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SUMMARY

TECHNICAL PROBLEM

[0005] Pseudo-moving bed chromatography requires a complex system and equipment and is more expensive than chromatography using a single column. Thus, there is a need for a method that efficiently produces a target polyunsaturated fatty acid ester composition at high purity without the need for complex equipment such as pseudo-moving bed chromatography.

SOLUTION TO PROBLEM

[0006] The inventors of the present disclosure have unexpectedly found that a multi-stage separation method using a first column chromatography and a second column chromatography can efficiently produce a target polyunsaturated fatty acid in view of the above problem.

The present disclosure is directed to the following method for producing a polyunsaturated fatty acid ester composition from a fatty oil composition.

[0007] [1-1] A method for producing a polyunsaturated fatty acid ester composition from a fatty oil composition, the method including:

(a) subjecting the fatty oil composition to a first column chromatography using a first column to obtain an eluent containing the target substance, wherein all or part of at least one component is removed by the first column chromatography, wherein a retention time at a peak top of the at least one component corresponds to a relative value of 1.8 to 3.5, wherein the relative value is based on a retention time at a peak top of a target substance in the first column chromatography which is set to 1.0, wherein the target substance is a polyunsaturated fatty acid ester;

(b) subjecting the eluent containing the target substance obtained by the first column chromatography to a second column chromatography using a second column to obtain an

containing the target substance obtained by the first column chromatography is an eluent from the first column until an end time of elution of the target substance.

[0056a] In an aspect, the present disclosure provides a method for producing a polyunsaturated fatty acid ester composition from a fatty oil composition, the method comprising:

(a) subjecting the fatty oil composition to a first column chromatography using a first column to remove all or part of at least one component, wherein a retention time at a peak top of the at least one component corresponds to a relative value of 1.8 to 3.5, wherein the relative value is based on a retention time at a peak top of a target substance in the first column chromatography which is set to 1.0, wherein the target substance is a polyunsaturated fatty acid ester;

(b) subjecting an eluent containing the target substance obtained by the first column chromatography to a second column chromatography using a second column to remove all or part of at least one component, wherein a retention time at a peak top of the at least one component corresponds to a relative value of 0.90 to 1.1, wherein the relative value is based on a retention time at a peak top of the target substance in the second column chromatography which is set to 1.0; and

(c) concentrating an eluent obtained by the second column chromatography to obtain a composition containing the target substance such that the obtained composition contains the target substance in an amount of 95 wt% or higher.

ADVANTAGEOUS EFFECTS

[0057] In the present disclosure, it has been found that a polyunsaturated fatty acid ethyl ester (e.g., EPA ethyl ester, DGLA ethyl ester, ARA ethyl ester)-rich composition can be efficiently produced in a shorter time and using less solvent than when using a single column. The present disclosure can remove fatty acids such as C20:0 ethyl ester.

DESCRIPTION OF EMBODIMENTS

[0058] In one aspect of the invention, a "composition" may contain one or more PUFAs or ester derivatives thereof. The PUFA- or its ester derivative-containing composition can be

obtained from a raw material containing polyunsaturated fatty acids as constituent fatty acids. For example, a suitable fatty oil composition may be obtained from a raw material composition containing esterified natural oils and fats, including plant and animal oils and fats, and from a raw material composition containing esterified oils obtained from genetically modified plants, animals, or microorganisms including yeast or filamentous fungi. Examples of the filamentous fungi include Mortierella sp. microorganisms, from which a composition containing esterified oils can be obtained. Examples include compositions containing ethyl-esterified fish oils, algae and microalgae oils, or plant oils such as Borago oil, Echium oil, and Oenothera oil. In one embodiment, the raw material composition is a composition containing ethyl-esterified fish oil. In another embodiment, the raw material composition is a composition containing ethyl-esterified algae oil.

[0059] In one aspect of the present invention, the fatty oil composition is obtained by subjecting a raw material composition to distillation.

In one aspect of the present invention, a polyunsaturated fatty acid ester composition is a composition obtained by the method of the present invention, and is a composition containing a polyunsaturated fatty acid ester that is a target substance. The target substance polyunsaturated fatty acid ester may be an ester (e.g., an ethyl ester) of any of eicosapentaenoic acid (EPA), dihomo-γ-linolenic acid (DGLA), or arachidonic acid (ARA).

CLAIMS

1. A method for producing a polyunsaturated fatty acid ester composition from a fatty oil composition, the method comprising:

(a) subjecting the fatty oil composition to a first column chromatography using a first column to remove all or part of at least one component, wherein a retention time at a peak top of the at least one component corresponds to a relative value of 1.8 to 3.5, wherein the relative value is based on a retention time at a peak top of a target substance in the first column chromatography which is set to 1.0, wherein the target substance is a polyunsaturated fatty acid ester;

(b) subjecting an eluent containing the target substance obtained by the first column chromatography to a second column chromatography using a second column to remove all or part of at least one component, wherein a retention time at a peak top of the at least one component corresponds to a relative value of 0.90 to 1.1, wherein the relative value is based on a retention time at a peak top of the target substance in the second column chromatography which is set to 1.0; and

(c) concentrating an eluent obtained by the second column chromatography to obtain a composition containing the target substance such that the obtained composition contains the target substance in an amount of 95 wt% or higher.

2. The method according to claim 1, wherein a loading interval is shorter than in a case of column chromatography using a single column with a length that is a sum of lengths of the first and second columns, wherein the loading interval is a period from starting one run of column chromatography to reaching a state wherein starting a subsequent run of column chromatography becomes possible.

3. The method according to claim 1 or 2, wherein the at least one component having the retention time at the peak top corresponding to a relative value of 1.8 to 3.5 is at least one component other than the polyunsaturated fatty acid ester in the fatty oil composition, wherein the relative value is based on a retention time at a peak top of the target substance in

the first column chromatography which is set to 1.0.

4. The method according to any one of claims 1 to 3, wherein the all or part of the at least one component that is removed in step (b), is at least one component having a retention time at a peak top corresponding to a relative value of 0.93 to 1.09, wherein the relative value is based on a retention time at a peak top of the target substance in the second column chromatography which is set to 1.0.

5. The method according to any one of claims 1 to 4, wherein the first column can be switched between a connected state with the second column so that the eluent from the first column is injected into the second column and a non-connected state with the second column.

6. The method according to any one of claims 1 to 5, wherein the first column chromatography and the second column chromatography are each reversed-phase column chromatography.

7. The method according to any one of claims 1 to 6, wherein the first column chromatography and the second column chromatography use an identical mobile phase.

8. The method according to any one of claims 1 to 7, wherein the second column has a larger amount of packed stationary phase than the first column, or has the same inner diameter as the first column and is longer than the first column.

9. The method according to any one of claims 1 to 8, wherein the target substance is an ethyl ester of any of eicosapentaenoic acid (EPA), dihomo-γ-linolenic acid (DGLA), or arachidonic acid (ARA).

10. The method according to any one of claims 1 to 9, wherein the at least one component, all or part of which is removed by the first column chromatography, is an ester of any of C22:0, C20:0, C18:0, or C20:1.

11. The method according to any one of claims 1 to 10, wherein the polyunsaturated fatty acid ester composition contains the target substance in an amount of 96 wt% or more.

12. The method according to any one of claims 1 to 11, wherein steps (a), (b) and (c) are repeated twice or more.

13. The method according to any one of claims 1 to 12, wherein the target substance is an

eicosapentaenoic acid ester and the at least one component, all or part of which is removed by the second column chromatography, is an ester of stearidonic acid, C19:4, or C21:5.

14. The method according to any one of claims 1 to 12, wherein the target substance is a dihomo-γ-linolenic acid ester and the at least one component, all or part of which is removed by the second column chromatography, is an ester of C20:4n-6.

15. The method according to any one of claims 1 to 12, wherein the target substance is an arachidonic acid ester and the at least one component, all or part of which is removed by the second column chromatography, is an ester of C18:3n-6.