



(22) Date de dépôt/Filing Date: 2001/11/02
(41) Mise à la disp. pub./Open to Public Insp.: 2002/05/07
(30) Priorité/Priority: 2000/11/07 (10055092.4) DE

(51) Cl.Int.⁷/Int.Cl.⁷ C12P 17/02, A61K 7/46, A23L 1/221,
C11B 9/00, C07D 307/12, C07D 309/10

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(54) Titre : METHODE DE PREPARATION D'OXYDE DE LINALOL OU DE MELANGES CONTENANT DE L'OXYDE DE LINALOL

(54) Title: PROCESS FOR THE PREPARATION OF LINALOOL OXIDE OR OF LINALOOL OXIDE-CONTAINING MIXTURES

(57) Abrégé/Abstract:

The present invention relates to a process for the preparation of linalool oxide or of linalool oxide-containing mixtures, in which linalool or linalool-containing mixtures are oxidized in the presence of enzymes.



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PROCESS FOR THE PREPARATION OF LINALOOL OXIDE OR OF
LINALOOL OXIDE-CONTAINING MIXTURES

ABSTRACT OF THE DISCLOSURE

The present invention relates to a process for the preparation of linalool oxide or of linalool oxide-containing mixtures, in which linalool or linalool-containing mixtures are oxidized in the presence of enzymes.

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FIELD OF THE INVENTION

The present invention relates to a process for the preparation of linalool oxide or of linalool oxide-containing mixtures.

BACKGROUND OF THE INVENTION

5 Linalool oxide is an aroma material, which has been known for a long time and is used in a large number of aromas. Linalool oxide, moreover, occurs both in the furanoid form as a 5-membered ring (2-methyl-2-vinyl-5-(α -hydroxyisopropyl)tetrahydrofuran, CAS 60047-17-8; cis and trans: CAS 5989-33-3 and 34995-77-2), and in the pyranoid form as
10 6-membered ring (2,2,6-trimethyl-6-vinyltetrahydro-2H-pyran-3-ol, CAS 14049-11-7; cis and trans: CAS 14009-71-3 and 39028-58-5). It occurs naturally in essential oils such as rosewood oil and osmanthus abs. and fruits (Volatile Compounds in Food, Qualitative and Quantitative Data, 7th
15 Netherlands) and has as a flowery-earthy, slightly bergamot-like odor (Bauer, Garbe, Surburg, Common Fragrance and Flavor Materials, second edition, VCH, Weinheim 1990, p. 114). The flavor impression of linalool oxide is in the citrus, apricot, blueberry, flowery direction.

The person skilled in the aroma sector distinguishes three areas:
20 natural, nature-identical and synthetic aromas. Synthetic aromas are of only minor interest because consumer acceptance is not particularly high. One example of the preparation of a nature-identical aroma material is described in JP A 59225176. This publication relates to the production of linalool oxide. In this case, unnatural starting materials are converted by
25 chemical synthesis using strong mineral acids into linalool oxide. Such a product can be categorized only as "nature-identical", but not as natural, and must, therefore, not be used to produce natural aromas.

Natural aromas, and thus also the natural aroma materials present therein, are to be categorized as having the highest value. This is also shown clearly by the considerably higher prices, which can be obtained for natural aromas. In addition, consumer interest in natural products and thus, also in natural aromas required for their production is increasing.

It is therefore an aim of the aroma industry to have natural building blocks for natural aromas available in sufficient quantity and quality. However, it is usually not economical to isolate these individual building blocks from natural extracts.

One example of the production of linalool oxide by fermentation is disclosed by Demyttenaere (Demyttenaere, J.C.R., 1998, Biotransformation of terpenes by fungi for the production of bioflavors, Meded. – Fac. Landbouwkd. Toegepaste Biol. Wet., 63 (4a), 1321-1324, and Demyttenaere, J.C.R.; Willemen, H.M., 1998, Biotransformation of linalool to furanoid and pyranoid linalool oxides by *Aspergillus niger*, Phytochemistry, 47 (6), 1029-1036). The biotransformation of terpene alcohols with the aid of *Aspergillus niger* and *Penicillium* strains is described therein. However, this process is very complicated to carry out, the conversion is very slow, and the yields of linalool oxide are only 20-33%.

In addition, the use of enzymes for epoxidation of double bonds in fatty acids and alkenes is known (1. PCT WO 91/04333. 2. Jarvie, A.W.P.; Overton, N.; St. Pourcain, C.B., 1998, Enzymatic epoxidation of polybutadiene, Chem. Comm., 177-178. 3. Lemoult, S.C.; Richardson, P.F.; Roberts, S.M., 1995, Lipase catalyzed Baeyer-Villiger Reactions, J. Chem. Soc. Perkin Trans. I, 89-91. 4. Rüsçh, M.; Warwel, S., 1996, Peroxy fatty acids: lipase catalyzed preparation and epoxidation, Lipid Technology, 7, 77-80. 5. Miurra, Y.; Yamane, T., 1997, Epoxidation of alkanes and cycloalkanes by microbial lipases immobilized with photo crosslinkable resin prepolymer, Biotechnol. Lett., 19, 7, 611-613). This involves epoxidation of double bonds analogous to the chemical reaction with a peroxycarboxylic acid. In these cases, the enzyme lipase (E.C. 3.1.1.3, CAS No. 9001-62-1, for example from *Pseudomonas cepacia*,

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DSM 3959, *Candida antarctica* DSM 3855, DSM 3908, DSM 3909, *Humicola lanuginosa*, DSM 3819, DSM 4109, *Humicola brevispora*, DSM 4110, *Humicola brevis* var. *thermoidea*, DSM 4111, *Humicola isolens*, DSM 1800) catalyzes, in the presence of hydrogen peroxide, intermediate
5 formation of the peroxy-carboxylic acid (WO 91/04333).

Numerous publications disclose the use of lipases on the laboratory or manufacturing scale, essentially for esterifications or transesterifications. However, none of these descriptions describes the preparation of furanoid or pyranoid linalool oxide by an enzymatic route.

10 SUMMARY OF THE PRESENT INVENTION

It is an object of the present invention now to produce linalool oxide or a linalool oxide-containing mixture, which can be categorized as natural and can be used to produce natural aromas. An additional aim is for the yields to be distinctly higher than in the state of the art to date. A particular
15 aim is to achieve, in some instances, yields above 90%.

A process for the preparation of linalool oxide or of linalool oxide-containing mixtures has been found and is characterized in that linalool or linalool-containing mixtures are oxidized in the presence of an enzyme.

DETAILED DESCRIPTION OF THE INVENTION

20 It is possible to use in the process according to the present invention both D-linalool and L-linalool. These substances may be in pure form or in the form of a mixture. It is likewise possible to employ the linalools mixed with other substances.

Suitable other substances are essential oils, absolutes or extracts.
25 In a preferred embodiment, linalool can be present in the essential oils such as rosewood oil, coriander oil, basil oil, lavender oil etc. It is also possible in these cases for D-linalool or L-linalool to be present as single substances in the reaction medium. A mixture of D-linalool and L-linalool in the reaction medium is, of course, likewise suitable for the purposes
30 according to the present invention.

The enzymes employed according to the present invention are preferably hydrolases, specifically lipases or proteases. Lipases are suitable and particularly preferred according to the present invention. In

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particular, a mobilized lipase is preferred, for example Chirazym C2 L2 from Roche Diagnostics, Penzberg, Germany, or Novozym 435 from Novo Nordisk, Denmark.

5 The enzymes are preferably those which can be obtained with the aid of the following microorganisms: *Pseudomonas cepacia*, for example DSM 3959, *Candida antarctica*, for example DSM 3855, DSM 3908, DSM 3909, *Humicola lanuginosa*, for example DSM 3819, DSM 4109, *Humicola brevispora*, for example DSM 4110, *Humicola brevis* var. *thermoidea*, for example DSM 4111, *Humicola isolens*, for example DSM 1800.

10 Yeasts are preferred as enzyme producers. *Candida* species in the form of pure cultures or mixed cultures of these yeasts are more preferred for this purpose, especially the *Candida antarctica*. Accordingly, lipases from *Candida antarctica* are most preferred for the process according to the present invention.

15 In the process according to the present invention, the oxidation of linalool was preferably carried out in the presence of said enzymes and of an oxidizing agent. Hydrogen peroxide is preferred. The oxidizing agent can be added before or during the oxidation process. It is likewise possible according to the present invention for the medium to have such a
20 composition and/or for the reaction conditions to be chosen such that the required oxidizing agent is formed during the progress of the process according to the present invention. In particular, the formation of oxidizing agent can be carried out with the use of enzymes. Examples thereof, are oxidases, for example, glucose oxidase. It is also possible to use as non-
25 enzymatic catalysts for preparing the hydrogen peroxide.

It is, thus, possible in the variant according to the present invention, for example, to choose the combination of the medium and of the enzymes such that the hydrogen peroxide is produced in situ during the process. This may involve the hydrogen peroxide production being started before
30 the oxidation process begins, or the hydrogen peroxide production being allowed to take place in situ during the oxidation process. It is possible according to the present invention, for example, for the hydrogen peroxide

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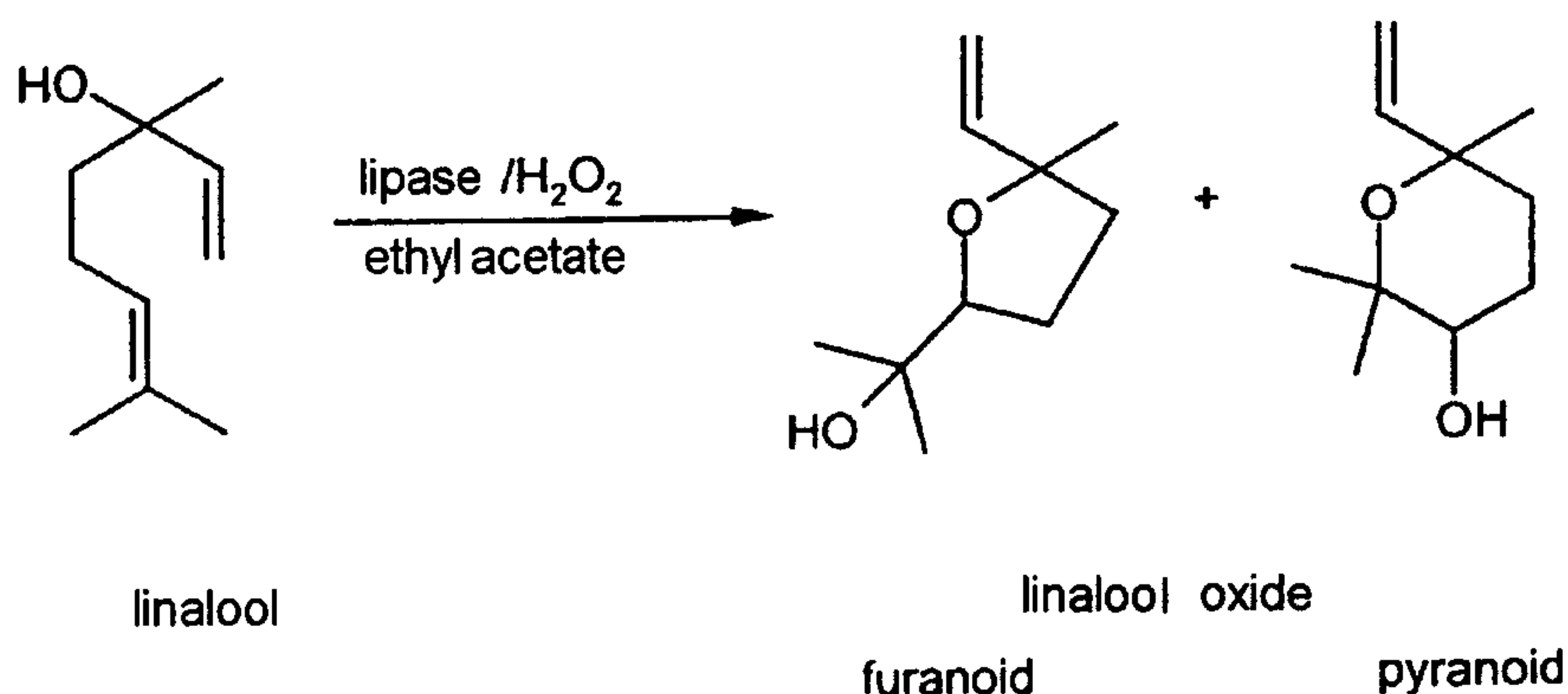
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to be formed enzymatically by oxidizing glucose in the aqueous medium with the enzyme glucose oxidase.

In addition to the use of enzymes and oxidizing agents it is often also possible to add a solvent to the reaction solution. Solvents suitable according to the present invention are acidic esters, preferably ethyl acetate. Apart from the esters, also suitable are carboxylic acids able to form peroxy-carboxylic acids. Examples thereof, are acetic acid, formic acid, benzoic acid. Acetic acid is more preferred.

The preferred solvents according to the present invention are those able to form peracids with the aid of the enzymes in the presence of hydrogen peroxide. One example, thereof, is ethyl acetate which forms peracetic acid as an intermediate in the presence of hydrogen peroxide with the aid of lipases.

In a form, which is particularly preferred according to the present invention, the following reaction accordingly takes place:



Surprisingly, it is possible, with the process according to the present invention described, to prepare linalool oxide, which can be categorized as natural and therefore, can be used to produce natural aromas. In addition, it has surprisingly emerged that natural linalool oxide can be produced in yields of more than 90% in a straight-forward manner. This means that the process according to the present invention displays a considerable advantage compared with previously disclosed fermentation processes

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which operate with the aid of microorganisms, and is accordingly suitable for the industrial production of natural linalool oxide.

The linalool oxide can be used according to the present invention directly or in aromas for a wide variety of foodstuffs. This is possible in particular where it is intended to produce "citrus", "apricot" and "blueberry" flavors.

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EXAMPLETABLE 1:

Item	Quantity	Starting material
1	100.0 ml	Ethyl acetate, nat.
2	7.7 g	Linalool, nat.
3	6.0 ml	Hydrogen peroxide, (H ₂ O ₂ , 30%)
4	0.1 g (245 units)	Lipase Chirazym C2 L2 (Roche Diagnostics) or Novozym 435 (Novo Nordisk)

Procedure:

Ethyl acetate (Item 1) is introduced into a 2 l three-necked flask with paddle stirrer, and linalool (Item 2), hydrogen peroxide (H₂O₂, 30% strength, Item 3) and enzyme (Item 4) are added while stirring slowly. The mixture is stirred slowly (about 100-120 rpm) at room temperature. The process is accompanied by analysis of the composition by GC every 1-2 days.

Result:

With the GC results listed in Table 2, the percentage areas for acetic acid were calculated (except in the case of acetic acid).

TABLE 2

	Linalool (GC-%)	Linalool oxide F, trans and cis (GC-%)	Linalool oxide P, trans and cis (GC-%)	Linalool oxide-epoxide (GC-%)	Acetic acid (GC-%)
Precursor	99.3	--	--	--	--
1 day	5.1	32.9	4.2	57.1	19.7
2 days	0	56.5	6.5	36.3	22.1
5 days	0	79.8	9.1	9.0	24.2
7 days	0	83.3	9.6	5.6	25.4

F: furanoid / **P:** pyranoid

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The total yield of furanoid and pyranoid linalool oxide is about 93%.

Purification by distillation (10-plate column) of the linalool oxide-containing organic phase results in a product with a purity of 99% (GC) furanoid linalool oxide. This is organoleptically comparable in taste and
5 odor with the nature-identical standard.

Although the invention has been described in detail in the foregoing for the purpose of illustration, it is to be understood that such detail is solely for that purpose and that variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention except as
10 it may be limited by the claims.

WHAT IS CLAIMED IS:

1. A process for the preparation of linalool oxide or linalool oxide-containing mixtures comprising the step of oxidizing linalool or linalool-containing mixtures in the presence of an enzyme to form a reaction mixture.
5
2. A process according to Claim 1, wherein D-linalool, L-linalool or essential oils comprising D-linalool or L-linalool, or mixtures of these substances are employed.
3. A process according to Claim 1, wherein enzymes from yeasts or bacteria are employed.
10
4. A process according to Claim 3, wherein enzymes from *Candida antarctica* strains, *Pseudomonas* sp., *Humicola* sp., *Mucor miehi*, or mixed cultures containing these yeasts are used.
5. A process according to Claim 4, wherein enzymes from *Candida antarctica* are employed.
15
6. A process according to Claim 3, wherein said enzyme is a lipase.
7. A process according to Claim 1, wherein oxidation is carried out in the presence of one or more oxidizing agents.
- 20 8. A process according Claim 7, wherein the oxidizing agent is added before or during the process.
9. A process according to Claim 7, wherein the oxidizing agent is generated in the reaction mixture before or during the process.
10. A process according to Claim 7, wherein the oxidizing agent is generated in the presence of a catalyst before or during the oxidation process.
25
11. A process according to Claim 1, wherein said enzymes are employed as catalysts
12. A process according to Claim 1, wherein said enzyme is glucose oxidase (GOD).
30
13. A process according to Claim 7, wherein the oxidizing agent is added in quantities of from 0.1 to 80% to the reaction medium.

14. A process according to Claim 7, wherein said oxidizing agent is hydrogen peroxide.

15. A process according to Claim 1, wherein solvents are added to the reaction medium.

5 16. A process according to Claim 15, wherein said solvent is a carboxylic acid or carboxylic ester.

17. A process according to Claim 16, wherein said solvent is ethyl acetate or acetic acid.

10 18. Linalool oxide prepared by a process for the preparation of linalool oxide or linalool oxide-containing mixtures comprising the step of oxidizing linalool or linalool-containing mixtures in the presence of an enzyme to form a reaction mixture.

15 19. Linalool oxide according to Claim 18, wherein D-linalool, L-linalool or essential oils comprising D-linalool or L-linalool, or mixtures of these substances are employed.

20. Linalool oxide according to Claim 18, wherein enzymes from yeasts or bacteria are employed.

20 21. Linalool oxide according to Claim 20, wherein enzymes from *Candida antarctica* strains, *Pseudomonas* sp., *Humicola* sp., *Mucor miehi*, or mixed cultures containing these yeasts are used.

22. Linalool oxide according to Claim 21, wherein enzymes from *Candida antarctica* are employed.

23. Linalool oxide according to Claim 18, wherein said enzyme is a lipase.

25 24. Linalool oxide according to Claim 18, wherein oxidation is carried out in the presence of one or more oxidizing agents.

25. Linalool oxide according Claim 24, wherein the oxidizing agent is added before or during the process.

30 26. Linalool oxide according to Claim 24, wherein the oxidizing agent is generated in the reaction mixture before or during the process.

27. Linalool oxide according to Claim 24, wherein the oxidizing agent is generated in the presence of a catalyst before or during the oxidation process.

28. Linalool oxide according to Claim 18, wherein said enzymes are employed as catalysts

29. Linalool oxide according to Claim 18, wherein said enzyme is glucose oxidase (GOD).

5 30. Linalool oxide according to Claim 24, wherein the oxidizing agent is added in quantities of from 0.1 to 80% to the reaction medium.

31. Linalool oxide according to Claim 24, wherein said oxidizing agent is hydrogen peroxide.

10 32. Linalool oxide according to Claim 18, wherein solvents are added to the reaction medium.

33. Linalool oxide according to Claim 32, wherein said solvent is a carboxylic acid or carboxylic ester.

34. Linalool oxide according to Claim 33, wherein said solvent is ethyl acetate or acetic acid.

15 35. A food product comprising an aroma material which comprises linalool oxide prepared by a process for the preparation of linalool oxide or linalool oxide-containing mixtures comprising the step of oxidizing linalool or linalool-containing mixtures in the presence of an enzyme to form a reaction mixture.

20 36. A fragrance composition comprising linalool oxide prepared by a process for the preparation of linalool oxide or linalool oxide-containing mixtures comprising the step of oxidizing linalool or linalool-containing mixtures in the presence of an enzyme to form a reaction mixture.

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37. A process for the preparation of linalool oxide which comprises forming a reaction mixture of linalool and an enzyme that oxidizes linalool to linalool oxide.

38. A process according to Claim 37, wherein the
5 reaction mixture is free of any living microorganism.

39. A process according to Claim 37 or 38, wherein the reaction mixture also contains an oxidizing agent.

40. A process according to Claim 39, wherein the oxidizing agent is generated *in situ*.

10 41. A process according to Claim 40, wherein the oxidizing agent is hydrogen peroxide that is generated *in situ* by means of an enzyme.

42. A process according to Claim 41, wherein the enzyme is glucose oxidase.

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