

Description

Title of Invention: FOOD OR BEVERAGE COMPOSITION CONTAINING PEPTIDE AND/OR SALT THEREOF, PRODUCTION METHOD THEREOF, USE OF HYDROLYZED COLLAGEN TYPE II, COMPOSITION FOR INHIBITING BONE RESORPTION, AND USE OF CHICKEN EXTRACT

Technical Field

[0001] The present invention relates to a food or beverage composition containing a peptide and/or a salt thereof, and a method of producing the same. The present invention relates to use of a hydrolyzed collagen type II containing a peptide and/or a salt thereof for the production of an anti-inflammatory composition. The present invention relates to a peptide and/or a salt thereof for the production of a composition for inhibiting bone resorption. The present invention relates to a composition for inhibiting bone resorption. The present invention relates to use of chicken extract for the production of a composition for inhibiting bone resorption.

Background Art

- [0002] Collagen known as gelatin has been widely used in the food field. Since collagen which is an animal protein is a main component of dermis and connective tissue, collagen has been recently drawing attention also in the medical field and the cosmetic field. Generally, orally consumed high molecular weight collagen cannot be efficiently utilized in the body. Recently, low molecular weight collagen peptides obtained by hydrolysis of high molecular weight collagen have been developed in order to facilitate intake of collagen in the body, and foods and/or beverages containing such a collagen peptide have also been developed.
- [0003] Inflammation is a phenomenon in which histamine, kinins, and the like are released by damaged cells, which causes vasodilation, increased capillary permeability, and aggregation of macrophages at an inflammatory site, resulting in increased blood flow at an infected site, edema, transfer of immune cells and antibodies, pain, fever, or the like.
- [0004] Recently, components capable of inhibiting expression of a protein related to inflammation have been studied to provide effective relief of inflammation. While anti-inflammatory drugs having various mechanisms, such as non-steroidal anti-inflammatory drug (NSAID) and steroidal anti-inflammatory drugs (SAID), have been developed, these drugs may have side effects. Thus, there is still a demand for components that are safer and that have anti-inflammatory action.

- [0005] For example, Patent Literature 1 discloses an anti-inflammatory composition containing, as an active component, a peptide derived from telomerase having anti-inflammatory activity.
- [0006] Osteoporosis is a disease that increases bone vulnerability due to low bone mass to increase bone fracture risk. Usually, bone remodeling is performed in which old bone is absorbed by osteoclasts and new bone is created by osteoblasts. Osteoporosis occurs due to an imbalance of such bone remodeling.
- [0007] Treatment of osteoporosis uses medicines such as female hormones and bisphosphonates. Dietary materials such as soy isoflavone, vitamin K, and calcium have been reported to improve bone metabolism and bone density. Yet, it is said that as many as 45% of patients with osteoporosis stop taking drugs as prescribed within a year because of anxiety about cost and side effects. Thus, there is a demand for a component that can be taken orally to safely and easily improve bone metabolism and increase bone density.
- [0008] For example, Patent Literature 2 discloses an agent to improve bone metabolism or bone density, the agent containing fungus body of *Bacillus* microorganisms and/or a culture thereof as an active component.

Citation List

Patent Literature

- [0009] PTL 1: JP 2015-525768 T
PTL 2: JP 2017-226649 A

Summary of Invention

Technical Problem

- [0010] The present invention aims to provide a novel food or beverage composition containing a peptide.
- The present invention aims to provide a food or beverage composition having anti-inflammatory action.
- The present invention aims to provide a food or beverage composition having bone resorption inhibitory action.
- The present invention aims to provide a novel composition for inhibiting bone resorption.

Solution to Problem

- [0011] The present inventors found that a composition containing a peptide consisting of an amino acid sequence represented by the SEQ ID NO: 1 and/or a salt thereof has bone resorption inhibitory action.
- The present inventors also found that a hydrolyzed collagen type II of chicken cartilage containing a peptide consisting of an amino acid sequence represented by the

SEQ ID NO: 1 and/or a salt thereof has anti-inflammatory activity.

The present inventors also found that chicken extract has bone resorption inhibitory action.

[0012] Specifically, the present invention is defined as follows.

(1) A food or beverage composition containing a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) and/or a salt thereof.

(2) The food or beverage composition according to (1) above, wherein the peptide and/or a salt thereof is a peptide derived from hydrolyzed collagen type II of chicken cartilage and/or a salt thereof.

(3) The food or beverage composition according to (1) or (2) above, wherein the food or beverage composition contains hydrolyzed collagen type II of chicken cartilage.

(4) The food or beverage composition according to any one of (1) to (3) above, further containing an animal extract and/or a plant extract.

(5) The food or beverage composition according to (4) above, wherein the animal extract is chicken extract.

(6) The food or beverage composition according to (5) above, wherein the food or beverage composition contains carnosine and/or anserine and/or one or more salts thereof.

(7) The food or beverage composition according to (6) above, wherein the weight ratio of the total weight of the carnosine, anserine and salts thereof in terms of carnosine and anserine to the weight of the peptide and/or a salt thereof in terms of peptide (total of carnosine and anserine/peptide) is 1,000,000/1 to 100/1.

(8) The food or beverage composition according to any one of (1) to (7) above, wherein the food or beverage composition is used to reduce inflammation.

(9) The food or beverage composition according to any one of (1) to (8) above, wherein the food or beverage composition inhibits the production of at least one cytokine selected from the group consisting of macrophage inflammatory protein-1 (MIP-1), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-12 (IL-12) and regulated on activation, normal T cell expressed and secreted (RANTES).

(10) The food or beverage composition according to any one of (1) to (9) above, wherein the food or beverage composition is used to prevent or alleviate inflammatory conditions or diseases.

(11) The food or beverage composition according to any one of (1) to (10) above, wherein the food or beverage composition is used to inhibit bone resorption activity of osteoclasts.

(12) The food or beverage composition according to any one of (1) to (11) above,

wherein the food or beverage composition is used to prevent or alleviate conditions or diseases associated with bone loss.

(13) The food or beverage composition according to (12) above, wherein the conditions or diseases associated with bone loss include osteoporosis and osteopenia.

(14) A method of producing a food or beverage composition, including: adding a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) and/or a salt thereof.

(15) The method according to (14) above, wherein the adding includes adding hydrolyzed collagen type II of chicken cartilage, the hydrolyzed collagen type II containing the peptide and/or a salt thereof.

(16) The method according to (14) or (15) above, further including adding an animal extract and/or a plant extract.

(17) The method according (16) above, wherein the animal extract is chicken extract.

(18) The method according (17) above, wherein the chicken extract contains carnosine and/or anserine and/or one or more salts thereof.

(19) Use of a hydrolyzed collagen type II of chicken cartilage containing a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) and/or a salt thereof for the production of an anti-inflammatory composition.

(20) Use of a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) and/or a salt thereof for the production of a composition for inhibiting bone resorption.

(21) A composition for inhibiting bone resorption, containing chicken extract as an active component.

(22) The composition for inhibiting bone resorption according (21) above, wherein the composition contains carnosine and/or anserine and/or one or more salts thereof.

(23) Use of chicken extract for the production of a composition for inhibiting bone resorption.

Advantageous Effects of Invention

[0013] The present invention provides a novel food or beverage composition containing a peptide and/or a salt thereof. The food or beverage composition of the present invention can be used to reduce inflammation and joint pain. The food or beverage composition of the present invention can also be used to inhibit bone resorption activity. The present invention also provides a novel composition for inhibiting bone resorption, containing chicken extract as an active component. A peptide consisting of an amino acid sequence represented by the SEQ ID NO: 1 and/or a salt thereof and chicken extract can be consumed as a food or beverage or the like, and are also ad-

vantageous in terms of high safety.

Brief Description of Drawings

- [0014] [fig.1]Fig. 1 is a flow chart summarizing a method of preparing hydrolyzed collagen type II of chicken cartilage.
- [fig.2]Fig. 2 is a graph showing an effect of hydrolyzed collagen type II of chicken cartilage (HCII) and chicken extract (CE) added to inflammation-induced cells on inhibition of the production of inflammatory marker MIP-1 β .
- [fig.3]Fig. 3 is a graph showing a synergistic effect of a combination of hydrolyzed collagen type II of chicken cartilage (HCII) and chicken extract (CE) on inhibition of the production of inflammatory marker MIP-1 β .
- [fig.4]Fig. 4 is a graph showing an effect of fraction P1 and fraction P2 among seven fractions obtained by fractionation of hydrolyzed collagen type II of chicken cartilage (HCII) on inhibition of the production of inflammatory marker MIP-1 β .
- [fig.5]Fig. 5 is a graph showing an effect of hydrolyzed collagen type II of chicken cartilage (HCII) added to inflammation-induced cells on inhibition of the production of inflammatory markers.
- [fig.6]Fig. 6 is a graph showing an effect of hydrolyzed collagen type II of chicken cartilage (HCII) and chicken extract (CE), a synergistic effect of a combination of HCII and CE, and a synergistic effect of a combination of a peptide GPEGAPGKD and CE on inhibition of the production of inflammatory marker MCP-1.
- [fig.7-1]Figs. 7-1(a), (b) and (c) are graphs showing synergistic effects of a combination of HCII and CE and a combination of a peptide GPEGAPGKD and CE on inhibition of the production of inflammatory markers IL-6, IL-8 and IL-9.
- [fig.7-2]Figs. 7-2(d), (e) and (f) are graphs showing synergistic effects of a combination of HCII and CE and a combination of a peptide GPEGAPGKD and CE on inhibition of the production of inflammatory markers MCP-1, MIP-1 β and RANTES.
- [fig.8]Fig. 8 is a graph showing an effect of hydrolyzed collagen type II of chicken cartilage (HCII) on inhibition of bone resorption activity.
- [fig.9]Fig. 9 is a graph showing effects of hydrolyzed collagen type II of chicken cartilage (HCII), fractions and combinations of fractions (specifically, each of fractions P1 to P5; a combination of fraction P6 and fraction P7; a combination of fraction P1 and fraction P2; a combination of fraction P3 and fraction P4; and a combination of fractions P1 to P7) among seven fractions obtained by fractionation of hydrolyzed collagen type II of chicken cartilage (HCII) on inhibition of bone resorption activity.
- [fig.10]Fig. 10 is a graph showing effects of hydrolyzed collagen type II of chicken cartilage (HCII), a combination of fraction P1 and fraction P2 of HCII, and a peptide consisting of an amino acid sequence represented by the SEQ ID NO: 1

(GPEGAPGKD) on inhibition of bone resorption activity.

[fig.11]Fig. 11 is a graph showing a dose-dependent effect of chicken extract (CE) on inhibition of bone resorption activity.

[fig.12]Fig. 12(a) is a graph showing an effect of 14-day intake with hydrolyzed collagen type II of chicken cartilage (HCII) on VAS pain score on Days 7 and 14 in each treatment group for per-protocol population (n=151). Fig. 12(b) is a graph showing an effect of 14-day intake with hydrolyzed collagen type II of chicken cartilage (HCII) on VAS pain score on Days 7 and 14 in each treatment group for subjects doing less than 10th percentile of total resistance training period (n=8).

[fig.13]Fig. 13 is a graph showing an effect of 24-week intake of a combination of hydrolyzed collagen type II of chicken cartilage (HCII) and chicken extract (CE) on left hip bone mass.

Description of Embodiments

[0015] According to an embodiment of the present invention, the food or beverage composition of the present invention contains a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) and/or a salt thereof (hereinafter the "peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1)" is sometimes referred to as "Pep 1" or "GPEGAPGKD").

[0016] <Peptide>

Pep 1 may be a peptide that can be obtained by hydrolysis of an animal/plant protein or the like, or may be an artificially synthesized peptide. Preferably, Pep 1 is a peptide derived from hydrolyzed collagen type II, more preferably a peptide derived from hydrolyzed collagen type II of chicken cartilage.

Pep 1 may be purified before use. Alternatively, Pep 1 in the form of Pep 1-containing hydrolyzed collagen type II of chicken cartilage may be contained in the composition. In a preferred embodiment, the food or beverage composition of the present invention contains hydrolyzed collagen type II of chicken cartilage. The food or beverage composition of the present invention, when containing hydrolyzed collagen type II of chicken cartilage, exhibits higher anti-inflammatory action and higher bone resorption inhibitory action.

[0017] Pep 1 can be contained, in the food or beverage composition of the present invention, in the form of a salt with an inorganic acid or an organic acid or a salt with an inorganic base or an organic base. Such an acid or a base can be selected based on the application of the salt. In view of application to foods and beverages, the following dietary acceptable salts are preferred. Examples of the inorganic acid salt include hydrochloride, nitrate, sulfate, methanesulfonate, and p-toluenesulfonate. Examples of

the organic acid salt include salts with dicarboxylic acids such as oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, and salts with monocarboxylic acids such as acetic acid, propionic acid, and butyric acid. Examples of the inorganic base include hydroxides, carbonates, and bicarbonates of sodium, lithium, calcium, magnesium, and aluminium and ammonia. Examples of the salt with the organic base include mono-, di-, or tri-alkylamine salts such as salts of methylamine, dimethylamine, and triethylamine, mono-, di-, or tri-hydroxyalkylamine salts, guanidine salt, and N-methylglucosamine salt.

[0018] <Hydrolyzed collagen type II of chicken cartilage>

Preferably, the food or beverage composition of the present invention contains hydrolyzed collagen type II of chicken cartilage.

Hydrolyzed collagen type II of chicken cartilage (hereinafter, the "hydrolyzed collagen type II of chicken cartilage" is sometimes referred to as "HCII") can be obtained by hydrolysis of collagen type II with an enzyme or the like. The collagen type II can be extracted from chicken cartilage by a known method. The hydrolyzed collagen type II of chicken cartilage for use in the present invention can be prepared from cartilage by a method usually used in this field.

For example, the hydrolyzed collagen type II can be obtained by treating the chicken cartilage with an enzyme. Specifically, the hydrolyzed collagen type II can be prepared by a pre-treatment step (1) in which chicken cartilage is heated in a liquid, and a step (2) in which the chicken cartilage after the pre-treatment step is treated with an enzyme. The enzyme for use in the step (2) is not limited as long as it is one usually used in this field. Examples include collagenase, papain, bromelain, actinidine, ficin, cathepsin, pepsin, chymosin, trypsin, protease, subtilisin, amino peptidase, endopeptidase and exopeptidase and enzyme preparations obtained by mixing these enzymes. The method of preparing hydrolyzed collagen type II is not limited to the enzyme treatment method.

[0019] The hydrolyzed collagen type II of chicken cartilage may be a solution obtained by hydrolysis of chicken cartilage, a concentrate or dry powder of the solution, or a purified product of the concentrate or dry powder. The purified product of the hydrolyzed collagen type II of chicken cartilage may be obtained by, for example, subjecting a solution obtained by hydrolysis of chicken cartilage to ultrafiltration, membrane treatment, liquid separation operation, or fraction treatment with resin or the like so as to increase the purity. After increasing the purity of the hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof, the purified product may be formed into powder by freeze-drying or spray-drying, for example.

[0020] The hydrolyzed collagen type II of chicken cartilage is a peptide mixture that usually contains Pep 1 and/or a salt thereof, and can be regarded as a collagen peptide derived

from the collagen type II.

The weight average molecular weight of the hydrolyzed collagen type II of chicken cartilage is preferably 100 to 20,000, more preferably 2,000 to 8,000, still more preferably 3,000 to 7,000. The molecular weight and weight average molecular weight can be measured by Eurofins HPAEC-PAD method.

[0021] In an embodiment, a fraction containing Pep 1 or a combination of a fraction containing Pep 1 and one or more of other fractions, which can be obtained by fractionating hydrolyzed collagen type II of chicken cartilage by molecular weight by a method such as gel filtration, can be used in the food or beverage composition of the present invention.

The fraction obtained by fractionating hydrolyzed collagen type II of chicken cartilage for use in the food or beverage composition of the present embodiment is preferably one having a molecular weight of less than 3100 and having weight average molecular weight of 1200 to 1400 (fraction 1) and one having a molecular weight of less than 2600 and having weight average molecular weight of 950 to 1150 (fraction 2). In the present embodiment, the food or beverage composition preferably contains a combination of a fraction 1 and a fraction 2. Pep 1 is usually contained in the fractions 1 and 2, which is derived from hydrolyzed collagen type II of chicken cartilage.

[0022] Preferably, the food or beverage composition of the present invention further contains an animal extract and/or a plant extract.

Examples of the animal extract include extracts of mammals such as cattle, pigs, sheep, and goats; birds such as poultry, quail, ducks, geese; shellfish; insects; fish; mollusks; and crustaceans. Examples of the plant extract include extracts of tea, fruit juice and concentrate thereof, vegetable extract and concentrate thereof, soy and nuts extract.

Of these animal extracts and plant extracts, the extract is preferably an animal extract, more preferably a bird extract, still more preferably a poultry extract, particularly preferably chicken extract. The food or beverage composition of the present invention, when containing chicken extract, exhibits a higher inflammation reducing effect.

[0023] <Chicken extract>

The chicken extract (hereinafter sometimes referred to as "CE") for use in the present invention may be an extract that can be obtained by heating chicken meat used as a raw material in a liquid, or a commercial product. The raw material may contain bone, cartilage, legs, or the like, but preferably, the raw material does not contain the head or internal organs.

[0024] Examples of the commercial product of the chicken extract (CE) include "Brand's Essence of Chicken (BEC) (produced by Suntory Beverage & Food Asia Pte Ltd)",

"Scotch™ Essence of Chicken (produced by Scotch Industrial (Thailand) Co., Ltd.)", "Quaker Essence of chicken (produced by Standard Foods Corporation (Taiwan) Co., Ltd.)", "Chicken stock and broth of SWANSON™ Produced by Campbell Soup Company (NYSE:CPB)", "Drip Chicken Essence produced by Eu Yan Sang International Ltd. (Singapore)", "Boned Chicken Tonic produced by Eu Yan Sang International Ltd. (Singapore)", "Boiled Essence of Chicken produced by Lao Xie Zhen Co. Ltd. (Taiwan)". Any of such commercial products may be used, but use of Brand's Essence of Chicken (BEC) is preferred.

[0025] When producing chicken extract for use in the present invention by hot water extraction, the chicken extract can be produced by a method that is usually used in this field. For example, normal pressure extraction and/or pressurized extraction is performed using a liquid at a temperature of 100°C or higher, preferably 125°C or higher, and the resulting extract is treated with a membrane or filtered, whereby chicken extract can be produced. Specifically, the extract is obtained by a pre-treatment step (3) in which chicken meat is heated in a liquid to remove water-soluble protein in the chicken meat, and a step (4) in which the liquid is replaced with a fresh liquid after the pre-treatment and the chicken meat is heated again. The heat treatment in each of the step (3) and the step (4) is preferably performed in a solvent in order to prevent burning of the chicken meat serving as a raw material. The solvent is preferably water, ethanol, or a mixture of these, for example.

The chicken extract encompasses a liquid extract obtained by the method described above; a diluted solution, concentrate, or dry powder of the liquid extract; and purified products of these. The purified products may be obtained by, for example, subjecting a chicken extract liquid to ultrafiltration, membrane treatment, liquid separation operation, or fraction treatment with resin or the like so as to increase the purity. After increasing the purity of the chicken extract, the purified product may be formed into powder by freeze-drying or spray-drying, for example.

[0026] Preferably, the chicken extract for use in the present invention contains carnosine and/or anserine and/or one or more salts thereof. Carnosine is β -alanyl $\cdot$ histidine, which is a dipeptide of β -alanine and histidine. Anserine is β -alanyl $\cdot$ 1-methylhistidine in which histidine is methylated.

Examples of the carnosine salts and anserine salts include the same salts as those for Pep 1 described above.

[0027] When the food or beverage composition of the present invention contains carnosine and/or a salt thereof, for example, the amount of carnosine and/or a salt thereof in terms of carnosine in the composition is preferably 0.00001 wt% or more, more preferably 0.0001 wt% or more, and is preferably 10 wt% or less, more preferably 1 wt% or less. In an embodiment, for example, the amount of carnosine in the com-

position is preferably 0.00001 to 10 wt%, more preferably 0.0001 to 1 wt%.

[0028] When the food or beverage composition of the present invention contains anserine and/or a salt thereof, for example, the amount of anserine and/or a salt thereof in terms of anserine in the composition is preferably 0.00001 wt% or more, more preferably 0.0001 wt% or more, and preferably 10 wt% or less, more preferably 1 wt% or less. In an embodiment, for example, the amount of anserine in the composition is preferably 0.00001 to 10 wt%, more preferably 0.0001 to 1 wt%.

[0029] Carnosine and anserine can be quantitated by HPLC, for example. More preferably, the food or beverage composition of the present invention contains carnosine and anserine.

[0030] Preferably, the weight ratio of the total weight of the carnosine, anserine and salts thereof in terms of carnosine and anserine to the weight of Pep 1 and/or a salt thereof in terms of peptide (total of carnosine and anserine/Pep 1) is 1,000,000/1 to 1/15000. The weight ratio is more preferably 1,000,000/1 to 100/1, still more preferably 100,000/1 to 1,000/1.

[0031] Preferably, the weight ratio of the hydrolyzed collagen type II of chicken cartilage (in terms of solids) to the total of carnosine, anserine and salts thereof in terms of carnosine and anserine (HCII/total of carnosine and anserine) is 20/1 to 1/5. The weight ratio is more preferably 15/1 to 1/3.

[0032] The amount of each component of the food or beverage composition of the present invention is not limited, and can be set according to the form or the like of the composition.

In an embodiment, the amount of Pep 1 and/or a salt thereof in the food or beverage composition of the present invention is preferably 0.00001 wt% or more, more preferably 0.0001 wt% or more, and preferably 1 wt% or less, more preferably 0.1 wt% or less in terms of Pep 1. In an embodiment, the amount of Pep 1 and/or a salt thereof in the composition is preferably 0.00001 to 1 wt%, more preferably 0.0001 to 0.1 wt%.

[0033] In an embodiment, the amount of hydrolyzed collagen type II of chicken cartilage (in terms of solids) in the food or beverage composition of the present invention is preferably 0.1 wt% or more, more preferably 0.5 wt% or more, and preferably 99 wt% or less, more preferably 90 wt% or less. In an embodiment, the amount of hydrolyzed collagen type II of chicken cartilage (in terms of solids) in the composition is preferably 0.1 to 99 wt%, more preferably 0.5 to 90 wt%.

Herein, the amount of hydrolyzed collagen type II of chicken cartilage contains the amount of Pep 1.

[0034] In an embodiment, for example, the amount of chicken extract (in terms of solids) in the food or beverage composition of the present invention is preferably 0.1 wt% or

more, more preferably 0.5 wt% or more, and preferably 99 wt% or less, more preferably 90 wt% or less. In an embodiment, for example, the amount of chicken extract (in terms of solids) in the composition is preferably 0.1 to 99 wt%, more preferably 0.5 to 90 wt%.

- [0035] Examples of the food or beverage include general foods or beverages, foods with function claims, health-promoting foods, foods for special dietary uses, dietary supplements, health supplements, and general supplements. The form of the food or beverage is not limited. For example, it may be a solid food or a liquid food. A beverage is preferred.
- [0036] The food or beverage composition of the present invention may contain dietary acceptable additives such as various diluents, acidulants, antioxidants, stabilizers, preservatives, flavouring or masking agents, emulsifiers, pigments, seasonings, pH adjusters, and nutritional enhancers.
- [0037] The food or beverage composition of the present invention is preferably for non-therapeutic use. The "non-therapeutic" is a concept that does not include medical activities, i.e., a concept that does not include methods of surgery, therapy or diagnosis of humans.
- [0038] <Anti-inflammatory composition>
In an embodiment, the food or beverage composition of the present invention can be used to reduce inflammation. The food or beverage composition of the present invention may be an anti-inflammatory composition, or an anti-inflammatory composition containing Pep 1 and/or a salt thereof as an active component.
- [0039] In order to achieve an anti-inflammatory effect contemplated by the present invention, hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof and, optionally, chicken extract, are added to a food or beverage composition, in the same manner as described above for the food or beverage composition. The hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof and chicken extract may be used directly, or a concentrate, dry powder, or purified product thereof may be added as described above, as long as the effect of the present invention is not impaired. The same additives as described above can be used.
- [0040] The intake of the food or beverage composition of the present invention is not limited. In an embodiment, when the food or beverage composition of the present invention is fed to achieve an anti-inflammatory effect, the intake may be suitably set according to the body weight of the subject and the like, as long as the inflammation reducing effect can be achieved.
- [0041] In an embodiment, when the food or beverage composition of the present invention is fed to a human (adult), the intake of Pep 1 and/or a salt thereof in terms of Pep 1 is preferably 0.001 mg or more, more preferably 0.01 mg or more, and also preferably

200 mg or less, more preferably 100 mg or less, per 60 kg body weight per day. In an embodiment, the intake of Pep 1 and/or a salt thereof in terms of Pep 1 by a human (adult) is preferably 0.001 to 200 mg, more preferably 0.01 to 100 mg, per 60 kg body weight per day.

- [0042] In an embodiment, when the food or beverage composition of the present invention is fed to a human (adult), the intake of hydrolyzed collagen type II of chicken cartilage (in terms of solids) is preferably 0.01 mg or more, more preferably 0.1 mg or more, and also preferably 4000 mg or less, more preferably 3000 mg or less, per 60 kg body weight per day. In an embodiment, the intake of hydrolyzed collagen type II of chicken cartilage by a human (adult) is preferably 0.01 to 4000 mg, more preferably 0.1 to 3000 mg, per 60 kg body weight per day.

Herein, the intake of hydrolyzed collagen type II of chicken cartilage includes the amounts of Pep 1 and/or a salt thereof.

- [0043] In an embodiment, when the food or beverage composition of the present invention is fed to a human (adult), the intake of chicken extract (in terms of solids) is preferably 0.1 mg or more, more preferably 1 mg or more, and also preferably 15000 mg or less, more preferably 13000 mg or less, per 60 kg body weight per day. In an embodiment, the intake of chicken extract (in terms of solids) by a human (adult) is preferably 0.1 to 15000 mg, more preferably 1 to 13000 mg, per 60 kg body weight per day. The intake of chicken extract includes the amounts of carnosine, anserine and salts thereof.

- [0044] In an embodiment, when the food or beverage composition of the present invention is fed to a human (adult), the total intake of carnosine, anserine and salts thereof in terms of carnosine and anserine is preferably 0.001 mg or more, more preferably 0.01 mg or more, and also preferably 500 mg or less, more preferably 400 mg or less, per 60 kg body weight per day. In an embodiment, when carnosine and anserine are fed to a human (adult), the total intake of carnosine, anserine and salts thereof in terms of carnosine and anserine is preferably 0.001 to 500 mg, more preferably 0.01 to 400 mg, per 60 kg body weight per day.

- [0045] In an embodiment, the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof and the above amount of chicken extract are preferably fed at least once per day, for example, at once or in several times (e.g., two or three times) per day. In an embodiment, preferably, the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof and the above amount of chicken extract are fed to a human. In an embodiment, the composition of the present invention can be used to feed the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof and the above amount of chicken extract to a human, per 60 kg body weight per day.

[0046] In an embodiment, when the food or beverage composition of the present invention is fed to achieve the anti-inflammatory effect, preferably, the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof and the above amount of chicken extract are fed.

[0047] The food or beverage composition of the present invention inhibits the production of cytokines such as macrophage inflammatory protein-1 (MIP-1), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-12 (IL-12) and regulated on activation, normal T cell expressed and secreted (RANTES). The food or beverage composition of the present invention is particularly highly effective in inhibiting the production of MIP-1 β , MCP-1, IL-6, IL-7, IL-8, IL-9, IL-12 and RANTES.

Inflammation in a living body can be reduced by inhibiting the production of the cytokine.

[0048] The food or beverage composition of the present invention can be used to prevent or alleviate inflammatory conditions or diseases. The inflammatory conditions or diseases are, for example, conditions or diseases caused by inflammation or conditions or diseases accompanied by inflammation. Examples of such conditions or diseases include collagen diseases such as arthritis and rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, tendonitis, sciatica, intervertebral hernia, stenosis, myelopathy, back pain, facet joint pain, carpal tunnel syndrome, tarsal tunnel syndrome, post-lumbar surgery pain syndrome, AIDS, arteriosclerosis, asthma, arthritis, diabetes, hepatitis, stroke, dementia, muscle wasting, viral infection, skin aging including photoaging, cancer, aging, allergic diseases, Parkinson's disease, cerebral infarction, cataract, epilepsy, spinal cord injury, retinopathy of prematurity, nephropathy, peptic ulcer, pancreatitis, ulcerative colitis, myocardial infarction, adult respiratory distress syndrome, emphysema, vasculitis, edema, diabetes complications, UV damage, altitude sickness, porphyria, burns, frostbite, contact dermatitis, shock, multiple organ failure, DIC, fatigue, sarcopenia (muscle weakness), mitochondrial dysfunction, Alzheimer's disease, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis and systemic lupus erythematosus (lupus). The food or beverage composition of the present invention is preferably used to prevent or alleviate these diseases. In particular, the food or beverage composition is preferably used to prevent or alleviate diseases such as osteoarthritis, rheumatoid arthritis and psoriatic arthritis.

Herein, prevention of conditions or diseases encompasses prevention of disease onset, delay of disease onset, reduction in disease incidence, reduction of risk of disease onset, and the like. Alleviation of conditions or diseases encompasses recovery of the subject from conditions or diseases, alleviation of conditions or disease symptoms, improvement of conditions or disease symptoms, delay or prevention of

progress of conditions or diseases, and the like.

[0049] The subject to which the food or beverage composition of the present invention is fed (which can also be referred to as a "subject") is not limited. The subject is preferably a human or non-human mammal, more preferably a human.

In an embodiment, the subject may be one needing or wanting inhibition of inflammation. Such a subject may be, for example, one needing or wanting prevention or alleviation of inflammation or one needing or wanting prevention or alleviation of inflammation of inflammatory conditions or diseases. In an embodiment, the subject in the present invention may be a middle-aged or older person. The food or beverage composition of the present invention can also be used by a healthy person, for example, for the purpose of prevention of conditions that can be prevented or alleviated by reducing inflammation.

[0050] The food or beverage composition of the present invention may be labeled with function claims stating that the effect is exerted by reducing inflammation. Such a label is also referred to as a label with function claims, the contents of the label are not limited. Examples of such function claims on the label include "relief of joint pain", "reduction of joint pain", "management of knee conditions", "maintenance of knee health", "improvement of joint health", "improvement of joint mobility", and other function claims equivalent to those mentioned above.

In an embodiment of the present invention, the food or beverage composition of the present invention is preferably a food or beverage labeled with the function claims described above, and a beverage is more preferred. The label may describe use of the food or beverage composition of the present invention to achieve the above functions. The label may be added to the food or beverage composition itself or a container or a package of the food or beverage composition.

[0051] <Composition for inhibiting bone resorption>

In another embodiment, the food or beverage composition of the present invention can be used to inhibit bone resorption activity of osteoclasts. The food or beverage composition of the present invention can be used as a composition for inhibiting bone resorption. The present invention also encompasses a composition for inhibiting bone resorption containing Pep 1 and/or a salt thereof as an active component.

[0052] In order to achieve a bone resorption inhibitory effect contemplated by the present invention, hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof is added to a food or beverage composition, in the same manner as described above for the anti-inflammatory composition. The hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof may be used directly, or a concentrate, dry powder, or purified product thereof may be added as described above, as long as the effect of the present invention is not impaired. The same

additives as described above can be used.

- [0053] When the food or beverage composition of the present invention is used to inhibit bone resorption activity, the intake is not limited. The intake of the food or beverage composition of the present invention may be suitably set according to the body weight of the subject and the like, as long as the bone resorption activity inhibitory effect can be achieved.
- [0054] When the food or beverage composition of the present invention is fed to a human (adult) in order to inhibit bone resorption activity, the amount and the frequency of intake of the Pep 1 and/or a salt thereof and the hydrolyzed collagen type II of chicken cartilage may be the same as those of the anti-inflammatory composition described above. In an embodiment, the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof is preferably fed to a human. In an embodiment, the food or beverage composition of the present invention can be used to feed the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 to a human (60 kg body weight) per day.
- [0055] In an embodiment, when the food or beverage composition of the present invention is fed to achieve bone resorption inhibitory effect, the above amount of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof is preferably fed.
- [0056] The food or beverage composition of the present invention inhibits bone resorption activity of osteoclasts. Inhibition of bone resorption activity of osteoclasts leads to reduced or alleviated bone loss. The reduction in bone loss includes maintenance of bone mass and delay or cessation of the progress of bone loss. Alleviation of bone loss includes a recovery of bone mass, an increase in bone mass, and a reduction in the degree of bone loss.
- [0057] The food or beverage composition of the present invention can be used to prevent or alleviate conditions or diseases associated with bone loss. Examples of such conditions or diseases include osteoporosis and osteopenia. The food or beverage composition of the present invention is preferably used to prevent or alleviate such diseases.
- [0058] In an embodiment, the subject may be one needing or wanting inhibition of bone resorption activity. Examples of such a subject include those needing or wanting prevention or alleviation of conditions or diseases associated with bone loss. In an embodiment, the subject in the present invention may be a middle-aged or older person. The composition of the present invention can also be used by a healthy person, for example, for the purpose of prevention of conditions that can be prevented or improved by inhibiting bone resorption activity.
- [0059] The food or beverage composition of the present invention may be labeled with function claims stating that the effect is exerted by inhibiting bone resorption activity.

Examples of such function claims on the label include "to increase bone density", "to replenish bone mass", "to maintain the health of bone", "to prevent bone loss", and other function claims equivalent to those mentioned above.

In an embodiment of the present invention, the food or beverage composition of the present invention is preferably a food or beverage labeled with the function claims described above, and a beverage is more preferred. The label may describe use of the food or beverage composition of the present invention to achieve the above function. The label may be added to the food or beverage composition itself or a container or a package of the food or beverage composition.

[0060] <Production method>

The present invention also relates to a method of producing a food or beverage composition, including adding a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) (Pep 1) and/or a salt thereof.

In the adding, Pep 1 and/or a salt thereof can be added by itself to a food or beverage composition, but it is preferred to add hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof to produce a food or beverage composition. In this case, for example, a hydrolysate obtained by hydrolysis of collagen type II of chicken cartilage with an enzyme or the like can be directly used for adding to the food or beverage composition. As described above, a concentrate, dry powder, or a purified product thereof may be added, as long as the effect of the present invention is not impaired.

[0061] In the production method of the present invention, preferably, the method further includes adding an animal extract and/or plant extract.

In the production method of the present invention, the order of adding raw materials is not limited. For example, Pep 1 and/or a salt thereof or hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof may be first placed in a container, followed by addition of an animal extract and/or a plant extract. Alternatively, an animal extract and/or a plant extract may be first placed in a container, followed by addition of Pep 1 and/or a salt thereof or hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof.

Of these animal extracts and plant extracts, the extract is preferably an animal extract, more preferably a bird extract, still more preferably a poultry extract, particularly preferably chicken extract.

In the production method of the present invention, a preferred embodiment of the hydrolyzed collagen type II of chicken cartilage is as described above. A preferred embodiment of the chicken extract is as described above.

In the production method of the present invention, preferably, the chicken extract

contains carnosine and/or anserine and/or one or more salts thereof.

[0062] In the production method of the present invention, commercially available chicken extract or chicken extract produced by hot water extraction can be directly used for adding to Pep 1 and/or a salt thereof or hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof. Yet, when the chicken extract is in the form of a concentrate, dry powder, or a purified product of the concentrate or the dry powder, the chicken extract may be diluted, dissolved, or the like in a liquid such as water, ethanol, or a mixture of water and ethanol, and then added to Pep 1 and/or a salt thereof, hydrolyzed collagen type II of chicken cartilage, or the like. When the hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof is in the form of a concentrate, dry powder, or a purified product thereof, such a product can be similarly diluted, dissolved, or the like in the liquid or the like before adding. Alternatively, such a product may be added without being diluted, dissolved, or the like in advance, and then blended with a liquid so as to be diluted, dissolved, or the like in the liquid.

[0063] The food or beverage composition produced by the production method of the present invention can contain the above-described additives, in addition to Pep 1 and/or a salt thereof, the hydrolyzed collagen type II of chicken cartilage and the chicken extract.

[0064] The present invention also relates to use of a hydrolyzed collagen type II containing a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) (Pep 1) and/or a salt thereof for the production of an anti-inflammatory composition.

Examples of the anti-inflammatory composition include a composition similar to the above-described food or beverage composition containing the hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof. The same chicken extract and the same additives as described above can be used.

[0065] The present invention also relates to use of a peptide consisting of an amino acid sequence represented by Gly-Pro-Glu-Gly-Ala-Pro-Gly-Lys-Asp (SEQ ID NO: 1) (Pep 1) and/or a salt thereof for the production of a composition for inhibiting bone resorption.

Examples of the composition for inhibiting bone resorption include a composition similar to the above-described food or beverage composition containing Pep 1 and/or a salt thereof. Pep 1 and/or a salt thereof can be used in the form of hydrolyzed collagen type II of chicken cartilage which contains Pep 1 and/or a salt thereof. The same additives as described above can be used.

[0066] The present invention also relates to a composition for inhibiting bone resorption containing chicken extract as an active component.

The composition for inhibiting bone resorption of the present invention has an effect

of inhibiting bone resorption of osteoclasts.

The composition of the present invention can exhibit the bone resorption inhibitory effect because it contains chicken extract as an active component.

- [0067] The chicken extract and its preferred embodiments are as described above. The composition may contain the above-described additives, if desired. The composition for inhibiting bone resorption of the present invention may further contain the hydrolyzed collagen type II of chicken cartilage.
- [0068] In an embodiment, for example, the amount of chicken extract (in terms of solids) in the composition for inhibiting bone resorption of the present invention is preferably 0.1 wt% or more, more preferably 0.5 wt% or more, and also preferably 99 wt% or less, more preferably 90 wt% or less. In an embodiment, for example, the amount of chicken extract (in terms of solids) in the composition is preferably 0.1 to 99 wt%, more preferably 0.5 to 90 wt%.
- [0069] Preferably, the composition for inhibiting bone resorption of the present invention contains carnosine and/or anserine and/or one or more salts thereof. In an embodiment, for example, the amount of carnosine and/or a salt thereof in terms of carnosine in the composition of the present invention is preferably 0.00001 wt% or more, more preferably 0.0001 wt% or more, and also preferably 10 wt% or less, more preferably 1 wt% or less. In an embodiment, for example, the amount of carnosine in the composition is preferably 0.00001 to 10 wt%, more preferably 0.0001 to 1 wt%.
- [0070] In an embodiment, for example, the amount of anserine and/or a salt thereof in terms of anserine in the composition for inhibiting bone resorption of the present invention is preferably 0.00001 wt% or more, more preferably 0.0001 wt% or more, and also preferably 10 wt% or less, more preferably 1 wt% or less. In an embodiment, for example, the amount of anserine in the composition is preferably 0.00001 to 10 wt%, more preferably 0.0001 to 1 wt%.
- [0071] The intake of the composition for inhibiting bone resorption of the present invention is not limited. The intake may be suitably determined according to the body weight of the subject or the like, as long as the effect of inhibiting bone resorption of mature osteoclasts can be achieved.
- [0072] In an embodiment, when the composition for inhibiting bone resorption of the present invention is fed to a human (adult), the intake of chicken extract (in terms of solids) is preferably 0.1 mg or more, more preferably 1 mg or more, and also preferably 15000 mg or less, more preferably 13000 mg or less, per 60 kg body weight per day. In an embodiment, the intake of chicken extract (in terms of solids) by a human (adult) is preferably 0.1 to 15000 mg, more preferably 1 to 13000 mg, per 60 kg body weight per day. The intake of chicken extract includes the amounts of carnosine and anserine.

- [0073] In an embodiment, when the composition for inhibiting bone resorption of the present invention is fed to a human (adult), the total intake of carnosine, anserine and salts thereof in terms of carnosine and anserine is preferably 0.001 mg or more, more preferably 0.01 mg or more, and also preferably 500 mg or less, more preferably 400 mg or less, per 60 kg body weight per day. In an embodiment, the total intake of carnosine, anserine and salts thereof in terms of carnosine and anserine by a human (adult) is preferably 0.001 to 500 mg, more preferably 0.01 to 400 mg, per 60 kg body weight per day.
- [0074] In an embodiment, the above amount of chicken extract is preferably fed at least once per day, for example, at once or in several portions (e.g., two or three times) per day. In an embodiment, the above amount of chicken extract is preferably fed to a human. In an embodiment, the composition of the present invention can be used to feed the above amount of chicken extract to a human (60 kg body weight) per day. Preferred examples of the subject to which the composition for inhibiting bone resorption is fed are as described above.
- [0075] The present invention also relates to use of chicken extract for the production of the composition for inhibiting bone resorption.
- Examples of the composition for inhibiting bone resorption include compositions similar to the food or beverage composition containing chicken extract. The same chicken extract and the same additives as described above can be used.
- Example**
- [0076] The present invention is more specifically described below with reference to examples. The present invention is not limited to these examples.
- [0077] 1. Inflammation reducing effect
- Raw materials, reagents, and the like used in the following examples and comparative examples are as follows.
- <Preparation of hydrolyzed collagen type II of chicken cartilage>
- Fig. 1 shows a flow chart briefly describing a HCII preparation method. First, frozen chicken cartilage was thawed in water at 40°C, and cleansed in water at 40°C (1 hour). Next, the wash water was discarded, and fresh water was poured into a 1200L pot as to be 3 times amount to the chicken cartilage. The water was heated to the optimal working temperature for enzyme treatment, and the washed chicken cartilage was immersed therein to perform enzyme treatment for several hours. After the enzyme treatment, the pot containing the chicken cartilage was heated to 90°C or higher, and the temperature was held at 90°C or higher for 30 minutes, whereby the enzyme used in the earlier enzyme treatment was inactivated. The resulting mixture (the liquid and the enzyme-treated chicken cartilage) was filtered. The resulting liquid was concentrated. Lastly, the resulting concentrate was spray-dried at 200°C, whereby HCII

powder was prepared.

[0078] <Measuring for the molecular weight of the HCII powder>

The molecular weight distribution of the HCII obtained was measured by Eurofins HPAEC-PAD method. Table 1 below shows the molecular weight distribution of the HCII obtained. The HCII obtained had a weight average molecular weight of 4582 which was obtained by calculating with usually used method in this field.

[0079]

[Table 1]

Molecular weight Mass Range	Percentage of Molecules	Assumed Mass of Molecule	Total Mass Type of Molecule	Weight Fraction Type of Molecule (NiMi)/Σ(NiMi)	Weight Molecular Weight WiMi
	Ni(%)	Mi(Da)	NiMi		WiMi
<500 D	57.0	500	285	0.221	110.47
500 - 1000 D	23.0	750	172.5	0.134	100.29
1000 - 3000 D	14.0	2000	280	0.217	434.11
3000 - 5000 D	1.0	4000	40	0.031	124.03
5000 - 10000 D	1.1	7500	82.5	0.064	479.65
> 10000 D	4.3	10000	430	0.333	3333.33
			Σ(NiMi)=1290		Σ(WiMi)=4581.88

[0080] <Fractionation of HCII fractions and identification of peptide>
(Material & Method for HCII Fractionation and Bioactive Identification)
(Materials)

Formic acid was purchased from Tokyo Chemical Industry Co. Ltd. Ultrapure water was obtained from Merck Milli-Q water purification system. The synthetic peptide GPEGAPGKD was obtained from GenScript.

[0081] (Preparative Gel Permeation Chromatography (GPC))

Fractionation of HCII was carried out using a preparative VERITY 271 HPLC system (Gilson). HCII was dissolved in ultrapure water to prepare a 10 mg/mL (w/v) solution. After centrifugation at 14000 rpm for 5 min, 1000 μ L aliquot was injected onto a preparative GPC column (BioSep 5 μ m SEC-s2000 145 Å LC column 300 x 21.2 mm (Phenomenex)) attached to a guard column (SecurityGuard PREP Cartridge C12 15 x 21.2 mm ID (Phenomenex)) and eluted isocratically with eluent A (ultrapure water: formic acid = 100: 0.1 (v/v)) for 30 min at the rate of 5 mL/min. The chromatogram was observed at 214 nm.

Fractions were collected every 0.5 min between 8 min to 28 min using a GX series fraction collector (Gilson), the resulted 40 individual fractions were subsequently pooled into 7 fractions, P1 to P7 (P1: 8.5-12.0 min, P2: 12.0-13.5 min, P3: 13.5-14.5 min, P4: 14.5-16.0 min, P5: 16.0-17.5 min, P6: 17.5-19.0 min, P7: 19.0-27.5 min). The pooled fractions were evaporated to dryness in a freeze dryer (ScanVac) and the dried samples were stored at -20°C until further use.

[0082] Table 2 shows the molecular weight of each fraction obtained above. In Table 2, “Mw” and “Mp” indicate weight average molecular weight and peak molecular weight, respectively. As shown in Table 2, fraction 1 (P1) and fraction 2 (P2) were composed of molecules having an average molecular weight of 1359(P1) and 1092(P2).

The molecular weights of fractions of HCII were measured by HPLC Gel filtration method under the following conditions.

Instrument: Agilent 1100 Series

Detection: UV 214 nm

Flow rate: 1mL/min

Mobile phase: Isocratic 0.1 mM Sodium phosphate buffer at pH 6.8

Running time: 20 minutes

Column: Biosep™ 5 μ m SEC-s2000 145 Å LC Column 300 x 7.8 mm

[0083]

[Table 2]

Fraction	Mw	Mp
P1	1359	1626
P2	1092	1494
P3	1035	956
P4	784	783
P5	422	213
P6	211	225

[0084] (In-silico Search of Peptides that lead to GPEGAPGKD)

Fractions P1 to P7 were subjected to LC-MS analysis on an Agilent HPLC 1290 series-coupled TripleTOF 5600 (AB Sciex) with a Duo Spray Turbo V ion source and a gas generator (Peak Scientific Ltd.). The major components were separated with a UHPLC Guard Zorbax EclipsePlusC18 2.1 x 5 mm 1.8 μ m (Agilent), and eluted with eluent A (ultrapure water: formic acid = 100: 0.1 (v/v)) and eluent B (acetonitrile: formic acid = 100: 0.1 (v/v)), with the following linear gradient: 0-0.5 min: 100% eluent A; 0.5-7.5 min: 100-65% eluent A; 7.5-10.0 min: 65-0% eluent A; 10.0-13.0 min: 0% eluent A; 13.0-13.1 min: 0-100% eluent A; 13.1-15.0 min: 100% eluent A. The experiment was conducted under Independent Data Acquisition (IDA) method in position mode with the collision energy (CE) and declustering potential (DP) of the MS were optimized as 10.0 V and 80 V respectively. The analysis was done by PeakView (AB Sciex). The mass spectroscopy data was subsequently analyzed and searched using Protein Pilot (AB Sciex) attached with Uniprot KB fasta file using gallus gallus as key word. The data was further processed with shortlisting of candidates and the confirmation was done with peptides purchased from Genscript.

[0085] (Detection and Quantification of Peptide Markers by LC-MS Analysis)

Detection and quantification of GPEGAPGKD peptide was performed on an LC-MS. LC-MS analysis was performed on an Agilent HPLC 1290 series-coupled TripleTOF 5600 (AB Sciex) with a Duo Spray Turbo V ion source and a gas generator (Peak Scientific Ltd.). The mass spectrometer uses electrospray ionization and multiple reaction monitoring (MRM) in positive ion mode at unit mass resolution, with the following parameters: Collision Energy Spread (CES): 10; Ion Release Delay (IRD): 67; Ion Release Width (IRW): 25; Ion Source Gas: 40; Curtain Gas (CUR): 30; Temperature: 500.0; Ion Spray Voltage Floating (ISVF): 5500). MS compound-dependent parameter settings such as declustering potential (DP) and collision energy (CE) were optimized accordingly for different peptides. Analyst 1.5.2 (AB Sciex) was used for equipment control, data acquisition, and data processing.

[0086] GPEGAPGKD was reconstituted in ultrapure water and injected on to a ZIC-HILIC

3.5 μm 100Å 2.1 x 50 mm column (Merck), with a SeQuant ZIR-HILIC Guard Fitting PEEK coated 14 x 1 mm column (Merck), and eluted with eluent A (ultrapure water: formic acid = 100: 0.1 (v/v)) and eluent B (acetonitrile: formic acid = 100: 0.1 (v/v)), with the following linear gradient: 0-0.5 min: 10% eluent A; 0.5-5.5 min: 10-45% eluent A; 5.5-10 min: 45-70% eluent A; 10-12 min: 70-80% eluent A; 12-13.5 min: 80-10% eluent A; 13.5-15 min: 10% eluent A. The injection volume was 10 μL at the flow rate of 300 $\mu\text{L}/\text{min}$ and UV absorbance of the eluents was monitored at 214 nm. The collision energy (CE) and declustering potential of the MS were optimized as 20 V and 85 V respectively. GPEGAPGKD was detected using Q1/Q3 ion transitions at m/z 414.2/641.3 Th with the retention time of 7.1 min as quantifier and qualifier. A calibration curve was constructed with 15.25, 31.5, 62.5, 125, 250 ng/mL of GPEGAPGKD standard. HCII was reconstituted as 500 $\mu\text{g}/\text{mL}$ solution as the working solution. The quantification of the GPEGAPGKD in HCII and its GPC fractions was done by MultiQuant (AB Sciex).

Pep 1 was contained in the fractions P1 and P2 of HCII, and the content of Pep 1 was $53.7 \pm 0.007 \mu\text{g} / \text{g}$ based on 1 g of HCII.

[0087] <Quantification of carnosine and anserine>

Quantification of carnosine and anserine in the chicken extract was performed by HPLC under the following conditions.

Carnosine standard stock was prepared by adding and dissolving carnosine powder in deionized water to a carnosine concentration of 2.50 mg/ml. Anserine standard stock was prepared by adding and dissolving L-anserine nitrate powder in deionized water to an L-anserine concentration of 3.96 mg/ml.

The weight of L-anserine was calculated by the following formula.

$$\text{L-anserine (g)} = 0.792 \times \text{L-anserine nitrate (g)}$$

<Analysis conditions of HPLC>

Device: High performance liquid chromatography system with UV detector (Agilent 1100 produced by Agilent)

Column: Zorbax 300-SCX 4.6 mm ID x 250 mm (Agilent)

Mobile phase: 50 mM potassium dihydrogen phosphate

Flow rate: 1.0 mL/min

Flow channel: channel A (50 mM potassium dihydrogen phosphate), channel B (acetonitrile), channel D (deionized water)

UV detector wavelength: 210 nm

Sample injection volume: 10 μL

[0088] <Evaluation method of anti-inflammatory activity>

In Examples 1 to 3, the effect of inhibiting the production of inflammatory markers was evaluated by the following method.

(Raw materials and reagents)

Chicken extract (CE): Brand's Essence of Chicken (Suntory Beverage & Food Asia Pte Ltd, carnosine content in 1 ml: 0.94 mg/ml, anserine content 1 ml: 1.9 mg/ml)

Chondrocyte medium (CM), fetal bovine serum (FBS), chondrocyte growth supplement (CGS), and penicillin/streptomycin (P/S): ScienCell Research Laboratories
IL-1 β : R&D Systems

Poly-L-lysine (PLL) coated 96-well plates (Corning)

[0089] <Cell culture and pre-treatments>

Human chondrocytes (HC-a, ScienCell Research Laboratories) were isolated from human articular cartilage and were maintained in CM supplemented with 5% FBS, 1% CGS and 1% P/S. Cells were seeded in PLL-coated 96-well plate at a cell density of 4000 cells/well and incubated overnight at 37°C in a humidified gas chamber containing 5% CO₂. The chondrocytes were washed once with PBS and pre-treated with HCII, chicken extract (CE), a combination of HCII and CE, a fraction fractionated from HCII, or GPEGAPGKD at various concentrations for 24 hours, and were further treated for 24 hours by adding 25 ng/mL IL-1 β . The cells were centrifuged at 1100 g for 5 minutes and the supernatant was then used for cytokine analysis. For comparison, cells treated with IL-1 β (IL-1 β -treated cells) without being pre-treated with HCII, CE, fractions fractionated from HCII, or GPEGAPGKD were prepared.

[0090] <Multiplex cytokine analysis>

The Pro-Human Cytokine Multiplex Assays (Bio-Rad) was used to analyze the cytokines in the culture media. The following 27-plex analyses were performed: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin (CCL11), macrophage colony-stimulating factor (M-CSF), interferon (IFN)- γ , monocyte chemotactic protein 1 (MCP-1; CCL2), macrophage inflammatory protein-1 α (MIP-1 α ; CCL3), MIP-1 β (CCL4), regulated on activation, normal T cell expressed and secreted (RANTES) (CCL5), TNF- α , and vascular endothelial growth factor (VEGF). Multiplex assays were carried out according to the manufacturers' instructions and run on the Luminex xPONENT for MAGPIX platform. Bio-Plex Manager version 6.0 was used for data processing. Cytokine and chemokine concentrations were calculated by reference to the standard curve. The sensitivity of the multiplex kit was < 5 pg/mL.

[0091] <Statistical analyses>

Statistical analysis was conducted using GraphPad Prism version 5.0 (GraphPad). All results were expressed as mean $\pm$ standard deviation. Statistical analysis was performed by analysis of variance (ANOVA) followed by posthoc Tukey's multiple comparison test. Data was considered to be significant when $p < 0.05$.

[0092] <Example 1 and Comparative Examples 1 and 2>

To assess the influence of HCII and chicken extract (CE) on the inflammatory marker MIP-1 β produced by the human chondrocytes, the following four different conditions were compared: cells before treatment (untreated control), IL-1 β -treated cells (Comparative Example 1), IL-1 β + HCII-treated cells (Example 1), and IL-1 β + CE-treated cells (Comparative Example 2). HCII-treated cells and CE-treated cells were pre-treated with HCII or CE. In the pre-treatment, the HCII concentration was 0.5 mg/ml or 2.5 mg/ml, and the CE concentration was 1.25 mg/ml or 6.25 mg/ml.

As shown in Fig. 2, IL-1 β 25 ng/mL induced inflammation in human chondrocytes leading to a significant increase in level of MIP-1 β as compared to untreated cells (control). Pre-treatment of the cells with HCII and CE reduced the level of MIP-1 β induced by IL-1 β . There is also a dose-dependent effect of HCII and CE on inhibition of the production of MIP-1 β . In Fig. 2, data is represented as means $\pm$ SD (n = 3). * denotes significant difference with p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001 against IL-1 β (IL-1 β -treated cells) unless otherwise indicated.

[0093] <Example 2> Synergistic effect of combination of HCII and CE

To assess a synergistic effect of a combination of HCII and CE on inhibition of the production of an inflammatory marker MIP-1 β , pre-treatment was performed with HCII alone, CE alone, or a combination of HCII and CE. In the pre-treatment, the HCII concentration was 0.5 mg/ml and the CE concentration was 1.25 mg/ml.

As shown in Fig. 3, a combination of HCII 0.5 mg/mL and CE 1.25 mg/mL provided a synergistic effect of reducing inflammation, as compared to treatment with HCII or CE alone. Data is represented as means $\pm$ SD (n = 3). * denotes significant difference with p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001 against IL-1 β (IL-1 β -treated cells) unless otherwise indicated.

[0094] <Example 3> Effect of fractions isolated from HCII on control of inflammatory marker

Among the seven fractions isolated from HCII, P1 and P2 were used. The cells were pre-treated with IL-1 β and HCII, P1 or P2 (each concentration of HCII, P1 and P2 was 5 mg/mL) as in Example 1 to compare the effect.

Fig. 4 shows the results. Data is represented as means $\pm$ SD (n = 3). * denotes significant difference with p < 0.05, ** denotes p < 0.01, *** denotes p < 0.001 against IL-1 β (IL-1 β -treated cells) unless otherwise indicated.

[0095] <Example 4 and Comparative Example 3>

To assess the influence of HCII on the inflammatory markers IL-6, IL-7, IL-9 and IL-12, the following three different conditions were compared: cells before treatment (untreated control), IL-1 β -treated cells (Comparative Example 3), and IL-1 β + HCII-treated cells (Example 4). HCII-treated cells were pre-treated with HCII. In the pre-treatment, the HCII concentration was 0.5 mg/ml or 2.5 mg/ml.

As shown in Fig. 5, IL-1 β 25 ng/mL induced inflammation in human chondrocytes leading to a significant increase in level of IL-6, IL-7, IL-9 or IL-12 as compared to untreated cells (control). Pre-treatment of the cells with HCII reduced the level of IL-6, IL-7, IL-9 and IL-12 induced by IL-1 β . There is also a dose-dependent effect of HCII on inhibition of the production of IL-9 and IL-12. In Fig. 5, data is represented as means $\pm$ SD (n = 3). * denotes significant difference with p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001 against IL-1 β (IL-1 β -treated cells) unless otherwise indicated.

[0096] <Example 5> Synergistic effect of combination of GPEGAPGKD and CE on inhibition of the production of inflammatory marker MCP-1

To assess a synergistic effect of a combination of GPEGAPGKD and CE on inhibition of the production of an inflammatory marker MCP-1, pre-treatment was performed with IL-1 β and a combination of GPEGAPGKD and CE as in Example 1. In the pre-treatment, GPEGAPGKD concentration was 2.5 mg/ml and the CE concentration was 6.25 mg/ml.

Fig. 6 shows the results. Data is represented as means $\pm$ SD (n=3). * denotes significant difference with p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001 against IL-1 β (IL-1 β -treated cells) unless otherwise indicated.

[0097] <Example 6> Synergistic effect of combination of GPEGAPGKD and CE on inhibition of the production of inflammatory markers

To assess a synergistic effect of a combination of GPEGAPGKD and CE on inhibition of the production of inflammatory markers IL-6, IL-8, IL-9, MCP-1, MIP-1 β and RANTES, pre-treatment was performed with IL-1 β and a combination of GPEGAPGKD and CE as in Example 1. In the pre-treatment, GPEGAPGKD concentration was 25 mg/ml, 125 mg/ml or 250 mg/ml and the CE concentration was 6.25 mg/ml.

Fig. 7-1(a), Fig. 7-1(b), Fig. 7-1(c), Fig. 7-2(d), Fig. 7-2(e) and Fig. 7-2(f) show the results. Data is represented as means $\pm$ SD (n = 3). * denotes significant difference with p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001 against IL-1 β (IL-1 β -treated cells) unless otherwise indicated.

[0098] 2. Bone resorption inhibitory effect (hydrolyzed collagen type II of chicken cartilage)

Raw materials, reagents, and the like used in the following examples are as follows.

The same hydrolyzed collagen type II of chicken cartilage as the one prepared and used in the examples and the like for "1. Inflammation reducing effect" above was used.

Osteolyse Assay Kit (Human Collagen), Osteoclast Precursors (OCP), OCP Bulletkit which includes OCP Basal Medium and OCP Growth Medium SingleQuots Kit were bought from Lonza.

The OCP Growth Medium SingleQuots Kit consisting of the Fetal Bovine Serum, L-glutamine, Penicillin/Streptomycin, macrophage colony-stimulating factor and Soluble RANK Ligand (RANKL) was provided by Lonza.

The Osteolyse Assay Kit (Human Collagen) also came with a 96 well OsteoLyse Plate, Fluorophore-releasing reagent and a 96-well black assay plate.

[0099] Bone resorption inhibitory effect was evaluated by the following methods in the following examples.

<Cell culture and Treatments>

Primary Human Osteoclast Precursors (OCP, Cat. #2T-110) were thawed and seeded at 10,000 cells per well in the 96-well OsteoLyse plate provided by the OsteoLyse Assay Kit (Cat. # PA-1500). The Primary Human Osteoclast Precursors were maintained in OCP Basal Medium (Cat. #PT-8201) along with 10% FBS, 2 mM L-glutamine, 100 units/ml and 100 µg/ml of Penicillin/Streptomycin respectively. RANK Ligand and M-CSF were added to this growth medium at 66 ng/ml and 33 ng/ml respectively in order to complete the differentiation medium for the Primary Human Osteoclast Precursors. The precursor cells were incubated for 7 days at 37°C in a humidified gas chamber containing 5% CO₂ to fully differentiate into functional mature osteoclasts. The morphology of the cells was examined under the microscope to ensure complete differentiation of the precursors into multi-nucleated mature osteoclasts. Undifferentiated cells were set aside as positive control and were maintained in growth medium in the absence of RANK Ligand. On day 7, treatment was initiated by adding peptide GPEGAPGKD 50 mg/ml, HC II 10 mg/ml and different fractions isolated from HCII at two concentrations i.e., 5 mg/ml and 10 mg/ml, to fresh differentiation medium. The cells were incubated for another 3 days at 37°C in a humidified gas chamber containing 5% CO₂.

[0100] <Bone resorption analysis>

The Bone Resorption assay was carried out according to the manufacturers' instructions. This assay was a direct measure of the release of the matrix metalloproteinases into the resorption lacuna of the osteoclasts. The OsteoLyse Cell Culture Plate was coated with fluorophore-derivatized human bone matrix (europium-conjugated collagen) and the resorptive activity of the osteoclasts, as reflected by Eu-labelled collagen fragments, was measured using the microplate reader (Tecan, Infinite 200 Pro). Fluorophore Releasing Reagent was added to a 96-well plate at 200 µL per well and 10 µL of cell culture supernatant was transferred and mixed well with the Fluorophore Releasing Reagent. Fluorescence was measured at 340 nm excitation wavelength and 615 nm emission wavelength over a 400 µ seconds time period after an initial delay of 400 µ seconds. A time-course study was done by sampling the supernatant at 24, 48 and 72 hours after treatment of the cells.

[0101] <Statistical analyses>

Statistical analysis was conducted using GraphPad Prism version 5.0 (GraphPad). All results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed by unpaired T-test and data was considered to be significant when P-value, $p < 0.05$.

[0102] <Example 7> Inhibition of bone resorption activity by HCII

To assess the influence of HCII on the bone resorption activity of mature osteoclasts, we compared four different conditions: undifferentiated cells (positive control), differentiated osteoclasts before treatment (untreated control), HCII-treated osteoclasts (HCII 6 mg/ml).

As shown in Fig. 8, mature osteoclasts were treated for 72 hours with HCII at dosages; 6 mg/ml. HCII significantly reduced bone resorption activity as compared to untreated control at 6 mg/mL for HCII. Data is represented as means $\pm$ SD ($n = 3$). * denotes significant difference with $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$ against untreated control unless otherwise indicated.

[0103] <Example 8> Inhibition of bone resorption activity by seven different HC II fractions

HCII was subjected to purification and separated into 7 fractions (P1 to P7) to investigate the bioactive(s) contributing to the efficacy of HCII in reducing bone resorption activity. Each fraction was added in an amount of 5 mg/mL. There was a significant reduction in bone resorption activity when cells were treated with HCII 5 mg/mL for 72 hours (Fig. 9). None of the fractions were effective when treated alone at a dosage equivalent to that of HCII. Yet, when fraction 1 and fraction 2 (P1 + P2) were treated in combination, there was a synergistic effect as demonstrated by a significant reduction in bone resorption activity as compared to untreated control.

Data is represented as means $\pm$ SD ($n = 3$). * denotes significant difference with $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ against untreated control unless otherwise indicated.

[0104] <Example 9> Inhibition of bone resorption activity by peptide GPEGAPGKD

Presence of a peptide GPEGAPGKD was discovered in the combination of fractions 1 and 2 (P1 + P2) and the efficacy was validated. Cells were treated with combination of HCII at 5 mg/mL, P1 and P2 fractions at 5 mg/ml or peptide GPEGAPGKD at 50 mg/ml for 72 hours. Similar to Example 4, both HCII and P1 + P2 at 5 mg/mL prevented bone resorptive activity of the osteoclasts compared to untreated control. Peptide GPEGAPGKD exhibited significant reduction in bone resorption at 10 times the dosage of HCII or P1 + P2 fraction (Fig. 10).

Data is represented as means $\pm$ SD ($n = 3$). * denotes significant difference with $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$ against untreated control unless otherwise indicated.

[0105] HCII inhibited bone resorption activity of mature osteoclasts in a dose-dependent

manner. The combination of fractions 1 and 2 significantly prevented bone resorption activity when compared to untreated cells. Peptide GPEGAPGKD found in the combination of fractions 1 and 2 also reduced bone resorption activity. Thus, it is likely that peptide GPEGAPGKD is one of the bioactive(s) that contributes partially to the inhibition of bone resorptive activity of HCII.

[0106] 3. Bone resorption inhibitory effect (chicken extract)

<Example 10> Inhibition of bone resorption activity by chicken extract

Inhibition of bone resorption activity of mature osteoclasts was examined as in Example 7, except that 7.5 mg/ml and 15 mg/ml of chicken extract was used instead of HCII.

When cells were treated with CE, a significant reduction in bone resorption activity was found at 7.5 mg/mL and 15 mg/mL as compared to untreated control (Fig. 11). * denotes significant difference with $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ against untreated control unless otherwise indicated.

[0107] <Example 11> A Randomized, Double-blind, Four-arm Pilot Study to Evaluate the Effects of HCII and CE on knee pain and bone mass

This pilot study was carried out in a single-center, and as double-blind, randomized, placebo-controlled study.

[0108] (Subject enrolment and randomization)

A total of 160 subjects between the ages of 45 to 75 were selected using inclusion and exclusion criteria summarized in Table 3. Subjects were randomly assigned to four groups i.e. "Placebo", "Glucosamine", "HCII" and "HCII and CE". Each group included 40 subjects.

[0109]

[Table 3]

Criteria	Description
Inclusion	Male and female at 45 to 75 years of age
	Subject has a body mass index range of 18.0 to 30.0 kg/m ² , inclusive, and weighs at least 40 kg at screening visit
	Subject agrees not to participate in another interventional study while participating in the present study, defined as signing the informed consent form until completion of the study
	Experience knee pain $\geq$ 3 months
	WOMAC total pain score $\geq$ 6 evaluated by walking on a flat surface, descending and/or ascending stairs
	Subject has mild-to-moderate knee osteoarthritis (OA) at grade 1 to 3 per Kellgren-Lawrence grading system
	Experience loss in muscular strength or physical performance greater than 1 year
	Be willing to refrain from hormone therapy and dietary supplements such as calcium, vitamin D, supplements enriched with proteins, omega-3, omega-6, glucosamine, or chondroitin during the entire study
Exclusion	Active viral infection or bacterial infection based on clinical observations
	Subjects with history of rheumatoid arthritis or any other inflammatory arthritis
	Chronic medical conditions including renal dysfunctions, psychiatric disorders, or diabetes (or HbA1c $>$ 6.5%)
	History of stroke or myocardial infarction, mental retardation, or schizophrenia
	Gout
	Paget disease of bone or spinal disc herniation
	Subjects intolerant or allergic to protein-based food or supplement
	Anti-osteoporotic therapy in the past year
	Intra-articular injection during previous 3 months (6 months for hyaluronic acid) applied at target knee joint (most painful knee joint at Screening visit)
	Current corticosteroid therapy
	Those expecting knee arthroscopy or arthroplasty
	Life-threatening pathology (such as cancer) in remission for less than 1 year or still ongoing
	Psychological or linguistic incapability to sign the informed consent
	History of allergy to chicken meat
	Pregnant or lactating women
	Suspected or known alcohol abuse or addiction (consume $\geq$ 14 drinks of alcohol per week; 1 drink: 355mL beer or 150mL wine)
	Subjects with any other conditions or diseases that investigator considers not appropriate to be entered in the study
	Subjects with active pathology that could confound interpretation of knee pain or any conditions that interfere with adherence

[0110] (Investigational product)

“Placebo” group took a mixture of 6.8g of maltodextrin and 7mg of xanthan gum.

“Glucosamine” group took 1.5g of glucosamine hydrochloride. Glucosamine hydrochloride acted as an active comparator.

“HCII” group took BRAND’S Collagen Hydrolysate (Suntory Beverage & Food Asia Pte Ltd), a hydrolyzed chicken sternal cartilage extract composed of a naturally occurring matrix of hydrolyzed collagen type II (HCII) and low molecular weight chondroitin sulfate and hyaluronic acid (HA). This product includes HCII containing peptide GPEGAPGKD. Each bottle with a volume of 68mL contained 2g of HCII providing naturally occurring composition of HCII (66.5%), depolymerized

chondroitin sulfate (18%) and HA (11%). Uncharacterized components of sternal cartilage account for the remaining 4.5%.

“HCII and CE” group took a mixture of 70g of BRAND’S Essence of chicken (BEC) (dry weight: 5-6g) and 2g of HCII in 68mL. All test products were prepared as liquid products in glass bottles, which were isocaloric, identical in appearance and equivalent in flavour and texture. The participants had to take the investigational product once daily in the morning after meal.

Subjects were allowed to continue on concomitant medication or supplements deemed not to affect the outcome of the study. Analgesics or painkillers were allowed as a rescue medication under prescription. Proportion of days covered (PDC) of painkillers was calculated based on the records in the Concomitant Medication of Painkiller section and defined as the percentage of painkillers covered days over the total number of days in the interval between visits. Any treatment or dietary supplement that could support joint, bone and muscle health including hormone therapy (growth hormone, progesterone, estrogen, or testosterone), calcium and vitamin D, supplements enriched with amino acids, peptides, proteins, omega-3, omega-6, glucosamine or chondroitin were prohibited throughout the study duration.

[0111] (Procedure)

The study consisted of a screening visit (28 days before baseline visit), followed by a baseline visit (Baseline visit was Day 0. Screening and baseline visits can be on the same day), and 3 follow-up visits (Week 8, 16, and 24). Subjects were screened from Day -28 to Day 0 to determine the eligibility for the study. Intake of test product was taken starting from the day following the baseline visit for 168 days (24 weeks) consecutively. For 168 days (24 weeks) consecutively, subjects took one bottle of test product daily in the morning (after meal). Intake compliance was recorded on a diary card.

The visual analogue scale (VAS) of knee pain was scored at Day 0, Day 7 and Day 14 post-intake.

Bone mass assessment was done at Baseline (Day 0) and Week 24 only.

During the study period, subjects were recommended to do the resistance training (not mandatory) twice a week at home, 30 minutes each time following the training schedule. Training was recorded on a diary card. Food dietary in the prior week before visits was recorded using a food questionnaire.

[0112] (Treatment compliance)

Compliance, regarding the intake of the investigational product, was checked by the collection of unused products and the daily records of the consumption kept by the subjects. Compliance was defined by the percentage of assigned doses that were actually consumed over number of days between visits. A 70% of consumption was

considered as compliant.

[0113] (Exercise programme)

All subjects were encouraged to do the resistance training for 30 minutes twice a week at home following the training manual provided at baseline visit. Compliance on the training programme were recorded on a diary card and were calculated using the following formula:

[0114] [Math.1]

$$\text{Compliance(\%)} = \frac{\text{Sum of minutes of training (actual)}}{\text{Sum of minutes of training (plan)}} \times 100$$

[0115] A compliance rate of $\geq 50\%$ was considered as compliant.

[0116] (Visual Analogue scale (VAS) of knee pain)

The visual analogue scale (VAS) was scored from 0 to 100 mm, where 0 indicates no pain and 100 the worst pain ever. At Day 0, Day 7 and Day 14 post-intake, respondents were asked to specify the level of pain felt by indicating a position along a continuous line by drawing a straight line on the scale.

[0117] (Assessment Bone Mass)

Dual-energy X-ray absorptiometry (DXA) was performed at baseline (Day 0) and week 24 to assess the bone mass (BM) of left hip bones.

[0118] (Tolerability and safety)

Spontaneous reported adverse events were recorded throughout the study. Vital signs were monitored at every visit. For assessment of safety of the investigational products, serum and urine of all participants were evaluated at each visit of the study duration.

[0119] (Statistical analyses)

Per-protocol statistical analyses were performed, according to the a priori statistical analysis plan. Randomized subjects with intake compliance rate $\geq 70\%$ were included in the per-protocol analyses. Analyses of safety parameters were performed based on the subjects who have taken at least 28 bottles of the study product. Dichotomous variables were reported using percentages, whereas continuous variables were reported as mean and SD. Comparisons of categorical variables were performed with chi-square test, whereas the Kruskal-Wallis test was used to compare differences between groups for continuous variables.

Repeated measures analysis of variance (ANOVA) was performed using mixed-effect models used for testing mean difference in changes between the study groups, with the intake-by-visit interaction as fixed effect factors, for continuous primary and secondary endpoints. All results were considered to be statistically significant if the corresponding p-value was below 0.05. If the intake-by-visit interaction term was significant, post-hoc, pairwise comparisons between treatment groups were performed and adjusted p-values reported. Variables which were expected to influence endpoints

were included in the models as factors. Gender and gender*visit interaction term was included as a factor in joint health analysis, and gender and resistance training compliance and their respective interaction terms were included as factors in bone health analyses.

In addition, subgroup analysis was done for subjects doing resistance training less than 10th percentile of the total training period. An independent statistician performed the analyses using SAS version 9.4 (Cary, USA).

[0120] <Results>

(Baseline characteristics and treatment compliance)

A total of 160 subjects were enrolled and 151 subjects completed the study and were included in the statistical analysis (PP). Nine subjects dropped out during the study; there were no significant differences in the number of the drop-outs between the groups. None of the drop-outs were related to any side effects caused by the intake of the investigational products or placebo. No adverse events were noted. No clinically significant changes were observed in the serum biochemistry markers and urinalysis.

Overall, there was no statistically difference in the compliance rate between all four groups (Table 4). To evaluate the homogeneity of the data, all parameters were compared between four arms. There was no statistically significant difference between the study groups at baseline (Table 4).

[0121]

[Table 4]

	HCII+CE (n=37)	HCII (n=38)	Glucosamine (n=38)	Placebo (n=38)
Age (years)	57.3 ± 7.14	59.8 ± 7.66	59.1 ± 8.22	56.4 ± 6.42
Male/female ratio (female %)	4:33 (89.2)	8:30 (78.9)	6:32 (84.2)	5:33 (86.8)
Menopause (%)		73.3	75	60.6
Kellgren-Lawrence grading system for knee OA (%)				
	Probable	81.1	57.9	68.4
	Mild	10.8	34.2	28.9
Moderate	8.1	7	7.9	2.6
Treatment compliance(%)	98.8	98.9	98.9	99.9

[0122] (Changes in VAS of knee pain)

After 14 days of intake, pairwise comparison between HCII and placebo group was statistically significant as HCII group had less pain than placebo ($p=0.021$), measured with VAS pain scores. Compared to Day 0 pain scores, HCII users had a -11.1% on Day 7 and -25% on Day 14 from baseline for HCII group (Fig. 12(a), Table 5). In fact, placebo group showed a trend in experiencing increased pain over the 14-day period, with an increment of 33.3% on Day 7 and 13.3% on Day 14 in pain score from Day 0.

Furthermore, sub-group analysis for subjects doing less than 10th percentile of total resistance training period showed that placebo group experienced significantly increased pain score of 200% from baseline at Day 14 compared to HCII ($p=0.021$) group, with 8.7% from Day 0 respectively (Fig. 12(b), Table 6).

In Tables 5 and 6, “P-value (\$)” means p value between groups; “(a)” and “(k)” denote methods used to determine the p value((a)= One-way ANOVA, (k)=Kruskal-Wallis test); “*” denotes statistical significance; “95% C.I.” denotes 95% Confidence Interval. In Figs. 12(a), 12 (b) and 13 mentioned below, two numerical values in parentheses for each data are “mean - standard deviation” and “mean + standard deviation”.

[0123] [Table 5]

	HCII (n=38)	Placebo (n=38)	P-value(\$)
Day 0 (Baseline)			
Number	38	38	0.135(k)
Mean ±SD	3.6 ± 2.14	3.0 ± 1.77	
Median	3.1	2.5	
Range	(0.2, 8.0)	(0.5, 7.3)	
95% C.I.	(2.91, 4.32)	(2.44, 3.61)	
Day 7			
Number	38	38	0.050*(k)
Mean ±SD	3.2 ± 2.17	4.0 ± 2.17	
Median	2.5	3.1	
Range	(0.0, 8.6)	(0.7, 8.0)	
95% C.I.	(2.52, 3.94)	(3.27, 4.70)	
Day 14			
Number	38	38	0.039*(k)
Mean ±SD	2.7 ± 1.98	3.4 ± 2.11	
Median	2.3	3	
Range	(0.0, 8.6)	(0.5, 8.3)	
95% C.I.	(2.09, 3.39)	(2.75, 4.14)	

[0124] [Table 6]

	HCII (n=2)	Placebo (n=2)	P-value(\$)
Day 0 (Baseline)			
Number	2	2	0.135(a)
Mean ±SD	2.3 ± 0.37	1.9 ± 1.25	
Median	2.3	1.9	
Range	(2.1, 2.6)	(1.0, 2.8)	
95% C.I.	(-0.97, 5.65)	(-9.32, 13.18)	
Day 7			
Number	2	2	0.395(a)
Mean ±SD	3.3 ± 1.25	5.7 ± 0.74	
Median	3.3	5.7	
Range	(2.4, 4.2)	(5.2, 6.3)	
95% C.I.	(-7.97, 14.53)	(-0.89, 12.35)	
Day 14			
Number	2	2	0.155(a)
Mean ±SD	2.5 ± 0.88	5.7 ± 0.74	
Median	2.5	5.7	
Range	(1.9, 3.1)	(5.2, 6.3)	
95% C.I.	(-5.44, 10.44)	(-0.89, 12.35)	

[0125] (Changes in bone mass)

After 24-week of intake, there was no significant difference in the change in the bone mass between any two groups. However, subgroup analysis of subjects in the 10th percentile or under of total resistance training period demonstrated that at week 24 (intake term: $p=0.009$), those taking the combination of HCII+CE had significantly increased left hip bone mass of 7.4% from Day 0 compared to those taking placebo ($p=0.01$) (Fig. 13, Table 7).

In Table 7, “P-value (\$)” means p value between groups; “(a)” denotes method used to determine the p value ((a)= One-way ANOVA); “*” denotes statistical significance; “95% C.I.” denotes 95% Confidence Interval.

[0126] [Table 7]

	HCII+CE (n=2)	Placebo (n=2)	P-value(\$)
Day 0 (Baseline)			
Number	2	2	0.283(a)
Mean ± SD	-2.7 ± 0.71	-0.9 ± 0.21	
Median	-2.7	-0.9	
Range	(-3.2, -2.2)	(-1.0, -0.7)	
95% C.I.	(-9.05, 3.65)	(-2.76, 1.06)	
Week 24			
Number	2	2	0.391(a)
Mean ± SD	-2.5 ± 0.71	-1.0 ± 0.14	
Median	-2.5	-1	
Range	(-3.0, -2.0)	(-1.1, -0.9)	
95% C.I.	(-8.85, 3.85)	(-2.27, 0.27)	

[0127] (Conclusion)

In conclusion, HCII was well-tolerated and provided a quick and significant symptomatic relief in patients suffering from osteoarthritic pain. Compared to placebo, intake with 2g of HCII significantly reduced knee joint pain in just 14 days. On the other hand, combination of HCII and CE significantly increased left hip bone mass in subjects who did minimal amount of resistance training. In vitro studies suggest that mechanism of action may be through modification of underlying disease processes, particularly inhibition of bone resorption and inflammation that leads to localized pain sensation. Taken together, HCII and a combination of HCII and CE may be considered as a safe and efficacious complement to current medical and dietary options in the management of OA symptoms.

Industrial Applicability

[0128] The present invention provides a novel food or beverage composition containing a peptide and/or a salt thereof. The food or beverage composition of the present invention can be used to reduce inflammation and joint pain. The food or beverage composition of the present invention can also be used to inhibit bone resorption activity. The present invention also provides a novel composition for inhibiting bone

resorption, containing chicken extract as an active component. A peptide consisting of an amino acid sequence represented by the SEQ ID NO: 1 and/or a salt thereof and chicken extract can be consumed as a food or beverage or the like, and are also advantageous in terms of high safety.