) and methyl *cis*-13-docosenoate (methyl erucate) were acquired as ≥ 95 percent materials from Croda (Gouda, The Netherlands). According to GC analysis (uncorrected) the purity of the C18:1 or C22:1 methyl ester was 95.2 or 95.0%, respectively. In the case of the methyl oleate, the impurities present were 3.6% of C18:2 methyl esters and also, in total, 1.2% of the saturated methyl esters methyl hexadecanoate, octadecanoate and eicosanoate (methyl palmitate, stearate and arachidate), i.e. 98.8% unsaturated, i.e. cleavable methyl esters. In the case of the methyl erucate, the impurities comprised 1.3% of C22:2 methyl esters, 0.7% of C20:1 methyl esters and 1.0% of C24:1 methyl esters, and, in total, 2.0% of the saturated methyl esters methyl eicosanoate, docosanoate and tetracosanoate (methyl arachidate, behenate and lignocerate), i.e. 98.0% of the starting material were unsaturated, cleavable methyl esters.

A 0.912 molal two-phase mixture of a solution of 80.0 g of methyl *cis*-13-docosenoate (methyl erucate) in 160 ml of 2-methyl-2-butanol and 30 ml of water is provided in a conical 250 ml three-neck receiver with bottom discharge (Teflon stopcock). Via the latter, a hose pump is attached, via the middle (NS29-) standard ground glass joint, a trickle bed glass column (800 × 30 mm) filled with Raschig rings (6 × 6 × 0.65 mm) with top and bottom gas feed and top gas discharge. Via one side (NS14-) standard ground glass joint an internal thermometer is attached, the other standard ground glass joint is free (sampling point). Using the pump, the two-phase mixture is now circulated at room temperature via the column (flow rate: 120 ml/min). Counter to the flow of solution, a reactive gas of an O₂/O₃ mixture is introduced from below the column from an ozone generator (1500 ml/min (stp) O₂ stream, maximum generator throughput). From above the column, CO₂ is mixed as carrier gas or flushing gas into the O₃-depleted O₂ discharge (likewise 1500 ml/min (stp)). The off-gas stream flows via an interconnected cold trap with ice cooling through 100 ml of a 5 percent by weight aqueous potassium iodide solution as ozone indicator. For sampling, a few drops of the reaction mixture are removed by pipette,

taken up in 2 ml of ethyl acetate/water (1:1), briefly extracted by shaking, and the organic phase is then analyzed by GC (15 m DB-1 column).

After 65 min, complete mixing of the two phases occurs, after 110 min the reaction is complete (> 99.5% conversion or < 0.5% remaining starting material), after which the pump is switched off and the introduction of the two gases is ended. The temperature of the reaction mixture increases over the entire time by significantly less than 10°C.

According to GC analysis, the products present in the resulting reaction solution are nonanal and methyl 13-oxodecanoate in equal parts in a relative purity of > 95%; the products of the further oxidation of these two compounds, nonanoic acid (pelargonic acid) and 13-hydroxy-13-oxomethyltridecanoate (methyl brassylate), are each found in < 5% in the solution.

The product solution is discharged from the apparatus, the emptied apparatus is rinsed with in each case 160 ml of 2-methyl-2-butanol and tetrahydrofuran and the resulting solution is divided into three equal parts each of 200 ml. To each of the three solutions is now added 100 ml of a freshly prepared aqueous solution of beef liver catalase in phosphate buffer solution (pH = 7.0), the end concentration of the beef liver catalase in the aqueous solution in the first case being 0% by weight (blank sample), in the second case being 0.05% by weight, and in the third case being 1.0% by weight. All three solutions are rinsed again with 100 ml buffer solution. The two-phase mixtures obtained in this way are stirred vigorously at room temperature, and after 1, 2, 4, 9 and 22 h, 40 ml samples are taken in each case and analyzed as to the content of reactive oxygen.

For this, the sample is boiled at reflux under protective gas (N₂) in the presence of excess sodium iodide. After cooling, the resulting iodide is titrated potentiometrically with a thiosulphate solution: to the sample of the sample weighed into the Erlenmeyer flask is firstly added dry ice, then 80 ml of 1-propanol, 4 ml of acetic acid, and 20 ml of isopropanolic sodium iodide solution. After a reflux condenser has been attached, nitrogen is introduced via the condenser. The reaction mixture is then left to stand for half an hour at room temperature and then boiled for 10 minutes. After cooling, ca. 20 ml of water are flushed through the condenser then the mixture is titrated with 0.01 mol/l of sodium thiosulphate solution to the equivalence point. In parallel, a blank-value determination is carried out in the same way.

The corresponding results can be found in **Tab. 1** and **Fig. 1**.

Tab. 1: Concentration of reactive oxygen species following the addition of different amounts of catalase to a reaction mixture with ozonolysis products and methyl erucate

Run time in h	0% by weight lyophilized catalase	0.05% by weight lyophilized catalase	1.00% by weight lyophilized catalase
0	0.58	0.58	0.66
1	0.34	0.22	0.12
2	0.35	0.21	0.08
4	0.43	0.16	0.05
9	0.36	0.13	0.04
22	0.25	0.11	0.03

It is therefore clearly evident that, compared with the blank sample, the use of a solution of just 0.05% by weight of beef liver catalase reduces the fraction of potentially oxidizing constituents in the organic product solution by a factor > 2 ; when using a solution of 1% by weight beef liver catalase, even a factor of almost 10 results.

Particularly the rapid decrease at the start of the treatment clearly shows that, with the help of such a treatment, it is possible to stabilize product solutions against further oxidation of very labile compounds such as aliphatic aldehydes in this sense.

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Claims

1. A method for the ozonolysis of unsaturated fatty acids and derivatives thereof, comprising:

a) ozonolysis of the unsaturated fatty acid or derivative thereof in an organic phase

5 comprising an organic solvent; and

b) contacting the organic phase with an aqueous phase comprising catalase and optionally, a buffer;

wherein the unsaturated fatty acid or the derivative thereof comprises at least one linear carbon chain of at least eight carbon atoms, and

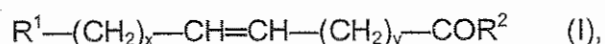
10 wherein at room temperature, the organic solvent separates as an organic phase when in the presence of an aqueous phase.

2. The method according to claim 1, further comprising contacting an aqueous phase with the organic phase during the ozonolysis.

15

3. The method according to claim 2, wherein the aqueous phase contacting the organic phase during ozonolysis is separated and removed from the organic phase prior to contacting the organic phase with the aqueous phase comprising catalase.

20 4. The method according to claim 1, wherein the unsaturated fatty acid or the derivative thereof is a compound of formula (I):



wherein

25 R^1 is selected from the group consisting of hydrogen, $HO-$, $HS-$, $CHO-$, R^2OC- , unsubstituted or substituted alkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl,

R^2 is selected from the group consisting of $HO-$, substituted or unsubstituted alkoxy, alkenoxy, alkynoxy and cycloalkoxy having one to seven carbon atoms, $-NR^3R^4$, aryloxy and benzyloxy,

R^3 and R^4 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, 30 alkynyl, aryl and benzyl, and

x and y , are each, independently, 0 or a larger integer.

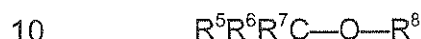
5. The method according to claim 4, wherein

R^1 is hydrogen,

R² is HO—, methoxy or ethoxy, and
a sum of x and y is nine or more.

6. The method according to claim 1, wherein the fatty acid or the derivative is at least one
5 selected from the group consisting of oleic acid, erucic acid and esters thereof.

7. The method according to claim 1, wherein the organic solvent is at least one selected from
the group consisting of compounds of formula:



or intramolecular ethers thereof,

wherein

R⁵, R⁶ and R⁷ are each independently selected from the group consisting of hydrogen, an alkyl
group of one to seven carbon atoms, a cycloalkyl group of one to seven carbon atoms, an
15 unsubstituted aryl group and a substituted aryl group, and

R⁸ is selected from the group consisting of hydrogen, an alkyl, cycloalkyl, hydroxyalkyl or
alkoxyalkyl group having one to seven carbon atoms, an unsubstituted aryl group, a substituted
aryl group and a benzyl group.

20 8. The method according to claim 7, wherein the organic solvent is at least one a compound of
formula



wherein R⁵, R⁶ and R⁷ are independently selected from the group consisting of hydrogen,
25 methyl, ethyl, propyl, butyl and pentyl.

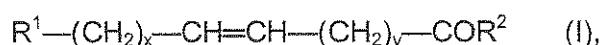
9. The method according to claim 7, wherein at least one of R⁵, R⁶ and R⁷ is methyl.

10. A method for the ozonolysis of unsaturated fatty acids and derivatives thereof, comprising:

- 30 a) ozonolysis of the unsaturated fatty acid or derivative thereof in an organic phase comprising
an organic solvent; and
b) contacting the organic phase with an aqueous phase comprising catalase and optionally, a
buffer;

wherein the unsaturated fatty acid or the derivative thereof comprises at least one linear carbon chain of at least eight carbon atoms and,
 wherein the organic solvent is 2-methyl-2-butanol.

- 5 11. The method according to claim 1, wherein a volume per cent of the aqueous phase in contact with the organic phase, is from more than 0 to 50 percent of a total volume of the two phases.
12. The method according to claim 1, wherein the aqueous phase comprising catalase is in
 10 contact with the organic phase during the ozonolysis.
13. The method according to claim 1, wherein the catalase is beef liver catalase or a variant thereof.
- 15 14. The method according to claim 1, wherein the aqueous phase comprises a buffer and the pH of the aqueous phase before contacting with the organic phase, is from 3 to 11.
15. The method according to claim 14, wherein the pH of the aqueous phase before contacting
 20 with the organic phase, is from 5 to 8.
16. A method for removing reactive oxygen species from a reaction mixture comprising ozonolysis products of an unsaturated fatty acid or a derivative thereof and an organic solvent, comprising:
 contacting the reaction mixture with a catalase
 25 wherein said organic solvent, in the presence of an aqueous phase at room temperature, forms an organic phase separate from the aqueous phase.
17. The method according to claim 16, wherein the unsaturated fatty acid or the ester thereof is a compound of formula (I):
 30



wherein

R^1 is selected from the group consisting of hydrogen, $HO-$, $HS-$, $CHO-$, R^2OC- , unsubstituted or substituted alkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl,

R^2 is selected from the group consisting of HO—, substituted or unsubstituted alkoxy, alkenoxy, alkynoxy and cycloalkoxy having one to seven carbon atoms, $—NR^3R^4$, aryloxy and benzyloxy, R^3 and R^4 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl and benzyl, and

5 x and y, are each, independently, 0 or a larger integer.

18. The method according to claim 16, wherein

the organic solvent is selected from the group consisting of compounds having a formula:



or intramolecular ethers thereof,

wherein

R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, an alkyl group of one to seven carbon atoms, a cycloalkyl group of one to seven carbon atoms, an

15 unsubstituted aryl group and a substituted aryl group, and

R^8 is selected from the group consisting of hydrogen, an alkyl, cycloalkyl, hydroxyalkyl or alkoxyalkyl group having one to seven carbon atoms, an unsubstituted aryl group, a substituted aryl group and a benzyl group.

20 19. The method according to claim 18, wherein the organic solvent is 2-methyl-2-butanol.

Ozonolysis of unsaturated fatty acids and derivatives thereof**Abstract**

- 5 The invention relates to a method for the ozonolysis of unsaturated fatty acids and derivatives thereof, comprising the steps a) ozonolysis of a fatty acid or of a derivative thereof in an organic phase comprising an organic solvent, and b) contacting the organic phase with an aqueous phase comprising catalase and preferably a buffer, where the fatty acid or the derivative thereof has a linear chain having at least eight carbon atoms, and the use of catalase for removing
10 reactive oxygen species from a reaction mixture comprising ozonolysis products of an unsaturated fatty acid.

(No suitable figure)