Description

Title of Invention: ~~PROTEIN-CONTAINING COMPOSITION AND METHOD FOR PRODUCING SAME~~AGENT FOR IMPROVING BOWEL MOVEMENT AND METHOD OF PRODUCING THE SAME

Technical Field

[0001] The present invention relates to a protein-containing composition that contains a protein which was contained in cacao beans at a high content, and a method of producing the protein-containing composition. More specifically, the present invention relates to an agent for improving bowel movement, and a method of producing the agent.

Background Art

[0002] Chocolate and cocoa have been studied not only as a food material for enjoying its taste but also as a food material having an effective function on an organism. Further, cacao mass obtained by removing husks from cacao beans and grinding the resultant product also contains functional components such as an antioxidant and dietary fiber, and hence has been studied not only as a raw material for chocolate or cocoa but also as a material for a food with nutrient function claims (see Patent Literatures 1 and 2 listed below).

In particular, a cacao extract with an increased concentration of an antioxidant or the like has been expected to have various

effects (see Patent Literatures 3 to 6 listed below).

[0003] Cacao beans contain not only an antioxidant and dietary fiber but also a protein. However, the protein content of cacao beans is lower than that of soybeans or the like. Therefore, the use of the protein in cacao beans has not attracted attention. For example, Patent Literatures 3 to 6 describe that an extract containing polyphenols at a concentration of 25 wt% or more is obtained from unroasted cacao beans which are unfermented or less fermented, but do not describe an attempt to increase the protein content of the extract.

Citation List

Patent Literature

[0004] [Patent Literature 1] JP 07-213251 A

[Patent Literature 2] JP 06-321796 A

[Patent Literature 3] JP 2010-524993 W

[Patent Literature 4] JP 2010-524995 W

[Patent Literature 5] JP 2010-526036 W

[Patent Literature 6] JP 2010-540670 W

Summary of the Invention

Problems to be Solved by the Invention

[0005] The protein content of cacao beans is lower than that of soybeans or the like. Therefore, in order to increase the protein content of a food or the like produced by using an extract or the

like from cacao beans as a material containing a protein of cacao beans, it is necessary to use a large amount of the extract or the like from the cacao beans. However, in this case, a bitter taste and an astringent taste (i.e. sustained harshness) derived from cacao beans may change taste and flavor of the food or the like. For this reason, the use of the protein-containing material obtained by using cacao beans for various foods and drinks, pharmaceuticals, chemical products, and the like has been limited. In addition, cacao beans contain physiologically active substances such as caffeine, theobromine, and the like. Therefore, when an extract itself from cacao beans is used, the above-mentioned physiologically active substances are also added, possibly resulting in unexpected physiological effects. Accordingly, it has been impossible to add an extract itself from cacao beans in a large amount to foods and drinks, pharmaceuticals, chemical products, and the like.

[0006] In addition, cacao beans or cacao mass contains cacao butter at a high content, and a fatty ingredient of cacao beans transfers to an extract or the like from cacao beans or cacao mass. Therefore, a phenomenon in which the extract or the like turns into an oily form by a change in temperature has been observed. Accordingly, the handling of the extract or the like as a protein-containing material has been cumbersome. Further, there has been a problem in that the addition of the extract or the like to foods or the like as a material containing a protein of cacao beans means the addition of a fatty ingredient of cacao beans to

foods or the like together with the protein.

Further, the effective use of a residue obtained after extraction of functional components such as an antioxidant contained in cacao beans has been desired.

[0007] An object of the present invention is to provide an agent utilizing a protein-containing composition that has an increased content of protein obtained from cacao beans, is substantially free of a fatty ingredient, caffeine, theobromine, and polyphenols, and has no bitter taste and astringent taste (i.e. sustained harshness), and a method of producing the protein-containing composition.

Means for Solving the Problem

[0008] The inventors of the present invention have studied to solve the above-mentioned problems. As a result, the inventors have found that the problems can be solved by a protein-containing composition having an increased content of a protein derived from cacao beans, which can be utilized as an agent for improving bowel movement. The inventors have also found that roasted cacao beans and a residue obtained after extraction of functional components from cacao beans also contain the protein derived from cacao beans and hence can be used as a material of an agent for improving bowel movement.

[0009] That is, the present invention provides an agent for improving bowel movement, which contains a protein-containing composition (hereinafter sometimes abbreviated as the

protein-containing composition of the present invention) which includes a protein obtained from cacao beans at a weight ratio of 42 to 70% based on the total solids of the composition. The protein-containing composition of the present invention is useful as a material for various foods and drinks, pharmaceuticals, chemical products, and the like, because the composition has an increased content of protein which was contained in cacao beans, is substantially free of a fatty ingredient derived from cacao beans, and has almost no bitter taste and astringent taste.

[0010] The present invention also provides a method of producing an agent for improving bowel movement (hereinafter sometimes referred to as the production method of the protein-containing composition of the present invention) which includes a protein-containing composition containing a protein from cacao beans at a high content, including: (a) an extraction step of extracting a protein from a raw material containing the protein from cacao beans under an alkaline condition to obtain an extract containing the protein; and (b) a precipitation step of precipitating the protein from the extract obtained in the step (a) under an acidic condition to obtain the protein-containing composition.

[0011] The method of producing a protein-containing composition according to the present invention preferably further includes (c) a defatting step of removing a fatty ingredient which was contained in cacao beans before the step (a) and/or after the step (b). In this case, it is possible to provide a

protein-containing composition substantially free of a fatty ingredient from cacao beans.

[0012] Preferred conditions for the extraction of the protein in the step (a) are as follows. The pH is preferably 8 to 13, and more preferably 10 to 12. The temperature is preferably 20 to 100°C, more preferably 30 to 100°C, even more preferably 40 to 100°C, and particularly preferably 50 to 100°C. When the protein is extracted under these conditions, the protein content of the protein-containing composition can be raised.

[0013] Preferred conditions for the precipitation of the protein in the step (b) are as follows. The pH is preferably 2 to 6, and more preferably 2 to 5. The temperature is preferably 0 to 40°C, and more preferably 0 to 20°C. When the protein is precipitated under these conditions, the protein content of the protein-containing composition can be raised.

[0014] In the case where the removal of the fatty ingredient in the step (c) is carried out by using water, the pH is preferably 2 to 7, more preferably 3 to 7, and even more preferably 4 to 6, and the temperature is preferably 30 to 90°C, and more preferably 40 to 70°C.

In the case where the removal of the fatty ingredient in the step (c) is carried out by using an organic solvent, preferred examples of the organic solvent include ethanol, an aqueous solution of ethanol, diethyl ether, and hexane. One kind of those organic solvents may be used alone, or two or more kinds thereof may be used in combination.

[0015] The lower limit of the weight ratio of the protein from cacao beans in the protein-containing composition of the present invention is 42%, preferably 44%, more preferably 46%, more preferably 48%, more preferably 50%, more preferably 52%, even more preferably 54%, and particularly preferably 56% in terms of weight ratio in the total solid content of the composition. When the weight ratio of the protein is large, even the addition of a small amount of the composition of the present invention to a food or the like can increase the protein content of the food or the like.

The upper limit of the weight ratio of the protein from cacao beans in the protein-containing composition of the present invention is 70%, more preferably 65%, and most preferably 60% in terms of weight ratio in the total solid content of the composition in consideration of the easiness of setting of various conditions in the production method of the present invention.

Advantageous Effects of the Invention

[0016] The protein-containing composition of the present invention (hereinafter sometimes abbreviated as the composition of the present invention) is used as an agent for improving bowel movement ~~has a high content of protein obtained from cacao beans~~.

In addition ~~Therefore~~, even the addition of a small amount of the composition of the present invention to a food or the like can increase the protein content of the food or the like, because the composition of the present invention has a high content of protein

obtained from cacao beans.

In addition, the composition of the present invention has a high content of protein obtained from cacao beans, has almost no bitter taste and astringent taste (i.e. sustained harshness) derived from cacao beans, and has no taste and smell. Further, the composition of the present invention is substantially free of a fatty ingredient from cacao beans. Therefore, the composition of the present invention does not turn into an oily form by a change in temperature and is very easy to handle. Consequently, the protein-containing composition of the present invention can be used advantageously as a protein-containing material that does not change the taste and flavor, the amount of the fatty ingredient, and the like, for constituting various foods and drinks, pharmaceuticals, chemical products, and the like.

[0017] In addition, according to the method of producing a protein-containing composition of the present invention (hereinafter sometimes abbreviated as the production method of the present invention), it is possible to provide a protein-containing composition that has an increased content of protein obtained from cacao beans in the protein-containing composition, is substantially free of a fatty ingredient, caffeine, theobromine, and polyphenols from cacao beans, and has almost no bitter taste and astringent taste. This enables a wide application of the composition of the present invention, whereas a conventional composition containing a protein obtained from cacao beans has not been used as a food

material because the conventional composition has the fatty ingredient, bitter taste, and astringent taste derived from cacao beans, or has not been added in a large amount because the conventional composition has a high content of physiologically active substances such as caffeine and theobromine. Further, roasted cacao beans and a residue after the extraction of a functional component such as an antioxidant contained in cacao beans may be used effectively to obtain the composition of the present invention.

Description of Embodiments

[0018] Hereinafter, embodiments of the present invention are described in detail.

[0019] <Step (a)>

A raw material used in the step (a) of the production method of the present invention is not particularly limited as long as the raw material contains a protein from cacao beans. Examples of the raw material include cacao beans (roasted or unroasted), cacao nibs obtained by removing husks of cacao beans (roasted or unroasted), cacao mass obtained by grinding the cacao nibs, cacao powder (alkalized or unalkalized) obtained by removing cacao butter from the cacao mass through compression, defatted cacao mass obtained by removing cacao butter with defatting means such as warm water, an organic solvent, or the like, and residues obtained after extraction of an antioxidant or the like from the above-mentioned raw materials shown as examples (for examples, a residue or the

like obtained after extraction by a method described in JP 07-213251 A). One kind of those raw materials may be used alone, or two or more kinds thereof may be used in combination.

[0020] In the step (a), in order to extract the protein from a raw material containing a protein from cacao beans under an alkaline condition, an alkali (which is an agent for alkalizing a solution and is also referred to as alkalizing agent), which is one of examples of extraction means, may be added to a suspension obtained by suspending the raw material in water. The condition of the extraction of the protein is not particularly limited as long as the solution is alkaline, but from the viewpoint of increasing the protein content of the composition of the present invention, the condition is preferably pH 8 to 13, more preferably pH 9 to 13, even more preferably pH 10 to 12, and particularly preferably pH 10.5 to 12.

The alkali used herein is not particularly limited as long as the alkali can adjust the pH of the solution to pH 8 to 13, and may be used in a form of a solid or an aqueous solution. Examples of the alkali include sodium hydroxide, potassium carbonate, trisodium phosphate, and the like. One kind of those alkalis may be used alone, or two or more kinds thereof may be used in combination.

[0021] The lower limit of the temperature for the extraction of the protein is not particularly limited, but from the viewpoint of increasing the protein content of the composition of the present invention, the temperature is preferably 20°C, more preferably 30°C, more preferably 40°C, more preferably 50°C, even more preferably

70°C, and particularly preferably 80°C. The upper limit of the temperature for the extraction of the protein is not particularly limited, but from the viewpoint of reaching a plateau in an increase of the protein content of the composition of the present invention when the temperature exceeds 80°C, the upper limit is preferably 100°C, more preferably 95°C, and particularly preferably 90°C.

The time for the extraction of the protein is preferably 30 minutes to 48 hours, and more preferably 30 minutes to 24 hours. When the time for the extraction is 30 minutes or more, the protein content of the composition of the present invention can be raised sufficiently. When the time for the extraction is 48 hours or less, the extraction can be carried out efficiently.

[0022] After the extraction of the protein, solids-liquid separation by filtration or the like may be carried out to obtain a supernatant containing the protein from cacao beans.

It is to be noted that, in a conventional technology for the extraction of a protein under a usual alkaline condition, the extraction is carried out under a mildly alkaline condition at a temperature of less than 50°C in order to avoid alkaline denaturation and thermal denaturation of the protein in the process of the extraction. However, in a preferred embodiment of the production method of the present invention, the extraction of the protein is carried out under a strong alkaline condition of pH 8 or more at a temperature as high as 50°C or more.

[0023] <Step (b)>

In the step (b), in order to obtain the protein-containing composition under an acidic condition, an acid may be added to the supernatant containing the protein from cacao beans obtained in the step (a) to precipitate the protein. The condition of the precipitation of the protein is not particularly limited as long as the solution is acidic, but from the viewpoint of increasing the protein content of the composition of the present invention, the condition is preferably pH 2 to 6, more preferably pH 2 to 5, even more preferably pH 2 to 4, and particularly preferably pH 2 to 3.5.

The acid used herein is not particularly limited as long as the acid can adjust the pH of a solution to pH 2 to 6, and may be used in a form of a solid or an aqueous solution. Examples of the acid include: inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid, and the like; and organic acids such as citric acid, malic acid, tartaric acid, and the like. One kind of those acids may be used alone, or two or more kinds thereof may be used in combination.

From the viewpoint of promoting the precipitation, the temperature for the precipitation of the protein is preferably 0 to 40°C, more preferably 0 to 20°C, and particularly preferably 0 to 10°C.

After the precipitation of the protein, operations such as solid-liquid separation by filtration or the like, washing with water, drying, and the like may be carried out to obtain the

protein-containing composition of the present invention.

[0024] <Step (c)>

In the case where the raw material containing the protein from cacao beans used in the step (a) contains a fatty ingredient (for example, cacao mass contains about 55% of fatty ingredients, and cacao powder contains about 20% of fatty ingredients), the production method of the present invention preferably further includes a step (c) (i.e. a defatting step) at the time of one selected from the following (A) to (C): (A) only before the step (a); (B) only after the step (b); and (C) both before the step (a) and after the step (b). The above-mentioned (A) to (C) are sometimes collectively referred to as "before the step (a) and/or after the step (b)"). The step (c) can provide a protein-containing composition substantially free of a fatty ingredient from cacao beans.

In the case where the step (c) is carried out before the step (a), the fatty ingredient is removed from the raw material containing the protein from cacao beans. In the case where the step (c) is carried out after the step (b), the fatty ingredient is removed from the protein-containing composition obtained in the step (b).

In both of the case where the step (c) is carried out before the step (a) and the case where the step (c) is carried out after the step (b), the same method of removing the fatty ingredient is employed except that the objects from which fatty ingredients are removed are different from each other. Therefore, the details of the step (c) is described below with taking the case where the step

(c) is carried out before the step (a).

[0025] The fatty ingredient may be removed from the raw material containing the protein from cacao beans by, for example, suspending the raw material in a solvent for removing the fatty ingredient such as water, an organic solvent or the like, extracting the fatty ingredient, and removing a supernatant containing the fatty ingredient by solid-liquid separation by filtration or the like to obtain a solid in which the fatty ingredient decreases.

The condition of the defatting is not particularly limited, but for example, in the case of using water, the condition is preferably pH 2 to 7, and more preferably pH 3 to 6. The acid used herein is not particularly limited as long as the acid can adjust the pH of the solution to pH 2 to 7, and may be used in a form of a solid or an aqueous solution. Examples of the acid include: inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid and the like; and organic acids such as citric acid, malic acid, tartaric acid and the like. One kind of those acids may be used alone, or two or more kinds thereof may be used in combination.

[0026] In the case of using an organic solvent for removing the fatty ingredient, examples of the organic solvent include ethanol, an aqueous solution of ethanol, diethyl ether, hexane and the like. One kind of those organic solvents may be used alone, or two or more kinds thereof may be used in combination. In the case where the composition of the present invention is a food material or the like, among those organic solvents, ethanol and an aqueous solution

of ethanol are preferred from the viewpoint of safety.

Defatting with water and defatting with an organic solvent may be carried out in combination. In addition, defatting with water and/or defatting with an organic solvent may be repeated.

The temperature for the defatting is preferably 30 to 90°C in the case of using water, or 20 to 90°C in the case of using an organic solvent. In both of the case of using water and the case of using an organic solvent, the temperature for the defatting is more preferably 40 to 70°C. The time for the defatting is preferably 10 minutes to 24 hours, and more preferably 30 minutes to 2 hours.

After the defatting, for example, solid-liquid separation by filtration or the like may be carried out to obtain a raw material in which the fatty ingredient decreases.

[0027] A usual cacao extract obtained by a conventional technology contains caffeine, theobromine, polyphenols, and the like from cacao beans. However, the protein-containing composition of the present invention is substantially reduced in caffeine, theobromine, and polyphenols.

Examples

[0028] Hereinafter, the present invention is described by way of Examples. The present invention is not limited to the following Examples. It is to be noted that, in Examples and Comparative Examples shown below, "%" means "w/v%" (weight in 100 ml (unit: g)), "wt%" means "w/w%", and "vol%" means "v/v%."

[0029] Protein contents described in Examples and Comparative Examples shown below were determined by an official method, i.e., improved macro Kjeldahl method (nitrogen-protein conversion factor: 6.25). Lipid contents were determined by an official method, i.e., acid digestion method. Details of these methods have been described in "Official test method and standard test method discussion, Food hygiene inspection guideline, Physics and chemistry reviews 2005" (edited by Ministry of Health, Labour and Welfare, published by Japan Food Hygiene Association).

[0030] 1. Study on step (a)

(1) pH

Water was added to cacao powder to prepare a suspension, and the suspension was adjusted to pH 4 with hydrochloric acid, was stirred at 50°C for 30 minutes, and was centrifuged to remove a supernatant containing a fatty ingredient. Water was added again to the resultant precipitate to prepare a suspension, and the above-mentioned defatting was carried out again.

Subsequently, water was added again to the resultant precipitate to prepare a suspension, and the pH of the suspension was adjusted with an aqueous solution of sodium hydroxide to: 1) pH 8 (Comparative Example 1); 2) pH 9 (Comparative Example 2); 3) pH 10 (Example 1); or 4) pH 10.5 (Example 2). Then, the suspension was stirred at 50°C for 1 hour, and after that, was centrifuged to obtain a supernatant containing a protein.

The resultant supernatant was filtered, and then the filtrate

was cooled on ice to adjust the liquid temperature to 4°C. After that, the pH of the filtrate was adjusted to pH 3.5 with hydrochloric acid. The supernatant was removed by centrifugation, and the resultant precipitate was washed with water and freeze-dried, thereby obtaining a protein-containing composition containing a protein from cacao beans.

[0031] (2) pH and temperature

In the same manner as in Example 2, defatting was carried out under an acidic condition, and water was added again to the resultant precipitate to prepare a suspension.

Subsequently, the suspension was adjusted with an aqueous solution of sodium hydroxide to: 1) pH 11 and then stirred at 80°C for 1 hour (Example 3); 2) pH 11 and then stirred at 100°C for 1 hour (Example 4); 3) pH 12 and then stirred at 80°C for 1 hour (Example 5); 4) pH 12 and then stirred at 100°C for 1 hour (Example 6); 5) pH 13 and then stirred at 80°C for 1 hour (Comparative Example 3); or 6) pH 13 and then stirred at 100°C for 1 hour (Comparative Example 4), followed by centrifugation, thereby obtaining a supernatant containing a protein.

Further, in the same manner as in Example 1, the protein was precipitated under an acidic condition, thereby obtaining a protein-containing composition containing a protein from cacao beans.

Table 1 shows the protein contents of the compositions obtained above.

[0032] [Table 1]

	Protein content		Protein content		Protein content
Example 1	44	Example 4	57	Comparative Example 1	17
Example 2	56	Example 5	58	Comparative Example 2	26
Example 3	58	Example 6	59	Comparative Example 3	38
				Comparative Example 4	37

Each of the values in Table 1 represents the protein content (g) in 100 g of the composition.

[0033] (3) Recovery rate of solid content

In the same manner as in Example 2, defatting was carried out under an acidic condition, and water was added again to the resultant precipitate to prepare a suspension.

Subsequently, the suspension was adjusted with an aqueous solution of sodium hydroxide to: 1) pH 11 and then stirred at 50°C for 1 hour (Example 7); 2) pH 11 and then stirred at 60°C for 1 hour (Example 8); 3) pH 11 and then stirred at 70°C for 1 hour (Example 9); 4) pH 11.5 and then stirred at 50°C for 1 hour (Example 10); 5) pH 11.5 and then stirred at 60°C for 1 hour (Example 11); 6) pH 11.5 and then stirred at 70°C for 1 hour (Example 12); 7) pH 12 and then stirred at 50°C for 1 hour (Example 13); 8) pH 12 and then stirred at 60°C for 1 hour (Example 14); or 9) pH 12 and then stirred at 70°C for 1 hour (Example 15), followed by centrifugation, thereby obtaining a supernatant containing a protein.

Further, in the same manner as in Example 1, the protein was precipitated under an acidic condition, thereby obtaining a

protein-containing composition containing a protein from cacao beans.

Table 2 shows protein contents, recovery rates of solid contents, and molecular weight distributions of the compositions obtained above.

[0034] The recovery rates of solid contents as shown in Table 2 were calculated by the following method.

Recovery rate of solid content (wt%) = [Weight of freeze-dried protein-containing composition (g) / Weight of raw material (g)]×100

The molecular weight distribution was determined by the following method.

First, each of the compositions obtained in Examples was accurately weighed as a sample in an amount of 0.25 g, 30 mL of water were added thereto, and the mixture was stirred well. After that, the pH of the mixture was adjusted to pH 10.5 with a 1 N aqueous solution of sodium hydroxide, and then the mixture was stirred at 50°C for 30 minutes. Further, the pH of the mixture was adjusted to pH 7.0 with 1 N hydrochloric acid, and then water was added thereto so that the final volume was 50 ml, thereby preparing a solution containing 0.5% of a sample.

An aqueous solution containing 0.1 vol% of trifluoroacetic acid, and an acetonitrile solution containing 0.1 vol% of trifluoroacetic acid were added to the above-mentioned solution

containing 0.5% of the sample to obtain a mixture including 0.1% of the sample and 45 vol% of acetonitrile, followed by centrifugation (10,000×g), thereby obtaining a supernatant. The resultant supernatant was filtered through a filter with a pore size of 0.45 μm, thereby preparing a sample solution.

The sample solution was analyzed by gel permeation chromatography (GPC). The solution was divided into a high-molecular-weight fraction (6.5 kDa or more) and a low-molecular-weight fraction (307 Da or more and less than 6.5 kDa) based on a commercially available molecular weight marker, and a molecular weight distribution was calculated as shown in Table 2. Each of the values in Table 2 represents the ratio (%) of area of the fraction of the test solution in the total area.

<Measurement conditions>

·Column for analysis: Tosoh, G4000PWXL, 7.8 mm×300 mm (or equivalent thereof)

·Column temperature: 40°C, detection: UV-Vis 190 to 600 nm

·Mobile phase: water:acetonitrile (volume ratio)=55:45 (containing 0.1 vol% of trifluoroacetic acid)

·Flow rate of mobile phase: 0.5 mL/min, injection amount: 10 μL of solution containing 0.1% of the sample

<Molecular weight markers (each having a final concentration of 0.1%)>

Aprotinin (molecular weight: 6,500)

Glutathione (molecular weight: 307)

[0035] [Table 2]

	Protein content (g/100 g of composition)	Recovery rate of solid content (w/w%)	Molecular weight distribution	
			High-molecular-weight fraction area ratio (%)	Low-molecular-weight fraction area ratio (%)
Example 2	56	11	67	23
Example 7	54	9	63	37
Example 8	57	18	70	30
Example 9	58	22	74	26
Example 3	58	22	74	26
Example 10	56	13	71	29
Example 11	60	22	72	28
Example 12	60	25	72	28
Example 13	53	17	57	43
Example 14	59	26	74	26
Example 15	59	29	72	28
Example 5	58	28	69	31

Low-molecular-weight fraction: 307 Da or more and less than 6.5 kDa; High-molecular-weight fraction: 6.5 kDa or more

[0036] (4) Extraction time

In the same manner as in Example 2, defatting was carried out under an acidic condition, and water was added again to the resultant precipitate to prepare a suspension.

Subsequently, the pH of the suspension was adjusted with an aqueous solution of sodium hydroxide to pH 12, and then the suspension was stirred at 70°C for: 1) 4 hours (Example 16); or 2) 8 hours (Example 17), followed by centrifugation, thereby obtaining a

supernatant containing a protein.

Further, in the same manner as in Example 1, the protein was precipitated under an acidic condition, thereby obtaining a protein-containing composition containing a protein from cacao beans.

Table 3 shows the protein contents, the recovery rates of solid content, and the molecular weight distributions of the compositions obtained above.

[0037] [Table 3]

	Protein content (g/100 g of composition)	Recovery rate of solid content (w/w%)	Molecular weight distribution	
			High-molecular-weight fraction area ratio (%)	Low-molecular-weight fraction area ratio (%)
Example 15	59	29	72	28
Example 16	53	27	72	28
Example 17	50	27	68	33

Low-molecular-weight fraction: 307 Da or more and less than 6.5 kDa; High-molecular-weight fraction: 6.5 kDa or more

[0038] 2. Study on step (b)

(1) pH

In the same manner as in Example 2, defatting under an acidic

condition and protein extraction under an alkaline condition were carried out.

Subsequently, the resultant supernatant was filtered, and the filtrate was cooled on ice to adjust the liquid temperature to 4°C. After that, the pH of the filtrate was adjusted with hydrochloric acid to: 1) pH 4 (Example 18); or 2) pH 5 (Example 19), or was adjusted with sulfuric acid to: 3) pH 2 (Example 20).

Then, the supernatant was removed by centrifugation, and the resultant precipitate was washed with water and freeze-dried, thereby obtaining a protein-containing composition containing a protein from cacao beans.

[0039] (2) Kind of acid

In the same manner as in Example 2, defatting under an acidic condition and protein extraction under an alkaline condition were carried out.

Subsequently, the resultant supernatant was filtered, and the filtrate was cooled on ice to adjust the liquid temperature to 4°C. After that, the pH of the filtrate was adjusted to pH 3.5 with: 3) sulfuric acid (Example 21); 4) phosphoric acid (Example 22); 5) citric acid (Example 23); 6) malic acid (Example 24); or 7) tartaric acid (Example 25). Then, the supernatant was removed by centrifugation, and the resultant precipitate was washed with water and freeze-dried, thereby obtaining a protein-containing composition containing a protein from cacao beans.

Table 4 shows the protein contents of the compositions obtained

above.

[0040] [Table 4]

	Protein content		Protein content		Protein content
Example 2	56	Example 20	56	Example 23	55
Example 18	54	Example 21	53	Example 24	57
Example 19	42	Example 22	54	Example 25	59

Each of the values in Table 4 represents the protein content (g) in 100 g of the composition.

[0041] 3. Study on step (c)

(1) Defatting condition

First, in the case of no defatting (Example 26), water (liquid temperature: 50°C) was added to cacao powder to prepare a suspension (pH: 6), and the suspension was used without additional treatments for the subsequent protein extraction. In the case of defatting with an organic solvent (Example 27), an aqueous solution containing 95 vol% of ethanol was added to cacao powder to prepare a suspension, and the suspension was stirred at 50°C for 1 hour and was filtered to remove the supernatant containing a fatty ingredient. The aqueous solution containing 95 vol% of ethanol was added again to the resultant precipitate to prepare a suspension, and the above-mentioned defatting was carried out further twice.

Subsequently, in the same way as in Example 2, protein extraction under an alkaline condition and protein precipitation under an acidic condition were carried out. Then, the supernatant

was removed by centrifugation, and the resultant precipitate was washed with water and freeze-dried, thereby obtaining a protein-containing composition containing a protein from cacao beans.

[0042] (2) Defatting order

In the same way as in Example 26, protein extraction under an alkaline condition and protein precipitation under an acidic condition were carried out without defatting, and the supernatant was removed by centrifugation.

Subsequently, in Example 28, the aqueous solution containing 95 vol% of ethanol was added to the resultant precipitate to prepare a suspension, and defatting was carried out in the same way as in Example 27. On the other hand, in Example 29, the resultant precipitate was freeze-dried, and the aqueous solution containing 95 vol% of ethanol was added to prepare a suspension, followed by defatting in the same way as in Example 27.

Then, the resultant precipitate was dried, thereby obtaining a protein-containing composition containing a protein from cacao beans.

Table 5 shows protein contents and lipid contents of the compositions obtained above.

[0043] [Table 5]

	Protein content	Lipid content

Example 2	56	1.5
Example 26	50	7.7
Example 27	46	0.7
Example 28	54	1.1
Example 29	50	3.8

Each of values in Table 5 represents the protein content (g) or the lipid content (g) in 100 g of the composition.

[0044] 4. Study on raw material

A residue obtained by extracting functional components from cacao powder was subjected to defatting under an acidic condition, protein extraction under an alkaline condition, and protein precipitation under an acidic condition in the same way as in Example 2, thereby obtaining a protein-containing composition containing a protein from cacao beans (Example 30).

A residue obtained by extracting functional components from cacao mass prepared by using unfermented cacao beans was subjected to defatting under an acidic condition, protein extraction under an alkaline condition, and protein precipitation under an acidic condition in the same way as in Example 2, thereby obtaining a protein-containing composition containing a protein from cacao beans (Example 31).

Table 6 shows the protein contents of the compositions obtained above.

[0045] [Table 6]

	Protein content
Example 2	56

Example 30	49
Example 31	56

Each of the values in Table 6 represents the protein content (g) in 100 g of the composition.

[0046] 5. Caffeine content and theobromine content

The content of caffeine and the content of theobromine were determined by a quantitative analysis for solid contents of the compositions obtained in Examples using commercially available caffeine and theobromine as standards with reference to a method described in AOAC Official Methods of Analysis (1990) 980.14 "Theobromine and Caffeine in Cacao Products Liquid Chromatographic Method."

First, each of the compositions obtained in Examples 2, 26, 27, and 31 was accurately weighed in a centrifuge tube as samples, and 30 mL of petroleum ether were added thereto to obtain a mixture. The mixture was stirred well and centrifuged, and the supernatant was discarded. The resultant precipitate (i.e. a defatted sample) was transferred to a conical flask, and water was added thereto so that the total volume was about 100 mL. After that, the mixture was heated in a water bath having a temperature of 100°C for 25 minutes, and then was cooled immediately. Then, 10 mL of an aqueous solution containing 2 wt% of zinc sulfate and 10 mL of an aqueous solution containing 1.8 wt% of barium hydroxide were added thereto, and the resultant was stirred and then allowed to stand still. After that, the resultant was adjusted to have a volume of 200 mL with

water to obtain a mixture. The mixture was heated again in the water bath having a temperature of 100°C for 10 minutes, and the mixture after heating was filtered, thereby obtaining a sample solution.

The sample solution was analyzed by high-performance liquid chromatography. Also, commercially available caffeine and theobromine were used to perform the same treatment and analysis as above, and a calibration curve was created to calculate the caffeine content and the theobromine content of each test solution. Table 7 shows the results.

<Measurement conditions>

·Column for analysis: Waters, µ-Bondapak C18, 10 µm, 4 mmID×300 mm (or equivalent thereof)

·Mobile phase: water:acetonitrile (volume ratio)=85:15

·Flow rate of mobile phase: 1.0 mL/min, detection: UV 273 nm

[0047] [Table 7]

	Caffeine content (mg/g)	Theobromine content (mg/g)
Example 2	0.0	0.3
Example 26	0.5	2.4
Example 27	0.0	0.0
Example 31	0.0	1.3
Cacao powder	3.7	23.6

Each of the values in Table 7 represents the caffeine content (mg) or the theobromine content (mg) in 1 g of the composition.

[0048] 6. Total polyphenol content

A total polyphenol content was determined by a Prussian blue

method. The solid contents of the compositions obtained in Examples 2 and 26 were quantitatively analyzed using commercially available epicatechin as a standard with reference to a method described in Martin L. Price and Larry G. Butler, J. Agric Food Chem., Vol. 25 No.6, 1268-1273 (1977).

First, the resultant compositions were defatted by a treatment with n-hexane, and were subjected to extraction with an aqueous solution containing 50 wt% of methanol, thereby preparing test solutions. Subsequently, 100 μL of each of the test solutions was added to 50 mL of distilled water to obtain a diluted solution. After that, 3 mL of a solution of 0.1 M ammonium iron(III) sulfate-0.1 N hydrochloric acid was added to the diluted solution while stirring. After 20 minutes from the addition of the solution, 3 mL of an aqueous solution of 8 mM potassium hexacyanoferrate(III) was added thereto. Furthermore, after 20 minutes from the addition of the aqueous solution, an absorbance at 720 nm was measured.

Also, a solution obtained by adding 100 μL of a solvent, which is the same as the solvent used for preparing the above-mentioned test solutions and is an aqueous solution containing 50 wt% of methanol, to 50 mL of distilled water, and a solution of commercially available (-)-epicatechin in methanol were treated in the same way as above to create calibration curves, and a total polyphenol content of each of the test solutions was calculated. Table 8 shows the results.

[0049] [Table 8]

	Total polyphenol content (w/w%)
Example 2	1.4
Example 26	2.7
Cacao powder	7.4

Each of the values in Table 8 represents the total polyphenol content (wt%) of the composition.

[0050] <Test Example>

1. Evaluation in mice (confirmation of the effect of improving bowel movement)

Six-week-old BALB/c female mice (six mice for each group) were fed for 14 days by being allowed to ingest water and feed freely. The mice in the control group were allowed to ingest standard feed (in accordance with AIN93G, containing no cellulose, supplemented with 5 wt% corn starch). The mice in the protein-containing composition addition group were allowed to ingest feed obtained by adding the protein-containing composition obtained in Example 2 (alternative to corn starch) in an amount of 5 wt% to the standard feed.

Then, the body weights (g) of the mice were measured at the start of breeding ("Day 0" in Table 9), and after 7 days ("Day 7" in Table 9) and 14 days ("Day 14" in Table 9) from the start of breeding. Also, the amount of the feed (g/mouse/day) from the 6th day to the 7th day ("Day 7" in Table 9), and the amount of the feed (g/mouse/day) from the 13th day to the 14th day ("Day 14" in Table 9) after the start of breeding were measured. Further, the feces

from the 9th day to the 11th day (i.e. three days) after the start of breeding were collected and freeze-dried, and the amounts of the feces (g/mouse/day) were measured.

Table 9 shows the values obtained as above.

[0051] [Table 9]

	Average body weight (g)			Amount of feed ingested (g/mouse/day)		Amount of feces (g/mouse/day)
	Day 0	Day 7	Day 14	Day 7	Day 14	
Control group	21.7±0.6	23.0±0.3	23.8±0.4	2.9	2.9	0.08
Protein-containing composition addition group	21.6±0.4	22.4±0.4	23.4±0.5	2.8	3.0	0.23

[0052] In Table 9, in the protein-containing composition addition group, increases or decreases in the average body weights and feed amounts were not observed compared with those of the control group, but the amounts of feces increased about three-fold.

[0053] 2. Evaluation of resultant protein-containing composition

The protein-containing compositions obtained in Example 2 and Example 26 were found to be dark brown flowable powder which was more blackish compared with cacao powder.

The compositions were evaluated by tasting (Table 10) and found to have no cacao flavor detected in cacao powder and no other flavors. In addition, the compositions were found to have no bitter taste and astringent taste (i.e. sustained harshness) detected in cacao powder and no other tastes (e.g. sweet taste, sour taste, salty taste, and umami taste).

[0054] [Table 10]

	Example 2	Example 26	Cacao powder
Flavor of cacao	-	-	++
Bitter taste	-	-	++
Astringent taste	-	-	+
Color tone	Blackish dark brown (dark chocolate color)	Blackish dark brown (dark chocolate color)	Dark brown (milk chocolate color)

Flavor of cacao: ++: strong, +: weak, -: not detected

Bitter taste: ++: strong, +: weak, -: not detected

Astringent taste: ++: strong, +: weak, -: not detected

[0055] As mentioned above, the composition substantially free of a fatty ingredient, caffeine, theobromine, and polyphenols which were contained in cacao beans and having an increased content of protein from cacao beans, and the production method for the composition were able to be established. In addition, according to the production method for the protein-containing composition, the residue obtained after extraction of functional components such as an antioxidant from cacao beans was able to be used efficiently.

Further, the effect of improving bowel movement of the composition of the present invention was able to be confirmed.

Claims

1. An agent for improving bowel movement ~~A protein-containing composition~~, comprising a protein-containing composition which comprises a protein obtained from cacao beans at a weight ratio of 42 to 70% based on the total solids of the composition.

2. A method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to claim 1, comprising:

(a) an extraction step of extracting a protein from a raw material comprising a protein obtained from cacao beans under an alkaline condition to obtain an extract containing the protein; and

(b) a precipitation step of precipitating the protein from the extract obtained in the step (a) under an acidic condition to obtain the protein-containing composition.

3. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to claim 2, wherein the step (a) is carried out under the condition of pH 8 to 13 and 20 to 100°C.

4. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to claim 3, wherein the step (a) is carried out under the condition of pH 10 to 12 and 50 to 100°C.

5. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to any one of

claims 2 to 4, wherein the step (b) is carried out under the condition of pH 2 to 6 and 0 to 40°C.

6. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to claim 5, wherein the step (b) is carried out under the condition of pH 2 to 5 and 0 to 20°C.

7. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to any one of claims 2 to 6, further comprising:

(c) a defatting step of removing a fatty ingredient of cacao beans before the step (a) and/or after the step (b).

8. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to claim 7, wherein the step (c) is carried out by using water under the condition of pH 2 to 7 and 30 to 90°C.

9. The method of producing the agent for improving bowel movement ~~protein-containing composition~~ according to claim 7 or 8, wherein the step (c) is carried out by using one or more kinds of organic solvents selected from the group consisting of ethanol, an aqueous solution of ethanol, diethyl ether, and hexane.

~~10. A protein-containing composition, which is produced by the method of producing the protein-containing composition according to any one of claims 2 to 9.~~

~~11. An agent for improving bowel movement, comprising the protein-containing composition according to claim 1 or 10.~~