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(54) Title: TEA BEVERAGE CONTAINING PEPTIDE, AND GREEN TEA EXTRACT, AND PREPARATION PROCESSES THEREOF

(57) Abstract: The present invention relates to a tea beverage, comprising a green tea extract containing no more than 500 ppm of catechins in all forms and no more than 80 ppm of catechins in gallate form, 100-1000 ppm of an alkali metal hexametaphosphate, and no more than 2400 ppm of a peptide. It also relates to a process for preparing a tea beverage, comprising extracting green tea leaves with water to obtain a green tea extraction liquid including no more than 500 ppm of catechins in all forms, treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate form, adding 100-1000 ppm of an alkali metal hexametaphosphate and no more than 2400 ppm of a peptide to the green tea extract. It further relates to a green tea extract and its preparation process.



TEA BEVERAGE CONTAINING PEPTIDE, AND GREEN TEA EXTRACT, AND PREPARATION PROCESSES THEREOF

5 **Technical Field**

The present inventions relates to a tea beverage containing a peptide, a green tea extract, and their preparation processes.

Background Art

10 Consumers are showing more and more interest in substances with anti-aging, energy supplying, relaxing, beauty enhancing, wound healing, anti-UV effects etc. Thus, functional beverages are increasingly popular.

Tea drinks are one of the beverages that have experienced fast growth in the soft drinks market in
15 Asia. To further promote such drinks, efforts have been made to add health additives and tonic substances, such as a peptide, or collagen, to tea drinks. Collagen has been proved to produce anti-aging, energy supplying, relaxing, beauty enhancing, wound healing, anti-UV effects, etc. However, owing to the natural components contained in tea, tea beverages sometimes do not display clear appearance, for instance, tea cream often forms while they cool down, especially
20 when the tea beverages contain other additives, such as collagen and vitamins. Tea cream is a complex mixture of many components, for example, polyphenols, caffeine, peptide, protein and polysaccharides. It not only produces an unattractive appearance but also damages both taste and color of a tea beverage; it also makes it difficult to add more peptides into a tea beverage.

25 Japanese Laid-open Patent Application Hei 11-308965 discloses a tea beverage which shows decreased turbidity. It is prepared by treating a tea extract with chlorogenic acid esterase or with both chlorogenic acid esterase and tannase.

Japanese Laid-open Patent Application No. 2004-321105A discloses a tea extract obtained by
30 treating a tea with tannase, addressing the problem of tea extract displaying bitterness and astringency. The extract has an improved taste and flavor. It includes, based on the solids

contained therein, 20-40 wt.% of non-polymerized catechins; and, based on the non-polymerized catechins, 40-60 wt.% of non-polymerized catechins in the gallate forms; and has a pH of 4.5-5.3 when the solids is at 2.0wt.%.

- 5 Japanese Laid-open Patent Application No. 2007-319008A discloses a tea beverage containing collagen peptides and its preparation process. The disclosed tea beverage containing collagen peptides shows less turbidity and little sediments, is stable and has a good taste and flavor. It is prepared from a tea extract treated with tannase, and contains 0.05-5% (W/V) collagen peptides preferably with a weight average molecular weight less than 4,000.

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Contents of the Invention

The invention provides a tea beverage containing, by weight based on the total weight of the beverage,

- a green tea extract containing no more than 500 ppm of catechins in all forms and no more
15 than 80 ppm of catechins in gallate forms;
--100-1,000 ppm of an alkali metal hexametaphosphate, and
--no more than 2,400 ppm of a peptide.

The invention also provides a process for preparing a tea beverage, comprising

- 20 --extracting a green tea leave with water to obtain a green tea extraction liquid including no more than 500 ppm of catechins in all forms;
--treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms;
--adding 100-1,000 ppm of an alkali metal hexametaphosphate and no more than 2,400 ppm
25 of a peptide to the green tea extract.

The present invention also provides a green tea extract, containing no more than 500 ppm of catechins in all forms and no more than 80 ppm of catechins in gallate forms.

- 30 The present invention also provides a process for preparing a green tea extract, comprising
--extracting a green tea leave with water to obtain a green tea extraction liquid including no

more than 500 ppm of catechins in all forms; and

--treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms.

- 5 The tea beverage and green tea extract of the invention show clear appearance, for instance, they suffer no creaming forming, even in storage, and can accommodate further addition of additives without compromising its quality.

Modes of carrying out the invention

- 10 For the purpose of the invention, "catechins" refers to, and is intended to include, catechin and its derivatives, including but not limited to catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, gallocatechin gallate, and a complex compound or polymer formed by any of catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, and gallocatechin
15 gallate.

- For the purpose of the invention, "catechins in gallate forms" refers to, and is intended to include, a compound, complex compound or polymer including therein both catechin or its derivative, and gallic acid or its derivative, including but not limited to catechin gallate, epicatechin gallate,
20 epigallocatechin gallate, and gallocatechin gallate.

- For the purpose of the invention, collagen, peptide or a hydrolysate thereof refers to, and is intended to include, a collagen, peptide or a derivative thereof obtained by hydrolyzing a collagen, peptide or a derivative thereof, optionally under the action of an acid, base, or enzyme.
- 25 Unlimited examples of collagen, peptide or a hydrolysate thereof include fish collagen peptide, soy peptide, milk peptide, albumin, egg protein peptide, whey peptide, maize peptide and so on. The peptides from food protein not only have good nutrition, but also have bio-function including antihypertensive activity, immunomodulating activity, and so on, and thus are preferred.

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For the purpose of the invention, unless otherwise indicated in explicit terms, any content in

percentage or ppm of a component is by weigh, based on the weight of the product of which the component is a part, such product including, for example, tea beverage, green tea extraction liquid and green tea extract.

5 The inventive tea beverage comprises

-- a green tea extract containing no more than 500 ppm of catechins in all forms and no more than 80 ppm of catechins in gallate forms;

--100-1,000 ppm of an alkali metal hexametaphosphate, and

--no more than 2,400 ppm of a peptide.

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The inventive tea extract comprises

---no more than 500 ppm of catechins in all forms, and

---no more than 80 ppm of catechins in gallate forms.

15 It is to be understood that the inventive tea beverage can be prepared from the inventive green tea extract, by adding further components to the latter.

In a preferred embodiment of the invention, the inventive tea beverage or the inventive green tea extract comprises no more than 450 ppm of catechins in all forms. Normally, the inventive tea beverage or the inventive green tea extract comprises 150-500 ppm of catechins in all forms, and more preferably, comprises 160-450 ppm of catechins in all forms, and even more preferably 180-400 ppm of catechins in all forms. Catechins may be present in gallate forms and in other forms, for example as catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, and gallocatechin gallate.

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In a preferred embodiment of the invention, the inventive tea beverage or the inventive green tea extract comprises no more than 70 ppm of catechins in gallate forms, for example, as catechin gallate, epicatechin gallate, epigallocatechin gallate, and gallocatechin gallate. Normally, the inventive tea beverage or the inventive green tea extract comprises 5-80 ppm of catechins in gallate forms, and more preferably, comprises 10-70 ppm, and even more preferably 10-45 ppm, of catechins in gallate forms. It is to be understood that, in the inventive tea beverage or the

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inventive green tea extract, the “no more than 80 ppm of catechins in gallate forms” is a part of the “no more than 500 ppm of catechins in all forms”, and this also applies to their respective preferred contents.

- 5 In a preferred embodiment of the invention, the inventive tea beverage comprises 100-1,000 ppm, more preferably 150-800 ppm, still more preferably 200-600 ppm, and even still more preferably 250-500 ppm, of an alkali metal hexametaphosphate. Preferably, long chain alkali metal hexametaphosphate is used in the present invention. As alkali metal hexametaphosphate, sodium hexametaphosphate (SHMP) is preferably used.

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- In a preferred embodiment of the invention, the inventive tea beverage comprises no more than 2,300 ppm of a peptide. Normally, it comprises 800-2,400 ppm of a peptide, and preferably, 1,000-2,300 ppm, and even more preferably 1,200-2,300 ppm, of a peptide. Peptide can be a collagen or a hydrolysate thereof, for example one selected from the group consisting of collagen, 15 gelatin or collagen hydrolysates, or other peptides from plant sources or animal sources. The origin of collagen is not particularly limited, as long as they can be used in the field of food industry. Example of collagen hydrolysates are from cattle, pig, fish, etc. Other peptides may be those from vegetables or animals. For example, peptides from soy, albumin, whey, maize, etc. may be used as said other peptides. Fish collagen hydrolysates have been proved to have good 20 effect in green tea system. As for the weight average molecular weight of the peptide, 5,000 or less (for example 600-5,000) is preferred. More preferably, the weight average molecular weight of the peptide is 4,500 or less (for example 800-4,500), and 1,000-3,800 are even more preferred.

- It would be appreciated by those skilled in the art that the inventive tea beverage also includes 25 other components conventionally present in a tea beverage, such as polyphenols, vitamin C or a salt thereof, and solvent, especially water. The beverage can also includes other additives, such as table salt, sweetener, flavorant, antioxidants, ester species, organic acid, organic acid salt, inorganic acid salts, mineral, pigments, emulsifier, preservatives, seasoning substance, acidulant, fruit-juice extract, vegetable extract, nectar extract, pH adjuster, and quality stabilizer. Besides 30 SHMP and the peptide, the tea beverage further comprises other additives at an amount of no more than 8% in total, preferably no more than 5% (based on the total weight of the resulting tea

beverage).

Preferably, in the inventive tea beverage, the weight ratio of the peptides to alkali metal hexametaphosphate is lower than 6.5, more preferably lower than 6.0. In a preferred embodiment
5 of the invention, the weight ratio of the peptides to alkali metal hexametaphosphate is 2.5-6.5, more preferably 3.0-6.0.

To the extent that any resulting tea beverage includes components within the most generic ranges as defined in the present invention, the tea beverage of the invention may contain other tea
10 extracts, such as those from black teas.

In the inventive tea beverage and the green tea extract, tea cream forming is inhibited, especially if SHMP is present therein, thus making a clear tea beverage and a clear green tea extract.

15 The invention also provides a process for preparing a tea beverage, comprising

- extracting a green tea leave with water to obtain a green tea extraction liquid including no more than 500 ppm of catechins in all forms;

- treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms;

- 20 --adding 100-1,000 ppm of an alkali metal hexametaphosphate and no more than 2,400 ppm of a peptide to the green tea extract.

The present invention also provides a process for preparing a green tea extract, comprising

- extracting a green tea leave with water to obtain a green tea extraction liquid including no
25 more than 500 ppm of catechins in all forms; and

- treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms.

It is to be understood that the process for preparing the inventive tea beverage includes the
30 process for preparing the inventive green tea extract.

The green tea leave used in the inventive processes can be any commercially available green tea. Green tea which may be used to prepare such extract, includes but is not limited to, West Lake Longjing (from Zhejian province), Tieguangying (from Fujian province), White tea, Oolong tea, Jasmine green tea, Huangshan Maofeng (from Anhui province), Dongting Biluochun (from Hunan province), Jianye, Emei Chunyu ((from Sichuan province)), Xinyang Maojian (from Henan province), Laoshan Green Tea (from Shandong province), Rizhao Green Tea (from Shandong province), Hanzhong Xianhao (from Shanxi province), Tianzhu Jianhao, Yuexi Cuilan, Lushan Yunwu Tea (from Jiangxi province) , Yingshan Yunwu Tea (from Hubei province), and the like, all being popular and commercially available in China. To extract the green tea leave with water, green tea leave is contacted, for instance, is infused or impregnated, with water at a temperature of 40-90°C for about 5-40 minutes, producing a green tea extraction liquid. The water used can be up to 150 fold of the volume of the green tea leave, preferably up to 80 fold, more preferably up to 40 fold, and even more preferably up to 30 fold. If necessary, the mixture is separated by, for example, filtration or centrifugation, so as to separate the green tea extraction liquid from residual green tea leave. After the infusion or impregnation is completed, the green tea extraction liquid may be diluted by additional water or concentrated by evaporation, thus keeping catechins in all forms at a concentration of no more than 500 ppm, preferably no more than 450 ppm. Normally, the green tea extraction liquid comprises 150-500 ppm of catechins in all forms, and more preferably, comprises 160-450 ppm of catechins in all forms, and even more preferably 180-400 ppm.

The obtained green tea extraction liquid is then treated with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms, and preferably, no more than 70 ppm of catechins in gallate forms, for example, as catechin gallate, epicatechin gallate, epigallocatechin gallate, and gallocatechin gallate. Normally, the green tea extract includes 5-80 ppm of catechins in gallate forms, and more preferably, comprises 10-70 ppm, and even more preferably 10-45 ppm, of catechins in gallate forms. It is to be understood that, “no more than 80 ppm of catechins in gallate forms” is a part of the “no more than 500 ppm of catechins in all forms”, and this also applies to their respective preferred contents. To treat the green tea extraction liquid with tannase, tannase is added to the green tea extraction liquid at a weight ratio of catechins in all forms in the green tea extraction liquid to tannase of 0.5-7.5:1, preferably

0.75-5:1, and more preferably 1-4.5:1. Or alternatively, to treat the green tea extraction liquid with tannase, tannase is added to the green tea extraction liquid at a weight ratio of catechins in gallate forms in the green tea extraction liquid to tannase of 0.45-4.0:1, preferably 0.5-3.5:1, and more preferably 0.6-3.0:1. Usually, tannase is added in an amount of 30-420 ppm. More preferably, tannase is added in an amount of 50-350 ppm, and most preferably 60-320 ppm. Tannase is reacted with the green tea extraction liquid at a temperature of 40-55°C, and more preferably is 45-50°C, for a period of 0.1 to 2 hours, and even more preferably 0.15 to 1 hours. After the reaction, the mixture is cooled down to a temperature less than 35°C, and preferably less than 30°C.

Tannase used in the invention can be derived from microorganisms, such as *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, etc. Commercially available tannase may be used, for example, "Tannase Sankyo" (trade name, available from Sankyo Life Tech Company, Japan), "Tannase" (trade name, available from KIKKOMAN CORP., Japan), and "Biotannase" (trade name, available from Kerry Bio-Science, Ireland).

After the green tea extraction liquid is treated with tannase, 100-1,000 ppm, preferably 150-800 ppm, still more preferably 200-600 ppm, and even still more preferably 250-500 ppm, of an alkali metal hexametaphosphate, preferably sodium hexametaphosphate, is added to the green tea extract.

After an alkali metal hexametaphosphate is added, no more than 2,400 ppm of a peptide is added to the green tea extract; preferably, no more than 2,200 ppm of a peptide is added to the treated green tea extract. Normally, 800-2,400 ppm of a peptide, and preferably, 1,000-2,300 ppm, and even more preferably 1,200-2,300 ppm, of a peptide is added. The peptide added can be collagen or a hydrolysate thereof, for example one selected from the group consisting of gelatin, collagen or a hydrolysates thereof, or other peptides from plant sources or animal sources; preferably, the peptide is one selected from the group consisting of gelatin, collagen or a hydrolysates thereof. The origin of peptide is not particularly limited, as long as they can be used in the field of food industry. Example of collagen or a hydrolysates are from cattle, pig, fish, etc. Other peptides may be those from vegetables or animals. For example, peptides from soy, albumin, whey, maize, etc.

may be used as peptide. Fish collagen hydrolysates is popular, except for its fishy aroma; however, as the inventive beverage offers wide latitude to receive additives without compromising its clear appearance, for example without creating cream or residues, flavorant or masking agent can be added, such as flavorant with fruit or vegetable flavor. As for the weight
5 average molecular weight of the peptide, 5,000 or less (for example 600-5,000) is preferred. More preferably, the weight average molecular weight of the peptide is 4,500 or less (for example 800-4,500), and 1,000-3,800 are even more preferred.

Besides SHMP and the peptide, other additives may be added at an amount of no more than 8%
10 in total, preferably no more than 5% (based on the total weight of the resulting tea beverage). Said other additives may include polyphenols, vitamin C or a salt thereof, table salt, sweetener, flavorant, antioxidants, ester species, organic acid, organic acid salt, inorganic acid salts, mineral, pigments, emulsifier, preservatives, seasoning substance, acidulant, fruit-juice extract, vegetable extract, nectar extract, pH adjuster, and quality stabilizer. They can be added alone or in
15 combination

Sweetener can be added into the tea beverage. Typical examples are sugar such as glucose, fructose, glycyrrhizin, stevia, aspartame, fructose oligosaccharide, galactosaccharide, etc. Sucrose is particularly preferred as the sweetener in the present invention. With a view to
20 reducing calories, the suitable amount of sweetener can be 0-6 wt%, more preferably lower than 4wt%, and most preferably between 2-3.2 wt%.

Vitamin C or a salt thereof may added into the tea beverage. The suitable total amount of them is 0-1 wt%, preferably 0-0.5 wt% and more preferably 0-0.2 wt%.

25 Solvents used in the present invention are those commonly used in the beverage or drinks, and most commonly being water.

Preferably, in the tea beverage, the weight ratio of the peptides to alkali metal
30 hexametaphosphate is lower than 6.5, more preferably lower than 6.0. In a preferred embodiment of the invention, the weight ratio of the peptides to alkali metal hexametaphosphate is 2.5-6.5,

more preferably 3.0-6.0.

After alkali metal hexametaphosphate, peptide, and optionally other additives are added into the green tea extract, the tea beverage is sterilized at a temperature normally used in the food processing industry, for example 70-140°C, more preferably 75-100°C, and most preferably 85-89°C. This sterilization process can be carried out for 0.5 to 1.5 minutes.

The final tea beverage of the present invention has a pH value of about 5.4 to 6.7.

The present tea beverage and green tea extract are clear product respectively and are stable after storage under a temperature of 38°C for at least 2 months.

The tea beverage according to the present invention can be converted to tea powder, beauty products, etc.

Examples

Analytical methods used in the examples

● Determination of tea polyphenols, gallic acid, caffeine and catechins (different forms)

Tea polyphenols, gallic acid, caffeine and catechins in tea beverage or green tea extract are measured by HPLC/PDA (High pressure liquid chromatography/Photodiode Array Detector): tea beverage or green tea extract sample are filtered with a 0.22 µm PTFE (polytetrafluoroethylen) filter, and then determined by HPLC. External standards (all from Sigma, USA) are used for quantitative measurement. Procedure as follows: take 5 ml test sample (before taking test sample, vibrate the sample gently; and plus ultrasonic treatment about 1 min when there is air bubbles), and filter the sample with a 0.22 µm PTFE filter, and then conduct HPLC analysis on the sample. Column: Atlantis T3, 3 µm, Φ4.6 mm×150 mm; Mobile phase A: water containing 20 µg/ml EDTA (Ethylenediamine tetraacetic acid) and 0.1% (by volume) TFA (trifluoroacetic acid); Mobile phase B: methanol containing 20 µg/ml EDTA and 0.1% TFA.

- Determination of peptide in green tea extract or tea beverage

Peptide (or proteins) in green tea extract or tea beverage are measured by Kjeldahl Method: a peptide is an organic compound containing nitrogen. Samples are heated and digested with concentrated sulfuric acid, copper sulfate, and potassium sulfate. Peptide is broken down into ammonia, which in turn can be changed into ammonium sulfate with sulfuric acid. After alkaline environment distillation, ammonia is distilled and absorbed with boric acid; it is titrated with hydrochloric acid. According to the quantity of hydrochloric acid, determine the content of nitrogen in the sample, and the quantity of peptide in the sample is calculated by multiplying the determined content of nitrogen by a factor: with respect to tea beverage or green tea extract, the factor is 6.25. The procedures are as follows: in the case of non-pulp beverage sample, without shaking, put 5.00 ± 0.01 g of a sample into a 250 ml tube. After drying the sample in the tube, add 10 ml sulfuric acid and one piece of copper sulfate-potassium sulfate digestion tablet (Kjeltabs) (commercially available from FOSS Ltd., UK), and then heat the mixture up to 420°C for 60 min.

- Determination of turbidity

Analysis of turbidity was performed with a Hach 2100AN Nephelometer, using the photorefractive tube of 2.4 cm. The measurement unit was NTU (Nephelometric turbidity unit). Each sample was measured three times and the average value was calculated.

Turbidity of a green tea extract or a tea beverage is used as the measurement of clearance of the respective green tea extract or tea beverage. If a beverage or an extract has a turbidity of less than 20, it is judged as being clear, showing no cream-forming.

- Determination of color (LAB)

Hunter L^* , a^* , b^* of samples were measured by a spectrophotometer (Model: Minolta Spectrophotometer CM-3500d). Measurements were performed with the pattern of penetration.

An average of three values was taken per sample. And the color difference ΔE^* was calculated according to the CIE 1976:

$$\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

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● Determination of ascorbic acid (VC)

Determination of ascorbic acid in beverage or extract by HPLC/PDA: Pipette some homogeneous sample whose VC content is within linear range to a 25 ml volumetric flask, mix it
 10 with some deionized water, add 1.0 ml 5% metaphosphoric acid, and dilute the solution with deionized water. Filter the diluted solution with a 0.45 μ m PTFE syringe filter into 1.5 ml amber vial before injection into HPLC-DAD (Waters e2695 HPLC-DAD with Atlantis T3, 5 μ m, Φ 4.6 mm $\times$ 250 mm).

15 **Preparation of the green tea extracts or beverages**

Green tea leave was extracted in a 30-55 fold volume of water at a temperature of 80-90°C for 10-25 minutes to produce a green tea extraction liquid; residual green tea leave was filtered out through a 300 mesh sieve. The extraction liquid was cooled down to a temperature no higher
 20 than 15°C in ice-water for further use, or cooled down to 50°C for immediate further use.

The extraction liquid was reacted with tannase (available from Kerry Bio-Science, USA, Biotannase sample NO. S1006001), and then cooled down to 15°C in ice-water. The cooled extraction liquid was subjected to centrifuging at 5000 rpm for 15 minutes at a temperature of
 25 15°C in a Himac CR 21G, high speed refrigerated centrifuge. The supernatant was collected for further use. The supernatant was determined for contents of components and, if necessary, concentrated by evaporation or diluted with water, to give a green tea extract. Green tea extracts were subjected to analysis.

30 To the supernatant (i.e. green tea extract), were added other ingredients to produce a tea beverage product. The other ingredients were collagen, peptide or its hydrolysate, and honey, SHMP (long chain), VC, sodium ascorbate and NaHCO_3 (all were food-grade products). The final tea

beverages were sterilized at a temperature of 87-89°C. After being cooled down to room temperature, the beverages were subjected to analysis.

The peptides used (in the examples and tables, all the contents of peptides are calculated based on the pure peptides respectively) are selected from the following:

Fish gelatin: liquid fish collagen hydrolysates, available from Hubei Reborn Biotech Co., Ltd, China, specification: protein > 58.3%, weight average molecular weight 4,800;

Royoung-fish liquid, available from RABJ Co., Ltd, Japan, specification: protein > 58.3%, weight average molecular weight 3,500;

Hydrolysate of collagen from pig: hydrolysate of collagen from pig hide: ARTHREDTM, available from Cargill Texturizing Solutions, USA, specification: protein 88%, weight average molecular weight 3,000;

Hydrolysate of collagen from cattle: hydrolysate of collagen from cattle hide, ARTHREDTM, available from Cargill Texturizing Solutions, USA, specification: protein 93%, weight average molecular weight 3,500;

Soy peptide, available from Tallyho Functional peptide, China, Specification: protein > 80%, weight average molecular weight 3,500;

Albumin, available from Tallyho Functional peptide, China, specification: protein > 80%, weight average molecular weight 1,800;

Whey hydrolysate, available from Arla Foods Ingredients, Denmark, specification: protein > 80%, weight average molecular weight 1,500;

Maize peptide, available from Tallyho Functional peptide, China, specification: protein > 90%, weight average molecular weight 2,800.

Examples 1-8 relate to the preparation of green tea extraction liquids and green tea extracts.

5 Comparative example 1 and Examples 2

By using a commercially available green tea, green tea extract was prepared and the results are summarized in table 1 (the ratio of the total catechins in the green tea extraction liquid to the tannase added is 2.62, and the ratio of the catechins in gallate forms in the green tea extraction
10 liquid to the tannase added to the extract is 1.50).

Table 1 Green tea extraction liquid and green tea extracts

Examples		Comparative 1	2
Preparation conditions			
Green tea leaves	Types	West Lake Longjing Tea, Pan-fried and slightly fermented tea, from Zhejiang Province, China; commercially available	
	Amounts	125 g	
Extraction	Water volume	40 times of that of tea leaves	
	Temperature	80 °C	
	Time	15 min	
Tannase Treatment	Types	Non	Tannase
	Amounts	Non	125 ppm
	Temperature	Non	50 °C
	Time	Non	15 min
Extraction products			
Compositions (in ppm)		Green tea extraction liquid	Green tea extracts
TTP		719.33 ppm	708 ppm
Caffeine		101.64 ppm	94.95 ppm
VC		321.13 ppm	319.7 ppm
Catechins	C	22.07 ppm	9.19 ppm
	CG	22.09 ppm	0
	EC	11.93 ppm	57.8 ppm
	ECG	17.51 ppm	0
	EGC	32.48 ppm	190.4 ppm
	EGCG	68.21 ppm	7.12 ppm
	GC	66.57 ppm	30.07 ppm
	GCG	79.45 ppm	2.04 ppm
	GA	7.03 ppm	12.74 ppm
	Total of catechins	327.34 ppm	309.36 ppm
	Total of catechin gallates	187.26 ppm	9.16 ppm
Properties			
pH		6.14	5.66
Turbidity (ntu)		4.76	10.88
LAB value (ΔE^*)		2.89	1.25

Meanings of the abbreviations:

TTP: tea polyphenols

5 VC: ascorbic acid or vitamin C

C: (-)-catechin

CG: Catechin 3-gallate

EC: (-)-epicatechin

ECG (-)-epicatechin gallate

5 EGC: (-)-epigallocatechin

EGCG: (-)-epigallocatechin gallate

GC: (+)-gallocatechin

GCG: gallocatechin 3-gallate

GA: gallic acid

10

Comparative example 3 and Examples 4

Comparative example 1 and example 2 were repeated, except that some conditions were varied.

The results are summarized in table 2 (the ratio of the total catechins in the green tea extraction

15 liquid to the tannase added is 1.22, and the ratio of the catechins in gallate forms in the green tea extraction liquid to the tannase added to the extract is 0.71).

Table 2 Green tea extraction liquid and green tea extracts

Examples		Comparative 3	4
Preparation conditions			
Green tea leaves	Types	Xinyang Maojian, from Henan Province, China; commercially available	
	Amounts	100 g	
Extraction	Water volume	45 times of that of tea leaves	
	Temperature	85 °C	
	Time	20 min	
Tannase Treatment	Types	Non	Tannase
	Amounts	Non	270 ppm
	Temperature	Non	45 °C
	Time	Non	15 min
Extraction products			
Compositions (in ppm)		Green tea extraction liquid	Green tea extracts
TTP		690.56 ppm	680.4 ppm
Caffeine		98.65 ppm	91.95 ppm
VC		298.13 ppm	280.2 ppm
Catechins	C	18.15 ppm	8.30 ppm
	CG	20.15 ppm	0
	EC	12.84 ppm	61.3 ppm
	ECG	19.52 ppm	0
	EGC	34.52 ppm	178.3 ppm
	EGCG	67.33 ppm	7.06 ppm
	GC	65.87 ppm	28.56 ppm
	GCG	85.30 ppm	1.44 ppm
	GA	5.98 ppm	14.87 ppm
	Total of catechins	329.66 ppm	299.83 ppm
	Total of catechin gallates	192.3 ppm	8.50 ppm
Properties			
pH		6.78	6.57
Turbidity (ntu)		6.70	12.30

Comparative example 5 and Examples 6

Comparative example 1 and example 2 were repeated, except that some conditions were varied.

The results are summarized in table 3 (the ratio of the total catechins in the green tea extraction liquid to the tannase added is 4.07, and the ratio of the catechins in gallate forms in the green tea

5 extraction liquid to the tannase added to the extract is 2.38).

Table 3 Green tea extraction liquid and green tea extracts

Examples		Comparative 5	6
Preparation conditions			
Green tea leaves	Types	Laoshan Green Tea, from Shandong Province, China, commercially available	
	Amounts	80 g	
Extraction	Water volume	50 times of that of tea leaves	
	Temperature	90 °C	
	Time	25 min	
Tannase Treatment	Types	Non	Tannase
	Amounts	Non	75 ppm
	Temperature	Non	40 °C
	Time	Non	30 min
Extraction products			
Compositions (in ppm)		Green tea extraction liquid	Green tea extracts
TTP		680.34 ppm	659.2 ppm
Caffeine		94.30 ppm	91.74 ppm
VC		289.33 ppm	280.3 ppm
Catechins	C	16.57 ppm	6.70 ppm
	CG	18.50 ppm	0
	EC	11.35 ppm	57.2 ppm
	ECG	17.25 ppm	0
	EGC	30.50 ppm	173.5 ppm
	EGCG	65.53 ppm	7.21 ppm
	GC	63.25 ppm	30.23 ppm
	GCG	77.32 ppm	1.85 ppm
	GA	5.24 ppm	9.43 ppm
	Total of catechins	305.51 ppm	286.12 ppm
	Total of catechin gallates	178.6 ppm	9.06 ppm
Properties			
pH		6.25	5.83
Turbidity (ntu)		5.20	11.37

Comparative example 7 and Examples 8

Comparative example 1 and example 2 were repeated, except that some conditions were varied.

The results are summarized in table 4 (the ratio of the total catechins in the green tea extraction liquid to the tannase added is 1.20, and the ratio of the catechins in gallate forms in the green tea extraction liquid to the tannase added to the extract is 0.68).

Table 4 Green tea extraction liquid and green tea extracts

Examples		Comparative 7	8
Preparation conditions			
Green tea leaves	Types	Lushan Yunwu Tea, from Jiangxi Province, China; commercially available	
	Amounts	120 g	
Extraction	Water volume	30 times of that of tea leave	
	Temperature	90 °C	
	Time	25 min	
Tannase Treatment	Types	Non	Tannase
	Amounts	Non	400 ppm
	Temperature	Non	40 °C
	Time	Non	10 min
Extraction products			
Compositions (in ppm)		Green tea extraction liquid	Green tea extracts
TTP		720.16 ppm	680.4 ppm
Caffeine		108.57 ppm	91.95 ppm
VC		335.36 ppm	280.2 ppm
Catechins	C	28.83 ppm	37.15ppm
	CG	31.86 ppm	0
	EC	18.34ppm	85.82 ppm
	ECG	23.81 ppm	0
	EGC	52.76 ppm	143.96 ppm
	EGCG	106.84 ppm	49.77 ppm
	GC	95.02 ppm	105.48 ppm
	GCG	110.64 ppm	22.39 ppm
	GA	12.07 ppm	30.08 ppm
	Total of catechins	480.17 ppm	474.65 ppm
	Total of catechin gallates	273.15 ppm	72.16 ppm
Properties			
Turbidity (ntu)		7.80	14.35

Examples 9-16 relate to the preparation of tea beverages and their measurements

Examples 9-11 and comparative example 12

Tea beverages were prepared by using the green tea extracts from examples 2, 4, 6 and
5 comparative example 1 (if necessary, with dilution by water).

Table 5 Beverages compositions

Examples		9	10	11	Com. 12
Compositions					
Green tea extracts	From example	2	4	6	Com. 1
	Total of catechins	309.36 ppm	299.83 ppm	286.12 ppm	327.34 ppm
	Total of catech in gallates	9.16 ppm	8.50 ppm	9.06 ppm	187.26 ppm
SHMP		500 ppm	230 ppm	450 ppm	500 ppm
Peptide	Source	Fish gelatin	Hydrolysate of collagen from pig	Hydrolysate of collagen from cattle	Fish gelatin
	Weight average molecular weight	4,800	3,000	3,500	4,800
	Contents	1,600 ppm	1,200 ppm	2,300 ppm	1,600 ppm
Other Peptide	Source	Soy peptide	Maize peptide		Soy peptide
	Weight average molecular weight	3,500	2,800		3,500
	Contents	500 ppm	100 ppm	0	500 ppm
Bee honey		50 ppm	0	90 ppm	50 ppm
VC		100 ppm	80 ppm	75 ppm	100 ppm
Sodium ascorbate		400 ppm	450 ppm	420 ppm	400 ppm
NaHCO ₃		75 ppm	0	65 ppm	75 ppm
Properties					
Turbidity (ntu)		8.88	7.95	8.35	80.48
LAB value (L*)		97.88	not measured		95.08
LAB value (ΔE^*)		1.25			2.89

The above results in table 5 confirmed that the beverages of the invention could significantly
10 inhibit turbidity caused by added collagen or its hydrolysate and other peptide.

LAB values of ΔE^* proved that the inventive beverage showed improved color.

The tea beverage of example 9 was stored at 38 °C and analyzed for its stability. The results are

summarized in tables 6-7. For comparison purpose, a commercially available ready-to-drink tea beverage Yuanye (brandname, avallbale from Coco-Cola China Beverage Ltd., China) was also subjected to experimentation.

5

Table 6 PH values of the tea beverage of example 9 during its storage

Products	Day 0	Day 30	Day 60	Day 90
Yuanye	6.2	6	5.83	5.66
Example 9	5.66	5.42	5.52	5.48

Table 7 Stability of the tea beverage of example 9

Time		Day 0	Day 30	Day 60
Compositions (in ppm)				
TTP		708	696.2	701.95
Caffeine		94.95	93.19	97.01
VC		319.7	229.34	211.06
Catechins	C	9.19	9.16	10.09
	CG	0	0	0
	EC	57.8	33.0	30.26
	ECG	0	0	0
	EGC	190.4	104.84	91.65
	EGCG	7.12	5.63	5.05
	GC	30.07	34.6	38.56
	GCG	2.04	2.34	3.62
	GA	12.74	58.8	66.92
	Total of catechins	309.36	248.37	246.15
	Total of catechin gallates	9.16	7.97	8.67
VE (tocopherol or vitamin E)		13.8	14.5	8.40
Protein (peptides) (as measured)		1200 ppm	1100 ppm	1100 ppm
Turbidity (ntu)		8.88	12.30	11.80

10 Examples 13-14 and comparative example 15

Tea beverages were prepared by using the green tea extracts from example 8 and comparative example 7 (if necessary, with dilution by water).

Table 8 Beverages compositions

Examples		13	14	Com. 15
Compositions				
Green tea extracts	From example	8	8	Com. 7
	Total of catechins	474.65 ppm	474.65 ppm	480.17 ppm
	Total of catech in gallates	72.16 ppm	72.16 ppm	273.15 ppm
SHMP		850 ppm	700 ppm	850 ppm
Peptide	Source	Fish gelatin	Hydrolysate of collagen from pig	Fish gelatin
	Weight average molecular weight	4,800	3,000	4,800
	Contents	1,800 ppm	2,200 ppm	1,500 ppm
Other Peptide	Source	Soy peptide	Maize peptide	Soy peptide
	Weight average molecular weight	3,500	2,800	3,500
	Contents	500 ppm	100 ppm	700 ppm
Bee honey		50 ppm	0	50 ppm
VC		100 ppm	80 ppm	100 ppm
Sodium ascorbate		400 ppm	450 ppm	400 ppm
NaHCO ₃		75 ppm	0	75 ppm
Properties				
Turbidity (ntu)		16.89	14.76	93.45

Example 16

5

With the green tea extract of example 2, different beverages were prepared and subjected to analysis.

1. Influence of SHMP (ppm) on the appearance of beverages

- 10 Samples 16-1 (i.e. first one in example 16) and 16-2, a blank sample including no peptide, a control-1 sample including no peptide or SHMP and a control-2 sample including peptide but no SHMP were prepared according to the procedures under **Preparation of the green tea extracts or beverages**, in a 500 ml volume respectively, and were used to demonstrate the green tea appearance of the beverages. The peptide used was hydrolysate of collagen from pig, as
- 15 mentioned in the above, weight average molecular weight was 3,000. The results are as follows:

Table 9 Influence of SHMP (ppm) on the appearance of beverages

Samples	Peptide (g)	SHMP (ppm)	Scum	Turbidity	Creamy	Clear	Dark (tea color)	Sediment
blank	0	400	-	-	-	+++	+++	-
Control 1	0	0	-	10.88	++	++	+	++
Control 2	0.8	0	-	++	++	++	+	++
16-1	0.8	300	-	-	-	-	++	-
16-2	0.8	500	-	-	-	-	++	-

+++ : very serious (as compared with control 1);

++ : serious (idem);

+ : unacceptable (idem);

5 +/- : detectable (idem);

- : not at all (idem).

From the above table, it can be seen that without SHMP, the green tea extract of example 2 could not contain peptide without forming cream.

2. Tea beverages including different peptides

10 With different peptides, different beverages were prepared and subjected to analysis.

Table 10 Beverages with different peptides and their turbidities
(0.8 g peptides per 500 ml beverage)

No.	Sample (peptide)	SHMP (g)	Turbidity (ntu)	pH
1	Control (without peptide)	0.25	5.44	6.32
2	Fish gelatin	0.20	17.5	6.23
3	Royoung – fish liquid	0.25	8.89	6.20
4	Soy peptide	0.15	17.8	6.23
5	Albumin	0.22	7.38	6.29
6	Whey hydrolysate	0.18	12.7	6.24
7	Maize peptide	0.14	19.2	6.13
8	Blank-1 (with Royoung– fish liquid but without SHMP)	0	200.0	6.25

The results show that the green tea extract of example 2 can form a clear tea beverage with all
 15 the listed peptides, if SHMP was used at a content in the range of the present invention. However, if no SHMP was used, the resulting beverage with a peptide displayed turbidity, even scrutinized by naked eyes; sample Blank-1 became more turbid within 2 weeks at room temperature, and formed a coacervated phase within 1 month and thus the beverage was phase-separated.

Claims

1. A tea beverage, comprising

- a green tea extract containing no more than 500 ppm of catechins in all forms and no more
- 5 than 80 ppm of catechins in gallate forms;
- 100-1,000 ppm of an alkali metal hexametaphosphate, and
- no more than 2,400 ppm of a peptide.

2. The tea beverage according to claim 1, comprising 160-450 ppm of catechins in all forms.

10

3. The tea beverage according to claim 1, comprising 10-70 ppm of catechins in gallate forms.

4. The tea beverage according to claim 1, comprising 250-500 ppm of an alkali metal hexametaphosphate.

15

5. The tea beverage according to claim 1, comprising 1000-2,300 ppm of a peptide.

6. The tea beverage according to claim 1, wherein the weight ratio of the peptide to alkali metal hexametaphosphate is lower than 6.5.

20

7. The tea beverage according to claim 1, wherein the weight ratio of the peptide to alkali metal hexametaphosphate is 2.5-6.5.

8. The tea beverage according to claim 1, wherein the alkali metal hexametaphosphate is sodium

25 hexametaphosphate.

9. The green tea extract according to claim 1, wherein the weight average molecular weight of the peptide is 5,000 or less.

30 10. The green tea extract according to claim 11, wherein the weight average molecular weight of

the peptide is 800-4,500.

11. A green tea extract, comprising no more than 500 ppm of catechins in all forms and no more than 80 ppm of catechins in gallate forms.

5

12. The green tea extract according to claim 11, comprising 160-450 ppm of catechins in all forms.

13. The green tea extract according to claim 11, comprising 10-70 ppm of catechins in gallate forms.

10

14. A process for preparing a tea beverage, comprising

--extracting a green tea leave with water to obtain a green tea extraction liquid including no more than 500 ppm of catechins in all forms;

15 --treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms;

--adding 100-1,000 ppm of an alkali metal hexametaphosphate and no more than 2,400 ppm of a peptide to the green tea extract.

20 15. The process according to claim 14, wherein the green tea is selected from the group consisting of West Lake Longjing, Tieguangying, white tea, Oolong tea, Jasmine green tea, Huangshan Maofeng, Dongting Biluochun, Jianye, Emei Chunyu, Xinyang Maojian, Laoshan Green Tea, Rizhao Green Tea, Hanzhong xianhao, Tianzhu Jianhao, Yuexi Cuilan, Lushan Yunwu Tea, and Yingshan Yunwu Tea.

25

16. The process according to claim 14, wherein the green tea extraction liquid is treated with tannase at a temperature of 40-55°C for a period of 0.1 to 2 hours at a weight ratio of catechins in all forms in the green tea extraction liquid to tannase of 0.5-7.5:1.

30 17. The process according to claim 16, wherein the green tea extraction liquid is treated with tannase at a weight ratio of catechins in all forms in the green tea extraction liquid to tannase of

1-4.5:1.

18. The process according to claim 14, wherein the green tea extraction liquid is treated with tannase at a temperature of 40-55°C for a period of 0.1 to 2 hours at a weight ratio of catechins in gallate forms in the green tea extraction liquid to tannase of 0.45-4.0:1.

19. The process according to claim 18, wherein the green tea extraction liquid is treated with tannase at a weight ratio of catechins in gallate forms in the green tea extraction liquid to tannase of 0.6-3.0:1.

20. The process according to claim 14, wherein the green tea extraction liquid is treated with tannase at a temperature of 40-55°C for a period of 0.1 to 2 hours in an amount of tannase of 30-420 ppm.

21. The process according to claim 20, wherein the green tea extraction liquid is treated with tannase in an amount of tannase of 60-320 ppm.

22. The process according to claim 14, wherein the weight average molecular weight of the peptide is 5,000 or less.

23. The process according to claim 22, wherein the weight average molecular weight of the peptide is 800-4,500.

24 The process according to claim claim 14, further comprising step of adding no more than 8% in total, based on the total weight of the tea beverage of one or more, one or more substances selected from the group consisting of polyphenols, vitamin C, sweetener, flavorant, antioxidants, ester species, organic acid, organic acid salt, inorganic acid salts, mineral, pigments, emulsifier, preservatives, seasoning substance, acidulant, fruit-juice extract, vegetable extract, nectar extract, pH adjuster, and quality stabilizer.

25. A process for preparing a green tea extract, comprising

--extracting a green tea leave with water to obtain a green tea extraction liquid including no more than 500 ppm of catechins in all forms; and

--treating the green tea extraction liquid with tannase to obtain a green tea extract including no more than 80 ppm of catechins in gallate forms.

5

26. The process according to claim 25, wherein the green tea extraction liquid is treated with tannase at a temperature of 40-55°C for a period of 0.1 to 2 hours at a weight ratio of catechins in all forms in the green tea extraction liquid to tannase of 0.5-7.5:1.

10 27. The process according to claim 26, wherein the green tea extraction liquid is treated with tannase at a weight ratio of catechins in all forms in the green tea extraction liquid to tannase of 1-4.5:1.

15 28. The process according to claim 25, wherein the green tea extraction liquid is treated with tannase at a temperature of 40-55°C for a period of 0.1 to 2 hours at a weight ratio of catechins in gallate forms in the green tea extraction liquid to tannase of 0.45-4.0:1.

20 29. The process according to claim 28, wherein the green tea extraction liquid is treated with tannase at a weight ratio of catechins in gallate forms in the green tea extraction liquid to tannase of 0.6-3.0:1.

30. The process according to claim 25, wherein the green tea extraction liquid is treated with tannase at a temperature of 40-55°C for a period of 0.1 to 2 hours in an amount of tannase of 30-420 ppm.

25

31. The process according to claim 30, wherein the green tea extraction liquid is treated with tannase in an amount of tannase of 60-320 ppm.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2011/000557

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A23F3/-;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, CNPAT, CNKI, ISI, GOOGLE: green tea, beverage?, extract+, isolate+, abstract+, catechin?, gallate+, hexametaphosphate?, SHMP, peptide, tannase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIAO Wen-jun et al., Study on technology of processing de-bitter tea extracts, Journal of Hunan Agricultural University (Natural Sciences). April 2007, vol.33, No.2, pages 208–212.	11-13,25-31
Y		1-10,14-24
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☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CN2011/000557

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INTERNATIONAL SEARCH REPORT

International application No.

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Continuation of: A. CLASSIFICATION OF SUBJECT MATTER of Second Sheet

A23F 3/14 (2006.01)i

A23F 3/16 (2006.01)i

A23F 3/18 (2006.01)i