the group consisting of (i) another fat or oil, (ii) a fatty acid ester with a lower alcohol and (iii) a fatty acid, to the action of a lipase in the form of an immobilized enzyme catalyst, with adjusting the water concentration in the reaction liquid during the reaction.

EP 245076 A2 discloses a process for the preparation of edible fats suitable for use in confectionary, by rearrangement of unsaturated glyceride oils and fats having a high oleate content using a lipase enzyme in the presence of saturated fatty acids or esters thereof (an “acidolysis reactant”). Preferably 1 to 5 moles of acidolysis reactant per mole of oil is used, more preferably 3-5 moles per mole. In the exemplified processes, the weight ratios of acidolysis reactant to oil are 1:1 (Example 1), 1:2.5 (Example 3), and 2.4:2.5 (Example 4).

Thus, known enzymatic processes for producing triglyceride compositions which contain a high SOS triglyceride content generally involve fractionation in order to achieve an appropriate composition of triglycerides (either dry fractionation or solvent fractionation). Solvent fractionation can produce products of good quality, but the cost for building this process is high, and the use of solvents can create safety and customer acceptance issues. Dry fractionation is a more economically feasible alternative, but not as efficient in the separation of SOS triglycerides. This results in the need for product to be recirculated, which adds to process costs. More recirculation also means more formation of by-products and therefore also a decreased overall yield. More than one fractionation step may be needed to achieve the desired quality. Accordingly, both types of fractionation are complex and costly processes steps which affect the process economy.

Thus, there is a need for a more efficient enzymatic transesterification process, yielding a triglyceride composition suitable for use as a cocoa butter equivalent, or a component thereof, with improved process economy, and fewer process steps, and where there is no need for fractionation of the triglyceride composition resulting from the enzymatic transesterification. It is also desirable to provide such an enzymatic transesterification process which can utilise high oleic oil as a starting material, and which therefore does not require the use of vegetable oils which already contain a high level of stearic and/or palmitic acid, such as Shea stearin or palm oil.

Summary of the invention

Aspects of the invention are defined in the independent claims. Some optional features of the invention are defined in the dependent claims.

The inventors have surprisingly found that, by using particular reactants and conditions in an enzymatic transesterification process, it is possible to provide a triglyceride composition which comprises a high level of SOS triglycerides, and which can be used as a cocoa butter equivalent, or a component thereof, after simply separating fatty acid esters and free fatty acids, without the need for fractionation of the triglyceride phase.

Accordingly, there is an exemplary process for increasing the SOS triglyceride content of a vegetable oil, wherein S represents stearic acid (C18:0) and palmitic acid (C16:0) residues and O represents oleic acid (C18:1) residues, said process comprising:

a) providing a reaction environment comprising:

i) an *sn*-1,3-specific lipase immobilised on a support,

ii) a vegetable oil, wherein the vegetable oil comprises at least 45% oleic acid fatty acid residues, based on total C6-C24 fatty acid residues, and wherein the vegetable oil has an oleic acid content in the *sn*-2 position of at least 75% by weight of the total *sn*-2 fatty acid residues of the vegetable oil, and

iii) an aliphatic alcohol ester of stearic acid, palmitic acid, or mixtures thereof, optionally mixed with stearic acid and/or palmitic acid, and

iv) water;

wherein the weight ratio of the aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to the vegetable oil (ii) is at least 4:1; and

wherein the water activity of the reaction environment is in the range 0.1 to 0.6;

b) heating the reaction environment to a temperature of 30-60°C to perform transesterification, thus obtaining a mixture comprising a triglyceride phase, fatty acid esters, and optionally free fatty acids; and

c) separating the fatty acid esters and, where present, free fatty acids from the mixture obtained in step (b) to obtain a triglyceride composition.

The process as disclosed herein can yield a triglyceride phase which has an SOS content of at least 65% by weight of the triglyceride phase. Moreover, undesirable production of SSO triglycerides can be minimised such that the triglyceride phase of the mixture obtained in step (b) has a weight ratio of SOS triglycerides to SSO triglycerides of at least 80:1. The undesirable production of SSS triglycerides can also be minimised, such that the triglyceride phase of the mixture can have an SSS triglyceride content of ≤4% by weight of the triglyceride phase. These amounts and ratios are calculated by weight on the basis of the triglyceride phase of the mixture. The triglyceride composition is suitable for use as a cocoa butter equivalent or a component thereof.

The steps of the process above may be followed by any of the three alternative steps d1), d2) and d3), where the fatty acid esters and, optionally free fatty acids, from step c) are returned to the reaction environment of step a) with the purpose of being used as substates for transesterification:

d1) recirculating fatty acid esters and, where present, free fatty acids separated in step (c) to the reaction environment;

d2) if the aliphatic alcohol ester (iii) comprises an aliphatic alcohol ester of stearic acid, optionally mixed with stearic acid, hydrogenating fatty acid esters and, where present, free fatty acids separated in step (c) and recirculating the hydrogenated fatty acid esters and free fatty acids to the reaction environment;

d3) separating fatty acid esters and, where present, free fatty acids separated in step (c) into:

a first fraction comprising stearate and/or palmitate ester, and optionally stearic acid and/or palmitic acid; and

a second fraction comprising oleate ester and optionally oleic acid; and

recirculating the first fraction to the reaction environment.

Where the aliphatic alcohol ester (iii) comprises an aliphatic alcohol ester of stearic acid, optionally mixed with stearic acid, the process may also comprise a further step (e3) of hydrogenating the second fraction to provide stearate ester and optionally stearic acid and recirculating the hydrogenated second fraction to the reaction environment.

The vegetable oil (ii) may be selected from High Oleic Rapeseed/canola oil, Olive oil, High Oleic Soybean oil, High Oleic Sunflower oil, High Oleic Safflower oil, Shea oil, or Rapeseed oil, and/or fractions or combinations thereof. Preferably the vegetable oil (ii) is selected from High Oleic Sunflower oil, High Oleic Safflower oil, and/or combinations or fractions thereof.

The process may further comprise using the triglyceride composition obtained in step (c) as a cocoa butter equivalent or a component thereof in the manufacture of a chocolate or chocolate-like product.

The invention may also provide triglyceride composition obtainable by the process.

The invention may also provide a chocolate or chocolate-like product comprising the triglyceride composition.

The invention may also provide a chocolate or chocolate-like product comprising the CBE.

Definitions

As used herein, the term “vegetable” shall be understood as originating from a plant or a single cell organism. Thus, vegetable oil or vegetable triglycerides are still to be understood as vegetable oil or vegetable triglyceride if all the fatty acids used to obtain said triglyceride or oil is of plant or single cell organism origin.

The term “oil” as used herein refers to glyceride fats and oils containing fatty acid acyl groups and does not imply any particular melting point. The term “fat” is used synonymously with “oil” herein.

Detailed description of exemplary embodiments of the invention

When describing the below exemplary embodiments, the present invention envisages all possible combinations and permutations of the below described embodiments with the above disclosed aspects.

An embodiment of the invention relates to a process for increasing the SOS triglyceride content of a vegetable oil, wherein S represents stearic acid (C18:0) and palmitic acid (C16:0) residues and O represents oleic acid (C18:1) residues, said process comprising:

a) providing a reaction environment comprising:

i) an *sn*-1,3-specific lipase immobilised on a support,

ii) a vegetable oil, wherein the vegetable oil comprises at least 45% oleic acid fatty acid residues, based on total C6-C24 fatty acid residues, and wherein the vegetable oil has an oleic acid content in the *sn*-2 position of at least 75% by weight of the total *sn*-2 fatty acid residues of the vegetable oil,

iii) an aliphatic alcohol ester of stearic acid, palmitic acid, or mixtures thereof, optionally mixed with stearic acid and/or palmitic acid, and

iv) water;

wherein the weight ratio of the aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to the vegetable oil (ii) is at least 4:1; and

wherein the water activity of the reaction environment is in the range 0.1 to 0.6;

b) heating the reaction environment to a temperature of 30-60°C to perform transesterification, thus obtaining a mixture comprising a triglyceride phase, fatty acid esters, and optionally free fatty acids; and

c) separating the fatty acid esters and free fatty acids from the mixture obtained in step (b) to obtain a triglyceride composition.

The triglyceride phase of the mixture obtained in step (b) may have an SOS triglyceride content of at least 65% by weight of the triglyceride phase and a weight ratio of SOS triglycerides to SSO triglycerides of at least 80:1. The SSO content of the triglyceride phase may be ≤1.2% by weight of the triglyceride phase. The SSS content of the triglyceride phase may be ≤4% by weight of the triglyceride phase, preferably ≤3% by weight of the triglyceride phase, more preferably ≤2% by weight of the triglyceride phase and most preferably ≤1.5% by weight of the triglyceride phase. The composition can therefore be used as a cocoa butter equivalent, or a component thereof, without the need for further fractionation of the triglyceride phase.

The SOS triglyceride, SSO triglyceride and SSS triglyceride content of the triglyceride phase can be determined using a non-aqueous reversed-phase HPLC method. A suitable method is described in “Non-aqueous reversed phase liquid chromatography with charged aerosol detection for quantitative lipid analysis with improved accuracy”, *Causevic, A. et al.,* Journal of Chromatography A, Vol. 1652, 2021, pages 1-11.

Stearate esters are preferred, because SOS triglycerides in which S represents stearic acid residues cannot be obtained in high amounts from widely available vegetable oils such as palm oil. The aliphatic alcohol ester of stearic acid or palmitic acid may be used in mixture with stearic acid and/or palmitic acid as free fatty acids. However, preferably, these free fatty acids are not used.

The reaction environment also comprises water. The presence of water is necessary to provide sufficient enzyme activity for high levels of SOS triglycerides to be obtained. However, when the level of water is too high, it has been found that diacylglycerides and SSO triglycerides are formed in higher amounts in the triglyceride phase which is obtained in step (b) of the process. It has been found that, to provide a high level of SOS triglycerides and a low level of diacylglycerides and SSO triglycerides, the water activity in step a) should be in the range 0.1-0.6, preferably 0.2- 0.4.

The process of the present invention may utilise a particularly high ratio of aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to vegetable oil (ii). It has been found that a high substrate ratio of at least 4:1 results in greater incorporation of stearic acid and palmitic acid in the sn-1 and sn-3 positions of the triglyceride, and therefore production of more SOS triglycerides. Surprisingly, the high substrate ratio, when combined with the other reaction conditions of process of the invention, may not increase the production of SSO and SSS triglycerides to an unacceptably high level. Accordingly, the triglyceride compositions which are produced by the process of the invention may be used as cocoa butter equivalents, or components thereof, without the need for further fractionation steps to separate SOS triglycerides from SSO and SSS triglycerides. Preferably, the substrate ratio is at least 5:1, more preferably at least 6:1, and even more preferably at least 7:1. Even higher substrate ratios are also contemplated within the present invention, such as at least 8:1 or at least 9:1. At very high substrate ratios, the efficiency of the process can be reduced by the need to remove large amounts of fatty acid ester and free fatty acids in step (c). Accordingly, the substrate ratio is typically at most 15:1, or at most 12:1, or at most 10:1.

The substrate ratio which is used in a particular process can be varied depending on the nature of the vegetable oil (ii). For example, when the vegetable oil has an oleic acid content in the *sn*-2 position which is particularly high, a high level of SOS triglycerides can be achieved by transesterification even when a substrate ratio is used which is towards the lower end of the specified range. In such cases, the use of substrate ratios towards the lower end of the specified range may be advantageous for reasons of process efficiency. Accordingly, in one embodiment, the vegetable oil has an oleic acid content in the *sn*-2 position of at least 90% or at least 95% by weight of the total *sn*-2 fatty acid residues of the vegetable oil and the weight ratio of the aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to the vegetable oil (ii) is in the range 4:1 to 7:1. Conversely, if the vegetable oil has an oleic acid content in the *sn*-2 position

Claims

1. A process for increasing the SOS triglyceride content of a vegetable oil, wherein S represents stearic acid (C18:0) and palmitic acid (C16:0) residues and O represents oleic acid (C18:1) residues, said process being performed in a reaction environment comprising:

   i) an *sn*-1,3-specific lipase immobilised on a support,

   ii) a vegetable oil, wherein the vegetable oil comprises at least 45% oleic acid fatty acid residues, based on total C6-C24 fatty acid residues, and wherein the vegetable oil has an oleic acid content in the *sn*-2 position of at least 75% by weight of the total *sn*-2 fatty acid residues of the vegetable oil,

   iii) an aliphatic alcohol ester of stearic acid, palmitic acid, or mixtures thereof, optionally mixed with stearic acid and/or palmitic acid, and

   iv) water;

   wherein the weight ratio of the aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to the vegetable oil (ii) is at least 4:1; and

   wherein the water activity of the reaction environment is in the range 0.1 to 0.6; the process comprising:

   a) heating the reaction environment to a temperature of 30-60°C to perform transesterification, thus obtaining a mixture comprising a triglyceride phase, fatty acid esters, and optionally free fatty acids; and

   b) separating the fatty acid esters and free fatty acids from the mixture obtained in step (a) to obtain a triglyceride composition.

2. The process of claim 1, wherein the triglyceride composition obtained in step (b) is not subjected to a fractionation step to provide a fractionated triglyceride composition having a further increased content of SOS triglycerides.

3. The process of claim 1 or claim 2, wherein the triglyceride phase of the mixture obtained in step (a) has an SOS triglyceride content of at least 65% by weight of the triglyceride phase.

4. The process of any preceding claim, wherein the triglyceride phase of the mixture obtained in step (a) has a weight ratio of SOS triglycerides to SSO triglycerides of at least 80:1.

5. The process of any preceding claim, wherein the triglyceride phase of the mixture obtained in step (a) has an SSO content of ≤1.2% by weight of the triglyceride phase.

6. The process of any preceding claim, wherein the water activity of the reaction environment is in the range 0.2 to 0.4.

7. The process of any preceding claim, wherein the vegetable oil comprises at least 50% oleic acid (C18:1) fatty acid residues, preferably at least 60% oleic acid (C18:1) fatty acid residues, and more preferably at least 70% oleic acid (C18:1) fatty acid residues by weight, based on total C6-C24 fatty acid residues.

8. The process of any preceding claim, wherein the vegetable oil has an oleic acid content in the *sn*-2 position of at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% by weight of the total *sn*-2 fatty acid residues of the vegetable oil.

9. The process of any preceding claim, wherein the vegetable oil is selected from High Oleic Rapeseed/canola oil, Olive oil, High Oleic Soybean oil, High Oleic Sunflower oil, High Oleic Safflower oil, Shea oil, or Rapeseed oil and/or fractions or combinations thereof.

10. The process of any preceding claim, wherein the vegetable oil is selected from High Oleic Sunflower oil, High Oleic Safflower oil and/or fractions or combinations thereof.

11. The process of any preceding claim, wherein the *sn*-1,3-specific lipase is a microbial lipase, such as a bacterial lipase or a fungal lipase.

12. The process of claim 11, wherein the *sn*-1,3-specific lipase is derived from a fungal species selected from *Rhizopus oryzae*, *Thermomyces lanuginosus*, and *Rhizomucor miehei*.

13. The process of any preceding claim, wherein the reaction environment is heated to a temperature of 35-45°C.

14. The process of any preceding claim, wherein the weight ratio of the aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to the vegetable oil (ii) is at least 5:1, such as at least 6:1 or at least 7:1.

15. The process of any preceding claim, wherein the vegetable oil has an oleic acid content in the *sn*-2 position of at least 90%, preferably at least 95% by weight of the total *sn*-2 fatty acid residues of the vegetable oil, and the weight ratio of the aliphatic alcohol ester and optional stearic acid and/or palmitic acid (iii) to the vegetable oil (ii) is in the range 4:1 to 7:1.

16. The process of any preceding claim, wherein the aliphatic alcohol ester of stearic acid or palmitic acid is an alkyl ester of stearic acid or palmitic acid or a mixture thereof, preferably a C_1-C_4 alkyl ester of stearic acid or palmitic acid or a mixture thereof, more preferably selected from the group consisting of methyl stearate, ethyl stearate, methyl palmitate, ethyl palmitate and mixtures thereof, most preferably selected from methyl stearate and ethyl stearate.

17. The process of any preceding claim, wherein the transesterification is performed as a batch process, as a fed-batch process, or as a continuous process.

18. The process of any preceding claim, further comprising:

c1) recirculating fatty acid esters and, where present, free fatty acids separated in step (b) to the reaction environment.

19. The process of any of claims 1 to 18, wherein the aliphatic alcohol ester (iii) comprises an aliphatic alcohol ester of stearic acid, optionally mixed with stearic acid, and wherein the process further comprises:

c2) hydrogenating fatty acid esters and, where present, free fatty acids separated in step (b) and recirculating the hydrogenated fatty acid esters and free fatty acids to the reaction environment.

20. The process of any of claims 1-18, further comprising:

c3) separating fatty acid esters and, where present, free fatty acids separated in step (b) into:

a first fraction comprising stearate and/or palmitate ester, and optionally stearic acid and/or palmitic acid; and

a second fraction comprising oleate ester and optionally oleic acid; and

recirculating the first fraction to the reaction environment.

21. The process of claim 20, wherein the aliphatic alcohol ester (iii) comprises an aliphatic alcohol ester of stearic acid, optionally mixed with stearic acid, and wherein the process further comprises:

d3) hydrogenating the second fraction to provide stearate ester and optionally stearic acid and recirculating the hydrogenated second fraction to the reaction environment.

22. The process of any of claims 18-21, wherein any of the following are bleached before being recirculated to the reaction environment:
the fatty acid esters and optionally free fatty acids separated in step (b);
the hydrogenated fatty acid esters and free fatty acids provided in step (c2);
the first fraction provided in step (c3); or
the second fraction provided in step (c3), either before or after hydrogenation.

23. The process of any preceding claim, further comprising using the triglyceride composition obtained in step (b) as a cocoa butter equivalent or a component thereof in the manufacture of a chocolate or chocolate-like product.

24. A triglyceride composition obtainable by the process of any of claims 1-22.

25. A cocoa butter equivalent which comprises 10-70% by weight of the triglyceride composition of claim 24.

26. A chocolate or chocolate-like product comprising the triglyceride composition of claim 24.