A METHOD OF SEPARATING PROTEIN FRACTIONS

References to Related Application

This application claims priority to Singapore application number 10202251369N filed with the Intellectual Property Office of Singapore on 13 October 2022, the contents of which are hereby incorporated by reference.

Technical Field

The present invention generally relates to a method of separating protein fractions from a protein sample. The present invention further relates to protein fractions obtained from the method.

Background Art

Plant proteins represent a promising solution to the escalating demand for proteins due to their long history of crop use and cultivation, lower cost of production, and better environmental sustainability. Beyond achieving high protein content, it has been recently observed that different plant protein fractions (e.g., albumin, vicilin and legumin) exhibit various techno-functionalities (i.e., solubility, emulsifying, foaming, and gelling), which could be captured for targeted food and nutrition applications.

One conventional method of fractionating proteins uses chromatographic techniques to separate protein fractions. However, this method requires expensive instruments which are generally difficult to operate. In addition, chromatographic instruments are generally not able to produce protein fractions on an industrial scale.

Another conventional method uses buffer systems to separate protein fractions. However, this method involves multiple dialysis steps, which is highly time- and labour-consuming. In addition, it is difficult to obtain protein fractions on an industrial scale based on multiple dialysis steps.

Accordingly, there is a need for a method of separating protein fractions from a protein sample that ameliorates one or more disadvantages mentioned above.

Summary

There is provided a method of separating protein fractions from a protein sample comprising the steps of:

(a) dispersing the protein sample in a deep eutectic solvent and optionally in the presence of an aqueous medium to form a dispersion, wherein the deep eutectic solvent comprises a hydrogen bond acceptor and a hydrogen bond donor; and

(b) treating the dispersion of the dispersing step (a) to form a treated dispersion comprising a first protein fraction and a second protein fraction, wherein:

(i) the first protein fraction is formed when the treating step (b) comprises treating the dispersion of the dispersing step (a) at an elevated temperature to form the first protein fraction and a supernatant, and the second protein fraction is formed when the treating step (b) further comprises treating the supernatant at an elevated pressure or precipitating via pH adjustment; or

(ii) the second protein fraction is formed when the treating step (b) comprises treating the dispersion of the dispersing step (a) at an elevated pressure to form the second protein fraction and a supernatant, and the first protein fraction is formed when the treating step (b) further comprises treating the supernatant at an elevated temperature or precipitating via pH adjustment.

In one aspect, there is provided a method of separating protein fractions from a protein sample comprising the steps of:

(a) dispersing the protein sample in a deep eutectic solvent and optionally in the presence of an aqueous medium to form a dispersion, wherein the deep eutectic solvent comprises a hydrogen bond acceptor and a hydrogen bond donor; and

(b) treating the dispersion of the dispersing step (a) to form a treated dispersion comprising a first protein fraction and a second protein fraction, wherein:

(i) the first protein fraction is formed when the treating step (b) comprises treating the dispersion of the dispersing step (a) at a temperature in the range of 80 ℃ and 100 ℃ to form the first protein fraction and a supernatant, and the second protein fraction is formed when the treating step (b) further comprises treating the supernatant at an elevated pressure or precipitating via pH adjustment; or

(ii) the second protein fraction is formed when the treating step (b) comprises treating the dispersion of the dispersing step (a) at an elevated pressure to form the second protein fraction and a supernatant, and the first protein fraction is formed when the treating step (b) further comprises treating the supernatant at an elevated temperature or precipitating via pH adjustment.

Advantageously, the deep eutectic solvent may separate a denaturation temperature of the first protein fraction, and a denaturation temperature of the second protein fraction. This allows the first protein fraction and the second protein fraction to be selectively denatured and aggregated by thermal or pressure treatments on a large scale. If a third protein fraction or a fourth protein fraction is to be obtained, the temperature selected for the thermal treatment will then be a value between the denaturation temperature of the first protein and that of the third protein fraction or the fourth protein fraction but one that is below the denaturation temperature of the second protein fraction.

In another aspect, there is provided a first protein fraction, a second protein fraction, a third protein fraction or a fourth protein fraction obtained from the method as described herein.

Advantageously, the first protein fraction, the second protein fraction, the third protein fraction and the fourth protein fraction may have an improved solubility when obtained from the present method as they have different compositions and structures compared with protein fractions obtained by conventional methods. The improved solubility allows the first protein fraction, the second protein fraction, the third protein fraction and the fourth protein fraction to be formulated into food products for different pH requirements.

Definitions

The following words and terms used herein shall have the meaning indicated:

The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that

Claims

1. A method of separating protein fractions from a protein sample comprising the steps of:

(a) dispersing the protein sample in a deep eutectic solvent and optionally in the presence of an aqueous medium to form a dispersion, wherein the deep eutectic solvent comprises a hydrogen bond acceptor and a hydrogen bond donor; and

(b) treating the dispersion of the dispersing step (a) to form a treated dispersion comprising a first protein fraction and a second protein fraction, wherein:

(i) the first protein fraction is formed when the treating step (b) comprises treating the dispersion of the dispersing step (a) at a temperature in the range of 80 °C and 100 °C to form the first protein fraction and a supernatant, and the second protein fraction is formed when the treating step (b) further comprises treating the supernatant at an elevated pressure or precipitating via pH adjustment; or

(ii) the second protein fraction is formed when the treating step (b) comprises treating the dispersion of the dispersing step (a) at an elevated pressure to form the second protein fraction and a supernatant, and the first protein fraction is formed when the treating step (b) further comprises treating the supernatant at an elevated temperature or precipitating via pH adjustment.

2. The method of claim 1, wherein the treated dispersion further comprises a third protein fraction that is formed by (c) adding an aqueous medium to the supernatant of the treating step (b)(i), wherein the treating step (b)(i) is treating the dispersion of dispersing step (a) at the temperature in the range of 80 °C and 100 °C only.

3. The method of claim 1, wherein the treated dispersion further comprises a fourth protein fraction that is formed by (c) adding an aqueous medium to the supernatant of the treating step (b)(ii), wherein the treating step (b)(ii) is treating the dispersion of dispersing step (a) at the elevated pressure only.

4. The method of any one of claims 1 to 3, wherein in the dispersing step (a), the hydrogen bond acceptor is selected from the group consisting of choline chloride, betaine, *N*-ethyl-2-hydroxy-*N*,*N*-dimethylethaninium chloride, 2-(chlorocarbonyloxy)-*N*,*N*,*N*-trimethylethanaminium chloride, *N*-benzyl-2-hydroxy-*N*,*N*-dimethylethanaminium chloride and combinations thereof.

5. The method of any one of claims 1 to 4, wherein in the dispersing step (a), the hydrogen bond donor is selected from the group consisting of glycerol, ethylene glycol, 1,3-butanediol, 1,4-butanediol, urea, acetamide, 1-methyl urea, 1,3-dimethyl urea, 1,1-dimethyl urea, thiourea, benzamide, malonic acid, benzoic acid, adipic acid, oxalic acid, succinic acid, citric acid and combinations thereof.

6. The method of any one of claims 1 to 5, wherein in the dispersing step (a), the deep eutectic solvent is selected from:

(i) choline chloride and glycerol at a molar ratio of 1:2;

(ii) choline chloride and ethylene glycol at a molar ratio of 1:2; or

(iii) choline chloride and 1,4-butanediol at a molar ratio of 1:3.

7. The method of any one of claims 1 to 6, wherein in the dispersing step (a), the dispersion comprises water at a weight percentage in the range of 50 weight% to 90 weight% based on the total weight of the deep eutectic solvent and the aqueous medium.

8. The method of any one of claims 1 to 7, wherein in the dispersing step (a), the dispersion comprises solids at a weight percentage of about 17 weight% based on the total weight of the dispersion.

9. The method of any one of claims 1 to 8, further comprising a step of heating the hydrogen bond acceptor and the hydrogen bond donor to form the deep eutectic solvent before the dispersing step (a).

10. The method of any one of claims 1 to 9, where elevated temperature is used in the treating step (b)(ii), the elevated temperature is a temperature in the range of 80 °C and 100 °C.

11. The method of any one of claims 1 to 10, where the temperature in the range of 80 °C and 100 °C is used in the treating step (b)(i) or the elevated temperature is used in the treating step (b)(ii), the temperature in the range of 80 °C and 100 °C of the treating step (b)(i) or the elevated temperature of the treating step (b)(ii) is conducted for a duration of about 15 minutes.

12. The method of any one of claims 1 to 11, where elevated pressure is used in the treating step (b), the elevated pressure is a pressure in the range of 400 MPa and 800 MPa.

13. The method of any one of claims 1 to 12, where elevated pressure is used in the treating step (b), the elevated pressure is conducted for a duration of about 5 minutes.

14. The method of any one of claims 2 to 13, further comprising a step of isolating the first protein fraction, the second protein fraction, the third protein fraction and/or the fourth protein fraction.

15. The method of any one of claims 1 and 4 to 14, wherein the precipitating via pH adjustment comprises adjusting a pH value of the supernatant of the treating step (b) to a value in the range of 4 and 5.

16. The method of any one of claims 2 to 14, wherein the aqueous medium added in the adding step (c) has a weight ratio to the supernatant of the treating step (b) in the range of 2:1 to 6:1.

17. The method of any one of claims 2 to 16, further comprising a step of purifying the first protein fraction and/or the second protein fraction after the treating step (b) or the third protein fraction or the fourth protein fraction after the adding step (c).

18. The method of any one of claims 2 to 17, further comprising a step of drying the first protein fraction and/or the second protein fraction after the treating step (b) or the third protein fraction or the fourth protein fraction after the adding step (c).

19. The method of any one of claims 1 to 18, wherein the protein sample is selected from the group consisting of pea, lentil, industrial hemp, chickpea, basil seed, pumpkin

seed, almond, soy, quinoa, nuts, textured vegetable, tempeh, rice, spirulina, peanut, legume, tofu, beans, seitan, nutritional yeast and combinations thereof.

20. A first protein fraction or a second protein fraction obtained from the method of any one of claims 1 to 19 or a third protein fraction obtained from the method of any one of claims 2 and 4 to 19 or a fourth protein fraction obtained from the method of any one of claims 3 to 19.