

DESCRIPTION

COMPOSITION FOR INHIBITING CARNOSINE DIPEPTIDASE

TECHNICAL FIELD

[0001] The present invention relates to a carnosine dipeptidase inhibiting composition. More particularly, this invention relates to a carnosine dipeptidase inhibiting composition comprising a particular compound such as polyphenol, use of a polyphenol and the like for inhibiting carnosine dipeptidases, and a method for inhibiting carnosine dipeptidases. This invention also relates to a composition comprising carnosine. More particularly, this invention relates to a composition comprising carnosine and a particular polyphenol.

BACKGROUND ART

[0002] Carnosine is a dipeptide composed of β -alanine and histidine, and found in high concentrations in muscle and nerve tissues of mammals such as humans. Carnosine is known to have various effects, including (1) proton buffering activity, (2) regulation of calcium secretion and calcium sensitivity, (3) antioxidant effect, (4) metal ion chelating effect, (5) acting as an extracellular histidine/histamine donor, (6) hyperglycemia ameliorating effect, (7) antiinflammatory effect, (8) skin wrinkle formation suppressing effect, and (9) promotion of recovery from fatigue. Carnosine is also reported to have other activities, including suppression of glycation end product production, suppression of cell death induced by cerebral ischemia, suppression of amyloid- β accumulation in Alzheimer's disease (AD) model mice, and immunomodulatory effect. As mentioned above, carnosine fulfills various functions in the living body, but degradation of carnosine by carnosine dipeptidases found in serum and different tissues prevents carnosine from exerting its functions.

[0003] It is known that there are two types of carnosine dipeptidases: serum carnosinase (carnosine dipeptidase 1; CNDP1) and tissue carnosinase (carnosine dipeptidase 2; CNDP2). Carnosine dipeptidases 1 and 2 are both known to have an activity to degrade dipeptides with a histidine residue at the carboxyl terminus (represented as X-His (where X is a naturally

occurring amino acid)) (NPL 1), and carnosine is one of those dipeptides degraded by these dipeptidases. Carnosine dipeptidases 1 and 2 differ in distribution in tissues and enzymatic properties, but are proteins sharing high homology with each other. Among them, carnosine dipeptidase 1 has been shown to be active only in the serum of higher primates (humans and larger monkeys), and not active in the serum of many other mammals (NPL 2).

[0004] With regard to inhibition of carnosine dipeptidase 1 activity, phenanthroline has for example been reported to act as an inhibitor for this peptidolytic enzyme (NPL 1). Also, it is known that while phenanthroline has carnosinase inhibitory activity, this inhibitor also has oral toxicity as a side effect. There have been almost no reports on other similar inhibitors, and very few carnosine degradation inhibitors focused on inhibition of carnosine dipeptidase 1 activity are presently known. With regard to carnosine dipeptidase 2, bestatin is for example known as an inhibitor for this enzyme (NPL 3). Also, β -alanine and derivatives thereof have been reported to be effective for inhibition of carnosine dipeptidase 2 (PTL 1).

[0005] Speaking of one of these enzymes, carnosine dipeptidase 1, diabetes-like symptoms, such as elevated fasting blood glucose and HbA1c levels at a younger age, and weight loss, were observed in db/db model mice transgenic for human carnosine dipeptidase 1 (NPL 4). Namely, it has been suggested that enhanced carnosine degradation by carnosine dipeptidase 1 may cause the onset of diseases. Therefore, inhibitors for carnosine dipeptidase 1 are considered as an approach to enhancing preventive efficacy against diabetes and various diseases caused by oxidative stress or glycation end product production, through effective delivery of L-carnosine into plasma, a target organ or any other organs.

[0006] It has been reported in some publications, including NPL 5, that the particular polymorphism ((CTG)_n) in the carnosine dipeptidase 1 gene is associated with susceptibility to developing diabetic nephropathy. In this connection, NPL 6 reported that homozygous (CTG)₅ recipients are at a low risk of developing diabetic nephropathy and have low carnosine dipeptidase 1 activity levels. Therefore, it is believed that suppressing carnosine dipeptidase 1 activity may be important in the maintenance of carnosine concentrations and effective for the prevention and treatment of associated diseases.

[0007] Further, a study of in vivo kinetics of carnosine after oral administration to humans has been reported in NPL 7. According to this literature, blood carnosine levels at different times after ingestion of 60 mg/kg carnosine varied considerably between subjects, and some subjects displayed no significant rise in blood carnosine levels as compared to those before ingestion (n = 17 of 25). The carnosine dipeptidase 1 activity levels and protein content in the group with increased carnosine levels were significantly lower than in the nonrespondent group. In light of the above, it is considered that suppression of carnosine dipeptidase 1 activity is highly likely to be effective for the maintenance of blood carnosine levels.

CITATION LIST

PATENT LITERATURE

[0008] PTL 1: International Patent Publication No. WO 2004/064866

NON-PATENT LITERATURES

[0009] NPL 1: *Molecules*, 2014, 19, 2299-2329

NPL 2: *Clin. Chim. Acta*, 1991, 196, 193-205.

NPL 3: *Biol. Chem. Hoppe Seyler*, 1988, 369, 1281-1286.

NPL 4: *Diabetes*, 2007, 56, 2425-2432. Epub 2007 Jun 29.

NPL 5: *Diabetes*, 2007, 56, 2410-2413.

NPL 6: *Diabetes*, 2005, 54, 2320-2327.

NPL 7: *Am. J. Physiol. Renal. Physiol.*, 2012, 302(12), F1537-F1544.

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0010] Since, as mentioned above, carnosine dipeptidases have various impacts in the body of mammals, particularly humans, it is very important to develop a technique for effectively inhibiting the activity of these enzymes. Also, it is known that while phenanthroline has carnosinase inhibitory activity, this inhibitor also has oral toxicity as a side effect. That being so, there is a strong desire that compositions for inhibiting carnosine dipeptidases should not only be capable of effectively inhibiting the activity of these enzymes but also be highly safe. Further, it is expected that the actions and effects of carnosine can be further

enhanced by combining carnosine with such a composition having carnosine dipeptidase inhibitory effect, since this combination leads to suppression of carnosine degradation in the composition.

[0011] Objects of the present invention consist in providing a carnosine dipeptidase inhibiting composition that is high in biological safety and contributes to the maintenance of the body's carnosine levels, and providing a composition that is high in biological safety and is capable of enhancing the pharmacological effects of carnosine through the use of carnosine dipeptidase inhibitory effect. Another object of this invention consists in providing use of such a composition for inhibiting carnosine dipeptidases, a method for inhibiting carnosine dipeptidases, and so forth.

SOLUTION TO PROBLEM

[0012] The present inventors have made intensive studies to achieve the aforementioned objects, and as a result found for the first time that polyphenols have carnosine dipeptidase inhibitory activity. The inventors also found that compositions comprising a particular polyphenol having the aforementioned activity and carnosine are novel. On the basis of these findings, the inventors have completed the present invention.

[0013] More specifically, the present invention is directed but not limited to the following.

- (1) A carnosine dipeptidase inhibiting composition comprising a polyphenol.
- (2) The composition as set forth in (1), wherein the polyphenol is selected from the group consisting of a flavonoid, a curcuminoid, a stilbenoid, a phenylethanoid, and a phenylpropanoid.
- (3) The composition as set forth in (2), wherein the flavonoid is selected from the group consisting of quercetin, myricetin, kaempferol, luteolin, a catechin compound, and apigenin.
- (4) The composition as set forth in (2), wherein the curcuminoid is curcumin.
- (5) The composition as set forth in (2), wherein the stilbenoid is resveratrol or piceatannol.
- (6) The composition as set forth in (2), wherein the phenylethanoid is hydroxytyrosol.
- (7) The composition as set forth in (2), wherein the phenylpropanoid is caffeic acid or ferulic acid.

- (8) The composition as set forth in any of (1) to (7), wherein the composition is intended for use in the prevention or amelioration of fatigue, skin wrinkle formation, a decline in cognitive function, diabetes, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism.
- (9) The composition as set forth in any of (1) to (8), wherein the composition is an agent.
- (10) The composition as set forth in any of (1) to (9), wherein the composition bears a claim regarding a function exerted through carnosine dipeptidase inhibition.
- (11) A composition comprising a polyphenol and carnosine, wherein the polyphenol is piceatannol or hydroxytyrosol.
- (12) The composition as set forth in (11), wherein the composition is intended for use in inhibition of carnosine dipeptidase activity.
- (13) The composition as set forth in (11) or (12), wherein the composition is intended for use in enhancement of a pharmacological effect of carnosine.
- (14) The composition as set forth in (13), wherein the pharmacological effect of carnosine is an anti-fatigue effect, a preventive or ameliorative effect on skin wrinkle formation, a cognitive function maintenance effect, a diabetes preventive or ameliorative effect, a suppressive effect on a decline in immune function, a preventive or ameliorative effect on blood vessel or tissue inflammation, an ameliorative effect on various diseases caused by oxidative stress or glycation end product production, a preventive or ameliorative effect on Alzheimer's disease, or a preventive or ameliorative effect on autism.
- (15) The composition as set forth in any one of (11) to (14), wherein the composition bears a claim regarding a function exerted through the pharmacological effect of carnosine.
- (16) The composition as set forth in (10) or (15), wherein the claim regarding a function is selected from the group consisting of "makes your body harder to get tired", "facilitates recovery from body fatigue", "prevents skin wrinkle formation", "ameliorates skin wrinkles and sags", "assists in beauty care", "suppresses a decline in cognitive function", "potentially helps maintain cognitive function", "suppresses a rise in blood glucose levels", "enhances

immune function”, “potentially exerts an antioxidative effect”, “reduces oxidative stress”, “potentially exerts an antiglycative effect”, “reduces glycative stress”, “suppresses blood vessel inflammation”, “potentially prevents or ameliorates Alzheimer’s disease”, and “potentially prevents or ameliorates autism”.

(17) The composition as set forth in any of (1) to (16), wherein the composition is in the form of solid or liquid.

(18) The composition as set forth in any of (1) to (17), wherein the composition is a food or beverage composition.

(19) Use of a polyphenol for inhibiting a carnosine dipeptidase.

(20) A method for inhibiting a carnosine dipeptidase using a polyphenol.

ADVANTAGEOUS EFFECT OF INVENTION

[0014] The present invention can provide a composition having excellent carnosine dipeptidase inhibitory effect. This invention can also provide a composition characterized in that the actions and effects of carnosine are enhanced through the use of carnosine dipeptidase inhibitory effect. Both of these compositions can exert a retarding effect on carnosine degradation through suppression of carnosine dipeptidase function, and thus enable effective delivery of higher concentrations of carnosine into plasma, a target organ or any other organs. Further, the carnosine-containing compositions of this invention can retard the degradation of not only carnosine contained in the compositions but also carnosine already present in the body.

[0015] As described above, the compositions of the present invention are all capable of enhancing the actions and effects of carnosine, and thus can be effective to enhance the known pharmacological effects inherent in carnosine (*i.e.*, preventive or ameliorative effects on a decline in cognitive function associated with schizophrenia, etc., diabetes, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress, Alzheimer’s disease, and autism). Further, since the compositions of this invention have carnosine dipeptidase inhibitory activity, said compositions can be used for the purpose of suppressing the degradation of not only carnosine but also all kinds of dipeptides having a

histidine residue at the carboxyl terminus (represented as X-His (where X is a naturally occurring amino acid)). Furthermore, since polyphenols that can be incorporated in the compositions of this invention are natural compounds occurring in plants, etc. and many of them can be used as food ingredients, it is considered that the compositions of this invention are high in safety and have significantly fewer side effects than conventional pharmaceutical products.

DESCRIPTION OF EMBODIMENTS

[0016] 1. Carnosine dipeptidase inhibiting composition

One aspect of the present invention is directed to a carnosine dipeptidase inhibiting composition comprising a polyphenol.

[0017] 1-1. Polyphenol(s)

As used herein, the term “polyphenol(s)” refers to a compound(s) having multiple phenolic hydroxyl groups (hydroxyl groups each attached to an aromatic hydrocarbon group such as benzene or naphthalene ring). Glycosides of such compounds are also included by the “polyphenol(s)”.

[0018] Examples of the polyphenol(s) to be incorporated in the present invention include, but are not particularly limited to, flavonoids, curcuminoids, stilbenoids, phenylethanoids, phenylpropanoids, or glycosides thereof.

[0019] Examples of flavonoids include, but are not limited to, flavonols, flavanols, flavones, flavanones, isoflavones, and anthocyanidins. Among them, flavonols, flavanols, and flavones are preferred in this invention. Specific examples of flavonols include, but are not limited to, quercetin, myricetin, azaleatin, fisetin, kaempferol, rhamnazin, and rhamnezin. Specific examples of flavanols include, but are not limited to, catechin compounds (including catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, and the like) and theaflavins. Specific examples of flavones include, but are not limited to, apigenin, luteolin, tangeretin, geosmin, and flavoxate. Specific examples of flavanones include, but are not limited to, eriodictyol, hesperetin, homoeriodictyol, and naringenin. Specific examples of isoflavones include, but are not limited to, coumestrol, daidzein,

daidzin, and genistein. Specific examples of anthocyanidins include, but are not limited to, cyanidin, delphinidin, malvidin, pelargonidin, and peonidin.

[0020] Specific examples of curcuminoids include, but are not limited to, curcumin, dimethoxycurcumin, and bisdimethoxycurcumin. Specific examples of stilbenoids include, but are not limited to, resveratrol, piceatannol, pinosylvin, and pterostilbene.

[0021] Specific examples of phenylethanoids include, but are not limited to, tyrosol and hydroxytyrosol.

[0022] Specific examples of phenylpropanoids include, but are not limited to, caffeic acid, ferulic acid, coumaric acid, cinnamic acid, lignin, and lignan.

[0023] As mentioned above, glycosides of the different compounds can also be used in the present invention. As used herein, the term “glycoside(s)” refers to a compound(s) formed by joining a hydroxyl group of a sugar to a non-carbohydrate compound. The sugar found in glycosides can be a monosaccharide or a di- or higher saccharide. The type of the sugar is also not particularly limited, and examples include, but are not limited to, aldoses such as glucose, mannose, galactose, fucose, rhamnose, arabinose and xylose, ketoses such as fructose, uronic acids such as glucuronic acid, galacturonic acid and mannuronic acid, as well as apiose and rutinose. The sugar used in glycosides can be a D-sugar or an L-sugar. Specific examples of glycosides of polyphenols include, but are not limited to, rutin, quercitrin, isoquercitrin, hyperoside, spiraeoside, myricitrin, azalein, astragalin, kaempferitrin, robinin, amurensin, apiin, daidzin, hesperidin, and naringin.

[0024] Among the examples of polyphenols listed above, quercetin is preferably used in the present invention. Quercetin, which may also be designated in different ways in Japanese, is a type of flavonols typically found in many plants such as citrus fruits, onions and buckwheat. Quercetin is also called “2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one” and registered under CAS Registry No. 117-39-5. Examples of quercetin glycosides include, but are not limited to, rutin, quercitrin, isoquercitrin, hyperoside, and spiraeoside.

[0025] Another polyphenol component preferred in the present invention is myricetin.

Myricetin is a type of flavonols typically found in fruits such as grape and berries, vegetables, and herbs. Myricetin is also called “3,3’,4’,5,5’,7-hexahydroxyflavone” and registered under CAS Registry No. 529-44-2. Examples of myricetin glycosides include, but are not limited to, myricitrin and myricetin 3-O-rutinoside.

[0026] Yet another polyphenol component preferred in the present invention is kaempferol. Kaempferol is a type of flavonols typically found in plants such as tea leaves, broccoli, cabbage and kale. Kaempferol is also called “3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one” and registered under CAS Registry No. 520-18-3.

[0027] Yet another polyphenol component preferred in the present invention is luteolin. Luteolin is a type of flavones typically found in plants such as celery, bell pepper, and *Perilla frutescens* var. *crispa*. Luteolin is also called “2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromenone” and registered under CAS Registry No. 491-70-3.

[0028] Yet another polyphenol component preferred in the present invention is a catechin compound. Catechin compounds are a type of flavanols typically found in abundance in tea leaves such as green tea and oolong tea. Specific examples of catechin compounds include, but are not limited to, catechin (CAS Registry Nos. 7295-85-4, 18829-70-4, 154-23-4), epicatechin (CAS Registry Nos. 490-46-0, 35323-91-2), gallocatechin (CAS Registry Nos. 3371-27-5, 970-73-0), epigallocatechin (CAS Registry No. 970-74-1), catechin gallate (CAS Registry No. 130405-40-2), epicatechin gallate (CAS Registry No. 1257-08-5), gallocatechin gallate (CAS Registry No. 4233-96-9) and epigallocatechin gallate (CAS Registry No. 989-51-5), with epigallocatechin gallate being particularly preferred.

[0029] Yet another polyphenol component preferred in the present invention is apigenin. Apigenin is a type of flavones typically found in vegetables such as celery and parsley. Apigenin is also called “5,7-dihydroxy-2-(4-hydroxyphenyl)-4-benzopyrone” and registered under CAS Registry No. 520-36-5. Examples of apigenin glycosides include, but are not limited to, apiin, apigetrin, vitexin, isovitexin, and rhoifolin.

[0030] Yet another polyphenol component preferred in the present invention is curcumin. As mentioned above, curcumin is a type of curcuminoids typically found in turmeric.

Curcumin is also called “(E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione” and registered under CAS Registry No. 458-37-7.

[0031] Yet another polyphenol component preferred in the present invention is resveratrol. As mentioned above, resveratrol is a type of stilbenoids typically found in grape skins.

Resveratrol is also called “(E)-5-(p-hydroxystyryl)resorcinol” or “(E)-5-(4-hydroxystyryl)benzene-1,3-diol” and registered under CAS Registry No. 501-36-0.

[0032] Piceatannol is a member of the group of compounds called stilbenoids, and typically found in plants such as Norway spruce (*Picea abies*) and coyure palm (*Aiphanes horrida*). Piceatannol is also called “3’,4’,3,5-tetrahydroxy-trans-stilbene astringinin” and registered under CAS Registry No. 10083-24-6. Examples of piceatannol glycosides include, but are not limited to, piceatannol-3’-O- β -D-glucopyranoside, and piceatannol-4’-O- β -D-glucopyranoside.

[0033] Yet another polyphenol component preferred in the present invention is ferulic acid. Ferulic acid is a type of cinnamic acid derivatives widely found in various plants such as rice, wheat and barley. Ferulic acid is also called “(E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid” and registered under CAS Registry No. 1135-24-6. γ -Oryzanols found in rice bran lipids are a generic term for esters formed by condensation between ferulic acid and sterols. It has been known that oral ingestion of γ -oryzanol results in a rise in blood levels of ferulic acid, a degradation product of γ -oryzanol.

[0034] Yet another polyphenol component preferred in the present invention is caffeic acid. Caffeic acid is a type of cinnamic acid derivatives widely found in plants. Caffeic acid is also called “(E)-3-(3,4-dihydroxyphenyl)-2-propenoic acid” and registered under CAS Registry No. 331-39-51. Chlorogenic acid, which is abundant in coffee beans, is a compound having the structure in which the carboxyl group of caffeic acid is condensed by dehydration with the hydroxyl group at position 3 of quinic acid. It has been known that oral ingestion of chlorogenic acid results in a rise in blood levels of caffeic acid, a degradation product of chlorogenic acid.

[0035] Yet another polyphenol component preferred in the present invention is

hydroxytyrosol. Hydroxytyrosol is a type of phenylethanoids typically found in plants such as olive leaves. Hydroxytyrosol is also called “4-(2-hydroxyethyl)-1,2-benzenediol” and registered under CAS Registry No. 10597-60-1. It has been known that oleuropein, which is typically found in olive leaves, is hydrolyzed or metabolized to produce hydroxytyrosol. [0036] In the present invention, one type of polyphenols, including the compounds listed hereinabove, may be used alone, or two or more types thereof may be used in combination. Polyphenols may be extracted from natural products such as plants and fruits using water (including hot water), an organic solvent or the like, or may be chemically synthesized. Extraction (including isolation, purification, etc.) or synthesis of polyphenols can be carried out using a method known to skilled artisans depending on, for example, the type of compound. A polyphenol prepared on one’s own or a commercially available one may be used. What is preferably used in this invention is, without particular limitation, a commercially available one. Also, a plant extract containing such a polyphenol as mentioned above may be used. The form of the polyphenol to be used is not particularly limited, and the polyphenol may be in free form or in the form of a solvate such as hydrate or ethanolate. Where the active component is exposed to blood, an ester of the active component and other components may be used, such as chlorogenic acid, γ -oryzanol or oleuropein. Further, a polyphenol-containing extract can be advantageously used.

[0037] 1-2. Other active components

1-2-1. Pentacyclic triterpenic acid(s)

In the present invention, a pentacyclic triterpenic acid may be incorporated in the carnosine dipeptidase inhibiting composition. The type of the pentacyclic triterpenic acid is not particularly limited, and examples include, but are not limited to, ursolic acid, maslinic acid, corosolic acid, betulinic acid and oleanolic acid. In this invention, ursolic acid is preferably used.

[0038] Ursolic acid, which may also be designated in a different way in Japanese, is found in many plants including apple, basil, bilberry, cranberry, elderflower, peppermint, rosemary, lavender, oregano, thyme, hawthorn, and prune. In particular, apple peels are rich in ursolic

acid. Ursolic acid is also called “3 β -hydroxy-12-ursen-28-oic acid” and registered under CAS Registry No. 77-52-1.

[0039] In the present invention, one type of pentacyclic triterpenic acid may be used alone, or two or more types thereof may be used in combination. Pentacyclic triterpenic acids may be extracted from natural products such as plants and fruits using water (including hot water), an organic solvent or the like, or may be chemically synthesized. Extraction (including isolation, purification, etc.) or synthesis of pentacyclic triterpenic acids can be carried out using a method known to skilled artisans depending on, for example, the type of compound. A pentacyclic triterpenic acid prepared on one's own or a commercially available one may be used. What is preferably used in this invention is a commercially available one. The form of the pentacyclic triterpenic acid to be used is not particularly limited, and the pentacyclic triterpenic acid may be in free form or in the form of a solvate such as hydrate or ethanolate.

[0040] 1-2-2. Anserine

In the present invention, anserine may be incorporated in the carnosine dipeptidase inhibiting composition. Anserine is a dipeptide found in the skeletal muscle and brain of mammals and birds. Since anserine is a dipeptide formed through a peptide linkage between β -alanine and 1-methyl-L-histidine, this dipeptide is also called “ β -alanyl-N-methylhistidine” or “ β -alanyl-1-N-methylhistidine”. Anserine is registered under CAS Registry No. 584-85-0.

[0041] Anserine may be isolated from animal tissues or may be chemically synthesized. Isolation or synthesis of anserine can be carried out using a method known to skilled artisans. As anserine, a product prepared on one's own or a commercially available one may be used. What is preferably used in the present invention is a commercially available one. The form of anserine to be used is not particularly limited, and anserine may be in the form of a salt. Examples of anserine salts include, but are not limited to, anserine hydrochloride, anserine nitrate, and anserine acetate.

[0042] 1-3. Carnosine dipeptidase(s) and carnosine dipeptidase inhibition

As used herein, the term “carnosine dipeptidase(s)” refers to a carnosine-degrading

enzyme(s) having a capability of degrading carnosine (L-carnosine) into β -alanine and histidine. The carnosine dipeptidase (carnosine-degrading enzyme) can be abbreviated as CNDP and is also called “carnosinase” or “carnosidase”. There are two types of carnosine dipeptidases: carnosine dipeptidase 1 (CNDP1), a serum carnosine-degrading enzyme; and carnosine dipeptidase 2 (CNDP2), a tissue carnosine-degrading enzyme. Among them, the carnosine dipeptidase to be inhibited in the present invention is preferably carnosine dipeptidase 1. Carnosine dipeptidases 1 and 2 are both known to have an activity to degrade not only carnosine but also all kinds of dipeptides having a histidine residue at the carboxyl terminus (represented as X-His (where X is a naturally occurring amino acid)).

[0043] As referred to herein, “carnosine dipeptidase inhibition (inhibiting)” refers to inhibition of the carnosine degradation activity of a carnosine dipeptidase. Carnosine dipeptidase inhibitory effect can be evaluated according to a known method. For example, when carnosine and a carnosine dipeptidase are brought into contact with each other, histidine is produced from carnosine, so that fluorescence specific to histidine can be determined by the presence of histidine. Thus, by observing a decrease in fluorescence intensity, carnosine dipeptidase inhibitory effect can be evaluated.

[0044] 1-4. Effects

As mentioned above, by inhibiting a carnosine dipeptidase, the levels of carnosine degraded by this enzyme in the body of mammals such as humans can be maintained, or a decline in carnosine levels can be suppressed. Carnosine has various effects, including: proton buffering activity; regulation of calcium secretion and calcium sensitivity; antioxidant effect; metal ion chelating effect; acting as an extracellular histidine/histamine donor; hyperglycemia ameliorating effect; antiinflammatory effect; promotion of recovery from fatigue; skin wrinkle formation suppressing effect; suppression of glycation end product production; suppression of cell death induced by cerebral ischemia; suppression of amyloid- β accumulation in Alzheimer’s disease (AD) model mice; and immunomodulatory effect. Therefore, the following effects can be expected: maintenance of high carnosine levels in the body leads to preventive or ameliorative effects on a decline in cognitive function associated

with schizophrenia, etc., or on Alzheimer's disease or autism; amelioration of hyperglycemia leads to preventive or ameliorative effects on diabetes or various diseases caused by oxidative stress or glycation end product production; reduction of inflammation leads to a preventive or ameliorative effect on blood vessel or tissue inflammation; and immune modulation leads to a preventive or ameliorative effect on a decline in immune function.

[0045] The content of a polyphenol or other active component in the carnosine dipeptidase inhibiting composition of the present invention is not particularly limited, as long as said content is adjusted to a level sufficient to achieve the desired effects of this invention, in consideration of *e.g.* the form and mode of administration. For example, the content of a polyphenol in the carnosine dipeptidase inhibiting composition of this invention can be set to not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. Also, the upper limit of this content can be set to, for example, not more than 50 wt.%, not more than 70 wt.%, or not more than 90 wt.%. The carnosine dipeptidase inhibiting composition of this invention can be in various forms, such as solid (*e.g.*, tablet) and liquid (*e.g.*, beverage packed in PET bottle). In particular, when the carnosine dipeptidase inhibiting composition of this invention is in solid form, the polyphenol content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the carnosine dipeptidase inhibiting composition of this invention is in liquid form, the polyphenol content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0046] When quercetin, myricetin, kaempferol, luteolin, apigenin, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, curcumin, resveratrol, piceatannol, hydroxytyrosol, ferulic acid, or caffeic acid is used as a polyphenol, the content of such a compound in the carnosine dipeptidase inhibiting composition of this invention can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. Also, the upper limit of the content of quercetin, myricetin, kaempferol, luteolin, apigenin, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, curcumin,

resveratrol, piceatannol, hydroxytyrosol, ferulic acid, or caffeic acid in the composition can be set to, for example, not more than 50 wt.%, not more than 70 wt.%, or not more than 90 wt.%. In particular, when the carnosine dipeptidase inhibiting composition of this invention is in solid form, the content of quercetin, myricetin, kaempferol, luteolin, apigenin, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, curcumin, resveratrol, piceatannol, hydroxytyrosol, ferulic acid, or caffeic acid in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the carnosine dipeptidase inhibiting composition of this invention is in liquid form, the content of quercetin, myricetin, kaempferol, luteolin, apigenin, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, curcumin, resveratrol, piceatannol, hydroxytyrosol, ferulic acid, or caffeic acid in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0047] When a pentacyclic triterpenic acid is used in the carnosine dipeptidase inhibiting composition of the present invention, the content of this component in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. Also, the upper limit of this content can be set to, for example, not more than 50 wt.%, not more than 70 wt.%, or not more than 90 wt.%. In particular, when the carnosine dipeptidase inhibiting composition of this invention is in solid form, the pentacyclic triterpenic acid content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the carnosine dipeptidase inhibiting composition of this invention is in liquid form, the pentacyclic triterpenic acid content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0048] When ursolic acid is used as a pentacyclic triterpenic acid, the ursolic acid content in the carnosine dipeptidase inhibiting composition of the present invention can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. Also, the upper limit of the ursolic acid content in the composition can

be set to, for example, not more than 50 wt.%, not more than 70 wt.%, or not more than 90 wt.%. In particular, when the carnosine dipeptidase inhibiting composition of this invention is in solid form, the ursolic acid content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the carnosine dipeptidase inhibiting composition of this invention is in liquid form, the ursolic acid content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0049] When anserine is used in the carnosine dipeptidase inhibiting composition of the present invention, the anserine content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. Also, this content can be set to, for example, not more than 50 wt.%, not more than 70 wt.%, or not more than 90 wt.%. In particular, when the carnosine dipeptidase inhibiting composition of this invention is in solid form, the anserine content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the carnosine dipeptidase inhibiting composition of this invention is in liquid form, the anserine content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0050] Unless otherwise specified, the term "wt.%" as used herein refers to "weight/weight percent (w/w%)". When any of the components is in the form of a salt, hydrate or the like, the content of this component shall be calculated in terms of its free form.

[0051] The content of a polyphenol or other active component can be measured by following a method known to skilled artisans -- for example, using LC-MS/MS, HPLC or the like under appropriately designed conditions depending on the types of components, etc.

[0052] 1-5. Other components

The carnosine dipeptidase inhibiting composition of the present invention may, depending on its form, contain any additives and/or any other commonly used components, in addition to the aforementioned components. Examples of such additives and/or common

components include, but are not limited to, physiologically active components, including vitamins such as vitamin E and vitamin C, minerals, nutrients, and flavorants, as well as other components added to make pharmaceutical preparations, such as excipients, binders, emulsifiers, isotonicizers, buffers, solubilizers, antiseptics, stabilizers, antioxidants, colorants, coagulants, and coating agents.

[0053] 1-6. Intended use

The carnosine dipeptidase inhibiting composition of the present invention is characterized by comprising a polyphenol or other active component as mentioned above, and thus it is expected that when the activity of a carnosine dipeptidase is inhibited by such a component, the levels of carnosine degraded by this enzyme in the body can be maintained at high levels, or a decline in carnosine levels can be suppressed. Effective prevention or amelioration of a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism can be achieved by maintenance of high levels of carnosine in the body. Therefore, the composition of this invention is a carnosine dipeptidase inhibiting composition intended for use in the prevention or amelioration of a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism. Based on these intended uses, the carnosine dipeptidase inhibiting composition of this invention can serve as a composition for use in the prevention or amelioration of a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism. Additionally, the term "prevention or amelioration" as used herein encompasses both of the concepts of "making the current state better" and "preventing the current state from getting worse"; thus, the terms "treatment", "restoration", "alleviation", "mitigation" and the like can also be included by this term.

[0054] The carnosine dipeptidase inhibiting composition of the present invention, which has an activity to inhibit carnosine dipeptidase 1 and/or carnosine dipeptidase 2, can also be used for the purpose of suppressing the degradation of not only carnosine but also all kinds of dipeptides having a histidine residue at the carboxyl terminus (represented as X-His (where X is a naturally occurring amino acid)).

[0055] The carnosine dipeptidase inhibiting composition of the present invention is provided in various forms, including but not particularly limited to, solid or liquid. Examples of solid compositions include, but are not limited to, tablets (including coated tablets), granules, powders, powdered drugs, and capsules; and the carnosine dipeptidase inhibiting composition of this invention can be formulated into such a solid pharmaceutical preparation according to a known method and can be provided as such. Examples of liquid compositions include, but are not limited to, oral solutions, suspensions, emulsions, syrups, and drinkable preparations; and the carnosine dipeptidase inhibiting composition of this invention can be formulated into such a liquid pharmaceutical preparation according to a known method and can be provided as such. Further, the carnosine dipeptidase inhibiting composition of this invention can be made into beverages (including beverages packed in PET bottle, soft drinks, and tea beverages), dairy products such as yogurt and lactobacillus beverages, seasonings, processed foods, desserts, confectionaries (*e.g.*, gum, candy, jelly), or the like. Such compositions can be taken directly or with water or the like. Furthermore, the carnosine dipeptidase inhibiting composition of this invention, after being prepared into an easy-to-mix form (*e.g.*, powder or granule form), can be used as an ingredient for *e.g.* pharmaceuticals, foods and beverages, but this is not the sole form of the carnosine dipeptidase inhibiting composition.

[0056] The carnosine dipeptidase inhibiting composition of the present invention can be provided in the form of, for example but not limited to, an agent. Such an agent can be provided directly as a composition or can be provided as a composition comprising said agent. In this invention, examples of such compositions include, but are not limited to, pharmaceuticals (pharmaceutical compositions), foods and beverages (including foods,

beverages, food and beverage compositions, food compositions, beverage compositions), and cosmetics (cosmetic compositions). Non-limiting examples of food compositions include, but are not limited to, functional foods, health supplements, foods with nutrient function claims, foods for special dietary uses, foods for specified health uses, nutritional supplements, dietetic foods, health foods, dietary supplements, and food additives.

[0057] The carnosine dipeptidase inhibiting composition of the present invention can be applied to both of therapeutic use (medical use) and non-therapeutic use (non-medical use). To be specific, the carnosine dipeptidase inhibiting composition of this invention can be used as a pharmaceutical, a quasi-drug, a cosmetic or the like, and also can be used as any other products that do not belong to any of the above groups under the Pharmaceutical Affairs Law, for example, as a composition that explicitly or implicitly appeals for a preventive or ameliorative effect on a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism.

[0058] In another aspect, the present invention relates to such a carnosine dipeptidase inhibiting composition as mentioned above which bears a label regarding a function exerted through carnosine dipeptidase inhibition. Examples of such a claim or function claim include, but are not particularly limited to, "makes your body harder to get tired", "facilitates recovery from body fatigue", "prevents skin wrinkle formation", "ameliorates skin wrinkles and sags", "assists in beauty care", "suppresses a decline in cognitive function", "potentially helps maintain cognitive function", "suppresses a rise in blood glucose levels", "enhances immune function", "potentially exerts an antioxidative effect", "reduces oxidative stress", "potentially exerts an antiglycative effect", "reduces glycative stress", "suppresses blood vessel inflammation", "potentially prevents or ameliorates Alzheimer's disease", and "potentially prevents or ameliorates autism", and other claims or function claims that can be regarded as being equivalent thereto. In this invention, such a claim or function claim may be borne by the composition *per se* or by a container or package of the composition.

[0059] The carnosine dipeptidase inhibiting composition of the present invention can be ingested by a suitable method depending on its form. The ingestion method is not particularly limited as long as the method permits the active component(s) of this invention to be transferred into the circulating blood. The carnosine dipeptidase inhibiting composition can be made into the form of, for example but not limited to, a solid preparation for oral use, a liquid preparation for oral use such as internal liquid agent or syrup, or a preparation for parenteral use such as injectable preparation, external preparation, suppository or percutaneous absorption preparation. Additionally, as used herein, the term “ingest(ion)” includes all modes of consumption, such as ingestion, taking or drinking.

[0060] The dose of the carnosine dipeptidase inhibiting composition of the present invention is not fixed and is determined as appropriate depending on the form and mode of administration, intended use, and the age, body weight and symptom of a patient or affected animal to be treated. The effective human intake of the composition of this invention is also not fixed and is not particularly limited. When the composition of this invention comprises a polyphenol as an active component, the effective human intake of the composition in terms of the weight of the polyphenol is for example not less than 10 mg, preferably not less than 30 mg, per 50 kg of human body weight per day. When the composition comprises a pentacyclic triterpenic acid as an active component, the effective human intake of the composition in terms of the weight of the pentacyclic triterpenic acid is for example not less than 10 mg, preferably not less than 30 mg, per 50 kg of human body weight per day. When the composition comprises anserine as an active component, the effective human intake of the composition in terms of the weight of anserine is for example not less than 10 mg, preferably not less than 30 mg, per 50 kg of human body weight per day. The composition of this invention can be administered in a dosage within the desired range in a single dose or divided doses per day. The duration of administration of the composition of this invention is also not fixed. Additionally, the effective human intake of the composition of this invention refers to the dosage of said carnosine dipeptidase inhibiting composition required for said composition to exhibit a beneficial effect in a human, and the type of an active

component to be included in the composition is not particularly limited by its effective human intake.

[0061] The subject to which the carnosine dipeptidase inhibiting composition of the present invention is applied is preferably a human, but may be a livestock animal such as cow, horse or goat, a companion animal such as dog, cat or rabbit, or a laboratory animal such as mouse, rat, guinea pig or monkey. When the composition of this invention is administered to an animal other than a human, the daily consumption dose of the composition in said animal, calculated based on the dose of about 20 g per mouse, varies with conditions such as the active component content in the composition, and the state, body weight, sex and age of the subject to which the composition is administered.

[0062] 1-7. Combination (combined use) with carnosine

The polyphenol or other active component as mentioned above can be used in combination with carnosine. Therefore, in one aspect, the present invention can provide a composition comprising a polyphenol or other active component as mentioned above in combination with carnosine (hereinafter also referred to as “the combined composition of the present invention”).

[0063] It can be expected that when such an active component is used in combination with carnosine, degradation of carnosine by a carnosine dipeptidase is retarded through the carnosine dipeptidase inhibitory effect of said active component and carnosine is effectively delivered into a target tissue or organ. It can be expected that combined use of the carnosine dipeptidase inhibiting composition of this invention with carnosine, aside from carnosine inherently present in the body, enables maintenance of higher carnosine levels in the body and effective enhancement of the effects of carnosine.

[0064] Since the combined composition of the present invention comprises a polyphenol or other active component as mentioned above, said combined composition can serve as a carnosine dipeptidase inhibiting composition. Also, since the combined composition of this invention is capable of enhancing the actions and effects of carnosine, said combined composition is preferred to be used for the intended purposes described above. In other

words, the combined composition of this invention is preferably a composition for use in the prevention or amelioration of a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism.

[0065] The combined composition of the present invention, like in the case of the aforementioned carnosine dipeptidase inhibiting composition, can be provided in the form of, for example but not particularly limited to, an agent (mixed agent). The combined composition of this invention can be provided in the form of a pharmaceutical (pharmaceutical composition), a food or beverage (including food, beverage, food and beverage composition, food composition, beverage composition) or the like, but the aforementioned forms are not the sole forms of the combined composition of this invention. Non-limiting examples of food compositions include, but are not limited to, functional foods, health supplements, foods with nutrient function claims, foods for special dietary uses, foods for specified health uses, nutritional supplements, dietetic foods, health foods, dietary supplements, and food additives.

[0066] As referred to in the present invention, carnosine is a dipeptide composed of β -alanine and histidine, and also called β -alanylhistidine. Carnosine includes all of D-form (D-carnosine), L-form (L-carnosine) or DL-form (DL-carnosine), but in this invention, carnosine is preferably L-form (L-carnosine) and DL-form (DL-carnosine), more preferably L-form (L-carnosine). Additionally, D-form (D-carnosine) is registered under CAS Registry No. 5853-00-9, and L-form (L-carnosine) is registered under CAS Registry No. 305-84-0.

[0067] The content of a polyphenol, a pentacyclic triterpenic acid, or anserine in the combined composition of the present invention is as mentioned above. The content of carnosine in the combined composition of this invention can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. Also, the upper limit of this content can be set to, for example, not more than 50

wt.%, not more than 70 wt.%, or not more than 90 wt.%. In particular, when the combined composition of this invention is in solid form, the carnosine content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the combined composition of this invention is in liquid form, the carnosine content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0068] The method for obtaining carnosine used in the present invention is not particularly limited, and both of natural carnosine derived from animal sources and synthetic carnosine obtained by a chemical synthesis method or the like can be used. In this invention, commercially available carnosine is preferably used. Also, the carnosine content in the combined composition of this invention is not particularly limited, as long as said content is adjusted to a level sufficient to achieve the desired effects of this invention, in consideration of *e.g.* the form and mode of administration.

[0069] 2. Use of a polyphenol or other active component for inhibiting carnosine dipeptidases

One aspect of the present invention relates to use of a polyphenol for inhibiting carnosine dipeptidases.

[0070] The polyphenol used in the present invention is preferably a flavonoid, a curcuminoid, a stilbenoid, a phenylethanoid, or a phenylpropanoid. Among flavonoids, quercetin, myricetin, kaempferol, luteolin, apigenin, or a catechin compound is preferred, with epigallocatechin gallate being particularly preferred. Among curcuminoids, curcumin is preferred. Among stilbenoids, resveratrol or piceatannol is preferred. Among phenylethanoids, hydroxytyrosol is preferred. Among phenylpropanoids, caffeic acid or ferulic acid is preferred.

[0071] Another aspect of the present invention relates to use of a pentacyclic triterpenic acid for inhibiting carnosine dipeptidases. Among pentacyclic triterpenic acids, ursolic acid is preferred. Yet another aspect of this invention relates to use of anserine for inhibiting carnosine dipeptidases.

[0072] The use according to the present invention includes, for example, use of a polyphenol, a pentacyclic triterpenic acid, anserine, or the like for the prevention or amelioration of a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism. Said use can be use in a human or a non-human animal, and can be therapeutic or non-therapeutic use. As referred to above, the term "non-therapeutic" has a concept that does not include medical practice, *i.e.*, acts of treating humans by therapy.

[0073] 3. Method for inhibiting carnosine dipeptidases

One aspect of the present invention relates to a method for inhibiting carnosine dipeptidases using a polyphenol.

[0074] The polyphenol used in the present invention is preferably a flavonoid, a curcuminoid, a stilbenoid, or a phenylpropanoid. Among flavonoids, quercetin, myricetin, kaempferol, luteolin, apigenin, or a catechin compound is preferred, with epigallocatechin gallate being particularly preferred. Among curcuminoids, curcumin is preferred. Among stilbenoids, resveratrol or piceatannol is preferred. Among phenylethanoids, hydroxytyrosol is preferred. Among phenylpropanoids, caffeic acid or ferulic acid is preferred.

[0075] Another aspect of the present invention relates to a method for inhibiting carnosine dipeptidases using a pentacyclic triterpenic acid. Among pentacyclic triterpenic acids, ursolic acid is preferred. Yet another aspect of this invention relates to a method for inhibiting carnosine dipeptidases using anserine.

[0076] Another mode of the aforementioned method is a method for inhibiting carnosine dipeptidases, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of a polyphenol, a pentacyclic triterpenic acid, or anserine as an active component. Among polyphenols, a flavonoid, a curcuminoid, a stilbenoid, a phenylethanoid, or a phenylpropanoid is preferred. Among flavonoids, quercetin, myricetin, kaempferol, luteolin, apigenin, or a catechin compound is preferred, with epigallocatechin gallate being particularly preferred. Among curcuminoids, curcumin is

preferred. Among stilbenoids, resveratrol or piceatannol is preferred. Among phenylethanoids, hydroxytyrosol is preferred. Among phenylpropanoids, caffeic acid or ferulic acid is preferred. Among pentacyclic triterpenic acids, ursolic acid is preferred.

[0077] In the aforementioned method, the subject in need of carnosine dipeptidase inhibition is for example a human, but this is not the sole example. As used herein, the phrase “therapeutically effective amount” refers to an amount of the carnosine dipeptidase inhibiting composition of this invention that is sufficient to inhibit the carnosine degradation activity of a carnosine dipeptidase in a subject receiving said composition, as compared to an individual not receiving said composition. The specific effective amount is not fixed and is determined as appropriate depending on, for example, the form and mode of administration, intended use, and the age, body weight and symptom of a subject.

[0078] In the method of the present invention, the polyphenol, pentacyclic triterpenic acid, or anserine can be administered as it is or in the form of a composition comprising said polyphenol, pentacyclic triterpenic acid, or anserine, so as to ensure that the aforementioned therapeutically effective amount is taken.

[0079] The method of the present invention permits inhibition of carnosine dipeptidases without causing any side effects. The method of this invention can serve, through its carnosine dipeptidase inhibition, as a method for preventing or ameliorating fatigue, skin wrinkle formation, a decline in cognitive function, diabetes, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer’s disease, or autism.

[0080] 4. Composition comprising a polyphenol and carnosine

One aspect of the present invention relates to a composition comprising a polyphenol and carnosine, wherein the polyphenol is piceatannol and/or hydroxytyrosol.

[0081] 4-1. Carnosine

Carnosine contained in the composition of the present invention is as described above. The carnosine content in the composition of this invention can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not

less than 0.01 wt.%. Also, the upper limit of this content can be set to, for example, not more than 50 wt.%, not more than 70 wt.%, not more than 80 wt.%, or not more than 90 wt.%. The composition of this invention can be in various forms, such as solid (*e.g.*, tablet) and liquid (*e.g.*, beverage packed in PET bottle). In particular, when the composition of this invention is in solid form, the carnosine content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the composition of this invention is in liquid form, the carnosine content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0082] The ratio of piceatannol to carnosine (piceatannol:carnosine) contained in the composition of the present invention is, by weight, for example from 1:1000 to 100:1, preferably from 1:100 to 50:1, more preferably from 1:10 to 10:1. Also, the ratio of hydroxytyrosol to carnosine (hydroxytyrosol:carnosine) contained in the composition of this invention is, by weight, for example from 1:1000 to 100:1, preferably from 1:100 to 50:1, more preferably from 1:10 to 10:1.

[0083] 4-2. Piceatannol and hydroxytyrosol

The polyphenol contained in the composition of the present invention is piceatannol and/or hydroxytyrosol. Piceatannol is as described above. Piceatannol has been reported to have various physiological effects, such as vascular aging-suppressing and fatigue-ameliorating effects in humans, eNOS (endothelial nitric oxide synthase) expression-promoting and vasodilatory effects at the laboratory animal level, and skin blemish and wrinkle formation-suppressing and UV-protecting effects at the cellular level. Piceatannol-containing materials are utilized as ingredients for health foods.

[0084] Hydroxytyrosol contained in the composition of the present invention is as described above. Hydroxytyrosol has been shown to have various physiological effects, such as skin-lightening, arteriosclerosis-preventing and antioxidant effects at the laboratory animal level, and vascular smooth muscle cell proliferation-suppressing, vascular endothelial wound healing, and oxidative stress cell protection effects at the cellular level. Hydroxytyrosol is

widely utilized as an active ingredient for health foods and cosmetics.

[0085] Piceatannol and hydroxytyrosol may both be glycosides thereof. The sugar found in the glycosides is not particularly limited, and can be a monosaccharide or a di- or higher saccharide. The type of the sugar is also not particularly limited, and examples include, but are not limited to, aldoses such as glucose, mannose, galactose, fucose, rhamnose, arabinose and xylose, ketoses such as fructose, uronic acids such as glucuronic acid, galacturonic acid and mannuronic acid, as well as apiose and rutinose. The sugar used in the glycosides can be a D-sugar or an L-sugar. Examples of glycosides of piceatannol include, but are not limited to, piceatannol-3'-O- β -D-glucopyranoside, and piceatannol-4'-O- β -D-glucopyranoside.

[0086] Piceatannol and hydroxytyrosol may be extracted from natural products such as plants and fruits using water (including hot water), an organic solvent or the like, or may be chemically synthesized. Extraction (including isolation, purification, etc.) or synthesis of these compounds can be carried out using a method known to skilled artisans depending on, for example, the types of compounds.

[0087] Piceatannol and hydroxytyrosol prepared on one's own, or commercially available ones may be used -- the sources of these polyphenols are not particularly limited. What are preferably used in this invention are, without particular limitation, commercially available ones. Also, a plant extract containing piceatannol and/or a plant extract containing hydroxytyrosol may be used. The forms of piceatannol and hydroxytyrosol are not particularly limited, and these polyphenols may be in free form or in the form of a solvate such as hydrate or ethanolate. Where the active component is exposed to blood, an ester of the active component and other components may be used, such as oleuropein.

[0088] The piceatannol content in the composition of the present invention can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. This content can also be set to, for example, not more than 50 wt.%, not more than 70 wt.%, not more than 80 wt.%, or not more than 90 wt.%. In particular, when the composition of this invention is in solid form, the piceatannol content in the

composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the composition of this invention is in liquid form, the piceatannol content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0089] The hydroxytyrosol content in the composition of the present invention can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%. This content can also be set to, for example, not more than 50 wt.%, not more than 70 wt.%, not more than 80 wt.%, or not more than 90 wt.%. In particular, when the composition of this invention is in solid form, the hydroxytyrosol content in the composition can be set to, for example, not less than 0.1 wt.%, preferably not less than 0.3 wt.%, more preferably not less than 1.0 wt.%. When the composition of this invention is in liquid form, the hydroxytyrosol content in the composition can be set to, for example, not less than 0.001 wt.%, preferably not less than 0.003 wt.%, more preferably not less than 0.01 wt.%.

[0090] When any of the components in the composition of the present invention is in the form of a salt, hydrate, glycoside or the like, the content of this component shall be calculated in terms of its free form. In this invention, as described above, the contents of different components can be measured by following a method known to skilled artisans.

[0091] When both piceatannol and hydroxytyrosol are used, the ratio of these polyphenols (piceatannol:hydroxytyrosol) contained in the composition can for example be, by weight, from 1:100 to 100:1, from 1:10 to 10:1, or from 1:5 to 5:1.

[0092] 4-3. Other components

The composition of the present invention may, depending on its form, contain any additives and/or any other common components, in addition to the aforementioned two polyphenols and carnosine. Examples of such additives and/or common components include, but are not limited to, physiologically active components, including vitamins such as vitamin E and vitamin C, minerals, nutrients, and flavorants, as well as other components

added to make pharmaceutical preparations, such as excipients, binders, emulsifiers, isotonizers, buffers, solubilizers, antiseptics, stabilizers, antioxidants, colorants, coagulators, and coating agents.

[0093] 4-4. Composition

The composition of the present invention is, for example but not particularly limited to, a pharmaceutical, food or beverage comprising carnosine and the aforementioned two polyphenols (*i.e.*, piceatannol and/or hydroxytyrosol), preferably a food or beverage comprising the same. The contents of carnosine and the aforementioned two polyphenols in such a pharmaceutical, food or beverage are as mentioned above.

[0094] When the composition of the present invention is a pharmaceutical, the route of administration of said composition is not particularly limited, but is preferably an oral route. Examples of the form of a pharmaceutical composition suitable for oral administration include, but are not limited to, solid preparations such as tablets (including coated tablets), granules, powders, powdered drugs and capsules, and liquid preparations such as oral solutions, suspensions, emulsions, syrups and drinkable preparations.

[0095] When the composition of the present invention is a food or beverage, said composition may be made into the form of health foods such as tablets (including coated tablets), granules, powders, powdered drugs and capsules, or may be made into the form of beverages (including beverages packed in PET bottle, soft drinks, and tea beverages), dairy products such as yogurt and lactobacillus beverages, seasonings, processed foods, desserts, confectionaries (*e.g.*, gum, candy, jelly), or the like.

[0096] The composition of the present invention is provided in various forms, including but not particularly limited to, solid or liquid. Specific examples of such forms are as mentioned above. The composition of this invention can be taken directly or with water or the like. Furthermore, the composition of this invention, after being prepared into an easy-to-mix form (*e.g.*, powder or granule form), can be used as an ingredient for *e.g.* pharmaceuticals, foods and beverages.

[0097] The composition of the present invention can be prepared, for example, by mixing

specified amounts of carnosine and the aforementioned two polyphenols (*i.e.*, piceatannol and/or hydroxytyrosol) with ingredients used to prepare a known pharmaceutical, food or beverage, and making the mixture into such a product by following a known preparation procedure, or can be prepared by adding specified amounts of the aforementioned two polyphenols and carnosine to a ready-made known pharmaceutical, food or beverage, and dissolving and/or suspending them therein. Also, a known pharmaceutical, food or beverage may inherently contain carnosine and the aforementioned two polyphenols -- such a product may be used as an ingredient to prepare the composition of this invention, if carnosine and the aforementioned two polyphenols as required in this invention are available in specified amounts from said product.

[0098] The composition of the present invention can be applied to both of therapeutic use (medical use) and non-therapeutic use (non-medical use). To be specific, the composition of this invention can be used as a pharmaceutical, a quasi-drug or a cosmetic or the like, and also can be used as any other products that do not belong to any of the above groups under the Pharmaceutical Affairs Law, for example, as a composition that explicitly or implicitly appeals for a preventive or ameliorative effect on a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism.

[0099] The subject to which the composition of the present invention is applied is preferably a human, but may be a livestock animal such as cow, horse or goat, a companion animal such as dog, cat or rabbit, or a laboratory animal such as mouse, rat, guinea pig or monkey. When the composition of this invention is administered to an animal other than a human, the consumption dose of the composition in said animal varies with conditions such as the active component content in the composition, and the state, body weight, sex and age of the subject to which the composition is applied.

[0100] 4-5. Effects and intended use

The composition of the present invention is characterized in that the composition

comprises carnosine, and piceatannol and/or hydroxytyrosol, and that piceatannol and hydroxytyrosol have carnosine dipeptidase inhibitory effect.

[0101] The term “carnosine dipeptidase(s)” is as described above. The carnosine dipeptidase to be inhibited in the present invention is preferably carnosine dipeptidase 1 (CNDP1), a serum carnosine-degrading enzyme.

[0102] The expression “carnosine dipeptidase inhibition (inhibiting)” is as described above. Since the composition of the present invention comprises piceatannol and/or hydroxytyrosol, the carnosine dipeptidase inhibitory effect of the polyphenols can retard carnosine degradation in the body and enables effective delivery of carnosine into a target tissue or organ. Thus, the composition of this invention can effectively enhance the physiological effects of not only carnosine contained in the composition but also carnosine inherently present in the body.

[0103] The physiological effects of carnosine are as described above in relation to the effects of carnosine. Based on its carnosine dipeptidase inhibitory effect, the composition of the present invention can serve as a composition for enhancing the physiological effects of carnosine (a composition intended for use in enhancement of the physiological effects of carnosine). Further, based on its capability of enhancing the physiological effects of carnosine, the composition of this invention can serve as a composition for preventing or ameliorating a decline in cognitive function, diabetes, fatigue, skin wrinkle formation, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism.

[0104] Furthermore, since the composition of the present invention has an activity to inhibit carnosine dipeptidase 1 and/or carnosine dipeptidase 2, said composition can be used for the purpose of suppressing the degradation of not only carnosine but also all kinds of dipeptides having a histidine residue at the carboxyl terminus (represented as X-His (where X is a naturally occurring amino acid)).

[0105] In another aspect, the present invention relates to a composition bearing a claim regarding a function exerted through a physiological effect of carnosine. Examples of such

a claim or function claim include, but are not particularly limited to, “makes your body harder to get tired”, “facilitates recovery from body fatigue”, “prevents skin wrinkle formation”, “ameliorates skin wrinkles and sags”, “assists in beauty care”, “suppresses a decline in cognitive function”, “potentially helps maintain cognitive function”, “suppresses a rise in blood glucose levels”, “enhances immune function”, “potentially exerts an antioxidative effect”, “reduces oxidative stress”, “potentially exerts an antiglycative effect”, “reduces glycative stress”, “suppresses blood vessel inflammation”, “potentially prevents or ameliorates Alzheimer’s disease”, and “potentially prevents or ameliorates autism”, and other claims or function claims that can be regarded as being equivalent thereto. In this invention, such a claim or function claim may be borne by the composition *per se* or by a container or package of the composition.

[0106] The composition of the present invention can be ingested by a suitable method depending on its form. The ingestion method is not particularly limited as long as the method permits the active components contained in the composition to be transferred into the circulating blood. The composition can be made into the form of, for example but not limited to, a solid preparation for oral use, a liquid preparation for oral use such as internal liquid agent or syrup, or a preparation for parenteral use such as injectable preparation, external preparation, suppository or percutaneous absorption preparation.

EXAMPLES

[0107] Hereunder, the present invention will be described in more detail by way of working examples, but these examples are not limitative of the scope of this invention. Skilled artisans could use this invention with various alternations or modifications being made thereto, and such alternations and modifications are also included in the scope of this invention.

[0108] Example 1 Investigation of inhibitory effects on carnosine dipeptidase 1 activity

The compounds used for evaluation were as follows: quercetin, myricetin, kaempferol, luteolin, apigenin (the above five were produced by Extrasynthese, France), catechin (produced by Nagara Science Co., Ltd.), epigallocatechin (produced by Kurita

Water Industries Ltd.), epicatechin, epigallocatechin gallate, curcumin, ferulic acid, caffeic acid (the above five are produced by Nacal Tesque, Inc.), resveratrol, ursolic acid (the above two were produced by Sigma), piceatannol, hydroxytyrosol (the above two were produced by Tokyo Chemical Industry Co., Ltd.), and anserine (produced by Wako Pure Chemical Industries, Ltd.). The human carnosine dipeptidase 1 used was Recombinant Human Carnosine Dipeptidase1/CNDP1 (produced by R&D Systems). Carnosine (produced by Tokyo Chemical Industry Co., Ltd.) was used to investigate the enzymatic reaction. The analysis of inhibitory effects on carnosine dipeptidase 1 activity was conducted at room temperature by following the procedure described below.

[0109] Each of 1.5 mL Eppendorf tubes was charged with 50 μ L of a solution of 2 ng/ μ L carnosine dipeptidase 1 dissolved in a buffer (50 mM Tris, pH 7.5) and 25 μ L of an aqueous solution containing one of the evaluation compounds, and then reaction was initiated by adding 25 μ L of a solution of 4 mM carnosine dissolved in the same buffer. After the reaction solution was incubated at room temperature for 60 minutes, the reaction was terminated by adding 50 μ L of an aqueous solution of 1% trichloroacetic acid (TCA) (produced by Sigma) dissolved in deionized water, followed by mixing by vortexing. To the sample obtained after the reaction, an aqueous 1.8 M sodium hydroxide solution (with 10% DMSO) containing 5 mg/mL o-phthaldialdehyde (OPA) (produced by Sigma) was added, and the solution was mixed and incubated again at room temperature for 30 minutes. L-histidine dilution series were prepared in buffer to cover the range of 15.625 to 250 μ M. Likewise, TCA and OPA were added to the sample and then the solution was incubated for 30 minutes, and the thus-obtained solution was used as a standard solution for calibration curve. Further, a solution prepared by adding water containing DMSO at the same concentration, instead of the aqueous solution containing one of the evaluation compounds, was used as a control. The whole amounts of each of the samples and the standard solution were added to a 96-well black plate, and the fluorescence intensity of histidine was measured with a fluorometer using an excitation wavelength of 360 nm and a fluorescence wavelength of 460 nm. The analysis was conducted on two samples (n=2) for each group.

[0110] With regard to the measurement results, corrected values were calculated by subtracting the fluorescence intensity for a sample prepared by adding a buffer instead of the enzyme (carnosine dipeptidase 1), and the percentages of the corrected values of fluorescence intensity in each of the evaluation compound groups relative to the corrected value of fluorescence intensity in control group, which was taken as 100%, were regarded as residual carnosine dipeptidase 1 activities (%). The results are shown in Tables 1 to 4.

[0111] [Table 1]

Flavonoids

	Conc. (μM)	Residual activity (%)	Standard deviation
Quercetin	2	85.8	4.4
	6	75.5	2.8
	20	60.6	5.0
	60	29.0	4.7
Myricetin	2	80.8	0.8
	6	57.0	1.7
	20	24.6	2.1
Kaempferol	2	96.6	8.9
	6	95.2	2.4
	20	90.5	6.7
Luteolin	2	101.8	3.5
	6	88.7	0.7
	20	77.1	0.5
Apigenin	2	86.1	10.9
	6	69.5	2.6
	20	11.3	4.0
Catechin	2	102.6	4.2
	6	105.4	1.4
	20	96.9	1.4
	60	74.4	0.0
Epicatechin	2	100.5	3.9
	6	97.2	2.9
	20	86.7	0.2
	60	68.3	1.7
Epigallocatechin	2	84.8	12.8
	6	85.8	4.1
	20	83.5	0.2
	60	68.0	0.1
Epigallocatechin gallate	2	24.8	0.4
	6	6.7	1.2
	20	4.9	1.3
	60	2.5	0.7

[0112] [Table 2]

Curcuminoid

	Conc. (μM)	Residual activity (%)	Standard deviation
Curcumin	2	101.7	3.2
	6	84.7	5.8
	20	29.7	3.4
	60	4.5	1.8

[0113] [Table 3]

Stilbenoids

	Conc. (μM)	Residual activity (%)	Standard deviation
Resveratrol	2	96.9	4.2
	6	89.9	1.4
	20	82.5	0.8
	60	62.0	1.8
Piceatannol	2	87.3	1.0
	6	95.9	1.7
	20	109.2	5.7
	60	54.5	4.2

Phenylethanoid, phenylpropanoids

	Conc. (μM)	Residual activity (%)	Standard deviation
Hydroxytyrosol	2	79.8	0.9
	6	80.3	0.5
	20	65.6	3.8
	60	59.8	2.4
Ferulic acid	2	102.1	2.5
	6	83.8	0.4
	20	69.2	3.7
	60	64.5	6.2
Caffeic acid	2	79.7	2.3
	6	75.5	2.8
	20	69.5	0.2
	60	46.5	0.5

[0114] [Table 4]

Other compounds than polyphenols

	Conc. (μ M)	Residual activity (%)	Standard deviation
Ursolic acid	2	95.7	1.6
	6	91.2	2.7
	20	50.6	0.6
	60	4.6	0.1
Anserine	2	64.2	4.1
	6	76.0	0.5
	20	40.5	1.1

[0115] The results given above revealed that all of the compounds shown in Tables 1 to 4 have an inhibitory effect on carnosine dipeptidase 1 activity. It was shown that since these compounds have carnosine dipeptidase inhibitory effect, compositions comprising any of said compounds in combination with carnosine have the potential to enhance the actions and effects of not only carnosine contained in said compositions but also carnosine inherently present in the body.

[0116] The following gives preparation examples of the compositions of the present invention.

(Preparation Example 1) Tablet

Epigallocatechin gallate	10 g
Vitamin E	60 g
Starch	223 g
Sucrose fatty acid ester	9 g
Silicon oxide	9 g

The aforementioned components were mixed and tableted on a single punch tableting machine to produce tablets with a diameter of 9 mm and a mass of 300 mg.

[0117] (Preparation Example 2) Granule

Curcumin	10 g
Carnosine	10 g
Tocopherol acetate	10 g
Silicic anhydride	20 g
Corn starch	110 g

The aforementioned powders were homogeneously mixed, then 100 mL of a 10% solution of hydroxypropyl cellulose in ethanol was added, and the mixture was kneaded, extruded and dried according to conventional procedures to yield granules.

[0118] (Preparation Example 3) Drinkable preparation

DL-sodium tartrate	0.1 g
Succinic acid	0.009 g
Liquid sugar	800 g
Citric acid	12 g
Vitamin C	10 g
Resveratrol	20 g
Vitamin E	20 g
Cyclodextrin	5 g
Emulsifier	5 g
Flavorant	15 g
Potassium chloride	1 g
Magnesium sulfate	0.5 g

The aforementioned components were mixed, and water was added to make the volume 1 L. This drinkable preparation was consumed at a dose of not less than 100 mL once.

[0119] (Preparation Example 4) Tablet

Piceatannol	10 g
Carnosine	10 g
Vitamin E	60 g
Starch	223 g
Sucrose fatty acid ester	9 g
Silicon oxide	9 g

The aforementioned components were mixed and tableted on a single punch tableting machine to produce tablets with a diameter of 9 mm and a mass of 300 mg.

[0120] (Preparation Example 5) Granule

Hydroxytyrosol	10 g
Carnosine	10 g
Tocopherol acetate	10 g
Silicic anhydride	20 g
Corn starch	110 g

The aforementioned powders were homogeneously mixed, then 100 mL of a 10% solution of hydroxypropyl cellulose in ethanol was added, and the mixture was kneaded, extruded and dried according to conventional procedures to yield granules.

[0121] (Preparation Example 6) Drinkable preparation

DL-sodium tartrate	0.1 g
Succinic acid	0.009 g
Liquid sugar	800 g
Citric acid	12 g
Vitamin C	10 g
Hydroxytyrosol	10 g
Carnosine	10 g
Vitamin E	20 g
Cyclodextrin	5 g
Emulsifier	5 g
Flavorant	15 g
Potassium chloride	1 g
Magnesium sulfate	0.5 g

The aforementioned components were mixed, and water was added to make the volume 1 L. This drinkable preparation was consumed at a dose of not less than 100 mL once.

[0122] (Preparation Example 7) Fruit juice

Frozen concentrated citrus unshu juice	5 wt.pts
High-fructose corn syrup	11 wt.pts
Citric acid	0.2 wt.pts
L-ascorbic acid	0.02 wt.pts
Piceatannol	0.06 wt.pts
L-carnosine	1 wt.pt
Flavorant	0.2 wt.pts
Coloring agent	0.1 wt.pt
Water	82.42 wt.pts

[0123] (Preparation Example 8) Yogurt

Raw milk (3.4% fat)	80 wt.pts
Dairy cream (50% fat)	8 wt.pts
Skimmed milk powder	1.5 wt.pts
Citric acid	0.08 wt.pts
Water	6.36 wt.pts
Yogurt starter	3 wt.pts
Piceatannol	0.06 wt.pts
L-carnosine	1 wt.pt

[0124] (Preparation Example 9) Lactobacillus beverage

Fermented milk containing 21% milk solids	14.76 wt.pts
High-fructose corn syrup	13.31 wt.pts
Pectin	0.5 wt.pts
Citric acid	0.08 wt.pts
Flavorant	0.15 wt.pts
Water	70.18 wt.pts
Hydroxytyrosol	0.02 wt.pts
L-carnosine	1 wt.pt

[0125] (Preparation Example 10) Lactobacillus beverage

Dairy cream (45% fat)	33.8 wt.pts
Skimmed milk powder	11 wt.pts
Granulated sugar	14.8 wt.pts
Sweetened egg yolk	0.3 wt.pts
Vanilla extract	0.1 wt.pt
Water	38.94 wt.pts
Hydroxytyrosol	0.06 wt.pts
L-carnosine	1 wt.pt

[0126] (Preparation Example 11) Coffee beverage

Granulated sugar	8 wt.pts
Skimmed milk powder	5 wt.pts
Caramel	0.2 wt.pts
Coffee extract	2 wt.pts
Flavorant	0.1 wt.pt
Polyglyceryl fatty acid ester	0.05 wt.pts
Common salt	0.05 wt.pts
Water	83.54 wt.pts
Piceatannol	0.06 wt.pts
L-carnosine	1 wt.pt

[0127] (Preparation Example 12) Alcoholic beverage

50 vol.% ethanol	8 wt.pts
Sugar	5 wt.pts
Fruit juice	0.2 wt.pts
Hydroxytyrosol	0.06 wt.pts
L-carnosine	1 wt.pt
Purified water	85.74 wt.pts

INDUSTRIAL APPLICABILITY

[0128] The present invention provides a carnosine dipeptidase inhibiting composition comprising a polyphenol or other active component. This invention also provides a composition comprising carnosine and a particular polyphenol having carnosine dipeptidase inhibitory effect. This invention provides a novel means that contributes to, for example, suppression of a rise in blood glucose levels, and thus is highly industrially applicable.

CLAIMS

1. A carnosine dipeptidase inhibiting composition comprising a polyphenol.
2. The composition according to claim 1, wherein the polyphenol is selected from the group consisting of a flavonoid, a curcuminoid, a stilbenoid, a phenylethanoid, and a phenylpropanoid.
3. The composition according to claim 2, wherein the flavonoid is selected from the group consisting of quercetin, myricetin, kaempferol, luteolin, a catechin compound, and apigenin.
4. The composition according to claim 2, wherein the curcuminoid is curcumin.
5. The composition according to claim 2, wherein the stilbenoid is resveratrol or piceatannol.
6. The composition according to claim 2, wherein the phenylethanoid is hydroxytyrosol.
7. The composition according to claim 2, wherein the phenylpropanoid is caffeic acid or ferulic acid.
8. The composition according to any one of claims 1 to 7, wherein the composition is intended for use in the prevention or amelioration of fatigue, skin wrinkle formation, a decline in cognitive function, diabetes, a decline in immune function, blood vessel or tissue inflammation, various diseases caused by oxidative stress or glycation end product production, Alzheimer's disease, or autism.
9. The composition according to any one of claims 1 to 8, wherein the composition is an

agent.

10. The composition according to any one of claims 1 to 9, wherein the composition bears a claim regarding a function exerted through carnosine dipeptidase inhibition.

11. A composition comprising a polyphenol and carnosine, wherein the polyphenol is piceatannol or hydroxytyrosol.

12. The composition according to claim 11, wherein the composition is intended for use in inhibition of carnosine dipeptidase activity.

13. The composition according to claim 11 or 12, wherein the composition is intended for use in enhancement of a pharmacological effect of carnosine.

14. The composition according to claim 13, wherein the pharmacological effect of carnosine is an anti-fatigue effect, a preventive or ameliorative effect on skin wrinkle formation, a cognitive function maintenance effect, a diabetes preventive or ameliorative effect, a suppressive effect on a decline in immune function, a preventive or ameliorative effect on blood vessel or tissue inflammation, an ameliorative effect on various diseases caused by oxidative stress or glycation end product production, a preventive or ameliorative effect on Alzheimer's disease, or a preventive or ameliorative effect on autism.

15. The composition according to any one of claims 11 to 14, wherein the composition bears a claim regarding a function exerted through the pharmacological effect of carnosine.

16. The composition according to claim 10 or 15, wherein the claim regarding a function is selected from the group consisting of "makes your body harder to get tired", "facilitates recovery from body fatigue", "prevents skin wrinkle formation", "ameliorates skin wrinkles

and sags”, “assists in beauty care”, “suppresses a decline in cognitive function”, “potentially helps maintain cognitive function”, “suppresses a rise in blood glucose levels”, “enhances immune function”, “potentially exerts an antioxidative effect”, “reduces oxidative stress”, “potentially exerts an antiglycative effect”, “reduces glycative stress”, “suppresses blood vessel inflammation”, “potentially prevents or ameliorates Alzheimer’s disease”, and “potentially prevents or ameliorates autism”.

17. The composition according to any one of claims 1 to 16, wherein the composition is in the form of solid or liquid.

18. The composition according to any one of claims 1 to 17, wherein the composition is a food or beverage composition.

19. Use of a polyphenol for inhibiting a carnosine dipeptidase.

20. A method for inhibiting a carnosine dipeptidase using a polyphenol.