

Yeast cells and methods for production of *E8,E10*-dodecadienyl coenzyme A, codlemone and derivatives thereof

Technical field

The present invention relates to yeast cells engineered for the production of *E8,E10*-dodecadienyl coenzyme A, codlemone (*E8,E10*-dodecadien-1-ol), and optionally its derivatives *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal. Methods for production of *E8,E10*-dodecadienyl coenzyme A, codlemone (*E8,E10*-dodecadien-1-ol), and optionally its derivatives *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal are also provided. Nucleic acid constructs useful for obtaining such yeast cells are also provided.

Background

Integrated Pest Management (IPM) is expected to play a major role for both increasing the crop yield and for minimizing environmental impact and enabling organic food production. IPM employs alternative pest control methods, such as mating disruption using pheromones, mass trapping using pheromones, beneficial insects, etc.

Pheromones constitute a group of diverse chemicals that insects (like other organisms) use to communicate with individuals of the same species in various contexts, including mate attraction, alarm, trail marking and aggregation. Insect pheromones associated with long-range mate finding are already used in agriculture and forestry applications for monitoring and control of pests, as a safe and environmentally friendly alternative to pesticides.

Pheromones represent a health- and environment-friendly alternative to pesticides. Dispensing sex pheromones in the fields or orchards disrupts insect communication and prevents mating; thus no fertile eggs will be laid and no larval damage will occur to the crops. This method is called "mating disruption". Pheromones are attractive alternatives to insecticides, because they are biodegradable, species-specific compounds, which neither harm beneficial species nor humans.

Application of insect pheromones for pest control became possible only after industrial-scale synthesis of pheromones started several decades ago. Nevertheless, the prices for chemically synthesized pheromones remain high and present a major barrier for expanding their usage in agriculture and forestry. Another drawback with the chemical production of pheromones is the requirement for toxic chemicals to be used as precursors, catalyzers and solvents, and large

amounts of organic waste generated during the purification. The current production methods based on complex chemical synthesis-based processes thus make the products prohibitively expensive for widespread use in many of the potential applications in agriculture and forestry.

5 There are several advantages to biological production methods as compared to chemical production methods. First, all the reactions are carried out by engineered cells at ambient temperatures in fermentation tanks instead of multiple chemical reaction steps requiring different precursors, catalyzers and conditions (often high temperatures and pressures).
10 Moreover, the engineered cells use cheap renewable materials, such as sugars or plant oils, instead of using multiple expensive specialty chemicals as precursors. While chemical reactions often suffer from low specificity, and thus require purification of intermediate compounds and extensive purification of the final product, biological reactions carried out by enzymes are typically very specific and formation of by-products is limited, thereby reducing the usage of organic solvents and other toxic chemicals for purification. Moreover, specific stereo-chemistry,
15 which is often important for pheromone activity, can be very difficult to achieve by chemical methods, while enzymatic methods can take advantage of enzymes specific for one of the cis- or trans- isomers.

20 A specific pheromone of interest is codlemone, a di-unsaturated fatty alcohol with the formula *E8,E10*-dodecadien-1-ol (*E8,E10*-C12:OH, CAS nr. 33956-49-9). Codlemone is a sex pheromone component of a number of species, and the main sex pheromone of *Cydia pomonella* (codling moth), which belongs to the order of *Lepidoptera* and is a major pest of apples, pears, plums, and other fruits.

25 Ding 2014 discloses plant cells in which desaturases were expressed, and tested to determine whether they could produce moth pheromones. By using degenerate PCR approach, three desaturase from *C. pomonella* were found (Ding et al. On the way of making plants smell like moths – a synthetic biology approach. Lund University, Faculty of Science, Department of Biology).
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Hence, there is a need for biological processes for production of insect pheromones, in particular codlemone. In addition to lower cost benefits, fermentation processes are inherently less hazardous and more environmentally friendly than chemical synthesis.

Summary

The invention is as defined in the claims.

5 Herein is provided a yeast cell capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, said yeast cell expressing at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA).

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Herein is provided a yeast cell capable of producing *E8,E10*-dodecadien-1-ol, said yeast cell expressing:

- 15 i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
- 20 ii) At least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.

25 Also provided is a method for producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:

- 30 i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
- ii) Optionally at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of

converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol,
thereby producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

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Also provided is a nucleic acid construct for modifying a yeast cell, said construct comprising:

- i) At least one first polynucleotide encoding at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
- ii) Optionally a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.

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Also provided is a method of monitoring the presence of pest or disrupting the mating of pest, said method comprising the steps of:

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- i) Producing *E8,E10*-dodecadien-1-ol and optionally *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal by the methods described herein;
- ii) Formulating said *E8,E10*-dodecadien-1-ol and optionally said *E8,E10*-dodecadienyl acetate and/or said *E8,E10*-dodecadienal as a pheromone composition; and
- iii) Employing said pheromone composition as an integrated pest management composition.

Also provided herein are *E8,E10*-dodecadienyl coenzyme A, *E8,E10*-dodecadien-1-ol, *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal obtainable by the methods described herein.

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Also provided herein is a kit of parts comprising instructions for use and:

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- a) the yeast cell described herein; and/or
- b) the nucleic acid construct described herein for modifying a yeast cell and optionally the yeast cell to be modified, wherein upon expression of the polynucleotides comprised within the nucleic acid construct, the modified yeast cell is capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

Description of the drawings

Figure 1. Proposed biosynthesis pathway for codlemone production (*E8,E10*-C12:OH) in yeast. ACC: acetyl-CoA-carboxylase; FA: fatty acids; FAS: fatty acid synthase; TE: thioesterase; FAA: Fatty acyl-CoA synthetase; L: lipids; FAE: fatty acid esters; FAD: fatty acyl desaturase; FAR: fatty acyl reductase; Comp. β -ox.: complete β -oxidation.

Figure 2. GC-MS analysis of FAME extracts from yeast transformed with (A) empty plasmid or (B) Cpo_CPRQ containing vector fed with 12:Me, (C) mass spectrum of *E9-12*:Me; GC-MS analysis of FAME extracts from yeast transformed with (D) empty plasmid or (E) Cpo_CPRQ fed with *E9-12*:Me, (F) mass spectrum of *E8,E10-12*:Me.

Detailed description

Definitions

Biopesticide: the term 'biopesticide' is a contraction of 'biological pesticide' and refers to several types of pest management intervention: through predatory, parasitic, or chemical relationships. In the EU, biopesticides have been defined as "a form of pesticide based on micro-organisms or natural products". In the US, they are defined by the EPA as "including naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs". The present disclosure relates more particularly to biopesticides comprising natural products or naturally occurring substances. They are typically created by growing and concentrating naturally occurring organisms and/or their metabolites including bacteria and other microbes, fungi, nematodes, proteins, etc. They are often considered to be important components of integrated pest management (IPM) programmes, and have received much practical attention as substitutes to synthetic chemical plant protection products (PPPs). The Manual of Biocontrol Agents (2009: formerly the Biopesticide Manual) gives a review of the available biological insecticide (and other biology-based control) products.

Cloud concentration: the term will herein be used to refer to the concentration of a surfactant, in particular non-ionic, or a glycol solution, in a solution above which, at a given temperature, a mixture of said surfactant and said solution starts to phase-separate, and two phases appear, thus becoming cloudy. For example, the cloud concentration of a surfactant in an aqueous solution at a given temperature is the minimal concentration of said surfactant which, when

mixed with the aqueous solution, gives rise to two phases. The cloud concentration can be obtained from the manufacturer of the surfactant, or it may be determined experimentally, by making a dosage curve and determining the concentration at which the mixture phase separates.

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Cloud point: The cloud point of a surfactant, in particular non-ionic, or a glycol solution, in a solution, for example an aqueous solution, is the temperature at which a mixture of said surfactant and said solution, for example said aqueous solution, starts to phase-separate, and two phases appear, thus becoming cloudy. This behavior is characteristic of non-ionic surfactants containing polyoxyethylene chains, which exhibit reverse solubility versus temperature behavior in water and therefore "cloud out" at some point as the temperature is raised. Glycols demonstrating this behavior are known as "cloud-point glycols". The cloud point is affected by salinity, being generally lower in more saline fluids.

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Codlemone: the term refers to a di-unsaturated alcohol with the formula *E8,E10*-dodecadien-1-ol (*E8,E10*-C12:OH). Codlemone is the main sex pheromone component of a number of species, among others *Cydia pomonella* (codling moth), which belongs to the order of *Lepidoptera* and is a major pest of apples, pears, plums, and other fruits. The terms "codlemone", "*E8,E10*-dodecadien-1-ol" and "*E8,E10*-C12:OH" will herein be used interchangeably.

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Desaturated: the term "desaturated" will be herein used interchangeably with the term "unsaturated" and refers to a compound containing one or more double or triple carbon-carbon bonds.

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Ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent: the term refers to a group of polyethoxylated, non-ionic surfactants which comprise or mainly consist of ethoxylated and propoxylated alcohols in C₁₆-C₁₈, for example CAS number 68002-96-0, also termed C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate or C₁₆-C₁₈ alcohols ethoxylated propoxylated polymer.

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Extractant: the term "extractant" as used herein refers to a non-ionic surfactant such as an antifoaming agent which facilitates recovery of hydrophobic compounds produced in a fermentation, in particular a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate such as simethicone and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

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5 Fatty acid: the term “fatty acid” refers to a carboxylic acid having a long aliphatic chain, i.e. an aliphatic chain between 4 and 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Most naturally occurring fatty acids are unbranched. They can be saturated, or desaturated.

10 Fatty alcohol acetate: the term will herein be used interchangeably with “fatty acetate” and refers to an acetate having a fatty carbon chain, i.e. an aliphatic chain between 4 and 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty alcohol acetates can be saturated or desaturated.

15 Fatty acyl-CoA: the term will herein be used interchangeably with “fatty acyl-CoA ester”, and refers to compounds of general formula R-CO-SCoA, where R is a fatty carbon chain. The fatty carbon chain is joined to the –SH group of coenzyme A by a thioester bond. Fatty acyl-CoAs can be saturated or desaturated, depending on whether the fatty acid which it is derived from is saturated or desaturated.

20 Fatty alcohol: the term “fatty alcohol” refers herein to an alcohol derived from a fatty acyl-CoA, having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty alcohols can be saturated or desaturated.

25 Fatty aldehyde: the term refers herein to an aldehyde derived from a fatty acyl-CoA, having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty aldehydes can be saturated or desaturated.

30 Heterologous: the term “heterologous” when referring to a polypeptide, such as a protein or an enzyme, or to a polynucleotide, shall herein be construed to refer to a polypeptide or a polynucleotide which is not naturally present in a wild type cell. For example, the term “heterologous $\Delta 9$ desaturase” when applied to *Yarrowia lipolytica* refers to a $\Delta 9$ desaturase which is not naturally present in a wild type *Y. lipolytica* cell, e.g. a $\Delta 9$ desaturase derived from *Drosophila melanogaster*.

35 Mixture of polyether dispersions: the term refers to a group of polyethoxylated non-ionic surfactants which comprise or mainly consist of a mixture of polyether dispersions, for example

organic antifoam 204 from Sigma Aldrich (product number A6426 and A8311, MDL number MFCD00130523).

5 Native: the term “native” when referring to a polypeptide, such as a protein or an enzyme, or to a polynucleotide, shall herein be construed to refer to a polypeptide or a polynucleotide which is naturally present in a wild type cell. The term will be used interchangeably with the term “endogenous”.

10 Pest: as used herein, the term ‘pest’ shall refer to an organism, in particular an animal, detrimental to humans or human concerns, in particular in the context of agriculture or livestock production. A pest is any living organism which is invasive or prolific, detrimental, troublesome, noxious, destructive, a nuisance to either plants or animals, human or human concerns, livestock, human structures, wild ecosystems etc. The term often overlaps with the related terms vermin, weed, plant and animal parasites and pathogens. It is possible for an organism to be a
15 pest in one setting but beneficial, domesticated or acceptable in another.

Pheromone: pheromones are naturally occurring compounds designated by an unbranched aliphatic chain (between 9 and 18 carbons) ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone. Pheromone compositions
20 may be produced chemically or biochemically, for example as described herein. Pheromones may thus comprise desaturated fatty alcohols, fatty aldehydes or fatty alcohol acetates, such as can be obtained by the methods and cells described herein.

25 Polyethoxylated surfactant: the term herein refers to polyethoxylated surfactants, i.e. non-ionic surfactants.

Polyethylene polypropylene glycol: the term refers to a group of polyethoxylated non-ionic surfactants which comprise or mainly consist of PEG-PPG-PEG block copolymer antifoaming agents, for example Kolliphor® P407 (CAS number 9003-11-6), also termed poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol).
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Reduced activity: the term “reduced activity” may herein refer to a total or a partial loss of activity of a given peptide, such as a protein or an enzyme. In some cases, peptides are encoded by essential genes, which cannot be deleted. In these cases, activity of the peptide
35 can be reduced by methods known in the art, such as down-regulation of transcription or translation, or inhibition of the peptide. In other cases, the peptide is encoded by a non-essential

gene, and the activity may be reduced or it may be completely lost, e.g. as a consequence of a deletion of the gene encoding the peptide. Reduced activity of an enzyme can also be achieved by repressing transcription of the gene encoding said enzyme as is known in the art, for example using a repressible promoter, by inhibiting the activity or by silencing at the translational level.

Saturated: the term "saturated" refers to a compound which is devoid of double or triple carbon-carbon bonds.

Simethicone: the term refers to a group of polyethoxylated non-ionic surfactants which comprise or mainly consist of simethicone, also termed simeticone (CAS number 8050-81-5), dimethyl polysiloxane, or activated Polymethylsiloxane. Simethicone is a silicone-based emulsion containing also 1.2-1.6% polyethylene glycol monostearate.

Surfactant: the term refers to compounds that lower the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, antifoaming agents, and dispersants. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant typically contains both a water-insoluble (or oil-soluble) component and a water-soluble component. Most commonly, surfactants are classified according to polar head group. A non-ionic surfactant has no charged groups in its head.

Titer: the titer of a compound refers herein to the produced concentration of a compound. When the compound is produced by a cell, the term refers to the total concentration produced by the cell, i.e. the total amount of the compound divided by the volume of the culture medium. This means that, particularly for volatile compounds, the titer includes the portion of the compound which may have evaporated from the culture medium, and it is thus determined by collecting the produced compound from the fermentation broth and from potential off-gas from the fermenter.

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Codlemone (E8,E10-C12:OH)

The biosynthesis of codlemone is based on acetyl-coenzyme A (CoA), which is carboxylated to malonyl-CoA; the reaction is catalyzed by acetyl-CoA-carboxylase (ACC). Malonyl-CoA and acetyl-CoA are precursors used by fatty acid synthase (FAS) to synthesize fatty acyl-CoAs up to a chain length of C16/C18. It was hypothesised that *C. pomonella* peroxisomal oxidases (POXs)

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catalyze the chain shortening (-2C) of C16:CoA via C14:CoA to C12:CoA (lauryl-CoA) (Ding, 2014). Evidence for a desaturase converting C12:CoA to *E*9-C12:CoA in *C. pomonella* has been found early on, but the gene coding for this desaturase has only been identified recently together with two more genes coding for additional desaturases

(Cpo_SPTQ/Cpo_NPVE/Cpo_CPRQ). A first desaturation step results in conversion of C12:CoA to *E*/Z9-C12:CoA, which in a second desaturation step is converted to *E*8,*E*10-C12:CoA (*E*8,*E*10-dodecadienyl coenzyme A). A fatty acyl reductase (FAR) then presumably reduces the diene *E*8,*E*10-C12:CoA to finally form codlemone (*E*8,*E*10-C12:OH). The gene coding for the FAR in *C. pomonella* has so far not been identified (Ding 2014, Löfstedt et al., 1988).

A proposed pathway for biosynthesis of codlemone is set out in Figure 1.

Production of codlemone

The present disclosure relates to yeast cells capable of producing *E*8,*E*10-dodecadienyl coenzyme A and optionally codlemone (*E*8,*E*10-C12:OH or *E*8,*E*10-dodecadien-1-ol) and to methods for production of codlemone (*E*8,*E*10-C12:OH or *E*8,*E*10-dodecadien-1-ol) in a yeast cell.

The inventors have designed a heterologous pathway (outlined in Figure 1 by way of example) for production of *E*8,*E*10-dodecadienyl coenzyme A and optionally *E*8,*E*10-dodecadien-1-ol in yeast.

Accordingly, herein is provided a method for production of *E*8,*E*10-dodecadienyl coenzyme A and optionally *E*8,*E*10-dodecadien-1-ol in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:

- i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting at least part of said fatty acyl-CoA to *E*8,*E*10-dodecadienyl coenzyme A (*E*8,*E*10-C12:CoA); and
 - ii) Optionally at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said *E*8,*E*10-dodecadienyl coenzyme A (*E*8,*E*10-C12:CoA) to *E*8,*E*10-dodecadien-1-ol,
- thereby producing *E*8,*E*10-dodecadienyl coenzyme A and optionally *E*8,*E*10-dodecadien-1-ol.

The present yeast cells and methods can thus be used to produce codlemone by producing *E8,E10*-dodecadienyl coenzyme A as described herein, which can then be converted to *E8,E10*-dodecadien-1-ol either in vivo by expressing a reductase in the yeast cell, or the *E8,E10*-dodecadienyl coenzyme A can be converted into a lipid such as a triacylglyceride or into a free fatty acid, which can then be recovered and converted to *E8,E10*-dodecadien-1-ol in vitro, as is known in the art, e.g. by contacting them with a reductase. In both cases, *E8,E10*-dodecadien-1-ol is produced.

10 Yeast cell

In a first step of the method, a yeast cell is provided, which can use acetyl-CoA and malonyl-CoA for the biosynthesis of longer acyl-CoAs. Any yeast cell capable of synthesising acyl-CoAs can be used for producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol as described herein. Alternatively, the yeast cell may be provided with suitable carbon sources as is known in the art. The yeast cell may be a non-naturally occurring yeast cell, for example a yeast cell which has been engineered to produce *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal as described herein.

Acetyl-CoA and malonyl-CoA can be converted to acyl-CoAs, in particular an acyl-CoA having a carbon chain length of 12. This can involve a step of converting dodecanoyl-CoA to dodecanoic acid (lauric acid), for example by the action of a native or heterologous acyl-CoA thioesterase (EC 3.1.2.20). The lauric acid can then be converted to dodecanoyl-CoA by the action of a native or heterologous fatty acyl-coenzyme A synthetase (FAA) (EC 6.2.1.3).

The yeast cell is thus also capable of converting acetyl-CoA and malonyl-CoA to fatty acyl-CoAs, in particular to a fatty acyl-CoA having a carbon chain length of 12. In some embodiments, the yeast cell thus expresses one or more fatty acyl-coenzyme synthetases (EC 6.2.1.3) and/or one or more acyl-CoA thioesterases (EC 3.1.2.20) capable of performing said reaction.

In some embodiments, the yeast cell is provided with lauric acid or methyl laureate or tri-lauroylglycerol or another fatty acid derivative in the culture medium. Where the yeast cell has been engineered to be able to shorten the carbon chain via β -oxidation as described in detail

below, the cell can be provided with oil or fat or any fatty acid derivative that has a carbon chain length longer than 12.

In some embodiments, the cell has been modified at the genomic level, e.g. by gene editing in the genome. The cell may also be modified by insertion of at least one nucleic acid construct such as at least one vector. The vector may be designed as is known to the skilled person to either enable integration of nucleic acid sequences in the genome, or to enable expression of a polypeptide encoded by a nucleic acid sequence comprised in the vector without genome integration.

In some embodiments of the disclosure, yeast or fungi of genera including, but not limited to, *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Saccharomyces* and *Yarrowia* are employed. In certain particular embodiments, organisms of species that include, but are not limited to, *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*, *C. tropicalis*, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C. japonica*, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M. ramanniana*, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium irregulare*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R. mucilaginis*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are used. In some embodiments, the yeast cell is a *Yarrowia lipolytica* cell or a *Saccharomyces cerevisiae* cell.

The yeast cell to be modified, which will also be referred to as the host cell, may express native enzymes which may have a negative impact on the titre of *E8,E10*-dodecadien-1-ol that can be obtained; the native enzymes may thus be inactivated by methods known in the art, such as gene editing. For example, the genes encoding the native enzymes having a negative impact on the titre may be deleted or mutated so as to lead to total or partial loss of activity of the native enzyme, as described herein below.

Desaturase

The present methods rely on the yeast cell expressing the necessary enzymes for converting a fatty acyl-CoA having a carbon chain length of 12 to *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol. The first enzyme needed for this is a desaturase, which is capable of introducing one or more double bonds in said fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA of carbon

chain length 12 and having one or more double bonds. The desaturated fatty acyl-CoA of carbon chain length 12 may be a mixture of desaturated fatty acyl-CoAs of carbon chain length 12; said mixture comprises *E8,E10*-C12:CoA, but typically also comprises the monounsaturated fatty acyl-CoAs *E9*-C12:CoA and *Z9*-C12:CoA. Thus in some embodiments the yeast cell

5 expresses a desaturase which is capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting at least part of said fatty acyl-CoA to *E8,E10*-C12:CoA (*E8,E10*-dodecadienyl coenzyme A). Desaturases of the EC class EC 1.14.19. are capable of performing such reactions.

10 The production of codlemone relies on two desaturation steps. These may be performed by one desaturase, for example Cpo_CPRQ or Gmo_CPRQ, mutants and functional variants thereof, as described herein below, or by two different desaturases. In embodiments with two different desaturases, at least one of the desaturases is Cpo_CPRQ, a mutant thereof or a functional variant thereof as described herein below. In other embodiments with two different desaturases,

15 at least one of the desaturases is Gmo_CPRQ, a mutant thereof or a functional variant thereof as described herein below. The other desaturase is capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, or can introduce at least one double bond in a fatty acyl-CoA of carbon chain length 14, which can then be shortened to a desaturated fatty acyl-CoA of carbon chain length 12 as detailed below in the section "Chain shortening". The

20 fatty acyl-CoA of carbon chain length 12 or 14 having one double bond can then be further desaturated by e.g. Cpo_CPRQ, the mutant or functional variant thereof.

The desaturase is preferably a heterologous desaturase. In some embodiments, the desaturase is Cpo_CPRQ (SEQ ID NO: 2), which is a desaturase naturally found in *C. pomonella*. As

25 demonstrated in example 16, Cpo_CPRQ expression alone is sufficient to produce *E8,E10*-C12:CoA. Expression of either Cpo_SPTQ or Cpo_NPVE alone did not result in production of *E8,E10*-C12:CoA. This finding is surprising in light of Ding 2014, in which functional assays of these three desaturases indicated that they work consecutively forming the conjugated double bonds in *C. pomonella* pheromone – this does not seem to be the case in yeast.

30 The heterologous desaturase may also be a functional variant of a heterologous desaturase such as Cpo_CPRQ, i.e. a variant which retains the ability to convert a fatty acyl-CoA having a carbon chain length of 12 to a desaturated fatty acyl-CoA of carbon chain length 12 such as *E8,E10*-C12:CoA. In some embodiments, the functional variant has at least 60% homology or

35 identity, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as

at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Cpo_CPRQ (SEQ ID NO: 2).

The heterologous desaturase may also be a functional variant of a heterologous desaturase such as Cpo_CPRQ, i.e. a variant which retains the ability to convert a fatty acyl-CoA having a carbon chain length of 12 to a desaturated fatty acyl-CoA of carbon chain length 12 such as *E8,E10*-C12:CoA. In some embodiments, the functional variant has at least 60% homology or identity, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Cpo_CPRQ (SEQ ID NO: 2).

The desaturase is preferably a heterologous desaturase. In some embodiments, the desaturase is Gmo_CPRQ (SEQ ID NO: 77), which is a desaturase naturally found in *Grapholita molesta*, or a functional variant thereof which retains the ability to convert a fatty acyl-CoA having a carbon chain length of 12 to a desaturated fatty acyl-CoA of carbon chain length 12 such as *E8,E10*-C12:CoA. In some embodiments, the functional variant has at least 60% homology or identity, such as at least 61% homology or identity, such as at least 62% homology or identity,

such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Gmo_CPRQ (SEQ ID NO: 78).

In some embodiments, the desaturase is expressed by introducing a nucleic acid which encodes said desaturase, as is known in the art. Such nucleic acid may be codon-optimised as is known in the art. In particular embodiments, the nucleic acid encoding the desaturase is as set forth in SEQ ID NO: 1, or is a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 1. In other embodiments, the nucleic acid encoding the desaturase is as set forth in SEQ ID NO: 78, or is a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least

72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%,
such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such
as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at
least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least
5 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such
as at least 98%, such as at least 99% to SEQ ID NO: 78.

10 In some embodiments, the yeast cell expresses several desaturases capable of introducing one
or more double bonds in a fatty acyl-CoA of carbon chain length 12. In such embodiments,
preferably at least one of the several desaturases is Cpo_CPRQ, a mutant thereof or a
functional variant thereof as detailed below. In other embodiments, preferably at least one of the
several desaturases is Gmo_CPRQ, a mutant thereof or a functional variant thereof. The other
desaturase may be for example Cpo_NPVE (accession number: AHW98355, SEQ ID NO: 67)
15 or Cpo_SPTQ (accession number: AHW98356, SEQ ID NO: 69), or functional variants thereof
having at least 65% homology or identity, such as at least 70% homology or identity, such as at
least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least
74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%,
such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such
20 as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at
least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least
95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%
homology or identity thereto. Such desaturases may be expressed in the yeast cell after
introduction of a nucleic acid, which may be codon-optimised for the yeast cell, for example a
25 nucleic acid as set forth in SEQ ID NO: 66 or SEQ ID NO: 68, or a homologue thereof having at
least 65% homology or identity, such as at least 70% homology or identity, such as at least 71%
homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as
at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least
83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,
30 such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such
as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at
least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or
identity thereto.

35 The yeast cell may be engineered to express several copies of the heterologous desaturase.
This can be done as is known in the art. The desaturase or desaturases may also be expressed

at a high level as is known in the art, for example by the use of a constitutive promoter leading to strong expression levels – such promoters are known in the art.

5 In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a Cpo_CPRQ mutant having a mutation at position 85. In some embodiments, the mutation is an S85A mutation. The desaturase may also be a functional variant of said mutant, and has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to a mutant Cpo_CPRQ having a mutation at position 85, such as an S85A mutant. In some embodiments, 15 the mutant is an S85T mutant.

In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a Cpo_CPRQ mutant having a mutation at position 82. In some embodiments, the mutation is an S82A mutation. The desaturase may also be a functional variant of said mutant, and has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to a mutant Cpo_CPRQ having a mutation at position 82, such as an S82A mutant. 20 25

In some embodiments, the yeast cell expresses two or more heterologous desaturases. Said two or more desaturases may be identical or different. In a particular embodiment the yeast cell expresses Cpo_CPRQ as set forth in SEQ ID NO: 2 and a mutant Cpo_CPRQ such as having a mutation at position 85, such as an S85A mutant. In some embodiments, the yeast cell expresses a Cpo_CPRQ (SEQ ID NO: 2), a mutant Cpo_CPRQ or a functional variant thereof having at least 65% homology or identity thereto, and also expresses another desaturase 30 35 capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12. In some embodiments, the yeast cell expresses Gmo_CPRQ as set forth in SEQ ID NO: 77 and

Cpo_CPRQ as set forth in SEQ ID NO: 2 or a mutant or functional variant thereof as described herein.

5 In some embodiments, the other desaturase is Cpo_NPVE as set forth in SEQ ID NO: 67, a mutant thereof or a functional variant thereof having at least 65% homology or identity thereto, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto. The yeast cell in some embodiments expresses Cpo_CPRQ, a mutant or functional variant thereof, and Cpo_NPVE, a mutant or functional variant thereof. In some embodiments, the yeast cell expresses Gmo_CPRQ, a mutant or functional variant thereof, and Cpo_NPVE or a mutant or functional variant thereof.

20 In other embodiments, the other desaturase is Cpo_SPTQ as set forth in SEQ ID NO: 69, a mutant thereof or a functional variant thereof having at least 65% homology or identity thereto, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto. The yeast cell in some embodiments expresses Cpo_CPRQ, a mutant or functional variant thereof, and Cpo_SPTQ, a mutant or functional variant thereof. In some embodiments, the yeast cell expresses Gmo_CPRQ, a mutant or functional variant thereof, and Cpo_SPTQ or a mutant or functional variant thereof.

In preferred embodiments, the at least one heterologous desaturase is Cpo_CPRQ or a mutant or functional variant thereof as described herein above.

35 Yeast cells which, beside the desaturase described herein, which can introduce one or two double bonds in a fatty acyl-CoA of carbon chain length 12, express a desaturase capable of

introducing at least one double bond in a fatty acyl-CoA of carbon chain length >12, such as of carbon chain length 14 or more, must also express other enzymes capable of reducing the carbon chain length of the desaturated fatty acyl-CoA of carbon chain length >12. This is detailed below in the section "Chain shortening".

5

The yeast cell may thus express a desaturase capable of introducing one or more double bonds in said fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA of carbon chain length 12 and having one or more double bonds, such as any of the desaturases described herein above, or functional variants thereof which retain the capability to convert a fatty acyl-CoA to a desaturated fatty acyl-CoA of carbon chain length 12. The yeast cell may further express a desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length >12, such as of carbon chain length 14 or more, or functional variants thereof which retain the capability to introduce at least one double bond in a fatty acyl-CoA of carbon chain length >12, such as of carbon chain length 14 or more.

10

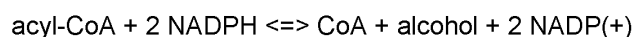
15

In order to test whether a desaturase or a functional variant thereof has the desired activity, methods known in the art can be employed. For example, the candidate enzyme to be tested can be introduced in the yeast cell, e.g. on a vector or in the genome of the yeast cell, incubating the yeast cell in an appropriate medium, extracting fatty alcohols and/or fatty acid methyl esters from the broth, and performing an analysis such as a GC-MS analysis to determine whether desaturated compounds are produced. It may be advantageous to test the activity in a yeast cell in which the native elongase gene(s) has/have been deleted. An example of such a procedure is described in example 4 or in Schneiter et al., 2000.

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Fatty acyl-CoA reductase (EC 1.2.1.84)

The terms "fatty acyl-CoA reductase", "reductase" and "FAR" will be used herein interchangeably. FARs catalyse the two-step reaction:



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wherein in a first step, the fatty acyl-CoA is reduced to a fatty aldehyde, before the fatty aldehyde is further reduced into a fatty alcohol in a second step. The fatty acyl-CoA may be a desaturated fatty acyl-CoA, in particular *E8,E10*-C12:CoA, which is then converted into *E8,E10*-dodecadien-1-ol.

The FARs capable of catalyzing such reaction are alcohol-forming fatty acyl-CoA reductases with an EC number 1.2.1.84. The yeast cells used in the present method may thus express a heterologous FAR capable of catalyzing the above reaction. Alternatively, the *E8,E10-C12:CoA* can be converted into *E8,E10-dodecadien-1-ol* after recovery of the *E8,E10-C12:CoA*, and
5 contacting said *E8,E10-C12:CoA* with a FAR in vitro.

The FAR is preferably be an insect FAR, such as a FAR native to an insect of the genus *Agrotis*, *Heliothis*, *Helicoverpa* or *Cydia*. For example, the FAR is native to *Agrotis segetum*, *Agrotis ipsilon*, *Heliothis subflexa*, *Helicoverpa assulta*, *Helicoverpa virescens* or *Cydia pomonella*.
10

In some embodiments the FAR is Ase_FAR (SEQ ID NO: 10), i.e. the FAR naturally occurring in *Agrotis segetum*. In some embodiments, the heterologous FAR is a functional variant of Ase_FAR, which retains the capability of converting *E8,E10-C12:CoA* to *E8,E10-dodecadien-1-ol*. For example, the functional variant has at least 65% homology or identity, such as at least
15 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least
20 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Ase_FAR (SEQ ID NO: 10).

In some embodiments the FAR is a mutant Ase_FAR, such as a mutant having a mutation at position 198 or 413. In some embodiments the Ase_FAR mutant is a T198A mutant. In other
25 embodiments, the Ase_FAR mutant is an S413A mutant.

In some embodiments, Ase_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Ase_FAR or the functional variant thereof. For example,
30 a nucleic acid as set forth in SEQ ID NO: 9 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or
35 identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as

at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 9.

In other embodiments, the FAR is Aip_FAR (SEQ ID NO: 61), i.e. the FAR naturally occurring in *Agrotis ipsilon*. In some embodiments, the heterologous FAR is a functional variant of Aip_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Aip_FAR (SEQ ID NO: 61).

In some embodiments, Aip_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Aip_FAR or the functional variant thereof. For example, a nucleic acid as set forth in SEQ ID NO: 60 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 60.

In other embodiments, the FAR is Hs_FAR (SEQ ID NO: 71), i.e. the FAR naturally occurring in *Heliothis subflexa*. In some embodiments, the heterologous FAR is a functional variant of Hs_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Hs_FAR (SEQ ID NO: 71).

In some embodiments, Hs_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Hs_FAR or the functional variant thereof. For example, a nucleic acid as set forth in SEQ ID NO: 70 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 70.

30

In other embodiments, the FAR is Has_FAR (SEQ ID NO: 73), i.e. the FAR naturally occurring in *Helicoverpa assulta*. In some embodiments, the heterologous FAR is a functional variant of Has_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such

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as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Has_FAR (SEQ ID NO: 73).

In some embodiments, Has_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Has_FAR or the functional variant thereof. For example, a nucleic acid as set forth in SEQ ID NO: 72 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 72.

In other embodiments, the FAR is Hv_FAR (SEQ ID NO: 75), i.e. the FAR naturally occurring in *Helicoverpa virescens*. In some embodiments, the heterologous FAR is a functional variant of Hv_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Hv_FAR (SEQ ID NO: 75).

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In some embodiments, Hv_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Hv_FAR or the functional variant thereof. For example, a nucleic acid as set forth in SEQ ID NO: 74 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 74.

In some embodiments, the yeast cell expresses a FAR from *Cydia pomonella*. In some embodiments, the FAR is Cpo_FAR (SEQ ID NO: 76), i.e. the FAR naturally occurring in *Cydia pomonella*. In some embodiments, the heterologous FAR is a functional variant of Cpo_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Cpo_FAR (SEQ ID NO: 76).

In some embodiments, Cpo_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Cpo_FAR or the functional variant thereof. For example, a nucleic acid as set forth in SEQ ID NO: 76 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology

or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 76.

In some embodiments, the FAR is Har_FAR (SEQ ID NO: 12), i.e. the FAR naturally occurring in *Helicoverpa armigera*. In some embodiments, the heterologous FAR is a functional variant of Har_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to Har_FAR (SEQ ID NO: 12).

In some embodiments, Har_FAR or a functional variant thereof is expressed by introducing a nucleic acid in the yeast cell encoding Har_FAR or the functional variant thereof. For example, a nucleic acid as set forth in SEQ ID NO: 11 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at

least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 11.

- 5 In some embodiments, the yeast cell expresses several copies of the FAR. For example, the FAR is expressed at high level as is known in the art.

In some embodiments, the yeast cell expresses a desaturase and a FAR as described herein. In specific embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional
10 variant thereof having at least 65% homology or identity thereto, and Ase_FAR (SEQ ID NO: 10) or a functional variant thereof having at least 65% homology or identity thereto. In some embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 65% homology or identity thereto, and the FAR is a mutant Ase_FAR such as a mutant having a mutation at position 198 or 413, for example a T198A mutant or an
15 S413A mutant. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a mutant having a mutation at position 85, for example an S85A mutant, and the FAR is Ase_FAR or a functional variant thereof. In other embodiments, the desaturase is an S85A Cpo_CPRQ mutant and the FAR is a mutant Ase_FAR such as a mutant having a mutation at position 198 or 413, for example a T198A mutant or an S413A mutant. In some embodiments, the
20 desaturase is two desaturases, such as two identical desaturases, for example Cpo_CPRQ or a mutant Cpo_CPRQ having a mutation at position 85, for example an S85A mutant, and the FAR is Ase_FAR or a functional variant thereof. In some embodiments, the desaturase is two desaturases, such as two identical desaturases, for example Cpo_CPRQ or a mutant Cpo_CPRQ having a mutation at position 85, for example an S85A mutant, and the FAR is a
25 mutant Ase_FAR such as a mutant having a mutation at position 198 or 413, for example a T198A mutant or an S413A mutant. In other embodiments, the desaturase is two different desaturases, for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is Ase_FAR or a functional variant thereof. In other embodiments, the desaturase is two different desaturases,
30 for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is a mutant Ase_FAR such as a mutant having a mutation at position 198 or 413, for example a T198A mutant or an S413A mutant. In other embodiments, the yeast cell expresses Gmo_CPRQ (SEQ ID NO: 77) and Ase_FAR or a mutant or functional variant thereof.

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In specific embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 65% homology or identity thereto, and Aip_FAR (SEQ ID NO: 61) or a functional variant thereof having at least 65% homology or identity thereto. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a mutant having a mutation at position 85, for example an S85A mutant, and the FAR is Aip_FAR or a functional variant thereof. In some embodiments, the desaturase is two desaturases, such as two identical desaturases, for example two Cpo_CPRQ desaturases or two mutant Cpo_CPRQ desaturases having a mutation at position 85, for example two S85A mutants, and the FAR is Aip_FAR or a functional variant thereof. In other embodiments, the desaturase is two different desaturases, for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is Aip_FAR or a functional variant thereof. In other embodiments, the yeast cell expresses Gmo_CPRQ (SEQ ID NO: 77) and Aip_FAR or a mutant or functional variant thereof.

In some embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 65% homology or identity thereto, and Hs_FAR (SEQ ID NO: 71) or a functional variant thereof having at least 65% homology or identity thereto. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a mutant having a mutation at position 85, for example an S85A mutant, and the FAR is Hs_FAR or a functional variant thereof. In some embodiments, the desaturase is two desaturases, such as two identical desaturases, for example two Cpo_CPRQ desaturases or two mutant Cpo_CPRQ desaturases having a mutation at position 85, for example two S85A mutants, and the FAR is Hs_FAR or a functional variant thereof. In other embodiments, the desaturase is two different desaturases, for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is Hs_FAR or a functional variant thereof. In other embodiments, the yeast cell expresses Gmo_CPRQ (SEQ ID NO: 77) and Hs_FAR or a mutant or functional variant thereof.

In specific embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 65% homology or identity thereto, and Has_FAR (SEQ ID NO: 73) or a functional variant thereof having at least 65% homology or identity thereto. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a mutant having a mutation at position 85, for example an S85A mutant, and the FAR is Hs_FAR or a functional variant thereof. In some embodiments, the desaturase is two desaturases, such as two identical desaturases, for example two Cpo_CPRQ desaturases or two mutant Cpo_CPRQ desaturases having a mutation at position 85, for example two S85A mutants, and the FAR is Hs_FAR or a

functional variant thereof. In other embodiments, the desaturase is two different desaturases, for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is Hs_FAR or a functional variant thereof. In other embodiments, the yeast cell expresses Gmo_CPRQ (SEQ ID NO: 77) and Has_FAR or a mutant or functional variant thereof.

In specific embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 65% homology or identity thereto, and Hv_FAR (SEQ ID NO: 75) or a functional variant thereof having at least 65% homology or identity thereto. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a mutant having a mutation at position 85, for example an S85A mutant, and the FAR is Hv_FAR or a functional variant thereof. In some embodiments, the desaturase is two desaturases, such as two identical desaturases, for example two Cpo_CPRQ desaturases or two mutant Cpo_CPRQ desaturases having a mutation at position 85, for example two S85A mutants, and the FAR is Hv_FAR or a functional variant thereof. In other embodiments, the desaturase is two different desaturases, for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is Hv_FAR or a functional variant thereof. In other embodiments, the yeast cell expresses Gmo_CPRQ (SEQ ID NO: 77) and Hv_FAR or a mutant or functional variant thereof.

In specific embodiments, the yeast cell expresses Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 65% homology or identity thereto, and Cpo_FAR (SEQ ID NO: 76) or a functional variant thereof having at least 65% homology or identity thereto. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a mutant having a mutation at position 85, for example an S85A mutant, and the FAR is Cpo_FAR or a functional variant thereof. In some embodiments, the desaturase is two desaturases, such as two identical desaturases, for example two Cpo_CPRQ desaturases or two mutant Cpo_CPRQ desaturases having a mutation at position 85, for example two S85A mutants, and the FAR is Cpo_FAR or a functional variant thereof. In other embodiments, the desaturase is two different desaturases, for example a Cpo_CPRQ desaturase and a mutant Cpo_CPRQ desaturase having a mutation at position 85, for example an S85A mutant, and the FAR is Cpo_FAR or a functional variant thereof. In other embodiments, the yeast cell expresses Gmo_CPRQ (SEQ ID NO: 77) and Cpo_FAR or a mutant or functional variant thereof.

In some embodiments, the yeast cell expresses a desaturase as described above, such as Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, a FAR as described herein

above, in particular Ase_FAR, Aip_FAR, Hs_AR, Has_FAR or Hv_FAR, or a mutant or a functional variant thereof having at least 65% homology or identity thereto, and also expresses another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, such as Cpo_NPVE (SEQ ID NO: 67) or Cpo_SPTQ (SEQ ID NO: 69),
5 a mutant or a functional variant thereof having at least 65% homology or identity to SEQ ID NO: 67 or SEQ ID NO: 69, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,
10 such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to SEQ ID NO: 67 or SEQ ID NO: 69.

15 In some embodiments, the FAR is not Har_FAR (FAR from *Helicoverpa armigera*, SEQ ID NO: 12). In some embodiments, the FAR is not Ta_FAR (FAR from *Tyto alba*, SEQ ID NO: 8).

The yeast cell may thus express a desaturase capable of introducing one or more double bonds in said fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA
20 to a desaturated fatty acyl-CoA of carbon chain length 12 and having one or more double bonds, such as any of the desaturases described herein above, or functional variants thereof which retain the capability to convert a fatty acyl-CoA to a desaturated fatty acyl-CoA of carbon chain length 12. The yeast cell may further express a desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length >12, such as of carbon chain length
25 14 or more, or functional variants thereof which retain the capability to introduce at least one double bond in a fatty acyl-CoA of carbon chain length >12, such as of carbon chain length 14 or more, as described above. Any of these yeast cells may further express a reductase as described herein above, or a functional variant thereof which retains reductase activity.

30 In order to test whether a reductase or a functional variant thereof has the desired activity, methods known in the art can be employed. For example, the candidate enzyme to be tested can be introduced in the yeast cell, e.g. on a vector or in the genome of the yeast cell, incubating the yeast cell in an appropriate medium, extracting fatty alcohols from the broth, and performing an analysis such as a GC-MS analysis to determine whether desaturated fatty
35 alcohols are produced. It may be advantageous to test the activity in a yeast cell in which the

native elongase gene(s) has/have been deleted. An example of such a procedure is described in example 4 or in Schneider et al., 2000.

Increased availability of precursors

5 In order to improve production of *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol and derivatives thereof, it may be advantageous to introduce additional modifications in the yeast cell in order to increase availability of the required precursors, in particular of *E8,E10*-C12:CoA. The yeast cell may thus be further modified with any of the modifications detailed below, in particular:

- 10
 - Expression of a heterologous cytochrome b5
 - Expression of a heterologous cytochrome b5 reductase
 - Expression of a hemoglobin
 - Inactivation of native elongase(s) resulting in total or partial loss of activity
 - Inactivation of native thioesterase(s) resulting in total or partial loss of activity
- 15
 - Inactivation or modification of activity of native fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s)
 - Expression of a heterologous thioesterase gene
 - Expression of a fusion protein of a fatty acyl synthase and of a thioesterase

20 An enzyme, for example an elongase, a thioesterase, a fatty aldehyde dehydrogenase, a fatty alcohol oxidase, a peroxisome biogenesis factor or a fatty acyl synthase, can be inactivated for example by introducing one or more mutations, including total or partial deletions, insertions, substitutions or non-sense or missense mutations, in the gene, for example in the coding sequence, promoter, Kozak sequence, terminator or other regulatory element. For example the
25 native promoter or the native terminator can be replaced by another, weaker promoter or by another terminator, respectively. Other inactivation methods resulting in partial or total loss of activity include repression of transcription as well as post-transcriptional inactivation, such as silencing, for example using an RNAi system or a CRISPR/Cas system resulting in the degradation of the relevant transcripts, thereby preventing or at least reducing translation, as
30 well as post-translational inactivation, such as inhibition of the protein. Enzyme activities can be otherwise modified, e.g. to modify properties of the enzymes such as intracellular localisation, or to increase activity, using methods known in the art.

35 Elongase activity can be tested by analysing the fatty acid profile, for example as described in Schneider et al., 2000.

Thioesterase activity can be tested by appropriate assays, such as the thioesterase activity assay described in Nancolas et al., 2017.

- 5 Fatty aldehyde dehydrogenase activity can be tested by appropriate assays, such as a fatty aldehyde degradation assay as described in Iwama et al., 2014.

Fatty alcohol oxidase activity can be tested by appropriate assays, such as a fatty alcohol degradation assay as described in Iwama et al., 2015.

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Peroxisome biogenesis factor activity can be tested by appropriate assays, such as growth assays of yeast cells expressing candidate fatty alcohol oxidases in a medium comprising fatty acids as sole carbon source.

- 15 Fatty acyl synthase activity can be tested by testing cell growth, as fatty acyl synthases are essential genes.

Any of said modifications can be combined, i.e. the yeast cell may comprise several of said modifications.

20 Expression of a heterologous cytochrome b5

One modification which the present inventors have found to be beneficial for production of codlemone and derivatives thereof is the expression of a heterologous cytochrome b5 in a yeast cell. This membrane bound hemoprotein functions as an electron carrier for several membrane bound oxygenases. As shown in the examples (example 6 in particular) expression of a
25 heterologous cytochrome b5 was found to increase availability of fatty acid methyl esters, in particular *E8,E10-C12:Me* and *E9/Z9-C12: Me*. Such a modification is thus expected to increase production of *E8,E10-dodecadienyl* coenzyme A and optionally desaturated fatty alcohols having a carbon chain length of 12, such as codlemone.

- 30 In some embodiments, the cytochrome b5 is a cytochrome b5 which is native to a *Lepidoptera* species. In particular embodiments, the cytochrome b5 is a cytochrome b5 from a *Helicoverpa* species, preferably a cytochrome b5 from *Helicoverpa armigera*, such as set forth in SEQ ID NO: 4, or a functional variant thereof having at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%,
35 such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such

as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.

The cytochrome b5 may be expressed at high level.

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The cytochrome b5 may be expressed by introducing a nucleic acid in the yeast cell encoding a cytochrome b5 or a homologue thereof. For example, a nucleic acid as set forth in SEQ ID NO: 3 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 3.

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In order to test whether a functional variant of a cytochrome b5 retains the desired activity, methods known in the art can be employed; for example, a spectrophotometric assay as described in Lamb et al., 1999.

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Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further expresses a heterologous cytochrome b5 as described herein. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and a cytochrome b5 as described herein above, such as the cytochrome b5 from *Helicoverpa armigera* (SEQ ID NO: 4) or a functional variant thereof. The yeast cell may, in addition to Cpo_CPRQ, a mutant or a

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functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

- 5 The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5 reductase, expression of a hemoglobin, mutation in native elongase gene(s) resulting in total or partial loss of activity, mutation in native thioesterase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s), expression of a heterologous thioesterase gene and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase.

Expression of a heterologous cytochrome b5 reductase (EC 1.6.2.2)

- 15 Another modification which may lead to increased production of E8,E10-dodecadienyl coenzyme A and optionally codlemone and derivatives thereof is the expression of a heterologous cytochrome b5 reductase (EC 1.6.2.2).

Cytochrome b5 reductase, also known as methemoglobin reductase, is an NADH-dependent enzyme converting methemoglobin to hemoglobin:

- 20 $\text{NADH} + \text{H}^+ + 2 \text{ ferricytochrome } b_5 = \text{NAD}^+ + 2 \text{ ferrocycytochrome } b_5$

- In some embodiments, the cytochrome b5 reductase is a cytochrome b5 reductase which is native to a *Lepidoptera* species. In particular embodiments, the cytochrome b5 reductase is a cytochrome b5 reductase from a *Helicoverpa* species, preferably a cytochrome b5 reductase from a *Helicoverpa* species such as *Helicoverpa armigera*, for example the cytochrome b5 reductase as set forth in SEQ ID NO: 24, or a functional variant thereof having at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.

- In order to test whether a functional variant of a cytochrome b5 reductase retains the desired activity, methods known in the art can be employed; for example, a spectrophotometric assay as described in Lamb et al., 1999.

The cytochrome b5 reductase may be expressed at high level.

5 The cytochrome b5 reductase may be expressed by introducing a nucleic acid in the yeast cell encoding said cytochrome b5 reductase or a homologue thereof. For example, a nucleic acid as set forth in SEQ ID NO: 23 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 23.

20 Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further expresses a heterologous cytochrome b5 reductase as described herein. The yeast cell may be further modified with any of the modifications described herein. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), Gmo_CPRQ (SEQ ID NO: 77), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and a cytochrome b5 reductase as described herein above, such as the cytochrome b5 reductase from *Helicoverpa armigera* (SEQ ID NO: 25) or a functional variant thereof. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof. The yeast cell may additionally express a cytochrome b5 as described herein above.

The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5, expression of a hemoglobin, mutation in native elongase gene(s) resulting in total or partial loss of activity, mutation in native thioesterase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s), expression of a heterologous thioesterase gene and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase.

Expression of a hemoglobin

Another modification which may be advantageous for production of *E8,E10*-dodecadienyl coenzyme A and optionally codlemone and derivatives thereof is the expression of a hemoglobin in the yeast cell, in particular a heterologous hemoglobin.

As shown in the examples, in particular example 6, expression of a hemoglobin in a yeast cell expressing a desaturase increased production of *E8,E10*-C12:Me and *E9,Z9*-C12:Me.

In some embodiments, the hemoglobin is a hemoglobin which is native to a *Vitreoscilla* species, such as *Vitreoscilla stercoraria*. In particular embodiments, the hemoglobin is as set forth in SEQ ID NO: 6, or a functional variant thereof having at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.

In order to test whether a functional variant of a hemoglobin retains the desired activity appropriate assays as known in the art, such as colorimetric assays, can be performed.

The hemoglobin may be expressed at high level.

The hemoglobin may be expressed by introducing a nucleic acid in the yeast cell encoding said hemoglobin or a homologue thereof. For example, a nucleic acid as set forth in SEQ ID NO: 5 is introduced, or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology

or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 5.

Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further expresses a hemoglobin as described herein. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), Gmo_CPRQ (SEQ ID NO: 77), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and a hemoglobin as described herein above, such as the hemoglobin from *Vitreoscilla stercoraria* (SEQ ID NO: 6) or a functional variant thereof. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5, expression of a cytochrome b5 reductase, mutation in native elongase gene(s) resulting in total or partial loss of activity, mutation in native thioesterase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s), expression of a heterologous thioesterase gene and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase.

Mutation in elongase gene(s)

Another modification which may be advantageous for production of *E8,E10*-dodecadienyl coenzyme A and optionally codlemone and derivatives thereof is the mutation of certain genes in the yeast cell, in particular mutation of one or more elongase genes, where the mutation
5 results in a partial or total loss of activity of the corresponding elongase. Elongases catalyse carbon chain extension of several molecules, including fatty acids. In some embodiments, the elongase is a medium chain acyl elongase. If a yeast cell is used which naturally comprises several genes encoding elongases, the yeast cell may be further engineered to comprise a mutation in one or more of said genes, resulting in partial or total loss of activity of the one or
10 more elongases.

In some embodiments, the yeast cell is a *Yarrowia lipolytica* cell and the elongase is encoded by the *ELO1* gene (SEQ ID NO: 13).

15 In some embodiments, the mutation is a deletion resulting in total loss of activity of the corresponding elongase. In other embodiments, the elongase is inactivated for example by introducing one or more mutations, including total or partial deletions, insertions, substitutions or non-sense or missense mutations, in the gene, for example in the coding sequence, promoter, Kozak sequence, terminator or other regulatory element. For example the native promoter or
20 the native terminator can be replaced by another, weaker promoter or by another terminator, respectively. Other inactivation methods resulting in partial or total loss of activity include repression of transcription as well as post-transcriptional inactivation, such as silencing, for example using an RNAi system or a CRISPR/Cas system resulting in the degradation of the relevant transcripts, thereby preventing or at least reducing translation, as well as post-
25 translational inactivation, such as inhibition of the protein. An example of how to test whether a protein retains elongase activity is described in example 4 or in Schneider et al., 2000.

Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further comprises one or more mutations in one or more
30 genes encoding an elongase, wherein said mutation results in partial or total loss of function, as described herein. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as
35 a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID

NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and may further comprise a mutation resulting in partial or total loss of activity of an elongase as described herein above. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome, expression of a heterologous cytochrome b5 reductase, expression of a hemoglobin, mutation in native thioesterase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s), expression of a heterologous thioesterase gene and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase.

Mutation in thioesterase gene(s)

Another modification which may be advantageous for production of E8,E10-dodecadienyl coenzyme A and optionally codlemone and derivatives thereof is the mutation of certain genes in the yeast cell, in particular mutation of one or more thioesterase genes, where the mutation results in a partial or total loss of activity of the corresponding thioesterase. If a yeast cell is used which naturally comprises several genes encoding thioesterases, the yeast cell may be further engineered to comprise a mutation in one or more of said genes, resulting in partial or total loss of activity of the one or more thioesterases.

In some embodiments, the yeast cell is a *Yarrowia lipolytica* cell and the thioesterase is encoded by YAL10_F14729g gene (SEQ ID NO: 19), YAL10_E18876g (SEQ ID NO: 54) or YAL10_D03597g (SEQ ID NO: 55). Thus in some embodiments the *Yarrowia lipolytica* cell comprises a mutation, such as a deletion, of the YAL10_F14729g gene (SEQ ID NO: 19) resulting in partial or total loss of the corresponding thioesterase. In other embodiments the *Yarrowia lipolytica* cell comprises a mutation, such as a deletion, of the YAL10_E18876g gene (SEQ ID NO: 54) resulting in partial or total loss of the corresponding thioesterase. In other embodiments the *Yarrowia lipolytica* cell comprises a mutation, such as a deletion, of the YAL10_D03597g (SEQ ID NO: 55) resulting in partial or total loss of the corresponding thioesterase. In some embodiments, the *Yarrowia lipolytica* cell comprises a mutation in several thioesterase genes. For example, the cell may comprise a mutation, such as a deletion, of

5 *YAL10_F14729g* (SEQ ID NO: 19) and of *YALI0_E18876g* (SEQ ID NO: 54); or a mutation, such as a deletion, of *YAL10_F14729g* (SEQ ID NO: 19) and of *YALI0_D03597g* (SEQ ID NO: 55); or a mutation, such as a deletion, of *YALI0_E18876g* (SEQ ID NO: 54) and of *YALI0_D03597g* (SEQ ID NO: 55). In some embodiments, the cell comprises a mutation, such as a deletion, of *YAL10_F14729g* (SEQ ID NO: 19), *YALI0_E18876g* (SEQ ID NO: 54) and *YALI0_D03597g* (SEQ ID NO: 55).

10 Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further comprises one or more mutations in one or more genes encoding a thioesterase, wherein said mutation results in partial or total loss of function, as described herein. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), Gmo_CPRQ (SEQ ID NO: 77) a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and one or more mutations in one or more genes encoding a thioesterase, wherein said mutation results in partial or total loss of function as described herein above, such as a mutation in one or more of *YAL10_F14729g* (SEQ ID NO: 19), *YALI0_E18876g* (SEQ ID NO: 54) and *YALI0_D03597g* (SEQ ID NO: 55). The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

25 The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5, expression of a heterologous cytochrome b5 reductase, expression of a hemoglobin, mutation in native elongase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s), expression of a heterologous thioesterase gene and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase.

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Additional modifications

The yeast cell may further comprise other modifications, such as at least one mutation resulting in reduced activity of enzymes involved in fatty acid metabolism. In some embodiments, activity of the native fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s) is modified, preferably the activity is reduced or abolished. For example, the yeast cell may further comprise one or more mutations in genes encoding a fatty aldehyde dehydrogenase, a fatty alcohol oxidase, and/or a peroxisome biogenesis factor. Any of these enzymes may be inactivated for example by introducing one or more mutations, including total or partial deletions, insertions, substitutions or non-sense or missense mutations, in the gene, for example in the coding sequence, promoter, Kozak sequence, terminator or other regulatory element. For example the native promoter or the native terminator can be replaced by another, weaker promoter or by another terminator, respectively. Other inactivation methods resulting in partial or total loss of activity include repression of transcription as well as post-transcriptional inactivation, such as silencing, for example using an RNAi system or a CRISPR/Cas system resulting in the degradation of the relevant transcripts, thereby preventing or at least reducing translation, as well as post-translational inactivation, such as inhibition of the protein. Enzyme activities can be otherwise modified, e.g. to modify properties of the enzymes such as intracellular localisation, or to increase activity, using methods known in the art.

In some embodiments, the yeast cell is a *Yarrowia lipolytica* cell as described herein above, further comprising a modification such as a mutation in at least one of *HFD1*, *HFD2*, *HFD3*, *HFD4*, *FAO1*, *GPAT* and *PEX10*, or a modification such as a mutation resulting in reduced activity of at least one protein having at least 60% homology or identity thereto, such as at least 65% homology or identity, such as at least 70% homology or identity, such as at least 75% homology or identity, such as at least 80% homology or identity, such as at least 81% homology or identity, such as at least 82% homology or identity, such as at least 83% homology or identity, such as at least 84% homology or identity, such as at least 85% homology or identity, such as at least 86% homology or identity, such as at least 87% homology or identity, such as at least 88% homology or identity, such as at least 89% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92% homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity thereto.

In *Yarrowia lipolytica*, the fatty aldehyde dehydrogenase Hfd1 is encoded by *HFD1* (YALI0_F23793g). It catalyses the oxidation of fatty aldehydes to fatty acids. As described in detail in application WO 2018/109163, reduced activity of Hfd1 results in increased titer of desaturated fatty alcohols in yeast cells. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation, such as a deletion, of *HFD1*, resulting in partial or total loss of activity of Hfd1. Reduction of activity of Hfd1 can be achieved by other methods as described herein.

The fatty aldehyde dehydrogenase Hfd2 is encoded by *HFD2* (YALI_0E15400g). It catalyses the oxidation of fatty aldehydes to fatty acids. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation, such as a deletion, of *HFD2*, resulting in partial or total loss of activity of Hfd2. Reduction of activity of Hfd2 can be achieved by other methods as described herein.

The fatty aldehyde dehydrogenase Hfd3 is encoded by *HFD3* (YALI0_A17875g). It catalyses the oxidation of fatty aldehydes to fatty acids. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation, such as a deletion, of *HFD3*, resulting in partial or total loss of activity of Hfd3. Reduction of activity of Hfd3 can be achieved by other methods as described herein.

In *Yarrowia lipolytica*, the fatty aldehyde dehydrogenase Hfd4 is encoded by *HFD4* (YALI0_B01298g). It catalyses the oxidation of fatty aldehydes to fatty acids. As described in detail in application WO 2018/109163, reduced activity of Hfd4 results in increased titer of desaturated fatty alcohols in yeast cells. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation, such as a deletion, of *HFD4*, resulting in partial or total loss of activity of Hfd4. Reduction of activity of Hfd4 can be achieved by other methods as described herein.

In some embodiments, the yeast cell further comprises a modification, for example a mutation, such as a deletion, resulting in partial or total loss of activity of a fatty aldehyde dehydrogenase having at least 60% homology or identity to Hfd1, Hfd2, Hfd3 or Hfd4, such as at least 65% homology or identity, such as at least 70% homology or identity, such as at least 75% homology or identity, such as at least 80% homology or identity, such as at least 81% homology or identity, such as at least 82% homology or identity, such as at least 83% homology or identity, such as at least 84% homology or identity, such as at least 85% homology or identity, such as at least 86% homology or identity, such as at least 87% homology or identity, such as at least

88% homology or identity, such as at least 89% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92% homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity to Hfd1, Hfd2, Hfd3 or Hfd4.

In *Yarrowia lipolytica*, the fatty alcohol oxidase Fao1 is encoded by *FAO1* (*YALI0B14014g*). Its deletion results in increased accumulation of ω -hydroxy fatty acids. As described in detail in application WO 2018/109163, reduced activity of Fao1 results in increased titer of desaturated fatty alcohols in yeast cells. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation, such as a deletion, of *FAO1*, resulting in partial or total loss of activity of Fao1. Reduction of activity of Fao1 can be achieved by other methods as described herein.

In some embodiments, the yeast cell further comprises a mutation, such as a deletion, resulting in partial or total loss of activity of a fatty alcohol oxidase having at least 60% homology or identity to Fao1, such as at least 65% homology or identity, such as at least 70% homology or identity, such as at least 75% homology or identity, such as at least 80% homology or identity, such as at least 81% homology or identity, such as at least 82% homology or identity, such as at least 83% homology or identity, such as at least 84% homology or identity, such as at least 85% homology or identity, such as at least 86% homology or identity, such as at least 87% homology or identity, such as at least 88% homology or identity, such as at least 89% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92% homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity to Fao1.

In *Yarrowia lipolytica*, the peroxisome biogenesis factor 10 Pex10 is encoded by *PEX10* (*YALI0C01023g*). As described in detail in application WO 2018/109163, reduced activity of Pex10 results in increased titer of desaturated fatty alcohols in yeast cells. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation, such as a deletion, of *PEX10*, resulting in partial or total loss of activity of Pex10. Reduction of activity of Pex10 can be achieved by other methods as described herein.

In some embodiments, the yeast cell further comprises a mutation, such as a deletion, resulting in partial or total loss of activity of a peroxisome biogenesis factor having at least 60% homology or identity to Pex10, such as at least 65% homology or identity, such as at least 70% homology or identity, such as at least 75% homology or identity, such as at least 80% homology or identity, such as at least 81% homology or identity, such as at least 82% homology or identity, such as at least 83% homology or identity, such as at least 84% homology or identity, such as at least 85% homology or identity, such as at least 86% homology or identity, such as at least 87% homology or identity, such as at least 88% homology or identity, such as at least 89% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92% homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity to Pex10.

15 In *Yarrowia lipolytica*, the glycerol-3-phosphate acyltransferase is encoded by *GPAT* (YALIO_C00209g). *GPAT* catalyzes the first reaction towards glycerolipids biosynthesis. The gene is essential in *Yarrowia lipolytica*. As described in detail in application WO 2018/109163, reduced activity of *GPAT* results in increased titer of desaturated fatty alcohols in yeast cells. A *Yarrowia lipolytica* cell according to the present disclosure may thus further comprise a mutation
20 of *GPAT*, resulting in partial or total loss of activity of *GPAT*. Reduction of activity of *GPAT* can be achieved by other methods as described herein.

In some embodiments, the yeast cell further comprises a mutation resulting in partial or total loss of activity of a glycerol-3-phosphate acyltransferase having at least 60% homology or identity to *GPAT*, such as at least 65% homology or identity, such as at least 70% homology or identity, such as at least 75% homology or identity, such as at least 80% homology or identity, such as at least 81% homology or identity, such as at least 82% homology or identity, such as at least 83% homology or identity, such as at least 84% homology or identity, such as at least 85% homology or identity, such as at least 86% homology or identity, such as at least 87% homology or identity, such as at least 88% homology or identity, such as at least 89% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92% homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity to *GPAT*.

Partial or total loss of activity of any of the above enzymes can also be achieved for example by introducing one or more mutations, including total or partial deletions, insertions, substitutions or non-sense or missense mutations, in the gene, for example in the coding sequence, promoter, Kozak sequence, terminator or other regulatory element. For example the native promoter or the native terminator can be replaced by another, weaker promoter or by another terminator, respectively. Other inactivation methods resulting in partial or total loss of activity include repression of transcription as well as post-transcriptional inactivation, such as silencing, for example using an RNAi system or a CRISPR/Cas system resulting in the degradation of the relevant transcripts, thereby preventing or at least reducing translation, as well as post-translational inactivation, such as inhibition of the protein. In order to determine whether a modification, such as a mutation or any modification described herein above, results in total or partial loss of activity, methods known in the art can be employed, such as detailed herein above. For example, in the case of a deletion or a modification leading to reduced transcription, amplification methods such as PCR may be employed to confirm absence of the relevant sequence. Protein expression may be investigated using appropriate assays, such as a Western blot or measuring expression levels using a marker such as a fluorescent marker.

It may also be advantageous for the yeast cell to express one or more modified fatty acyl synthases. This may help direct the metabolic flux towards production of desaturated products such as *E8,E10*-dodecadienyl coenzyme A and desaturated fatty alcohols and derivatives thereof, such as codlemone and derivatives thereof. Thus in some embodiments the yeast cell is further modified to express a fatty acyl synthase having a modified ketone synthase domain. In some embodiments, the yeast cell is a *Yarrowia lipolytica* cell as described herein, wherein the cell further expresses a modified fatty acid synthase complex. In one embodiment, the fatty acid synthase complex is modified by mutating the gene encoding the alpha subunit of the complex. In some embodiments, the mutation is in the gene encoding FAS2 (SEQ ID NO: 18). In other embodiments, the mutation is in the gene encoding FAS1 (SEQ ID NO: 16). The mutation may result in modification of one or more of residue 123 (L123) of SEQ ID NO: 16. The mutation may result in modification of one or more of residue 1220 (I1220), residue 1217 (M1217) or residue 1226 (M1226) of SEQ ID NO: 18, resulting in a variant FAS2. The skilled person will know how to design such mutations.

Preferably, a mutation in FAS2 results in an I1220F variant, an I1220W variant, an I1220Y variant or an I1220H variant of Fas2. In a specific embodiment, the mutation results in an I1220F variant. In some embodiments, the mutation results in an M1217F variant, an M1217W

variant, an M1217Y variant or an M1217H variant. In other embodiments, the mutation results in an M1226F variant, an M1226W variant, an M1226Y variant or an M1226H variant.

Preferably, a mutation in FAS1 results in an L123V variant.

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Yeast cells with more than one of the above mutations are also contemplated, such as two mutations or three mutations at residues I1220, M1217 or M1226 of FAS2, and/or one mutation at residue 123 of FAS1.

- 10 Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further comprises one or more modifications as described within the present section. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), Gmo_CPRQ (SEQ ID NO: 77), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an
- 15 S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and one or more modifications such as mutations resulting in
- 20 partial or total loss of function of one or more of Hfd1, Hfd2, Hfd3, Hfd4, Fao1 and Pex10 as described above, and/or further expresses one or more modified fatty acyl synthases as described above. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example
- 25 Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5, expression of a heterologous cytochrome b5 reductase, expression of a hemoglobin, inactivation of native elongase(s)

30 resulting in total or partial loss of activity, inactivation of native thioesterase(s) resulting in total or partial loss of activity, expression of a heterologous thioesterase gene and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase.

In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ

35 (SEQ ID NO: 2), Gmo_CPRQ (SEQ ID NO: 77) a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants

thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof and may further comprise mutations in: HFD1 and HFD2; HFD1 and HFD3; HFD1 and HFD4; HFD1 and FAO1; HFD1 and PEX10; HFD2 and HFD3; HFD2 and HFD4; HFD2 and FAO1; HFD2 and PEX10; HFD3 and HFD4; HFD3 and FAO1; HFD3 and PEX10; HFD4 and FAO1; HFD4 and PEX10; FAO1 and PEX10; HFD1, HFD2 and HFD3; HFD1, HFD2 and HFD4; HFD1, HFD2 and FAO1; HFD1, HFD2 and PEX10; HFD1, HFD3 and HFD4; HFD1, HFD3 and FAO1; HFD1, HFD3 and PEX10; HFD1, HFD4 and FAO1; HFD1, HFD4 and PEX10; HFD1, FAO1 and PEX10; HFD2, HFD3 and HFD4; HFD2, HFD3 and FAO1; HFD2, HFD3 and PEX10; HFD2, HFD4 and FAO1; HFD2, HFD4 and PEX10; HFD2, FAO1 and PEX10; HFD3, HFD4 and FAO1; HFD3, HFD4 and PEX10; HFD3, FAO1 and PEX10; HFD4, FAO1 and PEX10; HFD1, HFD2, HFD3 and HFD4; HFD1, HFD2, HFD3 and FAO1; HFD1, HFD2, HFD3 and PEX10; HFD1, HFD2, HFD4 and FAO1; HFD1, HFD2, HFD4 and PEX10; HFD1, HFD2, FAO1 and PEX10; HFD1, HFD3, HFD4 and FAO1; HFD1, HFD3, HFD4 and PEX10; HFD1, HFD3, FAO1 and PEX10; HFD1, HFD4, FAO1 and PEX10; HFD2, HFD3, HFD4 and FAO1; HFD2, HFD3, HFD4 and PEX10; HFD2, HFD3, FAO1 and PEX10; HFD2, HFD4, FAO1 and PEX10; HFD3, HFD4, FAO1 and PEX10; HFD1, HFD2, HFD3, HFD4 and FAO1; HFD1, HFD2, HFD3, HFD4 and PEX10; HFD1, HFD3, HFD4, FAO1 and PEX10; HFD2, HFD3, HFD4, FAO1 and PEX10; HFD1, HFD2, HFD3, HFD4, FAO1 and PEX10, or the afore-mentioned combinations of corresponding variants having at least 60% homology or identity thereto. In addition, the yeast cell may further express a modified fatty acyl synthase as described above, in particular a mutant Fas1 and/or a mutant Fas2. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

Expression of a heterologous thioesterase

It may be advantageous to further engineer the yeast cell by introducing a thioesterase, in particular a heterologous thioesterase. Thus in some embodiments, a nucleic acid encoding a thioesterase is introduced in the yeast cell, e.g. on a vector or by genomic integration. The thioesterase gene may be under the control of an inducible promoter, or under the control of a constitutive promoter. The nucleic acid encoding a thioesterase may be codon-optimised for the yeast cell, as is known in the art. In particular, the nucleic acid may be codon-optimised for a

Yarrowia cell, such as a *Yarrowia lipolytica* cell. The thioesterase may be expressed at high level as is known in the art.

5 In some embodiments, the thioesterase is derived from an organism selected from *Cuphea palustris*, *Cuphea hookeriana*, *Cinnamomum camphora*, or from *Escherichia coli*. In preferred
embodiments, the thioesterase is derived from *Escherichia coli* or *Cinnamomum camphora*. In
some embodiments, the thioesterase has at least 60% homology or identity to a thioesterase
selected from the thioesterase derived from *Cuphea palustris* as set forth in SEQ ID NO: 33, the
thioesterase derived from *Cuphea hookeriana* as set forth in SEQ ID NO: 57, the thioesterase
10 derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, and the thioesterase
derived from *Escherichia coli* as set forth in SEQ ID NO: 26. Preferably, the thioesterase has at
least 60% homology or identity to the thioesterase derived from *Cinnamomum camphora* as set
forth in SEQ ID NO: 35 or from *Escherichia coli* as set forth in SEQ ID NO: 26. In one
embodiment, the thioesterase has at least 60% homology or identity to the thioesterase derived
15 from *Cinnamomum camphora* as set forth in SEQ ID NO: 35. In another embodiment the
thioesterase has at least 60% homology or identity to the thioesterase derived from *Escherichia coli*
as set forth in SEQ ID NO: 26.

In another embodiment, the thioesterase has at least 60% homology or identity to the
20 thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, such as at
least 61% homology or identity, such as at least 62% homology or identity, such as at least 63%
homology or identity, such as at least 64% homology or identity, such as at least 65% homology
or identity, such as at least 66% homology or identity, such as at least 67% homology or
identity, such as at least 68% homology or identity, such as at least 69% homology or identity,
25 such as at least 70% homology or identity, such as at least 71% homology or identity, such as
at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least
76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%,
such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such
as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at
30 least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least
93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%,
such as at least 98%, such as at least 99%, such as 100% homology or identity to the
thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 35.

35 In another embodiment, the thioesterase has at least 60% homology or identity to the
thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 26, such as at least 61%

homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology or identity to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 26.

15 The nucleic acid encoding a thioesterase may be codon-optimised as is known in the art. In one embodiment, the yeast cell is a *Yarrowia* cell, preferably a *Yarrowia lipolytica* cell, and the nucleic acid is codon-optimised accordingly.

In one embodiment, the at least one thioesterase is encoded by a nucleic acid having at least 20 60% homology or identity to the nucleic acid encoding the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 34, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology or identity to the nucleic acid encoding the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 34.

35

In one embodiment, the at least one thioesterase is encoded by a nucleic acid having at least 60% homology or identity to the nucleic acid encoding the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 25, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology or identity to the nucleic acid encoding the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 25.

Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further expresses one or more thioesterases such as one or more heterologous thioesterases, as described herein. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), Gmo_CPRQ (SEQ ID NO: 77), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 4) and functional variants thereof, and one or more heterologous thioesterases such as the thioesterases set forth in SEQ ID NO: 33, SEQ ID NO: 57, SEQ ID NO: 35 and/or SEQ ID NO: 26, or functional variants thereof. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5, a heterologous cytochrome b5 reductase, expression of a hemoglobin, mutation in native elongase gene(s) resulting in total or

partial loss of activity, mutation in native thioesterase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s), and/or expression of a fusion protein of a fatty acyl synthase and of a thioesterase, as described herein above.

5

Expression of a fusion protein of a fatty acyl synthase and of a thioesterase

In some embodiments, the yeast cell further expresses a fusion protein of a truncated fatty acyl synthase and of a truncated thioesterase, such as the fusion protein as set forth in SEQ ID NO: 59 or a homologue thereof having at least 60% homology or identity thereto. This fusion protein is a fusion of a truncated version of Fas1 from *Y. lipolytica* and of a truncated version of the thioesterase TesA from *E. coli*. It can be expressed by introduction of a nucleic acid such as set forth in SEQ ID NO: 58. The fusion protein may be expressed at high level.

Thus in some embodiments, the yeast cell further expresses a fusion protein as set forth in SEQ ID NO: 59 or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 59.

In some embodiments, the yeast cell comprises a nucleic acid encoding said fusion protein such as the nucleic acid as set forth in SEQ ID NO: 58 or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity,

such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to SEQ ID NO: 58.

Thus in some embodiments, the yeast cell expresses a desaturase and a fatty acyl-CoA reductase as described above, and further expresses a fusion protein of a truncated fatty acyl synthase and of a truncated thioesterase, such as the fusion protein as set forth in SEQ ID NO: 59. In particular, the yeast cell may express one or more desaturases selected from Cpo_CPRQ (SEQ ID NO: 2), a mutant Cpo_CPRQ such as an S82 mutant or an S85 mutant, preferably an S85 mutant such as an S85A mutant, and functional variants thereof, and one or more reductases selected from Ase_FAR (SEQ ID NO: 10), a mutant Ase_FAR such as a T198 mutant or an S413 mutant, preferably a T198A mutant or an S413A mutant, Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12) and functional variants thereof, and a fusion protein of a truncated fatty acyl synthase and of a truncated thioesterase, such as the fusion protein as set forth in SEQ ID NO: 59 or a functional variant thereof. The yeast cell may, in addition to Cpo_CPRQ or Gmo_CPRQ, a mutant or a functional variant thereof, also express another desaturase capable of introducing at least one double bond in a fatty acyl-CoA of carbon chain length 12, as described above, for example Cpo_NPVE, Cpo_SPTQ, a mutant or a functional variant thereof.

The yeast cell may be further modified with any of the modifications described herein, in particular by expression of a heterologous cytochrome b5, expression of a heterologous cytochrome b5 reductase, expression of a hemoglobin, mutation in native elongase gene(s) resulting in total or partial loss of activity, mutation in native thioesterase gene(s) resulting in total or partial loss of activity, mutations in native gene(s) encoding fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s) and/or expression of a heterologous thioesterase gene, as described herein above.

Titer

The yeast cells disclosed herein are capable of producing *E8,E10*-dodecadien-1-ol with a titre of at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*-dodecadien-1-ol is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at least 0.5 mg/L, such as at least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

Methods for determining the titer are known in the art.

Production of E8,E10-dodecadienyl acetate

Codlemone can be further converted to *E8,E10*-dodecadienyl acetate; this can be done ex vivo, as is known in the art, e.g. by chemical conversion, or it can be done in vivo by the action of an acetyltransferase (EC 2.3.1.84) capable of converting at least part of the *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadienyl acetate.

In some embodiments, the yeast cell is thus engineered so that it overexpresses a native acetyltransferase and/or so that it expresses a heterologous acetyltransferase, which is optionally expressed at high level. The yeast cell is in such embodiments capable of producing *E8,E10*-dodecadien-1-ol and *E8,E10*-dodecadienyl acetate.

In some embodiments, the yeast cell expresses an acetyltransferase capable of converting at least part of the *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadienyl acetate, such as the Sc_At1 acetyltransferase (SEQ ID NO: 37) or a homologue thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least

78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%,
such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such
as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at
least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least
5 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to
SEQ ID NO: 37.

Expression of the acetyltransferase may be achieved by introducing a nucleic acid, which may
be codon-optimised for expression in the yeast cell, such as the nucleic acid as set forth in SEQ
10 ID NO: 36, or a homologue thereof having at least 60% homology or identity thereto, such as at
least 61% homology or identity, such as at least 62% homology or identity, such as at least 63%
homology or identity, such as at least 64% homology or identity, such as at least 65% homology
or identity, such as at least 66% homology or identity, such as at least 67% homology or
identity, such as at least 68% homology or identity, such as at least 69% homology or identity,
15 such as at least 70% homology or identity, such as at least 71% homology or identity, such as
at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least
76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%,
such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such
as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at
20 least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least
93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%,
such as at least 98%, such as at least 99% to SEQ ID NO: 36.

Thus in some embodiments, the yeast cell expresses a heterologous desaturase as described
25 herein above, a heterologous fatty acyl reductase as described herein above, and optionally any
of the above described additional modifications, and also expresses an acetyltransferase
capable of converting at least part of the produced *E8,E10*-dodecadien-1-ol into *E8,E10*-
dodecadienyl acetate.

30 The yeast cells disclosed herein may thus be capable of producing *E8,E10*- dodecadienyl
acetate with a titre of at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*- dodecadienyl
acetate is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at
least 0.5 mg/L, such as at least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L,
such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least
35 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least
100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L,

such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

5 Methods for determining the titer are known in the art.

Production of E8,E10-dodecadial

It may also be of interest to further convert at least part of the *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadial. This can be done by chemical conversion or by
10 further engineering the yeast cell.

In some embodiments, the yeast cell may be further engineered so that it is capable of converting at least part of the *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadial. This can be done by engineering the yeast cell so that it further expresses an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol
15 oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadial. The yeast cell is in such embodiments capable of producing *E8,E10*-dodecadien-1-ol and *E8,E10*-dodecadial.

20 A nucleic acid encoding an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadial, can thus be introduced in the yeast cell. The nucleic acid may be codon-optimised and may be expressed at high level.

25 Thus in some embodiments, the yeast cell expresses a heterologous desaturase as described herein above, a heterologous fatty acyl reductase as described herein above, and optionally any of the above described additional modifications, and also expresses an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into
30 *E8,E10*-dodecadial.

The yeast cells disclosed herein may thus be capable of producing *E8,E10*-dodecadial with a titre of at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*-dodecadial is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at least 0.5 mg/L, such as at
35 least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L,

such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

Methods for determining the titer are known in the art.

10 *Chain shortening*

In some embodiments, the yeast cell is further modified in order to increase availability of fatty acyl-CoAs of a given chain length by chain shortening. Without being bound by theory, such modifications are expected to increase availability of substrates having a desired carbon chain length, in particular having a carbon chain length of 12, whereby production of *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, and optionally of *E8,E10*-dodecadienyl acetate and of *E8,E10*-dodecadienal, can be increased. This can be achieved by reducing the activity of native acyl-CoA oxidases in a microbial production cell and by expressing specific acyl-CoA oxidases, desaturases, reductases, and acetyltransferases. Such modifications are described in detail in EP 19157910.1 (filed 19 February 2019 by same applicant).

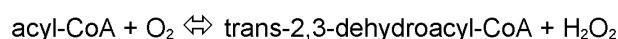
Thus in some embodiments, the yeast cell is any of the yeast cell described herein above, and further:

- i) has one or more mutations resulting in reduced activity of one or more native acyl-CoA oxidases; and
- ii) expresses at least one group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein the group of enzymes is capable of shortening a fatty acyl-CoA of a first carbon chain length X to a shortened fatty acyl-CoA having a second carbon chain length X' , wherein $X' \leq X-2$.

The activity of the acyl-CoA oxidases normally present in the yeast cell, i.e. the native enzyme(s), is in such embodiments reduced or abolished by mutating the genes encoding said enzyme(s) in the cell. In order to direct carbon chain shortening to obtain fatty alcohols and derivatives thereof of a desired carbon chain length, one or more acyl-CoA oxidase is expressed in the yeast cell. These acyl-coA oxidases may be native to the yeast cell, or they

may be derived from another organism. Genes encoding other enzymes required for oxidising a fatty acyl-CoA of a given chain length may also be introduced in the cell, if the cell does not express them already, or if increased activity or substrate specificity is desired. The acyl-CoA oxidase(s) thus expressed allow a fatty acyl-CoA to be oxidised and shortened to a fatty acyl-CoA having a shorter carbon chain length than the substrate. Thus in some embodiments, the reduced activity of the one or more native acyl-CoA oxidases is a reduced activity on acyl-CoAs having a carbon chain length smaller than X, such as smaller than X'.

The term acyl-CoA oxidase in the present disclosure refers to an enzyme such as an enzyme of EC number 1.3.3.6, capable of catalysing the following reaction:



This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-CH group of donor with oxygen as acceptor. The systematic name of this enzyme class is acyl-CoA:oxygen 2-oxidoreductase. Other names use include fatty acyl-CoA oxidase, acyl coenzyme A oxidase, and fatty acyl-coenzyme A oxidase.

The yeast cell of the present disclosure may be engineered starting from a yeast cell which has one or more native acyl-CoA oxidases. The modified yeast cell disclosed herein preferably has reduced activity of said one or more native acyl-CoA oxidases; this can be achieved by using a yeast cell which has one or more mutations resulting in reduced activity of at least one of its native acyl-CoA oxidases. The native acyl-CoA oxidases may be peroxisomal, mitochondrial or cytosolic. In some embodiments, the one or more mutations results in reduced activity of all the native acyl-CoA oxidases. By reduced activity it is to be understood that the yeast cell due to said mutations has reduced ability to catalyse the above reaction, in particular to convert an acyl-CoA to the corresponding trans-2,3-dehydroacyl-CoA. In some embodiments, "reduced capability" means that the ability to catalyse said reaction is abolished completely or partially. In some embodiments, "reduced capability" means that the ability to catalyse the reaction is limited to a subgroup of the substrates which can be used for the reaction under normal circumstances, i.e. by using enzymes having normal capability.

The yeast cell of the present disclosure may express at least one group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA. The group of enzymes comprises, besides the at least one acyl-CoA oxidase, the other enzymes required for converting a fatty acyl-CoA of a given carbon chain length to a fatty acyl-CoA of a shorter

carbon chain length. These other enzymes may preferably be native to the yeast cell; in such embodiments, only the introduction of a gene encoding an acyl-CoA oxidase is required for the yeast cell to express the group of enzymes.

5 In embodiments where the acyl-CoA oxidase is native to the yeast cell, said acyl-CoA oxidase may be modified as is known in the art, e.g. by the introduction of a promoter such as a constitutive or inducible promoter, or a promoter enabling overexpression of the acyl-CoA oxidase. The native acyl-CoA oxidase reintroduced in the first group of enzymes may be a mutated version with modified activity, such as modified substrate specificity and/or modified
10 activity such as increased reaction efficiency.

In other embodiments, the acyl-CoA oxidase is derived from another organism. The acyl-CoA comprised in the first group of enzymes may be an acyl-CoA oxidase derived from a yeast, a fungus, an insect, a mammalian, a bird or a plant, such as the at least one acyl-CoA oxidase of
15 the first group of enzymes is derived from a yeast, a fungus, an insect, a mammalian, a bird or a plant. For example, the acyl-CoA oxidase is derived from an organism of a genus selected from *Yarrowia*, *Saccharomyces*, *Agrotis*, *Arabidopsis*, *Aspergillus*, *Cucurbita*, *Homo*, *Paenarthrobacter* and *Rattus*, such as the at least one acyl-CoA oxidase of the first group of enzymes is derived from an organism of a genus selected from *Yarrowia*, *Saccharomyces*,
20 *Agrotis*, *Arabidopsis*, *Aspergillus*, *Cucurbita*, *Homo*, *Paenarthrobacter*, and *Rattus*. In some embodiments, the at least one first group of enzymes comprises an acyl-CoA oxidase derived from *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Agrotis segetum*, *Arabidopsis thaliana*, *Aspergillus nidulans*, *Cucurbita maxima*, *Homo sapiens*, *Paenarthrobacter ureafaciens* or *Rattus norvegicus*.

25 The acyl-CoA oxidase thus introduced in the yeast cell may be an acyl-CoA oxidase native to *Yarrowia lipolytica*, *Agrotis segetum*, *Arabidopsis thaliana*, *Aspergillus nidulans*, *Cucurbita maxima*, *Homo sapiens*, *Paenarthrobacter ureafaciens* or *Rattus norvegicus*. The yeast cell may be as described herein above.

30 The yeast cell of the present disclosure may thus express at least one group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein said at least one acyl-CoA oxidase is selected from the group Yli_POX1 (XP_504703), Yli_POX2 (XP_505264), Yli_POX3 (XP_503244), Yli_POX4 (XP_504475), Yli_POX5 (XP_502199),
35 Yli_POX6 (XP_503632), Ase_POX (SEQ ID NO: 39), Ath_POX1 (SEQ ID NO: 41), Ath_POX2 (SEQ ID NO: 43), Ani_POX (SEQ ID NO: 45), Cma_POX (SEQ ID NO: 47), Hsa_POX1-2 (SEQ

- ID NO: 49), Pur_POX (SEQ ID NO: 51), Sc_POX1 (SEQ ID NO: 31) and Rno_POX2 (SEQ ID NO: 53), or a functional variant thereof having at least 60% homology or identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.
- 10 In some embodiments, expression of the at least one acyl-CoA oxidase is achieved by introducing a nucleic acid encoding said at least one acyl-CoA oxidase. For example, the yeast expresses YALI0_E32835g coding for Yli_POX1, YALI0_F10857g encoding Yli_POX2, YALI0_D24750g encoding Yli_POX3, YALI0_E27654g encoding Yli_POX4, YALI0_C23859g encoding Yli_POX5, YALI0_E06567g encoding Yli_POX6, SEQ ID NO: 38 encoding Ase_POX, 15 SEQ ID NO: 40 encoding Ath_POX1, SEQ ID NO: 42 encoding Ath_POX2, SEQ ID NO: 44 encoding Ani_POX, SEQ ID NO: 46 encoding Cma_POX, SEQ ID NO: 48 encoding Hsa_POX, SEQ ID NO: 50 encoding Pur_POX, SEQ ID NO: 30 encoding Sc_POX1 or SEQ ID NO: 52 encoding Rno_POX2, or a homologue thereof having at least 60% homology or identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.
- 20
- 25 In some embodiments, $X'=12$.
- Suitable acyl-CoA oxidases are described in detail in WO 2020/169389 (filed 10 February 2020 by same applicant), in particular in the section "Acyl-CoA oxidase".
- 30
- In order to obtain *E8,E10*-dodecadienyl coenzyme A and optionally codlemone, in embodiments where chain shortening is taken advantage of, the yeast cell may thus, in addition to the at least one group of enzymes, also express an additional heterologous desaturase capable of introducing at least one double bond in E/Z conformations in a fatty acyl-CoA having a carbon 35 chain length X or X'. X or X' may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 carbon atoms. In some embodiments, the desaturase is capable of introducing at least one

double in E/Z conformations in a fatty acyl-CoA having a chain length of X', wherein X' is as defined above. Suitable desaturases are described in detail in WO 2020/169389 (filed 10 February 2020 by same applicant), in particular in the section "Desaturase (FAD)". In particular, desaturases capable of converting C14:CoA into Z11-C14:CoA and/or E11-C14:CoA are of interest. For example, the desaturases CroZ11 from *Choristoneura rosaceana* (SEQ ID NO: 63) or CpaE11 from *Choristoneura parallela* (SEQ ID NO: 65) or functional variants thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto, may be employed. The resulting Z11-C14:CoA and/or E11-C14:CoA may then be further chain shortened to give Z9-C12:CoA and/or E9-C12:CoA, which are then further desaturated by Cpo_CPRQ to give E8,E10-C12:CoA.

In such embodiments, the yeast cell thus expresses at least one desaturase as described herein above in the section "Desaturase", for example Cpo_CPRQ or Gmo_CPRQ, preferably Cpo_CPRQ, a mutant or functional variant thereof, and further expresses an additional heterologous desaturase capable of introducing at least one double bond in E/Z conformations in a fatty acyl-CoA having a carbon chain length X or X', where X and X' are as described above. In particular, the desaturase may be capable of introducing at least one double bond in E/Z conformations in a fatty acyl-CoA having a carbon chain length of 14, which can then be shortened as described herein above to a fatty acyl-CoA of carbon chain length 12 – this can then be further desaturated to E8,E10-C12:CoA, which can then be converted to E8,E10-dodecadien-1-ol by the action of the FAR as detailed herein above.

Methods for production of E8,E10-dodecadienyl coenzyme A, E8,E10-dodecadien-1-ol, E8,E10-dodecadienyl acetate and/or E8,E10-dodecadienyl acetate

5 The yeast cells described herein above can be used in a method for producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, which may be further converted into *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienyl acetate.

Method for production of *E8,E10*-dodecadien-1-ol

10 Herein is provided a method for producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:

- 15 i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
- 20 ii) Optionally at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol, thereby producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

25 The yeast cell may be any of the yeast cells described herein above.

The present methods preferably allow production of *E8,E10*-dodecadienyl coenzyme A with a titre of at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*-dodecadien-1-ol is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at least 0.5 mg/L, such as at least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such

as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

5 The present methods allow production of *E8,E10*-dodecadien-1-ol with a titre of at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*-dodecadien-1-ol is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at least 0.5 mg/L, such as at least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250
10 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

15 Methods for determining the titer are known in the art.

Method for production of *E8,E10*-dodecadienyl acetate

In some embodiments, the method further comprises the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate by expression of an
20 acetyltransferase or by chemical conversion. Accordingly, herein is disclosed a method for producing *E8,E10*-dodecadienyl acetate in a yeast cell, said method comprising the steps of:

- a) providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:
 - 25 i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
 - 30 ii) at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol,
- b) converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl
35 acetate.

In some embodiments, the *E8,E10*-dodecadienyl acetate is obtained by engineering the yeast cell as described herein above in "Production of *E8,E10*-dodecadienyl acetate".

5 In other embodiments, the conversion of *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadienyl acetate is performed chemically, as is known in the art. For example, the *E8,E10*-dodecadien-1-ol produced by the cell can be recovered, after which acetyl chloride is added to the *E8,E10*-dodecadien-1-ol, mixed and incubated, e.g. at room temperature, whereby at least part of the *E8,E10*-dodecadien-1-ol produced by the cell is converted into *E8,E10*-
10 dodecadienyl acetate.

In other embodiments, the yeast cell produces *E8,E10*-dodecadienyl coenzyme A, which can be converted into a lipid such as a triacylglyceride or into a free fatty acid, recovering said lipid or free fatty acid, which in turn can be converted to *E8,E10*-dodecadien-1-ol. *E8,E10*-dodecadien-
15 1-ol can then further be converted to *E8,E10*-dodecadien-1-ol in vitro, as described above. In such embodiments, conversion of *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-dodecadienyl acetate is performed chemically, as is known in the art.

The present methods may thus allow production of *E8,E10*-dodecadienyl acetate with a titre of
20 at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*-dodecadienyl acetate is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at least 0.5 mg/L, such as at least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at
25 least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

30 Methods for determining the titer are known in the art.

Method for production of *E8,E10*-dodecadienal

In some embodiments, the method further comprises the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal by further engineering the yeast cell or by

chemical conversion. Accordingly, herein is disclosed a method for producing *E8,E10*-dodecadial in a yeast cell, said method comprising the steps of:

- a) providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:
 - 5 i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
 - 10 ii) At least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol,
 - 15 b) converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadial.

In some embodiments, the *E8,E10*-dodecadial is obtained by engineering the yeast cell as described herein above in "Production of *E8,E10*-dodecadial".

20 In other embodiments, the method comprises a step of converting at least part of the *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadial by chemical conversion. The chemical conversion is based on the oxidation *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadial. Methods for performing this conversion are known in the art. Preferred methods are environmentally friendly and minimize the amount of hazardous waste.

25 In other embodiments, the yeast cell produces *E8,E10*-dodecadienyl coenzyme A, which can be converted into a lipid such as a triacylglyceride or into a free fatty acid, which can then be recovered and converted to *E8,E10*-dodecadien-1-ol in vitro, as described above. In such embodiments, conversion of *E8,E10*-dodecadien-1-ol produced by the cell into *E8,E10*-
30 dodecadial is performed chemically, as is known in the art.

Thus in some embodiments, the chemical conversion may be metal free, avoiding toxic heavy metal based reagents such as manganese oxides, chromium oxides (Jones ox. PDC, PCC) or ruthenium compounds (TPAP, Ley-Griffith ox.). In some embodiments, the conversion does not
35 involve reactions involving activated dimethyl sulfoxide such as the Swern oxidation or the Pfitzner-Moffat type. Such reactions may involve the stereotypic formation of traces of

intensively smelling organic sulfur compounds such as dimethyl sulfide which can be difficult to remove from the target product. In some embodiments, the method comprises a Dess-Martin reaction (Yadav et al., 2004, Meyer et al., 1994). In other embodiments, the chemical conversion comprises the oxidation with sodium hypochlorite under aqueous/organic two phase conditions (Okada et al., 2014; Tamura et al., 2012; Li et al., 2009). In some embodiments, the chemical oxidation can be performed with 1-chlorobenzotriazole in a medium of methylene chloride containing 25% pyridine (Ferrell and Yao, 1972).

Alternatively, the oxidation of *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadienal can be performed enzymatically by alcohol dehydrogenases. The skilled person will know how to carry out enzymatic oxidation. For example, enzymatic oxidation can be carried out by contacting purified enzymes, cell extracts or whole cells, with *E8,E10*-dodecadien-1-ol.

The methods disclosed herein thus in some embodiments allow production of *E8,E10*-dodecadienal with a titre of at least 0.2 mg/L. In some embodiments, the titre of *E8,E10*-dodecadienal is at least 0.25 mg/L, such as at least 0.3 mg/L, such as at least 0.4 mg/L, such as at least 0.5 mg/L, such as at least 0.75 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

Methods for determining the titer are known in the art.

Recovery

In some embodiments, the method further comprises a step of recovering the obtained products.

In some embodiments, the method is for production of *E8,E10*-dodecadien-1-ol and thus further comprises a step of recovering the produced *E8,E10*-dodecadien-1-ol. In other embodiments, the method is for production of *E8,E10*-dodecadienyl acetate and thus further comprises a step of recovering the produced *E8,E10*-dodecadienyl acetate. In other embodiments, the method is

for production of *E8,E10*-dodecadienal and thus further comprises a step of recovering the produced *E8,E10*-dodecadienal.

Methods for recovering the products obtained by the present methods are known in the art and may comprise an extraction with a hydrophobic solvent such as decane, hexane or a vegetable oil.

Alternatively, the methods described in application PCT/EP2020/076351 (filed on 22 September 2020 by same applicant) can also be used to recover the desired products. For example, said methods can be used to recover lipids such as triacylglycerides, or fatty acids, obtained from the conversion of *E8,E10*-dodecadienyl coenzyme A, or to recover the produced *E8,E10*-dodecadien-1-ol, the produced *E8,E10*-dodecadienyl acetate and/or the produced *E8,E10*-dodecadienal. Said methods take advantage of the addition of an extractant in the culture medium in an amount equal to or greater than its cloud concentration measured in an aqueous solution such as in the culture medium at the cultivation temperature, which greatly facilitates recovery of hydrophobic compounds such as fatty alcohols, fatty alcohol acetates and fatty aldehydes. Such methods can thus advantageously be used to facilitate recovery of the lipids such as triacylglycerides, or fatty acids, obtained from the conversion of *E8,E10*-dodecadienyl coenzyme A, of the *E8,E10*-dodecadien-1-ol, of the *E8,E10*-dodecadienyl acetate and of the *E8,E10*-dodecadienal produced by the present methods. In addition, the addition of an extractant in the culture medium was also found to generally increase titer of hydrophobic compounds produced by the cell, and to increase secretion of the produced hydrophobic compounds from the cell.

Thus in some embodiments, the medium used in the present methods comprises an extractant in an amount equal to or greater than its cloud concentration measured in an aqueous solution such as the culture medium at the cultivation temperature, wherein the extractant is a non-ionic surfactant, preferably a non-ionic ethoxylated surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate such as simethicone, fatty alcohol alkoxylates, polyethoxylated surfactants and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

The cloud concentration in an aqueous solution is determined at a given temperature, preferably at room temperature or at the temperature at which the fermentation is to be

performed, for example 30°C, or at room temperature. The term “extractant” as used herein refers to a non-ionic surfactant, in particular an antifoaming agent, which facilitates recovery of hydrophobic compounds produced in a fermentation. For example, the non-ionic surfactant is a non-ionic ethoxylated surfactant, for example a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate such as simethicone, fatty alcohol alkoxylates, polyethoxylated surfactants and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof. Example 7 of PCT/EP2020/076351 describes how to determine the cloud concentration of a surfactant.

Non-ionic surfactants which are suitable extractants and suitable amounts of said non-ionic surfactants are described in detail in application PCT/EP2020/076351 (filed on 22 September 2020 by same applicant), in particular in the section entitled “Non-ionic ethoxylated surfactant”.

In some embodiments, the culture medium used in the present methods thus comprises a non-ionic surfactant which is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, such as C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0), and the culture medium comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more.

In other embodiments, the culture medium used in the present methods comprises a non-ionic surfactant which is a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), and the culture medium comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, such as at least 11% vol/vol, such as at least 12% vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, or more.

In other embodiments, the culture medium used in the present methods comprises a non-ionic surfactant which is a mixture of polyether dispersions, such as antifoam 204, and the culture medium comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%,
5 such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more.

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In other embodiments, the culture medium used in the present methods comprises a non-ionic surfactant which comprises polyethylene glycol monostearate such as simethicone, and the culture medium comprises at least 1% vol/vol of polyethylene glycol monostearate or simethicone, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least
15 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol polyethylene glycol monostearate or simethicone, or more.

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In other embodiments, the culture medium used in the present methods comprises a non-ionic surfactant which is a fatty alcohol alkoxylate, and the culture medium comprises at least 1% vol/vol of fatty alcohol alkoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%,
25 such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol fatty alcohol alkoxylate or more. Suitable fatty alcohol alkoxylates include Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574.

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In other embodiments, the culture medium used in the present methods comprises a non-ionic surfactant which is Agnique BP420 (CAS number 68002-96-0), and the culture medium
35 comprises at least 1% vol/vol of Agnique BP420, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as

at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol Agnique BP420 or more.

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In some embodiments, the culture medium comprises the extractant in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more,

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and/or wherein the culture medium comprises the extractant in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration.

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The addition of an extractant, i.e. a non-ionic surfactant such as a polyethoxylated surfactant, for example any of the non-ionic surfactants, antifoaming agents or polyethoxylated surfactants described herein, results in the generation of an emulsion in the fermentation broth, where the hydrophobic compound produced by the microorganism, i.e. *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), the

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E8,E10-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal, is present in the emulsion. In embodiments where the present methods are performed with a culture medium comprising an extractant, the methods thus may also comprise a step of breaking the emulsion to recover a product phase comprising the extractant and the

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hydrophobic compound. Once the emulsion is broken, the fermentation broth is separated in three phases: a water phase, comprising mainly water and aqueous compounds, a phase comprising cells and cellular debris, and a product phase mainly comprising the extractant and the *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal. Thus a composition is obtained consisting of three

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phases. This is described in detail in application PCT/EP2020/076351 (filed on 22 September

2020 by same applicant), in particular in the section entitled "Product phase comprising the hydrophobic compound".

In some embodiments, most of the *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), and optionally most of the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal is present in the product phase. For example, at least 50% of the *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), and optionally of the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal is present in the product phase, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), and optionally of the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal is present in the product phase. In some embodiments, the product phase comprises at least 50% of the *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), and optionally the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), and optionally the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal initially present in the fermentation broth.

The step of breaking the emulsion may be performed as is known in the art, for example by submitting the emulsion to a step of phase separation, for example by centrifugation.

Following the step of breaking the emulsion, the product phase comprising the extractant and the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A, and optionally the *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal may be recovered from the composition. The method may in such embodiments further comprise the step of separating the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A, and optionally the *E8,E10*-dodecadien-1-ol, the *E8,E10*-

dodecadienyl acetate and/or the *E8,E10*-dodecadienal from the extractant. This can be performed by methods known in the art, such as by distillation, for example distillation under reduced pressure, or by column purification, or any other suitable method. The extractant may be recirculated to the fermentor or bioreactor.

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Nucleic acid constructs

Also provided is a nucleic acid construct for modifying a yeast cell, said construct comprising:

- i) At least one first polynucleotide encoding at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
- ii) Optionally a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.

The nucleic acid constructs can be used to obtain a yeast cell as described herein, i.e. a yeast cell capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol. The term "nucleic acid construct" may refer here to a single physical entity, i.e. a single molecule, for example a vector or a plasmid in which the first polynucleotide and optionally the second polynucleotide are comprised, or it may refer to a plurality of nucleic acid molecules, e.g. the first polynucleotide is comprised within one plasmid or vector and the second polynucleotide is comprised within another plasmid or vector.

The nucleic acid construct may further comprise one or more of:

- iii) a polynucleotide encoding a heterologous cytochrome b5, such as the polynucleotide as set forth in SEQ ID NO: 3 or a homologue thereof having at least 60% homology or identity thereto;
- iv) a polynucleotide encoding a heterologous cytochrome b5 reductase, such as the polynucleotide as set forth in SEQ ID NO: 23 or a homologue thereof having at least 60% homology or identity thereto;

- v) a polynucleotide encoding a hemoglobin, such as the polynucleotide as set forth in SEQ ID NO: 5 or a homologue thereof having at least 60% homology or identity thereto; and/or
- vi) a polynucleotide encoding a thioesterase, such as the polynucleotide as set forth in SEQ ID NO: 25 or SEQ ID NO: 34 or a homologue thereof having at least 60% homology or identity thereto.

The polynucleotides may comprise several copies of any of the above genes, and may be codon-optimised for proper expression in the yeast cell in which they are to be introduced.

In some embodiments, the at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) is Cpo_CPRQ (SEQ ID NO: 2) or a functional variant thereof having at least 60% homology or identity thereto, as described above. In such embodiments, the first polynucleotide comprises SEQ ID NO: 1 or a homologue thereof having at least 60% homology or identity thereto, as described herein above.

In some embodiments, the at least one heterologous desaturase is a mutant Cpo_CPRQ such as a Cpo_CPRQ mutant having a mutation at position 85, or a functional variant thereof having at least 60% homology or identity thereto. In some embodiments, the mutation is an S85A mutation. In some embodiments, the desaturase is a mutant Cpo_CPRQ such as a Cpo_CPRQ mutant having a mutation at position 82. In some embodiments, the mutation is an S82A mutation, or a functional variant thereof having at least 60% homology or identity thereto.

In other embodiments, the at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) is Gmo_CPRQ (SEQ ID NO: 77) or a functional variant thereof having at least 60% homology or identity thereto, as described above. In such embodiments, the first polynucleotide comprises SEQ ID NO: 78 or a homologue thereof having at least 60% homology or identity thereto, as described herein above.

- In some embodiments, the yeast cell expresses several desaturases capable of introducing one or more double bonds in a fatty acyl-CoA of carbon chain length 12. In such embodiments, preferably at least one of the several desaturases is Cpo_CPRQ, Gmo_CPRQ, a mutant thereof or a functional variant thereof as detailed herein, and the first polynucleotide comprises or consists of SEQ ID NO: 1 or a homologue thereof having at least 60% homology or identity thereto. The nucleic acid construct may in such embodiments comprise further first polynucleotides, each encoding a desaturase as described herein above. For example, the nucleic acid construct comprises a first polynucleotide encoding Cpo_CPRQ, Gmo_CPRQ or homologues thereof, and further comprises a further first polynucleotide encoding a further desaturase, preferably Cpo_NPVE (SEQ ID NO: 67) or Cpo_SPTQ (SEQ ID NO: 69), or a functional variant thereof having at least 60% homology or identity thereto. Thus in some embodiments, the further first polynucleotide comprises SEQ ID NO: 66 or SEQ ID NO: 68, or homologues thereof having at least 60% homology or identity thereto.
- The nucleic acid construct may also comprise a second polynucleotide encoding a FAR. The FAR is preferably be an insect FAR, such as a FAR native to an insect of the genus *Agrotis*, *Heliothis*, *Helicoverpa* or *Cydia*. For example, the FAR is native to *Agrotis segetum*, *Agrotis ipsilon*, *Heliothis subflexa*, *Helicoverpa assulta*, *Helicoverpa virescens* or *Cydia pomonella*.
- In some embodiments, the FAR is Ase_FAR (SEQ ID NO: 10), i.e. the FAR naturally occurring in *Agrotis segetum*. In some embodiments, the heterologous FAR is a functional variant of Ase_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. For example, the functional variant has at least 65% homology or identity thereto. In some embodiments the FAR is a mutant Ase_FAR, such as a mutant having a mutation at position 198 or 413. In some embodiments the Ase_FAR mutant is a T198A mutant. In other embodiments, the Ase_FAR mutant is an S413A mutant. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 9 or a homologue thereof having at least 60% homology or identity thereto.
- In other embodiments, the FAR is Aip_FAR (SEQ ID NO: 61), i.e. the FAR naturally occurring in *Agrotis ipsilon*. In some embodiments, the heterologous FAR is a functional variant of Aip_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 60 or a homologue thereof having at least 60% homology or identity thereto.

In other embodiments, the FAR is Hs_FAR (SEQ ID NO: 71), i.e. the FAR naturally occurring in *Heliothis subflexa*. In some embodiments, the heterologous FAR is a functional variant of Hs_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 70 or a homologue thereof having at least 60% homology or identity thereto.

In other embodiments, the FAR is Has_FAR (SEQ ID NO: 73), i.e. the FAR naturally occurring in *Helicoverpa assulta*. In some embodiments, the heterologous FAR is a functional variant of Has_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 72 or a homologue thereof having at least 60% homology or identity thereto.

In other embodiments, the FAR is Hv_FAR (SEQ ID NO: 75), i.e. the FAR naturally occurring in *Helicoverpa virescens*. In some embodiments, the heterologous FAR is a functional variant of Hv_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 74 or a homologue thereof having at least 60% homology or identity thereto.

In other embodiments, the FAR is Har_FAR (SEQ ID NO: 12), i.e. the FAR naturally occurring in *Helicoverpa armigera*. In some embodiments, the heterologous FAR is a functional variant of Har_FAR, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 13 or a homologue thereof having at least 60% homology or identity thereto.

In other embodiments, the FAR is a *Cydia pomonella* FAR, for example Cpo_FAR or a functional variant thereof, which retains the capability of converting E8,E10-C12:CoA to E8,E10-dodecadien-1-ol. In such embodiments, the second polynucleotide comprises or consists of SEQ ID NO: 75 or a homologue thereof having at least 60% homology or identity thereto.

In embodiments where it is desirable to express several FARs, the second polynucleotide may be a plurality of second polynucleotides each encoding one FAR.

In some embodiments, the nucleic acid construct comprises at least one further polynucleotide, which may be a different nucleic acid molecule than the first and/or second polynucleotides, or which may be part of the same nucleic acid molecule as the first and/or second polynucleotides.

- The further polynucleotide in some embodiments encodes a heterologous cytochrome b5, such as the cytochrome b5 as set forth in SEQ ID NO: 3 or a homologue thereof having at least 60% homology or identity thereto. In some embodiments, the cytochrome b5 is a cytochrome b5 which is native to a *Lepidoptera* species. In particular embodiments, the cytochrome b5 is a cytochrome b5 from a *Helicoverpa* species, preferably a cytochrome b5 from *Helicoverpa armigera*, such as set forth in SEQ ID NO: 4, or a functional variant thereof having at least 60% homology or identity thereto. In such embodiments, the further polynucleotide comprises or consists of SEQ ID NO: 3 or a homologue thereof having at least 60% homology thereto.
- 10 The further polynucleotide in some embodiments encodes a heterologous cytochrome b5 reductase, such as the cytochrome b5 reductase as set forth in SEQ ID NO: 24 or a homologue thereof having at least 60% homology or identity thereto. In some embodiments, the cytochrome b5 reductase is a cytochrome b5 reductase which is native to a *Helicoverpa* species. In particular embodiments, the cytochrome b5 reductase is a cytochrome b5 reductase from a *Helicoverpa* species, preferably a cytochrome b5 reductase from *Helicoverpa armigera*, such as set forth in SEQ ID NO: 24, or a functional variant thereof having at least 60% homology or identity thereto. In such embodiments, the further polynucleotide comprises or consists of SEQ ID NO: 23 or a homologue thereof having at least 60% homology thereto.
- 20 The further polynucleotide in some embodiments encodes a heterologous hemoglobin, such as the hemoglobin as set forth in SEQ ID NO: 6 or a homologue thereof having at least 60% homology or identity thereto. In some embodiments, the hemoglobin is an hemoglobin which is native to a *Vitreoscilla* species. In particular embodiments, the hemoglobin is a hemoglobin from *Vitreoscilla stercoraria*, such as set forth in SEQ ID NO: 6, or a functional variant thereof having at least 60% homology or identity thereto. In such embodiments, the further polynucleotide comprises or consists of SEQ ID NO: 5 or a homologue thereof having at least 60% homology thereto.
- 30 The further polynucleotide in some embodiments encodes a thioesterase, such as the thioesterase as set forth in SEQ ID NO: 6 or a homologue thereof having at least 60% homology or identity thereto. In some embodiments, the thioesterase is native to a *Cuphea* species, to a *Cinnamomum* species or to an *Escherichia* species. In particular embodiments, the thioesterase is a hemoglobin from *Cuphea palustris*, *Cuphea hookeriana*, *Cinnamomum camphora*, or *Escherichia coli*, such as set forth in SEQ ID NO: 33, SEQ ID NO: 57, SEQ ID NO: 35 or SEQ ID NO: 26, respectively, or a functional variant thereof having at least 60% homology or identity thereto. In such embodiments, the further polynucleotide comprises or

consists of SEQ ID NO: 34, SEQ ID NO: 56, SEQ ID NO: 34 or SEQ ID NO: 25 a homologue thereof having at least 60% homology thereto.

In some embodiments, the nucleic acid construct comprises a first polynucleotide as described above, and optionally a second polynucleotide as described above, and further expresses one or more further polynucleotide as described above. In some embodiments, the nucleic acid construct thus comprises the first polynucleotide and optionally the second polynucleotide, and further comprises one of:

- at least one further polynucleotide encoding a heterologous cytochrome b5; or
- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase; or
- at least one further polynucleotide encoding a hemoglobin; or
- at least one further polynucleotide encoding a thioesterase.

In other embodiments, the nucleic acid construct comprises the first polynucleotide and optionally the second polynucleotide, and further comprises:

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase;

or

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a hemoglobin;

or

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a thioesterase;

or:

- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase; and
- at least one further polynucleotide encoding a hemoglobin;

or:

- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase; and
- at least one further polynucleotide encoding a thioesterase;

or:

- at least one further polynucleotide encoding a hemoglobin; and
- at least one further polynucleotide encoding a thioesterase.

In other embodiments, the nucleic acid construct comprises the first polynucleotide and optionally the second polynucleotide, and further comprises:

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase;
- and
- at least one further polynucleotide encoding a hemoglobin;

or_

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase;
- and
- at least one further polynucleotide encoding a thioesterase;

or

- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase;
- and
- at least one further polynucleotide encoding a hemoglobin; and
- at least one further polynucleotide encoding a thioesterase;

or

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a hemoglobin; and
- at least one further polynucleotide encoding a thioesterase.

In some embodiments, the nucleic acid construct comprises the first polynucleotide and optionally the second polynucleotide, and further comprises all of:

- at least one further polynucleotide encoding a heterologous cytochrome b5; and
- at least one further polynucleotide encoding a heterologous cytochrome b5 reductase;
- and
- at least one further polynucleotide encoding a hemoglobin; and
- at least one further polynucleotide encoding a thioesterase.

The nucleic acid construct may further comprise additional polynucleotides for introducing in the yeast cell any of the additional modifications described herein above, in particular polynucleotides which upon introduction in the yeast cell result in modified activity of the native fatty aldehyde dehydrogenase(s), fatty alcohol oxidase(s), peroxisome biogenesis factor and/or fatty acyl synthase(s) is modified; preferably the activity is reduced or abolished.

The nucleic acid constructs may comprise additional elements required for or facilitating expression of the polynucleotides comprised therein, such as promoters, for example inducible, repressible or constitutive promoters, located upstream of the coding sequences comprised in the polynucleotides, as is known in the art.

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Formulation as a pheromone composition

In some embodiments, the present methods further comprise a step of formulating the *E8,E10*-dodecadien-1-ol, *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal produced by the yeast cell as a pheromone composition, as is known in the art.

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E8,E10-dodecadienyl coenzyme A, E8,E10-dodecadien-1-ol, E8,E10-dodecadienyl acetate and/or E8,E10-dodecadienal obtainable by the present methods

The present disclosure also provides *E8,E10*-dodecadienyl coenzyme A (or the lipid or free fatty acid obtained by converting *E8,E10*-dodecadienyl coenzyme A), *E8,E10*-dodecadien-1-ol, *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal obtainable by the present methods.

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When expressing insect desaturases and/or reductases in a yeast cell, the resulting mix of products, e.g. *E8,E10*-dodecadienyl coenzyme A and/or fatty alcohols comprising *E8,E10*-dodecadien-1-ol and produced by the cell will typically have a similar composition as the one produced in the pheromone glands of the insects. This allows for the production of pheromone mixes suitable for various insects instead of producing individual pheromone components in separate processes that then need to be mixed in appropriate proportions. Nevertheless, the resulting mixture of products, e.g. *E8,E10*-dodecadienyl coenzyme A and/or fatty alcohols may contain by-products characteristic of biological production.

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Thus in some embodiments where production of *E8,E10*-dodecadienyl coenzyme A is performed, the produced fatty acyl-CoAs comprise at least 1%, such as at least 2%, such as at least 3%, such as at least 4%, such as at least 5%, such as at least 10%, such as at least 15%, such as at least 20% of a desaturated fatty acyl-CoA having a desaturation at another position than the desired fatty acyl-CoA and/or at least 1%, such as at least 2%, such as at least 3%, such as at least 4%, such as at least 5%, such as at least 10%, such as at least 15%, such as at least 20% of the corresponding saturated fatty acyl-CoA.

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In embodiments where production of *E8,E10*-dodecadien-1-ol is performed, the produced fatty alcohols comprise at least 1%, such as at least 2%, such as at least 3%, such as at least 4%,

such as at least 5%, such as at least 10%, such as at least 15%, such as at least 20% of a desaturated fatty alcohol having a desaturation at another position than the desired fatty alcohol and/or at least 1%, such as at least 2%, such as at least 3%, such as at least 4%, such as at least 5%, such as at least 10%, such as at least 15%, such as at least 20% of the
5 corresponding saturated fatty alcohol. If the mix of fatty alcohols recovered from the fermentation broth is chemically oxidized into aldehydes or acetylated into acetates, then corresponding mixes of aldehydes and acetates are produced.

10 In some embodiments, the present methods are for production of *E8,E10*-dodecadienal. In some embodiments, the present yeast cells and methods result in production of a mixture of fatty aldehydes which comprises *E8,E10*-dodecadienal, but also comprises odd-chain fatty aldehydes. The term "odd-chain" fatty aldehydes refers to fatty aldehydes having a carbon chain length which is an odd number of carbon atoms, such as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 carbon atoms. The term "even-chain" fatty aldehydes refers to fatty aldehydes having a
15 carbon chain length which is an even number of carbon atoms, such as 8, 10, 12, 14, 16, 18, 20 or 22 carbon atoms.

In some embodiments, the present methods are for production of *E8,E10*-dodecadienyl acetate. In some embodiments, the present yeast cells and methods result in production of a mixture of
20 fatty alcohol acetates which comprises *E8,E10*-dodecadienyl acetate, but also comprises odd-chain fatty alcohol acetates. The term "odd-chain" fatty alcohol acetates refers to fatty alcohol acetates having a carbon chain length which is an odd number of carbon atoms, such as 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 carbon atoms. The term "even-chain" fatty alcohol acetates refers to fatty alcohol acetates having a carbon chain length which is an even number of carbon
25 atoms, such as 8, 10, 12, 14, 16, 18, 20 or 22 carbon atoms.

Pheromone composition

The *E8,E10*-dodecadien-1-ol, *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal produced by the yeast cell may be formulated as a pheromone composition, as is known in the
30 art. Such pheromone compositions may be used as integrated pest management products, which can be used in a method of monitoring the presence of pest or in a method of disrupting the mating of pest.

Pheromone compositions as disclosed herein may be used as biopesticides. Such compositions
35 can be sprayed or dispensed on a culture, in a field or in an orchard. They can also, as is known

in the art, be soaked e.g. onto a rubber septa, or mixed with other components. This can result in mating disruption, thereby preventing pest reproduction, or it can be used in combination with a trapping device to entrap the pests. Non-limiting examples of pests against which the present pheromone compositions can be used are: cotton bollworm (*Helicoverpa armigera*), striped
5 stemborer (*Chilo suppressalis*), diamond back moth (*Plutella xylostella*), cabbage moth (*Mamestra brassicae*), large cabbage-heart caterpillar (*Crocidolomia binotalis*), European corn stalk borer (*Sesamia nonagrioides*), currant clearwing (*Synanthedon tipuliformis*) and artichoke plume moth (*Platyptilia carduidactyla*). Accordingly, use of the present compositions on a culture can lead to increased crop yield, with substantially no environmental impact.

10 The relative amounts of the different compounds in the present pheromone compositions may vary depending on the nature of the crop and/or of the pest to be controlled; geographical variations may also exist. Determining the optimal relative amounts may thus require routine optimisation.

15 In some embodiments of the present disclosure, the pheromone composition may further comprise one or more additional compounds such as a liquid or solid carrier or substrate. For example, suitable carriers or substrate include vegetable oils, refined mineral oils or fractions thereof, rubbers, plastics, silica, diatomaceous earth, wax matrix and cellulose powder.

20 The pheromone composition may be formulated as is known in the art. For example, it may be under the form of a solution, a gel, a powder. The pheromone composition may be formulated so that it can be easily dispensed, as is known in the art.

25 *Kit*

Provided herein is a kit of parts for performing the present methods. The kit of parts may comprise a yeast cell "ready to use" as described herein. In one embodiment, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell, or a *Saccharomyces* cell such as a *Saccharomyces cerevisiae* cell.

30 Alternatively, the kit of parts may also comprise nucleic acid constructs encoding the activities of interest to be introduced in the yeast cell. The nucleic acid construct may be provided as a plurality of nucleic acid constructs, such as a plurality of vectors, wherein each vector encodes one or several of the desired activities. Useful nucleic acid constructs have been described
35 above.

The kit of parts may also comprise nucleic acid constructs useful for introducing mutations resulting in partial or total loss of function such as any of the mutations described herein above.

- 5 The kit of parts may optionally comprise the yeast cell to be modified.

In some embodiments, the kit of parts comprises all of the above.

Method for monitoring the presence of pest or disrupting the mating of pest

- 10 The *E8,E10*-dodecadien-1-ol and optionally *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal produced by the yeast cells and methods disclosed herein can be used in a method for monitoring the presence of pest or disrupting the mating of pest.

Accordingly, herein is also provided a method of monitoring the presence of pest or disrupting the mating of pest, said method comprising the steps of:

- 15 i) Producing *E8,E10*-dodecadien-1-ol and optionally *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal by the methods described herein;
- ii) Formulating said *E8,E10*-dodecadien-1-ol and optionally said *E8,E10*-dodecadienyl acetate and/or said *E8,E10*-dodecadienal as a pheromone composition; and
- 20 iii) Employing said pheromone composition as an integrated pest management composition.

Any of the yeast cells and methods described herein above can be used in such methods.

Examples

25 *Example 1: construction of biobricks*

All heterologous genes were synthesized by GeneArt (Life Technologies) in codon-optimized versions for *Yarrowia lipolytica*. All the genes were amplified by PCR using Phusion U Hot Start DNA Polymerase (ThermoFisher) to obtain the fragments for cloning into yeast expression vectors. The primers are listed in Table 1 and the resulting DNA fragments (BioBricks) are listed

30 in Table 2. The PCR products were separated on a 1%-agarose gel containing Midori Green Advance (Nippon Genetics Europe GmbH). PCR products of the correct size were excised from the gel and purified using the Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel).

Table 1 – primers

Primer name	Template	NCBI accession number	Hybridises at positions
PR141	pCfB4778	-	1806..1827
PR142	pCfB4778	-	3986..4010
PR1852	CEN.PK102.5B	NC_001139	920060..920080
PR1853	CEN.PK102.5B	NC_001139	920060..920080
PR8330	SEQ ID NO:55	-	1..22
PR8331	SEQ ID NO:55	-	1361..1377
PR8348	SEQ ID NO:54	-	1..21
PR8349	SEQ ID NO:54	-	1031..1047
PR10595	Yali0C	NC_006069	1244252..1244265
PR11110	pCfB6681	-	2306..2336
PR11111	pCfB6681	-	5129..5152 1..13
PR11138	pCfB4132	-	2894..2923
PR13163	pCfB3405	-	1680..1695
PR13494	pCfB3405	-	1133..1149
PR13495	pCfB3636	-	2621..2639
PR13513	pCfB4132	-	4160..4186
PR13514	pCfB4132	-	4168..4193
PR14149	Yali0A	NC_006067	2188554..2188574
PR14279	Yali0C	NC_006069	1244254..1244265
PR15521	Yali0C	NC_006069	1684182..1684200
PR15522	Yali0C	NC_006069	1685164..1685182
PR15781	Yali0A	NC_006067	2188556..2188574
PR15930	Yali0C	NC_006069	1243743..1243762
PR16463	Yali0B	NC_006068	1856737..1856756

PR16464	Yali0B	NC_006068	1857235..1857256
PR16465	Yali0B	NC_006068	1859087..1859109
PR16466	Yali0B	NC_006068	1859566..1859586
PR16594	SEQ ID NO:11	-	1..18
PR16595	SEQ ID NO:11	-	2735..2755
PR17976	Yali0E	NC_006071	3759199..3759218
PR17977	Yali0E	NC_006071	3759199..3759218
PR18066	SEQ ID NO:11	-	1..21
PR18214	Yali0C	NC_006069	1243743..1243761
PR18928	Yali0C	NC_006069	1244252..1244265
PR18233	Yali0C	NC_006069	1842838..1842857
PR18234	Yali0C	NC_006069	1842838..1842857
PR18239	Yali0C	NC_006069	568873..568892
PR18240	Yali0C	NC_006069	568873..568892
PR18241	Yali0D	NC_006070	2193231..2193250
PR18242	Yali0D	NC_006070	2193231..2193250
PR18253	Yali0E	NC_006071	1845879..1845898
PR18254	Yali0E	NC_006071	1845879..1845898
PR18255	Yali0E	NC_006071	2882070..2882089
PR18256	Yali0E	NC_006071	2882070..2882089
PR18930	Yali0C	NC_006069	1244252..1244265
PR20733	Yali0B	NC_006068	2567154..2567173
PR20734	Yali0B	NC_006068	2567154..2567173
PR20762	Yali0B	NC_006068	2566672..2566691
PR20763	Yali0B	NC_006068	2567146..2567162
PR20764	Yali0B	NC_006068	2567171..2567190

PR20765	Yali0B	NC_006068	2567645..2567662
PR20766	Yali0B	NC_006068	2567140..2567162
PR21723	SEQ ID NO:73	-	4..22
PR21724	SEQ ID NO:73	-	997..1014
PR21925	Yali0C	NC_006069	1664157..1664176
PR21660	Yali0E	NC_006071	3890734..3890752
PR21661	Yali0E	NC_006071	3891217..3891235
PR21662	Yali0E	NC_006071	3893283..3893298
PR21663	Yali0E	NC_006071	3893754..3893774
PR21664	Yali0F	NC_006072	1449003..1449023
PR21665	Yali0F	NC_006072	1449511..1449530
PR21666	Yali0F	NC_006072	1451632..1451653
PR21667	Yali0F	NC_006072	1452130..1452149
PR21668	Yali0D	NC_006070	3274947..3274966
PR21669	Yali0D	NC_006070	3275426..3275444
PR21670	Yali0D	NC_006070	3277548..3277567
PR21671	Yali0D	NC_006070	3277975..3277994
PR21672	Yali0E	NC_006071	3260813..3260833
PR21673	Yali0E	NC_006071	2773817..2773827
PR21674	Yali0E	NC_006071	3264041..3264062
PR21675	Yali0E	NC_006071	3263543..3263562
PR21676	Yali0C	NC_006069	3195071..3195092
PR21677	Yali0C	NC_006069	3195550..3195570
PR21678	Yali0C	NC_006069	3197667..3197688
PR21679	Yali0C	NC_006069	3198143..3198161
PR21717	SEQ ID NO: 68	-	4..21

PR21718	SEQ ID NO: 68	-	987..1005
PR21719	SEQ ID NO: 66	-	4..23
PR21720	SEQ ID NO: 66	-	1032..1050
PR21722	SEQ ID NO:1	-	1029..1047
PR21755	SEQ ID NO:71	-	1..19
PR21756	SEQ ID NO:71	-	1995..2013
PR21767	Yali0C	NC_006069	1663175..1663193
PR21768	Yali0C	NC_006069	1664158..1664176
PR21771	Yali0C	NC_006069	1663175..1663193
PR21868	Yali0D	NC_006070	2212116..2212130
PR21869	Yali0D	NC_006070	2210056..2210070
PR22075	Yali0C	NC_006069	1244246..1244265
PR22134	Yali0C	NC_006069	1244252..1244265
PR22187	Yali0E	NC_006069	1600091..1600110
PR22188	Yali0E	NC_006069	1601100..1601119
PR22191	Yali0E	NC_006069	1600091..1600110
PR22239	SEQ ID NO:5	-	1..21
PR22240	SEQ ID NO:5	-	420..441
PR22241	SEQ ID NO:3	-	1..20
PR22242	SEQ ID NO:3	-	363..384
PR22243	SEQ ID NO:23	-	1..24
PR22244	SEQ ID NO:23	-	945..969
PR22295	SEQ ID NO:1	-	4..17
PR22296	Yali0C	NC_006069	1243743..1243765
PR22342	SEQ ID NO:15	-	4..23
PR22343	SEQ ID NO:15	-	4877..4887

PR22344	SEQ ID NO:25	-	76..93
PR22345	SEQ ID NO:25	-	603..627
PR22847	SEQ ID NO:50	-	9..24
PR22848	SEQ ID NO:50	-	2146..2162
PR22915	Yali0F	NC_006072	993533..993552
PR22916	Yali0F	NC_006072	993533..993552
PR22917	Yali0F	NC_006072	992669..992688
PR22918	Yali0F	NC_006072	993146..993168
PR22919	Yali0F	NC_006072	994094..994117
PR22920	Yali0F	NC_006072	994595..994614
PR23176	Yali0E	NC_006071	2795526..2795545
PR23177	Yali0E	NC_006071	2795526..2795545
PR23285	Yali0E	NC_006071	2882054..2882073
PR23286	Yali0E	NC_006071	2882054..2882073
PR23412	Yali0F	NC_006072	1974881..1974900
PR23413	Yali0F	NC_006072	1974881..1974900
PR23414	Yali0F	NC_006072	1974682..1974726 1975796..1975840
PR23415	Yali0F	NC_006072	1974682..1974726 1975796..1975840
PR23423	Yali0E	NC_006071	2249605..2249624
PR23424	Yali0E	NC_006071	2249605..2249624
PR23425	Yali0E	NC_006071	2249902..2249946 2248697..2248741
PR23426	Yali0E	NC_006071	2249902..2249946 2248697..2248741
PR23429	Yali0D	NC_006070	455135..455154
PR23430	Yali0D	NC_006070	455135..455154

PR23431	Yali0D	NC_006070	455450..455494 454756..454800
PR23432	Yali0D	NC_006070	455450..455494 454756..454800
PR23468	SEQ ID NO:1	-	221..240
PR23469	SEQ ID NO:1	-	221..240
PR23472	SEQ ID NO:1	-	197..276
PR23473	SEQ ID NO:1	-	197..276
PR23613	SEQ ID NO:7	-	1..12
PR23615	SEQ ID NO:7	-	1566..1582
PR23632	SEQ ID NO:9	-	1..15
PR23633	SEQ ID NO:9	-	2752..2770
PR23685	SEQ ID NO:9	-	561..580
PR23686	SEQ ID NO:9	-	561..580
PR23687	SEQ ID NO:9	-	539..618
PR23688	SEQ ID NO:9	-	539..618
PR23689	SEQ ID NO:9	-	1293..1312
PR23690	SEQ ID NO:9	-	1293..1312
PR23691	SEQ ID NO:9	-	1250..1329
PR23692	SEQ ID NO:9	-	1250..1329
PR23693	SEQ ID NO:9	-	1373..1391
PR23694	SEQ ID NO:1	-	1..15
PR23695	SEQ ID NO:1	-	1029..1047
PR23698	Yali0B	NC_006068	2006545..2006564
PR23699	Yali0B	NC_006068	2006545..2006564
PR23700	Yali0B	NC_006068	2006501..2006590
PR23701	Yali0B	NC_006068	2006501..2006590

PR23938	SEQ ID NO:75	-	18..32
PR23939	SEQ ID NO:75	-	1373..1391
PR23940	SEQ ID NO:77/79/81	-	4..18/ 4..18/ 4..18
PR23941	SEQ ID NO:77/79	-	1344..1362/ 1344..1362
PR23942	SEQ ID NO: 80	-	1348..1371
attB1_Cpo_CPRQ_F	SEQ ID NO: 1 CpoCPRQ	-	1..30
attB2_Cpo_CPRQ_R	SEQ ID NO: 1 CpoCPRQ	-	1015..1047
PR23127	Yali0F	NC_006072	3823780..3823799
PR23218	Yali0C	NC_006069	763304..763285
PR22776	Yali0A	NC_006067	356505..356486
PR22777	Yali0A	NC_006067	356486..356505
PR22213	Yali0C	NC_006069	826768..826740
PR23702	SEQ ID NO:1	-	1032..1047
PR23703	SEQ ID NO: 9	-	1..15
PR23704	SEQ ID NO: 9	-	1359..1377
PR22536	Yali0F	NC_006072	2011914..2011929
PR22539	Yali0F	NC_006072	2013213..2013198
PR22534	Yali0F	NC_006072	3823853..3823869
PR22535	Yali0F	NC_006072	3824352..3824332
PR14148	Yali0E	NC_006071	784691..784708
PR15781	Yali0A	NC_006067	2188565.. 2188547
PR22744	Yali0A	NC_006067	356480..356458
PR22745	Yali0A	NC_006067	356511..356532
PR22684	Yali0A	NC_006067	355981..356001
PR22685	Yali0A	NC_006067	357012..356690

Table 2. DNA fragments (BioBricks) obtained by PCR using the indicated template and primers

Gene fragment name	Gene	Fw_primer	Rv_primer	Template DNA
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BB0410	Promoter Tdh3 from <i>S. cerevisiae</i>	PR1852	PR1853	CEN.PK102.5B
BB0684	Fatty acyl reductase from <i>A. segetum</i> codon optimized for <i>S. cerevisiae</i>	PR8330	PR8331	pCfB2341
BB0693	Fatty acid desaturase Cpo_CPRQ from <i>C. pomonella</i> codon optimized for <i>S. cerevisiae</i>	PR8348	PR8349	pCfB2339
BB1135	Easy clone vector backbone	PR11110	PR11111	pCfB6681
BB1338	HphSynMX cassette with universal EasyClone overhangs for use in knockout cassettes for <i>Y. lipolytica</i>	PR141	PR142	BB1339 BB1340
BB1339	HphMX_start	PR13513	PR13495	pCfB4346
BB1340	HphMX_end	PR11138	PR13514	pCfB4346
BB1341	NatSynMX_start	PR13513	PR13163	pCfB4253
BB1342	NatSynMX_end	PR13494	PR13514	pCfB4253
BB1346	loxP-PrTefintron-Nat-SCopti-Tcyc-loxP	PR141	PR142	BB1341 BB1342
BB1558	Promoter Exp from <i>Y. lipolytica</i>	PR15521	PR15522	ST6629
BB1688	Promoter Tefintron from <i>Y. lipolytica</i>	PR14279	PR15930	ST6629
BB1725	Long-chain alcohol oxidase from <i>Y. lipolytica</i>	PR16463	PR16464	ST6629
BB1726	Long-chain alcohol oxidase from <i>Y. lipolytica</i>	PR16465	PR16466	ST6629
BB1740	Fatty acyl reductase from <i>H. armigera</i> codon optimized for <i>Y. lipolytica</i>	PR16594	PR16595	pCfB5547
BB2068	Fatty acyl reductase from <i>H. armigera</i> codon optimized for <i>Y. lipolytica</i>	PR18066	PR16595	pCfB5547
BB2093	Promoter Tefintron from <i>Y. lipolytica</i>	PR10595	PR18214	ST6629
BB2311	Fatty acid synthase 2 from <i>Y. lipolytica</i>	PR20762	PR20763	ST6629
BB2312	Fatty acid synthase 2 from <i>Y. lipolytica</i>	PR20764	PR20765	ST6629

BB2313	Fatty acid synthase 2 from <i>Y. lipolytica</i>	PR20762	PR20765	BB2311 BB2312
BB2314	Fatty acid synthase 2 from <i>Y. lipolytica</i>	PR20762	PR20766	ST6629
BB2315	Fatty acid synthase 2 from <i>Y. lipolytica</i>	PR20762	PR20765	BB2312 BB2314
BB2646	Peroxisomal oxidase 1 from <i>Y. lipolytica</i>	PR21660	PR21661	ST6629
BB2647	Peroxisomal oxidase 1 from <i>Y. lipolytica</i>	PR21662	PR21663	ST6629
BB2648	Peroxisomal oxidase 1 from <i>Y. lipolytica</i>	PR21660	PR21663	BB2646 BB2647
BB2649	Peroxisomal oxidase 2 from <i>Y. lipolytica</i>	PR21664	PR21665	ST6629
BB2650	Peroxisomal oxidase 2 from <i>Y. lipolytica</i>	PR21666	PR21667	ST6629
BB2651	Peroxisomal oxidase 2 from <i>Y. lipolytica</i>	PR21664	PR21667	BB2649 BB2650
BB2652	Peroxisomal oxidase 3 from <i>Y. lipolytica</i>	PR21668	PR21669	ST6629
BB2653	Peroxisomal oxidase 3 from <i>Y. lipolytica</i>	PR21670	PR21671	ST6629
BB2654	Peroxisomal oxidase 3 from <i>Y. lipolytica</i>	PR21668	PR21671	BB2652 BB2653
BB2655	Peroxisomal oxidase 4 from <i>Y. lipolytica</i>	PR21672	PR21673	ST6629
BB2656	Peroxisomal oxidase 4 from <i>Y. lipolytica</i>	PR21674	PR21675	ST6629
BB2657	Peroxisomal oxidase 4 from <i>Y. lipolytica</i>	PR21672	PR21675	BB2655 BB2656
BB2658	Peroxisomal oxidase 5 from <i>Y. lipolytica</i>	PR21676	PR21677	ST6629
BB2659	Peroxisomal oxidase 5 from <i>Y. lipolytica</i>	PR21678	PR21679	ST6629

BB2660	Peroxisomal oxidase 5 from Y. lipolytica	PR21676	PR21679	BB2658 BB2659
BB2690	Fatty acid desaturase Cpo_SPTQ from C. pomonella codon optimized for Y. lipolytica	PR-21717	PR-21718	pBP7890
BB2691	Fatty acid desaturase Cpo_NPVE from C. pomonella codon optimized for Y. lipolytica	PR-21719	PR-21720	pBP7891
BB2719	Promoter Tef1 from Y. lipolytica	PR18928	PR18214	ST6629
BB2720	Promoter Tefintron from Y. lipolytica	PR18930	PR18214	ST6629
BB2721	Promoter Exp from Y. lipolytica	PR21767	PR21768	ST6629
BB2723	Promoter Exp from Y. lipolytica	PR21771	PR15522	ST6629
BB8012	Fatty acyl-CoA synthase from Y. lipolytica	PR21868	PR21869	ST6629
BB8018	Double promoter Exp/Tefintron from Y. lipolytica	PR18214	PR21925	BB2720 BB2723
BB8138	Promoter Yef3 from Y. lipolytica	PR22187	PR22188	ST6629
BB8141	Promoter Yef3 from Y. lipolytica	PR22191	PR22188	ST6629
BB8167	Fatty acyl reductase from H. armigera codon optimized for Y. lipolytica	PR22134	PR14149	pCfB5547
BB8168	Fatty acyl reductase from H. armigera codon optimized for Y. lipolytica	PR22075	PR15781	pCfB5547
BB8214	Hemoglobin from Vitreoscilla stercoraria codon optimized for Y. lipolytica	PR22239	PR22240	pBP8074
BB8215	Cytochrome b5 from H. armigera codon optimized for Y. lipolytica	PR22241	PR22242	pBP8075
BB8216	Cytochrome b5 reductase from H. armigera codon optimized for Y. lipolytica	PR22243	PR22244	pBP8076
BB8246	Promoter Tefintron from Y. lipolytica	PR10595	PR22296	ST6629

BB8247	Fatty acid desaturase Cpo_CPRQ from <i>C. pomonella</i> codon optimized for <i>Y. lipolytica</i>	PR22295	PR21722	pBP7892
BB8286	Shortened fatty acid synthase 1 from <i>Y. lipolytica</i>	PR22342	PR22343	ST6629
BB8287	Thioesterase from <i>Escherichia coli</i> codon optimized for <i>Y. lipolytica</i>	PR-22344	PR-22345	pCfB7680
BB8313	Hemoglobin from <i>V. stercoraria</i> codon optimized for <i>Y. lipolytica</i>	PR22187	PR22240	pBP8119
BB8526	Peroxisomal oxidase from <i>Paenarthrobacter ureafaciens</i>	PR22847	PR22848	pBP8308
BB8562	Fatty acid elongase 1 from <i>Y. lipolytica</i>	PR22917	PR22918	ST6629
BB8563	Fatty acid elongase 1 from <i>Y. lipolytica</i>	PR22919	PR22920	ST6629
BB8564	Fatty acid elongase 1 from <i>Y. lipolytica</i>	PR22917	PR22920	BB8562 BB8563
BB8724	Fatty acyl reductase from <i>H. armigera</i> codon optimized for <i>Y. lipolytica</i>	PR23005	PR15781	BB8614 BB2068
BB8790	Fatty acyl reductase from <i>T. alba</i> codon optimized for <i>Y. lipolytica</i>	PR23613	PR23615	pBP8751
BB8816	Fatty acyl reductase from <i>A. segetum</i> codon optimized for <i>Y. lipolytica</i>	PR23632	PR23633	pBP8777
BB8824	Fatty acid desaturase Cpo_CPRQ from <i>C. pomonella</i> codon optimized for <i>Y. lipolytica</i>	PR22295	PR21722	ST9072
BB8829	Fatty acyl reductase from <i>A. segetum</i> codon optimized for <i>Y. lipolytica</i>	PR23632	PR23693	pBP8782
BB8830	Fatty acid desaturase Cpo_CPRQ from <i>C. pomonella</i> codon optimized for <i>Y. lipolytica</i>	PR23694	PR23695	pBP7911

BB8923	Fatty acyl reductase from A. epsilon codon optimized for Y. lipolytica	PR23938	PR23939	pBP8925
BB8924	Fatty acyl reductase from Heliothis subflexa codon optimized for Y. lipolytica	PR23940	PR23941	pBP2344
BB8925	Fatty acyl reductase from Heliothis virescens codon optimized for Y. lipolytica	PR23940	PR23941	pBP2345
BB8926	Fatty acyl reductase from H. assulta codon optimized for Y. lipolytica	PR23940	PR23942	pBP2346
BB8618	Promoter Tefintron/GPD from Y. lipolytica	PR18214	PR22213	pCfB3465 ¹
BB8832	Fatty acid desaturase Cpo_CPRQ from C. pomonella codon optimized for Y. lipolytica	PR22295	PR23702	pBP7911
BB8833	Fatty acyl reductase from A. segetum codon optimized for Y. lipolytica	PR23703	PR23704	pBP8782
BB8386	Genomic region upstream of integration site	PR22536	PR22539	ST6629
BB1631	<i>Y. lipolytica</i> terminator regions of <i>pex20</i> and <i>lip2</i>	PR14148	PR15781	pCfB4586 ²
BB8387	Genomic region downstream of integration site	PR22534	PR22535	ST6629
BB8546	Vector backbone of pBP8375	PR22744	PR22745	pBP8375
BB8390	<i>gfp</i> expression cassette	PR22540	PR22541	pCfB5124 ²
BB8488	Genomic region upstream of integration site	PR22684	PR22744	ST6629
BB8489	Genomic region downstream of integration site	PR22745	PR22685	ST6629

¹Holkenbrink et al. 2020²Holkenbrink et al. 2017

Example 2: construction of plasmids

Integrative yeast vectors with USER cassette were linearized with FastDigest SfaAI (ThermoFisher) for 2 hours at 37°C and then nicked with Nb.BsmI (New England Biolabs) for 1 hour at 65°C. The resulting vectors containing sticky ends were separated by gel electrophoresis, excised from the gel, and gel-purified using the Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel). The DNA fragments were cloned into the so prepared vectors by USER-cloning as described in Holkenbrink et al., 2017. The reaction was transformed into chemically competent *E. coli* DHα cells and the cells were plated on Lysogeny Broth (LB) agar plates with 100 mg/L ampicillin. The plates were incubated overnight at 37°C and the resulting colonies were screened by colony PCR. The plasmids were purified from overnight *E. coli* liquid cultures and the correct cloning was confirmed by sequencing. The constructed vectors are listed in Table 3.

Table 3. Integrative expression vectors

Integrative expression vector name	Parent vector	DNA fragments cloned into parent vector
pCfB2227	-	-
pCfB2500	pCfB2227	BB0410 BB0693
pCfB2501	pCfB2190	BB0410 BB0684
pBP7909	pCfB6684	BB2719 BB2690
pBP7910	pCfB6684	BB2719 BB2691
pBP7911	pCfB6684	BB8246 BB8247
pBP7912	pCfB6684	BB2719 BB2693
pBP7980	pCfB6371	BB1688 BB1740
pBP8053	pCfB6677	BB8167 BB8168
pBP8100	pCfB6685	BB8214 BB8141

		BB2723 BB8215
pBP8114	pCfB6681	BB8012 BB8138
pBP8137	pCfB6679	BB8246 BB8247
pBP8175	pCfB6682	BB2093 BB8286 BB8287
pBP8193	pCfB6682	BB1558 BB8215
pBP8194	pCfB6682	BB8216 BB8141 BB2723 BB8215
pBP8212	pCfB6681	BB8313
pBP8350	pCfB6371	BB2721 BB8526
pBP8400	pCfB6371	BB1558 BB2709
pBP8781	pBP8660	BB2093 BB8790
pBP8782	pBP8660	BB2093 BB8816
pBP8795	pCfB6684	BB2093 BB8824
pBP8802	pBP8620	BB8825 BB8724
pBP8829	pBP8862	BB8018 BB8829 BB8830
pBP8926	pBP8660	BB2093 BB8923
pBP8931	pBP8660	BB2093 BB8924

pBP8932	pBP8660	BB2093 BB8925
pBP8933	pBP8660	BB2093 BB8926
pBP9361	pCfB6682	BB2719 BB2690
pBP8826	pBP8394	BB8018 BB8829 BB8830
pBP8828	pBP8263	BB8618 BB8832 BB8833
pBP8394	-	BB8546 BB1631
pBP8263	-	BB1135 BB8386 BB1631 BB8387
pBP8375	-	BB1135 BB8390 BB8488 BB8489

Example 3: Construction of strains

Yeast strains were constructed by transformation of DNA vectors as described in Holkenbrink et al., 2017. Integrative vectors were linearized with FastDigest NotI prior to transformation. When needed, helper vectors to promote the integration into specific genomic regions were co-transformed with the integrative plasmid or DNA repair fragments listed in Table 4. Strains were selected on yeast peptone dextrose (YPD) agar with appropriate antibiotics selection. Correct genotype was confirmed by colony PCR and when needed by sequencing. A *Y. lipolytica* wild-type strain was transformed with the plasmid pCfB6364 (EP19204554), leading to strain ST6029, then the genes *HFD1* (YALIO_F23793g), *HFD2* (YALIO_E15400g), *HFD3* (YALIO_A17875g), *HFD4* (YALIO_B01298g), *FAO1* (YALIO_B14014g), and *PEX10* (YALI1_C01416g) were deleted, leading to strain ST6629 (Borodina et al., 2018). Strains

ST6029 and ST6629 were used as parental strains to construct all other strains. The resulting strains are listed in Table 5.

Table 4. Helper vectors

Vector name	Selection marker	Parent vector	DNA fragments cloned into parent vector
pCfB5573	Hygromycin	-	BB1135 BB1338 BB1725 BB1726
pCfB5574	Nourseothricin N-acetyl transferase	-	BB1135 BB1346 BB1725 BB1726
pCfB6627	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR18233 PR18234
pCfB6630	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR18239 PR18240
pCfB6631	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR18241 PR18242
pCfB6637	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR18253 PR18254
pCfB6638	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR18255 PR18256

pCfB7088	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR20733 PR20734
pBP8032	Hygromycin	pCfB3431	BB1635 BB1636 PR18239 PR18240
pBP8033	Hygromycin	pCfB3431	BB1635 BB1636 PR18241 PR18242
pBP8034	Hygromycin	pCfB3431	BB1635 BB1636 PR18245 PR18246
pBP8035	Hygromycin	pCfB3431	BB1635 BB1636 PR18253 PR18254
pBP8161	Hygromycin	pCfB3431	BB1635 BB1636 PR18255 PR18256
pBP8185	Hygromycin	pCfB3431	BB1635 BB1636 PR17976 PR17977
pBP8406	Hygromycin	pCfB3431	BB1635 BB1636 PR22915 PR22916
pBP8535	Hygromycin	pCfB3431	BB1635 BB1636 PR23123 PR23124

pBP8575	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR23190 PR23191
pBP8568	Hygromycin	pCfB3431	BB1635 BB1636 PR23176 PR23177
pBP8623	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR23192 PR23193
pBP8634	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR23285 PR23286
pBP8650	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR23308 PR23309
pBP8657	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR21648 PR21649
pBP8674	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR23176 PR23177
pBP8704	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR23385 PR23386
pBP8713	Hygromycin	pCfB3431	BB1635 BB1636 PR23412 PR23413

pBP8716	Hygromycin	pCfB3431	BB1635 BB1636 PR23423 PR23424
pBP8717	Hygromycin	pCfB3431	BB1635 BB1636 PR23429 PR23430
pBP8734	Hygromycin	pCfB3431	BB1635 BB1636 PR23468 PR23469
pBP8813	Hygromycin	pCfB3431	BB1635 BB1636 PR23671 PR23672
pBP8815	Hygromycin	pCfB3431	BB1635 BB1636 PR23685 PR23686
pBP8817	Hygromycin	pCfB3431	BB1635 BB1636 PR23689 PR23690
pBP8871	Hygromycin	pCfB3431	BB1635 BB1636 PR23698 PR23699
pBP8645	Hygromycin	pCfB3431	BB1635 BB1636 PR23127 PR23128
pBP8328	Nourseothricin N-acetyl transferase	pCfB3405	BB1635 BB1636 PR22776 PR22777

Table 5. Yeast strains

Strain name	Modified intrinsic genes	Overexpressed gene(s)	Parent strain and (integrated DNA fragments)
ST6629	<i>hfd1Δ hfd2Δ hfd3Δ hfd4Δ fao1Δ pex10Δ</i>		
ST3332	Reference: CEN.PK102-5B	Cpo_CPRQ	CEN.PK102-5B (pBP2500)
ST8310	ΔHFD1 (YALI0_F23793g) ΔHFD2 (YALI0_E15400g) ΔHFD3 (YALI0_A17875g) ΔHFD4 (YALI0_B01298g) ΔFAO1 (YALI0_B14014g) ΔPEX10 (YALI0_C01023g)	VHb	ST6629 (pBP8212/pBP6637)
ST8311	ΔHFD1 (YALI0_F23793g) ΔHFD2 (YALI0_E15400g) ΔHFD3 (YALI0_A17875g) ΔHFD4 (YALI0_B01298g) ΔFAO1 (YALI0_B14014g) ΔPEX10 (YALI0_C01023g)	HarCyb5	ST6629 (pBP8193/pBP6627)
ST8406	ΔHFD1 (YALI0_F23793g) ΔHFD2 (YALI0_E15400g) ΔHFD3 (YALI0_A17875g) ΔHFD4 (YALI0_B01298g) ΔFAO1 (YALI0_B14014g) ΔPEX10 (YALI0_C01023g)	Cpo_CPRQ	ST6629 (pBP8137/pBP6638)
ST8411	Reference: ST8311	HarCyb5 Cpo_CPRQ	ST8311 (pBP8137/pBP8161)
ST8416	Reference: ST8310	VHb	ST8310 (pBP8137/pBP8161)

		Cpo_CPRQ	
ST8494	Reference: ST8406	Cpo_CPRQ 2x Har_FAR	ST8406 (pBP8053/pBP8034)
ST9060	Reference: ST8406 ΔELO1 (YALI0_F06754g)	Cpo_CPRQ	ST8406 (BB8564/pBP8406)
ST9061	Reference: ST8406 ΔYALI0_F14729g	Cpo_CPRQ	ST8406 (PR23414/PR23415/pBP8713)
ST9062	Reference: ST8406 ΔYALI0_E18876g	Cpo_CPRQ	ST8406 (PR23425/PR23426/pBP8716)
ST9063	Reference: ST8406 ΔYALI0_D03597g	Cpo_CPRQ	ST8406 (PR23431/PR23432/pBP8717)
ST9064	Reference: ST8406	Cpo_CPRQ CPO_SPTQ	ST8406 (pBP7909/pBP8033)
ST9065	Reference: ST8406	Cpo_CPRQ Cpo_NPVE	ST8406 (pBP7910/pBP8033)
ST9066	Reference: ST8406	2x Cpo_CPRQ	ST8406 (pBP7911/pBP8033)
ST9072	Reference: ST8406	Cpo_CPRQ_S85A	ST8406 (PR23472/PR23473/pBP8734)
ST9115	Reference: ST8411	HarCyb5 Cpo_CPRQ VHb	ST8411 (pBP8212/pBP6637)
ST9163	Reference: ST6029 ΔPOX5 (YALI0_C23859g)	-	ST6029 (BB2660/pBP8657)
ST9179	Reference: ST9163 ΔPOX3 (YALI0_D24750g)	-	ST9163 (BB2654/pBP8704)
ST9215	Reference: ST9179 ΔPOX1 (YALI0_E32835g)	-	ST9179 (BB2648/pBP8535)
ST9249	Reference: ST9066	2x Cpo_CPRQ Ta_FAR_w/o_SKL	ST9066 (pBP8781/pBP8674)
ST9250	Reference: ST9066	2x Cpo_CPRQ Ase_FAR	ST9066 (pBP8782/pBP8674)
ST9252	Reference: ST9215 ΔPOX4 (YALI0_E27654g)	-	ST9215 (BB2657/pBP8650)
ST9278	Reference: ST9060	2x Cpo_CPRQ	ST9060 (pBP7911/pBP6631)
ST9279	Reference: ST9060	Cpo_CPRQ Cpo_CPRQ_S85A	ST9060 (pBP8795/pBP6631)
ST9331	ΔPOX5 (YALI0_C23859g) ΔPOX3 (YALI0_D24750g) ΔPOX1 (YALI0_E32835g) ΔPOX4 (YALI0_E27654g) ΔPOX2 (YALI0_F10857g)	-	ST9252 (BB2651/pBP8813)

ST9335	Reference: ST9250	2x Cpo_CPRQ Ase_FAR_T198A	ST9250 (PR23687/PR23688/pBP8815)
ST9336	Reference: ST9250	2x Cpo_CPRQ Ase_FAR_S423A	ST9250 (PR23691/PR23692/pBP8817)
ST9355	Reference: ST9279	Cpo_CPRQ Cpo_CPRQ_S85A VHb HarCyb5	ST9279 (pBP8100/pBP8185)
ST9356	Reference: ST9355	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R	ST9355 (pBP8194/pBP6627)
ST9357	Reference: ST9356 ΔYALIO_F14729g	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R	ST9356 (PR23414/PR23415/pBP8713)
ST9358	Reference: ST9357	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R Ase_FAR	ST9357 (pBP8782/pBP8674)
ST9372	Reference: ST6029	Har_FAR	ST6029 (pBP7980/pBP8032)
ST9382	Reference: ST6029	Ase_FAR	ST6029 (pBP8782/pBP8674)
ST9387	Reference: ST9279	Cpo_CPRQ Cpo_CPRQ_S85A Ase_FAR	ST9279 (pBP8782/pBP8568)
ST9388	Reference: ST9387 FAS2(I1220F)	Cpo_CPRQ Cpo_CPRQ_S85A Ase_FAR	ST9387 (BB2313/pBP7088)
ST9395	Reference: ST9382	Ase_FAR Cpo_CPRQ	ST9382 (pBP7911/pBP8033)
ST9397	Reference: ST9388 FAS2(I1220F)	Cpo_CPRQ Cpo_CPRQ_S85A Ase_FAR FAS1'EcTesA'	ST9388 (pBP8175/pBP6627)
ST9398	Reference: ST9397 FAS2(I1220F)	Cpo_CPRQ Cpo_CPRQ_S85A Ase_FAR FAS1'EcTesA' FAA1	ST9397 (pBP8114/pBP8035)
ST9420	Reference: ST9387 FAS2(I1220W)	Cpo_CPRQ Cpo_CPRQ_S85A Ase_FAR	ST9387 (BB2315/pBP7088)
ST9421	Reference: ST9420 FAS2I1220W FAS1L123V	Cpo_CPRQ Cpo_CPRQ_S85A Ase_FAR	ST9420 (PR23700/PR23701/pBP8871)
ST9492	Reference: ST9372	Har_FAR Cpo_CPRQ	ST9372 (pBP8137/pBP8634)
ST9517	Reference: ST8406	Cpo_CPRQ	ST8406 (pBP8782/pBP8568)

		Ase_FAR	
ST9519	Reference: ST9331	Ase_FAR CPO_CPRQ	ST9331 (pBP8829/pBP8575)
ST9520	Reference: ST9519	Ase_FAR CPO_CPRQ Pur_POX	ST9519 (pBP8350/pBP8032)
ST9614	Reference: ST9519 Δ FAO1 (YALI0_B14014g)	Ase_FAR CPO_CPRQ	ST9519 (pBP5573)
ST9615	Reference: ST9520 Δ FAO1 (YALI0_B14014g)	Ase_FAR CPO_CPRQ Pur_POX	ST9520 (pBP5574)
ST9623	Reference: ST9357	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R AipFAR	ST9357 (pBP8926/pBP8674)
ST9628	Reference: ST9357	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R HsFAR	ST9357 (pBP8931/pBP8674)
ST9629	Reference: ST9357	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R HvFAR	ST9357 (pBP8932/pBP8674)
ST9630	Reference: ST9357	Cpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R HasFAR	ST9357 (pBP8933/pBP8674)
ST9631	Reference: ST3332	Cpo_CPRQ Ase_FAR	ST3332 (pBP2501)
ST10136	Reference: ST6629	Cpo_SPTQ	ST6629 (pBP9361/pBP6627)
ST10137	Reference: ST6629	Cpo_NPVE	ST6629 (pBP7910/pBP6631)
ST10138	Reference: ST9065	Cpo_CPRQ Cpo_NPVE Cpo_SPTQ	ST9065 (pBP9361/pBP6627)
<i>elo1Δ ole1Δ</i> (<i>Saccharomyces cerevisiae</i>)	Schneiter, 2000		<i>elo1Δ ole1Δ</i> (pYEX-CHT-CpoCPRQ)
<i>elo1Δ ole1Δ</i> <i>CpoCPRQ</i>	Reference: <i>elo1Δ</i> <i>ole1Δ</i>	CpoCPRQ	
ST9493	Reference: ST9357	2xCpo_CPRQ Cpo_CPRQ_S85A	ST9357 (pBP8826/pBP8328)

		VHb 2x HarCyb5 HarCyb5R AseFAR	
ST9494	Reference: ST9493	3xCpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R 2xAseFAR	ST9493 (pBP8828/pBP8645)
ST9495	Reference: ST9494	4xCpo_CPRQ Cpo_CPRQ_S85A VHb 2x HarCyb5 HarCyb5R 3xAseFAR	ST9494 (pBP8829/pBP8575)

Example 4: Cultivation of strains, extraction and analysis of fatty acid methyl esters and fatty alcohols

- 5 Strains were inoculated from a YPD agar plate (10 g/L yeast extract, 10 g/L peptone, 20 g/L glucose, 15 g/L agar agar) to an initial OD₆₀₀ of 0.1-0.2 into 2.5 mL YPG medium (10 g/L yeast extract, 10 g/L peptone, 40 g/L glycerol) in 24 well-plates (EnzyScreen). The plates were incubated at 28°C, shaken at 300 rpm. After 22 h, the plates were centrifuged for 5 min at 4°C and 3,000 xg. The supernatant was discarded and the cells were resuspended in 1.25 mL
- 10 production medium per well (Borodina et al., 2018). The medium was supplemented with 2.5 µL methyl dodecanoate. The plate was incubated for 28 hours at 28°C, shaken at 300 rpm.

- For analysis of fatty alcohols, 200 µL of the broth was extracted with 990 µL of ethyl acetate:ethanol (84:15) and 10 µL of Z10-17:Me (2 mg/mL) as internal standard. The samples
- 15 were vortexed for 20 sec and incubated for 1 h at room temperature, followed by 5 min of vortexing. 300 µL of H₂O was added to each sample. The samples were vortexed and centrifuged for 5 min at 21°C and 3,000 x g. The upper organic phase was analyzed via gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were performed on a Hewlett Packard 6890 GC coupled to a mass selective detector HP 5973. The GC was equipped with
- 20 an INNOWax column (30 m x 0.25 mm x 0.25 µm), and helium was used as carrier gas (average velocity: 33 cm/s). The MS was operated in electron impact mode (70eV), scanning between m/z 30 and 400, and the injector was configured in splitless mode at 220°C. The oven temperature was set to 80°C for 1 min, then increased at a rate of 10°C/min to 210°C, followed by a hold at 210°C for 15 min, and then increased at a rate of 10°C/min to 230°C followed by a

hold at 230°C for 20 min. Compounds were identified by comparison of retention times and mass spectra with those of reference compounds available in laboratory collection. Compounds were quantified by the Total Ion Current (TIC) recorded. Data were analyzed by the Agilent ChemStation software and iWork Numbers.

5

For analysis of the fatty acids, 1 mL of each vial was harvested by centrifugation for 5 min at 4°C and 3,000 xg. Each pellet was extracted with 1000 µL 1M HCl in Methanol (anhydrous). The samples were vortexed for 20 sec and placed in the 80°C water bath for 2 h. The samples were vortexed every 30 min for 10 sec. After cooling down of the samples to room temperature, 1000 µL of 1M NaOH in Methanol (anhydrous), 500 µL of NaCl saturated H₂O, 990 µL of hexane and 10 µL of Z10-17:Me (2 mg/mL) as internal standard were added. The samples were vortexed and centrifuged for 5 min at 21°C and 3,000 xg. The upper organic phase was analyzed via GC-MS as described above.

10

15 *Example 5: Production of E8,E10-C12:OH in Y. lipolytica*

Strain ST8494, derived from strain ST6629, expresses the *Helicoverpa armigera* fatty acyl reductase Har_FAR (in two copies) and the *Cydia pomonella* desaturase Cpo_CPRQ. Strain ST6629 is a *Y.lipolytica* strain engineered for decreased fatty alcohols degradation and storage lipid accumulation (Holkenbrink et al., 2020).

20

The strain was cultivated, extracted and analyzed as described in example 4, with the exception that for analysis of the formed fatty alcohols, six vials (à 1.25 mL) were combined and harvested by centrifugation for 5 min at 4°C and 3,000 g. The concentrations of fatty alcohols were calculated based on the internal standard.

25

Strain ST8494, combining the expression of the desaturase CpoCPRQ and fatty alcohol reductase HarFAR, in a strain engineered for lower fatty alcohol degradation showed the production of 4.4 mg/L E8,E10-C12:OH (Table 6).

30 **Table 6.** Concentrations of fatty alcohols in strain ST8494.

Strain	E9/Z9-C12:OH (mg/L)	E8,E10-C12:OH (mg/L)
ST8494	20.1	4.4

Example 6: Increased production of E8,E10-C12:Me and E8,E10-C12:OH in Y. lipolytica

Strain ST8406 is derived from strain ST6629 and additionally expresses the CpoCPRQ desaturase. Strain ST9066, derived from ST8406, expresses two copies of Cpo_CPRQ. The strains were cultivated, extracted and analysed as described in example 4. The concentrations of fatty acid methyl esters and fatty alcohols were calculated based on the internal standard (Tables 7-10).

The expression of an additional copy of the desaturase Cpo_CPRQ from *C. pomonella* (ST9066) led to a 2.8- and 1.5-fold increase in production of E8,E10-C12:Me and E9/Z9-C12:Me, respectively (Table 7). This shows that overexpression of the desaturase can lead to an increase in production of E8,E10-C12:Me and E9/Z9-C12:Me.

Table 7. Concentrations of fatty acid methyl esters in strains ST8406 and ST9066

Strain	E9/Z9-C12:Me (mg/L)	E8,E10-C12:Me (mg/L)
ST8406	3.81 ± 0.52	0.43 ± 0.00
ST9066	5.75 ± 1.12	1.22 ± 0.52

Strains ST8411 and ST8416 combining the expression of the desaturase Cpo_CPRQ from *C. pomonella* with either expression of the cytochrome b5 from *H. armigera* (HarCyb5, SEQ ID NO: 4) or with expression of hemoglobin from *V. stercoraria* (VHb, SEQ ID NO: 6), produced 18% and 22% more E8,E10-C12:Me, respectively, than the reference strain ST8406, only expressing the desaturase from *C. pomonella*. These strains also showed increased production of E9/Z9-C12:Me (Table 8). These data show that expression of a desaturase with a cytochrome b5 or with a hemoglobin can produce more E8,E10-C12:Me than a strain expressing only the desaturase.

Table 8. Concentrations of fatty acid methyl esters in strains ST8406, ST8411 and ST8416

Strain	E9/Z9-C12:Me (mg/L)	E8,E10-C12:Me (mg/L)
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ST8406	1.29 ± 0.20	0.22 ± 0.09
ST8411	1.60 ± 0.16	0.26 ± 0.03
ST8416	1.68 ± 0.10	0.27 ± 0.02

- Expression of the hemoglobin from *V. stercoraria* (VHb) (ST9115) in addition to the cytochrome b5 from *H. armigera* (HarCyb5) (strain ST8411) resulted in an additional 21% and 41% improvement in *E8,E10-C12:Me* and *E9/Z9-C12:Me* titre, respectively (Table 9). These data show that co-expression of a desaturase with a cytochrome b5 and a hemoglobin can produce more *E8,E10-C12:Me* and *E9/Z9-C12:Me* than a strain expressing only one of the three.

Table 9. Concentrations of fatty acid methyl esters in strains ST8406, ST8411 and ST9115

Strain	<i>E9/Z9-C12:Me</i> (mg/L)	<i>E8,E10-C12:Me</i> (mg/L)
ST8406	2.99 ± 0.64	0.34 ± 0.04
ST8411	3.40 ± 0.57	0.42 ± 0.00
ST9115	4.83 ± 0.62	0.51 ± 0.05

- Strain ST9250 expressing the fatty acyl reductase from *A. segetum* (Ase_FAR) showed production of C12:OH, *E9/Z9-C12:OH* and *E8,E10-C12:OH*, while strain ST9249 expressing the fatty acyl reductase from *T. alba* (Ta_FAR, SEQ ID NO: 8) only showed production of C12:OH (Table 10).

- Table 10. Concentrations of fatty alcohols in strains ST9066, ST9249 and ST9250. ND: not detected.**

Strain	C12:OH (mg/L)	<i>E9/Z9-C12:OH</i> (mg/L)	<i>E8,E10-C12:OH</i> (mg/L)
ST9066	ND	ND	ND
ST9249	12.91 ± 0.71	ND	ND
ST9250	12.37 ± 1.22	1.54 ± 0.52	0.66 ± 0.07

Example 7: Increased production of E8,E10-C12:Me in a $\Delta elo1$ Y. lipolytica strain

The intrinsic *Y. lipolytica* gene *ELO1* (YALI0_F06754g, SEQ ID NO: 13) was deleted in strain ST8406, leading to strain ST9060. The strains were cultivated, extracted and analysed as described in example 4. The concentrations of fatty acid methyl esters were calculated based on the internal standard (Table 11). Strain ST9060 showed a 2.2- and 1.6-fold increase in the production of *E8,E10-C12:Me* and *E9/Z9-C12:Me*, respectively, compared to strain ST8406. These data show that deletion of an elongase gene can increase production of *E8,E10-C12:Me* and *E9/Z9-C12:Me*.

Table 11. Concentrations of fatty acid methyl esters in strains ST8406 and ST9060

Strain	<i>E9/Z9-C12:Me</i> (mg/L)	<i>E8,E10-C12:Me</i> (mg/L)
ST8406	4.77 ± 0.65	0.63 ± 0.10
ST9060	7.40 ± 1.93	1.39 ± 0.33

Example 8: Increased production of E8,E10-C12:Me in a Y. lipolytica strain containing a deletion of the gene YALI0_F14729g, YALI0_E18876g or YALI0_D03597g

The intrinsic *Y. lipolytica* genes *YALI0_F14729g* (SEQ ID NO: 19), *YALI0_E18876g* (SEQ ID NO: 54) and *YALI0_D03597g* (SEQ ID NO: 55), all encoding putative thioesterases, were deleted in strain ST8406, leading to strains ST9061, ST9062 and ST9063, respectively. The strains were cultivated, extracted and analyzed as described in example 4. The concentrations of fatty acid methyl esters were calculated based on the internal standard (Table 12). Strain ST9061 showed an 1.6- and 1.7-fold increase in the production of *E8,E10-C12:Me* and *E9/Z9-C12:Me*, respectively, compared to strain ST8406. Strain ST9062 showed an 1.2- and 1.3-fold increase in the production of *E8,E10-C12:Me* and *E9/Z9-C12:Me*, respectively, compared to strain ST8406. Strain ST9063 showed a 1.1-fold increase in the production of *E8,E10-C12:Me* and *E9/Z9-C12:Me* compared to strain ST8406. These data show that deletion of an endogenous putative thioesterase can increase production of *E8,E10-C12:Me* and *E9/Z9-C12:Me*.

Table 12. Concentrations of fatty acid methyl esters in strains ST8406, ST9061, ST9062 and ST9063

Strain	<i>E9/Z9-C12:Me</i> (mg/L)	<i>E8,E10-C12:Me</i> (mg/L)
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ST8406	1.97 ± 0.12	0.29 ± 0.01
ST9061	3.35 ± 0.44	0.46 ± 0.06
ST9062	2.49 ± 0.34	0.35 ± 0.03
ST9063	2.24 ± 0.47	0.31 ± 0.04

Example 9: Production of E8,E10-C12:Me in Y. lipolytica strains containing amino acid modifications in the desaturase Cpo_CPRQ

The amino acid at position 85 in the protein Cpo_CPRQ was modified from serine (S) to alanine (A) in strain ST8406, leading to strain ST9072. The strains were cultivated, extracted and analysed as described in example 4. The concentrations of fatty acid methyl esters were calculated based on the internal standard (Table 13).

Strain ST9072, expressing Cpo_CPRQ_S85A, showed a 213% increased production of E8,E10-C12:Me compared to strain ST8406. These data show that Cpo_CPRQ can be engineered to increase production of E8,E10-C12:Me and E9/Z9-C12:Me.

Table 13. Concentrations of fatty acid methyl esters in strains ST8406 and ST9072

Strain	E9/Z9-C12:Me (mg/L)	E8,E10-C12:Me (mg/L)
ST8406	4.74 ± 0.26	0.75 ± 0.02
ST9072	9.37 ± 1.56	1.60 ± 0.16

Example 10: Production of E8,E10-C12:Me and E8,E10-C12:OH in Y. lipolytica strains containing an amino acid modification in the desaturase Cpo_CPRQ (S85A) in combination with other beneficial modifications

Strain ST9278, derived from ST9060, contains two copies of Cpo_CPRQ as well as ELO1 deletion. Strain ST9279, derived from ST9060, contains one copy of Cpo_CPRQ, one copy of Cpo_CPRQ_S85A as well as ELO1 deletion.

The strains were cultivated, extracted and analysed as described in example 4. The concentrations of fatty acid methyl esters were calculated based on the internal standard (Table 14).

Strain ST9278, expressing two copies of Cpo_CPRQ and having a deletion in the *ELO1* gene, showed a lower production of *E9/Z9-C12:Me* and *E8,E10-C12:Me* compared to strain ST9279, expressing one copy of Cpo_CPRQ, one copy of Cpo_CPRQ_S85A and having a deletion in the *ELO1* gene.

Table 14. Concentrations of fatty acid methyl esters in strains ST9060, ST9278, ST9279

Strain	<i>E9/Z9-C12:Me</i> (mg/L)	<i>E8,E10-C12:Me</i> (mg/L)
ST9060	12.63 ± 2.07	2.07 ± 0.10
ST9278	20.70 ± 2.46	5.47 ± 0.74
ST9279	21.91 ± 4.24	6.15 ± 1.62

Strain ST9355, derived from ST9279, expresses VHb and HarCyb5 in addition to other modifications. Strain ST9356, derived from ST9355, expresses HarCyb5 and HarCyb5 reductase (SEQ ID NO: 24) in addition to other modifications. Strain ST9357, derived from ST9356, contains a deletion of the intrinsic *Y. lipolytica* gene YALI0_F14729g in addition to other modifications. Strain ST9358, derived from ST9357, expresses Ase_FAR in addition to other modifications. Strain ST9387, derived from ST9279, expresses Ase_FAR in addition to other modifications. The strains were cultivated, extracted and analysed as described in example 4. The concentrations of fatty acid methyl esters and fatty alcohols were calculated based on the internal standard (Tables 15 and 16).

Table 15. Concentrations of fatty acid methyl esters in strains ST9279, ST9355, ST9356

Strain	<i>E9/Z9-C12:Me</i> (mg/L)	<i>E8,E10-C12:Me</i> (mg/L)
ST9279	12.6 ± 1.9	5.4 ± 0.7
ST9355	11.9 ± 0.7	6.4 ± 0.5
ST9356	12.8 ± 1.5	7.3 ± 0.1
ST9357	11.3 ± 0.1	7.2 ± 0.2
ST9358	11.1 ± 1.5	6.0 ± 1.2
ST9387	11.0 ± 3.2	4.0 ± 0.7

Table 16. Concentrations of fatty alcohols in strains ST9279, ST9355, ST9356, ST9357, ST9358 and ST9387

Strain	C12:OH (mg/L)	E9/Z9-C12:OH (mg/L)	E8,E10-C12:OH (mg/L)
ST9279	0 ± 0	0 ± 0	0 ± 0
ST9355	0 ± 0	0 ± 0	0 ± 0
ST9356	0 ± 0	0 ± 0	0 ± 0
ST9357	0 ± 0	0 ± 0	0 ± 0
ST9358	41.41 ± 0.34	4.73 ± 0.34	1.98 ± 0.01
ST9387	50.00 ± 1.10	7.59 ± 0.52	1.62 ± 0.29

- 5 These data show that beneficial modifications can be combined to achieve higher titres of E8,E10-C12:Me and E9/Z9-C12:Me as well as E8,E10-C12:OH and E9/Z9-C12:OH.

Example 11: Production of E8,E10-C12:OH in strains containing amino acid modifications in the reductase Ase_FAR

- 10 The amino acid at position 198 in the protein Ase_FAR is modified from threonine (T) to alanine (A) in strain ST9250, leading to strain ST9335. The amino acid at position 423 in the protein Ase_FAR is modified from serine (S) to alanine (A) in strain ST9250, leading to strain ST9336. The strains are cultivated, extracted and analyzed as described in example 4. The concentrations of fatty alcohols are calculated based on the internal standard.

15

Example 12: Production of E8,E10-C12:OH in Y. lipolytica strains containing amino acid modifications in the fatty acid synthase 1 (FAS1) and fatty acid synthase 2 (FAS2)

- The amino acid at position 1220 in the FAS2 (SEQ ID NO: 18) of *Y. lipolytica* is modified from isoleucine (I) to phenylalanine (F) in strain ST9387, leading to strain ST9388. The amino acid at position 1220 in the FAS2 of *Y. lipolytica* is modified from isoleucine (I) to tryptophan (W) in strain ST9387, leading to strain ST9420. The amino acid at position 123 in the FAS1 (SEQ ID NO: 16) of *Y. lipolytica* is modified from leucine (L) to valine (V) in strain ST9420, leading to strain ST9421. The strains are cultivated, extracted and analyzed as described in example 4,
- 20

except from that no methyl dodecanoate is added to the production medium. The concentrations of fatty alcohols are calculated based on the internal standard.

Example 13: Production of E8,E10-C12:OH in Y. lipolytica strains containing an amino acid modification in FAS2 of Y. lipolytica (FAS2(I1220F)) as well as a thioesterase from E. coli for C12 fatty acid formation

Strain ST9397 expresses a fusion of a truncated version of FAS1 from *Y. lipolytica* and a truncated version of the thioesterase TesA from *E. coli* (Xu et al., 2016) (SEQ ID NO: 59). Strain ST9397 is transformed with a plasmid containing the fatty acyl-CoA synthase from *Y. lipolytica*, leading to strain ST9398. The strains are cultivated, extracted and analyzed as described in example 4, with the exception that glass tubes were used and that fatty alcohols were extracted from the total broth. The concentrations of fatty alcohols are calculated based on the internal standard (Table 17).

The expression of the fatty acyl-CoA synthase from *Y. lipolytica* did not significantly affect the production of E8,E10-12:OH.

Table 17: Concentrations of E9/Z9-12:OH and E8,E10-12:OH in strain ST9397 and ST9398

Strain	E9/Z9-12:OH (mg/L)	E8,E10-C12:OH (mg/L)
ST9397	0.2 ± 0	0.1 ± 0
ST9398	0.2 ± 0	0.1 ± 0

Example 14: Production of E8,E10-C12:OH via chain shortening in peroxisomes in Y. lipolytica

To increase the amount of C12:CoA precursor in strain ST9395, the five endogenous peroxisomal oxidases of *Y. lipolytica*: POX1, POX2, POX3, POX4 and POX5 (YALI0_E32835g, YALI0_F10857g, YALI0_E32835g, YALI0_E27654g, YALI0_E27654g, respectively) are deleted and instead a heterologous peroxisomal oxidase as for example Cma_POX from *Cucurbita maxima* (SEQ ID NO: 47) is expressed.

To increase the amount of Δ 9-12:CoA precursor, the above mentioned strain expresses additionally a Δ 11-14 desaturase as for example CroZ11 from *Choristoneura rosaceana* (SEQ ID NO: 63) or CpaE11 from *Choristoneura parallela* (SEQ ID NO: 65). By this, Z/E11-14:CoA is

produced and is shortened to Z/E9-12:CoA, which is then further converted to E8,E10-C12:Me by desaturase Cpo_CPRQ (SEQ ID NO:1).

5 The strains are cultivated, extracted and analysed as described in example 4. The cultures of strains ST9600, ST9607 and ST9616 are supplemented with methyl myristate. The concentrations of fatty alcohols are calculated based on the internal standard.

Example 15: Production of E8,E10-C12:Me and E8,E10-C12:OH in Saccharomyces cerevisiae

10 The desaturase gene Cpo_CPRQ was amplified from cDNA of *Cydia pomonella* pheromone gland tissue using primer attB1_Cpo_CPRQ_F and attB1_Cpo_CPRQ_R.

The PCR product was separated by agarose gel electrophoresis and purified using the Wizard SV Gel and PCR Clean up system (Promega Biotech AB, Sweden). The purified DNA was
15 cloned into the pDONR221 vector by the Gateway Cloning technology (Life technologies). The resulting vector was confirmed by Sanger sequencing and the gene was subcloned into vector pYEX-CHT (Patel et al, 2003), which then was transformed into a *Saccharomyces cerevisiae* strain deficient of *OLE1* and *ELO1* (*MATa elo1::HIS3 ole1::LEU2 ade2 his3 leu2 ura3*) (Schneiter et al., 2000). For selection of positive transformants, the cells were cultivated on
20 synthetic complete medium containing 0.7% YNB (with ammonium sulfate), drop-out medium lacking uracil and leucine (Formedium LTD, England), 2% glucose, 1% tergitol (type Nonidet NP-40, Sigma-Aldrich, Sweden), 0.01% adenine (Sigma-Aldrich, Sweden) and 0.5 mM oleic acid (Sigma-Aldrich, Sweden). After incubation of the plates for four days at 30°C, individual colonies were inoculated into 10 ml selective medium. The cultures were incubated at 30°C for
25 48 h and used to inoculate 10 ml of selective medium containing 2 mM CuSO₄ with supplementation of 0.5 mM fatty acid methyl ester precursor to an OD₆₀₀ of 0.4. After 48 h of incubation the cells were harvested by centrifugation at 3000 rpm. The media supernatant was discarded and total lipids were extracted using 3.75 ml of methanol/chloroform (2:1, v/v), in a glass tube. One ml of HAc (0.15 M) and 1.25 ml of water were added and the tubes were
30 vortexed. The tubes were centrifuged at 2000 rpm for 2 min and the bottom chloroform phase was transferred to a fresh glass tube. To convert the lipids into fatty acid methyl esters (FAME), the solvent was evaporated under nitrogen flow. One ml of 2% sulfuric acid in methanol was added, the suspension was vortexed and incubated at 90°C for 1 h. Afterwards 1 ml of water was added, mixed and 1 ml of hexane was used to extract the FAMEs. The samples were
35 subjected to GC-MS analysis on a Hewlett Packard 6890 GC coupled to a mass selective

detector HP5973. The GC was equipped with a HP-88 column (30 m x 0.25 mm x 0.25 µm) and helium was used as carrier gas (average velocity: 33 ms). The MS was operated in electron impact mode (70eV), and the injector was configured in splitless mode at 220°C. The oven temperature was set to 80°C for 1 min, then increased at a rate of 10°C/min up to 210°C, followed by a hold of 210°C for 15 min, and then increased at a rate of 10°C/min up to 230°C, followed by a hold at 230°C for 20 min. As a reference standard, E8,E10-12:OAc was purchased from Bedoukian, USA and converted to the corresponding alcohol by hydrolysis using a 0.5 M solution of KOH in methanol. Fatty alcohols were oxidized to the corresponding acid with pyridinium dichromate in dimethylformamide as described (Bjostad and Roelofs, 1984)

The chromatograms in Figure 2 show that E9-12:Me and E8,E10-12:Me can be produced from 12:Me and E9-12:Me, respectively, in the *S. cerevisiae* strains expressing Cpo_CPRQ.

Example 16: Production of E8,E10-C12:Me by Cpo_SPTQ, Cpo_NPVE and Cpo_CPRQ in Y. lipolytica

Strain ST10136, derived from ST6629, expresses one copy of Cpo_SPTQ. Strain ST10137, derived from ST6629, expresses one copy of Cpo_NPVE. Strain ST9064, derived from ST8406, expresses one copy of Cpo_CPRQ and one copy of Cpo_SPTQ. Strain ST9065, derived from ST8406, expresses one copy of Cpo_CPRQ and one copy of Cpo_NPVE. Strain ST9066, derived from ST8406, expresses two copies of Cpo_CPRQ. Strain ST10138, derived from ST9065, expresses one copy of Cpo_CPRQ, one copy of Cpo_NPVE and one copy of Cpo_SPTQ.

The strains were cultivated, extracted and analyzed as described in example 4. The concentrations of fatty acid methyl esters were calculated based on the internal standard (Table 18).

The expression of Cpo_SPTQ (ST10136) did not lead to the production of E9-C12:Me, Z9-C12:Me or E8,E10-C12:Me. The expression of Cpo_NPVE (ST10137) led to the production of E9-C12:Me and Z9-C12:Me, but not E8,E10-C12:Me. The additional expression of Cpo_SPTQ or Cpo_NPVE in ST8406 (ST9064 and ST9065, respectively) did not lead to an increase in E8,E10-C12:Me. The expression of an additional copy of Cpo_CPRQ in ST8406 (ST9066) led to a 2.8- and 2.1-fold increase in production of E8,E10-C12:Me and E9/Z9-C12:Me, respectively. The combined expression of Cpo_CPRQ, Cpo_SPTQ and Cpo_NPVE (ST10138)

did not lead to an increase in *E8,E10-C12:Me* compared to ST8406. This shows that only the expression of *Cpo_CPRQ* leads to the production of *E8,E10-C12:Me*.

Table 18. Concentrations of fatty acid methyl esters in strains ST10136, ST10137, ST8406, ST9064, ST9065, ST9066 and ST10138

Strain	<i>E9-C12:Me</i> (mg/L)	<i>Z9-C12:Me</i> (mg/L)	<i>E8,E10-C12:Me</i> (mg/L)
ST10136	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ST10137	2.06 ± 0.89	3.82 ± 1.80	0.00 ± 0.00
ST8406	5.02 ± 0.88	0.33 ± 0.03	0.66 ± 0.15
ST9064	4.42 ± 0.00	0.29 ± 0.00	0.63 ± 0.00
ST9065	6.04 ± 0.22	2.29 ± 0.10	0.86 ± 0.10
ST9066	10.55 ± 0.07	0.58 ± 0.04	1.87 ± 0.06
ST10138	6.38 ± 0.99	2.66 ± 0.41	0.76 ± 0.06

Example 17: Production of E8,E10-C12:OH by expressing multiple copies of biosynthetic enzymes

Strain ST9358 is explained in Example 10. Strain ST9495 derives from strain ST9357 (described in Example 10) and expresses additional gene copies of the desaturase *Cpo_CPRQ* and the fatty acyl reductase *Ase_FAR*. The strains are cultivated, extracted and analyzed as described in example 4, with the exception that glass tubes were used and that fatty alcohols were extracted from the total broth. The concentrations of fatty alcohols are calculated based on the internal standard.

Table 19 shows that additional gene copies of *Cpo_CPRQ* and *Ase_FAR* can increase the production of *E8,E10-12:OH* to 7.1 mg/L.

Table 19. Concentrations of fatty alcohols in strains ST9358 and ST9495

Strain	<i>E9-C12:OH</i> (mg/L)	<i>E8,E10-C12:OH</i> (mg/L)
ST9358	0.3 ± 0.0	0.1 ± 0.0
ST9495	22.6 ± 4.5	7.1 ± 1.5

Example 18: Codlemone production by expression of various fatty acyl reductases

Strains ST9358 and ST9623 are derived from strain ST9357. They additionally express the fatty acyl reductase from *Agrotis segetum* and *Agrotis ipsilon*, respectively. The strains were cultivated, extracted and analysed as described in example 4.

- 5 The results in table 20 show that both fatty acyl reductases are able to produce E9-C12:OH and E8,E10-C12:OH.

Table 20. Concentrations of fatty alcohols in strains ST9357, ST9358 and ST9623

Strain	E9-C12:OH (mg/L)	E8,E10-C12:OH (mg/L)
ST9357	0 ± 0	0 ± 0
ST9358	1.1 ± 1.2	0.5 ± 0.5
ST9623	1.9 ± 0.3	0.6 ± 0.1

Sequences

SEQ ID NO	Description	Organism
SEQ ID NO: 1	DNA CPO_CPRQ desaturase (AHW98354) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Cydia pomonella</i>
SEQ ID NO: 2	PRT CPO_CPRQ desaturase (AHW98354)	<i>Cydia pomonella</i>
SEQ ID NO: 3	DNA cytochrome b5 (AAC33731) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Helicoverpa armigera</i>
SEQ ID NO: 4	PRT cytochrome b5 (AAC33731)	<i>Helicoverpa armigera</i>
SEQ ID NO: 5	DNA hemoglobin (AAT01097) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Vitreoscilla stercoraria</i>
SEQ ID NO: 6	PRT hemoglobin (AAT01097)	<i>Vitreoscilla stercoraria</i>
SEQ ID NO: 7	DNA fatty acyl reductase (NP_001289627) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Tyto alba</i>
SEQ ID NO: 8	PRT fatty acyl reductase (NP_001289627)	<i>Tyto alba</i>
SEQ ID NO: 9	DNA fatty acyl reductase (AGP26039) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Agrotis segetum</i>
SEQ ID NO: 10	PRT fatty acyl reductase (AGP26039)	<i>Agrotis segetum</i>

SEQ ID NO: 11	DNA fatty acyl reductase (ATJ44471) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Helicoverpa armigera</i>
SEQ ID NO: 12	PRT fatty acyl reductase (ATJ44471)	<i>Helicoverpa armigera</i>
SEQ ID NO: 13	DNA fatty acid elongase 1 (ELO1, YALI0_F06754g)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 14	PRT fatty acid elongase 1 (ELO1, XP_505094)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 15	DNA fatty acid synthase 1 (FAS1, YALI0_B15059g)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 16	PRT fatty acid synthase 1 (Fas1, XP_500912)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 17	DNA fatty acid synthase 2 (FAS2, YALI0_B19382g)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 18	PRT fatty acid synthase 2 (Fas2, XP_501096)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 19	DNA YALI0_F14729g	<i>Yarrowia lipolytica</i>
SEQ ID NO: 20	PRT XP_505426	<i>Yarrowia lipolytica</i>
SEQ ID NO: 21	DNA fatty acyl-CoA synthase (YALI0_D17864g)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 22	PRT fatty acyl-CoA synthase (XP_502959)	<i>Yarrowia lipolytica</i>
SEQ ID NO: 23	DNA cytochrome b5 reductase (XP_021183830) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Helicoverpa armigera</i>
SEQ ID NO: 24	PRT cytochrome b5 reductase (XP_021183830)	<i>Helicoverpa armigera</i>
SEQ ID NO: 25	DNA thioesterase (AAB40248) codon optimized for <i>Y. lipolytica</i> ; mRNA-coding sequence	<i>Escherichia coli</i>
SEQ ID NO: 26	PRT thioesterase (AAB40248)	<i>Escherichia coli</i>
SEQ ID NO: 27	DNA TEFintron promoter	<i>Yarrowia lipolytica</i>
SEQ ID NO: 28	DNA EXP promoter	<i>Yarrowia lipolytica</i>
SEQ ID NO: 29	DNA YEF3 promoter	<i>Yarrowia lipolytica</i>
SEQ ID NO: 30	DNA Peroxisomal oxidase POX1	<i>Saccharomyces cerevisiae</i>
SEQ ID NO: 31	PRT Peroxisomal oxidase POX1	<i>Saccharomyces cerevisiae</i>
SEQ ID NO: 32	DNA codon-optimized nucleotide sequence of thioesterase CpFATB2	<i>Cuphea palustris</i>
SEQ ID NO: 33	PRT thioesterase CpFATB2	<i>Cuphea palustris</i>
SEQ ID NO: 34	DNA	<i>Cinnamomum camphora</i>

	codon-optimised nucleotide sequence of thioesterase CcFatB1	
SEQ ID NO: 35	PRT thioesterase CcFatB1	<i>Cinnamomum camphora</i>
SEQ ID NO: 36	DNA Codon-optimised sequence of acetyltransferase Atf1	<i>Saccharomyces cerevisiae</i>
SEQ ID NO: 37	PRT Acetyltransferase Atf1	<i>Saccharomyces cerevisiae</i>
SEQ ID NO: 38	DNA Codon-optimised sequence of peroxisomal oxidase	<i>Agrotis segetum</i>
SEQ ID NO: 39	PRT Peroxisomal oxidase	<i>Agrotis segetum</i>
SEQ ID NO: 40	DNA Codon-optimised sequence of peroxisomal oxidase 1	<i>Arabidopsis thaliana</i>
SEQ ID NO: 41	PRT Peroxisomal oxidase 1	<i>Arabidopsis thaliana</i>
SEQ ID NO: 42	DNA Codon-optimised sequence of peroxisomal oxidase 2	<i>Arabidopsis thaliana</i>
SEQ ID NO: 43	PRT Peroxisomal oxidase 2	<i>Arabidopsis thaliana</i>
SEQ ID NO: 44	DNA Codon-optimised sequence of peroxisomal oxidase	<i>Aspergillus nidulans</i>
SEQ ID NO: 45	PRT Peroxisomal oxidase	<i>Aspergillus nidulans</i>
SEQ ID NO: 46	DNA Codon-optimised sequence of peroxisomal oxidase	<i>Cucurbita maxima</i>
SEQ ID NO: 47	PRT Peroxisomal oxidase	<i>Cucurbita maxima</i>
SEQ ID NO: 48	DNA Codon-optimised sequence of peroxisomal oxidase	<i>Homo sapiens</i>
SEQ ID NO: 49	PRT Peroxisomal oxidase	<i>Homo sapiens</i>
SEQ ID NO: 50	DNA Codon-optimised sequence of peroxisomal oxidase	<i>Paenarthrobacter ureafaciens</i>
SEQ ID NO: 51	PRT Peroxisomal oxidase	<i>Paenarthrobacter ureafaciens</i>
SEQ ID NO: 52	DNA Codon-optimised sequence of peroxisomal oxidase	<i>Rattus norvegicus</i>
SEQ ID NO: 53	PRT Peroxisomal oxidase	<i>Rattus norvegicus</i>
SEQ ID NO: 54	DNA YALI0_E18876g	<i>Yarrowia lipolytica</i>

SEQ ID NO: 55	DNA YALI0_D03597g	<i>Yarrowia lipolytica</i>
SEQ ID NO: 56	DNA Thioesterase	<i>Cuphea hookeriana</i>
SEQ ID NO: 57	PRT Thioesterase	<i>Cuphea hookeriana</i>
SEQ ID NO: 58	DNA Fusion of a truncated version of FAS1 from <i>Y. lipolytica</i> and a truncated version of the thioesterase TesA from <i>E. coli</i>	
SEQ ID NO: 59	PRT Fusion of a truncated version of FAS1 from <i>Y. lipolytica</i> and a truncated version of the thioesterase TesA from <i>E. coli</i>	
SEQ ID NO: 60	DNA Aip_FAR	<i>Agrotis ipsilon</i>
SEQ ID NO: 61	PRT Aip_FAR	<i>Agrotis ipsilon</i>
SEQ ID NO: 62	DNA CroZ11 desaturase	<i>Choristoneura rosaceana</i>
SEQ ID NO: 63	PRT CroZ11 desaturase	<i>Choristoneura rosaceana</i>
SEQ ID NO: 64	DNA CpaE11 desaturase	<i>Choristoneura parallela</i>
SEQ ID NO: 65	PRT CpaE11 desaturase	<i>Choristoneura parallela</i>
SEQ ID NO: 66	DNA Cpo_NPVE, codon-optimised for <i>Y. lipolytica</i>	<i>Cydia pomonella</i>
SEQ ID NO: 67	PRT Cpo_NPVE	<i>Cydia pomonella</i>
SEQ ID NO: 68	DNA Cpo_SPTQ, codon-optimised for <i>Y. lipolytica</i>	<i>Cydia pomonella</i>
SEQ ID NO: 69	PRT Cpo_SPTQ	<i>Cydia pomonella</i>
SEQ ID NO: 70	DNA, codon-optimized for <i>S. cerevisiae</i> ; mRNA-coding sequence Hs_FAR	<i>Heliothis subflexa</i>
SEQ ID NO: 71	PRT Hs_FAR	<i>Heliothis subflexa</i>
SEQ ID NO: 72	DNA, codon-optimized for <i>S. cerevisiae</i> ; mRNA-coding sequence Has_FAR	<i>Helicoverpa assulta</i>
SEQ ID NO: 73	PRT Has_FAR	<i>Helicoverpa assulta</i>
SEQ ID NO: 74	DNA Hv_FAR	<i>Helicoverpa virescens</i>
SEQ ID NO: 75	PRT Hv_FAR	<i>Helicoverpa virescens</i>
SEQ ID NO: 76	PRT Cpo_FAR	<i>Cydia pomonella</i>
SEQ ID NO: 77	PRT Gmo_CPRQ	<i>Grapholita molesta</i>

SEQ ID NO: 78	DNA Gmo_CPRQ	<i>Grapholita molesta</i>
SEQ ID NO: 79	DNA Gmo_CPRQ, modified	<i>Grapholita molesta</i>
SEQ ID NO: 80	DNA Cpo_FAR	<i>Cydia pomonella</i>

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Items

- 30 1. A yeast cell capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, said yeast cell expressing at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty
- 35 acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-

- dodecadienyl coenzyme A (*E8,E10*-C12:CoA), optionally wherein the yeast cell belongs to a genus selected from *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Saccharomyces* and *Yarrowia*, optionally wherein the yeast cell belongs to a species selected from *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*, *C. tropicalis*, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C. japonica*, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M. ramanniana*, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium irregulare*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R. mucilaginoso*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, preferably the yeast cell is a *Yarrowia lipolytica* cell or a *Saccharomyces cerevisiae* cell.
2. A yeast cell capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, said yeast cell expressing at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA).
3. The yeast cell according to any one of the preceding items, wherein the yeast cell is capable of producing *E8,E10*-dodecadien-1-ol, said yeast cell further expressing at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.
4. A yeast cell capable of producing *E8,E10*-dodecadien-1-ol, said yeast cell expressing:
- At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and
 - At least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least

part of said E8,E10-dodecadienyl coenzyme A (E8,E10-C12:CoA) to E8,E10-dodecadien-1-ol.

5. The yeast cell according to any one of the preceding items, wherein the yeast cell
5 belongs to a genus selected from *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*,
Lipomyces, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*,
Trichosporon, *Saccharomyces* and *Yarrowia*.
6. The yeast cell according to any one of the preceding items, wherein the yeast cell
10 belongs to a species selected from *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*,
C. tropicalis, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C.*
japonica, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M.*
ramanniana, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium*
irregulare, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R.*
15 *mucilaginosa*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, *Saccharomyces*
cerevisiae and *Yarrowia lipolytica*.
7. The yeast cell according to any one of the preceding items, wherein the yeast cell is of
the genus *Yarrowia* or *Saccharomyces*, preferably the yeast cell is a *Yarrowia lipolytica*
20 cell or a *Saccharomyces cerevisiae* cell.
8. The yeast cell according to any one of the preceding items, wherein the at least one
desaturase is Gmo_CPRQ (SEQ ID NO: 77) or Cpo_CPRQ (SEQ ID NO: 2), or a
functional variant thereof having at least 65% homology or identity, such as at least 70%
25 homology or identity, such as at least 71% homology or identity, such as at least 72%,
such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%,
such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%,
such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%,
such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%,
30 such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%,
such as at least 97%, such as at least 98%, such as at least 99% homology or identity to
SEQ ID NO: 77 or SEQ ID NO: 2, preferably the at least one desaturase is Cpo_CPRQ
or a functional variant thereof; or wherein the at least one desaturase is at least two
desaturases, wherein at least one of said two desaturases is Gmo_CPRQ (SEQ ID NO:
35 77) or Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 65%
homology or identity, such as at least 70% homology or identity, such as at least 71%

- homology or identity, such as at least 72%, such as at least 73%, such as at least 74%,
such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%,
such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%,
such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%,
5 such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%,
such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%,
such as at least 99% homology or identity to SEQ ID NO: 77 or SEQ ID NO: 2,
preferably the at least one desaturase is Cpo_CPRQ or a functional variant thereof, and
the other desaturase is a desaturase capable of introducing at least one double bond in
10 a fatty acyl-CoA having a carbon chain length of 12, such as a Z9-12 desaturase,
preferably Cpo_NPVE (SEQ ID NO: 67) or Cpo_SPTQ (SEQ ID NO: 69) or a functional
variant thereof having at least 65% homology or identity, such as at least 70% homology
or identity, such as at least 71% homology or identity, such as at least 72%, such as at
least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at
15 least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at
least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at
least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at
least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at
least 97%, such as at least 98%, such as at least 99% homology or identity to SEQ ID
20 NO: 67 or SEQ ID NO: 69.
9. The yeast cell according to any one of the preceding items, wherein the desaturase is a
mutant of Cpo_CPRQ having a mutation at position 85, such as an S85A mutation.
- 25 10. The yeast cell according to any one of the preceding items, wherein the at least one
heterologous desaturase is at least two different heterologous desaturases, such as
Cpo_CPRQ as set forth in SEQ ID NO: 2 and a mutant of Cpo_CPRQ having a mutation
at position 85 such as an S85A mutation.
- 30 11. The yeast cell according to any one of the preceding items, wherein the fatty acyl-CoA
reductase is selected from the group consisting of Ase_FAR (SEQ ID NO: 10), Aip_FAR
(SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR
(SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12), Cpo_FAR (SEQ ID NO: 76) and functional
variants thereof having at least 65% homology or identity, such as at least 70%
35 homology or identity, such as at least 71% homology or identity, such as at least 72%,
such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%,

such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%,
such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%,
such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%,
such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%,
such as at least 97%, such as at least 98%, such as at least 99% homology or identity
thereto.

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12. The yeast cell according to any one of the preceding items, wherein the fatty acyl-CoA
reductase is a mutant of Ase_FAR, such as having a mutation at position 198 or 413,
preferably a T198A mutation or an S413A mutation.

13. The yeast cell according to any one of the preceding items, wherein the heterologous
desaturase is expressed at high level.

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14. The yeast cell according to any one of the preceding items, wherein the heterologous
fatty acyl-CoA reductase is expressed at high level.

15. The yeast cell according to any one of the preceding items, wherein the yeast cell is
further modified to increase availability of *E8,E10-C12:CoA*.

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16. The yeast cell according to any one of the preceding items, further expressing a
heterologous cytochrome b5, such as a cytochrome b5 from a *Lepidoptera* species, such
as a cytochrome b5 from *Helicoverpa armigera*, preferably the cytochrome b5 HarCyb5
as set forth in SEQ ID NO: 4 or a functional variant thereof having at least 65%
homology or identity, such as at least 70% homology or identity, such as at least 71%
homology or identity, such as at least 72%, such as at least 73%, such as at least 74%,
such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%,
such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%,
such as at least 99% homology or identity thereto.

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17. The yeast cell according to any one of the preceding items, further expressing a
heterologous cytochrome b5 reductase (EC 1.6.2.2), such as a cytochrome b5
reductase from a *Lepidoptera* species, such as *Helicoverpa armigera*, preferably the
cytochrome b5 reductase is the cytochrome b5 reductase from *Helicoverpa armigera* as
set forth in SEQ ID NO: 24 or a functional variant thereof having at least 65% homology
or identity, such as at least 70% homology or identity, such as at least 71% homology or

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identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.

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18. The yeast cell according to any one of the preceding items, further expressing a hemoglobin, such as a hemoglobin from *Vitreoscilla stercoraria*, preferably the hemoglobin from *Vitreoscilla stercoraria* as set forth in SEQ ID NO: 6 or a functional variant thereof having at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.

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19. The yeast cell according to any one of the preceding items, further comprising a mutation of one or more genes encoding an elongase and resulting in a partial or total loss of elongase activity, such as a mutation of the *ELO1* gene (SEQ ID NO: 13) resulting in a partial or total loss of Elo1 activity, preferably wherein said mutation is a deletion.

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20. The yeast cell according to any one of the preceding items, further comprising a mutation of one or more genes encoding a thioesterase and resulting in a partial or total loss of thioesterase activity, such as a mutation of the *YAL10_F14729g* gene (SEQ ID NO: 19), a mutation of the *YALI0_E18876g* gene (SEQ ID NO: 54) or a mutation of *YALI0_D03597g* (SEQ ID NO: 55), preferably wherein said mutation is a deletion.

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21. The yeast cell according to any one of the preceding items, further comprising at least one modification such as at least one mutation resulting in reduced activity of at least one of Hfd1, Hfd2, Hfd3, Hfd4, Fao1, GPAT and Pex10, or having at least one modification such as at least one mutation resulting in reduced activity of at least one protein having at least 60% homology or identity thereto, such as at least 65% homology or identity, such as at least 70% homology or identity, such as at least 75% homology or identity, such as at least 80% homology or identity, such as at least 81% homology or identity, such as at least 82% homology or identity, such as at least 83% homology or identity, such as at least 84% homology or identity, such as at least 85% homology or identity, such as at least 86% homology or identity, such as at least 87% homology or

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identity, such as at least 88% homology or identity, such as at least 89% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92% homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity thereto.

22. The yeast cell according to any one of the preceding items, wherein the yeast cell further expresses a fatty acyl synthase variant having a modified ketone synthase domain, wherein said fatty acyl synthase variant is a variant of Fas1 (SEQ ID NO: 16) or Fas2 (SEQ ID NO: 18) such as a mutant Fas1 having a mutation at position 123, preferably an L123V mutation, or a mutant Fas2 having a mutation at position 1220, preferably an I1220F or an I1220W mutation.

23. The yeast cell according to any one of the preceding items, wherein the yeast cell further expresses a thioesterase such as a heterologous thioesterase, optionally wherein the thioesterase is expressed at high level.

24. The yeast cell according to item 23, wherein the thioesterase has at least 60% homology or identity to the thioesterase from *Cuphea palustris* as set forth in SEQ ID NO: 33, to the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 57, to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 26, preferably the thioesterase has at least 60% homology or identity to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 26.

25. The yeast cell according to any one of the preceding items, wherein the yeast cell further expresses a fusion protein of a truncated fatty acyl synthase and of a truncated thioesterase, such as the fusion protein as set forth in SEQ ID NO: 59 or a homologue thereof having at least 60% homology or identity thereto.

26. The yeast cell according to any one of the preceding items, wherein the yeast cell comprises a nucleic acid encoding said heterologous desaturase and a nucleic acid encoding said heterologous fatty acyl-CoA reductase.

27. The yeast cell according to item 26, wherein the nucleic acid encoding said heterologous desaturase and/or the nucleic acid encoding said heterologous fatty acyl-CoA reductase are present in a high copy number.
28. The yeast cell according to any one of items 26 to 27, wherein the nucleic acid encoding said heterologous desaturase is as set forth in SEQ ID NO: 1 or a homologue thereof having at least 60% homology or identity thereto, or as set forth in SEQ ID NO: 78 or a homologue thereof having at least 60% homology or identity thereto.
29. The yeast cell according to any one of items 26 to 28, wherein the nucleic acid encoding said heterologous fatty acyl-CoA reductase is as set forth in SEQ ID NO: 9 or a homologue thereof having at least 60% homology or identity thereto.
30. The yeast cell according to any one of the preceding items, wherein the yeast cell comprises a nucleic acid encoding said heterologous cytochrome b5, a nucleic acid encoding said heterologous cytochrome b5 reductase, a nucleic acid encoding said hemoglobin, a nucleic acid encoding said fatty acid synthase variant, a nucleic acid encoding said thioesterase, and/or a nucleic acid encoding said fusion protein.
31. The yeast cell according to item 30, wherein the nucleic acid encoding said heterologous cytochrome b5, the nucleic acid encoding said heterologous cytochrome b5 reductase, the nucleic acid encoding said hemoglobin, the nucleic acid encoding said fatty acid synthase variant, and/or the nucleic acid encoding said thioesterase are present in high copy number.
32. The yeast cell according to any one of the preceding items, wherein the nucleic acid encoding said heterologous desaturase, the nucleic acid encoding said heterologous fatty acyl-CoA reductase, the nucleic acid encoding said heterologous cytochrome b5, the nucleic acid encoding said heterologous cytochrome b5 reductase, the nucleic acid encoding said hemoglobin, the nucleic acid encoding said fatty acid synthase variant, and/or the nucleic acid encoding said thioesterase are codon-optimised for expression in the yeast cell.
33. The yeast cell according to any one of items 30 to 32, wherein the nucleic acid encoding said heterologous cytochrome b5 is as set forth in SEQ ID NO: 3 or a homologue thereof

5 having at least 60% homology or identity thereto, the nucleic acid encoding said heterologous cytochrome b5 reductase is as set forth in SEQ ID NO: 23 or a homologue thereof having at least 60% homology or identity thereto, the nucleic acid encoding said hemoglobin is as set forth in SEQ ID NO: 5 or a homologue thereof having at least 60% homology or identity thereto, and/or the nucleic acid encoding said thioesterase is as set forth in SEQ ID NO: 25 or SEQ ID NO: 34 or a homologue of SEQ ID NO: 25 or SEQ ID NO: 34 having at least 60% homology or identity thereto.

10 34. The yeast cell according to any one of the preceding items, wherein the yeast cell is capable of producing *E8,E10*-dodecadien-1-ol with a titer of at least 0.5 mg/L, such as at least 0.6 mg/L, such as at least 0.7 mg/L, such as at least 0.8 mg/L, such as at least 0.9 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as
15 at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

20 35. The yeast cell according to any one of the preceding items, wherein the yeast cell further expresses an acetyltransferase (EC 2.3.1.84) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate, whereby the yeast cell is capable of producing *E8,E10*-dodecadienyl acetate.

25 36. The yeast cell according to item 35, wherein the acetyltransferase is a heterologous acetyltransferase (AcT) expressed from said yeast cell or a native acetyltransferase overexpressed from said yeast cell.

30 37. The yeast cell according to any one of items 35 or 36, wherein the acetyltransferase is Sc_Atf1 (SEQ ID NO: 37) or a variant thereof having at least 60% homology or identity thereto, such as at least 61% homology or identity, such as at least 62% homology or identity, such as at least 63% homology or identity, such as at least 64% homology or identity, such as at least 65% homology or identity, such as at least 66% homology or identity, such as at least 67% homology or identity, such as at least 68% homology or identity, such as at least 69% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at
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least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% to Sc_Atf1 (SEQ ID NO: 37).

38. The yeast cell according to any one of the preceding items, wherein the yeast cell further expresses an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadialenal.

39. The yeast cell according to any one of the preceding items, wherein the yeast cell further:

- i) has one or more mutations resulting in reduced activity of one or more native acyl-CoA oxidases; and
- ii) expresses at least one group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein the group of enzymes is capable of shortening a fatty acyl-CoA of a first carbon chain length X to a shortened fatty acyl-CoA having a second carbon chain length X' , wherein $X' \leq X-2$.

40. The yeast cell according to item 39, wherein $X'=12$.

41. The yeast cell according to any one of items 39 to 40, wherein the yeast cell further expresses a desaturase capable of introducing at least one double bond in the fatty acyl-CoA of carbon chain length X , such as CroZ11 desaturase (SEQ ID NO: 63) or CpaE11 desaturase (SEQ ID NO: 65) or a functional variant thereof having at least 65% homology or identity, such as at least 70% homology or identity, such as at least 71% homology or identity, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity to SEQ ID NO: 63, SEQ ID NO: 65.

42. The yeast cell according to any one of items 39 to 42, wherein the native acyl-CoA oxidase of i) and/or the acyl-CoA oxidase of ii) is a peroxisomal acyl-CoA oxidase.

43. The yeast cell according to any one of items 39 to 41, wherein the at least one acyl-CoA oxidase of ii) is a native acyl-CoA oxidase or a heterologous acyl-CoA oxidase, which is optionally overexpressed compared to a reference yeast strain not expressing said at least one group of enzymes, preferably the at least one acyl-CoA oxidase of the group of enzymes of ii) is a heterologous acyl-CoA oxidase.

44. The yeast cell according to any one of items 39 to 43, wherein the group of enzymes of ii) comprises an acyl-CoA oxidase derived from an organism of a genus selected from *Yarrowia*, *Agrotis*, *Arabidopsis*, *Aspergillus*, *Cucurbita*, *Homo*, *Paenarthrobacter* and *Rattus* preferably the at least one first group of enzymes comprises an acyl-CoA oxidase derived from *Yarrowia lipolytica*, *Agrotis segetum*, *Arabidopsis thaliana*, *Aspergillus nidulans*, *Cucurbita maxima*, *Homo sapiens*, *Paenarthrobacter ureafaciens* or *Rattus norvegicus*, preferably the at least one acyl-CoA oxidase of the first group of enzymes is an acyl-CoA oxidase selected from the group consisting of Yli_POX1 (XP_504703), Yli_POX2 (XP_505264), Yli_POX3 (XP_503244), Yli_POX4 (XP_504475), Yli_POX5 (XP_502199), Yli_POX6 (XP_503632), Ase_POX (SEQ ID NO: 39), Ath_POX1 (SEQ ID NO: 41), Ath_POX2 (SEQ ID NO: 43), Ani_POX (SEQ ID NO: 45), Cma_POX (SEQ ID NO: 47), Hsa_POX1-2 (SEQ ID NO: 49), Pur_POX (SEQ ID NO: 51), Sc_POX1 (SEQ ID NO: 31) and Rno_POX2 (SEQ ID NO: 53), or a functional variant thereof having at least 60% homology or identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.

45. A method for producing E8,E10-dodecadienyl coenzyme A and optionally E8,E10-dodecadien-1-ol in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:

i) At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting

said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and

- ii) Optionally at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol,

thereby producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

46. The method according to item 45, wherein the yeast cell is as defined in any one of items 1 to 44.

47. The method according to any one of items 45 to 46, further comprising the step of converting the *E8,E10*-dodecadienyl coenzyme A into a lipid such as a triacylglyceride or into a free fatty acid, recovering said lipid or free fatty acid and converting said lipid or free fatty acid to *E8,E10*-dodecadien-1-ol.

48. The method according to any one of items 45 to 47, further comprising the step of recovering said *E8,E10*-dodecadien-1-ol.

49. The method according to any one of items 45 to 48, further comprising the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate by expression of an acetyltransferase or by chemical conversion.

50. The method according to item 49, wherein the acetyltransferase is a heterologous acetyltransferase (EC 2.3.1.84) expressed from said yeast cell or a native acetyltransferase overexpressed from said yeast cell, wherein said acetyltransferase is capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate, thereby further producing *E8,E10*-dodecadienyl acetate.

51. The method according to item 50, wherein the acetyltransferase is Sc_Atf1 (SEQ ID NO: 37) or a variant thereof having at least 75% homology or identity, such as at least 80% homology or identity, such as at least 85% homology or identity, such as at least 90% homology or identity, such as at least 91% homology or identity, such as at least 92%

homology or identity, such as at least 93% homology or identity, such as at least 94% homology or identity, such as at least 95% homology or identity, such as at least 96% homology or identity, such as at least 97% homology or identity, such as at least 98% homology or identity, such as at least 99% homology or identity, such as 100% homology or identity to Sc_At1 (SEQ ID NO: 37).

52. The method according to any one items 45 to 51, further comprising the step of recovering said *E8,E10*-dodecadienyl acetate.

53. The method according to any one items 45 to 52, further comprising the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal by expression of an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal, or by chemical conversion, thereby further producing *E8,E10*-dodecadienal.

54. The method according to item 53, further comprising the step of recovering said *E8,E10*-dodecadienal.

55. The method according to any one of items 45 to 54, wherein the medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution, wherein the extractant a non-ionic ethoxylated surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate such as simethicone, fatty alcohol alkoxylates, polyethoxylated surfactants and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.

56. The method according to item 55, wherein:

- the non-ionic ethoxylated surfactant is an ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agent, such as C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate (CAS number 68002-96-0), and wherein the culture medium comprises at least 1% vol/vol of C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such

as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol C₁₆-C₁₈ alkyl alcohol ethoxylate propoxylate, or more,

- 5 - the non-ionic ethoxylated surfactant is a polyethylene polypropylene glycol, for example Kolliphor® P407 (CAS number 9003-11-6), and wherein the culture medium comprises at least 10% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, such as at least 11% vol/vol, such as at least 12% vol/vol, such as at least 13% vol/vol, such as at least 14% vol/vol, such as at least 15% vol/vol, such as at least 16% vol/vol, such as at least 17% vol/vol, such as at least 18% vol/vol, such as at least 19% vol/vol, such as at least 20% vol/vol, such as at least 25% vol/vol, such as at least 30% vol/vol, such as at least 35% vol/vol of polyethylene polypropylene glycol such as Kolliphor® P407, or more,
- 10 - the non-ionic ethoxylated surfactant is a mixture of polyether dispersions, such as antifoam 204, and wherein the culture medium comprises at least 1% vol/vol of a mixture of polyether dispersions such as antifoam 204, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol of a mixture of polyether dispersions such as antifoam 204, or more; and/or
- 15 - the non-ionic ethoxylated surfactant is a non-ionic ethoxylated surfactant comprising polyethylene glycol monostearate such as simethicone, and wherein the culture medium comprises at least 1% vol/vol of polyethylene glycol monostearate or simethicone, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30% vol/vol polyethylene glycol monostearate or simethicone, or more;
- 20 - the non-ionic ethoxylated surfactant is a fatty alcohol alkoxylate, preferably selected from Plurafac® LF300 (CAS number 196823-11-7), Plurafac® LF1300 (68002-96-0), Plurafac® SLF180 (CAS number 196823-11-7), Dehypon® 2574 (CAS number 68154-97-2), and Imbentin SG/251 (CAS number 68002-96-0), preferably Plurafac® LF300 or Dehypon® 2574, and wherein the culture medium comprises at least 1% vol/vol of fatty
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alcohol alkoxylate, such as at least 1.5%, such as at least 2%, such as at least 2.5%,
such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 5%,
such as at least 6%, such as at least 7%, such as at least 8%, such as at least 9%, such
as at least 10%, such as at least 12.5%, such as at least 15%, such as at least 17.5%,
such as at least 20%, such as at least 22.5%, such as at least 25%, such as at least
27.5%, such as at least 30% vol/vol fatty alcohol alkoxylate or more;

- the non-ionic ethoxylated surfactant is Agnique BP420 (CAS number 68002-96-0), and
wherein the culture medium comprises at least 1% vol/vol of Agnique BP420, such as at
least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at
least 3.5%, such as at least 4%, such as at least 5%, such as at least 6%, such as at
least 7%, such as at least 8%, such as at least 9%, such as at least 10%, such as at
least 12.5%, such as at least 15%, such as at least 17.5%, such as at least 20%, such
as at least 22.5%, such as at least 25%, such as at least 27.5%, such as at least 30%
vol/vol Agnique BP420 or more.

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57. The method according to any one of items 45 to 56, wherein the culture medium
comprises the extractant in an amount greater than its cloud concentration by at least
50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at
least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such
as at least 500%, such as at least 750%, such as at least 1000%, or more, and/or
wherein the culture medium comprises the extractant in an amount at least 2-fold its
cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-
fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at
least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such
as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration,
such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud
concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold
its cloud concentration, such as at least 20-fold its cloud concentration, such as at least
25-fold its cloud concentration, such as at least 30-fold its cloud concentration.

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58. The method according to any one of items 45 to 57, wherein the *E8,E10*-dodecadienyl
coenzyme A is converted into a lipid or a free fatty acid, and wherein said lipid or free
fatty acid, said *E8,E10*-dodecadien-1-ol, and optionally said *E8,E10*-dodecadienyl
acetate and/or said *E8,E10*-dodecadienal produced by the yeast cell is present in an
emulsion in the fermentation broth, the method further comprising a step of breaking
said emulsion, thereby obtaining a composition comprising a product phase comprising

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the extractant and the lipid or free fatty acid, the *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal, optionally wherein:

- 5 - the step of breaking the emulsion comprises or consists of a step of phase separation, such as a step of centrifugation, of the fermentation broth, thereby obtaining a composition consisting of three phases: a water phase, a phase comprising cells and cellular debris, and the product phase comprising the extractant and the lipid or free fatty acid, *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal, and/or
- 10 - wherein the product phase comprises at least 50% of the lipid or free fatty acid, *E8,E10*-dodecadien-1-ol, and optionally of the *E8,E10*-dodecadienyl acetate and/or of the *E8,E10*-dodecadienal initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95%
15 or more.

59. The method according to any one of items 45 to 58, further comprising the steps of:

- 20 - recovering the lipid or free fatty acid, the *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal preferably by a distillation step such as a distillation under reduced pressure, or by a column purification,
- chemically converting at least part of the *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadienal and/or to *E8,E10*-dodecadienyl acetate,
- optionally, recovering said *E8,E10*-dodecadienal and/or to *E8,E10*-dodecadienyl acetate.

25 60. The method according to any one of items 45 to 59, further comprising the step of formulating the recovered *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal into a pheromone composition.

30 61. The method according to any one of items 45 to 60, wherein the pheromone composition further comprises one or more additional compounds such as a liquid or solid carrier or substrate.

62. A nucleic acid construct for modifying a yeast cell, said construct comprising:

- 35 i) At least one first polynucleotide encoding at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon

chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); and

- ii) Optionally a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.

63. The nucleic acid construct according to item 62, wherein:

a) the at least one desaturase is Gmo_CPRQ (SEQ ID NO: 77), Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 77 or SEQ ID NO: 2, preferably the at least one desaturase is Cpo_CPRQ or a functional variant thereof; or

b) the at least one desaturase is at least two desaturases, wherein at least one of said two desaturases is Gmo_CPRQ (SEQ ID NO: 77), Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2, preferably the at least one desaturase is Cpo_CPRQ or a functional variant thereof, and the other desaturase is a desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 12, such as a Z9-12 desaturase.

64. The nucleic acid construct according to any one of items 62 to 63, wherein the at least one heterologous desaturase is at least two desaturases, and wherein the other desaturase is selected from Cpo_NPVE (SEQ ID NO: 67), Cpo_SPTQ (SEQ ID NO: 69) and functional variants thereof having at least 60% homology or identity thereto, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as

at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 67 or SEQ ID NO: 69.

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65. The nucleic acid construct according to any one of items 62 to 64, wherein the first polynucleotide comprises SEQ ID NO: 1 or SEQ ID NO: 78, preferably SEQ ID NO: 1, or a homologue thereof having at least 60% homology or identity thereto, such as 61%,
such as at least 62%, such as at least 63%, such as at least 64%, such as at least 65%,
such as at least 66%, such as at least 67%, such as at least 68%, such as at least 69%,
such as at least 70%, such as at least 71%, such as at least 72%, such as at least 73%,
such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%,
such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%,
such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%,
such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%,
such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%,
such as at least 98%, such as at least 99% homology or identity to SEQ ID NO: 1 or
SEQ ID NO: 78, preferably to SEQ ID NO: 1.

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66. The nucleic acid construct according to any one of items 62 to 65, wherein the at least one heterologous desaturase is at least two heterologous desaturases, and wherein the first polynucleotide further comprises a nucleic acid as set forth in SEQ ID NO: 66 or
SEQ ID NO: 68, or a homologue thereof having at least 60% homology or identity
thereto, such as 61%, such as at least 62%, such as at least 63%, such as at least 64%,
such as at least 65%, such as at least 66%, such as at least 67%, such as at least 68%,
such as at least 69%, such as at least 70%, such as at least 71%, such as at least 72%,
such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%,
such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%,
such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%,
such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%,
such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%,
such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%,
such as at least 97%, such as at least 98%, such as at least 99% homology or identity
thereto.

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67. The nucleic acid construct according to any one of items 62 to 66, wherein the heterologous desaturase is as defined in any one of items 1 to 44.
- 5 68. The nucleic acid construct according to any one of items 62 to 67, wherein the at least one desaturase is a mutant of Cpo_CPRQ having a mutation at position 85, such as an S85A mutation.
- 10 69. The nucleic acid construct according to any one of items 62 to 68, wherein the heterologous fatty acyl-CoA reductase is as defined in any one of items 1 to 44.
- 15 70. The nucleic acid construct according to any one of items 62 to 69, wherein the second polynucleotide comprises or consists of SEQ ID NO: 9, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 11, SEQ ID NO: 76 and homologues thereof having at least 60% homology or identity thereto, such as at least 65%, such as at least 70%, such as at least 71%, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology or identity thereto.
- 20 71. The nucleic acid construct according to any one of items 62 to 70, further comprising one or more of:
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- iii) a polynucleotide encoding a heterologous cytochrome b5, such as the polynucleotide as set forth in SEQ ID NO: 3 or a homologue thereof having at least 60% homology or identity thereto;
 - iv) a polynucleotide encoding a heterologous cytochrome b5 reductase, such as the polynucleotide as set forth in SEQ ID NO: 23 or a homologue thereof having at least 60% homology or identity thereto;
 - 30 v) a polynucleotide encoding a hemoglobin, such as the polynucleotide as set forth in SEQ ID NO: 5 or a homologue thereof having at least 60% homology or identity thereto;
 - 35 vi) a polynucleotide encoding a fatty acyl synthase variant having a modified ketone synthase domain; and/or

- vii) a polynucleotide encoding a thioesterase, such as the polynucleotide as set forth in SEQ ID NO: 25 or SEQ ID NO: 34 or a homologue thereof having at least 60% homology or identity thereto.

5 72. The nucleic acid construct according to any one of items 62 to 71, wherein the heterologous cytochrome b5, the heterologous cytochrome b5 reductase, the hemoglobin, the fatty acyl synthase variant and/or the thioesterase are as defined in any one of items 1 to 44.

10 73. A method of monitoring the presence of pest or disrupting the mating of pest, said method comprising the steps of:

i) Producing *E8,E10*-dodecadien-1-ol and optionally *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal by the method according to any one of items 45 to 61;

15 ii) Formulating said *E8,E10*-dodecadien-1-ol and optionally said *E8,E10*-dodecadienyl acetate and/or said *E8,E10*-dodecadienal as a pheromone composition; and

iii) Employing said pheromone composition as an integrated pest management composition.

20 74. *E8,E10*-dodecadienyl coenzyme A, *E8,E10*-dodecadien-1-ol, *E8,E10*-dodecadienyl acetate and/or *E8,E10*-dodecadienal obtainable by the method according to any one of items 45 to 61.

75. A kit of parts comprising instructions for use and:

a) the yeast cell according to any one of items 1 to 44; and/or

25 b) the nucleic acid construct according to any one of items 62 to 72 for modifying a yeast cell and optionally a yeast cell to be modified, wherein upon expression of the polynucleotides comprised within the nucleic acid construct, the modified yeast cell is capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

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Claims

1. A yeast cell capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, said yeast cell expressing at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA), wherein:
- a) the at least one desaturase is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2; or
- b) the at least one desaturase is at least two desaturases, wherein at least one of said two desaturases is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2, and the other desaturase is a desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 12, such as a Z9-12 desaturase.
2. The yeast cell according to claim 1, wherein the at least one desaturase is at least two desaturases, and wherein the other desaturase is selected from Cpo_NPVE (SEQ ID NO: 67), Cpo_SPTQ (SEQ ID NO: 69) and functional variants thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 67 or SEQ ID NO: 69.

3. The yeast cell according to any one of the preceding claims, wherein the yeast cell belongs to a genus selected from *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Saccharomyces* and *Yarrowia*, optionally wherein the yeast cell belongs to a species selected from *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*, *C. tropicalis*, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C. japonica*, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M. ramanniana*, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium irregulare*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R. mucilaginosa*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, preferably the yeast cell is a *Yarrowia lipolytica* cell or a *Saccharomyces cerevisiae* cell.
4. The yeast cell according to any one of the preceding claims, wherein the yeast cell is capable of producing *E8,E10*-dodecadien-1-ol, said yeast cell further expressing at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.
5. The yeast cell according to any one of the preceding claims, wherein the desaturase is a mutant of Cpo_CPRQ having a mutation at position 85, such as an S85A mutation, and/or wherein the at least one heterologous desaturase is at least two different heterologous desaturases, such as Cpo_CPRQ as set forth in SEQ ID NO: 2 and a mutant of Cpo_CPRQ having a mutation at position 85 such as an S85A mutation.
6. The yeast cell according to any one of claims 4 to 5, wherein the fatty acyl-CoA reductase is selected from the group consisting of Ase_FAR (SEQ ID NO: 10), Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12), Cpo_FAR (SEQ ID NO: 76) and functional variants thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% r identity thereto, optionally wherein the fatty acyl-CoA

reductase is a mutant of Ase_FAR, such as having a mutation at position 198 or 413, preferably a T198A mutation or an S413A mutation.

7. The yeast cell according to any one of the preceding claims, further having one or more of the following:

- expressing a heterologous cytochrome b5, such as a cytochrome b5 from a *Lepidoptera* species, such as a cytochrome b5 from *Helicoverpa armigera*, preferably the cytochrome b5 HarCyb5 as set forth in SEQ ID NO: 4 or a functional variant thereof having at least 80% identity thereto, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto;
- expressing a heterologous cytochrome b5 reductase (EC 1.6.2.2), such as a cytochrome b5 reductase from a *Lepidoptera* species, such as *Helicoverpa armigera*, preferably the cytochrome b5 reductase is the cytochrome b5 reductase from *Helicoverpa armigera* as set forth in SEQ ID NO: 24 or a functional variant thereof having at least 80% identity thereto, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto,
- expressing a hemoglobin, such as a hemoglobin from *Vitreoscilla stercoraria*, preferably the hemoglobin from *Vitreoscilla stercoraria* as set forth in SEQ ID NO: 6 or a functional variant thereof having at least 80% identity thereto, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto,
- comprising a mutation of one or more genes encoding an elongase and resulting in a partial or total loss of elongase activity, such as a mutation of the *ELO1* gene (SEQ ID NO: 13) resulting in a partial or total loss of Elo1 activity, preferably wherein said mutation is a deletion,
- comprising a mutation of one or more genes encoding a thioesterase and resulting in a partial or total loss of thioesterase activity, such as a mutation of the *YAL10_F14729g* gene (SEQ ID NO: 19), a mutation of the *YAL10_E18876g* gene (SEQ ID NO: 54) or a mutation of *YAL10_D03597g* (SEQ ID NO: 55), preferably wherein said mutation is a deletion,
- comprising at least one mutation resulting in reduced activity of at least one of Hfd1, Hfd2, Hfd3, Hfd4, Fao1 and Pex10, or having a mutation resulting in reduced activity of at least one protein having at least 80% identity thereto, such as at least 81%, such as

- at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto,
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- expressing a fatty acyl synthase variant having a modified ketone synthase domain, wherein said fatty acyl synthase variant is a variant of Fas1 (SEQ ID NO: 16) or Fas2 (SEQ ID NO: 18) such as a mutant Fas1 having a mutation at position 123, preferably an L123V mutation, or a mutant Fas2 having a mutation at position 1220, preferably an
 - 10 I1220F or an I1220W mutation,
 - expressing a thioesterase such as a heterologous thioesterase, optionally wherein the thioesterase is expressed at high level, such as a thioesterase having at least 80% identity to the thioesterase from *Cuphea palustris* as set forth in SEQ ID NO: 33, to the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 57, to the thioesterase
 - 15 from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 26, preferably the thioesterase has at least 80% identity to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 26,
 - expressing a fusion protein of a truncated fatty acyl synthase and of a truncated
 - 20 thioesterase, such as the fusion protein as set forth in SEQ ID NO: 59 or a homologue thereof having at least 80% identity thereto.
8. The yeast cell according to any one of the preceding claims, further comprising at least one mutation resulting in reduced activity of at least one of Hfd1, Hfd2, Hfd3, Hfd4,
- 25 Fao1, GPAT and Pex10, or having at least one mutation resulting in reduced activity of at least one protein having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as
- 30 at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto.
9. The yeast cell according to any one of the preceding claims, wherein the yeast cell is capable of producing *E8,E10*-dodecadien-1-ol with a titer of at least 0.5 mg/L, such as at
- 35 least 0.6 mg/L, such as at least 0.7 mg/L, such as at least 0.8 mg/L, such as at least 0.9 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such

as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

10. The yeast cell according to any one of the preceding claims, wherein the yeast cell further expresses an acetyltransferase (EC 2.3.1.84) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate, whereby the yeast cell is capable of producing *E8,E10*-dodecadienyl acetate, preferably wherein the acetyltransferase is a heterologous acetyltransferase (AcT) expressed from said yeast cell or a native acetyltransferase overexpressed from said yeast cell, preferably wherein the acetyltransferase is Sc_At1f1 (SEQ ID NO: 37) or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to Sc_At1f1 (SEQ ID NO: 37).

11. The yeast cell according to any one of the preceding claims, wherein the yeast cell further expresses an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal.

12. The yeast cell according to any one of the preceding claims, wherein the yeast cell further:

- i) has one or more mutations resulting in reduced activity of one or more native acyl-CoA oxidases; and
- ii) expresses at least one group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein the group of enzymes is capable of shortening a fatty acyl-CoA of a first carbon chain length X to a shortened fatty acyl-CoA having a second carbon chain length X' , wherein $X' \leq X-2$, preferably wherein $X'=12$.

13. The yeast cell according to any one of the preceding claims, further expressing a desaturase capable of introducing at least one double bond in the fatty acyl-CoA of carbon chain length X, such as CroZ11 desaturase (SEQ ID NO: 63) or CpaE11 desaturase (SEQ ID NO: 65), or a functional variant thereof having at least 80% identity, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 63 or SEQ ID NO: 65.
14. A method for producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:
- At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); wherein:
 - the at least one desaturase is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2; or
 - the at least one desaturase is at least two desaturases, wherein at least one of said two desaturases is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at

least 99% identity to SEQ ID NO: 2, and the other desaturase is a desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 12, such as a Z9-12 desaturase;

and

- 5 ii) Optionally at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol,

10 thereby producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

15 15. The method according to claim 14, wherein the method further comprises the steps of converting the *E8,E10*-dodecadienyl coenzyme A into a lipid such as a triacylglyceride or into a free fatty acid, recovering said lipid or free fatty acid and converting said lipid or free fatty acid to *E8,E10*-dodecadien-1-ol.

20 16. The method according to any one of claims 14 to 15, wherein the method further comprises the step of recovering said *E8,E10*-dodecadien-1-ol, preferably wherein the yeast cell is as defined in any one of claims 1 to 13.

25 17. The method according to any one of claims 14 to 16, further comprising the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate by expression of an acetyltransferase or by chemical conversion, thereby further producing *E8,E10*-dodecadienyl acetate, and optionally further comprising the step of recovering said *E8,E10*-dodecadienyl acetate.

30 18. The method according to any one of claims 14 to 17, further comprising the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal by expression of an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal, or by chemical conversion, thereby further producing *E8,E10*-dodecadienal, and optionally further comprising the step of recovering said *E8,E10*-dodecadienal.

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19. The method according to any one of claims 14 to 18, wherein the medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution such as in the culture medium at the cultivation temperature, wherein the extractant a non-ionic ethoxylated surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate such as simethicone, fatty alcohol alkoxylates, polyethoxylated surfactants and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.
20. The method according to claim 19, wherein the medium comprises the extractant in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more, and/or wherein the culture medium comprises the extractant in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration, wherein the cloud concentration is measured in the medium, preferably at the cultivation temperature.
21. The method according to any one of claims 19 to 20, further comprising the step of converting the *E8,E10*-dodecadienyl coenzyme A into a lipid or a free fatty acid, and wherein said lipid or free fatty acid, said *E8,E10*-dodecadien-1-ol, and optionally said *E8,E10*-dodecadienyl acetate and/or said *E8,E10*-dodecadienal produced by the yeast cell is present in an emulsion in the fermentation broth, the method further comprising a step of breaking said emulsion, thereby obtaining a composition comprising a product phase comprising the extractant and the lipid or free fatty acid, the *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal.

22. The method according to claim 21, wherein the step of breaking the emulsion comprises or consists of a step of phase separation, such as a step of centrifugation, of the fermentation broth, thereby obtaining a composition consisting of three phases: a water phase, a phase comprising cells and cellular debris, and the product phase comprising the extractant and the lipid or free fatty acid, *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal.
23. The method according to any one of claims 21 to 22, wherein the product phase comprises at least 50% of the lipid or free fatty acid, *E8,E10*-dodecadien-1-ol, and optionally of the *E8,E10*-dodecadienyl acetate and/or of the *E8,E10*-dodecadienal initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95% or more.
24. The method according to any one of claims 21 to 23, further comprising the steps of:
- recovering the lipid or free fatty acid, the *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal preferably by a distillation step such as a distillation under reduced pressure, or by a column purification,
 - chemically converting at least part of the *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadienal and/or to *E8,E10*-dodecadienyl acetate,
 - optionally, recovering said *E8,E10*-dodecadienal and/or to *E8,E10*-dodecadienyl acetate.
25. The method according to any one of claims 14 to 24, further comprising the step of formulating the recovered *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal into a pheromone composition.