**Specification**

# FERMENTED MILK WITH A SUPPRESSED INCREASE IN ACIDITY, AND A METHOD FOR PRODUCING THE SAME

## [Technical Field]

[0001]

The present invention relates to fermented milk with a suppressed increase in acidity, and a method for producing the same.

## [Background Art]

[0002]

In fermented milk produced by lactic acid fermentation, live lactic acid bacteria are contained in the produced fermented milk (product). Accordingly, when actual products are, for example stored by refrigeration, a problem of quality deterioration occurs due to an increase in acidity in the fermented milk caused by live lactic acid bacteria. To date, in order to obtain fermented milk with a suppressed increase in acidity during refrigerated storage, a method of producing fermented milk using *Lactococcus lactis subsp lactis* that produces nisin has been disclosed (Patent Document 1). For the same purpose, there is provided a method forproducing fermented milk using a bacterial strain that produces antibacterial substances, or a cold-sensitive bacterial strain, which are lactic acid bacteria belonging to *Streptococcus thermophilus* (Patent Documents 2 and 3).

[0003]

In addition, by using specific lactic acid bacterial strains as a mixed starter, a method for producing fermented milk with a suppressed increase in acidity is also known. Patent Document 4 describes that fermented milk with a suppressed

increase in acidity is obtained by using lactic acid bacteria belonging to *Lactobacillus helveticus* and *Lactobacillus acidophilus* as a starter. Furthermore, in Patent Document 5, a method for producing fermented milk using a bacterial strain of *Lactobacillus bulgaricus* with suppressed acid production and a bacterial strain of *Streptococcus thermophilus* with generation of viscous products has been described.

[0004]

Meanwhile, several additives that promote the growth of lactic acid bacteria and bifidobacteria are known. Patent Document 6 discloses growth promoting agents and survival-improving agents for lactic acid bacteria wherein dead cells of lactic acid bacteria are used as an active ingredient; Patent Document 7 discloses growth promoting agents and survival-improving agents for lactic acid bacteria wherein κ-casein glycomacropeptides are used as an active ingredient. In addition, a growth promoting agent for bifidobacteria, wherein milk proteins that are hydrolyzed by a cell wall-enveloped protease PrtP are used as an active ingredient, has been known (Patent Document 8).

**[Prior Art Documents]**

**[Patent Documents]**

[0005]

Patent Document 1: JP B 2975148

Patent Document 2: JP A 7-32702

Patent Document 3: JP B 4331309

Patent Document 4: JP B 3013157

Patent Document 5: JP B 3101143

Patent Document 6: WO2008/001497

Patent Document 7: WO2011/027719

Patent Document 8: WO2009/150888

**[Summary of the Invention]**

**[Problems to Be Solved by the Invention]**

[0006]

However, when we intend to promote the growth of lactic acid bacteria and to reduce the fermentation time, then acidity during refrigerated storage tends to increase as well. Therefore, it is very difficult to suppress increases in acidity during refrigerated storage while promoting fermentation.

Accordingly, an object of the present invention is to provide fermented milk having moderately mild acidity with good palatability, and a method for producing the same, by promoting the fermentation of lactic acid bacteria and reducing the fermentation time, while suppressing an increase in acidity during refrigerated storage.

**[Means for Solving the Problems]**

[0007]

In order to solve the above problems, the present inventors have conducted extensive studies; and they have found that, in the production of fermented milk using a mixed starter, fermented milk with improved smooth curd texture wherein not only fermentation time is reduced, but also increases in acidity is suppressed during refrigerated storage can be obtained by blending a monoculture solution of *Lactobacillus bulgaricus* in a starting milk; as a result of further studies, the inventors have accomplished the present invention.

[0008]

Namely, the present invention relates to the following fermented milk with a suppressed increase in acidity, and a method for producing the same.

[1] A method for producing fermented milk by adding a starter to a starting milk, comprising a step of mixing a mixed starter and a monoculture solution of *Lactobacillus* lactic acid

bacteria.

[2] The method according to [1], wherein the mixed starter comprises *Lactobacillus* lactic acid bacteria and *Streptococcus* lactic acid bacteria.

[3] The method according to [1] or [2], wherein the *Lactobacillus* lactic acid bacteria are selected from the group consisting of *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, and *Lactobacillus gasseri*.

[4] The method according to [2] or [3], wherein the *Streptococcus* lactic acid bacteria are *Streptococcus thermophilus* or *Streptococcus macedonicus*.

[5] The method according to any one of [2] to [4], wherein the *Lactobacillus* lactic acid bacteria used in the monoculture solution are the same species of lactic acid bacteria that are used in the mixed starter.

[6] The method according to any one of [1] to [5], wherein the lactic acid bacteria in the monoculture solution are removed and/or sterilized.

[7] The method according to any one of [1] to [6], wherein the treatments of removal and/or sterilization are/is selected from centrifugation, filtration and heating.

[8] The method according to [7], wherein the heating of the monoculture solution is carried out simultaneously with heating of the starting milk.

[9] The method according to any one of [1] to [8], wherein the monoculture solution comprises, as its culture components, only the components contained in the starting milk.

[10] The method according to any one of [1] to [9], wherein the monoculture solution comprises raw milk, pasteurized milk, skim milk, whole milk powder, skim milk powder, concentrated whole milk and/or concentrated skim milk, as its culture components.

[11] The method according to any one of [1] to [10], wherein

the monoculture solution is added at a concentration of 2-15 wt%.

[12] A fermented milk produced by the method according to any one of [1] to [11].

**[Advantageous Effects of the Invention]**

[0009]

In the method for producing fermented milk of the present invention, the use of monoculture solution of lactic acid bacteria, in particular the use of monoculture solution in which bacterial bodies are removed and/or sterilized, enables reduction of fermentation time because the monoculture solution functions as an additive to promote fermentation (fermentation promoting agent). Compared to conventional technologies, the preparation method is simple as a fermentation promoting agent, so that it can be supplied at low cost. In addition, fermentation time may be further reduced by fermentation after deoxygenation.

[0010]

The monoculture solution of lactic acid bacteria of the present invention comprises, for example, peptides such as casein degradation products that promote fermentation; therefore, it can increase the number of bacteria such as *Streptococcus thermophilus*.

Meanwhile, since the monoculture solution of lactic acid bacteria of the present invention also comprises lactic acid, in particular D-lactic acid, the monoculture solution is considered to be able to suppress production of excess lactic acid in the fermentation process. Therefore, it is possible to suppress an increase in acidity during refrigerated storage while reducing the fermentation time, thereby providing fermented milk with excellent refrigerated storage stability.

[0011]

In addition, in the fermented milk obtained by the method

for producing fermented milk of the present invention, smoothness of the curd and texture are improved. Accordingly, with the method for producing fermented milk of the present invention, moderate and mild sour taste is maintained during refrigerated storage, and fermented milk, in particular plain yoghurt with excellent qualities such as texture and flavor can be provided.

[0012]

Furthermore, by preparing the monoculture solution of lactic acid bacteria using selected culture components, not only quality of fermented milk can be stabilized, but also necessity to indicate ingredients other than “dairy products” and “milk protein” on containers of products (fermented milk) is eliminated, in contrast to conventional fermentation promoting agent. Accordingly, the method of the present invention can be suitably used for commercial-scale production and sales of plain yoghurt and the like.

**[Brief Description of Drawings]**

[0013]

[Fig. 1] Figure 1 shows time-course changes in the amounts of D-lactic acid and L-lactic acid in yoghurt using low PA-4 starter (low PA-4 yoghurt) during fermentation and during refrigerated storage. Fermentation time is 6.75 h.

[Fig. 2] Figure 2 shows time-course changes in the amounts of D-lactic acid and L-lactic acid in low PA-4 yoghurt wherein milk peptide was blended at 0.05 wt%, during fermentation and during refrigerated storage. Fermentation time is 3.5 h.

[Fig. 3] Figure 3 shows time-course changes in the amounts of D-lactic acid and L-lactic acid in low PA-4 yoghurt wherein milk peptide was blended at 0.1 wt%, during fermentation and during refrigerated storage. Fermentation time is 3.25 h.

[Fig. 4] Figure 4 shows time-course changes in the amounts of

D-lactic acid and L-lactic acid in low PA-4 yoghurt wherein a monoculture solution of *L. bulgaricus* was blended at 5 wt%, during fermentation and during refrigerated storage. Fermentation time is 4 h.

[Fig. 5] Figure 5 shows time-course changes in the amounts of D-lactic acid and L-lactic acid in low PA-4 yoghurt wherein a monoculture solution of *L. bulgaricus* was blended at 10 wt%, during fermentation and during refrigerated storage. Fermentation time is 5 h.

**[Modes for Carrying out the Invention]**

[0014]

The method for producing fermented milk of the present invention includes a step of mixing a mixed starter and a monoculture solution of lactic acid bacteria. Preferably, a monoculture solution of *Lactobacillus* lactic acid bacteria is blended in a starting milk, and fermented with a mixed starter.

[0015]

The “fermented milk” referred to in the present specification includes the “fermented milk” defined under the Ministerial Ordinance on Milk and Milk Products Concerning Compositional Standards, etc. (Ministerial Ordinance on Milk, etc.). For example, the fermented milk is one obtained by fermenting milk such as raw milk, cow's milk, special milk, raw goat's milk, pasteurized goat's milk, raw sheep's milk, composition modified milk, low fat milk, skim milk or processed milk, or milk, etc. containing an equal or greater amount of milk solids-not-fat, with lactic acid bacteria or yeasts and then forming a paste or liquid, or frozen products thereof. They include various types of yoghurt, but in the present specification it is typically plain yoghurt.

[0016]

In general, a set type yoghurt such as plain yoghurt is

produced by charging a container with a starting milk and subsequently effecting fermentation (after-fermentation). On the other hand, a soft yoghurt or a drinking yoghurt is produced by subjecting fermented milk that has been fermented, to atomization or homogenization and then charging a container therewith (pre-fermentation).

[0017]

The “starting milk” in the present invention is a liquid-type or gel-type starting material used for the production of fermented milk such as yoghurt, and is also called yoghurt mix or fermented milk mix. In the present invention, a known starting milk may be used as appropriate. The starting milk includes that prior to pasteurization and that after pasteurization. Specific materials (starting materials) of the starting milk may include water, raw milk, pasteurized milk, skim milk, whole milk powder, skim milk powder, concentrated whole milk, concentrated skim milk, buttermilk, butter, cream, etc.

In addition, the starting milk may comprise whey protein concentrate (WPC), whey protein isolate (WPI), α-lactalbumin (α-La) and β-lactoglobulin (β-Lg).

[0018]

In order to subject the starting milk to lactic acid fermentation, the starting milk is blended (mixed) with a starter and fermented. As these starters, known starters may be used as appropriate, and lactic acid bacteria starters are preferred. As the lactic acid bacteria starter, one or more types from lactic acid bacteria, bifidobacteria, and yeasts, etc., generally used in the production of fermented milk may be used, such as *Lactobacillus bulgaricus* (*L. bulgaricus*), *Streptococcus thermophilus* (*S. thermophilus*), *Lactobacillus lactis* (*L. lactis*), *Lactobacillus gasseri* (*L. gasseri*), and *Bifidobacterium* (*Bifidobacterium*).

[0019]

"Mixed starter" in the present invention refers to a starter consisting of two or more types selected from lactic acid bacteria, bifidobacteria, and yeasts, etc.; it may be a mixed starter with a combination of lactic acid bacteria, bifidobacteria, and yeasts, etc., and may also be a mixed starter consisting solely of lactic acid bacteria. As such mixed starter, a mixed starter of lactic acid bacteria is preferable, and a mixed starter of lactic acid bacteria belonging to the genus *Lactobacillus* and genus *Streptococcus* is more preferable, and a mixed starter of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is furthermore preferable. As a mixed starter of lactic acid bacteria, it is possible to add other lactic acid bacteria and bifidobacteria such as *Bifidobacterium*.

[0020]

As the *Lactobacillus* lactic acid bacteria, *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, and *Lactobacillus gasseri* are preferable, and as the *Streptococcus* lactic acid bacteria, *Streptococcus thermophilus* and *Streptococcus macedonicus* are preferable. In particular, a mixed starter of *Lactobacillus bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*), which is standardized by the Codex Standard as a yoghurt starter, is preferably used.

[0021]

In the method for producing fermented milk of the present invention, an increase in acidity during refrigerated storage can be further suppressed by using a lactic acid bacteria starter showing slow fermentation. Such a lactic acid bacteria starter may be a mixed starter using lactic acid bacteria with which symbiosis cannot be established.

Specifically, a lactic acid bacteria starter requiring 5.5 h or more of fermentation time to achieve 0.73% of acidity

at fermentation temperature of 43°C is preferable. Examples of lactic acid bacteria used in such a lactic acid bacteria starter include *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Streptococcus thermophilus* and *Streptococcus macedonicus*, etc.

[0022]

As the amount of addition of a starter, 1-8 wt% is preferable, 2-6 wt% is more preferable, 2-4 wt% is furthermore preferable. In these cases, for example, the number of *Lactobacillus bulgaricus* in the starter is preferably $10^6$-$10^{12}$ cfu/mL, more preferably $10^7$-$10^{11}$ cfu/mL, and furthermore preferably $10^8$-$10^{10}$ cfu/mL. The number of *Streptococcus thermophilus* in the starter is preferably $10^7$-$10^{13}$ cfu/mL, more preferably $10^8$-$10^{12}$ cfu/mL, and furthermore preferably $10^9$-$10^{11}$ cfu/mL. As the method of adding (inoculating) a starter, there is a method of adding a bulk starter that has been prepared from a mother starter, and there is also a direct culture method in which frozen bacteria (concentrated frozen bacteria) are directly added.

[0023]

"Monoculture solution" in the present invention primarily means a culture solution in which a single type of microorganisms is cultured independently without mixing multiple types of microorganisms; typically, it means a monoculture solution in which a specific type of bacteria is solely cultured.

In the method for producing fermented milk of the present invention, the use of a monoculture solution of lactic acid bacteria is preferable. As the monoculture solution of lactic acid bacteria, those wherein lactic acid bacteria of the same genus are combined are preferable, those wherein lactic acid bacteria of the same genus and same species are combined are more preferable, and those wherein lactic acid bacteria of the same bacterial strain are furthermore preferable.

[0024]

In the method for producing fermented milk of the present invention, as the lactic acid bacteria used for monoculturing, *Lactobacillus* lactic acid bacteria are preferable, and *Lactobacillus* lactic acid bacteria selected from *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, and *Lactobacillus gasseri* are preferable, and *Lactobacillus bulgaricus* is especially preferable.

[0025]

In the method for producing fermented milk of the present invention, components contained in the monoculture solution are not particularly limited, and the use of culture components contained in the starting milk is preferable, and the use of only the culture components contained in the starting milk is more preferable. In addition, a liquid form is preferable as the monoculture solution; however, the form may also be a solid form or semi-solid form (such as gel) when the components of culture solution are substantially contained.

[0026]

As components for culturing lactic acid bacteria, the use of the following substances in a starting milk is preferable: raw milk, pasteurized milk, skim milk, whole milk powder, skim milk powder, concentrated whole milk, concentrated skim milk, buttermilk, butter, cream, whey protein concentrate (WPC), whey protein isolate (WPI), α-lactalbumin (α-La) and β-lactoglobulin (β-Lg), etc. It is more preferable to use raw milk, pasteurized milk, skim milk, skim milk powder, whole milk powder, concentrated whole milk, and/or concentrated skim milk.

When skim milk powder is used, it may be an aqueous solution containing 5 wt% or more of skim milk powder; the amount of reconstituted skim milk is preferably 7-15 wt%, more preferably 8-14 wt%, furthermore preferably 9-13 wt%.

[0027]

The amount of a monoculture solution blended in a starting

milk may be 1 wt% or more of the amount of the starting milk, preferably 2-15 wt%, more preferably 3-10 wt%, and furthermore preferably 4-8 wt%. At this time, the number of *Lactobacillus bulgaricus* in the monoculture solution is, for example, preferably $10^6$-$10^{12}$ cfu/mL, more preferably $10^7$-$10^{11}$ cfu/mL, and furthermore preferably $10^8$-$10^{10}$ cfu/mL.

[0028]

In the method for producing fermented milk of the present invention, as effective components contained in the monoculture solution, casein digest (peptide, amino acid, etc.) may be contained at 0.01 wt% or more of the monoculture solution, preferably at 0.02-0.2 wt%, more preferably at 0.03-0.15 wt%, and furthermore preferably at 0.05-0.1 wt%. In addition, D-lactic acid may be contained at 0.05 wt% or more of the monoculture solution, preferably at 0.1-2 wt%, more preferably at 0.4-1.6 wt%, and furthermore preferably at 0.8-1.2 wt%.

[0029]

In the method for producing fermented milk of the present invention, it is preferable to remove and/or sterilize bacterial bodies of the monoculture solution. At this time, as a process to remove and/or sterilize bacterial bodies of the monoculture solution, methods such as centrifugation, filtration, heating, ultraviolet irradiation, γ-ray irradiation, and ultrasonication may be used, but the methods are not particularly limited thereto. Preferable methods are centrifugation, filtration, and heating.

In addition, as a process to remove and/or sterilize bacterial bodies of the monoculture solution, the process can also be carried out simultaneously with the removal and/or sterilization of bacterial bodies of the starting milk.

[0030]

After removal and/or sterilization of bacterial bodies of a monoculture solution, the monoculture solution may comprise

dead bacterial bodies. Meanwhile, the monoculture solution may sometimes comprise live bacteria after removal and/or sterilization of bacteria; in such cases, preferably the amount of the live bacteria is within a range that they do not act as a starter.

[0031]

In the method for producing fermented milk of the present invention, as a step of mixing a monoculture solution and a mixed starter, it is preferable to almost simultaneously mix the starting milk, monoculture solution and mixed starter before fermentation, and it is more preferable to mix the starting milk with the monoculture solution, then mix the mixed starter. When the starting milk is mixed with the monoculture solution, the monoculture solution can be added to the starting milk either before or after pasteurization of the starting milk, or both before and after pasteurization of the starting milk. When the monoculture solution is added to the starting milk before its pasteurization, the monoculture solution is pasteurized together with the starting milk; therefore, when this monoculture solution comprises live bacteria, the amount of these live bacteria can be reduced to a level at which they do not act as a starter.

[0032]

In one embodiment of the method for producing fermented milk of the present invention, a deoxygenation step to deoxygenate the starting milk is comprised in the fermentation step.

The deoxygenation step is a step for decreasing or removing oxygen present in a starting milk. As a method for decreasing the dissolved oxygen concentration (DO) in a starting milk (deoxygenation method), for example, a method involving gas replacement with an inert gas such as nitrogen gas, helium, neon, argon and xyenon, a membrane separation method using an

oxygen permeating membrane, a method involving degassing at low pressure or under vacuum, etc. may be used. In the deoxygenation step, deoxygenation may be carried out until the amount of oxygen present in the starting milk (dissolved oxygen concentration) attains a degree of for example no greater than 5 ppm, preferably no greater than 3 ppm, and more preferably no greater than 2 ppm.

[0033]

The lower the dissolved oxygen concentration of the starting milk at the start of fermentation, the better. For example, when the temperature of the starting milk is approximately 40°C, it is preferably no greater than 5 ppm, and more preferably no greater than 3 ppm. Fermentation time can be reduced by decreasing the dissolved oxygen concentration upon start of the fermentation, and therefore, even when fermentation temperature is lowered, fermentation can be completed with fermentation time set at a relatively short time range of 3 to 7 hours. These deoxygenation steps are also described in Japanese Patent Nos. 3666871, 3644505, and 3968108.

[0034]

The deoxygenation step may be carried out for once or two or more times, at the time of preparing the starting milk, after homogenization and/or pasteurization of the starting milk, or after addition of a starter to the starting milk. However, since it is important to maintain a state in which dissolved oxygen concentration is decreased upon start of the fermentation, it is desirable that the deoxygenation step is carried out immediately before, immediately after, or at the same time as addition of a starter.

[0035]

In the method for producing fermented milk of the present invention, the fermentation temperature is preferably 30-50°C, more preferably 35-47°C, and furthermore preferably 40-45°C.

In addition, when deoxygenation of a starting milk is carried out in the fermentation step, since fermentation proceeds under a fairly low temperature, the fermentation temperature is preferably 30-39°C, more preferably 32-38°C, and furthermore preferably 34-37°C.

[0036]

In the method for producing fermented milk of the present invention, the fermentation time can be defined as the time taken until lactic acid acidity of fermented milk reaches approximately 0.7%. During fermentation, lactic acid acidity may be determined by, for example, titration using NaOH and a phenolphthalein indicator.

[0037]

In the method for producing fermented milk of the present invention, the fermentation time is preferably 2-24 h, more preferably 2-12 h, furthermore preferably 3-7 h, and particularly preferably 3-5 h.

In addition, when a deoxygenation step is carried out, the fermentation time is preferably 2-20 h, more preferably 2-10 h, furthermore preferably 3-6 h, and particularly preferably 3-4 h.

[0038]

In the method for producing fermented milk of the present invention, the addition of a monoculture solution to a starting milk provides mild sour taste, realizing the production of fermented milk with high palatability having improved natural sweetness and improved smooth mouth feel while maintaining suitable mild sour taste. In this case, since a mild sour taste as a fermented milk can be easily noticed, the product type is preferably yoghurt such as set type yoghurt, soft yoghurt and drinking yoghurt; because natural sweetness and smooth mouth feel stand out, it is more preferably set type yoghurt such as plain yoghurt.

[0039]

In the fermented milk of the present invention, an increase in acidity during refrigerated storage is suppressed. The rate of increase in acidity of the fermented milk of the present invention may be, when it is stored at 10°C for 7 days, 5% or less, preferably 0-5%, more preferably 0-3%, and furthermore preferably 0-2%.

[0040]

In the fermented milk of the present invention, the amount of fat is preferably 0-5 wt%, more preferably 0-4 wt%, and furthermore preferably 0-3 wt%. The amount of protein is preferably 7-15 wt%, more preferably 8-14 wt%, and furthermore preferably 9-13 wt%.

[0041]

The present invention is explained in detail by way of Examples, but such Examples are intended to exemplify the present invention and not to limit the present invention.

**[Examples]**

[0042]

[Example 1] Characteristics of lactic acid starter showing slow fermentation (low PA starter)

Yoghurt (fat content: 3.0%, SNF: 9.5%) was prepared using a mixed starter of lactic acid bacteria. Fermentation time, acidity increase and pH of this yoghurt during refrigerated storage are shown in Table 1. As the four types of mixed starters of lactic acid bacteria shown in Table 1, mixed starters consisting of *L. bulgaricus* (*Lactobacillus bulgaricus*) and *S. thermophilus* (*Streptococcus thermophilus*) were used.

[0043]

In the current examples, lactic acid bacteria starters having a tendency of slow progress of fermentation (5.5 h or

more of the fermentation time) and a suppressed increase in acidity after refrigerated storage (post acidification: PA) were used. These mixed starters of lactic acid bacteria are referred to as low PA starters in the current examples.

[0044]

[Table 1]

Table 1. Comparison of fermentation characteristics (43°C) and refrigerated storage stability (10°C) of low PA starters.

| Lactic acid bacteria starter | Fermentation time | Day 1 (Fresh) | | Day 7 | | Day 14 | |
|---|---|---|---|---|---|---|---|
| | | Acidity (%) | pH | Acidity (%) | pH | Acidity (%) | pH |
| Low PA-1 | 5 h 40 min | 0.74 | 4.61 | 0.78 | 4.48 | 0.83 | 4.40 |
| Low PA-2 | 6 h | 0.72 | 4.66 | 0.73 | 4.61 | 0.80 | 4.49 |
| Low PA-3 | 7 h 30 min | 0.72 | 4.59 | 0.75 | 4.52 | 0.80 | 4.46 |
| Low PA-4 | 7 h 20 min | 0.72 | 4.59 | 0.74 | 4.53 | 0.80 | 4.45 |

[0045]

[Experimental example 2] Fermentation-promoting effect of commercially available milk peptide

Commercially available milk peptide (CE 90GMM: Nippon Shinyaku Co., Ltd.) was blended in reconstituted skim milk (10% aqueous solution of skim milk powder) at 0-0.3%, and the mixture was heated (90°C, 10 min) to prepare a starting milk (medium). This starting milk was inoculated (added) with various types of low PA starters at 2.0%, and fermented (43°C) until lactic acid acidity of 0.73% was reached. Table 2 shows fermentation time and acidity after refrigerated storage (14 days).

[0046]

[Table 2]

Table 2. Effects of milk peptide on fermentation characteristics (43°C) and refrigerated storage stability (10°C).

| | CE90GMM (%) | Fermentation time (hr) | Acidity (%) day 14 |
|---|---|---|---|
| *S. thermophilus* alone (single strain) | 0.0 | 7.00 | 0.66 |
| | 0.1 | 4.50 | 0.85 |
| | 0.3 | 3.25 | 0.86 |

| | | | |
|---|---|---|---|
| Low PA-1 | 0.0 | 5.00 | 0.81 |
| | 0.1 | 4.00 | 0.88 |
| | 0.3 | 3.50 | 0.93 |
| Low PA-2 | 0.0 | 5.50 | 0.79 |
| | 0.1 | 4.00 | 0.92 |
| | 0.3 | 3.50 | 0.92 |
| Low PA-3 | 0.0 | 6.00 | 0.80 |
| | 0.1 | 4.00 | 0.90 |
| | 0.3 | 3.50 | 0.92 |
| Low PA-4 | 0.0 | 5.50 | 0.82 |
| | 0.1 | 3.50 | 0.96 |
| | 0.3 | 3.50 | 1.10 |

[0047]

By blending the milk peptide in the starting milk, the fermentation time was decreased for all cases of starters. When *S. thermophiles* alone (single strain), low PA-1, low PA-2, and low PA-3 were used as a starter, in order to complete the fermentation within a short period of 3.5 h, it was necessary to blend the milk peptide at 0.3% or more; and when low PA-4 was used as a starter, it was necessary to blend the milk peptide at 0.1% or more.

[0048]

When *S. thermophiles* alone (single strain) was used as a starter, then even when fermentation was completed within a short period of 3.5 h by blending the milk peptide at 0.3%, the acidity after refrigerated storage was as low as less than 0.9%. However, when low PA-1, low PA-2, low PA-3 and low PA-4 that are the starters intended for yoghurt were used as a starter, and when fermentation was completed within 3.5 h by blending the milk peptide at 0.3%, then the acidity after refrigerated storage became a high value of 0.9% or more, showing that an increase in acidity was not suppressed.

In the following examples, low PA-4, that has been confirmed to have a fermentation-promoting effect even when the amount of addition of milk peptide was minimum, was used as the lactic acid bacteria starter.

[0049]

[Example 3] Fermentation-promoting effect of culture solution of lactic acid bacteria

Various types of culture solution of lactic acid bacteria were blended at 2.0% in a modified milk (fat content: 3.0%, SNF: 9.5%), then the mixture was heated (90°C, 10 min) to prepare a starting milk (medium). This starting milk was inoculated (added) with the low PA-4 starter at 2.0%, and fermented (43°C) until lactic acid acidity of approximately 0.7% was reached.

[0050]

Table 3 shows the relationship between fermentation time and time-course changes in acidity. In the Table, "without addition" indicates the control without blending a culture solution of lactic acid bacteria.

[0051]

[Table 3]

Table 3. Effects of culture solution of lactic acid bacteria on fermentation characteristics (43°C).

| Fermentation time (min) | Culture solution | | | | | | |
|---|---|---|---|---|---|---|---|
| | Without addition | Bul.A | Therm.A | Bul.B | Therm.B | Bul.A+ Therm.A | Bul.B+ Therm.B |
| 150 | 0.40 | 0.45 | 0.42 | 0.46 | 0.43 | 0.44 | 0.43 |
| 200 | – | – | – | – | – | – | – |
| 210 | 0.50 | 0.58 | 0.52 | 0.56 | 0.52 | 0.52 | 0.53 |
| 240 | – | 0.62 | – | 0.61 | – | – | – |
| 270 | 0.57 | 0.65 | 0.61 | 0.65 | 0.60 | 0.62 | 0.63 |
| 300 | – | – | 0.67 | 0.69 | – | – | – |
| 306 | – | 0.69 | – | – | – | – | – |
| 336 | – | – | – | – | – | – | – |
| 330 | 0.65 | 0.70 | 0.67 | 0.72 | 0.68 | 0.67 | 0.68 |
| 360 | 0.69 | 0.73 | – | – | 0.69 | 0.69 | – |
| 390 | 0.72 | – | 0.70 | – | – | – | – |
| 400 | – | – | – | – | 0.70 | 0.71 | 0.71 |
| 420 | – | – | 0.73 | – | – | – | – |

*Numbers in the table represent lactic acid acidity (%).

[0052]

As the culture solution of lactic acid bacteria, a reconstituted skim milk (10% aqueous solution of skim milk powder) was inoculated (added) with the following bacteria at

2.0% each: *L. bulgaricus* A (Bul. A) alone or *L. bulgaricus* B (Bul. B) alone (single strain), *S. thermophilus* A (Therm. A) alone or *S. thermophilus* B (Therm. B) alone (single strain), a mixture of *L. bulgaricus* A and *S. thermophilus* A (mixed bacteria ), and a mixture of *L. bulgaricus* B and *S. thermophilus* B (mixed bacteria ); then the culture solutions were prepared by culturing (37°C, 16 h). Here, *L. bulgaricus* A and *S. thermophilus* A were isolated from Meiji Bulgaria Yoghurt LB81 plain, and *L. bulgaricus* B and *S. thermophilus* B were isolated from Meiji Bulgaria Fruits Yoghurt.

[0053]

In the cases of the monoculture solutions of *L. bulgaricus*, fermentation-promoting effects were observed compared to the control; however, in the cases of the monoculture solutions of *S. thermophilus* as well as the mixed culture solutions of *L. bulgaricus* and *S. thermophilus*, fermentation-promoting effects were not observed compared to the control.

[0054]

[Example 4] Fermentation-promoting effect of monoculture solution of *L. bulgaricus*

According to Example 3, fermentation-promoting effects were observed for the monoculture solutions of *L. bulgaricus*. Therefore, in order to investigate the effect of blending concentration of monoculture solution of *L. bulgaricus*, the monoculture solutions were blended at either 5% or 10% and fermentation was carried out (43°C) until lactic acid acidity of approximately 0.7% was reached.

Table 4 shows the relationship between fermentation time and time-course changes in acidity, and Table 5 shows an increase in acidity and pH during refrigerated storage.

[0055]

When the monoculture solution of *L. bulgaricus* was blended at either 5% or 10%, then fermentation time was decreased and

fermentation-promoting effect was observed (Table 4). In particular, when the monoculture solution of *L. bulgaricus* was blended at 5%, fermentation time was decreased remarkably, and strong fermentation-promoting effect was observed.

[0056]

[Table 4]

Table 4. Effects of monoculture solution of *L. bulgaricus* on fermentation characteristics (43°C).

| Fermentation time (min) | Culture solution | | |
|---|---|---|---|
| | Without addition | Bul. B 5% | Bul. B 10% |
| 150 | 0.39 | 0.54 | 0.52 |
| 200 | | | |
| 210 | 0.50 | 0.66 | 0.66 |
| 220 | – | – | – |
| 230 | – | – | – |
| 240 | – | 0.71 | 0.67 |
| 250 | – | – | – |
| 260 | – | – | – |
| 270 | 0.56 | – | 0.70 |
| 290 | – | – | – |
| 300 | – | – | 0.74 |
| 330 | 0.62 | – | – |
| 390 | 0.68 | – | – |
| 420 | 0.71 | – | – |

*Numbers in the table represent lactic acid acidity (%).

[0057]

When the monoculture solution of *L. bulgaricus* was blended at either 5% or 10%, the acidity during refrigerated storage was as low as less than 0.9%.

[0058]

[Table 5]

Table 5. Effects of monoculture solution of *L. bulgaricus* on refrigerated storage stability (10°C).

| | Day 1 (fresh) | | Day 7 | | Day 14 | |
|---|---|---|---|---|---|---|
| Culture solution | Acidity (%) | pH | Acidity (%) | pH | Acidity (%) | pH |
| Without addition | 0.73 | 4.60 | 0.77 | 4.54 | 0.79 | 4.39 |
| Bul. B 5% | 0.73 | 4.66 | 0.76 | 4.53 | 0.83 | 4.40 |
| Bul. B 10% | 0.73 | 4.63 | 0.78 | 4.52 | 0.82 | 4.35 |

[0059]

As shown above, by blending a monoculture solution of *L.*

*bulgaricus* at either 5% or 10%, it was found that increases in acidity after refrigerated storage can be suppressed while progress of fermentation is promoted.

Furthermore, by carrying out the fermentation after deoxygenation, fermentation time was further decreased and progress of fermentation was accelerated (3.5 h), resulting in successful preparation of plain yoghurt with excellent storage stability.

[0060]

[Example 5] Fermentation-promoting effect and acidity-increase suppressing effect in various types of yoghurt

The following five types of yoghurt were compared in terms of fermentation-promoting effect and acidity-increase suppressing effect, and characteristics of each type of yoghurt were investigated.

(1) Yoghurt using low PA-4 starter (low PA-4 yoghurt)
(2) Low PA-4 yoghurt in which milk peptide was blended at 0.05%
(3) Low PA-4 yoghurt in which milk peptide was blended at 0.1%
(4) Low PA-4 yoghurt in which monoculture solution of *L. bulgaricus* was blended at 5%
(5) Low PA-4 yoghurt in which monoculture solution of *L. bulgaricus* was blended at 10%

[0061]

Figures 1 to 5 show time-course changes in the amounts of D-lactic acid produced by *L. bulgaricus* and L-lactic acid produced by *S. thermophilus*, and the total amount of lactic acid (sum of the former two values), during preparation of yoghurt from the start of fermentation (43°C) to refrigerated storage (10°C).

[0062]

Here, when the total amount of lactic acid reaches 65 mM (lactic acid acidity of approximately 0.73%), this time point is designated to be the fermentation time and the fermentation

is terminated, and yoghurt is prepared and fermentation-promoting effect is investigated. Then the yoghurt is stored under refrigeration (10°C), and the effect of suppressing increases in acidity is investigated. Namely, fermentation characteristics (43°C) and refrigerated storage stability (10°C) during preparation of yoghurt are separately discussed.

Here, the amounts of D-lactic acid and L-lactic acid are measured by HPLC using chiral columns.

[0063]

<Fermentation-promoting effect of milk peptide (effect of decreasing fermentation time)>

In the above (2) (Fig. 2) and (3) (Fig. 3) wherein the growth promoting factor for *S. thermophilus*, i.e., milk peptide, was blended, the amount of L-lactic acid produced by *S. thermophilus* reached approximately 50 mM at 2.5 h after the start of fermentation. In contrast, in the control (1) (Fig. 1), the amount of L-lactic acid reached approximately 30 mM. That is, the amounts of L-lactic acid in (2) and (3) in which milk peptide was blended significantly increased compared to the control (1).

[0064]

On the other hand, the number of *S. thermophilus* in (2) and (3) in which milk peptide was blended did not increase during fermentation compared to the control (1). This suggests that milk peptide enhances metabolic capacity (ability to produce L-lactic acid) of individual *S. thermophilus*. That is, milk peptide is speculated to significantly affect the metabolic capacity of *S. thermophilus* rather than to increase their rate of cell division.

[0065]

Meanwhile, in (2) and (3) in which milk peptide was blended, while the number of *S. thermophilus* did not increase during

fermentation compared to the control (1), the number of *L. bulgaricus* increased by approximately 10 times during fermentation. Thus, it was considered that the number of *L. bulgaricus* increased during fermentation because milk peptide enhanced the metabolic capacity of *S. thermophilus* and thereby increased the amount of production of formic acid, i.e., the growth promoting factor for *L. bulgaricus*.

[0066]

<Fermentation-promoting effect of monoculture solution of *L. bulgaricus* (effect of decreasing fermentation time)>

In the above (4) wherein a monoculture solution of *L. bulgaricus* was blended (Fig. 4), at 3.5 h after the start of fermentation, the amount of L-lactic acid produced by *S. thermophilus* reached approximately 60 mM, whereas in (5) (Fig. 5), the amount of L-lactic acid reached approximately 55 mM. In contrast, in the control (1) (Fig. 1), the amount of L-lactic acid reached approximately 45 mM.

That is, in (4) wherein a monoculture solution of *L. bulgaricus* was blended, the amount of L-lactic acid increased compared to the control (1), whereas in (5), the amount of L-lactic acid increased slightly compared to the control (1).

[0067]

In the above (4) wherein a monoculture solution of *L. bulgaricus* was blended, fermentation time was sufficiently reduced. The reason for this was considered to be as follows: the amount of L-lactic acid increased due to the action between casein hydrolyzate derived from the culture solution and peptide, and also the amount of D-lactic acid derived from the culture solution increased; as a result, the total amount of lactic acid increased and the fermentation time was sufficiently reduced.

[0068]

In the above (5) wherein a monoculture solution of *L.*

*bulgaricus* was blended, the fermentation time was slightly reduced. The reason for this was considered to be as follows: because *S. thermophilus* is generally inferior in acid resistance, the metabolic capacity of *S. thermophilus* was reduced as a result of the increase in the amount of D-lactic acid derived from the culture solution to approximately 10 mM, and therefore a decrease in the fermentation time was not sufficient. Actually, in the above (5) wherein a monoculture solution of *L. bulgaricus* was blended, the amount of L-lactic acid produced by *S. thermophilus* was maintained at a low level during fermentation, compared to the control (1).

That is, in the above (5) wherein a monoculture solution of *L. bulgaricus* was blended, it was considered that, as a result of an increase in the amount of D-lactic acid derived from its culture solution, the amount of total lactic acid increased, and eventually the fermentation time was slightly reduced.

[0069]

<Effect of suppressing increases in acidity by milk peptide (Acidity-suppressing effect during refrigerated storage)>

In the above (2) wherein milk peptide as a growth promoting factor of *S. thermophilus* was blended (Fig. 2), the amount of increase in D-lactic acid (= “amount of D-lactic acid (D14)” — “amount of D-lactic acid (D1)”) was approximately 5 mM; in the above (3) (Fig. 3), the amount of increase in D-lactic acid was approximately 8 mM. In contrast, in the control (1) (Fig. 1), the amount of increase in D-lactic acid was approximately 1 mM.

[0070]

Meanwhile, in the above (2) wherein milk peptide was blended, the amount of increase in L-lactic acid (= “amount of L-lactic acid (D14)”— “amount of L-lactic acid (D1)”) was approximately 18 mM; in the above (3), the amount of increase in L-lactic acid was approximately 23 mM. In contrast, in the

control (1), the amount of increase in L-lactic acid was approximately 8 mM.

Thus, the following was considered: in the above (2) and (3) wherein milk peptide was blended, both the amount of D-lactic acid produced by *L. bulgaricus* and the amount of L-lactic acid produced by *S. thermophilus* increased during refrigerated storage compared to the control (1), so that increases in acidity could not be suppressed during refrigerated storage, and sour taste of the yoghurt became strong.

[0071]

At this time, in the above (2) and (3) wherein milk peptide was blended, the metabolic capacity of *S. thermophilus* was considered to be enhanced and the amount of L-lactic acid was increased compared to the control (1). Then, the amount of D-lactic acid increased because, milk peptide enhanced the metabolic capacity of *S. thermophilus* and increased the amount of production of formic acid, i.e., the growth promoting factor for *L. bulgaricus*, and as a result, the number of *L. bulgaricus* increased by approximately 10 times.

[0072]

<Effect of suppressing increases in acidity by monoculture solution of *L. bulgaricus* (Acidity-suppressing effect during refrigerated storage)>

In the above (4) (Fig. 4) and (5) (Fig. 5) wherein a monoculture solution of *L. bulgaricus* was blended, the amounts of D-lactic acid and L-lactic did not particularly increase during refrigerated storage compared to the control (1), so that an increase in acidity was suppressed during refrigerated storage, and sour taste of the yoghurt was not enhanced.

[0073]

In the above (4) wherein a monoculture solution of *L. bulgaricus* was blended, the number of *S. thermophilus* increased by approximately two folds compared to the control (1), which

moderately increased the amount of L-lactic acid produced by *S. thermophilus*; thus it was considered that the acidity did not increase excessively while fermentation time was reduced.

In the above (5) wherein a monoculture solution of *L. bulgaricus* was blended, the amount of D-lactic acid derived from the monoculture solution of *L. bulgaricus* increased compared to the control (1), resulting in an increase in acidity during fermentation and a decrease in the metabolic capacity of less acid-resistant *S. thermophilus*, which lead to a decrease in the amount of production of L-lactic acid; thus it was considered that the acidity did not increase excessively while fermentation time was reduced.

[0074]

Summary

Milk peptide enhances metabolic capacity of *S. thermophilus* and promotes fermentation; it also enhances the ability of *S. thermophilus* to produce L-lactic acid during fermentation and refrigerated storage; due to such symbiotic action, the milk peptide is considered to increase the number of *L. bulgaricus*. As a result, in yoghurt wherein milk peptide is blended, although fermentation time is shortened, acidity tends to increase during refrigerated storage; thus, it has been demonstrated that conventional fermentation-promoting agents are not able to simultaneously achieve the promotion of fermentation and the suppression of acidity increase during storage.

[0075]

In contrast, a monoculture solution of *L. bulgaricus* is considered to increase the total amount of lactic acid during fermentation and to reduce the fermentation time, by enhancing the amount of D-lactic acid while appropriately increasing the number of *S. thermophilus*. As a result, in yoghurt wherein a monoculture solution of *L. bulgaricus* is blended, the following

tendency has been demonstrated, that is, while fermentation is promoted, an acidity increase is suppressed during refrigerated storage and appropriate acidity can be maintained. Moreover, plain yoghurt wherein a monoculture solution of *L. bulgaricus* is blended shows improved natural sweetness and improved smooth mouth feel.

**[Industrial Applicability]**

[0076]

According to the method for producing fermented milk of the present invention, not only fermentation is promoted, but also an increase in acidity of the fermented milk obtained can be suppressed during refrigerated storage, and its quality such as flavor and physical properties can be improved. Therefore, the present method is useful for the commercial-scale production of plain yoghurt and others.

**[Claims]**

1. A method for producing fermented milk by adding a starter to a starting milk, comprising a step of mixing a mixed starter and a monoculture solution of *Lactobacillus* lactic acid bacteria.

2. The method according to Claim 1, wherein the mixed starter comprises *Lactobacillus* lactic acid bacteria and *Streptococcus* lactic acid bacteria.

3. The method according to Claim 1 or 2, wherein the *Lactobacillus* lactic acid bacteria are selected from the group consisting of *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, and *Lactobacillus gasseri*.

4. The method according to Claim 2 or 3, wherein the *Streptococcus* lactic acid bacteria are *Streptococcus thermophilus* or *Streptococcus macedonicus*.

5. The method according to any one of Claims 2 to 4, wherein the *Lactobacillus* lactic acid bacteria used in the monoculture solution are the same species of lactic acid bacteria that are used in the mixed starter.

6. The method according to any one of Claims 1 to 5, wherein the lactic acid bacteria in the monoculture solution are removed and/or sterilized.

7. The method according to any one of Claims 1 to 6, wherein the treatment of removal and/or sterilization are/is selected from centrifugation, filtration and heating.

8. The method according to Claim 7, wherein the heating of the monoculture solution is carried out simultaneously with heating of the starting milk.

9. The method according to any one of Claims 1 to 8, wherein the monoculture solution comprises, as its culture components, only the components contained in the starting milk.

10. The method according to any one of Claims 1 to 9, wherein the monoculture solution comprises raw milk, pasteurized milk, skim milk, whole milk powder, skim milk powder, concentrated whole milk and/or concentrated skim milk, as its culture components.

11. The method according to any one of Claims 1 to 10, wherein the monoculture solution is added at a concentration of 2-15 wt%.

12. A fermented milk produced by the method according to any one of Claims 1 to 11.