

SPECIFICATION

Method of Preparing Tea Extract and Tea Flavor

5 **Technical Field**

 This invention relates to an enzymatic treating method for preparing aroma-enriched tea extract and tea flavor.

Background Art

10 Tea has been used in various processed products in these years, and field of its application is yearly broadening, with steadily increasing use of that flavor. For example, it is used for tea beverage or milk beverage, functional drinks, confectionery such as cookies, cakes, jellies, and the like. Also use of tea flavor as perfume for
15 cosmetics or toiletry goods, and as skin lotion is increasing.

 While flavor or fragrance compounds can be used in these processed products, consumers who prefer natural materials are getting a majority in recent years and use of natural flavor extracted from tea leaves is desirable.

20 On the other hand, natural tea flavor is very delicate and easy to disappear in the process of making such products. For example, in production of beverages, packed or PET bottled drinks are heat-sterilized in their preparation process, either at the packing time or after packing. During the heat-sterilization, the flavor
25 components are deteriorated or decreased, and the drinks are not fully satisfactory in respect of flavor, compared with freshly made tea from tea leaves in the home.

 As a means to solve this problem, technology for recovering flavory substance obtained through distillation of tea leaves and
30 utilizing the same has been widely used in general. Methods proposed in the past in this connection include: a method comprising passing an inert gas through tea leaves and contacting the gas containing the flavor component with liquid at low temperature to condense the former (cf. JP Sho 61(1986)-254145A), instant powder
35 tea comprising tea extract obtained by extracting tea leaves with

water, extract obtained by distillation or extraction with liquefied carbon dioxide gas of tea leaves and powdered tea leaves (cf. JP Sho 63(1988)-3755A); a method comprising passing a humidified inert gas through humidified black tea or green tea to recover the volatile flavor component, dewatering and thereafter passing it through dry tea for re-flavoring (cf. JP Sho 63(1988)-137646A); a method comprising adding to dry matter containing the flavor component water of a temperature not higher than ambient temperature and collecting the gasified flavor component (cf. JP Hei 4(1992)-23895A); method comprising steam-distilling tea leaves and re-contacting the recovered flavor with untreated tea leaves, whereby producing recovered flavor little retaining hot-distillation odor (cf. JP Hei 8(1996)-116,882A); a method of steam-distilling in the presence of an antioxidant to obtain the flavor (cf. JP Hei 8(1996)-73886A); and the like. However, because those processes use ordinary tea leaves as starting materials for the flavor source, absolute quantity of free flavor component is limited and no substantial flavor enhancement can be expected. Thus, their results are not necessarily satisfactory.

On the other hand, research work in flavor component of tea is recently achieving rapid development, and studies for confirming presence of glycoside in tea extract, using a glycoside-hydrolysing enzyme such as β -glucosidase are under progress.

For example, it was suggested that the main flavor sources such as linalool, geraniol, benzyl alcohol, methyl salicylate, 2-phenylethanol and the like are present in tea leaves in the form of their glycosides (precursors) (cf. Phytochemistry, Vol.20, p. 2145 (1981) and Agric. Biol. Chem., Vol. 54, p.1023 (1990)); (z)-3-hexenol β -D-glucoside and benzyl alcohol β -D-glucoside were isolated from *Yabukita-cha* species (cf. Agric. Biol. Chem., Vol. 55, p. 1205 (1991) and Agric. Biol. Chem. Vol. 58, p. 592 (1994)); also from oolong tea, β -primeverosides (6-O- β -xylol β -D-glucosides) of geraniol, benzyl alcohol, linalool and 2-phenylethanol were isolated (cf. Phytochemistry, Vol. 33, p. 1373 (1993) and Biotech. Biochem. Vol. 58, p.1532 (1994)).

However, the central aim of above studies on glycosides was to analyze the mechanism of flavor generation during fermentation of

tea or glycosides in tea extracts. Hence they are insufficient in respect of effective utilization of all glycosides present in tea leaves.

Disclosure of the Invention

5 A chief object of the present invention is to provide tea extract and tea flavor in which the aroma is enhanced, by effectively extracting glycosides from tea leaves.

It is known; when tea leaves are extracted with water or hot water and the extract is treated with a glycoside-hydrolysing enzyme, 10 the glycoside decomposes and new flavor generates. Whereas, it is now discovered, surprisingly, that the amount of flavor components remarkably increases when tea leaves are acted on by glycoside-decomposing enzyme during and/or after a tannase treatment of tea leaves; and that the tannase treatment conducted in the presence of 15 protease can still further increase the amount of the flavor components. The present invention is thus completed.

Accordingly, therefore, the present invention provides a method for preparing tea extract with enhanced flavor, which is characterized by having glycoside-hydrolysing enzyme act on tea 20 leaves during and/or after treating the tea leaves with tannase and optionally further with protease.

The invention also provides a method for producing tea flavor, which comprises the first step of having glycoside-hydrolysing enzyme react with tea leaves during and/or after treating the tea leaves with 25 tannase, and the second step of subjecting the tea extract which is obtained in the first step to flavor-condensing treatment to obtain a flavor-enriched product.

According to the present invention, several tenfold stronger flavory substance compared with the products of heretofore practiced 30 methods of having glycoside-hydrolysing enzyme react with tea extract can be obtained, and the method can provide flavor-enhanced tea extract and tea flavor.

Brief Explanation of Drawings

35 Fig. 1 shows gas chromatograms of flavor components of

Example 1 and Comparative Example 1, as obtained by dynamic head space method.

Fig. 2 shows gas chromatograms of flavor-enriched products of Example 1 and Comparative Example 1, as obtained by solvent
5 extraction method.

Hereinafter the present invention is explained in further details.

As the tea leaves useful as starting materials in the method of
10 the present invention, for example, non-fermented tea such as green tea of middle grade, roasted tea, refined green tea, *Kabuse-cha*, *Ten-cha*, and the like (hereafter collectively referred to as "steamed tea"); non-fermented tea such as *Ureshino-Cha*, *Aoyagi-cha* and various Chinese tea ("cauldron-boiled tea"); semi-fermented tea such
15 as *Hoshu-cha*, *Tetsu-Kannon-cha*, oolong tea and the like; and fermented tea such as black tea, *Awa* coarse tea, *Goishi-cha*, pu-ehr tea and the like can be named. Those tea materials may be used as they are, but normally when they are given such treatment(s) as cutting, crushing, grinding or the like using devices used in food
20 processing before the enzyme treatment, formation of flavor component is further promoted to achieve more favorable effect. Of those, non-fermented and semi-fermented tea rich in glycosides, in particular, green tea black tea and oolong tea, are preferred.

As above, the present invention is characterized by having
25 tannin-hydrolysing enzyme, tannase, act on these tea leaves and, simultaneously with and/or after this tannase treatment, having glycoside- hydrolysing enzyme act on the tea material to enhance the flavor. Also by having one, two or more proteases act on the tea leaves simultaneously with the tannase treatment, the flavor can be
30 still more effectively enhanced. Combined use of at least two proteases can further heighten the effect of protease.

As tannase to be used for such an enzymatic treatment, any kind can be optionally used without any limitation, so long as it has tannin- hydrolysing activity. Specifically, for example, those
35 obtainable through the steps of solid or liquid culturing

tannase-producing microorganisms belonging to *Aspergillus*,
Penicillium, *Rhizopus*, *Mucor* and the like in a medium used for
cultivation of these filamentous fungi according to accepted practice,
and refining the resulting cultured product with or without further
5 intermediate processing, according to an accepted refining method.
Furthermore, commercially available tannases, for example, Tannase
Kikkoman (500 U/g) and Tannase Kikkoman (5000 U/g) (Kikkoman
Co.), Tannase Sankyo (500 U/g) (Sankyo Co.) and the like can be used.
Use rate of tannase differs depending on various factors such as
10 activity and cannot be generally specified, but normally, for example,
it can be used within a range of 0.1 – 50 U/g, preferably 0.5 – 20 U/g,
based on the weight of the tea material.

Also as glycoside-hydrolysing enzyme, for example,
 β -glucosidase, β -xylosidase, β -primeverosidase and the like can be
15 named.

Specific examples of β -glucosidase useful for the enzymatic
treatment of glycoside include those obtainable through the steps of
solid or liquid culturing β -glucosidase-producing microorganisms
belonging to *Aspergillus*, *Penicillium*, *Rhizopus*, *Pseudomonas*, *Pichia*
20 in a solid medium such as of wheat bran, rice bran or the like or a
liquid medium according to accepted practice, and refining the
resulting cultured product with or without further intermediate
processing, according to an accepted refining method. As
 β -glucosidase, those obtainable from vegetable such as vanilla beans,
25 raw tea leaves and the like, which are given a refining treatment can
also be used. Almond-derived emulsin commercialized by
Sigma-Aldrich Co., or those separated from β -glucosidase-containing
enzymatic preparations such as Cellulase A (Amanoenzyme Co.),
Cellulase T (Amanoenzyme Co.) and the like can also be used.
30 Specific examples of β -xylosidase include those obtainable through the
steps of solid or liquid culturing β -xylosidase-producing
microorganisms belonging to *Penicillium*, *Aspergillus*, *Rhizopus*,
Mucor or the like, in a solid medium such as of wheat bran, rice bran
or the like or a liquid medium according to accepted practice, and
35 refining the resulting cultured product with or without further

intermediate processing, according to an accepted refining method. These separated from black-koji mould (*Aspergillus niger*)-derived enzymatic preparation marketed by Sigma-Aldrich Co. or β -xylosidase-containing enzymatic preparation, Sumizyme ACH (Shin-nippon Chemical Industries) and the like can be used. As specific examples of β -primeverosidase, those obtainable through the steps of solid or liquid culturing β -primeverosidase-producing microorganisms belonging to *Cellulomanas*, *Penicillium*, *Aspergillus* or the like, in a solid medium such as of wheat bran, rice bran or the like or a liquid medium according to accepted practice, and refining the resulting cultured product with or without further intermediate processing, according to an accepted refining method, and those separated from vegetables such as raw tea leaves and refined can be named.

Use rate of these glycoside-hydrolysing enzymes differs depending on various factors such as titer and cannot be generally specified, but by way of an example, it may be within a range of 0.001 – 10 U/g, preferably 0.005 – 2 U/g, based on the weight of the starting tea material.

Protease is subject to no specific limitation, and one or more of animal-, vegetable-, or microorganism-derived proteases can be used either singly or in combination of two or more. For example, Protease A, Protease M, Protease P, *Umamizyme*, Peptidase R, Newlase A and Newlase F (koji mould-derived proteases, Amanoenzyme Co.); Sumizyme AP, Sumizyme LP, Sumizyme MP, Sumizyme FP and Sumizyme LPL (koji mould-derived proteases, Shin-nippon Chemical Industries, Co.); Protin FN (a koji mould-derived protease, Daiwa Kasei Industries, Co.); Denapsin 2P, Denazyme AP and XP-415 (koji mould-derived proteases, Nagase Chemtechs); Orientase 20A, Orientase ONS and Tetrase S (koji mould-derived proteases, Hankyu Bioindustries); Molsin F, PD Enzyme, IP Enzyme and AO Protease (koji mould-derived proteases, Kikkoman Co.); Sakanase, (a koji-mould-derived protease, Kaken Pharmaceuticals); Punchdase YP-SS, Punchdase NP-2 and Punchdase P (koji mould-derived proteases, Yakult Co.); Flavorzyme

(a koji mould-derived protease, Novozymes A/S); Kokulase SS, Kokulase P (koji mould-derived proteases, Sankyo Co.); VERON PS, COROLASE PN-L (koji mould-derived proteases, Röhm Enzyme Co.); Protease N, Protease NL, Protease S, Prolazer FG-F (bacteria-derived
 5 proteases, Amanoenzyme Co.); Protin P, Deskin, Depirace, Protin A, Thermoase (bacteria-derived proteases, Daiwa Kasei); Biopraser XL-416F, Biopraser SP-4FG, Biopraser SP-15FG (bacteria-derived proteases, Nagase Chemtechs); Orientase 90N, Nucleisin, Orientase 10NL; Orientase 22 BF (bacteria-derived proteases, Hankyu
 10 Bioindustries); Aloase AP-10 (bacteria-derived protease, Yakult Co.); Protamex, Neutrase, Alkalase (bacteria-derived proteases, Novozymes A/S); COROLASE N, COROLASE 7089, VERON W, VERON P (bacteria-derived proteases, Röhm Enzyme Co.); Enzyron NBS (bacteria-derived protease, Rakuto Kasei Industries); Alkali
 15 Protease GL 440, Purafect 4000L, Protease 899, Protex 6L (bacteria-derived proteases; Kyowa Enzyme Co.); Actinase AS, Actinase AF (actinomyces-derived proteases, Kaken Pharmaceuticals, Co.); Tasinase (actinomyces-derived protease, Kyowa Enzyme Co.); Papain W-40 (a vegetable-derived protease, Amanoenzyme Co.);
 20 Purified Papain for food (a vegetable-derived protease, Nagasechemtechs); other animal-derived pepsins, trypsins and the like can be named.

Use rate of these proteases differs depending on, e.g., activity of individual proteases and cannot be uniformly specified, but in
 25 general terms, for example, it may be within a range of 0.01 – 100 U/g, preferably 0.1 – 100 U/g, based on the weight of the starting tea material.

Above described enzymatic treatments of tea leaves can be conducted by those methods known per se, for example, by following
 30 the methods described in publications such as Official Gazette of the Patent Office, “Collection of Well Known Customary Technologies (flavor), Part 11, Food Spices”, The Japanese Patent Office (January 14, 2000) “2.1.7 Microorganisms · Enzyme Flavor” (pp. 46 – 57). An embodiment of the treatment is given hereafter as an example.

35 To 1 wt part of a tea material, 8 – 50 wt parts, preferably 10 –

30 wt parts, of water is added, sterilized at about 60 – about 121°C for about 2 seconds – about 20 minutes, cooled, and to which above-described tannase, a glycoside-hydrolysing enzyme and optionally protease are added, followed by the enzymatic treatment at
5 about 20 – about 60°C for about 30 minutes – about 24 hours. After the enzymatic treatment, the enzymes are deactivated by heating at about 60 – about 121 °C for about 2 seconds – about 20 minutes. Then the system is cooled and from which the tea leaves are separated by a suitable separation means such as centrifugation,
10 filtration through filter paper or the like, leaving clear tea extract.

Thus obtained tea extract may be converted into a forme of liquid concentrate by a suitable condensing means, for example, reduced pressure concentration, reverse osmotic membrane (RO membrane) concentration, freeze concentration and the like.
15 Furthermore, so obtained tea extract can be given an optional form such as paste, powder and the like, where desired.

Again, the tea extract obtained by the above-described method can be treated by flavor-enriching method such as, for example, steam distillation, solvent extraction, fat-extraction, membrane
20 concentration, resin adsorption, super critical extraction, concentration under reduced pressure and the like, to be given a form of enriched tea flavor. In particular, flavor-enriching by steam distillation method is convenient.

Steam distillation method comprises passing steam through
25 tea extract and condensing the flavor component distilling off as accompanied by the steam, together with the steam. For the steam distillation, any of distillation means such as steam distillation under pressure, ambient pressure or reduced pressure, or gas-liquid multicolumn alternating current contact distillation (spinning cone
30 column) can be adopted. More specifically, for example, steam is blown in from the bottom of steam distillation tank which is charged with tea extract as obtained in the above, and by cooling the distilled vapor with a cooler connected at the upper distilling side of the apparatus, the liquid distillate containing volatile flavor component
35 can be trapped as a condensate. Where necessary, a cold trap

charged with a cooling medium can be connected at the fore-end of this flavor-collecting device, which enables certain collection of volatile flavor component having still lower boiling point. Also when such steam distillation is conducted in the presence of an inert gas
5 such as nitrogen gas and/or an antioxidant such as vitamin C, deterioration of the flavor component under the heating can be effectively and conveniently prevented.

Thus obtained liquid distillate can be used for processed food as it is, or may further be concentrated by optional condensing means
10 to be given a form of flavor concentrate, where necessary. As such condensing means, for example, a method comprising adsorbing the liquid distillate onto a synthetic adsorbing agent and then eluting it using a suitable eluent, for example, ethanol, can be used. The synthetic adsorbing agent is subject to no special limitation and by
15 way of examples, styrene-divinylbenzene copolymer, ethylvinylbenzene-divinylbenzene copolymer, 2,6-diphenyl-9-phenyloxide polymer, methacrylic acid-diol polycondensation polymer, chemically bonded type silica gel (modified silica gel) obtained by chemically bonding to silica gel, for example,
20 alcohols, amines, silanes and the like, utilizing reactivity of silanol groups at the silica gel surface, and the like can be named. As preferred examples of such synthetic adsorbing agent, porous polymeric resins having a surface area of, for example, at least about 300 m²/g, preferably at least about 500 m²/g, and a pore diameter
25 distribution within a range of from about 10 Å to about 500 Å, in particular, from about 20 Å to about 200 Å can be named. As examples of commercially available porous polymeric resin meeting the above condition, HP resin (Mitsubishi Chemical), SP resin (Mitsubishi Chemical) and XAD-4 (Rohm & Haas) can be named.
30 Also methacrylic acid ester resins are available under the tradenames of, for example, XAD-7 and XAD-8 (Rohm & Haas).

As the treating means to adsorb the liquid distillate onto such a synthetic adsorbing agent, either a batch system or a column system can be utilized, while column system is preferred from the standpoint
35 of operability. As a method for the adsorption using a column system,

for example, the flavor component can be adsorbed by passing 10 – 1,000 volume times, preferably 20 – 500 volume times, the adsorbing agent of the liquid distillate through a column filled with an adsorbing agent as above-described, at a flow rate $SV = 1 - 100$, in particular, 2 – 50. Successively the adsorbing agent is washed with water, and thereafter 50 – 95 wt% aqueous ethanol solution is passed therethrough at a flow rate $SV = 0.1 - 10$, in particular, 0.2 – 5, to elute the flavor component adsorbed onto the adsorbing agent, to provide a water-soluble flavor concentrate.

As still other condensing means, for example, a method of extracting the liquid distillate with oils and fats, according to the accepted practice can be used. For oils and fats useful in that occasion there is no special limitation, and for example, any of vegetable oils and fats such as soybean oil, rice oil, sesame oil, peanut oil, corn oil, rape oil, coconut oil, palm oil and the like and their hydrogenated oils; animal oils and fats such as beef tallow, hog fat, fish oil and the like and their hardened oils; medium chain triglycerides (which hereafter may be referred to as MCT) and the like may be named. From the standpoint of stability of the flavor obtained, MCT can be named as the preferred example. As MCT, triglycerides of $C_6 - C_{12}$ medium-length chain fatty acids, such as caproic triglyceride, caprylic triglyceride, capric triglyceride, lauric triglyceride and their optional mixtures can be named. In particular, caprylic triglyceride, capric triglyceride and their optional mixtures are preferred. These MCT mixtures can be purchased from the market cheaply and easily.

Thus obtained tea extract and tea flavor can be used for beverage, in particular, tea drinks, milk beverage and functional beverage; and confectionery such as candies and cookies, cakes and jellies. They are also useful as perfume for cosmetics or toiletry goods and as skin lotion.

Hereinafter the present invention is more specifically explained, referring to working Examples and Comparative Examples.

Examples

Example 1

To 30 g of green tea leaves, 378 g of soft water (60°C) and 0.09 g
5 of sodium ascorbate were added and the system was sterilized by
heating to 80°C. After cooling the system to 40°C, 0.04 g of Tannase
(Kikkoman Co.), 0.1 g of Protease A (Amanoenzyme Co.), 5 units of
Emulsin (Sigma-Aldrich Co.) and 5 units of β -xylosidase
(Sigma-Aldrich Co.) were added, followed by 4 hours' standing at 40°C
10 to allow their reaction. Thereafter the tea leaves and the extract
were separated by filtration to provide 340 g of a green tea extract.

Comparative Example 1

Example 1 was repeated except that no enzyme was used, to
15 provide 341 g of a green tea extract.

(Flavor analysis)

Flavor analyses were conducted for those green tea extracts of
Example 1 and Comparative Example 1 by dynamic head space
20 method and solvent extraction method, to compare the flavor
difference brought about. Each of the analysis methods was as
follows.

Flavor analyzing method 1 (dynamic head space method)

25 Five (5) grams each of the samples as obtained in Example 1
and Comparative Example 1 was taken into a 500 mL two-neck flask,
and while maintaining the sample at 40°C, nitrogen gas was blown
into the sample from one of the necks through a capillary tube at a
flow rate of 50 mL/min, and the other neck was connected to a cooling
30 tube and an adsorbing agent (TENAX TA) at the fore-end of the tube,
allowing the flavor driven out of the sample to be adsorbed for 30
minutes. The adsorbing agent which adsorbed the flavor was heated
to release the flavor component in Thermo Desorption System (GERS
TEL Co.), and the components were subjected to a gas
35 chromatographic analysis under the following conditions.

Conditions for gas chromatographic analysis

Machine model: Hewlett-Packard Co. HP-6890

Column: Fused Silica Capillary

OV101 60 m × 0.25 mm

5 Column temp: 70 – 220°C (3°C/min.)

Injection temp.: 250°C

Detector temp.: 250°C

Carrier gas: N₂ 1.8 kg/cm²

10 Flavor analyzing method 2 (solvent extraction method)

In 300 g each of the samples as obtained in Example 1 and Comparative Example 1, 45 g of common salt was dissolved, and extracted three times with 105 mL of dimethylether. The organic layer was dried over magnesium sulfate and filtered. From each
15 filtrate the solvent was distilled off according to the accepted practice to provide a flavor concentrate. Thus obtained flavor concentrates were given a gas chromatographic analysis under the same conditions as employed in the flavor analyzing method 1.

Of the flavor components, those compounds which showed
20 particularly drastic increase in the flavor concentrates obtained upon the solvent extraction are shown in Table 1 by their names and their concentration levels.

TABLE 1

Components which showed particularly notable increase in flavor concentrate obtained by solvent extraction method and their concentration levels (ppb)

5

Compound	Comparative Example 1	Example 1
(3 <i>Z</i>)-hexenol	1.81	1432.35
(2 <i>E</i>)-hexenol	0.00	30.93
Hexanol	0.40	30.93
Geraniol	trace	135.31
Linalool	1.74	117.91
Linalool oxide (furanoid)	0.00	40.59
2-Phenylethanol	1.00	1080.55
Benzaldehyde	2.88	498.71
Benzyl alcohol	10.39	4735.85
Methyl salicylate	2.35	67.66

As shown in Table 1, compared with the green tea extract of Comparative Example 1, the amount of flavor did drastically increase in the green tea extract of Example 1.

10 The gas chromatogram obtained by the dynamic head space method is shown as Fig. 1, and that obtained by the solvent extraction method, as Fig. 2.

It can be understood from Fig. 1, when the flavor components are compared in terms of total integrated value on the gas chromatogram obtained from GC, GC-MS-measurements by the dynamic head space method, that the flavor component in Example 1 increased to about 2.6 times that of Comparative Example 1.

Also from Fig. 2 it can be understood that the yield of the flavor component in the flavor concentrate obtained by the extraction with the solvent from Example 1 extract was drastically higher, i.e., about 29 times that of the Comparative Example 1 extract.

Example 2

Example 1 was repeated except that 0.04 g of Tannase (Kikkoman Co.), 5 units of Emulsin (Sigma Aldrich Co.) and 5 units of β -xylosidase (Sigma Aldrich Co.) were used as the enzymes, to provide
5 338 g of a green tea extract.

Comparative Example 2

Example 1 was repeated except that 5 units of Emulsin (Sigma Aldrich Co.) and 5 units of β -xylosidase (Sigma Aldrich Co.) were used
10 as the enzymes, to provide 338 g of a green tea extract.

Comparative Example 3

Example 1 was repeated except that 0.04 g of Tannase (Kikkoman Co.) was used as the enzyme, to provide 337 g of a green
15 tea extract.

Comparative Example 4

Example 1 was repeated except that 0.04 g of Tannase (Kikkoman Co.) and 0.1 g of Protease A (Amanoenzyme Co.) were used
20 as the enzymes, to provide 339 g of a green tea extract.

(Functional evaluation)

Each of the extracts as obtained in Examples 1 and 2 and Comparative Examples 1 – 4 was given a gas chromatographic
25 analysis by the solvent extraction method. Also each extract was diluted by 10 times with ion-exchange water and given functional evaluation as to its flavor or relish, by ten well trained panelists. The result was as given in Table 2.

TABLE 2
Amount of flavor in the products of this invention and in control products and the products' functional evaluation

	Enzymatic treating conditions			Amount of flavor (ppb)	Functional evaluation
	tannase	glycoside-decomposing enzyme	protease		
Example 1	○	○	○	16394	Rather like roasted tea, intense green fresh flavor
Example 2	○	○	×	14550	Strong green fresh flavor
Comparative Example 1	×	×	×	565	Weak middle grade green tea-like flavor
Comparative Example 2	×	○	×	9656	Middle grade green tea-like note
Comparative Example 3	○	×	×	4672	Weak green note
Comparative Example 4	○	×	○	6886	Weak green note

As shown in Table 2, the products according to the present invention clearly contained greater amounts of flavor compared with those of Comparative Examples, and also had functionally excellent relish.

5

Example 3

Fifty (50) grams of Darjiling tea leaves and 750 g of soft water were mixed under stirring, heated to 80°C and thereafter cooled to 40°C. To the system 0.01 g of Tannase (Kikkoman Co.), 0.1 g of
10 Protease A (Amanoenzyme Co.) and 0.1 g of AROMAZYME (β -glucosidase, SHALIGAL Co.) were added and stirred at 40°C for 6 hours. The system was filtered through bleached cotton cloth to provide 690 g of the filtrate which was further sterilized at 90°C for 10 minutes, cooled to 40°C and centrifuged (centrifugal acceleration,
15 800G $\times$ 5 minutes). The supernatant was filtered using diatomaceous earth as the filter aid. Thus 660 g of clear filtrate (Bx 3.6°) was obtained.

Comparative Example 5

20 Example 3 was repeated except that no enzyme was used, to provide 620 g of black tea extract (Bx 2.5°).

Comparative Example 6

To 50 g of Darjiling tea leaves, 750 g of 100°C soft water was
25 added and mixed, followed by 5 minutes' standing and cooling to 40°C. The system was filtered through bleached cotton cloth to provide 645 g of the filtrate which was further sterilized at 90°C for 10 minutes, cooled to 40°C and centrifuged (centrifugal acceleration, 800G $\times$ 5 minutes). The supernatant was filtered using diatomaceous earth as
30 the filter aid. Thus 660 g of clear filtrate (Bx 1.5°) was obtained.

(Functional evaluation)

Flavor of the black tea extracts as obtained in Example 3 and Comparative Examples 5 and 6 were compared. The extracts were
35 each diluted to Bx 0.3° and their flavor was compared by five well

trained panelists. Each sample was evaluated by 5-grade system as to intensity of the flavor, flowery black tea note or taste and gorgeousness. The average points gained were as shown in Table 3.

5

TABLE 3

Functional evaluation of product of this invention and those of Comparative Examples

	Functional Evaluation			
	flavor intensity	flowery black tea note	gorgeous- ness	overall evaluation
Example 3 (product of this invention)	4.4	4.0	4.0	strong flavor, gorgeous, flowery
Comparative Example 5	3.6	3.0	2.4	modest flavor, lacking gorgeousness
Comparative Example 6	3.0	3.6	3.0	retaining black tea note but lacks strength

10 As shown in Table 3, Example 3 product of this invention had strong flavor and furthermore was gorgeous, exhibiting strong flavor inherent in the black tea, even as compared with Comparative Example 6 product. This strong and gorgeous flavor is considered to be attributable to the glycosides contained in the black tea leaves.

15

Example 4

A 3L-glass column was filled with 660 g of the extract as obtained in Example 3, and steam was fed into the column from its bottom (40 mesh wire net) under atmospheric pressure to conduct steam distillation. The steam containing the flavor as recovered from the column top was condensed in a cooling tube to provide 50 g (10% to the black tea) of an aqueous solution containing the flavor component.

20

Comparative Example 7

The extract (620 g) as obtained in Comparative Example 5 was treated in the manner similar to Example 4 to provide 50 g (10% to the black tea) of an aqueous solution containing the flavor component.

5

Comparative Example 8

The extract (660 g) as obtained in Comparative Example 6 was treated in the manner similar to Example 4 to provide 50 g (10% to the black tea) of an aqueous solution containing the flavor component.

10

(Example of flavoring canned black tea)

Ten (10) g of a black tea (BOP, Indonesian origin) was thrown into 300 g of 95°C hot water, and extracted for 3 minutes with occasional stirring, followed by solid-liquid separation by filtering through 200-mesh polyethylene filter cloth to provide 250 g of a black tea extract. After cooling the extract to 30°C, vitamin C and sodium bicarbonate were added thereto, followed by filtration. The filtrate was diluted to 1,000 g with water. To each 1,000 g of so prepared diluted tea extract, 0.2% of each flavor as prepared in Example 4 and Comparative Examples 7 and 8 was added and mixed, filled in 190 g-capacity cans and sterilized at 121°C for 10 minutes.

15

20

(Functional evaluation)

Above flavored black tea beverages were given functional evaluation by ten well trained panelists. The flavor or relish was evaluated, giving 5 points to non-flavor-added beverage and 10 points as the full mark. The result of the functional evaluation (total score given by the ten panelists) and characteristic relish of each were as shown in Table 4.

25

TABLE 4

Functional evaluation of product of this invention and those of Comparative Examples

	Functional Evaluation	
	point	Characteristics
no flavor addition	50	made to serve as Control, flat flavor unique to Indonesian tea
Example 4 (product of this invention)	85	floral note and body note inherent in Darjiling tea are imparted; natural and fresh mild flavor
Comparative Example 7	72	flavor strength increased but felt slightly astringent
Comparative Example 8	70	light flavor of the top note strengthened but body note insufficient as a whole

5

As shown in Table 4, the beverage to which the Example 4 product of the present invention was added could impart to the non-flavor-added product a light, fresh flavor as the top note well balanced with the body note. The product of the present invention also had strong flavor inherent in black tea and was clearly functionally excellent over the products of Comparative Examples 7 and 8.

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CLAIMS

1. Method for preparing tea extract with enhanced flavor, which is characterized by having glycoside-decomposing enzyme react with on tea leaves during and/or after treating the tea leaves with tannase.
2. The method as set forth in Claim 1, in which the glycoside-hydrolysing enzyme is at least one selected from β -glucosidase, β -primeverosidase and β -xylosidase.
3. The method as set forth in Claim 1 or 2, in which the use rate of the glucosidase-hydrolysing enzyme lies within a range of 0.001 – 10 U/g, based on the weight of the starting tea leaves.
4. The method as set forth in any one of Claims 1 – 3, in which the tannase treatment is carried out in the presence of one, two or more kinds of protease.
5. The method as set forth in Claim 4, in which the use rate of protease lies within a range of 0.01 – 100 U/g, based on the weight of the starting tea leaves.
6. The method as set forth in any one of Claims 1 – 5, in which the tea leaves are those of green tea, black tea or oolong tea.
7. The method as set forth in any of Claims 1 – 6, in which the use rate of tannase lies within a range of 0.1 – 50 U/g, based on the weight of the starting tea leaves.
8. A method for producing tea flavor, which comprises the first step of having glycoside-hydrolysing enzyme act on tea leaves during and/or after treating the tea leaves with tannase, and the second step of subjecting the tea extract which is obtained in the first step to flavor-condensing treatment to obtain a flavor concentrate.

9. The method as set forth in Claim 8, in which the flavor-condensing treatment is carried out by steam distillation.
10. The method as set forth in Claim 8 or 9, in which the liquid distillate obtained by the steam distillation is subjected to further flavor-condensation using a synthetic adsorbing agent.
11. The method as set forth in any one of Claims 8 – 10, in which the tea leaves are those of green tea, black tea or oolong tea.
12. Tea extract obtained by the preparation method as set forth in any one of Claims 1 – 7.
13. Tea flavor obtained by the preparation method as set forth in any one of Claims 8 – 11.