

Description

Title of Invention: METHOD FOR PRODUCING COMPOSITION, METHOD
FOR PRODUCING FOOD AND DRINK, AND FERMENTATION
COMPOSITION

5

Technical Field

[0001]

The present disclosure relates to a method for producing a composition,
a method for producing a food and/or beverage, and a fermentation
10 composition.

Background Art

[0002]

Isoflavone glycosides contained in soybean hypocotyls or soybean
15 hypocotyl extracts are converted into aglycone by β -glucosidase, and then
further metabolized into equol, equol derivatives, and the like. Equol has a
strong female hormonal physiological action, and thus its use for prevention
and improvement of menopausal symptoms and osteoporosis (Patent Document
1), prevention and treatment of skin aging and wrinkles (Patent Document 2),
20 alleviation of allergic symptoms (Patent Document 3), and the like has been
proposed.

Equol derivatives, particularly 5-hydroxyequol, are known to have an
antioxidant effect (Non-Patent Literature 1) and a life-extending effect (Non-
Patent Literature 2).

25

Citation List

Patent Document

[0003]

Patent Document 1: JP 2001-523258 T
30 Patent Document 2: JP 2002-511860 T
Patent Document 3: JP 4479505 B

Non-Patent Literature

[0004]

35 Non-Patent Literature 1: Archives Biochem. Biophys. vol. 356, pp133-
141 (1998)

Non-Patent Literature 2: J. Chin. Pharm. Sci. vol. 23, pp378-384 (2014)

Summary of Invention

Technical Problem

[0005]

The present inventor has produced a composition containing equol
5 and/or an equol derivative from an isoflavone glycoside, and has found that the composition browns by heating. In addition, it has been found that, when the composition as a raw material is mixed with a raw material containing protein and the like and heated to produce a food, the food browns by heating. This is considered to be due to a reaction of a reducing sugar and an amino acid, called
10 the Maillard reaction. In addition, there is a possibility that by-products such as acrylamide, which are said to have carcinogenic properties, are generated due to the Maillard reaction.

[0006]

An object of the present disclosure is to provide at least a method for
15 producing a composition containing equol and/or an equol derivative, which does not brown even when heated as it is or in the presence of an additional amino acid or the like.

Solution to Problem

20 [0007]

As a result of intensive studies, the present inventor has found that when an isoflavone glycoside is converted into an aglycone, glucose, which is a reducing sugar, is generated, and the glucose remains in the produced composition, and therefore, the Maillard reaction proceeds by heating, and the
25 composition browns. Furthermore, the present inventor has found that the above problems can be solved by decomposing the glucose contained in the composition.

That is, the present disclosure includes at least the following.

[0008]

30 [1] A method for producing a composition, the method including the following step (a); step (b1) and/or step (b2); and step (c):

(a) producing glucose and an isoflavone aglycone from an isoflavone glycoside;

(b1) allowing a microorganism having an equol-producing ability to
35 produce equol from the isoflavone aglycone;

(b2) allowing a microorganism having an equol derivative-producing ability to produce an equol derivative from the isoflavone aglycone; and

(c) decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in the composition, or 30 g/kg or less based on a solid content of the composition.

5 [2] The method according to [1], wherein the step (c) is decomposing the glucose by fermentation using a microorganism having a sugar assimilation ability.

[3] The method according to [2], wherein the microorganism having a sugar assimilation ability is one or more microorganisms selected from the group consisting of butyric acid bacteria, lactic acid bacteria, and yeasts.

10 [4] The method according to [3], wherein the microorganism having a sugar assimilation ability is one or more microorganisms selected from the group consisting of microorganisms belonging to the genus *Clostridium*, microorganisms belonging to the genus *Lactobacillus*, microorganisms belonging to the genus *Lactococcus*, microorganisms belonging to the genus
15 *Leuconostoc*, and microorganisms belonging to the genus *Saccharomyces*.

[5] The method according to [1], wherein the step (c) is decomposing the glucose using an enzyme that decomposes glucose.

[6] The method according to [5], wherein the enzyme that decomposes glucose is one or more selected from the group consisting of glucose oxidase
20 and glucose dehydrogenase.

[7] The method according to any of [1] to [6], wherein the step (b1) and/or the step (b2), and the step (c) are performed simultaneously.

[8] The method according to any of [1] to [7], wherein the isoflavone glycoside is daidzin, and the isoflavone aglycone is daidzein.

25 [9] The method according to any of [1] to [8], wherein the step (a) is allowing β -glucosidase to act on an isoflavone glycoside to produce glucose and an isoflavone aglycone.

[10] A method for producing a food and/or beverage, the method including the following step (a); step (b1) and/or step (b2); step (c); and step
30 (d):

(a) producing glucose and an isoflavone aglycone from an isoflavone glycoside;

(b1) allowing a microorganism having an equol-producing ability to produce equol from the isoflavone aglycone;

35 (b2) allowing a microorganism having an equol derivative-producing ability to produce an equol derivative from the isoflavone aglycone; and

(c) decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in a composition produced by a method including

step (a), step (b1) and/or step (b2), and step (c), or 30 g/kg or less based on a solid content of the composition; and

(d) producing a food and/or beverage using the composition produced by the method including step (a), step (b1) and/or step (b2), and step (c).

5 [11] A fermentation composition containing equol and/or an equol derivative, and containing substantially no glucose.

[12] The fermentation composition according to [11], wherein containing substantially no glucose is containing 0 g/L or more and less than 2.0 g/L of glucose.

10 [13] A fermentation composition containing equol and/or an equol derivative, and glucose, wherein

a content of the glucose is 30 g/kg or less based on a solid content of the fermentation composition.

[14] The fermentation composition according to any of [11] to [13],
15 wherein the fermentation composition is used for a food and/or beverage.

Advantageous Effects of Invention

[0009]

The present disclosure can achieve at least an effect that it is possible to
20 provide a method for producing a composition containing equol and/or an equol derivative, in which the amount of glucose is reduced, the Maillard reaction does not proceed and the composition does not brown even when heated as it is or in the presence of an additional amino acid or the like.

25 Description of Embodiments

[0010]

Each of the configurations, combinations thereof, and the like in each of the embodiments is an example, and additions, omissions, substitutions, and other changes of the configurations may be made as appropriate without
30 departing from the spirit of the present disclosure. The present disclosure is not limited by the embodiments and is limited only by the claims. Each embodiment disclosed in the present specification can be combined with any other feature disclosed herein.

A numerical range expressed by “to” implies a range including the
35 numerical values described before and after “to” as the lower limit value and the upper limit value. Specifically, “A to B” implies a value that is A or more and B or less.

[0011]

As used herein, the accession number of a strain beginning with the description DSM is the number assigned to a microorganism stored in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ).

5 The accession number of a strain beginning with the description JCM is the number assigned to a microorganism stored in Japan Collection of Microorganisms (National Research and Development Agency, RIKEN, Bioresource Research Center, Microbe Division, zip code: 305-0074, address: 3-1-1, Koyadai, Tsukuba-shi, Ibaraki-ken), and can be obtained from the same institution.

10 The accession number of a strain beginning with the description FERM is a number assigned to a microorganism stored in the International Patent Organism Depository of the National Institute of Advanced Industrial Science and Technology (currently, National Institute of Technology and Evaluation Patent Microorganisms Depository, zip code: 292-0818, address: Room 120, 2-15 5-8 Kazusakamatari, Kisarazu-shi, Chiba-ken, Japan), and can be obtained from the same institution.

The accession number of a strain beginning with the description KCCM is a number assigned to a microorganism stored in Korean Culture Center of Microorganisms (KCCM), and can be obtained from the same institution.

20 [0012]

In the present specification, "microorganisms" refer to eubacteria, archaeobacteria, or fungi, and does not include viruses, animals, and plants.

[0013]

25 In the present specification, the phrase "decomposing glucose" means that the reducing end of glucose is inactivated so that glucose and an amino acid or the like do not cause the Maillard reaction, and the phrase includes oxidation of glucose and conversion of glucose into another compound by metabolism.

30 The amino acid or the like is not particularly limited, as long as it is a compound capable of causing the Maillard reaction with glucose, and examples thereof include compounds containing an amino group. Examples of the compound containing an amino group include amino acids, peptides, and proteins.

[0014]

35 One embodiment of the present disclosure is a method for producing a composition, the method including the following step (a); step (b1) and/or step (b2); and step (c):

(a) step of producing glucose and an isoflavone aglycone from an isoflavone glycoside;

(b1) step of allowing a microorganism having an equol-producing ability to produce equol from the isoflavone aglycone;

5 (b2) step of allowing a microorganism having an equol derivative-producing ability to produce an equol derivative from the isoflavone aglycone; and

(c) step of decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in the composition, or 30 g/kg or less
10 based on a solid content of the composition.

[0015]

Step (a)

The production method according to the present embodiment includes step (a) of producing glucose and an isoflavone aglycone from an isoflavone
15 glycoside.

[0016]

The isoflavone glycoside refers to a glycoside of isoflavones. The isoflavones are one of the groups of polyphenols, and are flavonoids having an isoflavone as a basic backbone.

20 Isoflavones are contained in a large amount in plants of the family Fabaceae such as soybean, arrowroot (kudzu), red clover, and licorice. Examples of the isoflavones in the present embodiment include isoflavones derived from leguminous plants containing isoflavones. Specific examples thereof include isoflavones derived from soybeans (sometimes referred to as
25 "soybean isoflavone" or "soybean isoflavones" in the art and on the market, and they are treated as synonyms in the present disclosure), isoflavones derived from arrowroot (sometimes referred to as "Kudzu isoflavone" or "Kudzu isoflavones" in the art and on the market, and they are treated as synonyms in the present disclosure), isoflavones derived from red clover (sometimes
30 referred to as "red clover isoflavone" or "red clover isoflavones" in the art and on the market, and they are treated as synonyms in the present disclosure), and isoflavones derived from licorice (sometimes referred to as "licorice isoflavone" or "licorice isoflavones" in the art and on the market, and they are treated as synonyms in the present disclosure).

35 [0017]

Examples of the isoflavone glycoside include genistin, glycitin, and daidzin. In the present embodiment, the isoflavone glycoside may be a mixture of two or more isoflavone glycosides. For example, the isoflavone glycoside

may be a mixture of daidzin, glycitin, and genistin. Since these are contained in soybean hypocotyls, soybean hypocotyl extracts, and the like, for example, a soybean hypocotyl extract can be used as a mixture of isoflavone glycosides.

[0018]

5 Examples of the isoflavone aglycone include daidzein, 6-hydroxydaidzein, dihydroxydaidzein, genistein, glycitein, biochanin A, formononetin, orobol, and coumestrol. In the present embodiment, the isoflavone aglycone may be a mixture of two or more isoflavone aglycones. The term "isoflavone aglycone" in the present disclosure may be referred to as
10 "aglycones of isoflavones" and the like in the art and on the market, and they are treated as synonyms in the present disclosure.

[0019]

 In the present embodiment, it is preferable that the isoflavone glycoside is daidzin and the isoflavone aglycone is daidzein, or the isoflavone glycoside
15 is genistin and the isoflavone aglycone is genistein. It is more preferable that the isoflavone glycoside is daidzin and the isoflavone aglycone is daidzein.

[0020]

 Examples of the method for producing glucose and an isoflavone aglycone from an isoflavone glycoside include a method of allowing β -
20 glucosidase to act on an isoflavone glycoside, and a method of culturing a microorganism that produces β -glucosidase in an isoflavone glycoside-containing culture medium.

[0021]

β -glucosidase is an enzyme that catalyzes the hydrolysis reaction of β -
25 glycoside bonds of a sugar. The β -glucosidase is not limited, as long as it has the enzyme activity described above, and examples thereof include those derived from microorganisms belonging to the genus *Aspergillus*, the genus *Penicillium*, the genus *Rhizopus*, the genus *Pseudomonas*, the genus *Pichia*, and the genus *Lactococcus*, those derived from higher plants, and those derived
30 from animals. Commercially available products such as an enzyme preparation containing β -glucosidase, e.g., Pectinase G (available from Amano Enzyme Inc.), can also be used.

[0022]

 In order to allow β -glucosidase to act on the isoflavone glycoside, β -
35 glucosidase may be added to a solution containing the isoflavone glycoside. The amount to be added can be appropriately adjusted, and is usually 10 U or more and 3000 U or less, preferably 20 U or more and 1000 U or less, and more

preferably 50 U or more and 500 U or less relative to 1 g of the isoflavone glycoside.

[0023]

5 The conditions for allowing β -glucosidase to act on the isoflavone glycoside are not particularly limited, as long as β -glucosidase acts on the isoflavone glycoside to catalyze the release of glucose, but the temperature is usually 15°C or higher and 65°C or lower, preferably 20°C or higher and 55°C or lower, and more preferably 30°C or higher and 45°C or lower.

10 The pH is usually 2 or more and 9 or less, preferably 3 or more and 7 or less, and more preferably 4 or more and 6 or less.

[0024]

The microorganism that produces β -glucosidase is not particularly limited, and one or a plurality of microorganisms can be used regardless of the genus, species, and strain.

15 Examples thereof include microorganisms belonging to the genus *Aspergillus*, the genus *Lactococcus*, the genus *Streptococcus*, the genus *Lactobacillus*, the genus *Bifidobacterium*, the genus *Penicillium*, the genus *Pichia*, the genus *Pseudomonas*, and the genus *Rhizopus*.

20 Preferred examples thereof include microorganisms belonging to the genus *Aspergillus*, the genus *Lactococcus*, the genus *Streptococcus*, the genus *Lactobacillus*, and the genus *Bifidobacterium*.

25 Still more preferred examples thereof include microorganisms belonging to *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus aculeatus*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*.

[0025]

30 As the culture conditions of the microorganism that produces β -glucosidase, culture conditions usually used in the culture of the microorganism or culture conditions obtained by appropriately modifying the culture conditions can be used.

[0026]

35 As the culture liquid (culture medium), for example, a BHI culture medium available from Difco, an ANAEROBE BASAL BROTH (ABB) culture medium available from Oxoid Limited, a Wilkins-Chalgren Anaerobe Broth (CM0643) available from Oxoid Limited, and a GAM culture medium and a modified GAM culture medium available from Nissui Pharmaceutical Co., Ltd. can be used. As used herein, the culture medium refers to a solution in which microorganisms can grow, including a minimal medium, and does not include a

solution in which microorganisms cannot grow, for example, water, a salt solution, or a buffer.

[0027]

5 The isoflavone glycoside is added to the culture medium described above. The isoflavone glycoside can be added to the culture medium before and during culture of the microorganism.

The content of the isoflavone glycoside is not particularly limited, and is usually 0.5 g/L or more and 100 g/L or less, preferably 1 g/L or more and 50 g/L or less, and more preferably 2 g/L or more and 20 g/L or less.

10 [0028]

To the culture medium, a water-soluble organic material can be added as a carbon source. Examples of the water-soluble organic material include the following compounds. Examples thereof include sugars such as glucose, arabinose, sorbitol, fructose, mannose, sucrose, trehalose, and xylose; alcohols
15 such as glycerol; organic acids such as valeric acid, butyric acid, propionic acid, acetic acid, formic acid, fumaric acid, and succinic acid; and polysaccharides such as dextrin.

[0029]

20 The concentration of the organic material added in the culture medium as a carbon source can be appropriately adjusted for efficient growth. In general, the amount to be added can be selected from a range of from 0.1 to 10 wt/vol%.

[0030]

25 In addition to the carbon source described above, a nitrogen source can be added to the culture medium. Various nitrogen compounds that may be used ordinarily in fermentation can be used as the nitrogen source. Examples thereof include an inorganic nitrogen source and an organic nitrogen source.

30 Examples of the inorganic nitrogen source include ammonium salts and nitrates. The inorganic nitrogen source is preferably ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium hydrogen phosphate, potassium nitrate, or sodium nitrate.

35 Examples of the organic nitrogen source include amino acids (such as glutamic acid, arginine, and ornithine), oils and/or fats such as oleic acid, yeast extracts, peptones (e.g., polypeptone N and soybean peptone), meat extracts (e.g., Ehrlich bonito extract, Lab-Lemco powder, and bouillon), fish and shellfish extracts, liver extracts, digested serum powder, and fish oil. The organic nitrogen source is more preferably arginine, cysteine, citrulline, lysine, yeast extract, and peptones (e.g., polypeptone N).

[0031]

Furthermore, the growth and activity can be enhanced by adding, for example, cofactors such as vitamins and inorganic compounds such as various salts to the culture medium, in addition to the carbon source and the nitrogen source. Examples of plant- and animal-derived cofactors for microbial growth, such as inorganic compounds and vitamins, include the following.

[0032]

	Inorganic Compounds	vitamins
	Potassium dihydrogen phosphate	biotin
10	Magnesium sulfate	folic acid
	Manganese sulfate	pyridoxine
	Sodium chloride	thiamine
	Cobalt chloride	riboflavin
	Calcium chloride	nicotinic acid
15	Zinc sulfate	pantothenic acid
	Copper sulfate	vitamin B12
	Alum	thioctic acid
	Sodium molybdate	p-aminobenzoic acid
	Potassium Chloride	vitamin K
20	Boric acid and the like	
	Nickel chloride	
	Sodium tungstate	
	Sodium selenate	
	Ammonium iron(II) sulfate	
25	Sodium acetate trihydrate	
	Magnesium sulfate heptahydrate	
	Manganese sulfate tetrahydrate	

[0033]

Methods for producing a culture medium by adding these plant- and animal-derived cofactors for growth, such as inorganic compounds and vitamins, are well known. The culture medium can be a liquid, a semi-solid, or a solid. A preferred form of the culture medium is a liquid culture medium.

[0034]

The culture medium according to the present disclosure may contain dextrins. By culturing an anaerobic microorganism in a culture medium containing dextrins, a solution containing a functional substance and dextrins can be prepared without adding dextrins even when dextrins are required for a

culture liquid after the culture. The dextrans can be added to the culture medium before or during the culture of the microorganisms.

[0035]

5 In the case of anaerobic culture, the growth may be favorable by adding a reducing agent such as cysteine, cystine, sodium sulfide, sulfite, ascorbic acid, glutathione, thioglycolic acid, or rutin, or an enzyme that decomposes reactive oxygen species, such as catalase or superoxide mutase to the culture medium.

[0036]

10 In the case of anaerobic culture, the gas phase and aqueous phase during the culture preferably do not contain air or oxygen, and examples include gas and aqueous phases that contain nitrogen and/or hydrogen in any proportion, and gas and aqueous phases that contain nitrogen and/or carbon dioxide in any proportion. These may be supplied in gaseous form.

15 The proportion of hydrogen in the gas phase is not particularly limited, but is usually 0.5% or more and 100% or less, preferably 1.0% or more and 20% or less, and more preferably 2.0% or more and 10% or less.

[0037]

20 The pH of the culture medium is preferably 5.0 or more, more preferably 6.0 or more, and still more preferably 6.5 or more, and preferably 8.0 or less, and more preferably 7.5 or less.

The culture temperature is from 20°C to 45°C, more preferably from 25°C to 40°C, and even more preferably from 30°C to 37°C.

25 The pressure condition in an incubator is not particularly limited, as long as cells can grow, and is, for example, a range of 0.001 to 1 MPa, and preferably 0.01 to 0.5 MPa.

The culture time is usually from 8 to 340 hours, preferably from 12 to 170 hours, and more preferably from 16 to 120 hours.

[0038]

30 Step (b1)

The production method according to the present embodiment includes step (b1) of allowing a microorganism having an equol-producing ability to produce equol from the isoflavone aglycone, and/or step (b2) described below.

[0039]

35 Examples of the microorganism having an equol-producing ability include microorganisms belonging to the genus *Adlercreutzia*, microorganisms belonging to the genus *Atopobium*, microorganisms belonging to the genus *Bacteroides*, microorganisms belonging to the genus *Collinsella*,

microorganisms belonging to the genus *Coriobacterium*, microorganisms belonging to the genus *Cryptobacterium*, microorganisms belonging to the genus *Denitrobacterium*, microorganisms belonging to the genus *Eggerthella*, microorganisms belonging to the genus *Enterorhabdus*, microorganisms
5 belonging to the genus *Eubacterium*, microorganisms belonging to the genus *Gordonibacter*, microorganisms belonging to the genus *Lactococcus*, microorganisms belonging to the genus *Olsenella*, microorganisms belonging to the genus *Paraeggerthella*, microorganisms belonging to the genus *Ruminococcus*, microorganisms belonging to the genus *Slackia*, and
10 microorganisms belonging to the genus *Streptococcus*.
[0040]

Preferred are microorganisms belonging to *Adlercreutzia equolifaciens subsp. equolifaciens*, microorganisms belonging to *Adlercreutzia equolifaciens subsp. celatus*, microorganisms belonging to *Bacteroides ovatus*,
15 microorganisms belonging to *Eggerthella sp.*, microorganisms belonging to *Eubacterium sp.*, microorganisms belonging to *Lactococcus garvieae*, microorganisms belonging to *Paraeggerthella sp.*, microorganisms belonging to *Ruminococcus productus*, microorganisms belonging to *Slackia isoflavoniconvertens*, microorganisms belonging to *Slackia equolifaciens*,
20 microorganisms belonging to *Slackia sp.*, and microorganisms belonging to *Streptococcus intermedius*.
[0041]

More preferred examples include *Adlercreutzia equolifaciens subsp. equolifaciens* DSM 19450 strain, *Adlercreutzia equolifaciens subsp. celatus*
25 DSM 18785 strain, *Eggerthella sp.* KCCM 10490 strain, *Lactococcus garvieae* DSM 6783 strain, *Slackia isoflavoniconvertens* DSM 22006 strain, *Slackia equolifaciens* DSM 24851 strain, and *Slackia sp.* FERM AP-20729 strain.

The microorganisms described above may be used alone, or two or more thereof may be used, regardless of the genus, species, and strain.
30 [0042]

In the present embodiment, *Adlercreutzia equolifaciens subsp. equolifaciens* DSM 19450 strain is not limited to the same strain as the deposited strain, and may be a strain substantially equivalent to the deposited strain. The "strain substantially equivalent" refers to a strain that belongs to the
35 same genus or species as the deposited strain and has an equol-producing ability. The "strain substantially equivalent" refers to a microorganism in which the base sequence of the 16S rRNA gene has homology with 97% or more, preferably 97.5% or more, more preferably 98% or more, still more preferably

98.7% or more, and even more preferably 99% or more of the base sequence of the 16S rRNA gene of the deposited strain. Furthermore, as long as the effects of the present disclosure are not impaired, the microorganism having an equol-producing ability may be a strain bred from the deposited strain or a strain substantially equivalent to the deposited strain by mutation treatment, genetic recombination, selection of a natural mutant strain, or the like.

This also applies to the other deposited strains in the present specification.

[0043]

When the microorganism having an equol-producing ability produces β -glucosidase, the microorganism producing β -glucosidase may be the same as the microorganism having an equol-producing ability. In this case, step (a) and step (b1) are continuously performed in the same system.

[0044]

As the culture conditions, culture conditions usually used in the culture of the microorganism or culture conditions obtained by appropriately modifying the culture conditions can be used, and examples thereof include the same conditions as the culture conditions of the microorganism producing β -glucosidase.

[0045]

Step (b2)

The production method according to the present embodiment includes step (b1) and/or step (b2) of allowing a microorganism having an equol derivative-producing ability to produce the equol derivative from the isoflavone aglycone. Examples of the equol derivative include 5-hydroxyequol.

[0046]

When the equol derivative is 5-hydroxyequol, examples of the microorganism having a 5-hydroxyequol-producing ability include microorganisms belonging to the genus *Adlercreutzia*, the genus *Eggerthella*, and the genus *Slackia*.

The microorganism belonging to the genus *Adlercreutzia* is preferably a microorganism belonging to *Adlercreutzia equolifaciens*. In particular, *Adlercreutzia equolifaciens* subsp. *equolifaciens* DSM 19450 strain or *Adlercreutzia equolifaciens* subsp. *celatus* DSM 18785 strain is preferable.

[0047]

Examples of the microorganism belonging to the genus *Eggerthella* include microorganisms belonging to *Eggerthella* sp. In particular, *Eggerthella* sp. KCCM 10490 strain is preferable.

Examples of the microorganism belonging to the genus *Slackia* include microorganisms belonging to *Slackia isoflavoniconvertens*, microorganisms belonging to *Slackia equolifaciens*, and microorganisms belonging to *Slackia* *sp.*

5 [0048]

When the microorganism having an equol derivative-producing ability produces β -glucosidase, the microorganism producing β -glucosidase may be the same as the microorganism having an equol derivative-producing ability. In this case, step (a) and step (b2) are continuously performed in the same system.

10 [0049]

As the culture conditions, culture conditions usually used in the culture of the microorganism or culture conditions obtained by appropriately modifying the culture conditions can be used, and examples thereof include the same conditions as the culture conditions of the microorganism producing β -glucosidase.

15 [0050]

Step (c)

The production method according to the present embodiment includes step (c) of decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in the composition, or 30 g/kg or less based on a solid content of the composition. Decomposing glucose refers to converting glucose to a compound having a smaller number of carbons.

20

Step (c) may be a step of decomposing the glucose by fermentation using a microorganism having a sugar assimilation ability, or may be a step of decomposing the glucose using an enzyme that decomposes glucose.

25 [0051]

When step (c) utilizes fermentation using a microorganism having a sugar assimilation ability, the microorganism having a sugar assimilation ability is preferably one or more microorganisms selected from the group consisting of butyric acid bacteria, lactic acid bacteria, and yeasts. More preferred are one or more microorganisms selected from the group consisting of microorganisms belonging to the genus *Clostridium*, microorganisms belonging to the genus *Lactobacillus*, microorganisms belonging to the genus *Lactococcus*, microorganisms belonging to the genus *Leuconostoc*, and microorganisms belonging to the genus *Saccharomyces*.

30 [0052]

Examples of the microorganism belonging to the genus *Clostridium* include *Clostridium asparagiforme* DSM 15981 strain and *Clostridium bolteae* JCM 12243 strain.

Examples of the microorganism belonging to the genus *Lactobacillus* include *Lactobacillus brevis* DSM 20054 strain.

Examples of the microorganism belonging to the genus *Lactococcus* include *Lactococcus lactis* DSM 20481 strain.

5 Examples of the microorganism belonging to the genus *Leuconostoc* include *Leuconostoc mesenteroides subsp. mesenteroides* DSM 20343 strain. [0053]

When the microorganism having a sugar assimilation ability produces β -glucosidase, the microorganism producing β -glucosidase may be the same as
10 the microorganism having a sugar assimilation ability. In this case, step (a) and step (c) may be continuously performed in the same system. [0054]

The microorganism having a sugar assimilation ability may be of the same species as the microorganism having an equol-producing ability. That is,
15 the microorganism having a sugar assimilation ability may be a microorganism having an equol-producing ability and a sugar assimilation ability. In this case, step (b1) and step (c) are continuously performed in the same system. [0055]

The microorganism having a sugar assimilation ability may be of the same species as the microorganism having an equol derivative-producing
20 ability. That is, the microorganism having a sugar assimilation ability may be a microorganism having an equol derivative-producing ability and a sugar assimilation ability. In this case, the step (b2) and the step (c) are continuously performed in the same system. [0056]

As the culture conditions, culture conditions usually used in the culture of the microorganism or culture conditions obtained by appropriately modifying the culture conditions can be used, and examples thereof include the same conditions as the culture conditions of the microorganism producing β -glucosidase.
30 [0057]

When step (c) utilizes an enzyme that decomposes glucose, examples of the enzyme that decomposes glucose include glucose oxidase and glucose dehydrogenase. These may be used alone or in combination of two or more types. Glucose oxidase and glucose dehydrogenase are enzymes that catalyze
35 the oxidation reaction of β -D-glucose to D-glucono-1,5-lactone. [0058]

The glucose oxidase and the glucose dehydrogenase are not limited, as long as they have the enzyme activity described above, and examples thereof

include those derived from *Aspergillus niger* and those derived from *Gluconobacter oxydans*.

[0059]

In order to decompose glucose using the enzyme that decomposes glucose, the enzyme may be added to a solution (culture medium) containing glucose. The amount of the enzyme to be added can be appropriately adjusted, but is usually 1 U or more and 10000 U or less, preferably 10 U or more and 5000 U or less, and more preferably 100 U or more and 2000 U or less relative to 1 g of glucose.

10 [0060]

The temperature at which glucose is decomposed is not particularly limited, but is usually 15°C or higher and 60°C or lower, preferably 25°C or higher and 50°C or lower, more preferably 30°C or higher and 40°C or lower, and particularly preferably 35°C.

15 The pH at which glucose is decomposed is not particularly limited, but is usually 4 or more and 9 or less, preferably 4 or more and 7 or less, and more preferably 5 or more and 6 or less.

[0061]

When step (b1) step and step (b2) are performed, the order of these steps is not limited, and step (b2) may be performed after step (b1), step (b1) may be performed after step (b2), or step (b1) and step (b2) may be performed simultaneously.

[0062]

25 The order of step (b1) and/or step (b2) and step (c) is not limited, and step (c) may be performed after step (b1) and/or step (b2), step (b1) and/or step (b2) may be performed after step (c), or step (b1) and/or step (b2) and step (c) may be performed simultaneously.

Specific examples of the aspect in which step (b1) and/or step (b2) and step (c) are performed simultaneously include an aspect in which an enzyme that decomposes glucose is added to a culture medium containing glucose, an isoflavone aglycone, and a microorganism having an equol-producing ability, an aspect in which a microorganism having an equol-producing ability and a microorganism having a sugar assimilation ability are co-cultured in a culture medium containing glucose and an isoflavone aglycone, and an aspect in which a microorganism having an equol-producing ability and a sugar assimilation ability is cultured in a culture medium containing glucose and an isoflavone aglycone.

35 [0063]

In step (c), glucose is decomposed to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in the composition, or 30 g/kg or less based on the solid content of the composition.

5 In order to adjust the content of glucose to the above range, the culture conditions and the treatment conditions of the enzyme that decomposes glucose may be appropriately adjusted. For example, the culture time or the treatment time is prolonged.

[0064]

10 The content of glucose in the composition is preferably 0 g/L or more and less than 2.0 g/L, more preferably 0 g/L or more and 1.0 g/L or less, still more preferably 0 g/L or more and 0.5 g/L or less, and particularly preferably 0 g/L or more and 0.2 g/L or less. The content of glucose in the composition is preferably 30 g/kg or less, more preferably 10 g/kg or less, and still more preferably 4 g/kg or less based on the solid content of the composition. The
15 solid content is determined by measuring the remaining weight after drying the composition. Examples of the drying method include a reduced pressure heating drying method, an atmospheric pressure heating drying method, and a freeze drying method.

The content of glucose can be measured by an electrode method.

20 When the composition does not contain water, for example, in the case of a solid, water is added to the composition, glucose is dissolved in water, and then measurement is performed, whereby the glucose content based on the solid content can be measured.

[0065]

25 Additional Steps

The production method according to the present embodiment may include, for example, a step of quantifying the produced equol and/or equol derivative. The method can be in accordance with a routine method. For example, a portion of the culture liquid is collected, appropriately diluted, and
30 stirred well. Thereafter, the resultant product is filtered using a membrane such as a polytetrafluoroethylene (PTFE) membrane to remove insoluble matter, and the resultant product can then be quantified using high performance liquid chromatography (HPLC).

[0066]

35 The production method according to the present embodiment may include a step of recovering the produced equol and/or equol derivative. The recovery step includes a purification step, a concentration step, and the like. Examples of the purification treatment in the purification step include

sterilization of microorganisms by heat or the like; sterilization by microfiltration (MF), ultrafiltration (UF), or the like; removal of solid substances and polymer substances; extraction with an organic solvent, an ionic liquid, or the like; and adsorption and decolorization using a hydrophobic adsorbent, an ion-exchange resin, an activated carbon column, or the like. Examples of the concentration treatment in the concentration step include concentration by an evaporator, a reverse osmosis membrane, or the like. [0067]

Furthermore, the solution containing the produced equol and/or equol derivative can be formed into a powder by freeze drying, spray drying, or the like. In the formation of a powder, an excipient such as lactose, dextrin, or corn starch can be added. [0068]

Method for Producing Food and/or Beverage

Even when the composition produced by the production method according to the present embodiment is heated as it is or in the presence of an additional amino acid or the like, the Maillard reaction does not proceed and the composition does not brown, and thus, the composition can be suitably contained in a food and/or beverage. In the present specification, the food and/or beverage includes a supplement.

That is, another embodiment of the present disclosure is a method for producing a food and/or beverage, the method including the following step (a); step (b1) and/or step (b2); step (c); and step (d):

(a) step of producing glucose and an isoflavone aglycone from an isoflavone glycoside;

(b1) step of allowing a microorganism having an equol-producing ability to produce equol from the isoflavone aglycone;

(b2) step of allowing a microorganism having an equol derivative-producing ability to produce an equol derivative from the isoflavone aglycone; and

(c) step of decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in a composition produced by a method including step (a), step (b1) and/or step (b2), and step (c), or 30 g/kg or less based on a solid content of the composition; and

(d) step of producing a food and/or beverage using the composition produced by the method including step (a), step (b1) and/or step (b2), and step (c).

[0069]

The food and/or beverage produced by the production method according to the present embodiment contains equol and/or an equol derivative. The above food and/or beverage can be used as, for example, a food for specified health use, a nutritional supplement, a functional food, a food for sick persons, or a food additive (these include beverages) in addition to being used as a general food and/or beverage. The food and/or beverage may be prepared by, for example, adding an appropriate auxiliary agent to the composition, and then shaping into a form suitable for eating, such as a granular form, a particulate form, a tablet, a capsule, or a paste, using a typical means, and served for eating. The food and/or beverage may be added to various foods, for example, processed meat foods such as hams and sausages, processed marine foods such as fish sausages, breads, confectioneries, butters, powdered milk, fermented milk products, and beverages such as water, juices, milks, and cold beverages, and then used.

[0070]

The food and/or beverage may contain water, a protein, a carbohydrate, a lipid, a vitamin, a mineral, an organic acid, an organic base, a fruit juice, a flavor, or the like. Examples of the protein include animal and vegetable proteins, such as whole milk powders, skimmed milk powders, partially skimmed milk powders, casein, soy proteins, chicken egg proteins, and meat proteins; their hydrolysates; and butters. Examples of the carbohydrate include sugars, processed starches (dextrin, as well as soluble starches, British starch, oxidized starches, starch esters, starch ethers, and the like), and dietary fibers. Examples of the lipid include lard; and vegetable oils and/or fats, such as safflower oil, corn oil, rapeseed oil, coconut oil, and their fractionated oils, hydrogenated oils, and transesterified oils. Examples of the vitamin include vitamin A, carotenes, B-group vitamins, vitamin C, D-group vitamins, vitamin E, K-group vitamins, vitamin P, vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline, and folic acid. Examples of the mineral include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc, selenium, and whey minerals. Examples of the organic acid include malic acid, citric acid, lactic acid, and tartaric acid. Two or more types of these components may be used in combination. Synthetic products of these components and/or foods and/or beverages containing these components in large amounts may be used.

[0071]

As for step (a); step (b1) and/or step (b2); and step (c), the description in the method for producing a composition according to an embodiment of the

present disclosure is employed. The method for producing a food and/or beverage of the present embodiment may include additional steps described above.

[0072]

5 Step (d) of producing a food and/or beverage using the composition produced by the method including step (a), step (b1) and/or step (b2), and step (c) can be carried out according to a typical method. The amount of equol and/or the equol derivative blended, the blending method, and the blending timing can be appropriately selected. Furthermore, the above food and/or
10 beverage can be sealed in a container, such as a bottle, a bag, a can, a box, or a pack, as necessary.

[0073]

Fermentation Composition

 The method for producing a composition according to an embodiment of
15 the present disclosure can provide a composition containing equol and/or an equol derivative, in which the amount of glucose is reduced, and the Maillard reaction does not proceed even when the composition is heated as it is or in the presence of an additional amino acid or the like.

 That is, another embodiment of the present disclosure is a fermentation
20 composition containing equol and/or an equol derivative, and containing substantially no glucose. Alternatively, another embodiment of the present disclosure is a fermentation composition containing equol and/or an equol derivative, in which the content of the glucose is 30 g/kg or less based on a solid content of the fermentation composition. Examples of the equol derivative
25 include 5-hydroxyequol.

 Even when the fermentation composition according to the present embodiment is heated as it is or in the presence of an additional amino acid or the like, the Maillard reaction does not proceed and the fermentation composition does not brown, and thus, the fermentation composition can be
30 suitably contained in a food and/or beverage. That is, the fermentation composition can be used for a food and/or beverage.

[0074]

 As for the phrase "containing substantially no glucose", it is only required that the content of glucose is sufficiently low to the extent that
35 browning of the composition due to the Maillard reaction does not occur, and specific examples thereof include a case where the content of glucose is 0 g/L or more and less than 2.0 g/L.

[0075]

The content of glucose in the fermentation composition is preferably 0 g/L or more and less than 2.0 g/L, more preferably 0 g/L or more and 1.0 g/L or less, still more preferably 0 g/L or more and 0.5 g/L or less, and particularly preferably 0 g/L or more and 0.2 g/L or less. The content of glucose in the fermentation composition is preferably 30 g/kg or less, more preferably 10 g/kg or less, and still more preferably 4 g/kg or less based on the solid content of the composition. The solid content is determined by measuring the remaining weight after drying the fermentation composition. Examples of the drying method include a reduced pressure heating drying method, an atmospheric pressure heating drying method, and a freeze drying method.

The content of glucose can be measured by an electrode method.

When the fermentation composition does not contain water, for example, in the case of a solid, water is added to the fermentation composition, glucose is dissolved in water, and then measurement is performed, whereby the glucose content based on the solid content can be measured.

[0076]

The content of equol and/or the equol derivative is preferably 1 g/kg or more, more preferably 5 g/kg or more, and still more preferably 10 g/kg or more based on the solid content of the fermentation composition.

The content of equol and/or the equol derivative can be measured by HPLC described above.

[0077]

The fermentation composition may contain an additional component that may be contained in the culture medium described above.

[0078]

The above food and/or beverage containing the fermentation composition can be used as, for example, a food for specified health use, a nutritional supplement, a functional food, a food for sick persons, or a food additive (these include beverages) in addition to being used as a general food and/or beverage. The food and/or beverage may be prepared by, for example, adding an appropriate auxiliary agent to the composition, and then shaping into a form suitable for eating, such as a granular form, a particulate form, a tablet, a capsule, or a paste, using a typical means, and served for eating. The food and/or beverage may be added to various foods, for example, processed meat foods such as hams and sausages, processed marine foods such as fish sausages, breads, confectioneries, butters, powdered milk, fermented milk products, and beverages such as water, juices, milks, and cold beverages, and then used.

[0079]

The food and/or beverage may contain water, a protein, a carbohydrate, a lipid, a vitamin, a mineral, an organic acid, an organic base, a fruit juice, a flavor, or the like. Examples of the protein include animal and vegetable proteins, such as whole milk powders, skimmed milk powders, partially skimmed milk powders, casein, soy proteins, chicken egg proteins, and meat proteins; their hydrolysates; and butters. Examples of the carbohydrate include sugars, processed starches (dextrin, as well as soluble starches, British starch, oxidized starches, starch esters, starch ethers, and the like), and dietary fibers. Examples of the lipid include lard; and vegetable oils and/or fats, such as safflower oil, corn oil, rapeseed oil, coconut oil, and their fractionated oils, hydrogenated oils, and transesterified oils. Examples of the vitamins include vitamin A, carotenes, B-group vitamins, vitamin C, D-group vitamins, vitamin E, K-group vitamins, vitamin P, vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline, and folic acid. Examples of the minerals include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc, selenium, and whey minerals. Examples of the organic acid include malic acid, citric acid, lactic acid, and tartaric acid. Two or more types of these components may be used in combination. Synthetic products of these components and/or foods and/or beverages containing these components in large amounts may be used.

[0080]

The food and/or beverage can be produced by, for example, a commonly used method. The amount of equol and/or the equol derivative blended, the blending method, and the blending timing can be appropriately selected. Furthermore, the above food and/or beverage can be sealed in a container, such as a bottle, a bag, a can, a box, or a pack, as necessary.

Examples

[0081]

Hereinafter, the present disclosure will be described in further detail by way of specific examples; however, the present disclosure is not limited to these examples.

[0082]

Method for Measuring Glucose Concentration

1 mL of a liquid to be measured was collected, and cells were precipitated by centrifugation. The supernatant was filtered through a 0.45 μ m filter, and then the filtrate was analyzed under the following conditions. When

the result exceeded a measurement range (from 10 mg/dL to 600 mg/dL), the filtrate was diluted with milliQ water and analyzed.

Inspection apparatus: Glutest Neo Alpha available from SANWA KAGAKU KENKYUSHO CO., LTD.

5 Chip: Glutest Neo Sensor available from SANWA KAGAKU KENKYUSHO CO., LTD.

 The content based on the solid content (g/kg) was calculated by measuring the solid concentration (kg/L) of the liquid to be measured using an electronic moisture analyzer (MOC-120H available from Shimadzu Corporation) and dividing the glucose concentration (g/L) by the solid concentration.

[0083]

Method for Measuring Equol Concentration and Equol Derivative

 20 µL of a liquid to be measured was collected and 50-fold diluted with a diluent composed of ethanol:milliQ water = 70:30 (v/v). The diluted liquid was filtered through a 0.45 µm filter, and then the filtrate was analyzed under the following HPLC conditions.

 (S)-equol available from FUJIFILM Wako Pure Chemical Corporation was used as an equol standard, and 5-hydroxyequol available from Toronto Research Chemicals, Inc. was used as a 5-hydroxyequol standard.

HPLC Analysis Conditions

 Column: Phenomenex SYNERGI, 4 µm, POLAR-R, 150 mm × 4.6 mm

 Eluent: distilled water/methanol = 55/45 (v/v)

 Temperature: 40°C

25 Detection wavelength: 280 nm

 Flow rate: 1.0 mL/min

 Injection: 10 µL

 Time: 30 min

[0084]

30 Test Example 1

Preparation of Preculture Medium

 A preculture medium was prepared by dissolving 35.4 g of Anaerobe Basal Broth (ABB) medium available from Thermo scientific Inc. in 1 L of water. 10 mL of the preculture medium was dispensed into a test tube, and butyl rubber stoppers were put thereon. After gas replacement with nitrogen gas, sterilization was performed in an autoclave.

[0085]

Enzyme Treatment

To 16 g/L of soybean hypocotyl extract (containing 80% of isoflavone glycoside such as daidzin, glycitin, and genistin), 0.16 g/L of pectinase G Amano (available from Amano Enzyme Inc.) was added, and the mixture was kept at 50°C overnight with stirring to release sugars from the isoflavone glycoside.

[0086]

Preparation of Main Culture Medium

To the liquid subjected to the enzyme treatment, components were added to achieve final concentrations of 35.4 g/L of the ABB medium, 1 g/L of arginine, and 16 g/L of β -cyclodextrin, and 1 L of the mixture was dispensed into a 2 L volume mini jar. After replacement with nitrogen gas, sterilization was performed in an autoclave.

[0087]

Preculture

The microorganisms were inoculated into the preculture medium in a combination shown in Table 1, and then the atmosphere was replaced with an anaerobic gas and cultured at 37°C and 200 spm for 2 days.

As the microorganism having an equol-producing ability, any of *Adlercreutzia equolifaciens subsp. celatus* DSM 18785 strain (denoted as AC in the table) or *Adlercreutzia equolifaciens* DSM 19450 strain (denoted as AE in the table) was used. They also have an equol derivative- (in particular, 5-hydroxyequol-)producing ability.

As the microorganism having a sugar assimilation ability, *Clostridium asparagiforme* DSM 15981 strain (denoted as CA in the table), *Clostridium bolteae* JCM 12243 strain (denoted as CB in the table), *Lactobacillus brevis* DSM 20054 strain (denoted as LB in the table), *Lactococcus lactis* DSM 20481 strain (denoted as LL in the table), or *Leuconostoc mesenteroides subsp. mesenteroides* DSM 20343 strain (denoted as LM in the table) was used.

[0088]

Main Culture

The cells were inoculated from the preculture medium into the main culture medium, and then cultured at 37°C and 500 rpm for 2 days while being aerated with an anaerobic gas through a 0.22 μ m filter.

[0089]

Results

After the culture, the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration were measured. The results are

shown in Table 1. The glucose concentration expressed by "g/kg" indicates the glucose content based on the solid content.

[0090]

[Table 1]

Table 1

	Microorganism having equol- producing ability	Microorganism having sugar assimilation ability	Equol concentration (g/L)	5- Hydroxyequol concentration (g/L)	Glucose concentration (g/L)	Glucose concentration (g/kg)
Comparative Example 1	AC	-	1.79	0.27	3.8	58.5
Comparative Example 2	AE	-	1.78	0.26	3.9	60.1
Example 1	AC	CA	1.75	0.23	< 0.1	< 1.5
Example 2	AC	LB	1.81	0.28	< 0.1	< 1.5
Example 3	AE	CA	1.76	0.24	< 0.1	< 1.5
Example 4	AC	CB	1.75	0.24	< 0.1	< 1.5
Example 5	AC	LL	1.75	0.23	< 0.1	< 1.5
Example 6	AC	LM	1.74	0.23	< 0.1	< 1.5

5

[0091]

The results of Comparative Examples 1 and 2 and Example 1 to 6 indicate that equol and 5-hydroxyequol were produced by culturing a microorganism having an equol-producing ability. Furthermore, the results of Examples 1 to 6 indicate that co-culturing of a microorganism having a sugar assimilation ability assimilated sugar and no glucose remained in the culture liquid.

10

[0092]

Test Example 2

15 Preparation of Preculture Medium

The preculture medium was prepared in the same manner as in Test Example 1.

[0093]

Enzyme Treatment

20

To 16 g/L of soybean hypocotyl extract (containing 80% of isoflavone glycoside such as daidzin, glycitin, and genistin), 0.16 g/L of pectinase G

Amano was added, and the mixture was kept at 50°C overnight with stirring to release sugars from the isoflavone glycoside.

[0094]

Preparation of Main Culture Medium

5 To the liquid subjected to the enzyme treatment, components were added to achieve final concentrations of 35.4 g/L of the ABB medium, 1 g/L of arginine, and 16 g/L of β -cyclodextrin, and 50 mL of the mixture was dispensed into each vial, and butyl rubber stoppers were put thereon. After replacement with nitrogen gas, sterilization was performed in an autoclave.

10 [0095]

Preculture

The microorganism having an equol-producing ability shown in Table 2 was inoculated into the preculture medium, and then the atmosphere was replaced with an anaerobic gas and cultured at 37°C and 200 spm for 2 days.

15 [0096]

Main Culture

The cells were inoculated from the preculture into the main culture medium, and then aerated with an anaerobic gas and cultured at 37°C and 200 spm for 3 days.

20 Those obtained by culturing as described above were used as Comparative Examples 3 and 4, and the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration were measured.

[0097]

Yeast Treatment

25 To the culture liquids of Comparative Examples 3 and 4, 1 g of a bread yeast (SUPER CAMELLIA Dry Yeast, available from Nisshin Seifun Welna Inc.) was added per 100 mL of the culture liquid, and the mixtures were stored at 30°C overnight to provide Examples 7 and 8.

[0098]

30 Results

The measurement results of the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration in Comparative Examples 3 and 4 and Examples 7 and 8 are shown in Table 2.

[0099]

35 [Table 2]

Table 2

	Microorganism having equol- producing ability	Microorganism having sugar assimilation ability	Equol concentration (g/L)	5- Hydroxyequol concentration (g/L)	Glucose concentration (g/L)	Glucose concentration (g/kg)
Comparative Example 3	AC	-	1.80	0.27	4.3	66.2
Comparative Example 4	AE	-	1.80	0.28	4.1	63.2
Example 7	AC	Bread yeast	1.80	0.27	0.15	2.0
Example 8	AE	Bread yeast	1.80	0.28	0.18	2.4

[0100]

The glucose concentration was significantly decreased in Examples 7 and 8, which were after the yeast treatment, as compared with Comparative Examples 3 and 4, which were after the main culture. It is considered that glucose was assimilated by the yeast. The yeast-treated product had a smell unique to yeast and had a good flavor.

[0101]

Test Example 3

The liquids of Comparative Example 3 and Example 7 were centrifuged to remove the cells and insoluble components, and the supernatant was collected. The absorbance of the supernatant at 420 nm was measured. After the supernatant was heated at 80°C for 2 hours, the absorbance was measured in the same manner. The results are shown in Table 3.

[0102]

[Table 3]

Table 3

	Before heating	After heating
Comparative Example 3	0.88	1.40
Example 7	0.86	0.92

[0103]

In Comparative Example 3, the absorbance was increased by heating. It is considered that the Maillard reaction proceeded due to the remaining glucose, and browning occurred. In contrast, in Example 7, the glucose

concentration was decreased to 0.2 g/L or less by the action of the yeast, and thus, no increase in absorbance was observed even after the heating treatment.

[0104]

Test Example 4

- 5 Glycine was added to each of the liquids of Comparative Example 3 and Example 7 to achieve 2 g/L, the mixture was then centrifuged to remove the cells and insoluble components, and the supernatant was collected. The absorbance of the supernatant at 420 nm was measured. In addition, glycine was added to each of the liquids of Comparative Example 3 and Example 7 to achieve 2 g/L, the
10 mixture was then heated at 80°C for 2 hours, and the absorbance was measured in the same manner. The results are shown in Table 4.

[0105]

[Table 4]

Table 4

	Addition of glycine, before heating	Addition of glycine, after heating
Comparative Example 3	0.87	1.52
Example 7	0.87	0.92

- 15 [0106]

In Comparative Example 3, the absorbance was increased by heating. It is considered that the Maillard reaction proceeded due to the remaining glucose and the added glycine, and browning occurred. In contrast, in Example 7, the
20 glucose concentration was decreased to 0.2 g/L or less by the action of the yeast, and thus, no increase in absorbance was observed even after the addition of glycine and the heating treatment.

[0107]

Test Example 5

- The main culture was performed in the same manner as in Test Example
25 2. After the culture, the production of equol and 5-hydroxyequol was confirmed by HPLC analysis, and the glucose concentration was measured. After the remaining of glucose was confirmed, commercially available glucose oxidase (Amano Enzyme Inc.) and peroxidase (available from FUJIFILM Wako Pure Chemical Corporation) were added, and the mixture was stored at 30°C
30 overnight. Thereafter, the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration were measured. In Table 5, the culture liquids before the enzyme addition treatment are shown as Comparative

Examples 5 and 6, and the culture liquids after the enzyme addition treatment are shown as Examples 9 and 10.

[0108]

It is found that glucose is decomposed by the enzyme addition treatment and no glucose remains in the liquid after the treatment.

[0109]

[Table 5]

Table 5

	Microorganism having equol-producing ability	Enzyme treatment	Equol concentration (g/L)	5-Hydroxyequol concentration (g/L)	Glucose concentration (g/L)	Glucose concentration (g/kg)
Comparative Example 5	AC	-	1.80	0.27	4.2	64.7
Comparative Example 6	AE	-	1.80	0.28	4.3	66.2
Example 9	AC	Present	1.80	0.27	0.15	2.3
Example 10	AE	Present	1.80	0.28	0.18	2.8

[0110]

10 Test Example 6

Preparation of Preculture Medium

The preculture medium was prepared in the same manner as in Test Example 1.

Enzyme Treatment

15 To 16 g/L of soybean hypocotyl extract (containing 44% of isoflavone glycoside such as daidzin, glycitin, and genistin), 0.16 g/L of pectinase G Amano (available from Amano Enzyme Inc.) was added, and the mixture was kept at 50°C overnight with stirring to release sugars from the isoflavone glycoside.

[0111]

20 Preparation of Main Culture Medium

To the liquid subjected to the enzyme treatment, components were added to achieve final concentrations of 35.4 g/L of the ABB medium, 1 g/L of arginine, and 16 g/L of β -cyclodextrin, and 1 L of the mixture was dispensed into a 2 L volume mini jar. After replacement with nitrogen gas, sterilization was performed in an autoclave.

[0112]

Preculture

The microorganisms were inoculated into the preculture medium in a combination shown in Table 5, and then the atmosphere was replaced with an anaerobic gas and cultured at 37°C and 200 rpm for 2 days. The

5 microorganisms used were the same as those in Test Example 1.

Main Culture

The cells were inoculated from the preculture medium into the main culture medium, and then cultured at 37°C and 500 rpm for 2 days while being aerated with an anaerobic gas through a 0.22 µm filter.

10 Results

After the culture, the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration were measured. The results are presented in Table 6.

[0113]

15 [Table 6]

Table 6

	Microorganism having equol- producing ability	Microorganism having sugar assimilation ability	Equol concentration (g/L)	5- Hydroxyequol concentration (g/L)	Glucose concentration (g/L)	Glucose concentration (g/kg)
Comparative Example 7	AC	-	0.24	1.43	2.0	30.9
Comparative Example 8	AE	-	0.24	1.42	2.0	30.9
Example 11	AC	CA	0.23	1.42	< 0.1	< 1.5
Example 12	AC	LB	0.24	1.42	< 0.1	< 1.5
Example 13	AC	LL	0.24	1.41	< 0.1	< 1.5
Example 14	AE	CA	0.24	1.45	< 0.1	< 1.5
Example 15	AE	CB	0.25	1.45	< 0.1	< 1.5
Example 16	AE	LM	0.24	1.43	< 0.1	< 1.5

[0114]

The results of Comparative Examples 7 and 8 and Examples 11 to 16 indicate that equol and 5-hydroxyequol were produced by culturing the
20 microorganism having an equol-producing ability. Furthermore, the results of Examples 11 to 16 indicate that co-culturing of a microorganism having a sugar

assimilation ability assimilated sugar and no glucose remained in the culture liquid.

[0115]

Test Example 7

5 To the liquids of Comparative Examples 7 and 8, commercially available glucose oxidase (Amano Enzyme Inc.) and peroxidase (available from FUJIFILM Wako Pure Chemical Corporation) were added, and the mixture was stored at 30°C overnight. Thereafter, the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration were measured.

10 [0116]

(Results)

The culture liquids after the enzyme addition treatment were regarded as Examples 17 and 18, and the measurement results of the equol concentration, the 5-hydroxyequol concentration, and the glucose concentration in

15 Comparative Examples 7 and 8 and Examples 17 and 18 are shown in Table 7.

[0117]

[Table 7]

Table 7

	Microorganism having equol- producing ability	Enzyme treatment	Equol concentration (g/L)	5- Hydroxyequol concentration (g/L)	Glucose concentration (g/L)	Glucose concentration (g/kg)
Comparative Example 7	AC	-	0.24	1.43	2.0	30.9
Comparative Example 8	AE	-	0.24	1.42	2.0	30.9
Example 17	AC	Present	0.23	1.42	< 0.1	< 1.5
Example 18	AC	Present	0.24	1.42	< 0.1	< 1.5

[0118]

20 It is found that glucose is decomposed by the enzyme addition treatment and no glucose remains in the liquid after the treatment.

[0119]

Test Example 8

25 The liquids of Comparative Example 7 and Example 17 were centrifuged to remove the cells and insoluble components, and the supernatant was collected. The absorbance of the supernatant at 420 nm was measured. After the

supernatant was heated at 80°C for 2 hours, the absorbance was measured in the same manner. The results are shown in Table 8.

[0120]

[Table 8]

Table 8

	Before heating	After heating
Comparative Example 7	0.84	1.25
Example 17	0.83	0.84

5

[0121]

In Comparative Example 7, the absorbance was increased by heating. It is considered that the Maillard reaction proceeded due to the remaining glucose, and browning occurred. In contrast, in Example 17, the glucose
10 concentration was decreased to 0.1 g/L or less by the action of the enzyme, and thus, no increase in absorbance was observed even after the heating treatment.

Claims

[Claim 1]

A method for producing a composition, the method comprising the following step (a); step (b1) and/or step (b2); and step (c):

(a) producing glucose and an isoflavone aglycone from an isoflavone glycoside;

(b1) allowing a microorganism having an equol-producing ability to produce equol from the isoflavone aglycone;

(b2) allowing a microorganism having an equol derivative-producing ability to produce an equol derivative from the isoflavone aglycone; and

(c) decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in the composition, or 30 g/kg or less based on a solid content of the composition.

[Claim 2]

The method according to claim 1, wherein the step (c) is decomposing the glucose by fermentation using a microorganism having a sugar assimilation ability.

[Claim 3]

The method according to claim 2, wherein the microorganism having a sugar assimilation ability is one or more microorganisms selected from the group consisting of butyric acid bacteria, lactic acid bacteria, and yeasts.

[Claim 4]

The method according to claim 3, wherein the microorganism having a sugar assimilation ability is one or more microorganisms selected from the group consisting of microorganisms belonging to the genus *Clostridium*, microorganisms belonging to the genus *Lactobacillus*, microorganisms belonging to the genus *Lactococcus*, microorganisms belonging to the genus *Leuconostoc*, and microorganisms belonging to the genus *Saccharomyces*.

[Claim 5]

The method according to claim 1, wherein the step (c) is decomposing the glucose using an enzyme that decomposes glucose.

[Claim 6]

The method according to claim 5, wherein the enzyme that decomposes glucose is one or more selected from the group consisting of glucose oxidase and glucose dehydrogenase.

5 [Claim 7]

The method according to any one of claims 1 to 6, wherein the step (b1) and/or the step (b2), and the step (c) are performed simultaneously.

[Claim 8]

10 The method according to any one of claims 1 to 6, wherein the isoflavone glycoside is daidzin, and the isoflavone aglycone is daidzein.

[Claim 9]

15 The method according to any one of claims 1 to 6, wherein the step (a) is allowing β -glucosidase to act on an isoflavone glycoside to produce glucose and an isoflavone aglycone.

[Claim 10]

20 A method for producing a food and/or beverage, the method comprising the following step (a); step (b1) and/or step (b2); step (c); and step (d):

(a) producing glucose and an isoflavone aglycone from an isoflavone glycoside;

(b1) allowing a microorganism having an equol-producing ability to produce equol from the isoflavone aglycone;

25 (b2) allowing a microorganism having an equol derivative-producing ability to produce an equol derivative from the isoflavone aglycone; and

(c) decomposing the glucose to achieve a content of the glucose of 0 g/L or more and less than 2.0 g/L in a composition produced by a method comprising step (a), step (b1) and/or step (b2), and step (c), or 30 g/kg or less
30 based on a solid content of the composition; and

(d) producing a food and/or beverage using the composition produced by the method comprising step (a), step (b1) and/or step (b2), and step (c).

[Claim 11]

35 A fermentation composition comprising equol and/or an equol derivative, and comprising substantially no glucose.

[Claim 12]

The fermentation composition according to claim 11, wherein comprising substantially no glucose is comprising 0 g/L or more and less than 2.0 g/L of glucose.

5 [Claim 13]

A fermentation composition comprising equol and/or an equol derivative, and glucose, wherein

a content of the glucose is 30 g/kg or less based on a solid content of the fermentation composition.

10

[Claim 14]

The fermentation composition according to claim 11 or 13, wherein the fermentation composition is used for a food and/or beverage.