santalene, a terpene synthase which would produce >50% beta-santalene would be needed.

Furthermore, there is a constant need for aroma compositions with novel or improved olfactory properties.

The technical problem underlying the present disclosure shall be seen as the provision of means and methods complying with the aforementioned needs. The technical problem is solved by the examples characterized in the claims and herein below.

The present disclosure relates to a method for the manufacture of a composition comprising at least one santalene comprising the step of converting farnesyl pyrophosphate into at least one santalene, wherein said conversion is carried out by at least one polypeptide exhibiting santalene synthase activity, wherein said at least one polypeptide comprises (i) an amino acid sequence selected from the group consisting of:

a) an amino acid sequence as shown in any of SEQ ID NO: 1, 18, or 21 to 28;
b) an amino acid sequence which is at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences as shown in any of SEQ ID NO: 1, 18, or 21 to 28;
c) an amino acid sequence encoded by a nucleic acid sequence as shown in SEQ ID NO: 2 or 3 or 19;
d) an amino acid sequence encoded by a nucleic acid sequence which is at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequence as shown in SEQ ID NO: 2 or 3 or 19; and
e) an amino acid sequence of a fragment of any one of (a) to (d), said fragment encoding a polypeptide exhibiting a santalene synthase activity; or

(ii) an amino acid sequence selected from the group consisting of:

a) an amino acid sequence as shown in any one of SEQ ID NO: 4 to 17;
b) an amino acid sequence which is at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences as shown in SEQ ID NO: 4 to 17; and
c) an amino acid sequence of a fragment of (a) or (b), said fragment encoding a polypeptide exhibiting a santalene synthase activity.

In an aspect, there is provided a method for the manufacture of a composition comprising at least one santalene comprising the step of converting farnesyl pyrophosphate into at least one santalene, wherein said conversion is carried out by at least one polypeptide exhibiting santalene synthase activity, wherein said at least one polypeptide comprises an amino acid sequence selected from the group consisting of:

a) an amino acid sequence as shown in any of SEQ ID NO: 1, 18, or 21 to 28;
b) an amino acid sequence which is at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences as shown in any of SEQ ID NO: 1, 18, or 21 to 28;

c) an amino acid sequence encoded by a nucleic acid sequence as shown in any one of SEQ ID NOs: 2 or 3 or 19;

d) an amino acid sequence encoded by a nucleic acid sequence which is at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to a polypeptide encoded by the nucleic acid sequence as shown in any one of SEQ ID NOs: 2 or 3 or 19; and

e) an amino acid sequence of a fragment of any one of (a) to (d), said fragment encoding a polypeptide exhibiting a santalene synthase activity.

In another aspect, there is provided a composition comprising a mixture of beta-santalene and alpha-santalene with an excess of beta-santalene obtainable by the method as defined above, wherein the composition further comprises sesquithujene in detectable amounts and is substantially free of any one of cis-alpha-Bergamotene ((AS Nr. 18252-46-5), (E)-beta-Farnesene (CAS Nr. 18794-84-8), trans-beta-Bergamotene (CAS Nr.15438-94-5) or beta-Bisabolene (CAS Nr.495-61-4 ), or all of these, preferably substantially free of (E)-beta-Farnesene (CAS Nr. 18794-84-8).

In another aspect, there is provided a composition comprising a mixture of beta-santalene and alpha-santalene with an excess of beta-santalene obtainable as defined above, wherein said beta-santalene is present in the composition in a relative amount to total santalenes of at least about 50%, at least about 60 %, at least about 70%, preferably, between about 50% and about 80%, between about 60% and about 75%, between about 65% and about 70%, more preferably, at least about 67%, wherein the composition is substantially free of beta-farnesene and/or comprises sesquithujene.

In another aspect, there is provided the use of the polypeptide as defined above, the heterologous polynucleotide, the vector or gene construct as defined above, the host cell as defined above, or the non-vertebrate transgenic organism as defined above, for the manufacture of a composition comprising at least one santalene, preferably, beta-santalene, more preferably a mixture of beta-santalene and alpha-santalene, most preferably also comprising sesquithujene.

In another aspect, there is provided a method for manufacturing a composition comprising at least one santalol, preferably beta-santalol, comprising:

a) producing a composition comprising at least one santalene by the method as defined above, or providing a composition as defined above or as defined above; and

b) oxidising said at least one santalene, preferably, beta-santalene, into the respective alcohol in order to manufacture a composition comprising at least one santalol, preferably, beta-santalol.

In another aspect, there is provided a kit for the manufacture of a composition comprising at least one santalene comprising the polypeptide as defined above, the heterologous polynucleotide, the vector or gene construct as defined above, the host cell as defined above or the non-vertebrate transgenic organism as defined above.

In another aspect, there is provided a non-human host cell expressing a polypeptide exhibiting santalene synthase activity from the heterologous polynucleotide, the vector or gene construct as defined above, wherein the non-human host cell is transgenic for the nucleic acid encoding the santalene synthases as defined above.

In another aspect, there is provided a non-human or non-vertebrate transgenic organism expressing a polypeptide exhibiting santalene synthase activity from the heterologous polynucleotide, the vector or gene construct as defined above, wherein the non-human or non-vertebrate transgenic organism is transgenic for the nucleic acid encoding the santalene synthases as defined above.

In another aspect, there is provided an aroma composition comprising,

i) at least one composition as defined above or a composition as defined above or produced by any of the methods as defined above; and any of (ii) to (iv):
ii) at least one aroma chemical (X) other than a compound selected from the group consisting of beta-Santalene, alpha-Santalene, trans-alpha-Bergamotene, Sesquithujene and epi-beta-Santalene, or
iii) at least one non-aroma chemical carrier, or
iv) both of (ii) and (iii).

The composition manufactured by the method of the disclosure comprises at least one santalene as defined herein, preferably beta-santalene, or a mixture of both alpha-santalene and beta-santalene. If both alpha-santalene and beta-santalene are present, it is preferred that said composition comprises an excess of beta-santalene over alpha-santalene.

In one preferred example of the method of the disclosure, said composition comprising at least one santalene, is substantially free of any one or all of the following: cis-alpha-Bergamotene ((AS Nr. 18252-46-5), (E)-beta-Farnesene (CAS Nr. 18794-84-8), trans-beta-Bergamotene (CAS Nr.15438-94-5) and beta-Bisabolene (CAS Nr.495-61-4). Substantially free means that the composition contains no detectable amounts of cis-alpha-Bergamotene ((AS Nr. 18252-46-5), (E)-beta-Farnesene (CAS Nr. 18794-84-8), trans-beta-Bergamotene (CAS Nr.15438-94-5) and/or beta-Bisabolene (CAS Nr.495-61-4).

In another preferred example of the method of the disclosure, said composition comprising at least one santalene, in addition comprises Sesquithujene (CAS Nr. 58319-06-5) in detectable amounts. Preferably, detectable amounts of Sesquithujene (CAS Nr. 58319-06-5) means between and including 0.01 % and 3 % (mol/mol).

In still another preferred example of the method of the disclosure, said composition comprising at least one santalene, in addition comprises Sesquithujene (CAS Nr. 58319-06-5) in detectable amounts, and the composition is substantially free of any one or all of the following: cis-alpha-Bergamotene ((AS Nr. 18252-46-5), (E)-beta-Farnesene (CAS Nr. 18794-84-8), trans-beta-Bergamotene (CAS Nr.15438-94-5) and beta-Bisabolene (CAS Nr.495-61-4 ).

“%” in the context of the concentration of a solution as referred to herein means percentage (mol/mol) if not indicated otherwise.

It is to be understood that in the specification and in the claims, “a” or “an” can mean one or more of the items referred to in the following depending upon the context in which it is used. Thus, for example, reference to “an” item can mean that at least one of said item can be utilized.

As used in the following, the terms “have”, “comprise” or “include” are meant to have either a non-limiting meaning or a limiting meaning. Thus, having a limiting meaning these terms may refer to a situation in which, besides the feature introduced by these terms, no other features are present in an embodiment described, i.e. the terms have a limiting meaning in the sense of “consisting of” or “essentially consisting of”. Having a non-limiting meaning, the terms refer to a situation where besides the feature introduced by these terms, one or more other features are present in an embodiment described.

Further, as used in the following, the terms “preferably”, “more preferably”, “most preferably”, "particularly", "more particularly", “typically”, and “more typically” are used in conjunction with features in order to indicate that these features are preferred features, i.e. the terms shall indicate that alternative features may also be envisaged in accordance with the invention.

Further, it will be understood that the term “at least one” as used herein means that one or more of the items referred to following the term may be used in accordance with the invention. For example, if the term indicates that at least one item shall be used this may be understood as