Chapter 1

Probiotic Fermented Milk

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Abstract

Probiotic fermented milk is a product made by appropriate microbial growth using milk as the substrate which contains mainly live microorganisms. Fermented milk has been consumed for thousands of years worldwide, and the incorporation of probiotics has pushed it in a novel direction. The substrate selection includes cows, buffalo, goats, sheep, yak, horses, camel, and others' milk. The various substrate has their uniqueness, and typical traditional products, including kefir, koumiss, etc., are made from them. Further, the range of probiotics is vast, and commonly used genera contain *Lactobacillus* and *Bifidobacterium*. The primarily incorporated method is to inoculate it into the starter culture to co-ferment substrate with traditional fermentation culture. Other methods include fermenting substrate directly or adding it back into the product. The typical products include ambient-temperature fermented milk or probiotic fermented milk beverage. The basic processing method of probiotic fermented milk is similar to traditional fermented milk, where the incorporation of probiotics into the fermented milk product is unique due to the special incubation requirement of each probiotic. Commonly seen additives include sweetener, thickener (thickening technology), and prebiotics which were introduced in this chapter, which could give a comprehensive vision of the current fermented milk production and the indication of applying these additives to the fermented milk considering the existence of probiotics. Some novel and popular fermented milk products and their manufacturing methods were briefly introduced in this chapter, such as ambient-temperature fermented milk, roasted flavor fermented milk, and probiotic fermented milk beverage. General products' quality issues and legal compliance were also mentioned. Still, the most critical way to determine the manufacturing procedure and parameter is by running a pilot test based on the designation of the product, which could give a clear indication of the material, method, and post-manufacturing issues.

Key words Probiotic fermented milk, Manufacture process, Probiotics, Special milk, Sweetener, Prebiotics, Thickening technology

1 Introduction

Probiotic fermented milk is a product derived from traditional fermented milk. Fermented milk is a milk product made via appropriate microbial growth and/or enzymatic conversions of milk [1]. Here, the probiotic fermented milk should go further, where it requires the existence of probiotics in the fermented milk. It was recognized that probiotic fermented milk should contain live

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microorganisms [2]. However, the recent research regarding parabiotics and postbiotics broadened the scope of the products [3], where the importance of the viability of probiotics had been assimilated. In this chapter, *probiotic fermented milk*, referred in a broad sense, is a cluster of fermented milk products containing probiotic strains, live or not. More detailed introduction about parabiotics and postbiotics will be given in Chaps. 15 and 16. Fermented milk has been consumed for thousands of years. It was originated from various places, such as Mongolia, Egypt, Caucasian areas, etc., where multiple products were developed to fulfill the local requirements. For a clear written record, Greek and Roman are the first to mention this type of product (yogurt) in their history, around 100 BC [4]. For probiotics, its health effect had been realized and applied for dozens of centuries, combined with fermented milk consumption [5]. However, its mystery hadn't been revealed until modern times for their existence and taxonomy. In 1857, Pasteur discovered lactic acid bacteria (LAB) for their role in the fermentation of yogurt. In 1908, Elie Metchnikov proposed the idea of probiotics' health effect in his book *The prolongation of life: optimistic studies* (where the word "probiotic" haven't been proposed yet) [5, 6]. In 1953, German scientist Werner Kollath proposed the term "probiotic," which has been further used [7]. For currently admitted and used probiotic definition and effect, it was determined and published by FAO/WHO in 2001 and slightly modified in 2014 by Hill et al. [8] who confirmed that the *probiotic* should be "live microorganisms which could confer a health benefit on the host, when being administrated in appropriate amount." This definition differed the probiotic fermented milk from other traditional fermented milk (relatively different, traditionally used microorganisms for fermentation were sometimes regarded as probiotic in some situations), where the probiotic in the products should be capable of conferring benefit to humans after consumption. Firstly, the probiotic should tolerate gastric, bile, and intestinal fluid, and could colonize and proliferate in the gastrointestinal tract (GI tract). The safety and viability of probiotics are critical to the selection criteria, where the evaluation procedure has been clarified recently. China has published a new Group Standard names *Probiotic Food* by China National Food Industry Association (CNFIA) to define the requirement of probiotics used in food and the evaluation procedure to evaluate their safety and viability (T/CNFIA 131–2021) (*see* **Notes 1** and **2**) [9]. The standard also requires the precise strain number and source, and the completion of whole genome sequencing and random clinical trial to support its efficacy based on scientific articles. Other scholars also believe the probiotics used in the fermented milk (food) should exist in the GI tract originally, and genetically modified (GM) strain/species should not be used [10]. Meanwhile, there are a lot of strains or species that were tested and claimed to possess probiotic potential. Still, the authorities did not have explicit consensuses to determine which strain/species or groups can be regarded as probiotics. For example, China and Canada had a list showing the possibility of adding these species into foods as probiotics (Table 1).

Table 1
The list of microbial strains available to be used in foods in China and other countries [89–92]

Genera	Species
Bifidobacterium	*Bifidobacterium adolescentis*[*,^,#] *Bifidobacterium animalis* subsp. *animalis* [*,^,#] *Bifidobacterium animalis* subsp. *lactis*[*,^,#] *Bifidobacterium bifidum*[*,^,#] *Bifidobacterium breve*[*,^,#] *Bifidobacterium longum*[*,^,#] *Bifidobacterium longum* subsp. *Longum*[*,^,#!] *Bifidobacterium longum* subsp. *Infantis* *Bifidobacterium longum* subsp. *Suis*[*,^,#!]
Bacillus (*Assessed* case-by-case in AU)	*Bacillus subtilis*[^!] *Bacillus cereus*[^!]
Companilactobacillus	*Companilactobacillus farciminis* [#!]
Debaryomyce[%]	*Debaryomyces hansenii*[#!]
Enterococcus (*Assessed* case-by-case in AU)	*Enterococcus faecium*[^!] *Enterococcus faecalis*[^!]
Fructilactobacillus	*Fructilactobacillus sanfranciscensis* [#!]
Lacticaseibacillus	*Lacticaseibacillus casei*[#] *Lacticaseibacillus paracasei* [#] *Lacticaseibacillus rhamnosus*[#]
Lactiplantibacillus	*Lactiplantibacillus paraplantarum* [#!] *Lactiplantibacillus plantarum*[#]
Lactobacillus	*Lactobacillus acidophilus*[*,^,#] *Lactobacillus amylolyticus*[*,^,#!] *Lactobacillus crispatus*[*,^,#] *Lactobacillus delbrueckii*[#!] *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lactobacillus bulgaricus*)[#] *Lactobacillus delbrueckii* subsp. *Delbrueckii*[#!] *Lactobacillus delbrueckii* subsp. *Lactis*[#] *Lactobacillus gallinarum*[#!] *Lactobacillus gasseri*[#] *Lactobacillus helveticus*[#] *Lactobacillus johnsonii*[#] *Lactobacillus kefiranofaciens* subsp. *kefiranofaciens*[#]
Streptococcus	*Streptococcus salivarius* subsp. *thermophilus*
Lactococcus	*Lactococcus Lactis* subsp. *lactis* *Lactococcus cremoris* *Lactococcus Lactis* subsp. *Lactis* biovar diacetylactis
Latilactobacillus	*Latilactobacillus curvatus* [#] *Latilactobacillus sakei*

(continued)

Table 1 (continued)

Genera	Species
Lentilactobacillus	*Lentilactobacillus buchneri* [#!] *Lentilactobacillus hilgardii*[#!] *Lentilactobacillus kefiri* [#!]
Propionibacterium	*Propionibacterium freudenreichii* subsp. *Shermanii*[^,#] *Propionibacterium freudenreichii*[^,#!]
Acidipropionibacterium	*Acidipropionibacterium acidipropionici* [#]
Leuconostoc	*Leuconostoc mesenteroides* subsp. *Mesenteroides*[#] *Leuconostoc citreum*[#!] *Leuconostoc lactis*[#!] *Leuconostoc pseudomesenteroides*[#!]
Levilactobacillus	*Levilactobacillus brevis*[#!]
Ligilactobacillus	*Ligilactobacillus salivarius*[#]
Limosilactobacillus	*Limosilactobacillus fermentum* [^,#] *Limosilactobacillus mucosae* [#!] *Limosilactobacillus panis*[#!] *Limosilactobacillus pontis*[#!] *Limosilactobacillus reuteri* [*,#]
Loigolactobacillus	*Loigolactobacillus coryniformis* [#!]
Mammaliicoccus	*Mammaliicoccus vitulinus*
Oenococcus	*Oenococcus oeni*[#!]
Kluyveromyces[%]	*Kluyveromyces lactis*[#!] *Kluyveromyces marxianus*[#]
Pediococcus	*Pediococcus acidilactici*[#] *Pediococcus pentosaceus*[#]
Staphylococcus	*Staphylococcus xylosus* *Staphylococcus carnosus*
Saccharomyces[%]	*Saccharomyces bayanus*[#!] *Saccharomyces boulardii*[*,#!] *Saccharomyces cerevisiae*[#!] *Saccharomyces pastorianus*[#!]
Schizosaccharomyces[%]	*Schizosaccharomyces pombe*[#!]
Weizmanni	*Weizmannia coagulans*
Xanthophyllomyces[%]	*Xanthophyllomyces dendrorhous*[#!]

*: genera or species available in foods as probiotic (or showing health effect) in USA, symbols marked at species column, separated with other symbols using comma (,)

^: genera or species available in foods as probiotic (or showing health effect) in Australia (includes those that were not authorized by China, which was marked as ^!), symbols marked at species column, separated with other symbols using comma (,).

#: genera or species available in foods as probiotic (or showing health effect) in Canada (includes those that were not authorized by China, which was marked as #!), symbols marked at species column, separated with other symbols using comma (,).

%: yeast, marked at genera column.

Table 2
The approximate composition of various typical probiotic fermented milk products [47, 93–96]

	Yogurt	Kefir	Koumiss	Ymer (Denmark product)	Skyr (Iceland product)
Protein, %	5	3	2.2	5–6	12.7
Fat, %	7.5	0.2	1.9	3.5	0.2
Acidity, %	0.8	1			
Total solids, %	18.5	10.6–14.9		14.5	17.5
Carbohydrate, %		6	2.8	3.5	3.9
Alcohol, %		1	2.2		0.3–0.5
Ash, %		0.7			0.8
Others		1.97 g/L CO_2			

Many fermented milk could contain probiotics, such as yogurt, kefir, koumiss (kumys, kumis, kumiss, coomys), sour cream, and fermented buttermilk. Besides these traditional probiotic fermented milk products, some novel fermented dairy beverages containing probiotics have been developed recently, and the most famous one is Yakult®. The main difference among them is the product status (fluidity) and intrinsic microbial environment (multi vs. single strain) (*see* **Note 3**). They have different substrates, processing procedures, and storage conditions, where the most important is their proximate composition (Table 2). By the time of quality detection, the parameter measured had been regulated by the authorities from various countries. Table 3 summarizes the regulation parameters and numbers of the parameters which the products should achieve.

As mentioned above, the strict definition of probiotic fermented milk should contain live microorganisms in their product matrices. However, recent product development has combined the inactivation of live cells into the processing procedure to extend the shelf life or more stable quality, such as ambient-temperature yogurt (pasteurized fermented milk) and other products. They apply various live-cell inactivation methods to limit or eliminate the activity of viable microorganisms in the products to prolong the shelf life of the products for a farther distribution range or more manageable storage conditions. The inactivation methods include radiation, heating, high pressure, etc. (*see* **Note 4**). There are also coupled designs for these sterilized products about packaging material and style. General packaging uses plastic cups/containers (set) or bottles (stirred) to package fermented milk. For premium products, the glass jar is acceptable to package the product as well.

Table 3
The compilation of fermented milk standards from various countries [27, 38, 97–100]

	Codex Alimentarius	China	USA	Canada	Australia
Fat, %	$\leq 10^{acd}$, 15^{b}	3.1^{a}, 2.5^{e}	$\geq 3.25^{ab}$	——	——
Non-fat-solid, %	——	8.1^{a}	$\geq 8.25^{ab}$	$\geq 9.5^{b}$, 7.6^{e}, 6.5^{f}	——
Protein, %	Min 2.7^{abcd}	2.9^{a}, 2.3^{e}	——	$\geq 2.8^{b}$, 2.2^{f}	$\geq 3^{a}$ (cow's milk)
Acidity, %	Min 0.3^{a}, 0.6^{bc}, 0.7^{d}	——	$\geq 0.5^{a}$, 0.7^{b} (or $\leq$ pH 4.6)	$\geq 0.7^{b}$	$\leq$pH 4.5^{a}
Acidity, °T	——	70	——	——	——
Microbial load, cfu/g(mL)	Min $10^{6,\ ab}$ (total), $10^{4,\ cd}$ (yeast)	$\geq 10^{6,\ ae}$	$\geq 10^{7,a}$	$\geq 10^{7,b}$	$\geq 10^{6,\ a}$
Ethanol, %vol./w	Min 0.5^{d}	——	——	——	——
Document number	CXS 243–2003	GB19302–2010	FDA-21 CFR 131.112, 131.200	National Dairy Code, Part III	Australia New Zealand Food Standards 2.5.3, F2015L00413

[a]Fermented milk/Cultured milk.
[b]Yogurt, alternate culture yogurt, acidophilus milk (Yogurt: fermented milk using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as culture; alternate culture yogurt: using *Streptococcus thermophilus* and any *Lactobacillus* species; acidophilus milk: using *Lactobacillus acidophilus* as culture).
[c]Kefir.
[d]Kumys.
[e]Flavored fermented milk (with sugar or fruit component addition).
[f]Yogurt drink (drinkable fermented milk).
——: Not mentioned or required by such standards.

However, novel tetra packaging was developed to comply with the requirement of ambient-temperature fermented milk to assist its prolonged storage time. The shelf life of regular fermented milk (with or without probiotics) is around 21–28 days. For plastic packaged products, some of them can be shortened to 14 days (it is worth noting that the shelf life does not have a severe relationship with the preservation ability of LAB or the health effect of probiotics). The optimal storage condition of such products is around 4 °C, requiring fully cold-chain logistics. For ambient-temperature fermented milk, the shelf life can be extended to 6 months at ambient temperature (around 25 °C).

Moreover, there are vast amounts of products commercially available in the market. Still, they can be characterized according to several criteria, such as matrix status (set/stirred), product additive (natural, sweetened (flavored), nutritionally enhanced), post-fermentation processing (condensed, frozen, carbonized, spray-dried), fat content (full-fat, partially skimmed, skimmed, and Greek yogurt) [11]. Nevertheless, their material, main processing procedure, and packaging step are very similar, with a slight difference in additive, post-fermentation, and packaging steps. These will be described in detail in **Part III**.

2 Material

Materials used for probiotic fermented milk production can be divided into several groups: raw milk and milk substrate, starter culture and probiotic strains, sweetener and additive. They have different effects on the probiotic fermented milk, which should be focused on during processing.

2.1 Raw Milk and Milk Substrate

The substrate and primary material of probiotic fermented milk should be various milk originating from multiple breeds or species of mammals. Commonly seen dairy animal species include cows, goats, sheep, buffalo, donkeys, and camels, where cows are the most used for raw milk production. Bovine milk is the most consumed milk by humans. Various cattle breeds have been domesticated by humans for milk production (some of the breeds are for both milk and beef). These temperate breeds include Ayrshire, Guernsey, Brown Swiss, Shorthorn, Jersey, and Holstein Friesian. Among them, Holstein Friesian is the only most important breed for milk production. Holstein Friesian originated from the Netherlands and had been exported widely to the world due to its adaptability. It has excellent milk production capability, where its average milk yield is 25–35 kg/day [11]. This yield is far from other dairy breeds. Holstein Friesian has a lower milk fat content than other temperate breeds except for Shorthorn [11]. The typical appearance of Holstein Friesian is black and white color. Besides, other species have their characteristics, such as Jersey has a high milk fat (4.95%) content and dry matter (14.54%) content with low yield (19–25 kg/day), and shorthorn has a high protein-fat ratio but low yield as well (17–25 kg/day) [11]. Therefore, the selection of raw milk sources would significantly affect the final product's quality.

Notably, the quality of raw milk produced by different animals can be affected by various factors. Of which the most important and controllable are milking season, feeding (water and fodder), and equipment. The raw milk composition could be varied significantly following the milking season (lactation season) change, but the lactose in the milk could be stable. Protein and milk fat have a solid response to season change, where the lowest content occurred in summer (3.21% for protein, 4.1% for fat) and the highest content occurred in winter (3.38% for protein, 4.57% for fat), respectively [12]. It had been reported that the raw milk yield and composition were negatively related to environmental temperature [13–17]. This phenomenon is reasonable and explainable due to the Holstein Friesian originating from a cool area, which has a stress reaction to heat. Heat stress is one of the most significant issues in cows, especially Holstein Friesian husbandry. Lactation season could also affect raw milk yield and composition, whereas Holstein Friesian's lactation season could be over 200 days. Raw milk yield and composition have fluctuated over a long period [17]. The raw milk yield increases and reaches a peak during the early lactation period but goes lower following the lactation period [17]. The fat content has a real controversial tendency compared with yield [17]. It went lower at the beginning of the lactation period and turned to increase, accompanied by lactation progress [17]. Milk protein also has higher content at the beginning of the lactation period [17].

Water and forage feed could be crucial factors that impact the raw milk quality, where the contaminant and odor components, such as heavy metals, animal drugs, and toxins, could be transmitted to the milk through cow's milk secretion [18]. The type and quality of forage could also affect the milk fat content and composition, where the involvement of phytochemical composition in the forage attracts attention [19]. The feeding method could influence the quality of raw milk as well. Grazing cows have lower raw milk yield than feedlot cows, but the fat content in grazing cows' milk is higher than in feedlot cows' milk. The difference between the protein content is negligible [18, 20]. It is worth noting that the fatty acid composition in the milk produced from grazing or feedlot cow is also different. In summary, it is wise to determine the source of raw milk regarding the abovementioned factors before adopting it in fermented milk production for better product quality.

Apart from species, breed, and lactation season, and feeding material quality and method, milking sanitation and equipment are also critical to raw milk quality, especially microbial load. Essential sanitation of the cows' udder (or other dairy animals) is necessary as the microbe in the raw milk strongly correlates with teat skin sanitation. Research proved that the microbial composition is significantly different between raw milk and teat skin due to the both-way contamination. However, 92.1% of the bacteria in the raw milk come from the teats' skin (genetically connected) [21]. An efficient way to sanitize the udder is teat dipping (pre and post), in which the teat was sanitized via iodine solution. The same research also revealed that the microbial composition of teat skin is significantly similar to raw milk, which means the both-way contaminations were halted, and the microbial was only transferred from raw milk to teat [21]. This result proved that iodine sanitation is an efficient way to intercept teat-raw milk contamination. Sanitation of milk equipment is also a pivotal step in ensuring the quality of raw milk. Research indicates that appropriate sanitized equipment could reduce raw milk's thermophilic spore load [22]. Other factors that have relationships with low spore load include farming environment, husbandry scale, regular udder massage, and others [22]. These factors also confirmed that appropriate farming methods, feeding (fodder and silage), housing conditions, and even the cow's mood influence the raw milk quality, which needs attention.

Milking is an essential step for raw milk collection, where the equipment evolution has served this step well. Machine milking has far higher efficiency than manual milking, which has improved the raw milk yield significantly [11]. Recently, automatic milking equipment (robotic milking) was developed to avoid excess stress on cows and save human labor. This equipment ensures the animal welfare of cows and eases their nervousness, anxiety or other negative moods to prevent low-quality raw milk. Usually, the cows were tagged and managed via ear tag, where the information of each cow can be collected when they enter the milking robot for milk tracing. The cost of milking also decreased compared with traditional milking. This automated milking machine has attracted the attention of farmers from developed countries, such as the USA, Australia, The Netherlands, and New Zealand, to apply this system for better raw milk production.

After milking, the raw milk should be tested before production. Some standards or codes require the quality of raw milk. The most crucial parameters are microbial load and somatic cell count (SCC). For microbial load, the USA requires that the raw milk for direct consumption should not contain more than 15,000 total viable bacteria/mL and < 10 coliform bacteria/mL [23]; China has a 2×10^6/mL total viable microbial count limitation of raw milk, whereas the EU limited the total viable microbial count to 1×10^6/mL [24]. For somatic cells, it is not required by China, but the USA and EU had limited the count below 6×10^6 and 4×10^6 cells/mL, respectively [23, 25]. Somatic cell count (SCC) is vital for raw milk quality. It indicates the health status of cows or other dairy animals.

SCC was influenced by parities, calving season, and lactation period, and the yield will drop dramatically when the SCC goes higher [26]. Research proved that the composition of raw milk reached the lowest amount when the SCC exceeded 5×10^6/mL; hence the researcher recommended that the SCC in raw milk should not be above 5×10^6 cell/mL [26].

Besides the microbial count and SCC, many other parameters should be satisfied, including fat, protein, and non-fat milk solids in many countries. For industry raw milk collection, many essential tests need to be performed to ensure the quality of raw milk and perspective products. These include sensory tests, ethanol tests, clot-on-boil tests, titratable acidity, density test, microbial (dye reduction methods)/somatic cell/antibiotic test, composition determination, and adulterant tests [11]. Among them, the ethanol test is a rapid method to determine whether the raw milk is fresh or not, based on the acidity of raw milk [11]. This is a very fast and easy method to be applied in the industry due to the simple phenomenon, equipment, and indicative capability. For fresh raw milk, there will be no phenomenon when ethanol (68%, 70%, 72%) is added to the raw milk, where the coagulation of casein (protein) will occur when the raw milk deteriorates [11, 18]. Notably, a microbial/somatic cell/antibiotic test is necessary, especially for fermented milk production. Besides the microbial count, excess antibiotic in the raw milk is crucial for fermented milk production due to their inhibitory effect on the starter culture cultivation and growth, especially probiotic, which requires a strict environment. The source of antibiotics is vast, but it may come from cattle disease treatment, fodder additive residue, and milking contamination [18]. Addition of antibiotics purposely is rarely seen, but it affects the quality significantly, which needs strict regulation. Developed countries require that antibiotics should not be tested in raw milk [18]. However, a trace amount of antibiotics is still allowed in developing countries [27], indicating that raw milk should be appropriately tested and treated when applied to produce fermented milk in these countries.

After collection, pre-treatment should be performed to ensure the quality of raw milk for further production. Usually, pre-treatment includes filtration, purification, cooling, pre-pasteurization (optional), and deaeration (optional) [11]. Filtration and purification could efficiently remove physical contaminants and excessive microbial and somatic cells to reduce observable contaminants by the naked eye. However, rapid cooling is essential for the stable quality of raw milk during storage before processing. Usually, freshly collected milk has cow's body temperature, which should be cooled around 4–6 °C as soon as possible. The growth of microbes could be attenuated or inhibited at this temperature. If its temperature could be cooled down to 2–3 °C, the growth of the microorganism could be near completely halted, and it can be stored for about 7 days [11]. Pre-pasteurization should be performed if the raw milk is not used immediately to avoid quality deterioration.

2.2 Starter Culture and Probiotic Strains

Starter culture is essential for probiotic fermented milk production. It usually contained lactic acid and polysaccharides producers, such as *Lactobacillus* (L) and *Streptococcus* spp. (S). The ratio of L/S is around 1:1 or 1:2, where the overwhelming of *Lactobacillus* will result in excess lactic acid content and unacceptable flavor [11]. Detailed starter culture production will not be mentioned here. Still, the type of starter culture and production of starter culture are described in Table 4 and Fig. 1, respectively. It is worth noting that adding probiotics as a starter culture is the main method to incorporate probiotics into fermented milk. Hence, the cultivation of probiotics needs further attention. The synergistic or antagonistic bio-relationship between conventional starter culture (*Lactobacillus & Streptococcus* (L&S)) and probiotic could affect the success of fermentation [28]. For example, the difference between the growth rate of L&S and probiotic leads to desired microorganism cultivation failure, or the metabolites of each species could promote or inhibit the growth of other species (hydrogen peroxide, oxygen content, carbonized, etc.) [28–34].

Table 4
Various types of starter cultures used in fermented milk production [11, 40, 47]

Classification criteria	Type of starter culture	Notes
Preparation of Starter cultures	LAB pure culture Mother starter culture Bulk starter culture	Primary strains included in the culture (step 1) Proliferation of primary strains (step 2) Proliferation of mother culture, used for manufacture directly (step 3)
Strain composition of Starter cultures	Mixed strains starter culture Single strain starter culture Supplemented strains starter culture	Contains more than one strains for synergistic fermentation Contains only one strain, mixed when applying Contains one or more strains for special purpose, includes exopolysaccharides production, aroma component production, and probiotics
Status of Starter culture	Liquid starter culture Powder starter culture Frozen starter culture	Easy to operate and cheap, but the viability can be weakened Better viability and stability than liquid form Highly concentrated, highest viability, direct usage

LAB lactic acid bacteria
Steps 1, 2, 3: The steps required for starter culture application during production procedure. These steps were performed according to factories in situ application.

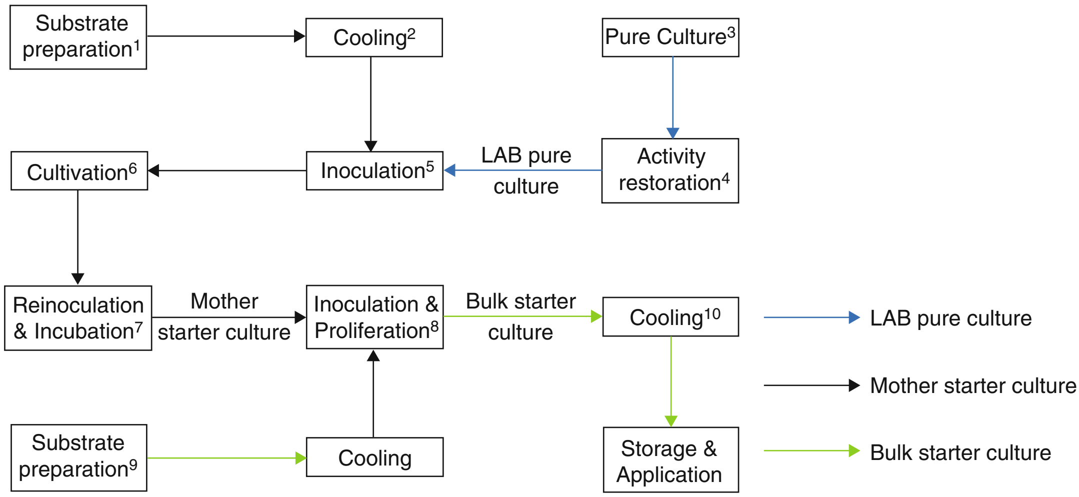

Fig. 1 The flow chart of starter culture preparation [11, 40]. (1) Reconstituted skimmed milk (10–12% solid), heated 90–95 °C for 30–40 min or 121 °C for 15 min. (2) Mesophilic culture: 20–30 °C; theromophilic culture: 42–45 °C. (3) 0–4 °C storage, subculture every 1–2 weeks; random purification is needed. (4) Restoration for 2–3 times. (5) 1–2% addition amount. (6) Temperature determination according to strain characteristics; Time: 3–20 h. (7) Same condition or 2–3 times. (8) At 42 °C, stop when acidity >0.8%. (9) Same substrate treatment condition, but using product raw material as substrate, 1–2% of total raw material. (10) Use within 6 h: 10–20 °C; use after 6 h: 4–5 °C

Due to the growth rate, the time and form of probiotic addition are crucial. As for the preservation of the viability of probiotics, many ways are used to protect probiotics and assist them in reaching the GI tract without severe weakening due to lactic acid in fermented milk or harsh condition in the GI tract. Encapsulation is a commonly used method to protect probiotics. Probiotics can be encapsulated (usually microencapsulated) in different wall materials or matrices to maintain viability (*see* Chap. 14 for more details). Different wall material has various properties, such as protection, lyse, texture alteration, etc. There is a study that showed that the addition of microencapsulated probiotics could affect the texture of yogurt (smoothness), which needs attention (alginate-starch as wall material, which can affect the texture) [35]. Other materials used for microencapsulation include whey protein (an useful by-product of cheese production), gellan gum (polysaccharides), etc. The microencapsulation method includes drop-out, emulsification, extrusion, coacervation, and others. Compared with extrusion, emulsification has a higher encapsulation rate [36]. Microencapsulated probiotics can shorten the fermentation time of fermented milk as well [36], but this phenomenon needs further clarification to differentiate between bacteria synergistic effect or microencapsulation promotion. Besides, the strong buffer capacity of the substrate (neutralized pH) or the firm texture of fermented milk (gel) (prevents acid contact with probiotics) can protect probiotics efficiently as well [28].

2.3 Sweetener and Additives

Many additives can be used in probiotic fermented milk, where the sweetener is the most important one. Sweeteners could provide a sweet taste to the consumer to assimilate or cover the harsh taste of lactic acid in the fermented milk. A commonly used sweetener is sugar (sucrose), which is accepted by most consumers. Recently, artificial sweeteners, such as sucralose and aspartame, were used to provide a more intense sweet taste and reduce cost. However, the health requirement of customers had forced the producer to replace artificial sweeteners with natural sweeteners, hence stevia, erythritol, and mogroside have come into sight of the producers. These selections have broadened the horizon of sweeteners from a health perspective and increased the acceptability and functionality of fermented milk. Besides, there are other additives, such as fruit components (jam, crushed or pulp), thickener/stabilizer/emulsifier, essence, pigment/colorant, etc. [11], that can be added into the fermented milk in accordance with local regulations.

It is worth noting that some unique carbohydrates, such as dietary fiber, resistance starch, oligosaccharides, and inulin, were added to the probiotic fermented milk to acquire its health benefit and probiotic promoting capability (synbiotic ability). These substances are called as prebiotics. Prebiotics is a type of food component that could not be digested by the endogenous host enzymes yet could exert benefit on the host by modulating gut microflora [37]. In this case, the type, purity, chain length, percentage of prebiotic, target probiotic/microflora, product formula and characteristic, and storage conditions need to be considered when applying prebiotic in probiotic fermented milk [37]. Prebiotics can significantly affect the probiotic viability and the physiochemical (texture and rheology), organoleptic and functional properties of the products [37]. However, the effect (positive, negative, or neutral) is still under debate, which needs more attention when utilizing them in the products [37]. More detailed availably of thickener (thickening technology) and prebiotic selection will be discussed in **Notes 5** and **6**.

3 Method

The production method of probiotic fermented milk is similar to yogurt production, which involves pre-treatment (standardization, pre-heating, homogenization, heating, cooling), inoculation, fermentation, additive addition, and packaging. The flow chart of the processing procedure is shown in Fig. 2. Here, it is notable that the order of fermentation, packaging, and additive use is different between set-fermented milk and stirred-fermented milk. Detailed order is shown in Fig. 2 as well. In the following paragraph, each step will be discussed separately, and the combination of such steps should be performed as per product and in situ requirements.

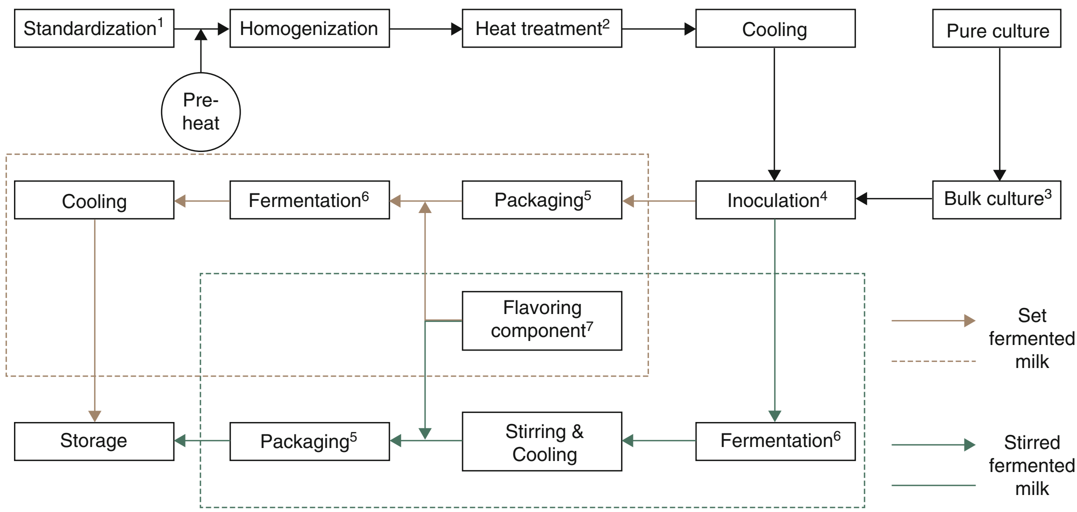

Fig. 2 The flow chart of fermented milk processing [11, 40, 47]. (1) Milk solid, includes protein, cream, thickener, sweeteners were added here; filtration may be applicable for unsolved substances, critical control point 2 (CCP 2) for both set and stirred fermented milk. (2) Significantly important for product quality control, CCP3 for both set and stirred fermented milk. (3) The hygienic condition of starter culture is important, CCP4 for both set and stirred fermented milk. (4) The control of hygienic condition and relative parameter is critical for this step, CCP5 for both set and stirred fermented milk. (5) The hygienic condition of environment and packaging container is critical, CCP6 for set fermented milk, CCP 7 for stirred fermented milk. (6) The fermentation temperature and time are critical for the success of products processing, CCP 7 for set-fermented milk, CCP 6 for stirred fermented milk. (7) Includes fruit component (pulp or jam), essence substances, etc

3.1 Pre-Treatment

Pre-treatment includes raw milk standardization, homogenization, heat treatment, and inoculation steps. Firstly, the raw milk pumped from the storage tank should be standardized to fulfill the requirement of local regulations where the factory resides, or the product will sell. In general, any product should satisfy the requirement of FAO/WHO regulation [38] for global distribution and retail selling. Fat and protein content should be less than 10% and more than 2.7%, respectively. Hence, any raw milk that does not meet this requirement should be standardized to achieve this limitation. Usually, the fermented milk fat content is between 0.5–3.0% [11], depending on whether it is skim or not, where the addition of cream is necessary to adjust this content to not only fulfill the regulation but also to guarantee the sensation of such product. Besides, the non-fat-solid of milk will be fortified, if necessary, whereas the skimmed milk powder should be used here. These components (cream, skimmed milk powder) can be provided within the factory from other product lines to utilize the by-product and make the best value of it. The sugar and stabilizer should be added here to favor the growth and fermentation of starter culture and possess desired texture of the product. Detailed additive addition will be discussed in the Subheading 3.4.

After standardization, milk should be pumped into the heater to pre-heat for homogenization. Appropriate heating could stabilize the fat globule in the milk for homogenization in case any undesired consequences occurred, such as fat separation (creaming) or incomplete homogenization. Homogenization aims to shrink the size of fat globules to prevent cream separation and unify fat distribution. Hence, the stability and consistency of the fermented milk could be improved. Further, this step could mix the ingredients added during standardization well to enhance the texture and mouthfeel of the final product [11, 39]. This step does not affect the growth of probiotics but could increase viable cell count [39]. In general, appropriate pressure should be 20–25 MPa at 60–65 °C [11]. However, slight modification should be applied in accordance with in situ, such as higher pressure for a higher amount of stabilizer or thickener. The time for homogenization varied significantly, which depends on the volume of milk to be homogenized.

The homogenization will not reduce the temperature of milk significantly, where it should be followed by further heating to sterilize the milk for fermentation. Any living microorganism will be killed during this step, but the spore may not be eliminated due to its heat resistance. However, the fermentation and growth of starter culture (including probiotic) could occupy the niche for spore growth, which make the product consumable. Meanwhile, heating could inactivate intrinsic antimicrobial components, such as some antimicrobial peptides or proteins, to favor the growth of starter culture [11]. Further, heating could denature whey protein to modify its tissue to improve viscosity and prevent whey separation [11]. Usually, an appropriate heating condition should be 90–95 °C for 5 min [40], where 120 °C for 3–5 s is acceptable, such as in Ultra High Temperature processed milk (UHT).

Scalding milk should be gradually and immediately cooled to ~40 °C for inoculation. Traditionally, yogurt fermentation uses 43 °C for fermentation, with a starter culture addition of 2–4% [11, 40]. However, this temperature should be modified when probiotics are incorporated into the starter culture for fermentation. The synergistic and antagonistic effect among bacteria or yeasts should be considered to obtain the best probiotic growth with acceptable product quality. Mostly, the optimal growth condition of probiotics is around 37 °C for many genera, such as *Lactobacilli*, *Bifidobacteria* [41], and the optimal condition of *Propionibacterium* is around 30 °C [42]. However, the starter culture bacteria (*Lactobacillus delbrueckii* subsp. *Bulgaricus*, and *Streptococcus thermophilus*) perform badly under this condition (lower lactic acid, volatile component, polysaccharides production, etc.), hence appropriate fermentation temperature modification should be determined previously during the pilot plant test before larger scale production, as well as the amount of addition, if the starter culture was developed by the fermented milk producer itself. Otherwise, sticking to starter culture instruction provided by the starter culture manufacturer (if applicable) is a wise decision to guarantee the success of fermentation. Previous thorough agitation is recommended for starter culture before addition for better performance [11]. Notably, the sterile operation is crucial for this step due to processing demand. There will be no more sterilization or pasteurization involved (generally, but there is ambient-temperature fermented milk available in the market, which is discussed in **Note 4**), where any contaminant (bacteria, yeast, mold, bacteriophage, etc.) introduced into the product will affect the quality of probiotic fermented milk significantly, hence causing severe consequences or results. This step and fruit pulp or jam addition (discussed in Subheading 3.4) are both critical control points of fermented milk production, which requires complete and careful administration.

3.2 Fermentation

Fermentation is the most critical step of fermented milk processing to obtain desired flavor and texture. The order of fermentation and packaging is decided by the desired fermented milk texture (set, stirred, or drinking). Here, we discussed fermentation firstly, then packaging, but the order can be changed. In general, starter culture contains *Lactobacillus delbrueckii* subsp. *bulgaricus* (L) and *Streptococcus thermophilus* (S) and requires a fermentation temperature around 41–42 °C for 2.5–4.0 h fermentation time (2–4% addition) [11]. However, introducing probiotics into the starter culture altered the appropriate fermentation temperature. As mentioned above, probiotic strains have the best performance when the temperature reaches 37 °C, but L&S cannot grow well at this temperature. Even the antimicrobial properties of probiotics could inhibit the growth of L&S, and the fermentation fails. Also, probiotic requires a longer fermentation time, from 8–9 h to 48 h, even some requires 72 h [42–45], this had led to a more difficult determination of fermentation time. Hence, an appropriate adjustment should be performed for fermentation conditions to facilitate the growth of both L&S and probiotic. For example, two-step fermentation is a practical way to ferment milk containing complicated microbial environments, such as kefir. Yoo et al. [46] developed a two-step fermentation method, which applied 37 °C for 9 h at the first step, and then 24 °C for 15 h for the second step. This method had acquired better sensory acceptance. Therefore, appropriate adjustment or separation of such fermented time or temperature could be applied to fit the growth of all the strains. Some probiotic strains can also grow at 40 °C, which is strain-specific, but this could also provide chances for producers to ferment milk at this temperature.

For set-fermented milk, the milk and starter culture mixture are packaged into the container firstly, which is plastic or glass, but the hygienic and aseptic conditions should be guaranteed before packaging. The packaged (sealed) products are placed in a warm room (fermentation room) which has appropriate spaces between the containers for better airflow [11, 40]. Stable temperature and shaking avoidance should be monitored during the whole procedure in case tissue breakdown or fermentation quality deteriorates [47]. The fermentation should be stopped when pH arrived at 4.6 and appropriate curdling happen in the container. At this time, immediate cooling is essential for controlling the acid content in the fermented milk. Generally, the temperature should be cooled down to 35 °C within 30 min, then 18–20 °C in the next 30–40 min, then 5 °C as soon as possible [11] and wait for distribution.

For stirred fermented milk, this fermentation step is carried out in a fermentation tank, which requires uniform temperature distribution in the tank due to the tank size. The upper and lower tank temperature difference should not exceed 1.5 °C [11]. When the fermentation is stopped (pH 4.2–4.5), immediate cooling is required to avoid excess acid production or flavor deterioration (*see* **Note** 7). However, cooling down should not be too fast, which could lead to curd shrink, and whey may be squeezed out of the curd [11]. The stirred fermented milk is agitated, in which mechanical force is involved in agitation. Appropriate control of such process is needed to maintain the tissue structure. Slow stirring, medium stirring temperature (10–25 °C), and desired pH (below 4.7) should be affirmed to maintain the tissue structure and avoid whey isolation due to mechanical force [11, 40]. Mechanical force may also occur when pumping the fermented milk for transportation due to turbulence, so slow pumping is needed to prevent any undesired results. The flow rate should be maintained below 0.5 m/s [11]. However, any parameters mentioned here are adjustable in accordance with the actual textural and other sensory properties of fermented milk products, where the pilot test is significantly essential to obtain the best parameter to favor the production.

3.3 Packaging and Storage

Packaging material can be plastic or glass, with different container shapes. Bottles, cups, bowls, or jars are all acceptable. It depends on the product or consumer demand. For set-fermented milk, the milk and starter culture mixture is packaged into retail containers/cups, with or without additives, to prepare for fermentation. However, for stirred-fermented milk, the fermented milk is packaged at 15–22 °C when mixed with additive or not [11, 40]. It is significantly vital to ensure aseptic and sterile condition during any packaging step, especially air cleanness. This is the rare step where products are exposed to and have contact with the outer environment, hence complete cleanness needs to be focused on to avoid introducing contaminants into the products.

Besides traditional packaging, the novel product pushed the development of packaging. There is a pasteurized fermented milk product commercially available in China, which uses Tetra Prisma® Aseptic to ensure the shelf life (6 months) (*see* Fig. 3). Other types

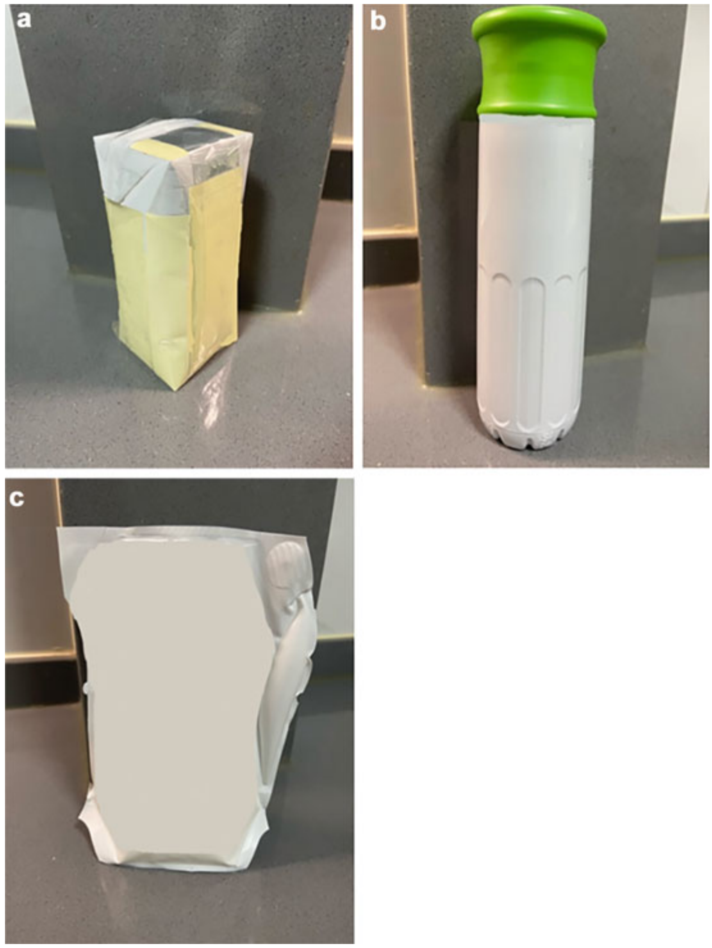

Fig. 3 (**a**) The photos of Tetra Prisma® Aseptic package, (**b**) plastic bottle for ambient-temperature fermented milk, and (**c**) Ecolean® package. *optional. (1) Skimmed milk powder is dissolved at 50–55 °C warm water where sterilization of such substrate is optional. (2) *Lactobacillus casei* Shirota as the culture seed was added into the substrate for incubation at 37 °C, and stopped when appropriate parameters were detected. (3) Culture base (fermented substrate) were stored at 5 °C after sweetened by syrup. (4): The sweetened culture base was mixed with sterilized water for better fluidity. (5): Bottles were made by food-grade polystyrene and transported with clean air for following selection step. Bottle selector makes the bottle oriented to the same direction for decorating (printing). The logo was printed on the bottle using instant-dry red ink (for sugar-reduced version, it is blue ink with more complicated decoration). (6) The content of each Yakult bottle is 100 mL, and the cap was made by aluminum foil which is easily opened. (7) The product was stored at 5 °C for following distribution, but it should be maintained at this temperature when selling and at home until consumption

of packaging are also available for this particular product, such as plastic bottles, which are rarely seen in traditional fermented milk packaging. Ecolean® air is also an available packaging for fresh stirred fermented milk due to its low weight, unique handle (air-filled), and suitability for straw use. The detailed production method of ambient-temperature fermented milk will be discussed in **Note 4**.

The storage condition of fermented milk products should be around 4–5 °C, where the storage step begins after packaging (stirred) or fermentation (set), depending on their production procedure. Usually, the storage time (shelf life) for traditionally fermented milk (yogurt) is around 28 days at 5 °C. However, research revealed that the *Lactobacillus acidophilus* could drop significantly after 21 days of storage at 5 °C, where *Lacticaseibacillus casei* have the highest viability retention [48]. These results indicate the incorporation of probiotics should be considered when examining shelf life to maintain the essential viability of probiotics.

3.4 Additive Addition

There are many additives available for fermented milk production. Common additives include fruit flavor components (mainly pulp or jam), sweetener, thickener/stabilizer, or other flavor ingredients, such as nuts or raisins. Detailed additive selection and commercially used novel additives will be discussed in **Notes 4–6**. Here, the procedure operation will be mainly introduced. As mentioned above, the milk-solid enhancers (protein, fat, etc.) are added at the beginning of production at the pre-treatment stage [11, 40, 47]. Protein is usually stored and sold as solid statues, where it needs to be added and solved into milk. Appropriate agitation is important to maintain the uniform milk texture and nutritious component distribution in the milk. Conversely, milk fat is usually added in liquid form (milk cream), which does not require long-time agitation. Excessive agitation or stirring would isolate fat and induce quality deterioration, such as unpleasant mouth sensation or lack of aroma. Sugars and other stabilizers are added at this stage for a better solution. Sugar (sucrose) is essential for certain microbial growth as well, making it a pivotal component to favor the growth of probiotics, especially for those non-lactose fermenters.

In general, fruit components or other flavor ingredients are added just before packaging [47] due to heat treatment could cause unexpected fouling in pipe or component degradation. However, this raised the hygiene issue when adding these ingredients. There is no more heat treatment following this addition, and the possibility of introducing contaminants needs to be controlled. As mentioned above, this is a critical control point of the whole processing procedure. Hence, complete sterilization of such ingredients should be guaranteed to ensure the safety and quality of desired products.

4 Notes

1. Several probiotics can be applied as starter cultures, where the commonly used probiotic species are listed in Table 5. It should be noted that the health effect of probiotics is strain-specific, hence the claim of strains on the label is necessary for legal compliance. However, as mentioned above, there is no precise list of probiotics in most countries, therefore a thorough evaluation of probiotics needs to be performed, especially for novel probiotic strains to ensure the availability of the strains. China has published a new probiotic standard (*Probiotic Food*, T/CNFIA 131–2021) which gives a good indication of the evaluation procedure applied to probiotic food. This evaluation

Table 5
Predominantly used thickeners/stabilizers, probiotics, prebiotics, and sweeteners in fermented milk products at present

Additive type	Name of the additive	Additive type	Name of the additive
Thickener	Acetylated distarch adipate Acetylated distarch phosphate[a] Agar[a] Carob bean gum Diacetyl tartaric acid ester of mono (di) glycerides (DATEM) Gelatin Gellan gum[a] Guar gum Hydroxypropyl distarch phosphate Lactic and fatty acid esters of glycerol Pectin[a] Starch	Sweetener	Acesulfame potassium Aspartame Erythritol[a] Isomaltose (palatinose) Jam[b] Lactase[b] Maltitol & maltitol syrup Mogroside Neotame Steviol glycosides[a] Sucralose Sucrose[a] Sweetened condensed milk[b] Xylitol
Probiotics	*Bifidobacterium* *Lacticaseibacillus paracasei* (Formerly *Lactobacillus paracasei*) *Lacticaseibacillus rhamnosus* (Formerly *Lactobacillus rhamnosus*) *Lactobacillus acidophilus* *Lactobacillus delbrueckii* subsp. *Bulgaricus* (*Lactobacillus bulgaricus*) *Lactococcus Lactis subsp. Cremoris* *Lactococcus Lactis* subsp. *Diacetylactis* *Lactococcus Lactis subsp. Lactis* *Leuconostoc mesenteroides* subsp. *Mesenteroides* *Streptococcus thermophilus*	Prebiotic	Plant powder Inulin Polydextrose Resistance dextrin Fructo-oligosaccharides

[a]could be used in ambient-temperature fermented milk
[b]the additive can provide sweetness but not a sweet additive (sweetener)

includes (1) probiotic species and strain identification with clear and well-known sources, (2) probiotic preservation method and safety evaluation, (3) whole-genome sequencing, which is peer-reviewed, and (4) in vivo or randomized clinical trial which supports its health effect [9].

2. Probiotic fermented milk has the potential to be claimed as food for a special purpose, but appropriate legal appliance and related clinical trial needs to be performed. For example, foods for health purposes need to be registered in China, and clearly label the registration number on the packaging bottle or other forms of packaging. Other countries have their regulations, where the individual analysis of local law, regulation, and policy is significantly vital for such claims. It should be mentioned that some products claim to provide nutrients to >3-year kids, but they are normal foods instead of foods for special purposes. The targeted population of the products limits the type of such product, which needs to be considered when developing products.

3. Dairy-based probiotic fermented beverage (probiotic beverage) is an alternative product that contains live probiotics but is more drinkable than traditional fermented milk. Yakult® is a popular product that is a representative of such products. It has a special and patented probiotic, *Lacticaseibacillus casei* Shirota, which was isolated by Minoru Shirota in 1930. The phenotype of this strain is similar to other *Lactobacillus* spp., and it is worth noting that it could grow at temperatures 15 °C–41 °C, but the optimal temperature is 37 °C [49], as mentioned above. *Lacticaseibacillus casei* Shirota is a sucrose fermenter [49], where possible sugar addition may involve in the production procedure, just before fermentation for better lactic acid production, but this depends on in situ application, not compulsory. The brief manufacturing procedure includes milk reconstitution, sterilization, fermentation, homogenization, flavoring, balancing (adding sterilized water to dilute the concentrated product), packaging, and further storage [49] (*see* Fig. 4). The uniqueness here is that probiotic beverages use skimmed milk powder to reconstituted milk as their fermentation substrate to minimize the effect of milk fat (fat isolation, as mentioned in **Note 6**) and control cost. Moreover, the sterilization procedure conferred brown color to the substrate, just like roasted fermented milk (*see* **Note 4**), due to such high temperature and time. Meanwhile, the fermentation temperature is maintained at 37 °C for better growth of the *Lacticaseibacillus casei* Shirota. This temperature differs from traditional fermented milk due to the simplified microbial environment (multi strains vs. individual strain).

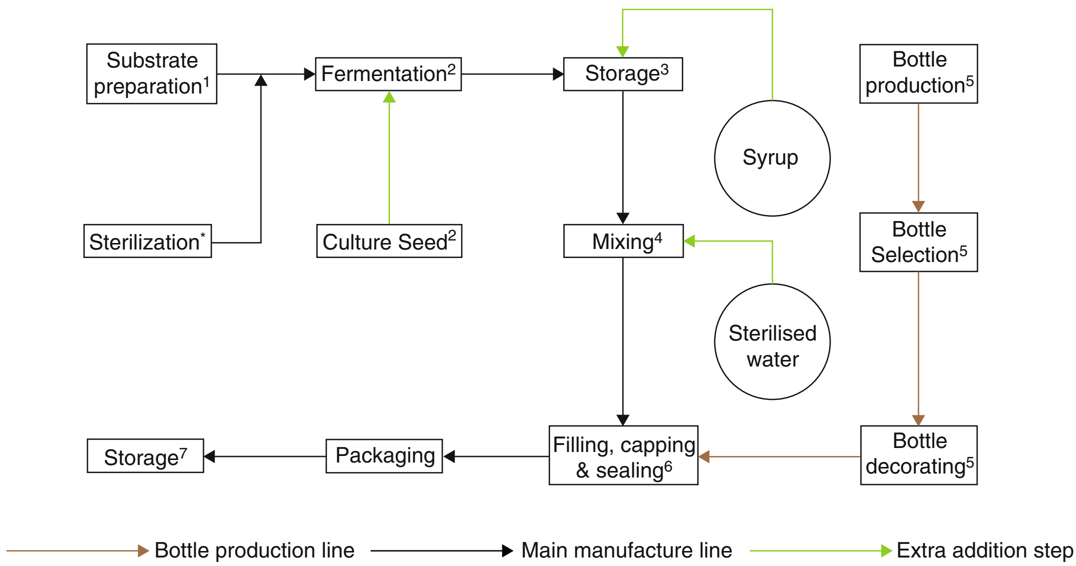

Fig. 4 The manufacturing flow chart of Yakult® [49]. *: optional (1) Skim milk powder is dissolved at 50–55 °C warm water where sterilisation of such substrate is optional. (2) Lactobacillus casei Shirota as the culture seed was added into the substrate for incubation at 37 °C, and stopped when appropriate parameters were detected. (3) Culture base (fermented substrate) were stored at 5 °C after sweetened by syrup. (4) The sweetened culture base was mixed with sterilised water for better fluidity. (5) Bottles were made by food-grade polystyrene and transported with clean air for following selection step. Bottle selector makes the bottle oriented to the same direction for decorating (printing). The logo was printed to the bottle using instant-dry red ink (for sugar-reduced version, it is blue ink with more complicated decoration). (6) The content of each Yakult bottle is 100 mL, and the cap was made by aluminum foil which is easily opened. (7) The product was stored at 5for following distribution, but it should be maintained at this temperature when selling and home storage until consumption

Additionally, balancing is a particular step for probiotic beverage production. This step added sterilized water into the product to dilute it and confer higher fluidity, making it more drinkable to mimic beverage status. This step differentiated Yakult® from traditional fermented milk products. Interestingly, the bottle of Yakult® is produced at the same factory by molding food-grade polystyrene [49]. This design made the packaging bottle controllable, but the Yakult® product should be stored avoiding light and kept at 4 °C due to the semi-transparent properties of the bottle. Other probiotic beverages have a very similar procedure, where the difference is the strain used. *Lacticaseibacillus paracasei* is the most used species for other probiotic beverages. However, the commercialization potential of *Lacticaseibacillus casei* Zhang is growing and has performed good ability to be utilized in yogurt and some health effect on rats [50–52].

4. As mentioned above, some unique products are commercially available in China with high sales. One of the most popular

products is pasteurized or sterilized fermented milk. It is also called ambient-temperature fermented milk, which indicates its most valued characteristic. It can be stored under ambient temperature with no quality deterioration for 6 months. The unusual step involved for this long storage is the following sterilization or pasteurization after fermentation [53, 54]. This step killed all available live microorganisms in the fermented milk, which stopped continuing fermentation usually occurring in unsterilized or unpasteurized fermented milk (traditional fermented milk). The pasteurization or sterilization method occurs by heating (72–121 °C, 4 s–20 min, usually 75 °C, 20 min) and high pressure (600–680 MPa, 10–40 min) [54]. However, inappropriate temperature or time for heating may cause color change or protein matrix breakdown, hence novel methods have been developed to perform this step, such as radiation and microwave [55, 56]. They all perform well for sterilization or second pasteurization (pasteurization is performed before fermentation). Meanwhile, the centrifugation in pre-treatment is another critical control point to remove the spores in the raw milk to avoid quality deterioration after packaging, just like traditional fermented milk production, where the parameters of the temperature and rotation speed is 50–65 °C and 4200–6300/min, respectively [54].

Despite having no live microorganisms in the fermented milk (including probiotics), the health effect of such a product may not be eliminated. Recently, the studies regarding postbiotics and parabiotics have been getting popular, and they are inactivated or killed probiotics [3], just like the microorganism in the pasteurized or sterilized fermented milk. Therefore, the health effect of postbiotics and parabiotics products cannot be determined, as not comparable with products containing live probiotics but needs more profound research to reveal the functional properties. The balance of nutritional values and the sale of the products need to be considered by manufacturers.

Another popular product, roasted-flavor fermented milk, has been developed based on Maillard reaction. This step was performed before homogenization, with the addition of glucose to promote the Maillard reaction to obtain the brown color and roasted flavor of raw milk [57]. Meanwhile, some concerns about this reaction are raised due to the production of harmful by-products, such as 5-hydroxymethylfurfural (HMF), glyoxal (GO), and methylglyoxal [58]. However, the harmful by-products all can be controlled after appropriate modification, such as keeping the product under 4 °C or setting a short shelf life [58]. Therefore, controlling such harmful by-products must be considered when developing new products using new technology or additives.

5. There are many thickening methods in fermented milk production. Thickening is significantly important due to its wide application in thickened yoghurt production in both stirred and set types. The easiest way to improve or thicken the fermented milk texture is by adding thickener into the product at the beginning of the milk treatment. Usually, this kind of additive needs thorough mixing to have a better solution; hence homogenisation could be a good step to achieve this under such high pressure, and heat treatment can be done simultaneously. The rationale for thickener is that it could absorb or bind more water to enhance the protein matrix's strength and improve texture [59]. Therefore, appropriate agents that could absorb more water or strengthen the protein matrix are selected to improve texture, such as polysaccharides or proteins or both.

   In general, most thickeners do not influence the viability or the survival rate of probiotics. However, natural ingredients and naturally produced additives have been selected to replace artificial thickeners to improve the health value of the product, where their prebiotic potential has been revealed for these substances. Carob bean gum and chia seed mucilage have been proven to be able to enhance fermented milk's texture, but have no significant impact on probiotic growth [60, 61]. The bitter almond gum exudate and its conjugates with sodium caseinate (SBAG-SC) had performed preservative ability on the viability of *Lactobacillus acidophilus*, La-5, but possess lower prebiotic potential compared with inulin, as well as a comparable ability for preventing phase separation to fermented milk compared with carboxymethylcellulose (CMC) [62]. In fact, multi-types of polysaccharides have been adopted as encapsulated wall material for the protection of probiotics. This trend indicates that the dispersion of such polysaccharides, such as alginate, xanthan gum, gum arabic, and maltodextrin, could not only enhance the firmness of the fermented milk but also protect probiotics from the digestion of the human stomach to reach the intestinal tract efficiently, via the matrix and gel formed by them [63]. Probiotic encapsulation using polysaccharides could have texture modification ability as well. Low methoxyl pectin encapsulated *Bifidobacterium breve* could improve the viscosity of yogurt and the hardness when the capsule was applied before fermentation [64]. This result shows that polysaccharides' preservation and texture modification ability can co-exist, but their effect may vary when applied under different forms.

Meanwhile, a starch-pectin blend, in which the ingredients were both commonly used in fermented milk as texture modifiers, could form resistant starch and slow-digestible starch via their interaction (starch and pectin), possessing a synergistic effect on probiotics growth [65]. This capacity has broadened the horizon that the impact of thickeners may not act alone, but the interaction between various thickeners could benefit the growth or viability of probiotics. In addition, some prebiotics (inulin, tragacanth gum, gellan gum) could improve the rheological properties of fermented milk, such as firmness and apparent viscosity, and weaken the syneresis as biopolymers [66]. The application of prebiotics to improve the viability or count of probiotics should consider their texture enhancement effect for better performance, or avoid undesired quality deterioration.

Some intrinsic components of the milk, especially fortified milk protein, could enhance the texture of fermented milk through crosslink formation with polysaccharides by the starter culture. Polymerized whey protein could be a good thickener for set fermented goat milk due to its good performance with the adjunction of pectin (PWP) [67]. PWP has properties like low syneresis, desirable viscosity and hardness, but its retention ability for the population of probiotics has added value to this mixture [67]. PWP could retain the viable cell of *Lactobacillus acidophilus* above 10^6 cfu/mL for 4 weeks, which proves its effect on probiotic retention [67]. Milk protein concentration can be regarded as a protecting agent for encapsulating *Lacticaseibacillus paracasei*, combined with gellan-caseinate [68] (Kia et al., 2018). This shell material could reduce syneresis as well [68]. This phenomenon shows its capability to maintain the viability of probiotics during storage alongside the elevation of textural quality.

Recently, many physical technologies have been developed and applied to realize this target. They remove an appropriate amount of water or whey to thicken the milk without adding anything extra to provide a "thick" mouth sensation to consumers. These technologies include flash evaporation [69], freeze concentration [70], centrifugation concentration, and membrane filtration [71]. Their core mechanism is that they could improve the content of dry matter to provide a thick sensation to the consumers, whereas the higher dry matter content could benefit the viability of probiotics as well [32].

6. Sweetener is another vital ingredient in fermented milk due to the harsh sensation of lactic acid and other organic acids. It could reduce and balance such sensations by providing sweetness to consumers. Some sweeteners could also promote the growth of probiotics. Traditionally, sucrose (sugar) has been

used as a sweetener with the highest acceptance among other sweeteners, where the growth of probiotics can also be maintained and controlled. However, the recent trend of fermented milk requires healthier ingredients, where the calorie needs to be controlled. Hence, artificial sweeteners and natural sweeteners (low glycemic index) have been developed to replace sugar as sweeteners. Most of the commonly used artificial sweeteners, such as aspartame, neotame, sucralose, sorbitol, and polynols (xylitol, erythritol, maltitol and isomalt), have no influence on the growth of probiotics[72–77], but their health concern mainly resides at metabolism aspects. For natural sweeteners, honey is a popular natural sweetener that consumers welcome and accept widely when replacing sugar. It is shown that the addition of honey does not affect the viability of *Streptococcus* in starter culture and could improve the viability retention of *Bifidobacterium animalis* BB-12, which show the suitability of honey to be used as a healthier natural sweetener compared with sugar[78]. Stevia (steviol glycoside) is a leaf extract of Stevia rebaudiana, a popular natural and low-calorie sweetener. It has been proved that it could enhance gel matrix and probiotic growth (*lactobacillus acidophilus*) with no harm to the sensory properties of fermented milk [79]. Stevia could also maintain the survival rate of *lacticaseibacillus casei* above 9 logs CFU/ml for 28 days of storage. Fermentable fibre addition (red beetroot) could assist its prebiotic performance[80], which was mainly observed in *lacticaseibacillus casei*'s growth promotion[81]. However, some researchers reported that stevia has a bitter aftertaste[82], which makes its usage need further attention. Iso-maltulose, also known as palatinose, is a product of an enzymatic reaction (glucosyltransferases) from sucrose[83]. It has both sweetness and prebiotic potential, which could favour the growth of probiotics, including *lactobacillus acidophilus*, *lactococcus* sp. and *bifidobacterium animalis*, with preservation of their biofunctions[83, 84]. This characteristic has broadened the horizon of this sweetener and makes it possible to be regarded as a prebiotic sweetener. This multifunctional property could reduce the ingredients added to the fermented milk and favour the growth of probiotics for better performance.

As mentioned above, prebiotics is a kind of non-digestible component of the host by endogenous enzymes that benefits the modulation of intestinal microflora. Prebiotics usually appear as a carbohydrate that does not digest in the small intestine but is fermented in the colon. Sweetener is typically a kind of carbohydrate as well, which makes it possess the high prebiotic potential to benefit the growth of probiotics. Mogroside is an extract from *Siraitia grosvenorii* (monk fruit) with high sweetness intensity. It is claimed that it has no effect on the fermented camel milk's organoleptic properties and could modulate the gut microbiota [85, 86]. Recent research revealed that the enzymatically modified mogroside combined with galactooligosaccharides (produced from mogroside and lactose combination by β-galactosidase) could improve the growth of gut microbiota, which includes *Bifidobacterium*, *Bacteroides*, *Enterococcus*, and *Clostridium coccoides* [87]. These examples indicate that using natural sweeteners has the advantages of low-calorie, prebiotic potential and high sweetness intensity, which makes them suitable to be used in many products and situations, especially probiotic fermented milk.

7. Many quality deteriorations may occur after product manufacturing and during storage. The deteriorations include texture, flavor, and color changes due to many factors. Here, we will briefly discuss some typical quality decline to give a comprehensive vision of quality control, but specified issue solution needs to be considered individually according to the situation. One of the most critical issues here is post-acidification. This phenomenon is mainly due to the growth of microorganisms during storage. Post-acidification can be affected by many factors, such as type of starter cultures, milk composition, temperature, and pH, homogenization and stirring, packaging material, and pre- and probiotics [88]. Here, the pre- and probiotic effects must be focused on due to their contradictory effect. Probiotics could metabolize some microbial inhibitory substances, such as bacteriocins or antimicrobial peptides, which could inhibit the growth of lactic acid producers. Hence, post-acidification can be assimilated. Further, the temperature set for probiotic growth may not favor the growth of lactic acid producers, where the pH dropping rate may be slowed under this condition, as mentioned above. However, prebiotics could accelerate the growth of many microorganisms, including lactic acid producers, as a promoting substance for microbial growth [88]. Even probiotics can produce more acid than usual [88]. Hence, controlling the production of such components is essential to ameliorate the post-acid effect. Other controlling methods mostly involve killing (or partially killing) the microorganisms in the fermented milk, but this does not meet some criteria of local regulations [88]. Hence, maybe the future direction could (1) genetically modify the microorganisms for reduced post-acidify capability [88] and (2) add live microorganisms (especially probiotics) back to the product after killing the intrinsic live cells and maintain the storage temperature at 4–5 °C for slowing the growth of such added microorganisms. However, the safety evaluation (such as antibiotic resistance, horizontal

gene transfer, etc.) would be more crucial to understanding the benefit of such products.

Other quality deterioration that can be discussed is based on the product. For example, set-fermented milk has issues with curd texture, whey isolation, undesired flavor, mold growth, and bad mouthfeel (sandy texture) [11]. For the curd texture and whey isolation, the main reason is the curd structure breakdown or inappropriate structure. The curd structure is affected by protein and polysaccharides cross-link. The protein content, protein quality (milk quality and composition), acid content, and microbial growth (polysaccharides production) may affect the structure. Among them, phage contamination may cause a significant quality issue by inhibiting the growth of microorganisms [11], which affect not only the curd but also other aspects, such as acid production, aroma component, etc. Phage is a type of virus with specificity to particular microorganism. It could lyse the microbial cell and kill them by leaching. Hence, the hygienic condition of the starter culture is important. Also, starter culture replacement and strain mixture are optional methods to avoid phage attacks due to their specificity [11]. The rest issues include undesired flavor, mold growth, and bad mouthfeel, possibly due to microbial contamination and raw milk quality deterioration (even mastitis milk has been used for manufacturing fermented milk products) [11]. Excessive hygienic practices and raw milk tests could prevent these issues.

Stirred fermented milk also has very similar issues to the set-counterpart. It is worth noting that stirring may introduce air into the product, which raises the possibility of whey isolation (due to air stratification) and microbial contamination (unclean air introduces yeast or mold into the product, especially the cross-contamination from other production lines which contain such contaminants) [11].

For probiotic beverages, the quality issues include live cell count, precipitation, fat isolation, flavoring ingredient quality, and microbial contamination [11]. Precipitation is a particular issue in probiotic beverages because observable precipitation in fermented milk is acceptable but not for beverages, hence appropriate methods to solve this issue are needed. Homogenization is an excellent way to mix ingredients and break protein (the main component of precipitate) to obtain a uniform beverage [11, 40]. This step is also used in Yakult® production [49]. Stabilizers and sugar are also available to facilitate homogenization [11, 40].

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