

DESCRIPTION

Title of Invention

YEAST EXTRACT WITH HIGH COPPER CONTENT, METHOD FOR
PRODUCING SAME, AND AGENT FOR MAINTAINING AND RESTORING
5 GREEN COLOR OF FOODS AND VEGETABLES

Technical Field

The present invention relates to a high-copper-content yeast extract which contains copper derived from a natural product in a high concentration, which is
10 excellent in water-solubility, and which is suitable for an oral or tube feeding composition (e.g., a fluid diet) or a food material capable of improving, for example, anemia and heart diseases associated with copper deficiency; a method for producing the same; food containing the high-copper-content yeast extract; 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
20 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.
25 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. The conventional copper-containing yeast is water-insoluble, so that turbidity and precipitation are caused when it is added to food. As a result, the conventional copper-containing yeast cannot be used in, especially, a soft drink, which is problematic.

In order to solve the above problems, there has been proposed a copper-containing yeast extract which is extracted from the copper-containing yeast. Extraction of a yeast 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 of a low mineral recovery rate (copper extraction rate). The methods of (2) and (3) have a problem that a yeast odor and an extract taste remain in the resultant yeast extract. The method of (3) has problems of a high cost and a residual enzyme in the resultant yeast extract.

Metals such as copper have a possibility to be used for restoring a green color of plants, but it has not been known that copper can maintain, among others, a green color for a long period of time. It has been required in, especially, food processing industry to maintain the green color of vegetables for a long period of time, because maintenance of the green color of vegetables affects shelf life of food and processed products thereof. However, a safe metal-containing material capable of being added to food has not been known, and maintenance of the green color of vegetables for a long period of time has not been achieved.

Therefore, there has been demanded a high-copper-content yeast extract; a method for efficiently producing the same; food (e.g., a fluid diet and a beverage) containing the high-copper-content yeast extract; and a green color-maintaining and

restoring agent having an effect of maintaining a green color of vegetables for a long period of time.

Citation List

5 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)

10 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

15 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 high-copper-content yeast extract which contains copper derived from a natural product in a high concentration, which is excellent in
20 water-solubility, which does not impair appearance of food containing it, which can be suitably used as an oral or tube feeding composition (e.g., a fluid diet or a beverage) and a food material, and which does not impair a flavor of food containing it; a method for producing the same; food; and a green color-maintaining and restoring agent for vegetables having an effect of maintaining a green color for a
25 long period of time.

Solution to Problem

Means for solving the problems are as follows.

<1> A method for producing a high-copper-content yeast extract, comprising:
extracting the high-copper-content yeast extract by suspending
copper-containing yeast into a solution containing a carboxylic acid, a carboxylic acid
5 salt, or both thereof to thereby obtain a suspension, and separating a solid
component and a liquid component of the suspension.

<2> The method for producing the high-copper-content yeast extract according to
<1>, wherein the carboxylic acid is a bivalent or higher carboxylic acid, and wherein
the carboxylic acid salt is a bivalent or higher carboxylic acid salt.

10 <3> The method for producing the high-copper-content yeast extract according to
<1> or <2>, wherein the carboxylic acid is a trivalent carboxylic acid, and wherein
the carboxylic acid salt is a trivalent carboxylic acid salt.

<4> The method for producing the high-copper-content yeast extract according to
any one of <1> to <3>, wherein a pH value of the suspension is in the range of 2.0 to
15 10.0.

<5> The method for producing the high-copper-content yeast extract according to
any one of <1> to <4>, wherein a total amount of the carboxylic acid and the
carboxylic acid salt is 2 mol or more relative to 1 mol of copper contained in the
yeast.

20 <6> The method for producing the high-copper-content yeast extract according to
any one of <1> to <5>, 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 thereby obtain
a suspension, and separating a solid component and a liquid component of the
suspension.

25 <7> The method for producing the high-copper-content yeast extract according to
<6>, wherein, in the hot-water treating, a phosphoric acid salt is added to the
hot-water.

<8> The method for producing the high-zinc-content yeast extract according to any one of <1> to <7>, wherein the separating the solid component and the liquid component of the suspension in the extracting is performed through filtration.

<9> The method for producing the high-zinc-content yeast extract according to any one of <6> to <8>, wherein the separating the solid component and the liquid component of the suspension in the hot-water treating is performed through filtration.

<10> A high-copper-content yeast extract, comprising:

0.2% by mass or more of copper derived from a yeast fungus body,

wherein a turbidity is 0.1 or less in terms of absorbance at a wavelength of 660 nm (O. D. 660) when 1 g of the high-copper-content yeast extract is dissolved or dispersed into 100 mL of water.

<11> The high-copper-content yeast extract according to <10> produced by the method for producing the high-copper-content yeast extract according to any one of <1> to <9>.

<12> The high-copper-content yeast extract according to <10> or <11>, wherein the yeast is edible yeast.

<13> The high-copper-content 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 and soy sauce yeast.

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

<15> The high-copper-content yeast extract according to any one of <10> to <14>, wherein the high-copper-content yeast extract is added to food.

<16> The high-copper-content yeast extract according to <13>, wherein the food is a fluid diet or a soft drink.

<17> Food, comprising:

the high-copper-content yeast extract according to any one of <10> to <16>.
<18> An agent for maintaining and restoring a green color of vegetables,
comprising:

the high-copper-content yeast extract according to any one of <10> to <14>.

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Advantageous Effects of Invention

The present invention can solve the existing problems, and can provide a high-copper-content yeast extract which contains copper derived from a natural product in a high concentration, which is excellent in water-solubility, which does
10 not impair appearance of food containing it, which is suitable as, for example, an oral or tube feeding composition (e.g., a fluid diet or a beverage) and a food material, and which does not impair a flavor of food containing it; a method for producing the same; food; and a green color-maintaining and restoring agent for vegetables having an effect of maintaining a green color for a long period of time.

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Brief Description of Drawings

FIG. 1 is a graph illustrating copper-elution rates with pure water, sodium chloride, and each carboxylic acid salt in Example 1 and Comparative Example 1.

FIG. 2 is a graph illustrating effects of a carboxylic acid or a carboxylic acid
20 salt on copper-elution rates in Example 2.

FIG. 3 is a graph illustrating an effect of pH of a suspension on a copper-elution rate in Example 3.

FIG. 4 is a graph illustrating a relationship between a concentration of a carboxylic acid buffer solution and a copper-elution rate in Example 4 and
25 Comparative Example 2.

FIG. 5 is a graph illustrating a relationship between an elution time and a copper-elution rate in Example 5.

FIG. 6A is one exemplary photograph illustrating water-solubilities of high-copper-content yeast extracts in Example 6-1 and Comparative Examples 3-1 and 3-2.

FIG. 6B is a photograph of aqueous solutions in FIG. 6A after centrifugation
5 at 3,000 rpm for 5 min.

FIG. 6C is one exemplary photograph illustrating solubilities into an apple juice solution of high-copper-content yeast extracts in Example 6-2 and Comparative Examples 3-3 and 3-4.

FIG. 6D is a photograph of apple juice solutions in FIG. 6C after
10 centrifugation at 3,000 rpm for 5 min.

FIG. 7A is a photograph illustrating test results of Example 8-1.

FIG. 7B is a photograph illustrating test results of Example 8-2.

FIG. 7C is a photograph illustrating test results of Example 8-3.

FIG. 7D is a photograph illustrating test results of Example 8-4.

15 FIG. 7E is a photograph illustrating test results of Example 8-5.

Description of Embodiments

(Production method of high-copper-content yeast extract)

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

<Extraction step>

The extraction step comprises suspending copper-containing yeast into a solution containing a carboxylic acid, a carboxylic acid salt, or both thereof to
25 thereby obtain a suspension, and separating a solid component and a liquid component of the suspension. The extraction step results in a high-copper-content liquid component (extract).

<<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 high as possible in the case where the high-copper-content 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 high-copper-content yeast extract, the copper content is preferably 0.01% by mass to 2% by mass, more preferably 0.5% by mass to 2% by mass, relative to dry mass of fungus body.

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 thereby 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.). An amount of copper to be added to the culture medium is not particularly limited and may be appropriately selected depending on the intended purpose, but it preferably achieves both of a high copper

utilization rate (uptake rate of copper into a fungus body) and a high yield based on sugar (growth rate).

Note that, a type of copper to be added, a type of a culture medium, and a culturing condition are not particularly limited and may be appropriately selected depending on the intended purpose.

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 intended 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 intended 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 intended purpose. Examples thereof include iron, magnesium, manganese, and zinc. These minerals may be contained alone or in combination in the yeast. These minerals are, in general, preferably contained in a high concentration, but the concentration varies depending on the intended purpose and cannot be unambiguously defined.

The yeast is particularly preferably edible yeast in the case where extract thereof 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 and 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 intended 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 fibuligera, Saccharomyces lipolytica, Rhodotorula rubra, and Pichia farinosa.

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

<<Carboxylic acid and carboxylic acid salt>>

The carboxylic acid is not particularly limited and may be appropriately selected depending on the purpose. The carboxylic acid may be a monovalent carboxylic acid or a bivalent or higher carboxylic acid. However, preferable is a bivalent or higher carboxylic acid, and more preferable is a trivalent or higher carboxylic acid, from the viewpoint of extraction efficiency of copper (copper-elution rate).

Examples of the monovalent carboxylic acid include formic acid, acetic acid,

propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pyruvic acid, and gluconic acid.

Examples of the bivalent carboxylic acid include oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, fumaric acid, maleic acid, tartaric acid,
5 oxaloacetic acid, and α -ketoglutaric acid.

Examples of the trivalent carboxylic acid include citric acid, isocitric acid, aconitic acid, and oxalosuccinic acid.

Among them, from the viewpoints of addition to food and copper-elution rate, preferable are citric acid, succinic acid, and tartaric acid, more preferable is citric
10 acid.

These may be used alone or in combination.

The carboxylic acid salt is not particularly limited and may be appropriately selected depending on the intended purpose. The carboxylic acid salt may be a monovalent carboxylic acid salt or a bivalent or higher carboxylic acid salt.
15 However, preferable is a bivalent or higher carboxylic acid salt from the viewpoint of extraction efficiency of copper, and more preferable is a trivalent or higher carboxylic acid salt from the viewpoint of higher extraction efficiency of copper. Examples of the carboxylic acid salt include salts of the above-described specific carboxylic acids. Among them, preferable are a citric acid salt, a succinic acid salt, and a tartaric acid
20 salt, more preferable is the citric acid salt.

These may be used alone or in combination.

A type of the salt is not particularly limited and may be appropriately selected depending on the intended purpose. Examples thereof include alkali metal salts such as a sodium salt and a potassium salt; and alkaline earth metal salts such
25 as a magnesium salt and a calcium salt. These may be used alone or in combination.

Either of the carboxylic acid or the carboxylic acid salt may be used.

Alternatively, both of the carboxylic acid and the carboxylic acid salt may be used.

An amount of the carboxylic acid or the carboxylic acid salt is not particularly limited and may be appropriately selected depending on the purpose. However, relative to 1 mol of copper contained in the yeast, the total amount of the carboxylic acid and the carboxylic acid salt is preferably 2 mol or more, and from the
5 viewpoint of extraction efficiency of copper, it is more preferably 10 mol or more. When the total amount is less than 2 mol, a copper-elution rate may be deteriorated.

A solvent used for a solution containing the carboxylic acid, the carboxylic acid salt, or both thereof (hereinafter may be referred to as "carboxylic acid buffer
10 solution") 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).

A concentration of the carboxylic acid buffer solution is not particularly limited and may be appropriately selected depending on the purpose. The
15 concentration is preferably high as possible from the viewpoint of extraction efficiency of copper. Specifically, the concentration is preferably 20 mmol/L or more, more preferably 50 mmol/L or more, further preferably 150 mmol/L or more.

The pH value of the carboxylic acid buffer solution is not particularly limited and may be appropriately selected depending on the intended purpose. However, it
20 is preferably in the range of pH 1.5 to 9, and from the viewpoint of extraction efficiency of copper, it is more preferably in the range of pH 3.5 to 8.5. Note that, the pH can be adjusted by varying a quantity ratio of the carboxylic acid and the carboxylic acid salt.

The pH of the suspension in the extraction step is not particularly limited
25 and may be appropriately selected depending on the intended purpose. However, it is preferably in the range of pH 2.0 to 10.0, and from the viewpoint of extraction efficiency of copper, it is more preferably in the range of pH 4.0 to 7.0.

An extraction time in the step is not particularly limited and may be appropriately selected depending on the purpose. However, from the viewpoint of extraction efficiency of copper, it is preferably 5 hours or more, more preferably 10 hours or more, further preferably 15 hours or more.

5 A temperature of the suspension in the extraction step is not particularly limited and may be appropriately selected depending on the purpose.

The copper-elution rate (hereinafter may be referred to as "extraction 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, the
10 copper-elution rate is preferably 20% by mass or more, more preferably 30% by mass or more, particularly preferably 60% by mass or more.

The copper-elution rate can be calculated according to the following equation.

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

A means of suspending the yeast into the solution containing the carboxylic acid, the carboxylic acid salt, or both thereof (carboxylic acid buffer solution) is not particularly limited and may be appropriately selected depending on the purpose.

20 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>>

25 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
5 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.

After extraction with the carboxylic acid buffer solution, the carboxylic acid
10 remains in the high-copper-content yeast extract as extraction traces. Therefore, the carboxylic acid contained in the high-copper-content yeast extract can be measured to determine whether the extraction with the buffer solution has been achieved. A method for measuring is not particularly limited. For example, an amount of the carboxylic acid (e.g., citric acid) can be measured by means of HPLC
15 (high performance liquid chromatography).

<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 a hot-water treatment step, a drying
20 step, a concentration step, and a dilution step. Among them, the hot-water treatment step is preferably comprised.

<<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 thereby obtain a suspension, and
25 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 high-copper-content 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,
5 the higher an effect of reducing the yeast odor and the yeast taste is. The temperature is more preferably 80°C to 120°C, further 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.

10 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-elution
15 rate in the subsequent extraction step. Examples thereof include salts other than carboxylic acid salts. Among them, 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
20 limited and may be appropriately selected depending on the intended 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 fungus body of the yeast.

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

The drying step comprises drying the high-copper-content yeast extract.

25 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

high-copper-content yeast extract (powdered high-copper-content yeast extract), which can be used in various applications described below. Note that, during the high-copper-content yeast extract is formed into the solid high-copper-content yeast extract, an excipient (e.g., dextrin) may be appropriately added.

5 The concentration step comprises concentrating the high-copper-content yeast extract. The dilution step comprises diluting the high-copper-content yeast extract.

A means of r concentrating and a means of diluting are not particularly limited and may be conventionally known methods.

10 (High-copper-content yeast extract)

A high-copper-content yeast extract of the present invention is an extract which contains 0.2% by mass or more of copper derived from a yeast, and which has a turbidity of 0.1 or less in terms of absorbance at a wavelength of 660 nm (O. D. 660) when 1 g of the extract is dissolved or dispersed into 100 mL of water.

15 A method for producing the high-copper-content yeast extract is not particularly limited and may be appropriately selected depending on the purpose. However, the extract can be suitably produced by the method of the present invention. That is, the high-copper-content yeast extract is preferably produced by subjecting the copper-containing yeast to extraction with a solution containing a
20 carboxylic acid, a carboxylic acid salt, or both thereof.

<Copper-content>

The copper-content in the high-copper-content yeast extract is preferably high as possible from the viewpoint of use of the extract as a copper-enriched food material. "High-copper-content," as used herein, means that copper derived from a
25 yeast is contained in a concentration of 0.2% by mass or more, preferably 0.5% by mass or more, more preferably 0.8% by mass or more.

Containing copper in such a high concentration allows the

high-copper-content yeast extract to be suitably used as an oral or tube feeding composition (e.g., a fluid diet or a beverage) and a food material.

Note that, the copper-content can be measured by known methods such as an atomic absorption spectrometry or an ICP emission spectrometric analysis.

5 <Turbidity>

The high-copper-content yeast extract of the present invention has a high water-solubility, so that an aqueous solution of the extract has a high transparency. The high-copper-content yeast extract has a turbidity of 0.1 or less, preferably 0.05 or less, more preferably 0.01 or less in terms of absorbance at 660 nm (O. D. 660) when 1 g of the is dissolved or dispersed into 100 mL of water. When the turbidity is more than 0.1, food to which the extract is added may get turbid and be discolored, which may impair appearance of the food. In addition, the high-copper-content yeast extract has insufficient water-solubility and may precipitate, which may be problematic in the case where the extract is used in food required to be transparent (in particular, soft drink).

A form of the high-copper-content 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 a powdered form (powder), a particle form, or a sheet form; and a semi-solid form such as a gel form or a slurry form. In the measurement of the turbidity, "1 g of the high-copper-content yeast extract" is in the form of a dried solid of which moisture content is 7% by mass or less. A means of drying is not particularly limited and may be the above-described methods. A drying condition is not particularly limited.

Note that, the turbidity can be measured as absorbance at 660 nm (O. D. 660) by using spectrophotometer. Example of the spectrophotometer includes U-2000 model (Hitachi, Ltd.).

<Application>

Application of a high-copper-content 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 from the viewpoint of excellent solubility. In addition, the extract is also preferably used in a food fermentation medium or as an agent for maintaining and restoring a green color of vegetables. The high-copper-content yeast extract of the invention can result in high-copper-content food, high-copper-content animal food, and high-copper-content feed.

A form of the high-copper-content yeast extract in use is not particularly limited and may be appropriately selected depending on the application. Examples thereof include a dried powder form (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 form or cream form). Note that, a method for forming the high-copper-content 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 high-copper-content 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, 5 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 high-copper-content yeast extract contained in the food is not particularly limited and may be appropriately selected depending on the 10 intended 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 15 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, 20 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.

25 (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 high-copper-content 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
5 maintain a green color of green plants such as green vegetables, tea leaves, and Matcha (green powdered tea). 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 high-copper-content yeast extract contained in the agent is
10 not particularly limited and may be appropriately selected depending on the intended application and purpose.

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

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Examples

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

(Example 1 and Comparative Example 1: elution of copper with carboxylic acid salt)

20 To 5 g (dry mass) of a high-copper-content yeast powder (MINERAL YEAST Cu1, copper-content: 11,200 ppm by mass, Oriental Yeast Co., Ltd.), was added 50 mL of each of aqueous solutions of carboxylic acid sodium salts described in Table 1 at 200 mmol/L, followed by stirring and diluting to 100 mL with water to thereby obtain a suspension. Note that, an amount of each of the carboxylic acid salts was
25 11.4 mol relative to 1 mol of copper contained in yeast to be subjected to extraction. Water or a 200 mmol/L aqueous solution of sodium chloride (table salt) was used as a control and was tested in the same manner as the suspension. The suspension was

measured for pH by means of a pH meter (MP230, METTLER-TOREDO Inc.).

Under stirring with a stirrer, 5 mL of the suspension was taken out into a test tube, followed by heating in a boiling water bath for 10 min. The resultant suspension is centrifuged at 3,000 rpm for 5 min. Only the supernatant (extraction liquid) was transferred to a test tube to thereby obtain yeast extract. The yeast extract was weighed.

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. Results are shown in Table 1 and FIG. 1.

The copper-content in the extract on a dry powder basis (% by mass) was measured as follows. That is, dextrin was added to the extraction liquid which had been subjected to extraction, followed by adjusting a solid content to 10% by mass. Then, the resultant was dried by means of SPRAY DRIER L-8 (OHKAWARA KAKOHKI CO., LTD.), and quantified for copper by the ICP emission spectrometric analysis using the ICP emission spectrophotometer (OPTIMA 2100DV, PerkinElmer Inc.). Results are shown in Table 1.

Table 1

	Type of extraction solvent	pH of suspension	Copper-elution rate (%)	Copper-content on dry powder basis (% by mass)
Comp. Ex. 1-1	Pure water	5.4	14	0.11
Comp. Ex. 1-2	Sodium chloride (table salt) aqueous solution	5.3	17	0.13
Ex. 1-1	Monosodium succinate aqueous solution	4.8	32	0.25
Ex. 1-2	Sodium L-tartrate aqueous solution	5.7	30	0.23
Ex. 1-3	Trisodium citrate aqueous solution	6.7	60	0.46

As shown in Table 1 and FIG. 1, use of carboxylic acid salts results in a high

copper-elution rate and a high-copper-content extract. This effect was the highest in the citrate, which is a trivalent carboxylic acid, followed by the succinate and the tartrate, which are bivalent carboxylic acids.

(Example 2: copper-elution rate by carboxylic acid or carboxylic acid salt)

MINERAL YEAST Cu1 powder (Oriental Yeast Co., Ltd.) was suspended into 300 mL of water to thereby obtain 40% by mass of cream. The resultant cream was heated for 10 min in a boiling water bath, followed by washing. The resultant precipitate was diluted to 300 mL with water. Then, 10 mL of the cream was added to and suspended into 10 mL of each of aqueous solutions containing citric acid, trisodium citrate, or both thereof (citric acid buffer solutions) described in Table 2, to thereby obtain a suspension. Note that, all of the citric acid buffer solutions had a concentration of 200 mmol/L, and the pH of each of the citric acid buffer solutions was adjusted by appropriately varying a quantity ratio of citric acid and trisodium citrate.

Two hours after suspending each of the citric acid buffer solutions, the suspension was subjected to centrifugation. The resultant supernatant (yeast extract) was measured for the copper-elution rate in the same manner as in Example 1. Additionally, the yeast extract was dried to thereby obtain dry powder, which was measured for the copper-content in the same manner as in Example 1. Results are shown in Table 2 and FIG. 2.

Table 2

	pH of aqueous solution containing citric acid, trisodium citrate, or both thereof (citric acid buffer solution)	pH of suspension	Copper-elution rate (%)	Copper-content on dry powder basis (% by mass)
Ex. 2-1	1.9 (only citric acid)	2.1	51.7	0.61
Ex. 2-2	5.0	5.1	53.0	0.63
Ex. 2-3	8.2 (only trisodium citrate)	6.6	66.0	0.78

As shown in Table 2 and FIG. 2, copper could be eluted at a high rate in any of the cases where the extraction solvent contains only carboxylic acid, the extraction solvent contains only a carboxylic acid salt, and the extraction solvent contains both carboxylic acid and a carboxylic acid salt.

5 (Example 3: effect of pH on copper-elution rate)

A suspension was prepared in the same manner as in Example 2, except that each of the aqueous solutions in the following Table 3 (citric acid buffer solutions, each having a concentration of 300 mmol/L). The pH of the citric acid buffer solution was adjusted by appropriately varying a quantity ratio of citric acid and
10 trisodium citrate.

Two hours after suspending each of the citric acid buffer solutions, the resultant suspension was subjected to centrifugation. The resultant supernatant (yeast extract) was measured for the copper-elution rate and the copper-content by mass of the dry powder of the extract in the same manner as in Example 1. Results
15 are shown in Table 3 and FIG. 3.

Table 3

	pH of aqueous solution containing citric acid, trisodium citrate, or both thereof (citric acid buffer solution)	pH of suspension	Copper-elution rate (%)	Copper-content on dry powder basis (% by mass)
Ex. 3-1	1.9 (only citric acid)	2.2	54.0	0.62
Ex. 3-2	2.5	2.7	54.5	0.63
Ex. 3-3	3.0	3.3	58.0	0.67
Ex. 3-4	3.5	3.7	60.6	0.70
Ex. 3-5	4.0	4.2	64.9	0.75
Ex. 3-6	4.5	4.7	69.1	0.79
Ex. 3-7	5.0	5.2	69.1	0.79
Ex. 3-8	5.5	5.7	72.8	0.84
Ex. 3-9	6.0	6.1	74.9	0.86
Ex. 3-10	6.5	6.4	77.7	0.89
Ex. 3-11	7.0	6.6	80.3	0.92

As shown in Table 3 and FIG. 3, there is a trend that the lower the pH of a

solvent used for extraction (carboxylic acid buffer solution) is, the higher the copper-elution rate is. However, a high copper-elution rate of 50% or more could be attained in a broad pH range, that is, the high-copper-content yeast extract could be obtained.

5 (Example 4 and Comparative Example 2: relationship between copper-elution rate and molar ratio of carboxylic acid to copper contained in yeast extract)

MINERAL YEAST Cu1 (manufactured by Oriental Yeast Co., Ltd.) was used as a raw material to test a relationship between the concentration of a carboxylic acid buffer solution and the extraction efficiency of copper. Trisodium citrate was
10 used as a carboxylic acid salt contained in an extraction solvent to prepare trisodium citrate buffer solutions having concentrations described in the following Table 4.

The yeast extracts were obtained in the same manner as in Example 2, except that each of the trisodium citrate buffer solutions having the concentrations described in the following Table 4 was used. The resultant yeast extracts were
15 measured for the copper-elution rate. Additionally, in the same manner as in Example 1, each of the extracts was dried to thereby obtain dry powder, which was measured for the copper-content by mass of the dry powder of the extract. Results are shown in the following Table 4 and FIG. 4.

Table 4

	Concentration of trisodium citrate aqueous solution (mmol/L)	Molar ratio (carboxylic acid/copper)	pH of suspension	Copper-elution rate (%)	Copper content on dry powder basis (% by mass)
Comp. Ex. 2	0	0	5.6	1.7	0.02
Ex. 4-1	50	2.2	6.4	50.1	0.53
Ex. 4-2	100	4.4	6.6	54.5	0.58
Ex. 4-3	150	6.6	6.6	59.5	0.63
Ex. 4-4	200	8.7	6.7	75.1	0.80
Ex. 4-5	250	10.9	6.7	78.4	0.83
Ex. 4-6	300	13.1	6.7	87.3	0.93
Ex. 4-7	350	15.3	6.8	86.4	0.92
Ex. 4-8	400	17.5	6.8	85.1	0.91
Ex. 4-9	450	19.7	6.8	82.9	0.88
Ex. 4-10	500	21.8	6.8	80.5	0.86

As shown in Table 4 and FIG. 4, the copper-elution rate depends on the molar ratio of copper to a carboxylic acid and salt thereof. When the total amount of the carboxylic acid and the carboxylic acid salt is 2 mol to 25 mol relative to 1 mol of copper in yeast to be extracted, the copper-elution rate is high, and the copper content of extract (dry powder) is also high.

(Example 5: effect of elution time on copper-elution rate)

MINERAL YEAST Cu1 powder (Oriental Yeast Co., Ltd.) was suspended into 300 mL of water to thereby obtain 40% by mass of cream. The resultant cream was heated for 10 min in a boiling water bath, followed by washing. The resultant precipitate was diluted to 300 mL with water. Then, 10 mL of the cream was added to and suspended into 10 mL of 200 mmol/L trisodium citrate aqueous solution.

A time from the above suspending to centrifugation (sampling); i.e., an elution time, was set to 1 hour, 2 hours, 18 hours, 21 hours, and 24 hours. After each of the elution times, the resultant suspension was subjected to centrifugation. The resultant supernatant (yeast extract) was measured for the copper-elution rate in the same manner as in Example 1. Additionally, the yeast extract was dried to

thereby obtain dry powder, which was measured for the copper-content in the same manner as in Example 1. Results are shown in Table 5 and FIG. 5.

Table 5

	Elution time (hour)	Copper-elution rate (%)	Copper-content on dry powder basis (% by mass)
Ex. 5-1	1	54.0	0.65
Ex. 5-2	2	58.5	0.70
Ex. 5-3	18	75.5	0.90
Ex. 5-4	21	74.9	0.90
Ex. 5-5	24	76.0	0.91

5 As shown in Table 5 and FIG. 5, the longer the elution time is, the higher the copper-elution rate is, and a copper-elution rate of about 75% could be attained for an elution time longer than 15 hours.

(Example 6 and Comparative Example 3: evaluation of solubility)

The high-copper-content yeast extract obtained in Example 4-6 was mixed
 10 with dextrin so that the amount of the extract became 0.5% by mass, to thereby prepare high-copper-content yeast extract powder. One gram of the powder was diluted to 100 mL with water or apple juice (beverage containing 10% by volume apple juice, ASAHI SOFT DRINKS CO., Ltd.) to thereby prepare 1% (mass/volume) solutions of the copper yeast extract. The prepared solutions were each measured
 15 for turbidity by measuring absorbance at a wavelength of 660 nm (O. D. 660) by means of a spectrophotometer. The spectrophotometer was U-2000 model (Hitachi, Ltd.). The aqueous solution was observed for the presence or absence of a precipitate after centrifugation at 3,000 rpm for 5 min.

As a control, the following were used: a high-copper-content yeast extract
 20 (SOLUBLE COPPER YEAST, Grow Company, Inc.), which is a conventional product; and unextracted MINERAL YEAST Cu1 (Oriental Yeast Co., Ltd.). These controls were used to prepare solutions so that the copper content became the same as the

above solution, and the control solutions each were measured for turbidity and evaluated for the presence or absence of a precipitate. Results are shown in Table 6 and FIGs. 6A to 6D.

Table 6

	Sample	Solvent	O. D. 660 (1% solution)	Visual evaluation	Visual evaluation after centrifugation
Ex. 6-1	High-copper-content yeast extract	Water	0.05	Aqueous solution with high clarity	Precipitate was not confirmed
Comp. Ex. 3-1	Conventional product	Water	0.29	Slight turbidity was confirmed	Precipitate was confirmed
Comp. Ex. 3-2	MINERAL YEAST Cu1	Water	15.28	Suspended	Precipitate was confirmed
Ex. 6-2	High-copper-content yeast extract	Apple juice	0.03	Aqueous solution with high clarity	Precipitate was not confirmed
Comp. Ex. 3-3	Conventional product	Apple juice	0.17	Slight turbidity was confirmed	Precipitate was confirmed
Comp. Ex. 3-4	MINERAL YEAST Cu1	Apple juice	12.70	Suspended	Precipitate was confirmed

5

The aqueous solution of Example 6-1 had significantly lower turbidity than the aqueous solution of Comparative Example 3-1. As can be seen from FIG. 6A, it was visually confirmed that the aqueous solution of Example 6 had significantly higher clarity than the aqueous solution of Comparative Example 3-1. As can be seen from FIG. 6B, after centrifugation at 3,000 rpm for 5 min, the aqueous solution of Example 6-1 contained no precipitate and was a solution with high clarity. In contrast, the aqueous solution of Comparative Example 3-1 contained a precipitate.

10

The same results could be obtained in Examples 6-2 and Comparative Examples 3-2 and 3-3 each using apple juice instead of water.

15

Therefore, the high-copper-content yeast extract of the present invention has higher solubility than the conventional product, so that it can be added to, for example, the fluid diet or the beverage. Additionally, the extract of the invention does not cause turbidity and discoloration, so that it can be added to, in particular, a soft drink having a high transparency.

20

(Example 7 and Comparative Example 4: evaluation of odor and taste)

The following were prepared: high-copper-content yeast extract powder prepared by mixing with dextrin the high-copper-content yeast extract obtained in Example 4-6 so that the amount of the high-copper-content yeast extract became 0.5% by mass; a 1% (mass/volume) aqueous solution of the powder; a 1% solution prepared by dissolving the powder in apple juice so that the concentration of the powder became 1% (mass/volume); a solution (hereinafter referred to as “milk”) prepared by dissolving the powder in an aqueous solution of modified milk powder (CHIL MIL, MORINAGA MILK INDUSTRY CO., LTD.) so that the concentration of the powder became 1% (mass/mass), the aqueous solution of modified milk powder being prepared by dissolving or suspending 14 g of the modified milk powder into 100 mL of water; and a mixture prepared by adding the powder to a nutritional adjustment food (MEIBALANCE (yogurt taste), Meiji Co., Ltd.) so that the concentration of the powder became 1% (mass/mass) (hereinafter the mixture is referred to as “fluid diet”).

<Evaluation method>

The above high-copper-content yeast extract, the 1% aqueous solution, the 1% solution (apple juice), the milk, and the fluid diet were evaluated for odors (e.g., yeast odor and extract odor) and tastes (e.g., yeast taste and extract taste), which are distinctive of yeast or yeast extract, by 6 evaluators.

Each sample was ranked for evaluation items by 6 evaluators according to the following criteria, assuming that the high-copper-content yeast extract was ranked as 3 for each of the evaluation items, followed by averaging. Note that, as a control, the following were used: a high-copper-content yeast extract (SOLUBLE COPPER YEAST, Grow Company, Inc.), which is a conventional product; and unextracted MINERAL YEAST Cu1 (Oriental Yeast Co., Ltd.). These controls were used to prepare samples so that the copper content became the same as the above prepared samples, and the control samples were similarly evaluated. Results are

shown in Table 7.

-Evaluation criteria for odor-

5: very strong

4: strong

5 3: comparable

2: weak

1: very weak

-Evaluation criteria for taste-

5: very strong

10 4: strong

3: comparable

2: weak

1: very weak

Table 7

	Sample	Powder	1% (mass/volume) aqueous solution		1% (mass/volume) solution (apple juice)		Milk		Liquid food	
		Odor	Odor	Taste	Odor	Taste	Odor	Taste	Odor	Taste
Ex. 7	High-copper-content yeast extract	3	3	3	3	3	3	3	3	3
Comp. Ex. 4-1	Conventional product	4	3	4	3	4	3	3	3	4
Comp. Ex. 4-2	MINERAL YEAST Cu1	5	4	5	4	5	3	4	3	4

15

As can be seen from Table 7, the high-copper-content yeast extract of Example 7 was evaluated to have odors (e.g., yeast odor and extract odor) significantly lower than those of Comparative Example 4-1 (conventional product) in the powder, the 1% aqueous solution, and the 1% solution (apple juice); and to have yeast taste and extract taste both significantly lower in the milk and the fluid diet. Therefore, the high-copper-content yeast extract of the invention was found to have reduced odor and taste which were distinctive of yeast or yeast extract, so that the

20

high-copper-content yeast extract does not impair a flavor of food containing it.
(Example 8-1: evaluation of effect of maintaining and restoring green color of green pepper)

A test substance used was high-copper-content yeast extract powder
5 prepared by mixing with dextrin the high-copper-content yeast extract obtained in Example 4-6 so that the amount of the extract became 0.5% by mass.

A commercially available green pepper was cut into pieces and added to a 1 % by volume aqueous solution of brewed vinegar (Mizkan Group Corporation), and then subjected to discoloration treatment by boiling at 65°C to 70°C for 30 min.

10 Thereafter, the discolored green pepper was transferred to an immersion liquid prepared so that the concentration of the test substance became 2% by mass, and immersed therein overnight (16 hours). The immersed green pepper was taken out, then the immersion liquid was boiled, and the green pepper was immersed again in the immersion liquid. The green pepper was boiled for 15 min after transferred,
15 followed by removal of the boiled water with a colander and cooling with running water.

The resultant cool green pepper was photoirradiated at a light intensity of 800 luxes at 10°C, and observed for the green color of the green pepper at the start of photoirradiation, 5 days after the start of photoirradiation, and 10 days after the
20 start of photoirradiation. FIG. 7A shows a state of the green pepper at each of the points of time. Table 8 shows measurement results obtained by means of the color difference meter (CR-400, manufactured by Konica Minolta, Inc.).

Note that, as controls, a green pepper which had not been subjected to the discoloration treatment and had been immersed into an immersion liquid free of the
25 test substance (hereinafter may be referred to as "without treatment") and a green pepper which had been treated only with the brewed vinegar (only discoloration treatment) were subjected to the test in the same manner (hereinafter may be

referred to as "vinegar only"). Note that, the observation in the case of "vinegar only" was performed at the start of photoirradiation and 5 days after the start of photoirradiation.

Table 8

	At the start of photoirradiation			5 days after the start of photoirradiation			10 days after the start of photoirradiation		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Without treatment	44.9	-7.1	10.9	45.2	-6.5	11.1	45.3	-5.5	8.7
Ex. 8-1	45.1	-7.1	9.6	42.9	-7.4	9.8	42.9	-7.1	10.0
Vinegar only	43.4	-3.2	15.6	44.4	-1.4	15.0	*	*	*

5

Note that, in the measurement by the color difference meter, the larger a value of "L*" is, the brighter the color is, and the smaller the value of "L*" is, the darker the color is. The larger a value of "a*" is, the more red the color is, and the smaller the value of "a*" is, the more green the color is. The larger a value of "b*" is, the more yellow the color is, and the smaller the value of "b*" is, the more blue the color is.

As can be seen from FIG. 7A and Table 8, in Example 8-1 using the high-copper-content yeast extract of the invention, the a* value was hardly changed even 10 days after the start of photoirradiation, that is, the green color was not discolored, confirming its excellent green color-maintaining and restoring effect. In contrast, in the case of without treatment, the a* value was increased at 5 days after the start of photoirradiation.

(Example 8-2: evaluation of effect of maintaining and restoring green color of green tea paste)

The photoirradiation test was performed in the same manner as in Example 8-1, except that the test object to be subjected to the photoirradiation test was changed to a Matcha paste prepared in the following manner. Results are shown in FIG. 7B and Table 9.

<Preparation of Matcha paste>

Commercially available Matcha powder (S-ASAHINA No. 4, Meiyo Co., Ltd.) (10.4 g), the test substance (0.8 g), citric acid (0.5 g), and water (88.3 mL) were mixed together, and the resultant mixture was sterilized with heating at 121°C for 60 min, to thereby prepare a Matcha paste.

Note that, as controls, a Matcha paste which had not been subjected to the sterilization treatment with heating and had been immersed into an immersion liquid free of the test substance (hereinafter may be referred to as “without heating”) a Matcha paste which had been subjected to the sterilization treatment with heating and had been immersed into an immersion liquid not containing the test substance (hereinafter may be referred to as “with heating”).

Table 9

	At the start of photoirradiation			5 days after the start of photoirradiation			10 days after the start of photoirradiation		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Without heating	38.8	-5.4	15.2	37.3	-3.1	17.5	37.6	-2.0	16.9
With heating	39.3	-0.2	13.9	37.3	-0.3	15.9	37.7	-0.3	15.5
Ex. 8-2	36.6	-5.7	11.3	34.9	-5.9	12.6	35.4	-5.8	12.4

As can be seen from FIG. 7B and Table 9, in Example 8-2 using the high-copper-content yeast extract of the invention, the a* value was hardly changed even 10 days after the start of photoirradiation, that is, the green color was not discolored, confirming its excellent green color-maintaining and restoring effect. In contrast, in the case of without heating, the a* value was increased at 5 days after the start of photoirradiation.

(Example 8-3: evaluation of effect of maintaining and restoring green color of broccoli)

A test substance used was a mixture prepared by mixing with dextrin the high-copper-content yeast extract obtained in Example 4-6 so that the amount of the

high-copper-content yeast extract became 0.5% by mass.

A commercially available frozen broccoli (BROCCOLI, Life Foods Co., Ltd.) was charged into an immersion liquid prepared so that the concentration of the test substance became 4.0% by mass, and immersed therein at 4°C overnight (16 hours). The immersed broccoli was taken out, then the immersion liquid was boiled, and the broccoli was immersed again in the immersion liquid. The broccoli was boiled for 15 min after transferred, followed by removal of the boiled water with a colander and cooling with running water.

The resultant cool broccoli was photoirradiated at a light intensity of 800 luxes at 10°C, and observed for the green color of the broccoli at the start of photoirradiation, 5 days after the start of photoirradiation, and 10 days after the start of photoirradiation. FIG. 7C shows a state of the green pepper at each of the points of time. Table 10 shows measurement results by using the color difference meter (CR-400, Konica Minolta, Inc.).

Note that, as a control, broccoli had been immersed into an immersion liquid free of the test substance (hereinafter may be referred to as “without addition”) was subjected to the test in the same manner (hereinafter may be referred to as “without addition”).

Table 10

	At the start of photoirradiation			5 days after the start of photoirradiation			10 days after the start of photoirradiation		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Without addition	51.3	-13.2	21.0	57.1	-4.0	15.2	56.5	-3.4	15.7
Ex. 8-3	53.1	-12.3	19.3	54.7	-11.5	12.1	55.1	-10.8	11.0

As can be seen from FIG. 7C and Table 10, in Example 8-3 using the high-copper-content yeast extract of the present invention, the a* value was hardly changed even 10 days after the start of photoirradiation, that is, the green color was not discolored, confirming its excellent green color-maintaining and restoring effect.

In contrast, in the case of without addition, the a^* value was increased at 5 days after the start of photoirradiation.

(Example 8-4: evaluation of effect of maintaining and restoring green color of broccoli)

5 The test was performed in the same manner as in Example 8-3 to evaluate an effect of maintaining and restoring a green color, except that the frozen broccoli was changed to frozen snow peas (KINUSAYA, KOHYO Co., Ltd.) and the concentration of the test substance in the immersion liquid was changed to 5.0% by mass. Results are shown in FIG. 7D and Table 11.

10 Table 11

	At the start of photoirradiation			5 days after the start of photoirradiation			10 days after the start of photoirradiation		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Without addition	48.5	-13.9	23.3	53.2	-3.8	19.7	52.6	-3.2	18.7
Ex. 8-4	50.9	-13.0	22.4	52.3	-12.8	15.8	52.1	-13.2	14.7

As can be seen from FIG. 7D and Table 11, in Example 8-4 using the high-copper-content yeast extract of the present invention, the a^* value was hardly changed even 10 days after the start of photoirradiation, that is, the green color was not discolored, confirming its excellent green color-maintaining and restoring effect. In contrast, in the case of without addition, the a^* value was increased at 5 days after the start of photoirradiation.

(Example 8-5: evaluation of effect of maintaining and restoring green color of broccoli)

20 The test was performed in the same manner as in Example 8-3 to evaluate an effect of maintaining and restoring a green color, except that the frozen broccoli was changed to frozen kidney beans (INGEN, Nichirei Foods Co., Ltd.) and the concentration of the test substance in the immersion liquid was changed to 5.0% by mass. Results are shown in FIG. 7E and Table 12.

Table 12

	At the start of photoirradiation			5 days after the start of photoirradiation			10 days after the start of photoirradiation		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Without addition	44.8	-12.7	17.1	45.3	-4.5	14.1	46.6	-2.4	13.6
Ex. 8-5	43.0	-10.6	15.4	44.3	-10.4	9.4	43.8	-10.8	10.5

As can be seen from FIG. 7E and Table 12, in Example 8-5 using the high-copper-content yeast extract of the invention, the a* value was hardly changed even 10 days after the start of photoirradiation, that is, the green color was not discolored, confirming its excellent green color-maintaining and restoring effect. In contrast, in the case of without addition, the a* value was increased at 5 days after the start of photoirradiation.

From the results of Examples 8-1 to 8-5, the extract of the present invention was found to have a green color-maintaining and restoring effect on various green plants.

Industrial Applicability

The high-copper-content yeast extract of the present invention contains copper derived from a natural product in a high concentration, is excellent in water-solubility, and does not impair a flavor of food containing it, and hence can be suitably used as, for example, an oral or tube feeding composition (e.g., a fluid diet or a beverage), a food material, various food additives such as an agent for maintaining and restoring a green color of vegetables, and a food fermentation medium.

In the method of the invention for producing a high-copper-content yeast extract, the copper-elution rate of copper eluted from a copper-containing yeast is high, and it is possible to efficiently produce a high-copper-content yeast extract.

CLAIMS

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

extracting the high-copper-content yeast extract by suspending copper-containing yeast into a solution containing a carboxylic acid, a carboxylic acid salt, or both thereof to
5 thereby obtain a suspension, and separating a solid component and a liquid component of the suspension to thereby obtain the liquid component.

2. The method for producing the high-copper-content yeast extract according to claim 1, wherein the carboxylic acid is a bivalent or higher carboxylic acid, and wherein the carboxylic acid salt is a bivalent or higher carboxylic acid salt.

10 3. The method for producing the high-copper-content yeast extract according to claim 1 or 2, wherein the carboxylic acid is a trivalent carboxylic acid, and wherein the carboxylic acid salt is a trivalent carboxylic acid salt.

4. The method for producing the high-copper-content yeast extract according to any one of claims 1 to 3, wherein a pH value of the suspension is 2.0 to 10.0.

15 5. The method for producing the high-copper-content yeast extract according to any one of claims 1 to 4, wherein a total amount of the carboxylic acid and the carboxylic acid salt is 2 mol or more relative to 1 mol of copper contained in the yeast.

6. The method for producing the high-copper-content yeast extract according to any one of claims 1 to 5, further comprising, prior to the extracting, hot-water treating the yeast
20 by suspending the yeast into hot-water of 60°C to 120°C to thereby obtain a suspension, and separating a solid component and a liquid component of the suspension to thereby obtain the solid component,

wherein the solid component is the copper-containing yeast used in the extracting.

7. The method for producing the high-copper-content yeast extract according to any
25 one of claims 1 to 6,

wherein the high-copper-content yeast extract comprises

0.2% by mass or more of copper derived from a yeast fungus body,

wherein a turbidity of the high-copper-content yeast extract is 0.1 or less in terms of absorbance at a wavelength of 660 nm (O. D. 660) when 1 g of the high-copper-content yeast extract is dissolved or dispersed into 100 mL of water.

8. A method for producing food,

5 wherein the high-copper-content yeast extract obtained by the method according to any one of claims 1 to 7 is used.

9. A method for producing an agent for maintaining and restoring a green color of vegetables,

10 wherein the high-copper-content yeast extract obtained by the method according to any one of claims 1 to 7 is used.