Comprising more than 30,000 compounds, terpenes are produced predominantly by plants. In light of this, further production systems for terpenes are required as alternatives for plant sources.

Recently, Otto et al. (Microb Cell Fact (2019) 18: 205) established a multistep metabolic pathway in the yeast S. cerevisiae to produce abscisic acid. In another study, the biosynthetic pathway to abscisic acid via ionylideneethane has been described in the fungus Botrytis cinerea, in a study by Inomata and co-wokers (Phytochemistry. 2004 Oct;65(19):2667-78. doi: 10.1016/j.phytochem.2004.08.025.). However, this pathway to abscisic acid has not been used to produce ionylideneethane and alpha-ionone, for industrial applications. Ionylideneethane has not been considered as an aroma compound so far, and the use of the abscisic acid synthesis pathway for the production of aroma compounds has not been reported by these authors. Furthermore, it was not known that ionylideneethane could also be a useful precursor for vitamin A production.

It is an object of the present disclosure to provide new aroma chemicals. These should have pleasant organoleptic properties. It is a further object of the present disclosure to provide substances which can be used as an aroma chemical in ready-to-use compositions. In particular, odor-intensive substances having a pleasant odor are sought. Furthermore, they should be combinable with other aroma chemicals, allowing the creation of novel advantageous sensory profiles. In addition, these aroma chemicals should be obtainable from readily available starting materials, allowing their fast and economic manufacturing.

In one aspect, there is provided the use of alpha-ionylideneethane as an aroma compound.

In another aspect, there is provided the use of an alpha-ionylideneethane synthase in the production of one or more aroma compounds.

In another aspect, the present disclosure refers to a method for preparing one or more aroma compounds, comprising

a) providing farnesyl diphosphate and an alpha-ionylideneethane synthase, preferably an alpha-ionylideneethane synthase as defined above, under conditions suitable for the alpha-ionylideneethane synthase to produce alpha-ionylideneethane,
b) converting farnesyl diphosphate to alpha-ionylideneethane, in vitro or in a host cell,
c) optionally, converting alpha-ionylideneethane to one or more further aroma compounds,
d) isolating alpha-ionylideneethane and/or the optionally one or more further aroma compounds and,
e) optionally, purifying alpha-ionylideneethane and/or the optionally one or more further aroma compounds.

In another aspect, the present disclosure refers to a method for scenting a product, particularly for imparting and/or enhancing an odor or flavor, in which at least one alpha-ionylideneethane

synthase as defined above, wherein the method includes the step of preparing one or more aroma compounds according to the methods as defined above, followed optionally by a step of purification of the one or more aroma compounds and a subsequent step of scenting a product with the one or more aroma compounds.

In another aspect, the present disclosure refers to an aroma compound or composition and/or fragrance composition and/or perfumed or fragranced product, comprising:

i) at least an alpha-ionylideneethane as defined above;

ii) optionally, at least one further aroma compound different from i), and

iii) optionally, at least one diluent.

In another aspect, the present disclosure refers to a perfumed or fragranced product comprising at least an alpha-ionylideneethane as defined above.

In another aspect, the present disclosure refers to a method for producing alpha-ionone (4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one), comprising the steps in the following order:

a) contacting farnesyl diphosphate with at least one alpha-ionylideneethane synthase as defined above, under conditions suitable to produce at least one alpha-ionylideneethane;

b) producing the at least one alpha-ionylideneethane;

c) exposing the at least one alpha-ionylideneethane produced in step b) to conditions suitable for oxidative cleavage of alpha-ionylideneethane to produce alpha-ionone; and

d) optionally, isolating the alpha-ionone produced in step c).

In another aspect, the present disclosure refers to a host cell for producing alpha-ionone (4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one), wherein the host cell comprises farnesyl diphosphate and a heterologous alpha-ionylideneethane synthase as defined above, wherein the host cell is preferably a bacterial cell, a yeast cell, a fungal cell, an algal cell, a cyanobacterial cell, a non-human animal cell, a non-human mammalian cell, or a plant cell, and the host cell is suitable for oxidative cleavage of alpha-ionylideneethane to produce alpha-ionone.

In another aspect, there is provided the use of a host cell comprising farnesyl diphosphate and a heterologous nucleic acid encoding an alpha-ionylideneethane synthase as defined above, for

(i) producing alpha-ionylideneethane, preferably 2Z,4E-alpha-ionylideneethane (1,5,5-trimethyl-6-[(1E,3Z)-3-methyl-penta-1,3-dienyl]cyclohexene), preferably as an aroma

ingredient or aroma compound, a precursor of an aroma substance or as a precursor of vitamin A;
(ii) producing alpha-ionone, preferably R-alpha-ionone;
(iii) producing vitamin A;
(iv) converting alpha-ionylideneethane to alpha-ionone;
(v) converting alpha-ionylideneethane to vitamin A;
(vi) for heterologous reconstitution of a terpene or terpenoid;
(vii) for producing an industrial product, preferably an aroma composition, flavour or fragrance, a pharmaceutical composition, an agricultural composition, animal feed, a human nutritional product, a cosmetic, a colorant (carotenoid) or a radical scavenger; and /or
(viii) a fermentative production system for producing a sesquiterpene.

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

The present disclosure relates to a method for preparing one or more aroma compounds, comprising:
a) providing farnesyl diphosphate and an alpha-ionylideneethane synthase, under conditions suitable for the alpha-ionylideneethane synthase to produce alpha-ionylideneethane,
b) converting farnesyl diphosphate to alpha-ionylideneethane, in vitro or in a host cell,
c) optionally, converting alpha-ionylideneethane to one or more further aroma compounds,
d) isolating alpha-ionylideneethane and the optionally one or more further aroma compounds and,
e) optionally, purifying alpha-ionylideneethane and the optionally one or more further aroma compounds.

One example of the disclosure relates to a method for preparing one or more aroma compounds, comprising providing farnesyl diphosphate and an alpha-ionylideneethane synthase, converting farnesyl diphosphate to alpha-ionylideneethane, in the presence of farnesyl diphosphate and an alpha-ionylideneethane synthase, in vitro or in a host cell, optionally converting all or part of the alpha-ionylideneethane to one or more further aroma compounds, isolating alpha-

ionylideneethane and the optionally one or more further aroma compounds and, optionally, purifying alpha-ionylideneethane and the optionally one or more further aroma compound. Preferably, at least one aroma compound is alpha-ionylideneethane, more preferably, the alpha-ionylideneethane is 2Z,4E-alpha-ionylideneethane (E,Z alpha-ionylideneethane = 1,5,5-trimethyl-6-[(1E,3Z)-3-methyl-penta-1,3-dienyl]cyclohexene). Preferably, at least one of the further aroma compounds is alpha-ionone, preferably R-alpha-ionone, and more preferably the method of the disclosure is a method for the preparation of alpha-ionylideneethane and alpha-ionone and, optionally, one or more aroma compounds other than alpha-ionylideneethane and alpha-ionone.

In a preferred example of the method of the disclosure for preparing one or more aroma compounds, the method includes the further steps of exposing all or part of the produced at least one alpha-ionylideneethane to conditions suitable for oxidative cleavage of alpha-ionylideneethane to produce at least one alpha-ionone, preferably R-alpha-ionone, and converting all or part of the at least one alpha-ionylideneethane to alpha-ionone, preferably R-alpha-ionone, preferably by chemical or enzymatical oxidative cleavage of alpha-ionylideneethane.

The method of the disclosure for preparing one or more aroma compounds, such as the aroma compound alpha-ionylideneethane, can be carried out, in vitro or in a host cell. It comprises the provision of farnesyl diphosphate and one or more alpha-ionylideneethane synthase(s) as defined herein. Farnesyl diphosphate is provided as a substrate for the one or more alpha-ionylideneethane synthase(s). The method further comprises the conversion of farnesyl diphosphate to alpha-ionylideneethane by said one or more alpha-ionylideneethane synthase(s). The thus produced alpha-ionylideneethane is isolated and, optionally, purified.

Thanks to the present inventors, ionylideneethane could be identified as an aroma compound. This finding could not be expected because ionylideneethane was not considered as an aroma compound, thus far. Surprisingly, it has been found by the present inventors that alpha-ionylideneethane can be used for preparing one or more aroma compounds which convey a note of Floral-Violet and / or Woody-Orris /Iris Root to a perfume, fragrance or aroma.

In addition, the present inventors found that ionylideneethane could also be a useful precursor for vitamin A production which has not yet been reported in the prior art. Furthermore, the present inventors advantageously found that this part of the abscisic acid synthesis pathway can be used for the industrial-scale production of aroma compounds for aroma chemical compositions of the disclosure which is a novel and surprising finding as well.

Alpha-ionylideneethane is a sesquiterpenoid.

A compound description of 2E,4E-alpha-Ionylideneethane can be found, e.g., in https://pubchem.ncbi.nlm.nih.gov/compound/101359914. 2Z,4E-alpha-Ionylideneethane is