

DESCRIPTION

Title of Invention: METHOD FOR PRODUCING FERMENTED MILK HAVING
IMPROVED PHYSICAL PROPERTIES

5

Technical Field
[0001]

10 The present invention relates to a method for producing
fermented milk that is excellent in quality, in which a change in
physical properties caused by coagulation of milk protein during
chilled storage or distribution (occurrence of syneresis, an
increase in particle size of milk protein, or thus-developed
grainy texture) is suppressed.

Background Art
15 [0002]

It is known that milk-derived protein in fermented milk,
such as yogurt, coagulates during chilled storage or refrigerated
transport (distribution), and that its physical properties
(texture) are accordingly changed. Specifically, it is known that
20 the coagulation of milk protein causes syneresis, and that the
particles of milk protein generated due to coagulation become
larger over time, which leads to a gritty feeling in the mouth;
thus, both the appearance and flavor deteriorate, which results
in a decrease in quality.

25 [0003]

In order to solve the problem, PTL 1 proposes a method
of homogenization treatment in which a specific amount of whey
hydrolysate is added when producing fermented milk; PTL 1
discloses that this method can suppress coagulation and
30 precipitation of suspended milk protein particles. PTL 2 to 5
also disclose that peroxidase is added together with a lactic
acid bacteria starter to a mix of starting milk materials of
fermented milk to ferment the mix; thus, whey separation and
protein coagulation that occur during the storage or distribution
35 process are suppressed, thereby producing fermented milk products

with a fine and smooth texture. However, there is no teaching regarding the use of glucose oxidase.

[0004]

It has been heretofore known that glucose oxidase has
5 an antibacterial effect. The antibacterial effect is attributed to the fact that glucose generates hydrogen peroxide when oxidized by glucose oxidase. As a method for producing fermented milk using this antibacterial effect of glucose oxidase, PTL 6
10 discloses a method for producing an antibody-containing fermented food product, in which glucose oxidase is added to raw milk as a hydrogen-peroxide generator to kill harmful microorganisms in the raw milk to prevent the antibody being deactivated by heating, and subsequently fermentation is performed using lactic acid bacteria. In PTL 6, glucose oxidase is disclosed together with
15 its substrate, glucose, merely as a hydrogen-peroxide generator, i.e., a bactericide. Moreover, in the production method disclosed in PTL 6, heating treatment at 63°C or over must be avoided to prevent the antibody from being deactivated; thus, the coagulation of milk protein does not become a problem. In other
20 words, suppressing a change in physical properties caused by coagulation of milk protein is not an agenda in PTL 6, and the use of glucose oxidase to address such a problem is therefore not suggested.

Citation List

25 Patent Literature

[0005]

PTL 1: JP2007-006738A

PTL 2: JP62-228224A

PTL 3: JP6-276933A

30 PTL 4: JP10-262550A

PTL 5: JP10-099019A

PTL 6: JP2007-053930A

Summary of Invention

Technical Problem

35 [0006]

An object of the present invention is to provide a method for producing fermented milk in which a change in physical properties caused by coagulation of milk protein, which occurs during chilled storage or distribution, is suppressed; and
5 specifically a method for producing fermented milk in which occurrence of syneresis, an increase in particle size of milk protein, or thus-developed grainy texture is suppressed. Further, an object of the present invention is to provide a method for suppressing the change in physical properties caused by the
10 coagulation of milk protein of fermented milk.

Solution to Problem

[0007]

To solve the problem, the present inventors conducted extensive research, and discovered that, as compared to
15 the case where no glucose oxidase is added, adding glucose oxidase and allowing reaction in the presence of its substrate in the production process of fermented milk significantly suppresses syneresis or an increase in particle size of milk protein, which occurs along with coagulation of milk protein. The present
20 inventors confirmed that the method is convenient as it can be applied to the current production process of fermented milk; moreover, the method does not negatively affect the original flavor of fermented milk, and maintains an appropriate level of physical properties and excellent flavor required of fermented
25 milk for a longer period of time in a stable manner. Consequently, the present inventors completed the present invention.

[0008]

Specifically, the present invention relates to the embodiments described below.

30 [0009]

I. Method for producing fermented milk having improved physical properties

I-1: A method for producing a fermented milk comprising the following three steps:

35 (1) preparing a fermented milk mix using one or more

starting milk materials;

(2) heat-pasteurizing the fermented milk mix; and

(3) adding a starter to the heat-pasteurized fermented milk mix to ferment the mix,

5 wherein, in performing steps (1) to (3) above, at least one of the following operations (A) to (C) is carried out:

(A) adding glucose oxidase to said one or more starting milk materials to prepare the fermented milk mix in step (1), wherein said one or more starting milk materials contain a
10 substance that can serve as a substrate for the glucose oxidase;

(B) adding glucose oxidase and a substance that can serve as a substrate for the glucose oxidase to the heat-pasteurized fermented milk mix before step (3); and

(C) adding glucose oxidase and a substance
15 that can serve as a substrate for the glucose oxidase to the fermented milk after step (3).

[0010]

I-2: The method according to I-1, wherein the starter is a combination of *Lactobacillus bulgaricus* and *Streptococcus*
20 *thermophilus*.

[0011]

I-3: The method according to I-1 or I-2, which is a method for producing a fermented milk that undergoes less change in physical properties caused by coagulation of milk protein than a fermented
25 milk produced without using glucose oxidase.

[0012]

I-4: The method according to any one of I-1 to I-3, wherein the change in physical properties caused by coagulation of milk protein is at least one selected from the group consisting of
30 occurrence of syneresis (water separation), an increase in particle size of milk protein (and/or fat), and development of grainy texture of the fermented milk.

[0013]

I-5: The method according to any one of I-1 to I-4, wherein the
35 fermented milk is yogurt.

[0014]

I-6: A fermented milk obtained by any method of I-1 to I-5, wherein the change in physical properties caused by coagulation of milk protein is suppressed.

5 [0015]

II. Method for improving physical properties of fermented milk

II-1: A method for suppressing a change in physical properties caused by coagulation of milk protein in a fermented milk produced by a method comprising the following steps:

- 10 (1) preparing a fermented milk mix using one or more starting milk materials;
- (2) heat-pasteurizing the fermented milk mix; and
- (3) adding a starter to the heat-pasteurized fermented milk mix to ferment the mix,

15 wherein, in performing steps (1) to (3) above, at least one of the following operations (A) to (C) is carried out:

 (A) adding glucose oxidase to said one or more starting milk materials to prepare the fermented milk mix in step (1), wherein said one or more starting milk materials contain a

20 substance that can serve as a substrate for the glucose oxidase;

 (B) adding glucose oxidase and a substance that can serve as a substrate for the glucose oxidase to the heat-pasteurized fermented milk mix before step (3); and

 (C) adding glucose oxidase and a substance

25 that can serve as a substrate for the glucose oxidase to the fermented milk after step (3).

[0016]

II-2. The method according to II-1, wherein the starter is a combination of *Lactobacillus bulgaricus* and *Streptococcus*

30 *thermophilus*.

[0017]

II-3: The method according to II-1 or II-2, which suppresses a change in physical properties caused by coagulation of milk protein, unlike the method for producing a fermented milk without

35 using glucose oxidase.

[0018]

II-4: The method according to any one of II-1 to II-3, wherein the change in physical properties caused by coagulation of milk protein is at least one selected from the group consisting of
5 occurrence of syneresis (water separation), an increase in particle size of milk protein (and/or fat), and development of grainy texture of the fermented milk.

[0019]

II-5: The method according to any one of II-1 to II-4, wherein
10 the fermented milk is yogurt.

Advantageous Effects of Invention

[0020]

The method of the present invention enables production of fermented milk that undergoes significantly less change in
15 physical properties caused by coagulation of milk protein during chilled storage or distribution, specifically, occurrence of syneresis, an increase in particle size of milk protein, or thus-developed grainy texture, than fermented milk produced without adding glucose oxidase thereto.

20 [0021]

The method of the present invention is convenient as it can be applied to the current production process of fermented milk; moreover, the method does not negatively affect the original flavor of fermented milk while maintaining an
25 appropriate level of the physical properties and excellent flavor required of fermented milk for a longer period of time in a stable manner.

Description of Embodiments

30 [0022]

I. Method for producing fermented milk having improved physical properties

The method for producing a fermented milk of the present invention comprises at least the following three steps:

35 (1) preparing a fermented milk mix using one or more

starting milk materials;

(2) heat-pasteurizing the fermented milk mix; and

(3) adding a starter to the heat-pasteurized fermented milk mix to ferment the mix,

5 wherein, in performing steps (1) to (3) above, at least one of the following operations (A) to (C) is carried out:

(A) adding glucose oxidase to said one or more starting milk materials to prepare the fermented milk mix in step (1), wherein said one or more starting milk materials contain a
10 substance that can serve as a substrate for the glucose oxidase;

(B) adding glucose oxidase and a substance that can serve as a substrate for the glucose oxidase to the heat-pasteurized fermented milk mix before step (3); and

(C) adding glucose oxidase and a substance
15 that can serve as a substrate for the glucose oxidase to the fermented milk after step (3).

[0023]

As used herein, "fermented milk" means "the products which are obtained by fermenting milk, or milk, etc. containing
20 an equal or greater amount of milk solids-not-fat with lactic acid bacteria or yeast and then forming a paste or liquid, or the frozen product" as defined in the Ministerial Ordinance on Milk and Milk Products Concerning Compositional Standards, etc. Fermented milk includes yogurt (milk solids-not-fat: 8% or more,
25 lactic acid bacteria count or yeast count: 10 million or more per 1 mL, as defined by an Ordinance of the Ministry of Health, Labour and Welfare of Japan), fermented milk drink under the category of milk product (milk solids-not-fat content: less than 8%, lactic acid bacteria count or yeast count: 10 million or more
30 per 1 mL, as defined by an Ordinance of the Ministry of Health, Labour and Welfare of Japan), and fermented milk drink (milk solids-not-fat content: less than 3%, lactic acid bacteria count or yeast count: 10 million or more per 1 mL, as defined by an Ordinance of the Ministry of Health, Labour and Welfare of Japan).
35 A preferable fermented milk is yogurt.

[0024]

According to the preparation method, yogurt is classified into two types: "advance fermentation-type," and "subsequent fermentation-type." The former, advance fermentation-type yogurt, is produced by a method comprising the steps of: fermenting a fermentation mix in a tank, the fermentation mix being prepared by adding a fermented milk starter to a raw milk material; squishing the produced curd; and packing the squished curd into individual-portion containers optionally with a gelatinizing agent. The latter, subsequent fermentation-type yogurt, is produced by the method comprising the steps of: adding a fermented milk starter to a raw milk material to prepare a fermentation mix; and packing the fermentation mix into individual-portion containers to ferment the mix in the containers. Yogurt intended in the present invention includes these two types of yogurt, "advance fermentation-type" yogurt and "subsequent fermentation-type" yogurt. In the case of "subsequent fermentation-type" produced through fermentation in individual-portion containers, operation (C) cannot be performed; i.e., glucose oxidase or the like cannot be added to fermented milk after fermentation step (3). Thus, when producing "subsequent fermentation-type" yogurt, the production method of the present invention is carried out by performing operation (A) in step (1) above, or by performing operation (B) before step (3). However, in the case of "advance fermentation-type" produced by packing into individual-portion containers after fermentation, there is no such limitation, and at least one of the following operations can be performed: operation (A) in step (1); operation (B) before step (3); or operation (C) after step (3).

[0025]

Hereinafter, production steps (1) to (3) of the present invention will be explained in order.

(1) Step of preparing a fermented milk mix using a starting milk material

The term "starting milk material" refers to a dairy

product that is used as a raw material of fermented milk. A starting milk material is suitably selected from, for example, raw milk, milk that has been pasteurized (pasteurized milk), skim milk, whole milk powder, skim milk powder, butter, buttermilk, cream, whey protein concentrate (WPC), whey protein isolate (WPI), etc., depending on the type of fermented milk to be produced. These can be used in a combination of two or more; for example, for producing calorie-reduced yogurt with 8% or more of a milk solids-not-fat content, raw milk and/or milk that has been pasteurized (pasteurized milk) and skim milk and/or a skim milk powder may be combined.

[0026]

A "fermented milk mix" is a raw material preparation that is a mixture of raw materials of fermented milk. Such a fermented milk mix is prepared by optionally adding to and blending with the aforementioned starting milk materials, water, a sweetening additive such as saccharides, typically sucrose, or a sweetener, and a flavor, followed by dissolution optionally with heating. Further, a stabilizer (gelatinizer), such as gelatin, agar, carrageenan, guar gum, low methoxyl pectin, or high methoxyl pectin, can be optionally added thereto. The proportions of raw materials contained in a fermented milk mix are not particularly limited insofar as the milk solids-not-fat content satisfies the proportion predetermined for each type of fermented milk (yogurt: not less than 8%, fermented milk drink under the category of milk product: less than 8%, fermented milk drink: less than 3%). For example, when using raw milk (and/or pasteurized milk, etc.) and a skim milk powder as starting milk materials, it is preferable that the proportion of raw milk (and/or pasteurized milk) is greater than 0 wt% and not more than 100 wt%, preferably greater than 0 wt% and not more than 90 wt%, more preferably 20 to 80 wt%, still more preferably 40 to 80 wt%; and that the proportion of a skim milk powder is greater than 0 wt% and not more than 12 wt%, preferably 1 to 10 wt%, more preferably 2 to 8 wt%, and still more preferably 2 to 6 wt%. The

production method in which operation (A) is carried out in step
(1) is one embodiment of the present invention, and is
characterized in that when preparing the aforementioned fermented
milk mix, glucose oxidase is added to and mixed with the
5 fermented milk mix as one of the raw materials of fermented milk.
[0027]

At this stage, when a substance that can serve as a
substrate for the glucose oxidase is not yet contained in the
fermented milk mix, a substance that can serve as a substrate for
10 the glucose oxidase is added to and mixed with the fermented milk
mix together with glucose oxidase. When glucose oxidase is added
to a fermented milk mix that has been prepared with heating, the
enzyme may be deactivated. For this reason, when adding glucose
oxidase, it is preferable to cool the fermented milk mix to a
15 temperature at which the enzyme is not deactivated, preferably to
room temperature ($25\pm 5^{\circ}\text{C}$) or below, more preferably to 15°C or
below.
[0028]

As used herein, the "substance that can serve as a
20 substrate for the glucose oxidase" refers not only to a substrate
for the glucose oxidase (glucose), but also a substance that
generates the substrate (glucose). The substance that generates
glucose specifically includes a combination of an enzyme that
generates glucose and its substrate, such as the combination of
25 lactase and lactose; however, it is not particularly limited
thereto.
[0029]

For glucose oxidase, a commercially available glucose
oxidase preparation can be used. Examples of commercially
30 available glucose oxidase preparations include enzyme
preparations distributed by Shin Nihon Kagaku Kogyo Co., DKSH
Japan K.K., Shonan Wako Junyaku, Nagase Seikagaku Kogyo, Kyowa
Enzyme, and Danisco Cultor Japan Ltd. (all are based in Japan).
[0030]

35 For example, when using glucose oxidase and glucose as

part of starting milk materials, the proportion of glucose oxidase (approx. 2000 to 4000 units/kg) in a fermented milk mix is 0.1 to 1 wt%, preferably 0.1 to 0.5 wt%, more preferably 0.15 to 0.3 wt%, and still more preferably 0.15 to 0.2 wt%; and the
5 proportion of glucose is 1 to 5 wt%, preferably 1 to 4 wt%, more preferably 1.5 to 3 wt%, and still more preferably 1.5 to 2.5 wt%.
[0031]

For lactase, a commercially available lactase preparation can be used. Similarly to the aforementioned oxidase
10 preparation, examples of commercially available lactase preparations include enzyme preparations distributed by Shin Nihon Kagaku Kogyo Co., Amano Enzyme Inc., Daiwa Fine Chemicals Co., Ltd., Nagase Chemtex Corporation, and Godo Shusei Co., Ltd. (all are based in Japan).

15 [0032]

For example, when using lactase and lactose as part of starting milk materials, the proportion of lactase (approx. 4000 to 6000 units/kg) in a fermented milk mix is 0.01 to 0.1 wt%, preferably 0.02 to 0.1 wt%, more preferably 0.03 to 0.08 wt%, and
20 still more preferably 0.03 to 0.05 wt%; and the proportion of lactose is 2 to 10 wt%, preferably 2 to 8 wt%, more preferably 3 to 6 wt%, and still more preferably 3 to 5 wt%.
[0033]

A fermented milk mix to which lactase has been added
25 and mixed may be allowed to stand undisturbed in advance (preliminary static incubation) at a temperature of 0 to 40°C, preferably 1 to 30°C, more preferably 3 to 15°C, and still more preferably 5 to 10°C. Although not particularly limited, this static incubation time period is ordinarily 1.5 hours or more,
30 preferably 1.5 to 24 hours, more preferably 2 to 16 hours, still more preferably 2 to 6 hours, and particularly more preferably 2 to 4 hours.

[0034]

As described above, a fermented milk mix to which
35 glucose oxidase has been added and mixed in the presence of

glucose that can serve as a substrate for glucose oxidase, or in the presence of an enzyme that generates the glucose and its substrate (e.g., lactase and its substrate, lactose) is allowed to stand undisturbed at a temperature of 0 to 40°C, preferably 1 to 30°C, more preferably 3 to 15°C, and still more preferably 5 to 10°C. Although not particularly limited, this static incubation time period is ordinarily 1.5 hours or more, preferably 1.5 to 24 hours, more preferably 2 to 16 hours, still more preferably 2 to 6 hours, and particularly more preferably 2 to 4 hours. When adding an enzyme that generates glucose, its substrate, and glucose oxidase to a fermented milk mix substantially at the same time, the fermented milk mix may be immediately allowed to stand undisturbed under the same conditions as described above without performing the preliminary static incubation as mentioned in the previous paragraph.

[0035]

(2) Step of heat-pasteurizing a fermented milk mix

The fermented milk mix prepared in step (1) is subsequently subjected to pasteurization by heating (the mix refers to both the mix prepared by carrying out operation (A) and the mix prepared by not carrying out operation (A)).

[0036]

The heating temperature and the heating duration are not particularly limited insofar as intended pasteurization can be performed. It suffices if the temperature of the fermented milk mix itself is at least 90°C or over, preferably about 95°C, and the pasteurization method includes, but is not limited to, a method comprising treating the fermented milk mix at 90 to 100°C for 1 to 5 minutes, and a method comprising treating the fermented milk mix at 90 to 95°C for 1 to 3 minutes.

[0037]

The production method in which operation (B) is carried out before step (3) is one embodiment of the present invention, and is characterized in that glucose oxidase is added to and mixed with the fermented milk mix that has been heat-pasteurized

in step (2) according to the above-described method.

[0038]

A fermented milk mix to be used in the embodiment is preferably the mix prepared without carrying out operation (A) in step (1). Thus, at this stage, when a substance that can serve as a substrate for the glucose oxidase is not yet contained in the fermented milk mix, a substance that can serve as a substrate for the glucose oxidase is added to and mixed with the fermented milk mix together with glucose oxidase. In this case, when glucose oxidase is added to the fermented milk mix that remains at a high temperature, the enzyme may be deactivated. Thus, when adding glucose oxidase, it is preferable to cool the fermented milk mix in advance to a temperature at which the enzyme is not deactivated, preferably to room temperature ($25\pm 5^{\circ}\text{C}$) or below, and more preferably to 15°C or below.

[0039]

As used herein, the "substance that can serve as a substrate for the glucose oxidase" is as mentioned above; and the amount of glucose oxidase and lactase to be used, the source thereof, and the amount of glucose and lactose to be used are also as mentioned above.

[0040]

(3) Step of adding a fermented milk starter to a heat-pasteurized fermented milk mix to ferment the mix

The fermented milk mix that has been heat-pasteurized in step (2) is subsequently subjected to fermentation treatment (the mix refers to the mix prepared by carrying out operation (A), the mix prepared by carrying out operation (B), and the mix prepared by carrying out neither of operations (A) and (B)).

[0041]

The "starter" refers to a seed strain, such as a lactic acid bacterium or yeast, which is inoculated into a fermented milk mix to ferment the mix. Although a known starter can be suitably used in the present invention, a preferable starter is a lactic acid bacteria starter. As a lactic acid bacteria starter,

one or at least two species selected from *Lactobacillus bulgaricus* (*L. bulgaricus*), *Streptococcus thermophilus* (*S. thermophilus*), *Lactobacillus lactis* (*L. lactis*), *Lactobacillus gasseri* (*L. gasseri*), *Bifidobacterium*, or other lactic acid bacteria that are typically used in the production of fermented milk may be used. Among them, a lactic acid bacteria starter based on a mixed starter of *Lactobacillus bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*), which are defined as a yogurt starter in the Codex Alimentarius, can be preferably used. While using this lactic acid bacteria starter as a base, other lactic acid bacteria such as *Lactobacillus gasseri* (*L. gasseri*) and *Bifidobacterium* may be further added, taking into consideration the fermentation temperature and fermentation conditions for the desired fermented milk.

[0042]

The additive amount of a starter may be suitably determined with reference to the additive amount of starters adopted in known methods for producing fermented milk. The inoculation method for a starter is not particularly limited, and any method conventionally used in the production of fermented milk may be suitably used.

[0043]

The fermentation treatment conditions can be suitably determined, taking into consideration the type of fermented milk, the desired flavor, and the type of starter to be used. Examples include a fermentation method in which the temperature of a fermentation chamber (fermentation temperature) is maintained in the range of 30 to 50°C, and static fermentation is performed in the fermentation chamber. Under such temperature conditions, lactic acid bacteria normally become active, and fermentation can therefore be carried out effectively. The fermentation temperature is typically about 30 to 50°C, preferably about 35 to 45°C, and more preferably about 37 to 43°C.

[0044]

The fermentation time can be suitably determined and adjusted with a view to achieving a predetermined lactic acid acidity of a fermented milk mix. The lactic acid acidity of the "advance fermentation-type" yogurt is about 1.5 to 2%, and the lactic acid acidity of the "subsequent fermentation-type" yogurt is about 0.7 to 0.8%, for example. The fermentation time is typically about 1 hour or more to about 12 hours or less, preferably about 2 hours or more to about 5 hours or less, and more preferably about 3 hours or more to about 4 hours or less.

10 [0045]

When the lactic acid acidity of the "advance fermentation-type" yogurt and the lactic acid acidity of the "subsequent fermentation-type" yogurt reach about 1.5 to 2% and about 0.7 to 0.8%, respectively, fermentation should be terminated by cooling to, for example, 15°C or below, preferably 0 to 10°C, and more preferably 3 to 7°C.

[0046]

In the case of "advance fermentation-type" yogurt, it is possible to carry out operation (C) after step (3).

20 Specifically, glucose oxidase can be added to and mixed with fermented milk that has been prepared in advance. The production method including operation (C) is one embodiment of the production method according to the present invention. A fermented milk mix intended here is preferably the mix that has been prepared by carrying out neither operation (A) in step (1) nor operation (B) before step (3).

25 [0047]

At this stage, when fermented milk prepared in advance does not include a substance that can serve as a substrate for the glucose oxidase, a substance that can serve as a substrate for the glucose oxidase is added to and mixed with the fermented milk together with glucose oxidase. As used herein, the "substance that can serve as a substrate for the glucose oxidase" is as mentioned above, and the amount of glucose oxidase and lactase to be used, the source thereof, and the amount of glucose

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and lactose to be used are also as mentioned above.

[0048]

Fermented milk intended by the present invention can be produced through steps (1) to (3) described above. When fermented
5 milk is "advance fermentation-type" yogurt, the incubation process in step (3) is performed using a tank (tank incubation). After tank incubation, fermented and thus-coagulated curd is squished by stirring, followed by pasteurizing, cooling, emulsifying and aging as necessary. Thereafter, the resulting
10 mixture is packed into market containers (individual-portion containers), and prepared as a fermented milk product. In contrast, when fermented milk is "subsequent fermentation-type" yogurt, the fermented milk mix is packed into market containers before the incubation process of step (3), and the fermented milk
15 mix in the market containers (individual-portion containers) are subjected to the incubation process of step (3) (in-container incubation). After in-container incubation, the resulting product is cooled (cold storage) and prepared as a fermented milk product.
[0049]

20 II. Method for improving physical properties of fermented milk

The method for improving physical properties of a fermented milk according to the present invention is a method for suppressing a change in the physical properties caused by coagulation of milk protein in a fermented milk product produced by the method
25 comprising at least the following three steps:

(1) preparing a fermented milk mix using one or more starting milk materials;

(2) heat-pasteurizing the fermented milk mix; and

(3) adding a starter to the heat-pasteurized fermented
30 milk mix to ferment the mix,

wherein, in performing steps (1) to (3) above, at least one of the following operations (A) to (C) is carried out:

(A) adding glucose oxidase to said one or more starting milk materials to prepare the fermented milk mix in step

35 (1), wherein said one or more starting milk materials contain a

substance that can serve as a substrate for the glucose oxidase;

(B) adding glucose oxidase and a substance that can serve as a substrate for the glucose oxidase to the heat-pasteurized fermented milk mix before step (3); and

5 (C) adding glucose oxidase and a substance that can serve as a substrate for the glucose oxidase to the fermented milk after step (3).

[0050]

In the method of the present invention described above,
10 the treatments and conditions of each step and operation, and the materials used in each step and operation may be the same as those used in the section "I. Method for producing fermented milk having improved physical properties."

[0051]

15 As will be shown in the Examples below, the fermented milk produced and treated as described above underwent significantly less change in physical properties caused by coagulation of milk protein than the fermented milk produced without carrying out any of operations (A) to (C), i.e.,
20 fermented milk that has been produced by not using glucose oxidase (hereinafter referred to as "control fermented milk"). The change in physical properties caused by coagulation of milk protein is at least one selected from occurrence of syneresis, an increase in particle size of milk protein, and development of
25 grainy texture of fermented milk. The grainy texture of fermented milk is presumably, although not limited thereto, a phenomenon caused by an increase in particle size of milk protein. The change in physical properties of any fermented milk increases (worsens) over time during storage; however, the fermented milk
30 of the present invention exhibited significantly less increase over time (change in physical properties) than the control fermented milk. Therefore, the method of the present invention is useful to keep fermented milk excellent in quality (appearance and texture) for as long as possible, at least until the best-
35 before date. As far as the best-before date of yogurt is

concerned, the duration during which the fermented milk maintains excellent quality is about 10 days from the manufacturing date, preferably 12 days or longer, and more preferably 14 days or longer.

5 Examples

[0052]

Hereinafter, the present invention will be described in detail with reference to Experimental Examples and Examples.

However, the present invention is not limited thereto, and

10 various modifications can be added based on known techniques.

[0053]

Experimental Example 1

1-1: Production of fermented milk

Following the formulation shown in Table 1, four kinds
15 of fermented milk 1 to 4 were prepared.

[0054]

Table 1

Table 1. Formulation of a raw milk material (yogurt mix) (1)

Raw Material	Fermented milk 1 (Comparative Example 1)	Fermented milk 2 (Example 1)	Fermented milk 3 (Comparative Example 2)	Fermented milk 4 (Example 2)
Raw milk [% (w/w)] (Meiji Dairies Corp)	50.00	50.00	50.00	50.00
Skim milk powder [% (w/w)] (Meiji Dairies Corp)	5.45	5.45	5.44	5.44
Unsalted butter [% (w/w)] (Meiji Dairies Corp)	1.32	1.32	1.32	1.32
Glucose [% (w/w)] (Sanmatsu Kogyo Co., Ltd.)			2.00	2.00
Lactase [% (w/w)] (Godo Shusei Co., Ltd.) (5000 unit/g, GODO-YNL)		0.040		
Glucose Oxidase [% (w/w)] (Shin Nihon Kagaku Kogyo Co.) (2000 unit/g, Sumizyme PGO)		0.175		
Glucose Oxidase [% (w/w)] (Shonan Wako Junyaku) (230 unit/mg, test reagent)				0.00014
Starter [% (w/w)] of Lactobacillus bulgaricus OLL1073R-1 and Streptococcus thermophilus OLS3059 (Meiji Dairies Corp)	2.00	2.00	2.00	2.00
Water [% (w/w)]	41.23	41.02	39.24	39.24
Sum [% (w/w)]	100	100	100	100

[0055]

20 Specifically, as shown in Table 1, the starting milk materials (fat content: 3.0 wt%, milk solids-not-fat: 9.7 wt%), water, and glucose, which was added only to prepare fermented

milk 3 and 4, were mixed and stirred with heating at 60°C to allow them to dissolve. After cooling to 5°C, lactase and/or glucose oxidase was added to and mixed with each mixture of fermented milk 2 and 4, according to the formulation shown in Table 1. Each prepared fermented milk mix was allowed to stand undisturbed at a temperature of about 5°C for about 14 hours, and then subjected to pasteurization by heating until each fermented milk mix reached a temperature of 95°C. Each heat-pasteurized fermented milk mix was cooled to about 40°C (approx. 43°C), and 2 wt% of a lactic acid bacteria starter (a mixed starter of *Lactobacillus bulgaricus* OLL1073R-1 and *Streptococcus thermophilus* OLS3059) was inoculated therein. Each resulting mixture was packed into market containers, followed by static incubation in an incubation room at a temperature of about 40°C (approx. 43°C). When the lactic acid acidity reached 0.72%, each mixture was placed in a refrigerator at a temperature of about 5°C to terminate fermentation by cooling.

[0056]

Lactobacillus bulgaricus OLL1073R-1 (*Lactobacillus delbrueckii* subspecies *bulgaricus* OLL1073R-1) used in the present invention is deposited with the International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology, under accession No. FERM P-17227 (Identification Name: *Lactobacillus delbrueckii* subspecies *bulgaricus* OLL1073R-1: accession date (receipt date) February 19, 1999).

[0057]

Moreover, *S. thermophilus* OLS3059 (*Streptococcus thermophilus* OLS3059) used in the present invention is deposited with the International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology under accession No. FERM P-15487 (Identification Name: *Streptococcus thermophilus* OLS3059, accession date (receipt date): February 29, 1996).

[0058]

1-2: Evaluation of each fermented milk

Each of the thus-produced fermented milk 1 to 4 was stored while undisturbed at a temperature of about 5°C for 16 days. Following the procedure below, the syneresis rate (%), the particle size of milk protein (μm), the acidity (%), the pH (20°C), the curd tension (g), and the viscosity (Pa·s) were measured over time (the beginning of production (day 1); and on day 8 and day 16 since the beginning of production).

(a) Measurement of syneresis rate of fermented milk

40 g of each fermented milk (10°C) was individually packed into a centrifuge tube (internal diameter: 20 mm), and treated with a centrifugal separator (about 180 G, radius gyration: 160 mm, revolving speed: 1,000 rpm, processing time: 10 minutes). Subsequently, the weight of each supernatant was measured, and the weight percentage (%) of the supernatant to the total weight (40 g) of each fermented milk was determined to be the syneresis rate.

(b) Measurement of particle size of fermented milk

The particle size (μm) of coagulated particles contained in each fermented milk was measured with a laser diffraction particle size analyzer (produced by Shimadzu Corporation: SALD-2000).

(c) Measurement of acidity (lactic acid acidity) of fermented milk

After phenolphthalein was added to each fermented milk as an indicator, the acidity was measured using sodium hydrate (decinormal solution) as a titrant.

(d) Measurement of hardness of fermented milk (curd tension, CT)

The hardness (g) of each fermented milk was measured with a Neo Curd meter M302 (produced by I Techno Engineering Co., formerly Iio Electronic Co.). For example, by filling a small container with 100 g of fermented milk (10°C) and running a yogurt knife (a disc with a diameter of about 20 mm) thereinto, hardness (curd tension) and smoothness can be evaluated with this curd meter.

(e) Measurement of viscosity of fermented milk

The viscosity of each fermented milk was measured with a rotational B-type viscometer (produced by Toki Sangyo: TV-10M).

For example, by filling a small container with 100 g of fermented milk (10°C), running a No. 4 rotor (code M23) therein and rotating the rotor (30 rpm, 30 seconds), viscosity can be measured with this viscometer.

5 [0059]

Table 2 shows the results of the measurement.

[0060]

Table 2

Table 2. Temporal change in syneresis and particle size of fermented milk (yogurt) (1)

Evaluation Item	Fermented milk 1 (Comparative Example 1)	Fermented milk 2 (Example 1)	Fermented milk 3 (Comparative Example 2)	Fermented milk 4 (Example 2)
Syneresis [%] (Day 1: fresh product)	15.2	7.1	10.7	9.6
Syneresis [%] (Day 8)	21.8	8.2	19.6	10.0
Syneresis [%] (Day 16)	25.7	8.3	22.5	15.0
Particle size [μm] (Day 1)	20.98	11.58	18.79	14.29
Particle size [μm] (Day 8)	30.98	13.48	24.12	15.67
Particle size [μm] (Day 16)	35.97	14.46	35.63	20.62
Acidity [%] (Day 1)	0.82	0.81	0.79	0.82
Acidity [%] (Day 8)	1.01	0.98	0.95	0.97
Acidity [%] (Day 16)	1.03	0.99	0.97	0.97
pH (20°C) [-] (Day 1)	4.44	4.45	4.39	4.32
pH (20°C) [-] (Day 8)	4.20	4.24	4.17	4.12
pH (20°C) [-] (Day 16)	4.12	4.15	4.14	4.14
Hardness [g] (Day 1)	44.8	32.5	50.9	47.5
Hardness [g] (Day 8)	62.8	29.1	43.4	27.7
Hardness [g] (Day 16)	54.7	36.4	50.0	38.0
Viscosity [Pa·s] (Day 1)	2.74	3.53	2.63	2.82
Viscosity [Pa·s] (Day 8)	2.49	2.98	2.44	2.52
Viscosity [Pa·s] (Day 16)	1.59	2.27	2.25	2.25

10 [0061]

A sensory evaluation (flavor evaluation) was carried out by a panel of five experts. Table 3 shows the results (evaluation results).

[0062]

15 Table 3

Table 3. Temporal change in flavor of fermented milk (yogurt) (1)

Fermented milk 1 (Comparative Example 1) (Day 1)	Smooth texture, no slimy strings produced
Fermented milk 1 (Comparative Example 1) (Day 8)	Crisp texture, no slimy strings produced
Fermented milk 1 (Comparative Example 1) (Day 16)	Crisp texture, no slimy strings produced
Fermented milk 2 (Example 1) (Day 1)	Smooth texture, slimy strings produced
Fermented milk 2 (Example 1) (Day 8)	Smooth texture, not many slimy strings produced
Fermented milk 2 (Example 1) (Day 16)	Mellow texture, not many slimy strings produced
Fermented milk 3 (Comparative Example 2) (Day 1)	Creamy texture, no slimy strings produced
Fermented milk 3 (Comparative Example 2) (Day 8)	Light texture, less viscous
Fermented milk 3 (Comparative Example 2) (Day 16)	Light texture, less viscous
Fermented milk 4 (Example 2) (Day 1)	Smooth texture, not many slimy strings produced, shape is not retained as much
Fermented milk 4 (Example 2) (Day 8)	Smooth texture, not many slimy strings produced, less viscous
Fermented milk 4 (Example 2) (Day 16)	Smooth texture, not many slimy strings produced, less viscous

[0063]

As is apparent from the results, fermented milk 4 produced by adding to a fermented milk mix glucose oxidase and its substrate, glucose, and fermented milk 2 produced by adding to a fermented milk mix glucose oxidase and lactase that acts on lactose contained in starting milk materials to generate glucose exhibited lower syneresis rates and smaller particle sizes in the beginning of the production than fermented milk 1 and 3 that were produced without adding glucose oxidase thereto; and the time-dependent increase of the syneresis increase rate and particle size increase rate of fermented milk 4 and 2 was suppressed to a significantly lower level than that of fermented milk 1 and 3.

[0064]

Consequently, it was confirmed that allowing glucose oxidase to act in the presence of glucose during the production process of fermented milk significantly suppresses the change in physical properties caused by coagulation of milk protein, specifically the occurrence and progression of syneresis, and the occurrence and progression of coagulation of milk protein.

[0065]

Coagulation of milk protein results in a gritty feeling in the mouth. Since the method of the present invention significantly suppresses the occurrence and progression of coagulation of milk protein, the method can also suppress the

occurrence and progression of grainy texture of fermented milk, which leads to the gritty feeling in the mouth.

[0066]

Experimental Example 2

5 2-1: Production of fermented milk

Following the formulation shown in Table 4, three kinds of fermented milk 5 to 7 were prepared.

[0067]

Table 4

Table 4. Formulation of a raw milk material (yogurt mix) (2)

Raw Material	Fermented milk 5 (Comparative Example 3)	Fermented milk 6 (Comparative Example 4)	Fermented milk 7 (Example 3)
Pasteurized milk [% (w/w)] (Meiji Dairies Corp)	74.50	74.50	74.50
Skim milk powder [% (w/w)] (Meiji Dairies Corp)	2.89	2.89	2.89
Lactase [% (w/w)] (Godo Shusei Co., Ltd.) (5000 unit/g, GODO-YNL)		0.040	0.040
Glucose Oxidase [% (w/w)] (DKSH Japan K.K.) (4000 unit/g)			0.175
Starter of Meiji yogurt R-1 [% (w/w)] (Meiji Dairies Corp)	2.00	2.00	2.00
Water [% (w/w)]	20.61	20.57	20.40
Sum . [% (w/w)]	100	100	100

[0068]

Specifically, as shown in Table 4, the starting milk materials (fat content: 4.0 wt%, milk solids-not-fat: 8.8 wt%) water, lactase, which was added to prepare fermented milk 6, and

15 lactase and glucose oxidase, which were added only to prepare fermented milk 7, were mixed. Each fermented milk was prepared in the same manner as in Experimental Example 1, except that a mixed starter separated from "Meiji yogurt R-1" (produced by Meiji Dairies Corporation) was used as a lactic acid bacteria starter.

20 At this stage, the acidity of fermented milk 5, 6, and 7 after about 3 hours of the fermentation process was 0.62%, 0.59%, and 0.59%, respectively. The acidity of fermented milk 5, 6, and 7 after about 3.5 hours of the fermentation process was 0.74%, 0.74%, and 0.74%, respectively. This means that adding and mixing
25 lactase and/or glucose oxidase did not affect the fermentation process (the fermentation time etc.).

[0069]

2-2: Evaluation of each fermented milk

In the same manner as in Experimental Example 1, the syneresis rate (%), the particle size of milk protein (μm), the acidity (%), the pH (20°C), the hardness (curd tension) (g), and the viscosity (Pa·s) of the thus-produced fermented milk 5 to 7 were measured over time. Table 5 shows the results of the measurement. A sensory evaluation was carried out in the same manner as in Experimental Example 1. Table 6 shows the results of the evaluation.

[0070]

Table 5

Table 5. Temporal change in syneresis and particle size of fermented milk (yogurt) (2)

Evaluation Item	Fermented milk 5 (Comparative Example 3)	Fermented milk 6 (Comparative Example 4)	Fermented milk 7 (Example 3)
Syneresis [%] (Day 1: fresh product)	19.9	11.0	5.3
Syneresis [%] (Day 8)	20.1	16.3	5.5
Syneresis [%] (Day 16)	21.9	17.7	5.3
Particle size [μm] (Day 1)	19.64	19.31	12.93
Particle size [μm] (Day 8)	24.11	21.34	13.38
Particle size [μm] (Day 16)	29.54	28.03	16.75
Acidity [%] (Day 1)	0.84	0.82	0.81
Acidity [%] (Day 8)	0.99	0.98	0.97
Acidity [%] (Day 16)	1.06	1.01	0.98
pH (20°C) [-] (Day 1)	4.36	4.40	4.40
pH (20°C) [-] (Day 8)	4.08	4.10	4.14
pH (20°C) [-] (Day 16)	4.04	4.11	4.18
Hardness [g] (Day 1)	45.5	43.2	38.4
Hardness [g] (Day 8)	41.6	41.8	34.5
Hardness [g] (Day 16)	41.2	39.3	31.6
Viscosity [Pa·s] (Day 1)	2.52	2.79	3.10
Viscosity [Pa·s] (Day 8)	1.65	2.18	2.54
Viscosity [Pa·s] (Day 16)	1.44	1.86	2.32

[0071]

Table 6

Table 6. Temporal change in flavor of fermented milk (yogurt) (2)

Fermented milk 5 (Comparative Example 3) (Day 1)	Smooth texture, no slimy strings produced
Fermented milk 5 (Comparative Example 3) (Day 8)	Light texture, less viscous
Fermented milk 5 (Comparative Example 3) (Day 16)	Light texture, non-viscous
Fermented milk 6 (Comparative Example 4) (Day 1)	Smooth texture, no slimy strings produced
Fermented milk 6 (Comparative Example 4) (Day 8)	Light texture, less viscous
Fermented milk 6 (Comparative Example 4) (Day 16)	Light texture, non-viscous
Fermented milk 7 (Example 3) (Day 1)	Creamy texture, slimy strings produced
Fermented milk 7 (Example 3) (Day 8)	Creamy texture, not many slimy strings produced, less viscous
Fermented milk 7 (Example 3) (Day 16)	Creamy texture, not many slimy strings produced, less viscous

[0072]

As is apparent from the results, fermented milk 7 produced by adding to a fermented milk mix glucose oxidase and lactase that acts on lactose contained in starting milk materials to generate glucose exhibited a lower syneresis rate and a smaller particle size in the beginning of production than fermented milk 5 and 6 that were produced without adding glucose oxidase thereto, and the time-dependent increase of the syneresis increase rate and particle size increase rate of fermented milk 7 was suppressed to a significantly lower level than that of fermented milk 5 and 6.

[0073]

Experimental Example 3

15 3-1: Production of fermented milk

Following the formulation shown in Table 7, four kinds of fermented milk 8 to 11 were prepared.

[0074]

Table 7

Table 7. Formulation of a raw milk material (yogurt mix) (3)

Raw Material	Fermented milk 8 (Comparative Example 5)	Fermented milk 9 (Example 4)	Fermented milk 10 (Comparative Example 6)	Fermented milk 11 (Example 5)
Raw milk [% (w/w)] (Meiji Dairies Corp)	50.00	50.00	50.00	50.00
Skim milk powder [% (w/w)] (Meiji Dairies Corp)	5.18	5.18	5.18	5.18
Unsalted butter [% (w/w)] (Meiji Dairies Corp)	1.16	1.16	1.16	1.16
Lactase [% (w/w)] (Godo Shusei Co., Ltd.) (5000 unit/g, GODO-YNL)		0.040		0.040
Glucose Oxidase [% (w/w)] (DKSH Japan K.K.) (4000 unit/g)		0.175		0.175
Meiji Bulgaria plain type starter [% (w/w)] (Meiji Dairies Corp)	2.00	2.00		
Meiji Bulgaria soft type starter [% (w/w)] (Meiji Dairies Corp)			2.00	2.00
Water [% (w/w)]	41.66	41.45	41.66	41.45
Sum [% (w/w)]	100	100	100	100

[0075]

Specifically, as shown in Table 7, the starting milk materials (fat content: 3.0 wt%, milk solids-not-fat: 9.7 wt%), water, and glucose oxidase and lactase, which were added only to prepare fermented milk 9 and 11, were mixed. Each fermented milk was prepared in the same manner as in Experimental Example 1, except that a mixed starter separated from either a plain type or a soft type of "Meiji Bulgaria Yogurt" (produced by Meiji Dairies Corporation) was used as a lactic acid bacteria starter. At this stage, the acidity of fermented milk 8, 9, 10, and 11 after about 3 hours of the fermentation process was 0.62%, 0.64%, 0.62%, and 0.57%, respectively. The acidity of fermented milk 8, 9, and 10 after about 3.5 hours of the fermentation process was 0.71%, 0.72%, and 0.69%, respectively. The acidity of fermented milk 11 after about 3.75 hours of the fermentation process was 0.66%.

[0076]

3-2: Evaluation of each fermented milk

In the same manner as in Experimental Example 1, the syneresis rate (%), the particle size of milk protein (μm), the acidity (%), the pH (20°C), the hardness (curd tension) (g), and the viscosity (Pa·s) of the thus-produced fermented milk 8 to 11 were measured

over time. Table 8 shows the results of the measurement. In the same manner as in Experimental Example 1, a sensory evaluation was carried out. Table 9 shows the results of the evaluation.

[0077]

5 Table 8

Table 8. Temporal change in syneresis and particle size of fermented milk (yogurt) (3)

Evaluation Item	Fermented milk 8 (Comparative Example 5)	Fermented milk 9 (Example 4)	Fermented milk 10 (Comparative Example 6)	Fermented milk 11 (Example 5)
Syneresis [%] (Day 1: fresh product)	27.0	12.1	19.5	15.5
Syneresis [%] (Day 8)	28.8	15.8	22.6	17.3
Syneresis [%] (Day 16)	27.9	16.3	32.9	22.6
Particle size [μm] (Day 1)	30.56	21.81	23.05	19.92
Particle size [μm] (Day 8)	35.18	23.60	29.55	21.72
Particle size [μm] (Day 16)	36.75	27.70	36.83	23.59
Acidity [%] (Day 1)	0.81	0.80	0.79	0.84
Acidity [%] (Day 8)	0.94	0.93	0.90	1.01
Acidity [%] (Day 16)	0.98	0.95	0.96	1.01
pH (20°C) [-] (Day 1)	4.40	4.45	4.47	4.36
pH (20°C) [-] (Day 8)	4.16	4.20	4.25	4.11
pH (20°C) [-] (Day 16)	4.10	4.18	4.13	4.08
Hardness [g] (Day 1)	37.7	24.5	29.1	21.4
Hardness [g] (Day 8)	31.6	25.0	23.9	22.7
Hardness [g] (Day 16)	40.0	34.1	40.6	31.4
Viscosity [Pa·s] (Day 1)	1.39	2.58	1.61	1.63
Viscosity [Pa·s] (Day 8)	1.37	1.61	1.48	1.33
Viscosity [Pa·s] (Day 16)	1.37	1.62	1.49	2.73

[0078]

Table 9

Table 9. Temporal change in flavor of fermented milk (yogurt) (3)

Fermented milk 8 (Comparative Example 5) (Day 1)	Smooth texture, no slimy strings produced
Fermented milk 8 (Comparative Example 5) (Day 8)	Mellow texture, no slimy strings produced
Fermented milk 8 (Comparative Example 5) (Day 16)	Mellow texture, no slimy strings produced
Fermented milk 9 (Example 4) (Day 1)	Smooth texture, no slimy strings produced
Fermented milk 9 (Example 4) (Day 8)	Very mellow, no slimy strings produced
Fermented milk 9 (Example 4) (Day 16)	Very mellow, no slimy strings produced
Fermented milk 10 (Comparative Example 6) (Day 1)	Smooth texture, with mild flavor
Fermented milk 10 (Comparative Example 6) (Day 8)	Mellow texture, with mild flavor
Fermented milk 10 (Comparative Example 6) (Day 16)	Mellow texture, with mild flavor
Fermented milk 11 (Example 5) (Day 1)	Smooth texture, with mild and excellent flavor
Fermented milk 11 (Example 5) (Day 8)	Very mellow, with mild and excellent flavor
Fermented milk 11 (Example 5) (Day 16)	Very mellow, with mild and excellent flavor

[0079]

As is apparent from the results, fermented milk 9 and 11 that were produced by adding to a fermented milk mix glucose oxidase and lactase that acts on lactose contained in starting milk materials to generate glucose exhibited lower syneresis rates and smaller particle sizes in the beginning of the production than fermented milk 8 and 10 that were produced without adding glucose oxidase thereto, and the time-dependent increase of the syneresis increase rate and particle size increase rate of fermented milk 9 and 11 was suppressed to a significantly lower level than that of fermented milk 8 and 10. [0080]

Experimental Example 4

4-1: Production of fermented milk

Following the formulation shown in Table 10, two kinds of fermented milk 12 and 13 were prepared. [0081]

Table 10

Table 10. Formulation of a raw milk material (yogurt mix) (4)

Raw Material	Fermented milk 12 (Comparative Example 7)	Fermented milk 13 (Example 6)
Pasteurized milk [(w/w)] (Meiji Dairies Corp)	74.50	74.50
Skim milk powder [(w/w)] (Meiji Dairies Corp)	2.89	2.89
Lactase [(w/w)] (Godo Shusei Co., Ltd.) (5000 unit/g, GODO-YNL)	0.020	0.020
Glucose Oxidase [(w/w)] (DKSH Japan K.K.) (4000 unit/g)		0.175
Starter of Meiji yogurt R-1 [(w/w)] (Meiji Dairies Corp)	2.00	2.00
Water [(w/w)]	20.59	20.42
Sum [(w/w)]	100	100

In the case of fermented milk 13 (Example 6), glucose oxidase was added when lactase was added, and the mixture was then allowed to stand undisturbed at 5°C for 15 hours before being subjected to lactate fermentation.

[0082]

Specifically, as shown in Table 10, the starting milk materials (fat content: 4.0 wt%, milk solids-not-fat:8.8 wt%), water, lactase, which was added only to prepare fermented milk 12, and lactase and glucose oxidase, which were added only to prepare

fermented milk 13, were mixed. Each prepared fermented milk mix was allowed to stand undisturbed at a temperature of about 5°C for about 15 hours; thereafter, pasteurization by heating was performed until each fermented milk mix reached a temperature of 95°C. Each heat-pasteurized fermented milk mix was cooled to about 40°C (approx. 43°C), and 2 wt% of a lactic acid bacteria starter (a mixed starter of *Lactobacillus bulgaricus* OLL1073R-1 and *Streptococcus thermophilus* OLS3059) was inoculated thereinto. Each resulting mixture was packed into market containers, followed by static incubation in an incubation room at a temperature of about 40°C (approx. 43°C). When the lactic acid acidity reached 0.72%, the yogurt curd was squished with a scoopula, and placed in a refrigerator at a temperature of about 5°C to terminate fermentation by cooling.

[0083]

4-2: Evaluation of each fermented milk

In the same manner as in Experimental Example 1, the syneresis rate (%), the particle size of milk protein (μm), the acidity (%), the pH (20°C), and the viscosity (Pa·s) of the thus-produced fermented milk 12 and 13 were measured over time. In this experimental example, assuming a soft-type yogurt, the yogurt curd was squished; thus, the hardness was not measured. Table 11 shows the results of the measurement. In the same manner as in Experimental Example 1, a sensory evaluation was carried out. Table 12 shows the results of the evaluation.

[0084]

Table 11

Table 11. Temporal change in syneresis and particle size of fermented milk (yogurt) (4)

Evaluation Item	Fermented milk 12 (Comparative Example 7)	Fermented milk 13 (Example 6)
Syneresis [%] (Day 1 : fresh product)	13.7	8.7
Syneresis [%] (Day 8)	8.7	7.5
Syneresis [%] (Day 16)	11.4	15.0
Particle size [μm] (Day 1)	15.91	10.66
Particle size [μm] (Day 8)	17.91	11.06
Particle size [μm] (Day 16)	16.27	11.11
Acidity [%] (Day 1)	0.86	0.89
Acidity [%] (Day 8)	0.97	1.00
Acidity [%] (Day 16)	0.99	1.00
pH (20°C) [-] (Day 1)	4.27	4.25
pH (20°C) [-] (Day 8)	4.14	4.10
pH (20°C) [-] (Day 16)	4.14	4.09
Viscosity [Pa·s] (Day 1)	1.68	1.45
Viscosity [Pa·s] (Day 8)	1.92	1.56
Viscosity [Pa·s] (Day 16)	1.73	1.60

Assuming a soft-type yogurt, the curd was squished. Thus, the hardness was not measured.

[0085]

Table 12

Table 12. Temporal change in flavor of fermented milk (yogurt) (4)

Fermented milk 12 (Comparative Example 7) (Day 1)	Smooth texture, excellent flavor
Fermented milk 12 (Comparative Example 7) (Day 8)	Smooth texture, excellent flavor
Fermented milk 12 (Comparative Example 7) (Day 16)	Smooth texture, excellent flavor
Fermented milk 13 (Example 6) (Day 1)	Slightly viscous texture, excellent flavor
Fermented milk 13 (Example 6) (Day 8)	Slightly thick consistency, excellent flavor
Fermented milk 13 (Example 6) (Day 16)	Slightly thick consistency, excellent flavor

5

[0086]

As is apparent from the results, fermented milk 13 produced by adding to a fermented milk mix glucose oxidase and lactase that acts on lactose contained in starting milk materials to generate glucose exhibited a smaller particle size and a lower syneresis rate in the beginning of the production than fermented milk 12 produced without adding glucose oxidase thereto.

[0087]

Experimental Example 5

15 5-1: Production of fermented milk

Following the formulation shown in Table 13, three kinds of

fermented milk 14 to 16 were prepared.

[0088]

Table 13

Table 13. Formulation of a raw milk material (yogurt mix) (5)

Raw Material	Fermented milk 14 (Comparative Example 8)	Fermented milk 15 (Example 7)	Fermented milk 16 (Example 8)
Pasteurized milk [% (w/w)] (Meiji Dairies Corp)	74.50	74.50	74.50
Skim milk powder [% (w/w)] (Meiji Dairies Corp)	2.89	2.89	2.89
Lactase [% (w/w)] (Godo Shusei Co., Ltd.) (5000 unit/g, GODO-YNL)	0.020	0.020	0.020
Glucose Oxidase [% (w/w)] (DKSH Japan K.K.) (4000 unit/g)		0.175	0.035
Starter of Meiji yogurt R-1 [% (w/w)] (Meiji Dairies Corp)	2.00	2.00	2.00
Water [% (w/w)]	20.59	20.42	20.56
Sum [% (w/w)]	100	100	100

In the case of fermented milk 15 (Example 7), glucose oxidase was added when lactase was added, and the mixture was then allowed to stand undisturbed at 5°C for 15 hours before being subjected to lactate fermentation.

In the case of fermented milk 16 (Example 8), lactase was added, and the mixture was then allowed to stand undisturbed at 5°C for 15 hours. Thereafter, glucose oxidase was added to the mixture when the mixture was subjected to lactate fermentation.

5 [0089]

Specifically, as shown in Table 13, the starting milk materials (fat content: 4.0 wt%, milk solids-not-fat: 8.8 wt%), water, lactase, which was added only to prepare fermented milk 14, and lactase and glucose oxidase, which were added only to prepare fermented milk 15, were mixed. Each prepared fermented milk mix was allowed to stand undisturbed at a temperature of about 5°C for about 15 hours. Thereafter, pasteurization by heating was performed until each fermented milk mix reached a temperature of 95°C. Each heat-pasteurized fermented milk mix was cooled to about 40°C (approx. 43°C), and 2 wt% of a lactic acid bacteria starter (a mixed starter of *Lactobacillus bulgaricus* OLL1073R-1 and *Streptococcus thermophilus* OLS3059) was inoculated therein. Each resulting mixture was packed into market containers, followed by static incubation in an incubation room at a temperature of about 40°C (approx. 43°C). When the lactic acid

acidity reached 0.72%, each mixture was placed in a refrigerator at a temperature of about 5°C to terminate fermentation by cooling.

[0090]

5 In contrast, only lactase was added to and mixed with the mixture for fermented milk 16 at the beginning, following the formulation shown in Table 13. The thus-prepared fermented milk mix was allowed to stand undisturbed at a temperature of about 5°C for about 15 hours. Thereafter, pasteurization by heating was
10 performed until the fermented milk mix reached a temperature of 95°C. The heat-pasteurized fermented milk mix was then cooled to about 40°C (approx. 43°C), and glucose oxidase was added and mixed following the formulation shown in Table 13, while 2 wt% of a lactic acid bacteria starter (a mixed starter of *Lactobacillus*
15 *bulgaricus* OLL1073R-1 and *Streptococcus thermophilus* OLS3059) was inoculated thereinto at the same time. The mixture was packed into market containers, followed by static incubation in an incubation room at a temperature of about 40°C (approx. 43°C). When the lactic acid acidity reached 0.72%, the mixture was
20 placed in a refrigerator at a temperature of about 5°C to terminate fermentation by cooling. [0091]

5-2: Evaluation of each fermented milk

 In the same manner as in Experimental Example 1, the syneresis rate (%), the particle size of milk protein (μm), the
25 acidity (%), the pH (20°C), the hardness (curd tension) (g), and the viscosity (Pa's) of the thus-produced fermented milk 14 to 16 were measured over time. Table 14 shows the results of the measurement. A sensory evaluation was carried out in the same manner as in Experimental Example 1. Table 15 shows the results
30 of the evaluation.

[0092]

Table 14

Table 14. Temporal change in syneresis and particle size of fermented milk (yogurt) (5)

Evaluation Item	Fermented milk 14 (Comparative Example 8)	Fermented milk 15 (Example 7)	Fermented milk 16 (Example 8)
Syneresis [%] (Day 1 : fresh product)	9.1	5.7	8.8
Syneresis [%] (Day 8)	8.7	5.7	9.6
Syneresis [%] (Day 16)	8.6	6.3	11.2
Particle size [μ m] (Day 1)	12.59	8.26	9.42
Particle size [μ m] (Day 8)	16.54	9.39	
Particle size [μ m] (Day 16)	20.69	11.47	10.92
Acidity [%] (Day 1)	0.86	0.82	0.81
Acidity [%] (Day 8)	0.99	0.93	0.86
Acidity [%] (Day 16)	1.01	0.97	0.88
pH (20°C) [-] (Day 1)	4.33	4.37	4.43
pH (20°C) [-] (Day 8)	4.11	4.21	4.32
pH (20°C) [-] (Day 16)	4.11	4.19	4.29
Hardness [g] (Day 1)	51.5	45.9	36.0
Hardness [g] (Day 8)	45.7	41.8	35.2
Hardness [g] (Day 16)	44.7	40.7	33.4
Viscosity [Pa·s] (Day 1)	3.44	3.78	2.58
Viscosity [Pa·s] (Day 8)	2.39	3.00	1.85
Viscosity [Pa·s] (Day 16)	1.93	2.93	1.88

[0093]

Table 15

Table 15. Temporal change in flavor of fermented milk (yogurt) (5)

Fermented milk14 (Comparative Example 8) (Day 1)	Not many slimy strings produced, excellent flavor
Fermented milk14 (Comparative Example 8) (Day 8)	Not many slimy strings produced, relatively sourer
Fermented milk14 (Comparative Example 8) (Day 16)	Not many slimy strings produced, relatively sourer
Fermented milk15 (Example 7) (Day 1)	Slimy strings produced, excellent flavor
Fermented milk15 (Example 7) (Day 8)	Slimy strings produced, mild flavor
Fermented milk15 (Example 7) (Day 16)	Slimy strings produced, mild flavor
Fermented milk16 (Example 8) (Day 1)	Mellow, slimy texture, excellent flavor
Fermented milk16 (Example 8) (Day 8)	Mellow, slimy strings produced, mild flavor
Fermented milk16 (Example 8) (Day 16)	Mellow, slimy strings produced, mild flavor

5 [0094]

As is apparent from the results, fermented milk 15 and 16 that were produced by adding to a fermented milk mix glucose oxidase and lactase that acts on lactose contained in starting milk materials to generate glucose exhibited lower syneresis rates and smaller particle sizes in the beginning of the production than fermented milk 14 produced without adding glucose oxidase thereto, and the time-dependent increase of the syneresis increase rate and particle size increase rate of fermented milk

15 and 16 was suppressed to a significantly lower level than that of fermented milk 14.

[0095]

Experimental Example 6

5 6-1: Production of fermented milk

Following the formulation shown in Table 16, three kinds of fermented milk 17 to 19 were prepared.

[0096]

Table 16

Table 16. Formulation of a raw milk material (yogurt mix) (6)

Raw Material	Fermented milk 17 (Example 9)	Fermented milk 18 (Example 10)	Fermented milk 19 (Example 11)
Pasteurized milk [% (w/w)] (Meiji Dairies Corp)	74.50	74.50	74.50
Skim milk powder [% (w/w)] (Meiji Dairies Corp)	2.89	2.89	2.89
Lactase [% (w/w)] (Godo Shusei Co., Ltd.) (5000 unit/g, GODO·YNL)	0.020	0.020	0.020
Glucose Oxidase [% (w/w)] (DKSH Japan K.K.) (4000 unit/g)	0.175	0.175	0.175
Starter of Meiji yogurt R-1 [% (w/w)] (Meiji Dairies Corp)	2.00	2.00	2.00
Water [% (w/w)]	20.42	20.42	20.42
Sum [% (w/w)]	100	100	100
Enzyme reaction time [h]	2	6	16
Lactose [% (w/v)] (before fermentation)	3.85	2.64	1.12
Glucose [% (w/v)] (before fermentation)	0.77	1.43	2.03
Acidity [%] (before fermentation)	0.191	0.201	0.223
pH (20°C) [-] (before fermentation)	6.54	6.46	6.35

Glucose oxidase was added when lactase was added, and the mixture was allowed to stand undisturbed at 5°C for a predetermined time period before being subjected to lactate fermentation.

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[0097]

Specifically, as shown in Table 16, the starting milk materials (fat content: 4.0 wt%, milk solids-not-fat: 8.8 wt%), water, and additionally lactase and glucose oxidase were added and mixed. Each prepared fermented milk mix was allowed to stand undistributed at a temperature of about 5°C for a predetermined time period (about 2 hours, 6 hours, 16 hours), followed by pasteurization by heating until each fermented milk mix reached a temperature of 95°C. Each heat-pasteurized fermented milk mix was cooled to about 40°C (approx. 43°C), and 2 wt% of a lactic acid

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bacteria starter (a mixed starter of *Lactobacillus bulgaricus* OLL1073R-1 and *Streptococcus thermophilus* OLS3059) was inoculated thereinto. Each mixture was packed into market containers, followed by static incubation in an incubation room at a temperature of about 40°C (approx. 43°C). When the lactic acid acidity reached 0.72%, each mixture was placed in a refrigerator at a temperature of about 5°C to terminate fermentation by cooling. At this stage, the acidity of fermented milk 17, 18, and 19 after 4 hours of the fermentation process was 0.72%, 0.70%, and 0.71%, respectively. This means that adding and mixing lactase and glucose oxidase, and changing the reaction time of those enzymes within a predetermined time period did not affect the fermentation process (fermentation time, etc.)

[0098]

6-2: Evaluation of each fermented milk

In the same manner as in Experimental Example 1, the syneresis rate (%), the particle size of milk protein (μm), the acidity (%), the pH (20°C), the hardness (curd tension) (g), and the viscosity (Pa·s) of the thus-produced fermented milk 17 to 19 were measured over time. Table 17 shows the results of the measurement. A sensory evaluation was carried out in the same manner as in Experimental Example 1. Table 18 shows the results of the evaluation.

[0099]

Table 17

Table 17. Temporal change in syneresis and particle size of fermented milk (yogurt) (6)

Evaluation Item	Fermented milk 17 (Example 9)	Fermented milk 18 (Example 10)	Fermented milk 19 (Example 11)
Syneresis [%] (Day 1 : fresh product)	7.2	4.9	5.7
Syneresis [%] (Day 8)	6.9	6.2	5.7
Syneresis [%] (Day 16)	14.5	7.3	6.3
Particle size [μ m] (Day 1)	8.16	9.52	8.26
Particle size [μ m] (Day 8)	9.17	8.73	9.39
Particle size [μ m] (Day 16)	9.69	9.18	11.47
Acidity [%] (Day 1)	0.82	0.83	0.82
Acidity [%] (Day 8)	0.92	0.96	0.93
Acidity [%] (Day 16)	0.97	0.98	0.97
pH (20°C) [-] (Day 1)	4.43	4.38	4.37
pH (20°C) [-] (Day 8)	4.23	4.18	4.21
pH (20°C) [-] (Day 16)	4.19	4.17	4.19
Hardness [g] (Day 1)	46.1	45.7	45.9
Hardness [g] (Day 8)	38.2	42.5	41.8
Hardness [g] (Day 16)	39.1	39.1	40.7
Viscosity [Pa·s] (Day 1)	3.24	3.08	3.78
Viscosity [Pa·s] (Day 8)	2.15	2.73	3.00
Viscosity [Pa·s] (Day 16)	2.32	2.34	2.93

[0100]

Table 18

Table 18. Temporal change in flavor of fermented milk (yogurt) (6)

Fermented milk 17 (Example 9) (Day 1)	Slimy strings produced, excellent flavor
Fermented milk 17 (Example 9) (Day 8)	Slimy strings produced, mild flavor
Fermented milk 17 (Example 9) (Day 16)	Slimy strings produced, mild flavor
Fermented milk 18 (Example 10) (Day 1)	Slimy strings produced, excellent flavor
Fermented milk 18 (Example 10) (Day 8)	Slimy strings produced, mild flavor
Fermented milk 18 (Example 10) (Day 16)	Slimy strings produced, mild flavor
Fermented milk 19 (Example 11) (Day 1)	Slimy strings produced, excellent flavor
Fermented milk 19 (Example 11) (Day 8)	Slimy strings produced, mild flavor
Fermented milk 19 (Example 11) (Day 16)	Slimy strings produced, mild flavor

5 [0101]

As is apparent from the results, fermented milk 17 to 19 produced by adding to a fermented milk mix glucose oxidase and lactase that acts on lactose contained in starting milk materials to generate glucose exhibited low syneresis rates and small particle sizes in the beginning of the production, and the time-dependent increase of the syneresis increase rate and particle size increase rate of fermented milk 17 to 19 was suppressed to a significantly low level even in any predetermined enzyme reaction time setting (about 2 hours, 6 hours, and 16 hours).

CLAIMS

[Claim 1]

A method for producing a fermented milk, comprising the steps of:

- 5 (1) preparing a fermented milk mix using one or more starting milk materials;
- (2) heat-pasteurizing the fermented milk mix; and
- (3) adding a starter to the heat-pasteurized fermented milk mix to ferment the mix,

10 wherein, in performing step (1) above, the following operation (A) is carried out:

 (A) adding glucose oxidase to said one or more starting milk materials to prepare the fermented milk mix in step (1), wherein said one or more starting milk materials contain a

15 substance that can serve as a substrate for the glucose oxidase.

[Claim 2]

 The method according to Claim 1, wherein the starter is a combination of *Lactobacillus bulgaricus* and *Streptococcus*

20 *thermophilus*.

[Claim 3]

 The method according to Claim 1 or 2, which is a method for producing a fermented milk that undergoes less change in

25 physical properties caused by coagulation of milk protein than a fermented milk produced without adding glucose oxidase thereto.

[Claim 4]

 The method according to Claim 3, wherein the change in

30 physical properties caused by coagulation of milk protein is at least one selected from the group consisting of occurrence of syneresis, an increase in particle size of milk protein and/or fat, and development of grainy texture of the fermented milk.

[Claim 5]

The method according to Claim 1, wherein the fermented milk is yogurt.

5 [Claim 6]

A method for suppressing a change in physical properties caused by coagulation of milk protein in a fermented milk produced by a method comprising the following steps:

(1) preparing a fermented milk mix using one or more
10 starting milk materials;

(2) heat-pasteurizing the fermented milk mix; and

(3) adding a starter to the heat-pasteurized fermented milk mix to ferment the mix,

wherein, in performing step (1) above, the following
15 operation (A) is carried out:

(A) adding glucose oxidase to said one or more starting milk materials to prepare the fermented milk mix in step (1), wherein said one or more starting milk materials contain a substance that can serve as a substrate for the glucose oxidase.

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[Claim 7]

The method according to Claim 6, wherein the starter is a combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

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[Claim 8]

The method according to Claim 6, which suppresses a change in physical properties caused by coagulation of milk protein, unlike the method for producing a fermented milk without
30 using glucose oxidase.

[Claim 9]

The method according to Claim 6, wherein the change in physical properties caused by coagulation of milk protein is at
35 least one selected from the group consisting of occurrence of

syneresis, an increase in particle size of milk protein and/or fat, and development of grainy texture of the fermented milk.

[Claim 10]

5 The method according to Claim 6, wherein the fermented milk is yogurt.

[Claim 11]

10 The method according to any one of claims 1 to 5, wherein step (2) is a step of heat-pasteurizing the fermented milk mix at 90 to 100°C.

[Claim 12]

15 The method according to any one of claims 6 to 10, wherein step (2) is a step of heat-pasteurizing the fermented milk mix at 90 to 100°C.