DESCRIPTION

Title of Invention

YEAST EXTRACT CONTAINING COPPER, METHOD FOR MANUFACTURING SAME, FOOD PRODUCT, AND GREEN COLOR PRESERVING AND RESTORING AGENT FOR VEGETABLES

Technical Field

The present invention relates to a copper-containing yeast extract, a method for producing the same, food, and an agent for maintaining and restoring a green color of vegetables.

Background Art

According to the data recently published by Ministry of Health, Labour and Welfare, the estimated average copper requirement per day is about 0.7 mg in adult males (about 0.6 mg in adult females). Copper deficiency in the body can cause anemia and heart diseases, for example (see NPL 1). Therefore, there is a need to develop a safe food material for a fluid diet or a beverage which allows for efficient ingestion and absorption of copper into a body, and which can improve for example, anemia and heart diseases associated with copper deficiency.

Yeast has been utilized as a food material by human since a long time ago. For example, brewer's yeast has been utilized as a source of supply of dietary fiber, vitamin, or mineral. Especially, yeast into which copper has been uptaken (copper-containing yeast) or extract thereof is thought to be available as a safe copper-enriched food material.

However, in conventional copper-containing yeast, a distinctive odor such as a yeast odor and a distinctive taste such as a yeast taste are insufficiently reduced, making it unsatisfactory in use as food.

In order to solve the above problems, there has been proposed a copper-containing yeast extract which is extracted from the copper-containing yeast. Preparation of an extract from mineral-containing yeast (e.g., copper-containing yeast) has been performed by, for example, the following methods: (1) a hot-water extraction method, (2) an autolysis method, and (3) an enzymolysis method (see NPL 2). However, the methods of (1) to (3) have a problem that a recovery rate (hereinafter may be referred to as "extraction rate") of minerals is low. Among minerals, the extraction rate of copper is particularly low, and it has been strongly demanded to develop a technique of being able to increase the extraction rate thereof.

Citation List

Non-Patent Literature

NPL 1: "Dietary Reference Intakes for Japanese 2010," Ministry of Health, Labour and Welfare, the report from the Scientific Committee of "Dietary Reference Intakes for Japanese", published by DAI-ICHI SHUPPAN Co. Ltd., p. 231 – p. 233, Appendix XLIX (2010)

NPL 2: Hiroshi Sugimoto, "Transition of yeast extract production method (I) -from the trend of patent applications in Japan-," New Food Industry 1994, Vol. 36, No. 10, p. 41 – 48

Summary of Invention

Technical Problem

The present invention aims to meet the demand, break the status quo, solve the existing problems, and achieve the following objects. That is, an object of the invention is to provide: a method for producing a copper-containing yeast extract, the method being able to extract copper at a high extraction rate from copper-containing

yeast; a copper-containing yeast extract obtained by this method; food containing this copper-containing yeast extract; and an agent for maintaining and restoring a green color of vegetables.

Solution to Problem

Means for solving the problems are as follows.

<1> A method for producing a copper-containing yeast extract, comprising:

extracting the copper-containing yeast extract by suspending copper-containing yeast into a solution containing an amino acid or a salt thereof to obtain a suspension, and separating a solid component and a liquid component of the suspension,

wherein the amino acid or the salt thereof is water-soluble.

<2> The method for producing the copper-containing yeast extract according to <1>, wherein a pH value of an extraction liquid obtained in the extracting is lower than 4.0 or in the range of higher than 7.0 but lower than 11.0.

<3> The method for producing the copper-containing yeast extract according to <1> or <2>, wherein the amino acid or the salt thereof is at least one selected from the group consisting of histidine or a salt thereof, glycine or a salt thereof, threonine or a salt thereof, and glutamic acid or a salt thereof.

<4> The method for producing the copper-containing yeast extract according to any one of <1> to <3>, wherein the amino acid or the salt thereof contains histidine or a salt thereof.

<5> The method for producing the copper-containing yeast extract according to any one of <1> to <4>, wherein the amino acid or the salt thereof contains histidine or a salt thereof and at least one selected from the group consisting of glycine or a salt thereof, threonine or a salt thereof, and glutamic acid or a salt thereof.

<6> The method for producing the copper-containing yeast extract according to

any one of <1> to <5>, further comprising treating the copper-containing yeast with a cell wall-degrading enzyme.

<7> The method for producing the copper-containing yeast extract according to any one of <1> to <6>, wherein a copper-extraction rate expressed by a formula below is 40% or more:

Copper-extraction rate (%) = {Total amount (mass) of copper in the extract / Total amount (mass) of copper contained in the yeast used for extraction} × 100.

<8> The method for producing the copper-containing yeast extract according to <7>, wherein the copper-extraction rate is 50% or more.

<9> The method for producing the copper-containing yeast extract according to any one of <1> to <8>, further comprising, prior to the extracting, hot-water treating the yeast by suspending the yeast into hot-water of 60°C to 120°C to obtain a suspension, and separating a solid component and a liquid component of the suspension.

<10> The method for producing the copper-containing yeast extract according to <9>, wherein, in the hot-water treating, a phosphoric acid salt is added to the hot-water.

<11> A copper-containing yeast extract produced by the method for producing the copper-containing yeast extract according to any one of <1> to <10>.

<12> The copper-containing yeast extract according to <11>, wherein the yeast is edible yeast.

<13> The copper-containing yeast extract according to <12>, wherein the edible yeast is at least one selected from the group consisting of baker's yeast, brewer's yeast, wine yeast, sake yeast, and miso or soy sauce yeast.

<14> The copper-containing yeast extract according to <12>, wherein the edible yeast is Saccharomyces cerevisiae.

<15> The copper-containing yeast extract according to any one of <11> to <14>,

wherein the copper-containing yeast extract is added to food.

<16> Food, comprising:

the copper-containing yeast extract according to any one of <11> to <15>.

<17> An agent for maintaining and restoring a green color of vegetables, comprising:

the copper-containing yeast extract according to any one of <11> to <15>.

Advantageous Effects of Invention

The present invention can solve the existing problems, and can provide: a method for producing a copper-containing yeast extract, the method being able to extract copper at a high extraction rate from a copper-containing yeast; a copper-containing yeast extract obtained by this method; food containing this copper-containing yeast extract; and an agent for maintaining and restoring a green color of vegetables.

Brief Description of Drawings

FIG. 1 is a graph illustrating results of Test Example 1.

FIG. 2 is a graph illustrating results of Test Examples 2-1 to 2-4.

FIG. 3 is a graph illustrating results of Test Examples 3-1 and 3-2.

FIG. 4 is a graph illustrating results of Test Examples 4-1-1 to 4-3-2.

FIG. 5A is a photograph illustrating results of LEVELUP V8 CLEAR in Test Example 8.

FIG. 5B is a photograph illustrating results of a histidine hydrochloride extract in Test Example 8.

FIG. 5C is a photograph illustrating results of a glycine extract in Test Example 8.

FIG. 5D is a photograph illustrating results of a threonine hydrochloride

extract in Test Example 8.

FIG. 5E is a photograph illustrating results of a glutamic acid hydrochloride extract in Test Example 8.

Description of Embodiments

(Method for producing copper-containing yeast extract)

A method for producing a copper-containing yeast extract of the present invention comprises an extraction step; and, if necessary, further comprises other steps such as an enzymatic treatment step, a hot-water treatment step and a drying step.

<Extraction step>

The extraction step comprises suspending copper-containing yeast into a solution containing an amino acid or a salt thereof to obtain a suspension, and separating a solid component and a liquid component of the suspension. The extraction step results in a copper-containing liquid component (hereinafter may be referred to as "extraction liquid").

<<Yeast>>

The yeast is not particularly limited and may be appropriately selected depending on the purpose, as long as it contains copper in its body. A copper-content in the yeast is preferably as high as possible in the case where the copper-containing yeast extract is used as a copper-enriched food material. However, from the viewpoints of limitation of an amount of copper uptaken by the yeast and of an efficient production of the copper-containing yeast extract, the copper content is preferably 0.01% by mass to 10% by mass, more preferably 0.5% by mass to 2% by mass, relative to dry mass of yeast.

The copper content, as used herein, refers to an amount of copper contained in a yeast For example, the copper content is preferably kept at a high level even

after washing, for example, in the case where a yeast is washed with water. In such yeast, copper is not removed and remains within the yeast body even after washing, so that the copper is derived from the body and is highly safe. Therefore, in the case where the hot-water treatment (washing) is performed for reducing an odor and a taste which are distinctive of yeast, the yeast extract does not impair a taste of food containing it, and contains the copper in a high concentration. As a result, the yeast extract is suitable as a food material.

Note that, the copper content in the yeast can be measured by known methods such as an atomic absorption spectrometry.

The copper-containing yeast may be produced by culturing yeast in a copper-containing culture medium to allow copper to be uptaken into the yeast; or may be a commercially available product. Examples of the commercially available product include MINERAL YEAST Cu1 and YEAST MINERAL COPPER (both manufactured by Oriental Yeast Co., Ltd.).

The copper-containing yeast may be further pulverized. Even when the yeast is pulverized yeast, the copper content in the yeast is preferably high as possible. The copper content relative to dry mass of yeast in a precipitate fraction obtained through washing the pulverized yeast with, for example, water is preferably 70% by mass or more, more preferably 80% by mass or more, particularly preferably 90% by mass or more, relative to the copper content, relative to dry mass of yeast in an unpulverized yeast.

Note that, a method for pulverizing yeast is not particularly limited and may be appropriately selected depending on the purpose. For example, a physical pulverization treatment or a chemical pulverization treatment may be used. Specifically, suitable is a method using DINOMILL in which 0.5 mm-diameter beads are packed to 50% by volume.

A form of the copper-containing yeast is not particularly limited and may be

appropriately selected depending on the purpose. For example, the copper-containing yeast may be in the form of a wet mass or powder.

The yeast may further contain other minerals other than copper. The minerals are not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include iron, magnesium, manganese, and zinc. These minerals may be contained alone in the yeast or in combination of two or more kinds thereof in the yeast. These minerals are, in general, preferably contained in a high concentration, but the concentration varies depending on the purpose and cannot be unambiguously defined.

The yeast is particularly preferably edible yeast in the case where extract of the copper-containing yeast is used as a food material.

The edible yeast is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include baker's yeast, brewer's yeast, wine yeast, sake yeast, and miso or soy sauce yeast. Among them, the baker's yeast is particularly preferable.

A class of the edible yeast is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include the genus Saccharomyces, the genus Torulopsis, the genus Mycotorula, the genus Torulaspora, the genus Candida, the genus Rhodotorula, and the genus Pichia.

Specific examples of the edible yeast include Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Saccharomyces uvarum, Saccharomyces rouxii, Torulopsis utilis, Torulopsis candida, Mycotorula japonica, Mycotorula lipolytica, Torulaspora delbrueckii, Torulaspora fermentati, Candida sake, Candida tropicalis, Candida utilis, Hansenula anomala, Hansenula suaveolens, Saccharomycopsis fibligera, Saccharomyces lipolytica, Rhodotorula rubra, and Pichia farinosa.

Among them, preferable are Saccharomyces cerevisiae, and Saccharomyces carlsbergensis, particularly preferable is Saccharomyces cerevisiae.

<<Amino acid or salt thereof>>

The amino acid or the salt thereof is not particularly limited and may be appropriately selected depending on the purpose, as long as it is water-soluble.

Being water-soluble, as used herein, refers to solubility that it is soluble in water of 20±5°C in an amount of 1% by mass or more.

Specific examples of the amino acid or the salt thereof include histidine or a salt thereof, glycine or a salt thereof, threonine or a salt thereof, glutamic acid or a salt thereof, lysine or a salt thereof, arginine or a salt thereof, alanine or a salt thereof, valine or a salt thereof, proline or a salt thereof, serine or a salt thereof, and cysteine or a salt thereof. Among them, from the viewpoint of attaining more excellent copper extraction rates, histidine or a salt thereof, glycine or a salt thereof, threonine or a salt thereof, and glutamic acid or a salt thereof, with histidine or a salt thereof being more preferable.

The salt is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include a hydrochloride and a sodium salt.

The amino acid or the salt thereof may be used alone or in combination of two or more kinds thereof. Also, either the amino acid or the salt thereof may be used, or both the amino acid and the salt thereof may be used.

An aspect where two or more of the amino acid or the salt thereof are used is not particularly limited and may be appropriately selected depending on the purpose. From the viewpoints of attaining more excellent extraction rates and reducing the production cost, a preferable aspect is that histidine or a salt thereof is used in combination with at least one selected from the group consisting of glycine or a salt thereof, threonine or a salt thereof, and glutamic acid or a salt thereof.

In the aspect where the amino acids or the salts thereof are used in combination, a ratio between histidine or a salt thereof and at least one selected from

the group consisting of glycine or a salt thereof, threonine or a salt thereof, and glutamic acid or a salt thereof is not particularly limited and may be appropriately selected depending on the purpose.

An amount of the amino acid or the salt thereof in the suspension is not particularly limited and may be appropriately selected depending on the purpose, but is preferably 10 mmol/L or more, more preferably 10 mmol/L to 200 mmol/L.

A solvent used for a solution containing the amino acid or the salt thereof is not particularly limited and may be appropriately selected depending on the purpose. The solvent is typically water, and may be a mixed solution of the water and an organic solvent (e.g., alcohol).

The suspension may contain other ingredients than the amino acid or the salt thereof. The other ingredients may be appropriately selected depending on the purpose, as long as they do not impair the effects of the present invention. Examples thereof include a carboxylic acid or a salt thereof.

The carboxylic acid or the salt thereof is not particularly limited and may be appropriately selected depending on the purpose. Citric acid or a salt thereof is preferable since it can increase the extraction rate of copper when used in combination with the amino acid or the salt thereof.

The salt of citric acid is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include trisodium citrate.

An amount of the carboxylic acid or the salt thereof used is not particularly limited and may be appropriately selected depending on the purpose, as long as it does not impair the effects of the present invention.

The pH value of the solution containing the amino acid or the salt thereof is not particularly limited and may be appropriately selected depending on the purpose, but is preferably adjusted to a pH corresponding to a preferable pH value of an extraction liquid described below. A known method can be appropriately selected

as a method for adjusting the pH value of the solution containing the amino acid or the salt thereof.

The pH value of the suspension is not particularly limited and may be appropriately selected depending on the purpose, but is preferably adjusted to a pH corresponding to a preferable pH value of an extraction liquid described below. A known method can be appropriately selected as a method for adjusting the pH value of the suspension.

An extraction time in the extraction step is not particularly limited and may be appropriately selected depending on the purpose. However, from the viewpoint of copper extraction rate, it is preferably 5 min or more, more preferably 5 min to 24 hours.

A temperature of the suspension in the extraction step is not particularly limited and may be appropriately selected depending on the purpose. However, from the viewpoint of copper extraction rate, it is preferably 10°C or higher, more preferably 10°C to 110°C.

The copper-extraction rate (hereinafter may be referred to as "copper-elution rate") in the extraction step is not particularly limited and may be appropriately selected depending on the purpose, but is preferably high as possible. Specifically, it is preferably 40% or more, more preferably 50% or more, particularly preferably 60% or more.

The copper-extraction rate can be calculated according to the following formula.

Copper-extraction rate (%) = {Total amount (mass) of copper in the extract / Total amount (mass) of copper contained in the yeast used for extraction} × 100

<<Suspension>>

A means of suspending the yeast into the solution containing the amino acid or the salt thereof is not particularly limited and may be appropriately selected

depending on the purpose. For example, a known means of agitating or shaking may be used.

In the extraction step, the suspension may be further shaken. The condition is not particularly limited and may be appropriately selected depending on the purpose.

<<Separation of solid component and liquid component>>

A means of separating the solid component and the liquid component in the suspension is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include filtration or centrifugation.

A means of filtration is not particularly limited. Known filtration devices may be appropriately selected and used. Examples the devices include a filter press and a line filter. Note that, these may be used in combination.

A means of centrifugation is not particularly limited. Known centrifugation devices may be appropriately selected and used. The condition is not particularly limited and may be appropriately selected depending on an amount of the suspension. For example, in the case where the amount of the suspension is 5 mL, the centrifugation is performed at 3,000 rpm for 5 min.

The pH value of the extraction liquid obtained in the extraction step is not particularly limited and may be appropriately selected depending on the amino acid or the salt thereof to be used. However, from the viewpoint of copper extraction efficiency, it is preferably lower than 4.0, or in the range of higher than 7.0 but lower than 11.0.

In the case where histidine or a salt thereof is used as the amino acid or the salt thereof, the pH value of the extraction liquid is preferably lower than 4.0, or in the range of higher than 7.0 but lower than 11.0, more preferably in the range of higher than 7.0 but 10.0 or lower, particularly preferably in the range of 8.0 or higher but 9.5 or lower.

In the case where glycine or a salt thereof is used as the amino acid or the salt thereof, the pH value of the extraction liquid is preferably lower than 4.0, or in the range of higher than 7.0 but lower than 11.0, more preferably 3.0 or lower, or in the range of higher than 7.0 but 10.0 or lower, particularly preferably 2.3 or lower.

In the case where threonine or a salt thereof is used as the amino acid or the salt thereof, the pH value of the extraction liquid is preferably lower than 4.0, or in the range of higher than 7.0 but lower than 11.0, more preferably 2.7 or lower, or in the range of higher than 7.0 but 10.9 or lower, particularly preferably 2.0 or lower, or in the range of 8.0 or higher but 9.5 or lower.

In the case where glutamic acid or a salt thereof is used as the amino acid or the salt thereof, the pH value of the extraction liquid is preferably lower than 4.0, or in the range of higher than 7.0 but lower than 11.0, more preferably 3.3 or lower, or in the range of higher than 7.0 but 10.3 or lower, particularly preferably 3.0 or lower, or in the range of 8.5 or higher but 10.0 or lower.

The pH value of each solution in the extraction step will change during the time from the suspension to the extraction. Therefore, preferably, the pH value of each solution in the extraction step is appropriately adjusted so that the extraction liquid has the above preferable pH value.

<Other steps>

The other steps are not particularly limited and may be appropriately selected depending on the purpose, as long as they do not impair the effects of the present invention. Examples thereof include an enzymatic treatment step, a hot-water treatment step, a drying step, a concentration step, and a dilution step. Among them, the enzymatic treatment step and the hot-water treatment step are preferably comprised.

<<Enzymatic treatment step>>

The enzymatic treatment step comprises treating the copper-containing

yeast with a cell wall-degrading enzyme. The enzymatic treatment step can further improve the extraction rate of copper.

A timing at which the enzymatic treatment step is performed is not particularly limited and may be appropriately selected depending on the purpose. The enzymatic treatment step may be performed before the extraction step, simultaneously with the extraction step, or after the extraction step.

-Cell wall-degrading enzyme-

The cell wall-degrading enzyme is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include β-glucanase and a protease. Among them, β-glucanase is advantageous since it makes it easier for the extract to leak in the solvent.

The cell wall-degrading enzyme may be used alone or in combination of two or more kinds thereof.

An amount of the cell wall-degrading enzyme used is not particularly limited and may be appropriately selected depending on the purpose, but is preferably 4.05 YsU/mL to 405.0 YsU/mL.

The YsU is an activity unit. One unit of a power to degrade the cell wall of yeast (YsU) is defined as an amount of an enzyme at which a turbidity of a reaction liquid decreases by 1% per minute when the enzyme acts at 35°C on 1 mg of freeze-dried fungus body of Saccharomyces cerevisiae IFO 0309.

The Saccharomyces cerevisiae IFO 0309 is available from the Institute for Fermentation.

The temperature, pH, and time in the enzymatic treatment are not particularly limited and may be appropriately selected depending on the cell wall-degrading enzyme to be used.

<<Hot-water treatment step>>

The hot-water treatment step comprises suspending the yeast containing

copper into hot-water of 60°C to 120°C to obtain a suspension, and separating a solid component and a liquid component of the suspension, prior to the extraction step. Performing the hot-water treatment step prior to the extraction step is preferable, because the odor and the taste which are distinctive of yeast (i.e., yeast odor and yeast taste) can be reduced, and a taste of food to which the resultant copper-containing yeast extract has been added is not impaired.

A temperature of the hot-water is not particularly limited and may be appropriately selected depending on the purpose. The higher the temperature is, the higher an effect of reducing the yeast odor and the yeast taste is. Thus, the temperature is preferably 80°C to 120°C, more preferably 95°C to 120°C.

Note that, a method for suspending the yeast and a method for separating the solid component and the liquid component of the suspension are not particularly limited. For example, the methods described for the extraction step may be used.

In the hot-water treatment step, an extraction accelerator for accelerating extraction (removal) of the yeast odor and the yeast taste is preferably added to hot water in order to reduce the odor and taste which are distinctive of yeast. The extraction accelerator is not particularly limited and may be appropriately selected depending on the purpose, as long as it does not adversely affect the copper-extraction rate in the subsequent extraction step. Phosphoric acid salts are preferable because they are superior in extraction of the yeast odor, but are less likely to accelerate extraction of copper in the hot water step.

An amount of the extraction accelerator to be added is not particularly limited and may be appropriately selected depending on the purpose, but is preferably 5% by mass to 50% by mass, more preferably 20% by mass to 50% by mass, relative to dry mass of the yeast.

<<Drying step, concentration step, dilution step>>

The drying step comprises drying the copper-containing yeast extract.

A method for drying is not particularly limited and may be appropriately selected depending on the purpose. For example, SPRAY DRIER L-8 (OHKAWARA KAKOHKI CO., LTD.) may be used. The drying step can result in a solid (powder) of the copper-containing yeast extract. Note that, during the copper-containing yeast extract is formed into the solid copper-containing yeast extract, an excipient (e.g., dextrin) may be appropriately added.

The concentration step comprises concentrating the copper-containing yeast extract. The dilution step comprises diluting the copper-containing yeast extract.

A means of concentrating and a means of diluting are not particularly limited and may be known methods that are appropriately selected.

(Copper-containing yeast extract)

A copper-containing yeast extract of the present invention is produced by the method of the present invention for producing the copper-containing yeast extract.

Since the copper-containing yeast extract is produced by the method for producing the copper-containing yeast extract, it contains copper derived from a natural product at a high concentration. The copper-containing yeast extract is also excellent in water-solubility.

The copper content in the copper-containing yeast extract is not particularly limited and may be appropriately selected depending on the purpose, but is preferably 0.2% by mass or more, more preferably 0.5% by mass or more, particularly preferably 0.8% by mass or more.

A form of the copper-containing yeast extract is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include a liquid form (extraction liquid such as filtrate or supernatant) obtained through the extraction step; a solid form such as powder, particle, or sheet; and a semi-solid form such as gel or slurry.

<Application>

Application of the copper-containing yeast extract of the present invention is not particularly limited and may be appropriately selected depending on the purpose. The extract is preferably used as a food material to be added to food, animal food, or feed, particularly preferably as the food material. Among the food materials, the extract of the invention is preferably used in the fluid diet or the soft drink since it contains copper derived from a natural product at a high concentration and is also excellent in water-solubility. In addition, the extract is also preferably used, for example, in a food fermentation medium or as an agent for maintaining and restoring a green color of vegetables. The copper-containing yeast extract of the invention can result in copper-containing food, copper-containing animal food, and copper-containing feed.

A form of the copper-containing yeast extract in use is not particularly limited and may be appropriately selected depending on the application. Examples thereof include a dried powder (e.g., extract dried by means of, e.g., a spray drier), a solution form in which the extract is dissolved in a solvent, and a semi-solid form (e.g., a gel or cream). Note that, a method for forming the copper-containing yeast extract into the above-described forms is not particularly limited and can be performed by means of known devices according to known methods.

(Food)

Food of the present invention contains the copper-containing yeast extract of the invention; and, if necessary, further contains other ingredients.

The "food," as used herein, refers to those having a low possibility of being harmful to human health, and being ingested via oral or gastrointestinal administration in an ordinary social life, which is not limited by administrative classification such as food, medicine, or quasi drug. For example, the food encompasses a wide range of orally ingested general food, health food, food with health claims, quasi drug and medicine.

A type of the food is not particularly limited and may be appropriately selected depending on the purpose, but is preferably a fluid diet bread, confectionery such as a biscuit or a cracker, a processed marine product, a processed meat product, noodles, a seasoning such as miso, a processed vegetable product, a beverage such as juice, ices such as ice cream, and health food, particularly preferably the fluid diet or the beverage.

An amount of the copper-containing yeast extract contained in the food is not particularly limited and may be appropriately selected depending on the application and purpose.

The other ingredients are not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include auxiliary materials or additives generally used in production of the food.

The auxiliary materials or additives are not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include glucose, fructose, sucrose, maltose, sorbitol, stevioside, rubusoside, corn syrup, lactose, citric acid, tartaric acid, malic acid, succinic acid, lactic acid, L-ascorbic acid, dl-α-tocopherol, sodium erythorbate, glycerin, propylene glycol, glycerine fatty acid ester, polyglycerin fatty acid ester, sucrose fatty acid ester, sorbitan fatty acid ester, gum arabic, carrageenan, casein, gelatin, pectin, agar, vitamin B, nicotinic-acid amide, calcium pantothenate, amino acids, calcium salts, a pigment, a perfume, and a preservative.

An amount of the other ingredients is not particularly limited and may be appropriately selected depending on the purpose.

(Agent for maintaining and restoring green color of vegetables)

An agent for maintaining and restoring a green color of vegetables of the present invention contains the copper-containing yeast extract of the present invention; and, if necessary, further contains other ingredients.

Intended vegetables of the agent for maintaining and restoring a green color are not particularly limited and may be appropriately selected depending on the purpose, as long as they are edible green plants. For example, the agent can maintain a green color of green plants such as green vegetables, and tea leaves. It can also restore a faded color of, for example, frozen or refrigerated green vegetables, salted green vegetables, and commercially available pickled vegetables.

An amount of the copper-containing yeast extract contained in the agent is not particularly limited and may be appropriately selected depending on the application and purpose.

The other ingredients in the agent are not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include the above-described auxiliary materials or additives in food.

An amount of the other ingredients contained in the agent is not particularly limited and may be appropriately selected depending on the purpose.

An amount of the agent added to vegetables is not particularly limited and may be appropriately selected depending on the purpose.

Examples

Test Examples of the present invention now will be described, but the present invention is not limited thereto.

(Test Example 1: extraction of copper with various amino acids or salts thereof)

To 250 mg (dry mass) of a copper-containing yeast powder (MINERAL YEAST Cu1, copper-content: 11,422 ppm by mass, Oriental Yeast Co., Ltd.), were added 2.5 mL of 100 mmol/L Tris-HCl buffer (pH 7.6) and 2.5 mL of 100 mmol/L solution of each of amino acids or salts thereof shown in Table 1-1 (final concentration: 50 mmol/L), followed by stirring to suspend the copper-containing yeast, to obtain a suspension. Also, as a control, water or a 100 mmol/L Tris-HCl

buffer (pH 7.6) only (hereinafter may be referred to as "buffer") was similarly treated to obtain a suspension, which was tested in the same manner.

The suspension was maintained at room temperature (25°C) for 30 min, and was then centrifuged at 3,000 rpm for 5 min. The resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer, and the resultant supernatant was transferred to a test tube to obtain yeast extract. The yeast extract was weighed. The yeast extract (hereinafter may be referred to as "extraction liquid") was measured for pH value by means of a pH meter (MP230, METTLER-TOREDO Inc.).

Regarding the extraction rate of copper, the copper-content was determined by measuring copper in the yeast extract through an ICP emission spectrometric analysis using an ICP emission spectrophotometer (OPTIMA 2100DV, PerkinElmer Inc.), from which a rate of copper eluted to the extraction liquid was calculated from a formula below. Results are shown in Table 1 and FIG. 1.

Copper-extraction rate (%) = {Total amount (mass) of copper in the extract / Total amount (mass) of copper contained in the yeast used for extraction} × 100

Table 1

Amino acids or salts thereof	pH value of extraction liquid	Copper-extraction rate (%)
Water	5.4	10.8
Buffer	7.3	21.7
Histidine hydrochloride	5.6	57.6
Glycine	7.3	51.2
Threonine hydrochloride	7.2	42.2
Glutamic acid hydrochloride	3.1	48.5
Lysine hydrochloride	7.2	36.4
Arginine hydrochloride	7.1	29.8

In FIG. 1, "His" indicates the result of histidine hydrochloride, "Gly" indicates the result of glycine, "Thr" indicates the result of threonine hydrochloride,

"Glu" indicates the result of glutamic acid hydrochloride, "Lys" indicates the result of lysine hydrochloride, and "Arg" indicates the result of arginine hydrochloride.

As shown in Table 1 and FIG. 1, use of amino acids or salts thereof can extract copper at a high extraction rate. Among the amino acids or the salts thereof, the extraction rate of copper is higher when histidine hydrochloride, glycine, threonine hydrochloride, and glutamic acid hydrochloride are used, and use of histidine hydrochloride results in the highest extraction rate.

(Test Example 2-1: effect of pH value on extraction of copper by histidine hydrochloride)

A test was performed in the same manner as in Test Example 1-1, except that the 100 mmol/L Tris-HCl buffer (pH 7.6) used in Test Example 1-1 was changed to each of the solutions adjusted to give the pH values of extraction liquids described in Table 2-1, and histidine hydrochloride was used as the amino acid or the salt thereof, to obtain the pH values and the extraction rates of copper. Results are shown in Table 2-1 and FIG. 2.

Table 2-1

Solution	pH value of extraction liquid	Copper-extraction rate (%)	Remarks
2-1-1	1.9	66.4	pH value was adjusted with hydrochloric acid.
2-1-2	2.6	60.8	pH value was adjusted with hydrochloric acid.
2-1-3	3.1	51.3	pH value was adjusted with hydrochloric acid.
2-1-4	4.4	58.6	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-1-5	5.1	57.4	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-1-6	6.1	63.2	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-1-7	6.5	68.9	pH value was adjusted with sodium hydroxide.
2-1-8	9.3	81.2	pH value was adjusted with sodium hydroxide.
2-1-9	12.5	25.7	pH value was adjusted with sodium hydroxide.

(Test Example 2-2: effect of pH value on extraction of copper by glycine)

A test was performed in the same manner as in Test Example 1-1, except that the 100 mmol/L Tris-HCl buffer (pH 7.6) used in Test Example 1-1 was changed to each of the solutions adjusted to give the pH values of extraction liquids described in Table 2-2, and glycine was used as the amino acid or the salt thereof, to obtain the pH values and the extraction rates of copper. Results are shown in Table 2-2 and FIG. 2.

Table 2-2

Solution	pH value of extraction liquid	Copper-extraction rate (%)	Remarks
2-2-1	1.9	65.1	pH value was adjusted with hydrochloric acid.
2-2-2	2.9	51.6	pH value was adjusted with hydrochloric acid.
2-2-3	3.5	41.5	pH value was adjusted with hydrochloric acid.
2-2-4	5.1	36.9	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-2-5	5.8	38.3	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-2-6	6.8	44.5	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-2-7	8.0	51.7	pH value was adjusted with Tris and hydrochloric acid.
2-2-8	8.6	54.6	pH value was adjusted with Tris and hydrochloric acid.
2-2-9	8.8	56.8	pH value was adjusted with Tris and hydrochloric acid.
2-2-10	9.7	54.2	pH value was adjusted with sodium hydroxide.
2-2-11	11.0	36.7	pH value was adjusted with sodium hydroxide.
2-2-12	12.7	19.8	pH value was adjusted with sodium hydroxide.

(Test Example 2-3: effect of pH value on extraction of copper by threonine hydrochloride)

A test was performed in the same manner as in Test Example 1-1, except that the 100 mmol/L Tris-HCl buffer (pH 7.6) used in Test Example 1-1 was changed to each of the solutions adjusted to give the pH values of extraction liquids described in Table 2-3, and threonine hydrochloride was used as the amino acid or the salt

thereof, to obtain the pH values and the extraction rates of copper. Results are shown in Table 2-3 and FIG. 2.

Table 2-3

Solution	pH value of extraction liquid	Copper-extraction rate (%)	Remarks
2-3-1	1.9	66.3	pH value was adjusted with hydrochloric acid.
2-3-2	2.7	54.5	pH value was adjusted with hydrochloric acid.
2-3-3	3.3	43.3	pH value was adjusted with hydrochloric acid.
2-3-4	5.0	44.0	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-3-5	5.8	45.2	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-3-6	6.8	52.3	pH value was adjusted with sodium dihydrogenphosphate and sodium hydroxide.
2-3-7	7.9	58.4	pH value was adjusted with Tris and hydrochloric acid.
2-3-8	8.4	62.9	pH value was adjusted with Tris and hydrochloric acid.
2-3-9	8.7	61.4	pH value was adjusted with Tris and hydrochloric acid.
2-3-10	9.3	63.4	pH value was adjusted with sodium hydroxide.
2-3-11	10.8	54.7	pH value was adjusted with sodium hydroxide.
2-3-12	12.7	21.9	pH value was adjusted with sodium hydroxide.

(Test Example 2-4: effect of pH value on extraction of copper by sodium glutamate)

A test was performed in the same manner as in Test Example 1-1, except that the 100 mmol/L Tris-HCl buffer (pH 7.6) used in Test Example 1-1 was changed to each of the solutions adjusted to give the pH values of extraction liquids described in Table 2-4, and sodium glutamate was used as the amino acid or the salt thereof, to obtain the pH values and the extraction rates of copper. Results are shown in Table 2-4 and FIG. 2.

Table 2-4

Solution	pH value of extraction liquid	Copper-extraction rate (%)	Remarks
2-4-1	2.9	55.7	pH value was adjusted with hydrochloric acid.
2-4-2	4.2	40.1	pH value was adjusted with hydrochloric acid.
2-4-3	4.7	37.0	pH value was adjusted with hydrochloric acid.
2-4-4	5.0	35.7	pH value was adjusted with hydrochloric acid.
2-4-5	5.3	34.3	pH value was adjusted with hydrochloric acid.
2-4-6	5.4	37.1	pH value was adjusted with hydrochloric acid.
2-4-7	6.0	39.7	pH value was adjusted with sodium hydroxide.
2-4-8	6.5	44.9	pH value was adjusted with sodium hydroxide.
2-4-9	8.7	54.9	pH value was adjusted with sodium hydroxide.
2-4-10	9.8	55.2	pH value was adjusted with sodium hydroxide.
2-4-11	11.2	31.1	pH value was adjusted with sodium hydroxide.
2-4-12	12.9	19.5	pH value was adjusted with sodium hydroxide.

In FIG. 2, "white triangle" indicates the results of Test Example 2-1 (histidine hydrochloride), "black circle" indicates the results of Test Example 2-2 (glycine), "black square" indicates the results of Test Example 2-3 (threonine hydrochloride), and "white square" indicates the results of Test Example 2-4 (sodium glutamate).

From the results of Test Examples 2-1 to 2-4, when the pH value of the extraction liquid was lower than 4.0 or in the range of higher than 7.0 but lower than 11.0, more excellent extraction rates of copper were obtained.

Also, in the case where histidine hydrochloride was used as the amino acid or the salt thereof, more preferable results were obtained when the pH value of the extraction liquid was in the range of higher than 7.0 but 10.0 or lower, and particularly preferable results were obtained when it was in the range of 8.0 or higher but 9.5 or lower.

In the case where glycine or a salt thereof was used as the amino acid or the salt thereof, more preferable results were obtained when the pH value of the

extraction liquid was 3.0 or lower or in the range of higher than 7.0 but 10.0 or lower, and particularly preferable results were obtained when it was 2.3 or lower.

In the case where threonine hydrochloride was used as the amino acid or the salt thereof, more preferable results were obtained when the pH value of the extraction liquid was 2.7 or lower or in the range of higher than 7.0 but 10.9 or lower, and particularly preferable results were obtained when it was 2.0 or lower or in the range of 8.0 or higher but 9.5 or lower.

In the case where sodium glutamate was used as the amino acid or the salt thereof, more preferable results were obtained when the pH value of the extraction liquid was 3.3 or lower or in the range of higher than 7.0 but 10.3 or lower, and particularly preferable results were obtained when it was 3.0 or lower or in the range of 8.5 or higher but 10.0 or lower.

(Test Example 3-1: treatment with cell wall-degrading enzyme)

To 250 mg (dry mass) of a copper-containing yeast powder (MINERAL YEAST Cu1, copper-content: 11,422 ppm by mass, Oriental Yeast Co., Ltd.), were added 2.5 mL of 150 mmol/L NaOH and 2.5 mL of 100 mmol/L solution of histidine hydrochloride (final concentration: 50 mmol/L), followed by stirring to suspend the copper-containing yeast, to obtain a suspension.

The suspension was maintained at room temperature (25°C) for 60 min, and was then centrifuged at 3,000 rpm for 5 min. The resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer.

4.5 mL of 2% by mass enzyme liquid (see below for details) was added to the residue after the centrifugation, and the mixture was suspended and then allowed to react at 45°C for 180 min.

After completion of the reaction, the reaction mixture was centrifuged at 3,000 rpm for 5 min, and the resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer.

The resultant supernatant after the treatment with the amino acid and the resultant supernatant after the treatment with the enzyme were transferred to a test tube to obtain a yeast extract, which was measured for extraction rate of copper in the same manner as in Test Example 1-1. Results are shown in Table 3 and FIG. 3.

<Enzyme liquid>

67 mmol/L phosphate buffer (pH 7.7) containing β-glucanase (Amano Enzyme Inc., TUNICASE) adjusted to 80.1 YsU/mL

(Test Example 3-2: treatment with cell wall-degrading enzyme)

In the same manner as in Test Example 3-1, except that the treatment with the enzyme liquid in Test Example 3-1 was not performed, an extraction rate of copper was obtained. Results are shown in Table 3 and FIG. 3.

Table 3

	Copper-extraction rate (%)
Test Example 3-1	91.8
Test Example 3-2	70.3

As shown in Table 3 and FIG. 3, an extraction rate of copper is increased by performing the treatment with the amino acid or the salt thereof in combination with the treatment with the cell wall-degrading enzyme.

(Test Example 4: use in combinational with histidine hydrochloride)

<Test Example 4-1-1: glycine alone>

To 250 mg (dry mass) of a copper-containing yeast powder (MINERAL YEAST Cu1, copper-content: 11,422 ppm by mass, Oriental Yeast Co., Ltd.), was added 5.0 mL of 50 mmol/L glycine-containing liquid prepared in the following manner, followed by stirring to suspend the copper-containing yeast, to obtain a suspension.

The suspension was maintained at room temperature (25°C) for 30 min, and

was then centrifuged at 3,000 rpm for 5 min. The resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer, and was measured for extraction rate of copper in the same manner as in Test Example 1-1. Results are shown in Table 4-1 and FIG. 4.

<Glycine-containing liquid>

100 mmol/L NaOH solution containing 100 mmol/L glycine (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare glycine-containing liquid containing glycine at a concentration of 50 mmol/L.

<Test Example 4-1-2: use of glycine in combination with histidine hydrochloride>

A test was performed in the same manner as in Test Example 4-1-1, except that 5.0 mL of the 50 mmol/L glycine-containing liquid added in Test Example 4-1-1 was changed to 2.5 mL of the 50 mmol/L glycine-containing liquid and 2.5 mL of 50 mmol/L histidine hydrochloride-containing liquid prepared in the following manner. Results are shown in Table 4-1 and FIG. 4.

<Histidine hydrochloride-containing liquid>

100 mmol/L NaOH solution containing 100 mmol/L histidine hydrochloride (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare histidine hydrochloride-containing liquid containing histidine hydrochloride at a concentration of 50 mmol/L.

Table 4-1

	Amino acid and the like	Copper-extraction rate (%)
Test Example 4-1-1	Glycine alone	46.7
Test Example 4-1-2	Glycine and Histidine hydrochloride	65.1

<Test Example 4-2-1: threonine hydrochloride alone>

A test was performed in the same manner as in Test Example 4-1-1, except that 5.0 mL of the 50 mmol/L glycine-containing liquid used in Test Example 4-1-1

was changed to 5.0 mL of 50 mmol/L threonine hydrochloride-containing liquid. Results are shown in Table 4-2 and FIG. 4.

<Test Example 4-2-2: use of threonine hydrochloride in combination with histidine hydrochloride>

A test was performed in the same manner as in Test Example 4-1-2, except that 2.5 mL of the 50 mmol/L glycine-containing liquid used in Test Example 4-1-2 was changed to 2.5 mL of the 50 mmol/L threonine hydrochloride-containing liquid. Results are shown in Table 4-2 and FIG. 4.

Table 4-2

	Amino acid and the like	Copper-extraction rate (%)
Test Example 4-2-1	Threonine hydrochloride alone	53.5
Test Example 4-2-2	Threonine hydrochloride and Histidine hydrochloride	64.7

<Test Example 4-3-1: sodium glutamate alone>

A test was performed in the same manner as in Test Example 4-1-1, except that 5.0 mL of the 50 mmol/L glycine-containing liquid used in Test Example 4-1-1 was changed to 5.0 mL of 50 mmol/L sodium glutamate-containing liquid. Results are shown in Table 4-3 and FIG. 4.

<Test Example 4-3-2: use of sodium glutamate in combination with histidine hydrochloride>

A test was performed in the same manner as in Test Example 4-1-2, except that 2.5 mL of the 50 mmol/L glycine-containing liquid used in Test Example 4-1-2 was changed to 2.5 mL of the 50 mmol/L sodium glutamate-containing liquid. Results are shown in Table 4-3 and FIG. 4.

Table 4-3

	Amino acid and the like	Copper-extraction rate (%)
Test Example 4-3-1	Sodium glutamate alone	37.5
Test Example 4-3-2	Sodium glutamate and Histidine hydrochloride	61.5

From the results of Test Examples 4-1-1 to 4-3-2, using histidine hydrochloride or a salt thereof in combination with other amino acids or salts thereof could increase the extraction rate of copper.

(Test Example 5-1: effect of concentration on extraction of copper by histidine hydrochloride)

To 250 mg (dry mass) of a copper-containing yeast powder (MINERAL YEAST Cu1, copper-content: 11,422 ppm by mass, Oriental Yeast Co., Ltd.), was added 5.0 mL of histidine hydrochloride-containing liquid having various concentrations prepared in the following manner, followed by stirring to suspend the copper-containing yeast, to obtain a suspension.

The suspension was maintained at room temperature (25°C) for 60 min, and was then centrifuged at 3,000 rpm for 5 min. The resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer, and was measured for extraction rate of copper in the same manner as in Test Example 1-1. Results are shown in Table 5-1.

Note that, a test was performed in the same manner about the case where histidine hydrochloride was not contained.

<Histidine hydrochloride-containing liquid>

300 mmol/L NaOH solution containing 200 mmol/L histidine hydrochloride (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare histidine hydrochloride-containing liquid containing histidine hydrochloride at concentrations of 10 mmol/L, 20 mmol/L, 50 mmol/L, 100 mmol/L, and 200 mmol/L. Note that, 300 mmol/L NaOH solution

containing no histidine hydrochloride was also prepared.

Table 5-1

Concentration of histidine hydrochloride	Copper-extraction rate (%)
0 mmol/L	18.3
10 mmol/L	57.2
20 mmol/L	66.1
50 mmol/L	76.0
100 mmol/L	85.8
200 mmol/L	88.4

(Test Example 5-2: effect of concentration on extraction of copper by glycine)

A test was performed in the same manner as in Test Example 5-1, except that the histidine hydrochloride-containing liquid in Test Example 5-1 was changed to glycine-containing liquid having various concentrations prepared in the following manner. Results are shown in Table 5-2.

<Glycine-containing liquid>

75 mmol/L NaOH solution containing 200 mmol/L glycine (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare glycine-containing liquid containing glycine at concentrations of 10 mmol/L, 20 mmol/L, 50 mmol/L, 100 mmol/L, and 200 mmol/L. Note that, 75 mmol/L NaOH solution containing no histidine hydrochloride was also prepared.

Table 5-2

Concentration of glycine	Copper-extraction rate (%)
0 mmol/L	18.7
10 mmol/L	33.4
20 mmol/L	41.6
50 mmol/L	49.6
100 mmol/L	55.7
200 mmol/L	60.6

(Test Example 5-3: effect of concentration on extraction of copper by sodium glutamate)

A test was performed in the same manner as in Test Example 5-1, except that the histidine hydrochloride-containing liquid in Test Example 5-1 was changed to sodium glutamate-containing liquid having various concentrations prepared in the following manner. Results are shown in Table 5-3.

<Sodium glutamate-containing liquid>

75 mmol/L NaOH solution containing 200 mmol/L glycine (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare sodium glutamate-containing liquid containing sodium glutamate at concentrations of 10 mmol/L, 20 mmol/L, 50 mmol/L, 100 mmol/L, and 200 mmol/L.

Table 5-3

Concentration of sodium glutamate	Copper-extraction rate (%)
0 mmol/L	18.4
10 mmol/L	41.1
20 mmol/L	44.5
50 mmol/L	52.2
100 mmol/L	55.8
200 mmol/L	58.9

(Test Example 5-4: effect of concentration on extraction of copper by threonine hydrochloride)

A test was performed in the same manner as in Test Example 5-1, except that the histidine hydrochloride-containing liquid in Test Example 5-1 was changed to threonine hydrochloride-containing liquid having various concentrations prepared in the following manner. Results are shown in Table 5-4.

<Threonine hydrochloride-containing liquid>

300 mmol/L NaOH solution containing 200 mmol/L threonine hydrochloride

(pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare threonine hydrochloride-containing liquid containing threonine hydrochloride at concentrations of 10 mmol/L, 20 mmol/L, 50 mmol/L, 100 mmol/L, and 200 mmol/L.

Table 5-4

Concentration of threonine hydrochloride	Copper-extraction rate (%)
0 mmol/L	18.5
10 mmol/L	42.0
20 mmol/L	52.4
50 mmol/L	59.8
100 mmol/L	66.8
200 mmol/L	71.0

From the results of Test Examples 5-1 to 5-4, excellent extraction rates of copper could be obtained in a wide range of concentration of the amino acids or the salts thereof.

(Test Example 6-1: effect of temperature on extraction of copper by histidine hydrochloride)

To 250 mg (dry mass) of a copper-containing yeast powder (MINERAL YEAST Cu1, copper-content: 11,422 ppm by mass, Oriental Yeast Co., Ltd.), was added 5.0 mL of histidine hydrochloride-containing liquid containing histidine hydrochloride at 100 mmol/L prepared in the following manner, followed by stirring to suspend the copper-containing yeast, to obtain a suspension.

The suspension was maintained at room temperature (25°C), 50°C, 75°C or 100°C for 10 min, and was then centrifuged at 3,000 rpm for 5 min. The resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer, and was measured for extraction rate of copper in the same manner as in Test Example 1-1. Results are shown in Table 6-1.

<Histidine hydrochloride-containing liquid>

300 mmol/L NaOH solution containing 200 mmol/L histidine hydrochloride (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare histidine hydrochloride-containing liquid containing histidine hydrochloride at a concentration of 100 mmol/L.

Table 6-1

Treatment temperature	Copper-extraction rate (%)
25°C	82.3
50°C	80.6
75°C	82.3
100°C	82.6

(Test Example 6-2: effect of temperature on extraction of copper by glycine)

A test was performed in the same manner as in Test Example 6-1, except that the histidine hydrochloride-containing liquid in Test Example 6-1 was changed to glycine-containing liquid containing glycine at 100 mmol/L prepared in the following manner. Results are shown in Table 6-2.

<Glycine-containing liquid>

75 mmol/L NaOH solution containing 200 mmol/L glycine (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare glycine-containing liquid containing glycine at a concentration of 100 mmol/L.

Table 6-2

Treatment temperature	Copper-extraction rate (%)
25°C	43.9
50°C	46.3
75°C	45.8
100°C	44.9

(Test Example 6-3: effect of temperature on extraction of copper by threonine

hydrochloride)

A test was performed in the same manner as in Test Example 6-1, except that the histidine hydrochloride-containing liquid in Test Example 6-1 was changed to threonine hydrochloride-containing liquid containing threonine hydrochloride at 100 mmol/L prepared in the following manner. Results are shown in Table 6-3.

<Threonine hydrochloride-containing liquid>

300 mmol/L NaOH solution containing 200 mmol/L threonine hydrochloride (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare threonine hydrochloride-containing liquid containing threonine hydrochloride at a concentration of 100 mmol/L.

Table 6-3

Treatment temperature	Copper-extraction rate (%)
25°C	60.1
50°C	60.8
75°C	60.5
100°C	61.0

From the results of Test Examples 6-1 to 6-3, excellent extraction rates of copper could be obtained in a wide range of treatment temperature.

(Test Example 7-1: effect of treatment time on extraction of copper by histidine hydrochloride)

To 250 mg (dry mass) of a copper-containing yeast powder (MINERAL YEAST Cu1, copper-content: 11,422 ppm by mass, Oriental Yeast Co., Ltd.), was added 5.0 mL of histidine hydrochloride-containing liquid containing histidine hydrochloride at 100 mmol/L prepared in the following manner, followed by stirring to suspend the copper-containing yeast, to obtain a suspension.

The suspension was maintained at room temperature (25°C) for 10 min, 20 min, 40 min or 60 min, and was then centrifuged at 3,000 rpm for 5 min. The

resultant supernatant was filtrated through a filter for an ICP emission spectrophotometer, and was measured for extraction rate of copper in the same manner as in Test Example 1-1. Results are shown in Table 7-1.

<Histidine hydrochloride-containing liquid>

300 mmol/L NaOH solution containing 200 mmol/L histidine hydrochloride (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare histidine hydrochloride-containing liquid containing histidine hydrochloride at a concentration of 100 mmol/L.

Table 7-1

Treatment time	Copper-extraction rate (%)
10 min	82.3
20 min	81.7
40 min	84.2
60 min	83.7

(Test Example 7-2: effect of treatment time on extraction of copper by glycine)

A test was performed in the same manner as in Test Example 7-1, except that the histidine hydrochloride-containing liquid in Test Example 7-1 was changed to glycine-containing liquid containing glycine at 100 mmol/L prepared in the following manner. Results are shown in Table 7-2.

<Glycine-containing liquid>

75 mmol/L NaOH solution containing 200 mmol/L glycine (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare glycine-containing liquid containing glycine at a concentration of 100 mmol/L.

Table 7-2

Treatment time	Copper-extraction rate (%)
10 min	43.9
20 min	44.2
40 min	42.8
60 min	43.0

(Test Example 7-3: effect of treatment time on extraction of copper by threonine hydrochloride)

A test was performed in the same manner as in Test Example 7-1, except that the histidine hydrochloride-containing liquid in Test Example 7-1 was changed to threonine hydrochloride-containing liquid containing threonine hydrochloride at 100 mmol/L prepared in the following manner. Results are shown in Table 7-3.

<Threonine hydrochloride-containing liquid>

300 mmol/L NaOH solution containing 200 mmol/L threonine hydrochloride (pH value: about 9.5) was prepared. The above NaOH solution was diluted with 20 mmol/L NaOH solution to prepare threonine hydrochloride-containing liquid containing threonine hydrochloride at a concentration of 100 mmol/L.

Table 7-3

Treatment time	Copper-extraction rate (%)
10 min	60.1
20 min	61.0
40 min	60.2
60 min	60.5

From the results of Test Examples 7-1 to 7-3, excellent extraction rates of copper could be obtained in a wide range of treatment time.

(Test Example 8: evaluation of effect of maintaining and restoring green color of salted cucumber)

<Preparation of powder of copper-containing yeast extract>

Histidine hydrochloride, glycine, threonine hydrochloride, or glutamic acid hydrochloride was used as the amino acid or the salt thereof and treated in the same manner as in Test Example 1, to obtain a copper-containing yeast extract.

DL-malic acid and dextrin were added to the above copper-containing yeast extract, followed by drying using SPRAY DRIER L-8 (OHKAWARA KAKOHKI CO., LTD.), to prepare powder containing copper at 0.5% by mass (hereinafter may be referred to "histidine hydrochloride extract", "glycine extract", "threonine hydrochloride extract", or "glutamic acid hydrochloride extract").

Note that, as a positive control, LEVELUP V8 CLEAR (Oriental Yeast Co., Ltd.) was also used to prepare powder containing copper at 0.5% by mass.

<Green color-maintaining test>

A 2% aqueous solution (mass/volume) of the histidine hydrochloride extract, glycine extract, threonine hydrochloride extract, glutamic acid hydrochloride extract, or LEVELUP V8 CLEAR was prepared and heated to 90°C.

Thereafter, salted cucumber (Kissui Corporation) was charged in the solution, followed by heating under stirring at 90°C for 40 min.

After heating for 40 min, the heated water was removed and the salted cucumber was observed for color tone. Results are shown in FIG. 5A to FIG. 5E.

FIG. 5A illustrates results of LEVELUP V8 CLEAR, FIG. 5B illustrates results of the histidine hydrochloride extract, FIG. 5C illustrates results of the glycine extract, FIG. 5D illustrates results of the threonine hydrochloride extract, and FIG. 5E illustrates results of the glutamic acid hydrochloride extract.

As shown in FIG. 5A to FIG. 5E, the histidine hydrochloride extract, the glycine extract, the threonine hydrochloride extract, and the glutamic acid hydrochloride extract exhibited more excellent green color-maintaining effects than LEVELUP V8 CLEAR.

Industrial Applicability

The method of the present invention for producing a copper-containing yeast extract can extract copper at a high extraction rate, and can efficiently produce a copper-containing yeast extract containing copper derived from a natural product at a high concentration.

The copper-containing yeast extract of the present invention is suitable for, for example, an oral or tube feeding composition (e.g., a fluid diet and beverage), a food material, and an agent for maintaining and restoring a green color of vegetables. Use of the copper-containing yeast extract of the present invention as the oral or tube feeding composition or the food material can prevent or improve, for example, anemia and heart diseases associated with copper deficiency.

CLAIMS

1. A method for producing a copper-containing yeast extract, comprising:

extracting the copper-containing yeast extract by suspending copper-containing yeast into an aqueous solution containing an amino acid or a salt thereof to obtain a suspension, and separating a solid component and a liquid component of the suspension to obtain the liquid component,

wherein the amino acid or the salt thereof is water-soluble, and

wherein the amino acid or the salt thereof is at least one selected from the group consisting of histidine hydrochloride, glycine, threonine hydrochloride, glutamic acid hydrochloride, sodium glutamate, lysine hydrochloride, and arginine hydrochloride.

2. The method for producing the copper-containing yeast extract according to claim 1, wherein a pH value of an extraction liquid obtained in the extracting is lower than 4.0 or in the range of higher than 7.0 but lower than 11.0.

3. The method for producing the copper-containing yeast extract according to claim 1 or 2, wherein the amino acid or the salt thereof is at least one selected from the group consisting of histidine hydrochloride, glycine, threonine hydrochloride, and glutamic acid hydrochloride.

4. The method for producing the copper-containing yeast extract according to any one of claims 1 to 3, wherein the amino acid or the salt thereof contains histidine hydrochloride.

5. The method for producing the copper-containing yeast extract according to any one of claims 1 to 4, wherein the amino acid or the salt thereof contains histidine hydrochloride and at least one selected from the group consisting of glycine, threonine hydrochloride, and glutamic acid hydrochloride.

6. The method for producing the copper-containing yeast extract according to any one of claims 1 to 5, further comprising treating the copper-containing yeast

with a cell wall-degrading enzyme.

7. The method for producing the copper-containing yeast extract according to any one of claims 1 to 6, wherein a copper-extraction rate expressed by a formula below is 40% or more:

Copper-extraction rate (%) = {Total amount (mass) of copper in the extract / Total amount (mass) of copper contained in the yeast used for extraction} × 100.

8. The method for producing the copper-containing yeast extract according to claim 7, wherein the copper-extraction rate is 50% or more.

9. The method for producing the copper-containing yeast extract according to any one of claims 1 to 8, wherein a copper content in the copper-containing yeast extract is 0.2% by mass or more.

10. A method for producing food, comprising:

extracting the copper-containing yeast extract by suspending copper-containing yeast into an aqueous solution containing an amino acid or a salt thereof to obtain a suspension, and separating a solid component and a liquid component of the suspension to obtain the liquid component; and

adding the liquid component to the food,

wherein the amino acid or the salt thereof is water-soluble, and

wherein the amino acid or the salt thereof is at least one selected from the group consisting of histidine hydrochloride, glycine, threonine hydrochloride, glutamic acid hydrochloride, sodium glutamate, lysine hydrochloride, and arginine hydrochloride.

11. A method for maintaining and restoring a green color of vegetables, comprising:

extracting the copper-containing yeast extract by suspending copper-containing yeast into an aqueous solution containing an amino acid or a salt thereof to obtain a suspension, and separating a solid component and a liquid

component of the suspension to obtain the liquid component; and

adding the liquid component to the vegetables,

wherein the amino acid or the salt thereof is water-soluble, and

wherein the amino acid or the salt thereof is at least one selected from the group consisting of histidine hydrochloride, glycine, threonine hydrochloride, glutamic acid hydrochloride, sodium glutamate, lysine hydrochloride, and arginine hydrochloride.