

DESCRIPTION

Title of Invention

MANGANESE-RICH YEAST EXTRACT, METHOD FOR PRODUCING SAME,
AND USE OF MANGANESE-RICH YEAST EXTRACT

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Technical Field

The present invention relates to a manganese-rich yeast extract and a method for producing the extract; food and a composition for a culture medium for an edible lactobacillus containing the extract; a culture medium for an edible
10 lactobacillus containing the composition; and a method for culturing an edible lactobacillus using the culture medium.

Background Art

According to the data recently published by Ministry of Health, Labour and
15 Welfare, the adequate intake of manganese per day is 4.0 mg in adult males (3.5 mg in adult females). Manganese deficiency is presumed to cause miliaria crystalline which is a kind of dermatitis. Additionally, it is generally believed that bone metabolism, glycolipid metabolism (diabetes or adipose obesity), motor function, and skin metabolism are also affected by manganese deficiency, but not specific for
20 manganese deficiency (see NPL 1). Therefore, there is a need to develop a safe food material which allows for efficient ingestion and absorption of manganese into a body and which can improve, for example, dermatitis and a bone metabolic disorder associated with manganese deficiency.

Yeast has been utilized as a food material by human since a long time ago.
25 For example, there has been proposed a technology for allowing yeast to contain manganese in a high concentration (e.g., see PTL 1). Therefore, yeast into which manganese has been uptaken or extract thereof is thought to be available as a safe

manganese-enriched food material.

However, in conventional manganese-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 a food material. The conventional manganese-containing yeast is water-insoluble, so that turbidity and precipitation are caused when it is added to food. As a result, the conventional manganese-containing yeast cannot be used in, especially, a soft drink, which is problematic.

Extraction of a yeast extract from mineral-containing yeast (e.g., manganese-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 (e.g., see NPL 2).

However, the methods of (1) to (3) have a problem of a low mineral recovery rate (extraction rate). The methods of (2) and (3) have a problem that a yeast odor and an extract taste which are distinctive of a yeast extract 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.

In addition, there has been proposed to use the manganese-containing yeast as one component of a culture medium for an edible lactobacillus (e.g., see PTL 2).

However, the manganese-containing yeast is water-insoluble, and thus a lactobacillus may be contaminated with the manganese-containing yeast when the lactobacillus is collected from the culture medium after culturing, which is problematic. Therefore, there is needed a manganese-containing yeast extract which is water-soluble and which can be used as one component of a culture medium for an edible lactobacillus.

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. 234 – p. 236,
5 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

Patent Literature

10 PTL 1: Japanese Patent Application Laid-Open (JP-A) No. 2003-153685

PTL 2: JP-A No. 2009-225722

Summary of Invention

Technical Problem

15 The present invention aims to meet the demand, break the status quo, solve the existing problems, and achieve the following object. That is, an object of the invention is to provide a manganese-rich yeast extract which contains manganese derived from a natural product in a high concentration, which is excellent in water-solubility, which does not impair appearance of food containing it, which does
20 not impair a flavor of food containing it, and which can be used as a component of a culture medium for an edible lactobacillus; a method for producing the extract; food and a composition for a culture medium for an edible lactobacillus containing the extract; a culture medium for an edible lactobacillus containing the composition; and a method for culturing an edible lactobacillus using the medium.

Solution to Problem

Means for solving the problems are as follows.

- <1> A method for producing a manganese-rich yeast extract, comprising:
extracting the manganese-rich yeast extract by suspending
manganese-containing yeast in a solution containing a carboxylic acid, a carboxylate,
or both thereof to thereby obtain a suspension, and separating the suspension into a
5 solid component and a liquid component.
- <2> The method according to <1>, wherein the carboxylic acid is a monovalent to
trivalent carboxylic acid, and wherein the carboxylate is a monovalent to trivalent
carboxylate.
- <3> The method according to <1> or <2>, wherein a pH value of the suspension
10 during the extracting is in the range of 8.0 or less.
- <4> The method according to any one of <1> to <3>, wherein a total amount of
the carboxylic acid and the carboxylate is 0.5 mol or more relative to 1 mol of
manganese contained in the yeast.
- <5> The method according to any one of <1> to <4>, further comprising, prior to
15 the extracting, hot-water treating the yeast by suspending the yeast in hot-water of
60°C to 120°C to thereby obtain a suspension, and separating the suspension into a
solid component and a liquid component.
- <6> The method according to <5>, wherein, in the hot-water treating, a
phosphoric acid salt is added to the hot-water.
- 20 <7> A manganese-rich yeast extract, comprising:
0.2% by mass or more of manganese derived from yeast,
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 manganese-rich yeast extract is dissolved or
dispersed into 100 mL of water.
- 25 <8> The manganese-rich yeast extract according to <7> produced by the method
for producing a manganese-rich yeast extract according to any one of <1> to <6>.
- <9> The manganese-rich yeast extract according to <7> or <8>, wherein the yeast

is edible yeast.

<10> The manganese-rich yeast extract according to <9>, 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.

5 <11> The manganese-rich yeast extract according to <9>, wherein the edible yeast is Saccharomyces cerevisiae.

<12> The manganese-rich yeast extract according to any one of <7> to <11>, wherein the manganese-rich yeast extract is added to food.

10 <13> The manganese-rich yeast extract according to <12>, wherein the food is a fluid diet or a soft drink.

<14> Food, comprising:

the manganese-rich yeast extract according to any one of <7> to <13>.

<15> A composition for a culture medium for an edible lactobacillus, comprising: the manganese-rich yeast extract according to any one of <7> to <11>.

15 <16> A culture medium for an edible lactobacillus, comprising: the composition for a culture medium for an edible lactobacillus according to <15>.

<17> A method for culturing an edible lactobacillus, comprising:

20 culturing an edible lactobacillus using the culture medium for an edible lactobacillus according to <16>.

Advantageous Effects of Invention

25 The present invention can solve the existing problems, and can provide a manganese-rich yeast extract which contains manganese derived from a natural product in a high concentration, which is excellent in water-solubility, which does not impair appearance of food containing it, which does not impair a flavor of food containing it, and which can be used as a component of a culture medium for an

edible lactobacillus; a method for producing the extract; food and a composition for a culture medium for an edible lactobacillus containing the extract; a culture medium for an edible lactobacillus containing the composition; and a method for culturing an edible lactobacillus using the medium.

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Brief Description of Drawings

FIG. 1 is a graph illustrating manganese-extraction rates with pure water, sodium chloride, and each carboxylate in Test Example 1.

FIG. 2 is a graph illustrating manganese-extraction rates with each
10 carboxylate in Test Example 2.

FIG. 3 is a graph illustrating an effect of pH of a suspension on a manganese-extraction rate in Test Example 3.

FIG. 4 is a graph illustrating a relationship between a concentration of a carboxylic acid buffer solution and a manganese-extraction rate in Test Example 4.

FIG. 5A is one exemplary photograph illustrating water-solubilities of a
15 manganese-rich yeast extract powder, which is one aspect of the present invention, and MINERAL YEAST Mn₂ in Test Example 5.

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

FIG. 5C is one exemplary photograph illustrating solubilities into an apple
20 juice solution of a manganese-rich yeast extract powder, which is one aspect of the present invention, and MINERAL YEAST Mn₂ in Test Example 5.

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

FIG. 6A shows growth curves of A) Lactobacillus casei subsp. casei (NRIC
25 1042) in each medium in Test Example 7.

FIG. 6B shows pH transition curves corresponding to the growth curves in

FIG. 6A.

FIG. 6C shows growth curves of B) Lactobacillus brevis (NRIC 1684) in each medium in Test Example 7.

FIG. 6D shows pH transition curves corresponding to the growth curves in FIG. 6C.

FIG. 6E shows growth curves of C) Lactobacillus plantarum (NRIC 1067) in each medium in Test Example 7.

FIG. 6F shows pH transition curves corresponding to the growth curves in FIG. 6E.

FIG. 6G shows growth curves of D) Leuconostoc mesenteroides subsp. mesenteroides (NRIC 1541) in each medium in Test Example 7.

FIG. 6H shows pH transition curves corresponding to the growth curves in FIG. 6G.

FIG. 6I shows growth curves of E) Pediococcus pentosaceus (NRIC 0099) in each medium in Test Example 7.

FIG. 6J shows pH transition curves corresponding to the growth curves in FIG. 6I.

Description of Embodiments

(Production method of manganese-rich yeast extract)

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

<Extraction step>

The extraction step comprises suspending manganese-containing yeast in a solution containing a carboxylic acid, a carboxylate, or both thereof to thereby obtain a suspension, and separating the suspension into a solid component and a liquid

component. The extraction step results in a manganese-rich liquid component (extract).

<<Yeast>>

The yeast is not particularly limited and may be appropriately selected depending on the purpose, as long as it contains manganese in its body. A manganese-content in the yeast is preferably high as possible in the case where the manganese-rich yeast extract is used as a manganese-enriched food material. However, from the viewpoints of limitation of an amount of manganese uptaken by the yeast and of an efficient production of the manganese-rich yeast extract, the manganese content is preferably 0.01% by mass to 5% by mass, more preferably 1% by mass to 5% by mass, relative to dry mass of the yeast.

The manganese content in the yeast, as used herein, refers to an amount of manganese contained in a yeast body. For example, the manganese content is preferably kept at a high level even after washing, for example, in the case where the yeast is washed with water. In such yeast, manganese is not removed and remains within the body even after washing, so that the manganese 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 manganese in a high concentration. As a result, the yeast extract is suitable as a food material.

The manganese content in the yeast can be measured by known methods such as an atomic absorption spectrometry or an ICP emission spectrometric analysis.

The manganese-containing yeast may be produced by culturing yeast in a manganese-containing culture solution to thereby allow manganese to be uptaken into the yeast (e.g., see JP-A No. 2003-153685); or may be a commercially available

product. Examples of the commercially available product include MINERAL YEAST Mn, MINERAL YEAST Mn₂ and MINERAL YEAST Mn-F (Oriental Yeast Co., Ltd.). An amount of manganese to be added to the culture medium is not particularly limited and may be appropriately selected depending on the purpose, but it preferably achieves both of a high manganese utilization rate (uptake rate of manganese into yeast) and a high yield based on sugar (growth rate).

A type of manganese 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 purpose.

The manganese-containing yeast may be further pulverized. A degree to be pulverized is not particularly limited and may be appropriately selected depending on the purpose. For example, the manganese-containing yeast may be pulverized until unpulverized yeast body cannot be observed under a microscope. Even when the yeast is pulverized yeast, the manganese content in the yeast is preferably high as possible. The manganese content relative to dry mass of the 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 of the manganese content relative to dry mass of the yeast in an unpulverized yeast.

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 in a cylinder to 50% by volume.

A form of the manganese-containing yeast is not particularly limited and may be appropriately selected depending on the purpose. For example, the manganese-containing yeast may be in the form of a wet mass or powder.

The yeast may further contain minerals other than manganese. The minerals are not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include iron, copper, magnesium 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 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 or a composition for a culture medium for an edible lactobacillus.

The edible yeast is not particularly limited and may be selected from those known in the art. 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.

Examples of the edible yeast include Saccharomyces spp., Torulopsis spp., Mycotorula spp., Torulaspora spp., Candida spp., Rhodotorula spp., and Pichia spp.

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.

<<Carboxylic acid and carboxylate>>

The carboxylic acid is not particularly limited and may be appropriately selected depending on the purpose. However, preferable is a monovalent to

trivalent carboxylic acid from the viewpoint of a manganese-extraction rate (manganese-elution rate).

5 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, oxaloacetic acid, and α -ketoglutaric acid.

10 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 a manganese-extraction rate, preferable are succinic acid, citric acid, acetic acid, gluconic acid and tartaric acid.

The carboxylic acid may be used alone or in combination.

15 The carboxylate is not particularly limited and may be appropriately selected depending on the purpose. However, preferable is a monovalent to trivalent carboxylate from the viewpoints of a manganese-extraction rate (manganese-elution rate).

20 Examples of the carboxylate include salts of the above-described specific carboxylic acids.

Among them, preferable are a succinic acid salt, a citric acid salt, an acetic acid salt, a gluconic acid salt, and a tartaric acid salt from the viewpoints of addition to food and a manganese-extraction rate.

The carboxylate may be used alone or in combination.

25 A type of the salt is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include alkali metal salts such as a sodium salt and a potassium salt; and alkaline earth metal salts such as a

magnesium salt and a calcium salt. These may be used alone or in combination.

Either of the carboxylic acid or the carboxylate may be used. Alternatively, both of the carboxylic acid and the carboxylate may be used.

An amount of the carboxylic acid or the carboxylate is not particularly limited and may be appropriately selected depending on the purpose. However, relative to 1 mol of manganese contained in the yeast, the total amount of the carboxylic acid and the carboxylate is preferably 0.5 mol or more, more preferably 1.0 mol or more, and particularly preferably 2.0 mol or more from the viewpoint of a manganese-extraction rate.

A solvent used for a solution containing the carboxylic acid, the carboxylate, or both thereof (hereinafter may be referred to as "carboxylic buffer") 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 buffer is not particularly limited and may be appropriately selected depending on the purpose, but is preferably 25 mmol/L or more, more preferably 50 mmol/L or more, particularly preferably 100 mmol/L or more from the viewpoint of a manganese-extraction rate.

The pH value of the carboxylic buffer is not particularly limited and may be appropriately selected depending on the purpose. Note that, the pH can be adjusted by varying a quantity ratio of the carboxylic acid and the carboxylate.

The pH of the suspension in the extraction step is not particularly limited and may be appropriately selected depending on the purpose, but is preferably in the range of pH 8.0 or less, more preferably in the range of pH 7.0 or less, particularly preferably in the range of pH 6.5 or less from the viewpoint of extraction efficiency of manganese.

An extraction time in the extraction step is not particularly limited and may

be appropriately selected depending on the purpose.

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

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

The manganese-extraction rate can be calculated according to the following equation.

$$\text{Manganese-extraction rate (\%)} = \left\{ \frac{\text{Total amount (mass) of manganese in extract}}{\text{Total amount (mass) of manganese contained in yeast used for extraction}} \right\} \times 100$$

<<Suspension>>

A means of suspending the yeast in the solution containing the carboxylic acid, the carboxylate, or both thereof is not particularly limited. For example, 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 into solid component and liquid component>>

A means of separating the suspension into a solid component and a liquid component 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 of the devices include a filter press and a line filter. 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.

After extraction with the carboxylic buffer, the carboxylic acid remains in the manganese-rich yeast extract as extraction traces. Therefore, the carboxylic acid contained in the manganese-rich yeast extract can be measured to determine whether the extraction with the buffer has been achieved. A method for measuring is not particularly limited. For example, an amount of the carboxylic acid can be measured using HPLC (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 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 manganese in hot-water of 60°C to 120°C to thereby obtain a suspension, and separating the suspension into a solid component and a liquid component, 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 manganese-rich 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, but is preferably 80°C to 120°C,

more preferably 95°C to 120°C from the viewpoint of an excellent effect of reducing the yeast odor and the yeast taste.

A method for suspending the yeast and a method for separating the resultant suspension into the solid component and the liquid component 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 the hot water in order to reduce the odor and taste which are distinctive of yeast. The accelerator is not particularly limited and may be appropriately selected depending on the purpose, as long as it does not adversely affect the manganese-elution rate in the subsequent extraction step. Examples thereof include salts other than carboxylates. Among them, phosphates are preferable because they are superior in extraction of the yeast odor, but are less likely to accelerate extraction of manganese in the hot water step.

An amount of the 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 manganese-rich yeast extract.

A means of 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 manganese-rich yeast extract (powdered manganese-rich yeast extract), which can be used in various applications described below. During the drying step, an excipient (e.g., dextrin) may be appropriately added.

The concentration step comprises concentrating the manganese-rich yeast

extract. The dilution step comprises diluting the manganese-rich yeast extract.

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

(Manganese-rich yeast extract)

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

A method for producing the manganese-rich yeast extract is not particularly
10 limited and may be appropriately selected depending on the purpose. However, the extract can be suitably produced by the method of the invention. That is, the manganese-rich yeast extract is preferably produced by subjecting manganese-containing yeast to extraction with a solution containing a carboxylic acid, a carboxylate, or both thereof.

15 <Manganese-content>

The manganese-content in the extract is preferably high as possible from the viewpoint of use of the extract as a manganese-enriched food material.

“Manganese-rich,” as used herein, means that manganese derived from yeast is contained in a concentration of 0.2% by mass or more, preferably 0.5% by mass or
20 more, more preferably 1.0% by mass or more.

Containing manganese in such a high concentration allows the extract to be suitably used as an oral or tube feeding composition (e.g., a fluid diet or a beverage), a food material, or a composition for a culture medium for a lactobacillus.

The manganese-content can be measured by known methods such as an
25 atomic absorption spectrometry or an ICP emission spectrometric analysis.

<Turbidity>

The manganese-rich yeast extract of the present invention has a high

water-solubility, so that an aqueous solution of the extract has a high transparency. The 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 extract 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 manganese-rich 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 manganese-rich 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 manganese-rich 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 any of the above-described methods. The condition is not particularly limited.

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

<Application>

Application of a manganese-rich 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, feed or a composition for a culture medium for an edible lactobacillus described below, particularly preferably used as the food material or the composition for a

culture medium for an edible lactobacillus. 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. The manganese-rich yeast extract of the invention can result in manganese-rich food, manganese-rich animal food, and
5 manganese-rich feed, and a manganese-rich composition for a culture medium for an edible lactobacillus.

A form of the manganese-rich 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 using, e.g., a spray drier), a solution
10 form in which the extract is dissolved in a solvent, and a semi-solid form (e.g., a gel form or cream form). A method for preparing the manganese-rich yeast extract into the above-described forms is not particularly limited and can be performed using known devices according to known methods.

(Food)

15 Food of the present invention contains the manganese-rich 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
20 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
25 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 manganese-rich yeast extract contained in the food is not particularly limited and may be appropriately selected depending on the application and purpose.

5 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,
10 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
15 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.

(Composition for a culture medium for an edible lactobacillus)

20 A composition for a culture medium for an edible lactobacillus of the present invention contains the manganese-rich yeast extract of the invention; and, if necessary, further contains other ingredients.

The composition for a culture medium for an edible lactobacillus may consist of the extract or may comprise the extract and other ingredients.

25 The other ingredients are not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include ingredients contained in a culture medium for an edible lactobacillus described below.

(Culture medium for an edible lactobacillus)

A culture medium for an edible lactobacillus of the present invention contains the composition for a culture medium for an edible lactobacillus of the invention; and, if necessary, further contains other ingredients.

5 The culture medium is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include a GYP medium and a GYP medium without $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$.

The GYP medium has been commonly used as a basal medium for culturing a lactobacillus, and a composition thereof is as follows:

10 -Composition of GYP medium (pH 6.8) (per 100 mL)-

- Glucose . . . 1.0 g
- Yeast extract . . . 1.0 g
- Peptone . . . 0.5 g
- Na acetate $\cdot 3\text{H}_2\text{O}$. . . 0.2 g
- 15 · Salts solution (*1) . . . 0.5 mL
- Tween 80 solution (2.5 mg/mL aqueous solution) . . . 1.0 mL

*1: Composition of Salts solution (per 1 mL)

- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. . . 40 mg
- $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$. . . 2 mg
- 20 · $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. . . 2 mg
- NaCl . . . 2 mg

In the culture medium, the manganese-rich yeast extract can be used instead of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ contained in the GYP medium. The culture medium contains the manganese-rich yeast extract, so that it can be suitably used from the viewpoints
25 that the yeast body is not precipitated in the medium and that a lactobacillus is not contaminated with the yeast when the bacterium is collected from the culture medium. Such culture medium contains manganese derived from a natural

product, so that it can be safely used for culturing the edible lactobacillus.

(Method for culturing edible lactobacillus)

A method for culturing an edible lactobacillus of the present invention comprises culturing an edible lactobacillus in the culture medium of the invention.

5 <Edible lactobacillus>

The edible lactobacillus is not particularly limited and may be appropriately selected depending on the purpose, as long as it has been used for food. Various lactic acid bacteria can be used, and specific examples thereof include Lactobacillus, Leuconostoc, Pediococcus, and any species and strain belonging thereto. Among
10 them, preferable are Lactobacillus casei subsp. casei, Lactobacillus brevis, Lactobacillus plantarum, Leuconostoc mesenteroides subsp. mesenteroides, and Pediococcus pentosaceus.

<Culturing of edible lactobacillus>

In the case where the edible lactobacillus is cultured in the culture medium
15 for an edible lactobacillus of the present invention, the culture medium is preferably prepared and subjected to an autoclave sterilization treatment prior to culturing.

The culture medium may be prepared according to conventional methods. For example, in the case where the GYP medium without $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ is used, a solution having the same composition as the GYP medium except without
20 $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ may be prepared, to which the composition for a culture medium for an edible lactobacillus may be added.

In this case, an amount of the composition for a culture medium for an edible lactobacillus contained in the culture medium is not particularly limited and may be appropriately selected depending on the purpose, but is preferably an amount such
25 that a manganese (Mn) content (content in terms of Mn) is almost the same as that of in the common GYP medium. Alternatively, the manganese (Mn) content may be higher than that of in the GYP medium.

The autoclave sterilization treatment may be achieved according to conventional methods. The condition is not particularly limited and may be appropriately selected depending on the purpose.

5 An amount of the edible lactobacillus inoculated into the culture medium is not particularly limited and may be appropriately selected depending on the purpose, but is preferably about 0.01% (mass/volume) to about 30% (mass/volume) in the form of a wet mass from the viewpoints of properties, culture operability, and production efficiency of yeast.

10 In the case of a small-scale culture, the edible lactobacillus may be cultured by a shaking culture using, e.g., a Sakaguchi flask. The condition is not particularly limited, but is preferably about 100 rpm to about 200 rpm in amplitude of about 12 cm. In the case of a large-scale culture, a jar fermenter may be suitably used. The condition is not particularly limited and may be appropriately determined.

15 The culture condition is not particularly limited and may be appropriately selected depending on the purpose, but is preferably a temperature in the range of, e.g., about 28°C to about 33°C, time in the range of, e.g., about 1 hour to about 40 hours, an air flow rate in the range of, e.g., about 0 vvm to about 5 vvm, and an agitation rate in the range of, e.g., about 100 rpm to about 700 rpm. The pH value
20 of a culture solution may not be adjusted or may be adjusted to the range of about 4.0 to about 8.0. The pH value is adjusted to preferably the range of 7.0 or higher, more preferably the range of 7.0 to 8.0 at an initial stage of culture.

The bacterium may be cultured by a standing culture.

The bacterium may be precultured prior to culturing.

25 The edible lactobacillus obtained by culturing in the culture medium of the invention using the method for culturing of the invention may be used as it is, or may be isolated and washed to be suitably used for various foods.

The food is not particularly limited and may be appropriately selected depending on the purpose. Examples thereof include daily products such as yogurt, bread, confectioneries, noodles, daily dishes, and beverages.

5 Examples

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

(Test Example 1: extraction of manganese with carboxylate)

To 5 g (dry mass) of a manganese-rich yeast powder (MINERAL YEAST Mn₂,
10 manganese-content: 18,980 ppm by mass, Oriental Yeast Co., Ltd.), was added 50 mL of each of aqueous solutions of sodium carboxylate described in Table 1 at 200 mmol/L, followed by stirring and diluting to 100 mL with water to thereby obtain a suspension. An amount of each of the sodium carboxylate was 4.1 mol relative to 1 mol of manganese contained in yeast to be subjected to extraction. Water or a 200
15 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 using 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
20 suspension is centrifuged at 3,000 rpm for 5 min. Only supernatant was transferred to a test tube to thereby obtain a yeast extract. The extract was weighed.

The manganese-content was determined by measuring manganese in the extract through an ICP emission spectrometric analysis using an ICP emission
25 spectrophotometer (OPTIMA 2100DV, PerkinElmer Inc.), from which a rate of manganese extracted to the extract was calculated. Results are shown in Table 1 and FIG. 1.

The extract was dried to powder to thereby obtain a dry powder. The manganese-content on the dry powder basis (% by mass) was measured as follows. That is, dextrin was added to the extract which had been subjected to extraction, followed by adjusting a solid content to 10% by mass. Then, the resultant was dried using SPRAY DRIER L-8 (OHKAWARA KAKOHKI CO., LTD.), and quantified for manganese 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	Manganese extraction rate (%)	Manganese-content on dry powder basis (% by mass)
Test Example 1-1	Pure water	6.7	7	0.1
Test Example 1-2	Sodium chloride (table salt) aqueous solution	6.7	9	0.1
Test Example 1-3	Monosodium succinate aqueous solution	5.1	65	0.7
Test Example 1-4	Trisodium citrate aqueous solution	7.9	62	0.7

As shown in Table 1 and FIG. 1, use of carboxylates results in a high manganese-extraction rate and a manganese-rich extract.

(Test Example 2: extraction of manganese with carboxylate)

To 5 g (dry mass) of a manganese-rich yeast powder (MINERAL YEAST Mn₂, manganese-content: 18,980 ppm by mass, Oriental Yeast Co., Ltd.), was added 10 mL of each of aqueous solutions of sodium carboxylates described in Table 2 at 200 mmol/L, followed by stirring, adjusting to the specified pH, and diluting to 20 mL with water to thereby obtain a suspension. An amount of each of the sodium carboxylates relative to 1 mol of manganese contained in the yeast was as shown in Table 2. The pH was adjusted while being measured with the 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 supernatant was transferred to a test tube to thereby obtain yeast extract. The yeast extract was weighed.

The manganese-content was determined by measuring manganese in the extract through the ICP emission spectrometric analysis using the ICP emission spectrophotometer (OPTIMA 2100DV, PerkinElmer Inc.), from which a rate of manganese extracted to the extract was calculated. Results are shown in Table 2 and FIG. 2.

A dry powder of the extract was obtained and measured for a manganese-content in the extract in the same manner as in Test Example 1. Results are shown in Table 2.

Table 2

	Type of extraction solvent	Molar ratio (Carboxylate/Manganese)	pH of suspension	Manganese extraction rate (%)	Manganese content on dry powder basis (% by mass)
Test Example 2-1	Sodium acetate(anhydrous) aqueous solution	4.1	2.0	90	1.0
Test Example 2-2	Sodium gluconate aqueous solution	4.1	2.0	91	1.0
Test Example 2-3	Sodium L-Tartrate aqueous solution	4.1	2.0	90	1.0

As shown in Table 2 and FIG. 2, use of sodium acetate, sodium gluconate, and sodium L-tartrate as the carboxylates also results in a high manganese-extraction rate and a high manganese content in the extract. (Test Example 3: effect of pH on manganese-extraction rate)

MINERAL YEAST Mn₂ powder (Oriental Yeast Co., Ltd.) was suspended in 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 in 10 mL of each of aqueous solutions containing citric acid, trisodium citrate, or both thereof (citric buffers) described in Table 3, to thereby obtain a suspension. Note that, all of the citric buffers had a concentration of 200 mmol/L, and the pH of each of the citric buffers was adjusted by appropriately varying a quantity ratio of citric acid and trisodium citrate.

Two hours after suspending each of the citric buffers, the suspension was subjected to centrifugation. The resultant supernatant (yeast extract) was measured for the manganese extraction rate in the same manner as in Test Example 1. Additionally, a dry powder of the extract was obtained and measured for a manganese content in the extract in the same manner as in Test Example 1.

Results 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	Manganese extraction rate (%)	Manganese content on dry powder basis (% by mass)
Test Example 3-1	1.9 (only citric acid)	2.9	96	1.3
Test Example 3-2	3.0	3.5	91	1.2
Test Example 3-3	3.5	4.0	91	1.2
Test Example 3-4	4.0	4.4	86	1.2
Test Example 3-5	4.5	4.9	88	1.2
Test Example 3-6	5.0	5.5	87	1.2
Test Example 3-7	5.5	6.2	92	1.3
Test Example 3-8	6.0	6.9	74	1.0
Test Example 3-9	6.5	7.5	53	0.7
Test Example 3-10	7.0	7.9	47	0.6
Test Example 3-11	7.5	8.0	43	0.6
Test Example 3-12	8.2 (only trisodium citrate)	8.0	41	0.6

As shown in Table 3 and FIG. 3, there is a trend that the lower the pH of a solvent for extraction (carboxylic buffer) is, the higher the manganese-extraction rate is. However, a high manganese-extraction rate of 40% or higher could be attained in a broad pH range, that is, the manganese-rich yeast extract could be obtained. Manganese could be extracted at a high rate in all cases where the solvent contains only a carboxylic acid, both of a carboxylic acid and a carboxylate, and only a carboxylate, resulting in the manganese-rich yeast extract. (Test Example 4: effect of relationship between manganese content in yeast and total amount of carboxylic acid and carboxylate, on manganese-extraction rate)

MINERAL YEAST Mn₂ (Oriental Yeast Co., Ltd.) was used as a raw material to test a relationship between a total amount of a carboxylic acid and a carboxylate and the extraction efficiency of manganese. Citric acid was used as a carboxylic acid contained in an extraction solvent to prepare citric buffers having concentrations described in Table 4. The pH of each of the citric buffers was adjusted to 5.0 by appropriately varying a quantity ratio of citric acid and trisodium citrate.

Yeast extracts were obtained in the same manner as in Test Example 3, except that the citric buffers having the concentrations described in Table 4 were used. The yeast extracts were measured for the manganese-extraction rate. Additionally, a dry powder of each of the extracts was obtained and measured for a manganese-content on the dry powder basis in the same manner as in Test Example 1. Results are shown in Table 4 and FIG. 4. Note that, in FIG. 4, a "black diamond" denotes a manganese-extraction rate, and a "black square" denotes a pH of suspension.

Table 4

	Concentration of citric buffer (pH 5.0) (mmol/L)	Molar ratio (Total of citric acid and citrate /Manganese)	pH of suspension	Manganese extraction rate (%)	Manganese content on dry powder basis (% by mass)
Test Example 4-1	0	0	7.2	1	0.0
Test Example 4-2	25	0.5	6.5	33	0.5
Test Example 4-3	50	1.0	6.1	65	1.1
Test Example 4-4	75	1.5	5.9	77	1.2
Test Example 4-5	100	2.0	5.7	81	1.3
Test Example 4-6	125	2.6	5.5	79	1.3
Test Example 4-7	150	3.1	5.5	81	1.3
Test Example 4-8	175	3.6	5.4	84	1.4
Test Example 4-9	200	4.1	5.3	90	1.5

As shown in Table 4 and FIG. 4, the manganese-extraction rate varies depending on a molar ratio of a total of a carboxylic acid and a carboxylate to manganese. A very high manganese-extraction rate of 65% or higher could be attained in the case where the total of the carboxylic acid and the carboxylate was 1.0 mol or more relative to 1 mol of manganese contained in the yeast.

(Test Example 5: evaluation of solubility)

One gram of the manganese-rich yeast extract powder obtained in Test Example 4-9 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 a 1% (mass/volume) solution of the manganese-rich yeast extract (test solution).

The prepared solutions were each measured for turbidity by measuring absorbance at 660 nm (O. D. 660) using the spectrophotometer (U-2000 model, Hitachi, Ltd.).

The solutions were each observed for the presence or absence of a precipitate

after centrifugation at 3,000 rpm for 5 min.

MINERAL YEAST Mn₂ which had not been subjected to extraction (Oriental Yeast Co., Ltd.) was used to prepare control solutions having the same manganese content as the test solutions, and the control solutions each were measured for turbidity and evaluated for the presence or absence of a precipitate in the same manner as in the test solution. Results are shown in Table 5, FIG. 5A (aqueous solution prior to centrifugation), FIG. 5B (aqueous solution after centrifugation), FIG. 5C (apple juice solution prior to centrifugation), and FIG. 5D (apple juice solution after centrifugation).

Table 5

	Sample	Solvent	O. D. 660 (1% solution)	Visual evaluation	Visual evaluation after centrifugation
Test Example 5-1	Manganese-rich yeast extract	Water	0.010	Aqueous solution with high clarity	Precipitate was not confirmed
Test Example 5-2	MINERAL YEAST Mn ₂	Water	11.89	Suspended	Precipitate was confirmed (yeast)
Test Example 5-3	Manganese-rich yeast extract	Apple juice	0.010	Aqueous solution with high clarity	Precipitate was not confirmed
Test Example 5-4	MINERAL YEAST Mn ₂	Apple juice	10.20	Suspended	Precipitate was confirmed (yeast)

The aqueous solution of Test Example 5-1 had significantly lower turbidity than the aqueous solution of Test Example 5-2. As shown in FIG. 5A, it was visually confirmed that the aqueous solution of Test Example 5-1 had significantly higher clarity than the solution of Test Example 5-2. As shown in FIG. 5B, after centrifugation at 3,000 rpm for 5 min, the solution of Test Example 5-1 contained no precipitate and was a solution with high clarity. In contrast, the solution of Test Example 5-2 contained a precipitate.

The same results could be obtained in Test Examples 5-3 and 5-4 each using apple juice instead of water.

Therefore, the manganese-rich yeast extract of the present invention has

high solubility, so that it can be added to, for example, a fluid diet or a beverage. Additionally, the extract of the invention does not cause turbidity and discoloration, so that it does not impair appearance of food containing it and can be added to, in particular, a soft drink having a high transparency.

5 (Test Example 6: evaluation of odor and taste)

The following test samples were prepared: the manganese-rich yeast extract powder obtained in Example 4-9 (hereinafter referred to as "powder"); a 1% (mass/volume) aqueous solution of the powder; a solution prepared by dissolving the powder in apple juice (beverage containing 10% by volume apple juice, ASAHI SOFT
10 DRINKS CO., Ltd.) so as to have a concentration of 1% (mass/volume) (hereinafter referred to as "apple juice"); a solution prepared by dissolving the powder in an aqueous solution of modified milk powder (CHIL MIL, MORINAGA MILK INDUSTRY CO., LTD.) so as to have a concentration of 1% (mass/volume), the aqueous solution of modified milk powder being prepared by dissolving or
15 suspending 14 g of the modified milk powder in 100 mL of water (hereinafter referred to as "milk"); and a mixture prepared by adding the powder to a nutritional adjustment food (MEIBALANCE (yogurt taste), Meiji Co., Ltd.) so as to have a concentration of 1% (mass/volume) (hereinafter referred to as "fluid diet").

<Evaluation method>

20 The powder, the 1% aqueous solution of the powder, the apple juice, the milk, and the fluid diet were evaluated for odors ("extract odor" and "metal odor/unpleasant odor") and tastes ("extract taste" and "metal taste/off-taste/harsh taste"), which were distinctive of yeast or a yeast extract, by 6 evaluators. Note that, the powder of the manganese-rich yeast extract was evaluated only for odors.

25 MINERAL YEAST Mn₂ (Oriental Yeast Co., Ltd.) which had not been subjected to extraction was used to prepare control samples having the same manganese content as the test samples, and the control samples each were

evaluated in the same manner as the test samples.

<<Evaluation-1>>

For the powder, the 1% aqueous solution of the powder, and the apple juice, each sample (MINERAL YEAST Mn₂) was ranked for evaluation items by 6 evaluators according to the following criteria, assuming that the manganese-rich yeast extract was ranked as 3 (standard) for each of the evaluation items, followed by averaging. Results are shown in Tables 6-1 to 6-3.

-Evaluation criteria for odor ("extract odor" and "metal odor/unpleasant odor")-

5: very strong

4: strong

3: comparable

2: weak

1: very weak

-Evaluation criteria for taste ("extract taste" and "metal taste/off-taste/harsh taste")-

5: very strong

4: strong

3: comparable

2: weak

1: very weak

<<Evaluation-2>>

For the milk and the fluid diet, each sample (manganese-rich yeast extract and MINERAL YEAST Mn₂) was ranked for evaluation items by 6 evaluators according to the following criteria, assuming that a no-additive sample was ranked as 3 (standard) for each of the evaluation items, followed by averaging. Results are shown in Tables 6-4 and 6-5.

-Evaluation criteria for odor ("extract odor" and "metal odor/unpleasant odor")-

5: very strong

4: strong

3: comparable

2: weak

1: very weak

5 -Evaluation criteria for taste ("extract taste" and "metal taste/off-taste/harsh taste")-

5: very strong

4: strong

3: comparable

2: weak

10 1: very weak

Table 6-1

	Sample	Food	
		Powder	
		Extract odor	Metal odor/ Unpleasant odor
Test Example 6-1	Manganese-rich yeast extract	3.0	3.0
Test Example 6-2	MINERAL YEAST Mn2	3.5	3.7

Table 6-2

	Sample	Food			
		1% aqueous solution of powder			
		Extract odor	Metal odor/ Unpleasant odor	Extract taste	Metal taste/ Off-taste/ Harsh taste
Test Example 6-1	Manganese-rich yeast extract	3.0	3.0	3.0	3.0
Test Example 6-2	MINERAL YEAST Mn2	3.7	3.3	3.0	4.3

15 Table 6-3

	Sample	Food			
		Apple juice			
		Extract odor	Metal odor/ Unpleasant odor	Extract taste	Metal taste/ Off-taste/ Harsh taste
Test Example 6-1	Manganese-rich yeast extract	3.0	3.0	3.0	3.0
Test Example 6-2	MINERAL YEAST Mn2	3.7	3.3	3.8	4.3

Table 6-4

	Sample	Food			
		Milk			
		Extract odor	Metal odor/ Unpleasant odor	Extract taste	Metal taste/ Off-taste/ Harsh taste
Control	No additive	3.0	3.0	3.0	3.0
Test Example 6-1	Manganese-rich yeast extract	3.7	3.3	4.0	3.3
Test Example 6-2	MINERAL YEAST Mn2	4.2	3.7	4.0	4.5

Table 6-5

	Sample	Food			
		Fluid diet			
		Extract odor	Metal odor/ Unpleasant odor	Extract taste	Metal taste/ Off-taste/ Harsh taste
Control	No additive	3.0	3.0	3.0	3.0
Test Example 6-1	Manganese-rich yeast extract	3.2	3.0	3.5	3.0
Test Example 6-2	MINERAL YEAST Mn2	3.7	3.5	3.8	4.2

5 As shown in Tables 6-1 to 6-5, Test Example 6-1 containing the manganese-rich yeast extract, which is one aspect of the invention, could reduce the yeast extract odor to a greater extent than Test Example 6-2 containing MINERAL YEAST Mn2. Additionally, Test Example 6-1 containing the manganese-rich yeast extract, which is one aspect of the invention, was more excellent than Test Example 6-2 in terms of the metal odor/unpleasant odor which is a non-food odor. Therefore, a manganese-rich yeast extract of the invention was demonstrated not to impair a flavor of food containing it.

(Test Example 7: culture of edible lactobacillus)

15 The below-described 5 edible lactobacillus were cultured in the following 4 media. Specifically, the following 4 media were used: (1) a GYP medium (composition thereof was described below; hereinafter may be referred to as "GYP"), (2) a GYP medium containing no Mn (having the same composition as the GYP medium except without $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; hereinafter may be referred to as "Mn-free GYP"), (3) a GYP medium containing Mn-containing edible yeast (having the same

composition as the GYP medium except containing Mn-containing edible yeast (MINERAL YEAST Mn-F, Oriental Yeast Co., Ltd.) instead of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; hereinafter may be referred to as "manganese-containing yeast"), and (4) a GYP medium containing a Mn-rich yeast extract (having the same composition as the GYP medium except containing the manganese-rich yeast extract powder obtained in Test Example 4-9 instead of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; hereinafter may be referred to as "manganese-rich yeast extract"). The GYP medium had a Mn concentration from MnSO_4 of 2.5 mg/L. Therefore, the Mn-containing edible yeast and the manganese-rich yeast extract powder obtained in Test Example 4-9 were added to the media of (3) and (4), respectively, such that Mn contents thereof were substantially the same as that of in the GYP medium of (1).

The 4 media were adjusted in the pH value to 6.8 and then subjected to an autoclave treatment (at 121°C for 15 min) prior to inoculation of the edible lactobacillus.

<Edible lactobacillus>

The following 5 strains were cultured, all of which was a type strain provided by NODAI Culture Collection Center (NRIC).

A) *Lactobacillus casei* subsp. *casei* (NRIC 1042)

B) *Lactobacillus brevis* (NRIC 1684)

C) *Lactobacillus plantarum* (NRIC 1067)

D) *Leuconostoc mesenteroides* subsp. *mesenteroides* (NRIC 1541)

E) *Pediococcus pentosaceus* (NRIC 0099)

<Composition of GYP medium (pH 6.8) (per 100 mL)>

· Glucose . . . 1.0 g

· Yeast extract . . . 1.0 g

· Peptone . . . 0.5 g

· Na-acetate·3H₂O . . . 0.2 g

- Salts solution (*1) . . . 0.5 mL
- Tween 80 solution (2.5 mg/mL aqueous solution) . . . 1.0 mL

*1: Composition of Salts solution (per 1 mL)

- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. . . 40 mg
- $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$. . . 2 mg
- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. . . 2 mg
- NaCl . . . 2 mg

The 5 edible lactobacillus were inoculated into 5 GYP media (each 5 mL) in one scrape of a platinum loop, followed by a standing culture at 30°C for 48 hours (preculture-1). A total amount of each of the resultant 5 cultures was added to (inoculated into) 200 mL of the GYP medium, followed by a standing culture at 30°C for 48 hours (preculture-2). Thereafter, the media were washed off from the bacteria with saline to thereby obtain 5 suspensions. The suspensions were inoculated into the following 4 media: (1) the GYP medium, (2) the GYP medium containing no Mn, (3) the GYP medium containing Mn-containing edible yeast, and (4) the GYP medium containing a Mn-rich yeast extract, followed by a main culture. Each of the suspensions was inoculated in an amount so as to provide the O. D. 660 nm of 0.05. The main culture was a standing culture at 30°C. Culture solutions were sampled over time during culturing, and measured for the turbidity (O. D. 660 nm) and the pH value. Note that, the turbidity (O. D. 660 nm) was measured using the spectrophotometer (U-1000 model, Hitachi, Ltd.), and the pH value was measured using the pH meter (MP230, METTLER-TOREDO Inc.).

Measurement results for the turbidity (O. D. 660 nm) and the pH value and transitions thereof in a total of 20 types of culture solutions are shown in FIGs. 6A to 6J. In FIGs. 6A to 6J, a "black diamond" denotes the case of using (1) the GYP medium (GYP), a "black square" denotes the case of using (2) the GYP medium containing no Mn (Mn-free GYP), a "white square" denotes the case of using (3) the

GYP medium containing the Mn-containing edible yeast (manganese-containing yeast), and a "black circle" denotes the case of using (4) the GYP medium containing the Mn-rich yeast extract (manganese-rich yeast extract). FIG. 6A shows growth curves of A) Lactobacillus casei subsp. casei (NRIC 1042). FIG. 6B shows pH transition curves corresponding to the growth curves in FIG. 6A. FIG. 6C shows growth curves of B) Lactobacillus brevis (NRIC 1684). FIG. 6D shows pH transition curves corresponding to the growth curves in FIG. 6C. FIG. 6E shows growth curves of C) Lactobacillus plantarum (NRIC 1067). FIG. 6F shows pH transition curves corresponding to the growth curves in FIG. 6E. FIG. 6G shows growth curves of D) Leuconostoc mesenteroides subsp. mesenteroides (NRIC 1541). FIG. 6H shows pH transition curves corresponding to the growth curves in FIG. 6G. FIG. 6I shows growth curves of E) Pediococcus pentosaceus (NRIC 0099). FIG. 6J shows pH transition curves corresponding to the growth curves in FIG. 6I.

As clearly shown in FIGs. 6A to 6J, in the case of using the GYP medium containing the manganese-rich yeast extract powder obtained in Test Example 4-9 which is one aspect of the invention (the medium of (4)), various lactobacillus could be grown to the same extent as the case of using (1) the GYP medium or (3) the GYP medium containing the Mn-containing edible yeast.

In (4) the GYP medium containing the manganese-rich yeast extract powder, the manganese-rich yeast extract was contained, so that the yeast was not precipitated in the medium and the lactobacillus was not contaminated with the yeast when the bacterium was collected from the medium.

In (4) the GYP medium containing the manganese-rich yeast extract powder, manganese, which had not been permitted as a food additive, was not used, so that the resultant edible lactobacillus was highly safe and suitable for various foods.

Industrial Applicability

A manganese-rich yeast extract of the present invention contains manganese derived from a natural product in a high concentration, is excellent in water-solubility, does not impair appearance of food containing it, and does not impair a flavor of food containing it, and hence is suitable for an oral or tube feeding composition (e.g., fluid diet and beverage) or a food material capable of improving, e.g., dermatitis and a bone metabolic disorder associated with manganese deficiency. Also, the extract is suitable as a composition for a culture medium for an edible lactobacillus.

According to a method for producing a manganese-rich yeast extract of the invention, manganese can be extracted at a high rate from manganese-containing yeast, resulting in an efficient production of a manganese-rich yeast extract.

A composition for a culture medium for an edible lactobacillus of the invention is suitable as a component of a culture medium for an edible lactobacillus, and the culture medium is suitable for a method for culturing an edible lactobacillus which is available as food.

CLAIMS

1. A method for producing a manganese-containing yeast extract, comprising:
hot-water treating manganese-containing yeast by suspending the
5 manganese-containing yeast in hot-water of 60°C to 120°C to thereby obtain a first suspension,
and separating the first suspension into a solid component and a liquid component; and
extracting the manganese-containing yeast by suspending the solid component
separated in the hot-water treating in a solution containing a carboxylic acid, a carboxylate, or
both thereof to thereby obtain a second suspension, and separating the second suspension into
10 a solid component and a liquid component to thereby obtain the liquid component containing
manganese,
wherein a total of the carboxylic acid and the carboxylate is 1.0 mole (mol) or more
relative to 1 mole (mol) of manganese contained in the yeast.
- 15 2. The method for producing a manganese-containing yeast extract according to claim 1,
wherein the carboxylic acid is a monovalent to trivalent carboxylic acid, and wherein the
carboxylate is a monovalent to trivalent carboxylate.
3. The method for producing a manganese-containing yeast extract according to claim 1 or
20 2, wherein a pH value of the suspension during the extracting is in the range of 8.0 or less.
4. The method for producing a manganese-containing yeast extract according to claim 1 or
2, wherein the manganese-containing yeast extract is a manganese-containing yeast extract
containing 0.2% by mass or more of manganese derived from the yeast,
25 wherein a turbidity is 0.1 or less in absorbance at a wavelength of 660 nm (O. D. 660)
when 1 g of the manganese-containing yeast extract is dissolved or dispersed into 100 mL of
water.

5. A manganese-containing yeast extract produced by the method according to claim 1 or 2.

5 6. A food, comprising:
the manganese-containing yeast extract according to claim 5.

7. A composition for a culture medium for an edible lactobacillus, comprising:
the manganese-containing yeast extract according to claim 5.

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8. A culture medium for an edible lactobacillus, comprising:
the composition for a culture medium for an edible lactobacillus according to claim 7.

9. A method for culturing an edible lactobacillus, comprising:
15 culturing an edible lactobacillus using the culture medium for an edible lactobacillus
according to claim 8.