CLAIMS

1. A process for preparing (-)-Ambrox or a mixture comprising (-)-Ambrox, the process comprising enzymatically converting (*3E,7E*)-homofarnesol (EEH) or a mixture of isomers of homofarnesol comprising EEH to (-)-Ambrox or a mixture comprising (-)-Ambrox using a SHC/HAC enzyme variant,

wherein the SHC/HAC enzyme variant has an amino acid sequence having at least 90.0 % identity to SEQ ID NO: 1,

wherein the SHC/HAC enzyme variant has an increased enzymatic activity relative to SEQ ID NO: 10,

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 132, 224 and 432 of SEQ ID NO: 1 which are M132R, A224V and I432T respectively, and

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at a position corresponding to position 557 of SEQ ID NO: 1 and at least one position corresponding to position 81, 431 or 613 of SEQ ID NO: 1,

wherein one or more of the amino acid alterations at positions 81, 431, 557, or 613 are substitutions, preferably non-conservative substitutions,

wherein the substitution at the position corresponding to position 557 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid; and/or

wherein the substitution at the position corresponding to position 81 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a basic amino acid; and/or

wherein the substitution at the position corresponding to position 431 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a hydrophobic amino acid; and/or

wherein the substitution at the position corresponding to position 613 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid.

2. A process for preparing Ambra oxide or a mixture comprising Ambra oxide, the process comprising enzymatically converting (*E,E*)-Bishomofarnesol (BisEEH) or a mixture of isomers of bishomofarnesol comprising BisEEH to Ambra oxide or a mixture comprising Ambra oxide using a SHC/HAC enzyme variant,

wherein the SHC/HAC enzyme variant has an amino acid sequence having at least 90.0 % identity to SEQ ID NO: 1,

wherein the SHC/HAC enzyme variant has an increased enzymatic activity relative to SEQ ID NO: 10,

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 132, 224 and 432 of SEQ ID NO: 1 which are M132R, A224V and I432T respectively, and

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at a position corresponding to position 557 of SEQ ID NO: 1 and at least one position corresponding to position 81, 431 or 613 of SEQ ID NO: 1,

wherein one or more of the amino acid alterations at positions 81, 431, 557, or 613 are substitutions, preferably non-conservative substitutions,

wherein the substitution at the position corresponding to position 557 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid; and/or

wherein the substitution at the position corresponding to position 81 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a basic amino acid; and/or

wherein the substitution at the position corresponding to position 431 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a hydrophobic amino acid; and/or

wherein the substitution at the position corresponding to position 613 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid.

3. An SHC/HAC enzyme variant having an amino acid sequence having at least 90.0 % identity to SEQ ID NO: 1,

wherein the SHC/HAC enzyme variant has an increased enzymatic activity relative to SEQ ID NO: 10,

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 132, 224 and 432 of SEQ ID NO: 1 which are M132R, A224V and I432T respectively, and

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at a position corresponding to position 557 of SEQ ID NO: 1 and at least one position corresponding to position 81, 431 or 613 of SEQ ID NO: 1,

wherein one or more of the amino acid alterations at positions 81, 431, 557, or 613 are substitutions, preferably non-conservative substitutions,

wherein the substitution at the position corresponding to position 557 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid; and/or

wherein the substitution at the position corresponding to position 81 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a basic amino acid; and/or

wherein the substitution at the position corresponding to position 431 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a hydrophobic amino acid; and/or

wherein the substitution at the position corresponding to position 613 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid.

4. The process of claim 1 or 2 or the SHC/HAC enzyme variant of claim 3, wherein the SHC/HAC enzyme variant has an amino acid sequence having at least 95.0 % identity to SEQ ID NO: 1.

5. The process or SHC/HAC enzyme variant of any one of the preceding claims, wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 557 and 431 of SEQ ID NO: 1.

6. The process or SHC/HAC enzyme variant of any one of the preceding claims, wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 557 and 613 of SEQ ID NO: 1.

7. The process or SHC/HAC enzyme variant of claim 5 or 6, wherein the SHC/HAC enzyme variant amino acid sequence has a further amino acid alteration relative to SEQ ID NO: 1 at a position corresponding to position 81 of SEQ ID NO: 1.

8. The process or SHC/HAC enzyme variant of any one of the preceding claims, wherein :
the neutral hydrophilic amino acid is selected from the group consisting of cysteine, serine, threonine, asparagine and glutamine; and/or
the basic amino acid is selected from the group consisting of histidine, lysine and arginine; and/or
the hydrophobic amino acid is selected from the group consisting of methionine, valine, leucine and isoleucine.

9. The process or SHC/HAC enzyme variant of any one of the preceding claims, wherein:
the substitution at the position corresponding to position 557 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for threonine; and/or
the substitution at the position corresponding to position 81 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for histidine; and/or
the substitution at the position corresponding to position 431 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for leucine; and/or
the substitution at the position corresponding to position 613 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for serine.

10. The process or SHC/HAC enzyme variant of any one of claims 1-3 or 5-9, wherein the enzyme variant has at least 90% identity with SEQ ID NO:2 and has the following amino acid alterations at positions 132, 224, 432, 557 and 431: M132R, A224V, I432T, A557T and H431L; or
wherein the enzyme variant has at least 90% identity with SEQ ID NO:3 and has the following amino acid alterations at positions 132, 224, 432, 557 and 613: M132R, A224V, I432T, A557T and R613S; or
wherein the enzyme variant has at least 90% identity with SEQ ID NO:4 and has the following amino acid alterations at positions 81, 132, 224, 432, 557 and 613: Y81H, M132R, A224V, I432T, A557T and R613S; or
wherein the enzyme variant has at least 90% identity with SEQ ID NO:5 and has the following amino acid alterations at positions 81, 132, 224, 432, 557 and 431: Y81H, M132R, A224V, I432T, A557T and H431L; and wherein positions 81, 132, 224, 431, 432, 557, 613 correspond to positions of SEQ ID NO:1.

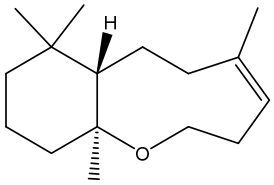
11. The process or SHC/HAC enzyme variant of claim 10, wherein the SHC/HAC enzyme variant has the following amino acid sequence: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, preferably SEQ ID NO: 3.

12. The process of any one of claims 1 to 2 and 4 to 11, wherein the enzymatic conversion takes place at a temperature in the range of 30ºC to 50ºC, for example from 40^0 to 50^0C, and/or at a pH in the range of 5 to 6.

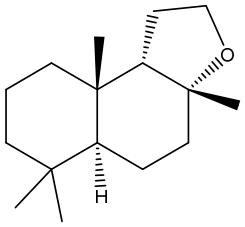
13. The process of any one of claims 1 to 2 and 4 to 12, wherein the process comprises culturing recombinant host cell that produces the SHC/HAC enzyme variant.

14. The process of claim 13, wherein the recombinant host cells comprise a nucleic acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9, preferably a nucleic acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 9.

15. The process of any one of claims 1 and 4 to 14, wherein (-)-Ambrox is produced in admixture with at least one or more of the by-products (II), (III), and (IV),



(II)



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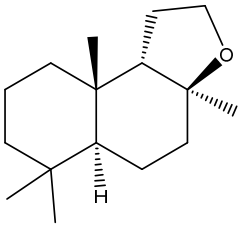

(IV)

16. The process or SHC/HAC enzyme variant of any one of the preceding claims, wherein the SHC/HAC enzyme variant provides an increased rate of EEH conversion over the first 6 hours or over the first 8 hours or over the first 12 hours of the reaction compared to SEQ ID NO: 10.