Chapter 7

Probiotics Consumption Increment through the Use of Whey-Based Fermented Beverages

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Additional information is available at the end of the chapter

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Abstract

Probiotics have been taking value over the last years due to its benefits in human health. Researchers have been looking for options in order to increase probiotics consumption, and one of the more nutritional choices is to use whey as a substrate in fermented beverages. Whey is a by-product liquid obtained during cheese processing. It is an economic source of protein, which provides multiple properties in foods. The main objective of this chapter was to carry out a complete review of important researches related to whey-based fermented beverages production. Researches show that probiotic micro-organisms have the ability to grow in whey properly, in such a way that they reach high concentrations, needed to achieve the probiotic effect that consumers are looking for. Certain substances, such as fruit pulps and carboxymethyl cellulose, have been used to improve viscosity, flavor among other important characteristics. Sensorial evaluations have been performed in order to assess consumers' impression, and they have been pleasantly accepted. Average shelf-life is 21 days. Through this review, it is known that whey is an excellent alternative to increment probiotic consumption, not only because it is an outstanding substrate for probiotic micro-organism's growth but also due to its excellent sensorial characteristics.

Keywords: whey, fermented beverage, acid lactic bacteria, probiotic, organoleptic characteristics

1. Introduction

During the last decades, the use of probiotics has been increasing, due to their important benefits in human health. Kollath, in [1], first defined the term "probiotic," when he suggested the term to denote all organic and inorganic food complexes as "probiotics," in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. In

1965, Lilly et al. [2] used the term "probiotic" to describe those substances secreted by an organism that stimulate the growth of another. Since then, this definition has been evolving remarkably, so that today, probiotics are defined as microbial dietary supplements, viable, selected, which when are introduced in sufficient amount, affect human organism beneficially through their effects on the intestinal tract [3]. On the other hand, Vasudha and Mishra [4] define them as alive microbial supplements, which beneficially affect the host by improving its intestinal microbial balance.

Dairy products have become a healthy alternative to increase probiotics consumption, developing fermented beverages based on milk, whey or their mixture. Whey has been less used than milk. However, it has wonderful physico-chemical characteristics that make it an excellent substrate to be used in the development of fermented beverages.

Whey is a green translucent liquid obtained by separating milk clot in cheese making process [5]. Its composition and characteristics depend on the technological process used and the type of milk. It is composed of 5% lactose, 93% water, 0.85% protein, 0.53% minerals, and 0.36% fat [6].

Its characteristics correspond to a fluid of yellowish green color, turbid, fresh taste, weakly sweet, acidic, with a content of nutrients of 5.5–7% that come from milk. It retains about 55% of total milk ingredients like lactose, soluble proteins, lipids, and mineral salts [7]. Whey is a by-product of high energetic and nutritional quality. For human being, it serves as an important source of vitamins, proteins, and carbohydrates.

Some statistical studies indicate that a significant portion of this waste is discarded to tributaries, resulting in an environmental problem due to its high biochemical oxygen demand. It physically and chemically affects the soil structure, decreasing the yield of agricultural crops, and polluting water because it depletes dissolved oxygen [7].

For the reasons explained above, dairy industry has been looking for alternatives for the use of this by-product, which is a high pollutant; however, it has a great nutritional value. Among the products of successful acceptance are fermented dairy drinks, refreshing beverages [8], protein concentrates [9], infant formulas [10], and others.

The processing of whey for beverages production began in the 1960s, and Rivella was the first fermented drink prepared from whey, made in Switzerland [11]. Whey products improve texture, reduce flavor and color, emulsify, stabilize, improve flow properties, and show many other functional properties that increase the quality of the products [12].

The main objective of this chapter is to carry out a complete review about fermented beverages based on whey inoculated with probiotics micro-organisms that have been produced around the world over the last years, focusing specially in important aspects such as sensorial and microbiological quality, shelf-life, and probiotic effects, showing that probiotics consumption can be increased through the use of whey as a substrate in this type of formulation, promoting it as a useful dairy by-product due to its excellent sensorial characteristics and its contribution in high quality organoleptic foods.

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2. Relevant aspects related to the use of whey as a substrate for the fermented beverages formulation

2.1. Whey physico-chemical characterization

The knowledge of whey physicochemical characterization is an important step in the use of this by-product in the dairy industry for different industrial processes. For this reason, most of the studies related to the use of whey propose a physico-chemical characterization in order to evaluate whether it meets the standards required to be used in technological processes.

In a very recent research, Molero et al. [13], carried out a physico-chemical characterization of whey obtained by cheese making process applying an artisanal method. It consisted of the determination of pH, titrated acidity, total solids, fat, protein according to the Venezuelan Standard COVENIN, and determination of lactose and minerals by analytical difference. The values obtained were statistically analyzed using a statistical package. The results classify whey as sweet, with excellent nutritional characteristics and attractive to be used in food technology for probiotic production, protein-fermented beverages, among other applications.

In an interesting research, Tirado et al. [14] carried out a physico-chemical characterization of whey derived from the production of coastal cheese. Fat analyzes were performed by Gerber method, lactose by the Lane and Eynon method (AOAC 923.09, 920.183b); the protein was analyzed by the Kjedahl method (AOAC 920,152); total solids by spectrophotometry; pH was determined by the method established in AOAC 945.10/90 and the acidity expressed as a percentage of lactic acid according to the Colombian Technical Standard. The values obtained were: fat 0%, lactose 3.69%, protein 2.29%, total solids 6.28%, acidity 0.08% lactic acid, and pH 6.5.

Linares et al. [15] showed similar results in the physico-chemical characterization of sweet whey samples, obtaining a pH of 6.84; acidity titrated of 0.11% (% lactic acid); protein between 0.6 and 1%; and ash 0.6%. De Paula et al. [16] performed a physico-chemical characterization of whey obtained from the manufacture of coastal cheese. This characterization was carried out using the following methods: acidity (AOAC 947.05/90), pH (AOAC 981.12/90), soluble solids (AOAC 932.12/90), total solids (AOAC 925,105/90), and lactose (FIL 28a/74). The following results were obtained: acidity (% lactic acid) 0.11; pH 6.58; total solids 6.83%, protein 0.98%; fat 0.4%; and lactose 4.54%.

Similarly, Montero et al. [17] carried out a whey fermentation with *Lactobacillus (L)* for feeding calves in the tropics. Researchers performed a physico-chemical characterization, determining pH, total protein, fat and total solids, tested by the Standard Method for examination of Dairy Products, 2004. The values obtained for these characteristics were in accordance with the Colombian standards established for this type of analysis.

In other studies, Londoño et al. [18] developed a fermented drink of fresh cheese whey inoculated with *L. casei*. Acidity, pH, viscosity, total solids, protein, fat, ash, lactose, minerals, soluble solids, reducing sugars, and moisture were determined for this purpose. Acidity, pH, total solids, protein, fat, ash, soluble solids, were determined using AOAC methods; lactose was determined using the Teles reagent method; reducing sugars by the method of 3-amino-5-nirosalicylic acid; mineral content was determined by spectrophotometric method of atomic absorption and viscosity by the Brookfield method. The values obtained were found within the established standards for these parameters.

On the other hand, Miranda et al. [19] carried out a physico-chemical characterization of sweet and acid whey produced in the cheese complex of Bayamo (Cuba). The authors determined acidity, pH, density, and fat content, following the guidelines of the Ministry of Agriculture of Cuba; lactose was determined by the phenol-sulfuric method; dry extract, crude protein, calcium and phosphorus were tested according to internationally recommended methods. The acid whey was distinguished by a lower pH and a higher acidity than the sweet. No significant differences were observed between the two varieties of whey for the remaining characteristics tested. All of them were within the specifications of quality established by the Cuban norm. Low acidity of whey benefits its quality, because it allows a better use for human and animal feeding. It is great important to know about dry extract in the evaluation of the quality of cheese whey as raw material, because it would indicate its water content: a greater amount of water makes whey has less nutritional value.

In this order of ideas, Sepúlveda et al. [20] developed a fermented beverage with the use of fresh whey with the addition of Maracuyá pulp. For this purpose, a physico-chemical characterization was performed, determining pH, viscosity, total solids, protein content, fat, and ash by AOAC methods; lactose was determined by the reactive method of Teles; calcium, sodium, and potassium were determined using the spectrophotometric method of atomic absorption. When comparing composite ranges obtained with data reported by Amiot et al. [21], Scott [22], Posati and Orr [23], and Morales et al. [24], similarities were observed with measures reported for total solids, lactose and protein; under these levels of composition a substrate for fermentation was guaranteed, influencing in the performance of beverage processing properly. On the other hand, fatty content in whey is directly linked to the cheese manufacturing conditions. The values obtained in this trial were considerably lower than that reported by Scott [22], who states that fat content for sweet whey ranges from 0.2 to 0.7%. The pH obtained was slightly higher than that reported by Spreer [25] for this type of whey.

From the studies explained above, it is a fact that whey's physico-chemical characteristics vary depending on the composition of the milk, the cheese making process and the type of cheese, which could determine the ultimate destination of this by-product.

2.2. Microbial cultures used to produce fermented beverages based on whey

The cultures most likely used are lactic acid bacteria (LAB), which play an important role in fermentation processes. They are widely used in food industry because of their involvement in texture, taste, smell and aroma, and development of fermented foods [26].

LABs may be contained in a group of micro-organisms named lactic cultures or starters [27]. They are used in dairy industry for fermented milks production, cheeses, butter, and other products that are required to be fermented [28]. LABs were referred to as probiotics in the 1960s.

The scientific interest in bacteria as protective agents against different diseases comes from the observation of Metchnikoff, who at the beginning of the twentieth century, emphasized the longevity and good health of the Bulgarian peasants, who consumed large quantities of yoghurt [29].

The observations of multiple scientists such as Trapp et al. [30] assumed that consumption of large quantities of foods rich in lactic acid bacteria, eliminated toxin-forming bacteria, while raising the proportion of lactic acid bacteria and intestinal flora, improved health and increased life expectancy. Since then, and throughout almost a hundred years of study, various authors have endeavored to know different functions of beneficial micro-organisms that populate the digestive tract.

Lilly et al. [2] used the term "probiotics" to describe those substances secreted by an organism that stimulates the growth of another, as opposed to the term "antibiotic," understood as any chemical compound used to eliminate or inhibit the growth of infectious organisms. Parker [31] was the first to use "probiotic" referring to organisms and substances that contribute to intestinal balance.

The definition of probiotics has evolved remarkably, so that today, they are defined as viable, microbial selected dietary supplements that, when they are introduced in sufficient amounts, affect the human organism through their effects on the intestinal tract [3]. Probiotics must meet some basic requirements to be selected in the development of commercial probiotic products. The most important requirements are: the probiotic micro-organism survives in the product, the physical and genetic stability during product storage is guaranteed, and all its essential properties that evidence its health benefits after consumption, are maintained during manufacture and storage of the product [32]. Laws et al. [33] states that the essential criteria for primer selection include acidification, aroma, taste, stability, and texture.

Many researches have been carried out with this class of micro-organisms, producing drinks of high microbiological and sensorial quality. Following the same idea, Molero et al. [34] formulated a probiotic fermented beverage based on whey, using a mixed culture of *L. acidophilus* and commercial yoghurt culture: *Streptococcus (S) thermophilus and L. bulgaricus*. Tirado et al. [14] produced a fermented whey milk drink using *S. salivarius* ssp. *thermophilus* and *L. casei*.

On the other hand, Linares et al. [15] evaluated the effect of different proportions of citrus pulp on the sensorial acceptability of a fermented and protein drink made from residual whey. For this purpose they used a lyophilized lactic culture of *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *L. casei*. Likewise, Martínez et al. [35] formulated a fermented cheese-whey drink adding maracuyá pulp. For this purpose, lactic ferments were used for direct inoculation: *S. thermophilus*, *L. delbrueckii* sub *bulgaricus*, and *Lactococcus lactis* sub *lactis.* Similarly, Vela et al. [36] developed a probiotic whey-based fermented beverage with mango pulp and almonds, using isolated colonies of *L. casei.*

In another study, Fiorentini et al. [37] evaluated the influence of different combinations of probiotic bacteria and different fermentation temperatures on the physico-chemical characteristics of fermented lactic beverages based on soybean and whey. For this purpose, a lyophilized probiotic culture was used, composed of *L. acidophilus* and *Bifidobacterium (B) bifidum*. A second culture was prepared with *B. lactis* and *S. thermophilus*.

In other studies, Pescuma et al. [38] developed fermented functional beverages based on whey, using lactic acid bacteria: strains of *L. acidophilus*, *L.* subsp. *bulgaricus*, and *S. thermophilus*. A similar approach was given by Legarová and Kousimska [39] who formulated a whey-based drink using the same starter culture.

Katechaki et al. [40] performed a research related to thermal drying of *L. delbrueckii* subsp. *bulgaricus* and its efficient use as starter culture in whey fermentation and in cheese process. This micro-organism is a thermophilic LAB, able to ferment lactose, glucose, and fructose [12]. It was isolated from Bulgarian yoghurt, from Germany. Montero et al. [17] used *L. casei* to ferment whey to feed calves, the culture contained 1×10^7 CFU/ml. De Castro et al. [41] evaluated the effect of the incorporation of oligofructose on the properties of fermented probiotic lactic beverages using a probiotic culture composed of *L. acidophilus*, *B.,* and *S. thermophilus*. Oligofructose is an oligosaccharide, obtained from the enzymatic hydrolysis of insulin [42]. It is a prebiotic whose use can bring functional benefits and can affect the sensory properties of the products significantly [43].

Similarly, Londoño et al. [18] worked on a fresh cheese fermented drink formulation, inoculated with *L. casei* as a probiotic culture. Other cultures used were *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*.

In another research, Gallardo et al. [44] evaluated taste and sensation in the mouth of beverages made from whey with addition of hydrocolloids. The functionality of hydrocolloids at low concentrations is that it enhances viscosity and prevents particles sedimentation. It also contributes to the microstructural properties of meals, based on its ability to confer structure to the continuous phase of the substrate, which depends on their solubility in water and/or their intermolecular associations [45]. Beverages were prepared with a commercial yoghurt starter culture, which consisted of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*.

Hernández et al. [46] worked on the preparation of a probiotic drink based on whey, using cultures of *L. reuteri* and *B. bifidum*. The first was preserved on LBS (*Lactobacillus*) agar at 4°C. Three subcultures were performed consecutively prior to the use of the strain in the experiment with 1% inoculum. The second was preserved in MRS medium. Two subcultures were performed before use the strain in the experiment with 1% of inoculum.

Following the same idea, Dalev et al. [47] evaluated the sensory quality of whey-based probiotic beverages. A probiotic culture was prepared with strains of *B. breve* ATCC 15700, *B. infantis* ATCC 15697, *B. animalis/lactis* J38, *L. plantarum* W42, *L. plantarum* IB, *L. casei Lc* and *S. thermophilus*.

Sepulveda et al. [20] prepared a fermented beverage with the use of fresh whey with the addition of Maracuyá pulp, using a traditional lactic acid culture, in a 1:1 ratio of *S. thermophilus* and *L. bulgaricus*. Oliveira et al. [48] developed a fermented lactic drink using four probiotic cultures using *S. thermophilus and L. delbrueckii* ssp. *bulgaricus*; *L. acidophilus* and *L. rhamnosus*.

Kéfir has also been used as a starter culture in the production of beverages from whey [49–51]. Kefir is made by inoculating milk with kefir grains. This grain is irregular and its size varies from 3 to 35 mm in diameter, contains lactic acid bacteria (*Lactobacillus*, *Lactococcus*, *Leuconostoc*), acetic acid bacteria, and yeast mixture, coupled with casein and sugar through a matrix of polysaccharides called kefirán [52].

Yeasts, such as *Kluyveromyces marxianus*, have also been used as crops. This yeast has been isolated from fruit, cheese, yoghurt [53], milk [54], and has been used in whey processing. It has the ability to hydrolyze lactose and ferment sugars efficiently [55]. Cóndor et al. [56] prepared a beverage from cheese whey using immobilized *Kluyveromyces Marxianus* cells. Padín and Díaz [57] worked on the alcoholic fermentation of whey, using this yeast and organic solvents as extractants. Dragone et al. [58] performed the characterization of volatile components in an alcoholic beverage produced from whey with *Kluyveromyces marxianus* ATCC22.

2.3. Technological process followed for whey-based probiotic fermented beverages production

Table 1 summarizes the review of whey-based fermented beverages. It tells the technological process used in drinks manufacture. It is interesting to observe how the use of probiotic micro-organism plays an important role. The tendency is to use this type of bacteria and the reason is its potential benefit to human health.

In the development of these beverages, authors have used additives in order to improve some organoleptic characteristics, for example oligofructose, hydrocolloids, processed fruits, and others. Regarding technological process, fermentation is the essence of the drink production. However, it can be changes related to raw material (e.g., whey powder, liquid whey, combination with soymilk or whole milk), prior bacteria isolation, among others. Dairy industry has thus diversified methods for producing whey-based beverages. In the following sections, the acceptance of these drinks can be evidenced based on the sensorial evaluations and their probiotic character based on the viable count.

2.4. Fermented whey-based beverage sensorial quality

In the process of making dairy drinks, it is essential that they have adequate sensory properties to ensure they are accepted by consumers. Sensory quality researches have been increased over the recent years.

In this order of ideas, Molero et al. [59] carried out a sensory evaluation of probiotic fermented beverages based on whey. Four treatments were developed using combinations of two stabilizers, carboxymethyl cellulose and unflavored gelatin and two starter cultures: *L. acidophilus* and a mixed culture with *L. acidophilus* and yoghurt micro-organisms. For these, a sensory evaluation in three phases was designed. In a first phase test, acceptance-rejection was applied to an untrained 30 people in order to select the essence of fruits that best suited to drinks. In second place, preference test was performed with three concentrations of sugar, applied to 30 people panel. In a third phase, smell, taste, overall acceptance, and consistency of the four treatments were evaluated using an acceptance degree test with 5-point hedonic scale to an untrained panel of 100 people. Fruit essence largely accepted by the panelists was the coconut.

Author	Micro-organism	Special additives	Fermentative process
Molero et al. [13, 34]	*Lactobacillus acidophilus* and commercial yoghurt culture		Whey was pasteurized at 65°C for 20 min and cooled to 38°C. Inoculation of micro-organisms. Fermentation was carried out controlling pH until 4.5
Tirado [14]	*Streptococcus salivarius* ssp. *thermophilus y Lactobacillus casei* ssp. *casei*		Mixture of whey, skim milk powder and sugar was pasteurized at 63°C for 30 min and cooling to 43°C. Inoculation of micro-organisms in equal proportions. Fermentation was carried out for 3 h at 40°C controlling pH up to 5.
Linares et al. [15]	Lactic culture: *Streptococcus thermophilus*, *Lactobacillus delbrueckii* sub. *bulgaricus*, and *Lactobacillus casei*	Citrus fruit pulps	Fruit juice mixed with whey, previously pasteurized at 70°C for 30 min and fermented at 42°C for 5 h. White sugar was added in order to standardize at 14 °Brix. The beverage obtained was pasteurized at 80°C for 15 s and packed at the same temperature
Martínez et al. [35]	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* sub. *bulgaricus y Lactococcus lactis*	Pasteurized maracuya pulp	Five different treatments with different Maracuya pulp percentages (5; 7,5; 10; 12 y 15%) and final value of 14 °Brix
Vela et al. [36]	*Lactobacillus casei*	Mango pulp (*Magnifera indica* var. *Tommy atkin*) and almonds (*Amygdalus communis*)	150 ml of pasteurized whey was taken and inoculated with *Lactobacillus casei*. They were incubated at 35°C for 48 h. pH was measured. Fully mature pulp was added in a 1:1 ratio. 12.5% (w/v) of previously crushed almonds were added
Teixeira et al. [51]	Kéfir grains		Kefir grains washed with distilled water and inoculated in 250 ml of whey, with a temperature of 25°C for 72 h
Fiorentini et al. [37]	Probiotic freeze-dried cultured of *L. acidophilus* y *Bifidobacterium bifidum*	Water-soluble soybean extract	Whey powder was dissolved in water in a 1:1 ratio right before use. Mixture of 40% whole milk, 30% whey of mozzarella cheese, 30% water-soluble extract of soybean and 10% of sugar. Heat treatment at 90°C for 5 min, cooling to fermentation temperature (37°C). Incubation until reaching a pH between 4.5 and 5. Cooling at 20°C, homogenized, distributed in plastic bottles and stored at 7°C for 21 days
Legarová et al. [39]	Commercial yoghurt starter culture: *L. delbrueckii bulgaricus* y *Streptococcus thermophilus*		Incubation at 43°C and cooling at 4°C.
Katechaki et al. [40]	*L. delbrueckii bulgaricus*	*Lactobacillus delbrueckii* subsp. *Bulgaricus drying*	Drying was carried out in convection ovens at 35, 45, and 55°C for 10 h. After drying remaining moisture was removed with further drying at 102°C. Fermentation was performed at 37°C for 3 days

Author	Micro-organism	Special additives	Fermentative process
Pescuma et al. [38]	*L. acidophilus*, *L. bulgaricus y Streptococcus thermophilus*		Incubation a 37°C for 24 h
Padín and Díaz [57]	*Kluyveromyces marxianus*	Organic solvents	Reconstituted whey powder at 20% w/w. 100 ml of reconstituted whey 10% v/v were inoculated with a *Kluyveromyces marxianus* culture, incubated at 30°C. After 8 h 100 ml of selected solvents were added (oleic acid and soybean oil) in a ratio of 1:1 by maintaining them under the same experimental conditions for 30 h
Montero et al. [17]	*L. casei*		100 ml of culture was taken to inoculate 900 ml of whey in a beaker and incubated for 24 h at 39°C. Fermented whey was poured into 9 l of fresh whey and fermented 24 h at 39°C in a convection oven. The 10 l of fermented whey were poured into 90 l of fresh whey and rested 24 h at room temperature
De Castro et al. [41]	*L. acidophilus, Bifidobacterium* y *Streptococcus salivarius thermophilus*	Oligofructose	Pasteurized milk with commercial sucrose was heat treated at 95°C for 5 min while liquid whey with oligofructose was heated to 65°C for 30 min. The temperature of the mixture was lowered to 40°C. The beverage was made with the addition of the lyophilized culture at 8.3 mg/100 ml. Fermentation occurred at 40°C. pH of 4.6 was monitored. The beverage was then cooled to 4°C
Londoño et al. [18]	*L. casei*. Other cultures: *L. delbrueckii bulgaricus* and *Streptococcus salivarius thermophilus*	Inverted sugar syrup; maracuyá pulp; carboxymethyl cellulose (CMC)	Inoculation was performed maintaining a pH of 5.8 and stirring for 3–5 min. Subsequently, the beverage was flavored with the addition of maracuya pulp, packed and stored at 4°C
Hernández et al. [46]	*L. reuteri* y *Bifidobacterium bifidum.*		Reconstituted whey (7%) with addition of 7% sucrose and 0.4% pectin. Three treatments were applied by inoculation of probiotic strains in different ratios. Incubation at 37°C and storage at 4°C for 30 days
Gallardo et al. [44]	Commercial yoghurt starter culture: *L. delbrueckii bulgaricus* and *Streptococcus thermophilus*	Hydrocolloids	Fermentation was carried out by inoculation (2% v/v) with the starter culture at 42°C until reaching a pH of 4.6
Dalev et al. [47]	*Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium animalis/lactis*, *L. plantarum*, *L. casei*, and *Streptococcus thermophilus*	Soy milk	Equal amounts of whey and soy milk. Fermentation at 37°C for 24 h until reaching a pH of 4.4–4.6. Drinks were cooled and supplemented with processed fruits

Author	Micro-organism	Special additives	Fermentative process
Oliveira et al. [48]	Probiotics cultures: yoghurt culture of *Streptococcus thermophilus* y *L. delbrueckii bulgaricus; L. rhamnosus*		Complete and skimmed pasteurized milk mixed with skim milk powder to obtain 130 g/l of total solids and 26 g/l of fat, supplemented with 20 g/l of casein hydrolyzate. Heat treatment at 90°C for 10 min cooled to 4°C and stored 24 h prior to use. Fermentation with incubation at 42°C until reaching a pH of 4.3
Sepulveda et al. [20]	*Streptococcus thermophilus* y *L. bulgaricus.*	Maracuyá pulp (*Passiflora edulis*) and Carboxymethyl cellulose	It was incubated 2 or 3 h, maintaining the temperature until reaching a pH of 4.6. Fermentation was stopped with a fast cooling of 4°C. Agitation to ensure that maracuya pulp and vitamin dosage were well incorporated into the mixture
Cóndor et al. [56]	*Kluyveromyces marxianus*		Fermentation at 30°C for 7 days, evaluating the fermentation kinetics with the reading of °Brix and pH

Table 1 Whey-based fermented beverage technological process review

The preferred concentration of sugar by the panelists was 6%. The best evaluated treatments for consistency and general acceptance were those who had carboxymethyl cellulose as a stabilizer. The best drink evaluated in reference to the taste was the one containing carboxymethyl cellulose and *L. acidophilus*. The four drinks were equally qualified in relation to the smell.

Similarly, Valencia et al. [60] carried out a sensorial evaluation to nutritional drinks based on pumpkin and whey, enriched with oats and passion fruit. They evaluated 12 beverage formulations considering color, aroma, taste, and acceptability. They performed the test with a panel of 26 school-aged children; each child received three 100 mL samples. They used a questionnaire with expression faces of pleasure or displeasure, corresponding with a hedonic scale of 1–5, being 1 the lowest score and 5, the highest score. They found significant differences between the results obtained for the samples analyzed, but all of them were very well accepted by the panelists.

De Paula et al. [16] performed a sensory evaluation of fermented beverage fermented from whey with and without Maracuyá pulp. They performed an order-preference test, using a panel of 59 consumer catheters, using note 1 for the most preferred and 5 for the least preferred. They coded the samples and presented them randomly in 50 mL beakers, finding that the combination of whey with passion fruit flavor was the most preferred beverage. In addition, they indicated that the panelists described the product as very good, novel, and interesting.

In another research, Vela et al. [36] evaluated a probiotic beverage based on whey with addition of Mango and Almond pulp, in a ratio of 1:1, using *L. casei* as a probiotic micro-organism. They applied a hedonic scale preference test to assess the level of impact on smell, taste, and texture with a group of 20 untrained judges. The scale used was structured with scores from 6 to 10; being 6 "I dislike much," 7 "I do not like," 8 "I do not like or dislike me," 9 "I like," and 10 "I like it a lot." The drink got a "I like" rating.

Legarová et al. [39] evaluated whey-based fermented beverages using the commercial yoghurt culture of *S. thermophilus* and *L. bulgaricus* to determine the effect of milk content on sensorial properties. They evaluated cold samples (4–6°C) using a panel of 15 people, an unstructured linear scale was designed. The samples prepared with whey and milk obtained the highest scores in all the descriptors, evidencing that a higher percentage of milk in the mixture, higher indexes of organoleptic quality in the drink.

Similarly, De Castro et al. [41] analyzed the sensorial acceptance of whey based fermented beverages with different concentrations of oligofructose (2 and 5%). They evaluated the samples after 72 h of preparation, using: (a) an untrained panel of 36 people, who were asked which drink they liked the most or did not like; and (b) an untrained panel of 50 people in one test of acceptability, using a structured hedonistic scale of 9 points (1—I do not like anything, 9—I like it very much) and a test of intention to buy using a scale of 5 points (1—definitely not going to buy it; 5—I will definitely buy it). Panelists preferred 2 and 5% oligofructose beverages compared to the control drink (without oligofructose), where acidity was an attribute mentioned by 41% of judges. Furthermore, in terms of acceptability, the average score for both drinks was over 7 points, evidencing that the variation in the oligofructose content added did not affect acceptance of the beverages.

On the other hand, Londoño et al. [18] carried out a sensorial evaluation of a fermented beverage made from fresh whey, using *L. casei* and commercial yoghurt culture of *L. bulgaricus* and *S. thermophilus*. They performed a drink acceptability test, using a panel of 80 people, considering a 9 points hedonic scale. The drink had a "like" rating. Pescuma et al. [38] evaluated the noble properties of whey-based functional beverages using LABs, which were made using commercial yoghurt culture of *L. bulgaricus* and *S. thermophilus* and a probiotic culture of *L. acidophilus*. They performed an acceptability test of the fermented drink, obtaining a grade of "I like."

Gallardo et al. [44] used a highly trained panel in the sensory evaluation of a whey-based fermented beverage with and without addition of hydrocolloids, with commercial yoghurt as an inoculum (0.02%).They performed the sensory evaluation under controlled conditions, following the ISO Standard 8589 (1988), using samples of 30 mL, in triplicate, on separate days. They used a 100 mm unstructured linear scale to measure the sensation of panelists leaving the sample for a maximum of 3 s before swallowing. According to the results, the addition of hydrocolloids affected the perception of the viscosity of fermented beverages substantially, evidencing the lack of a greasy sensation in the mouth.

In other studies, Hernández et al. [46] performed the sensory analysis on a reconstituted whey-based probiotic drink (7%) and pasteurized (80°C, 3 min) using three combinations (treatments) of *L. reuteri* and *B. bifidum* as inoculum T1: *L. reuteri* 1% and *B. bifidum* 0.5%, T2: *L. reuteri* 1% and *B. bifidum* 1%, T3: *L. reuteri* 2% and *B. bifidum* 0.5%. They used the triangle test and a panel of 10 people (8 women and 2 men) between 22 and 27 years old. They performed 12 tests per session, determining differences between treatments using a "d" value defined as the difference between intensities for two products valued in standard deviation. The triangle test results showed sensorial differences between treatments T1 and T2; however, T3 was different from the first two. To confirm this difference, they performed a test in which 109 consumers participated, finding that 56% expressed differences by T3, 34.86% by T2 and 9.17% showed no preference, selecting T3 as final product, which after a descriptive test, was cataloged with "very good" organoleptic characteristics.

Following the same idea, Dalev et al. [47] conducted a qualitative sensory analysis of fermented whey and soy milk beverages to five probiotic beverages based on whey and soy milk: (1) unfermented soy milk (control drink), (2) fermented drink with equal volume of whey and soy milk. Starter culture: *B. breve* and *L. casei*, (3) fermented drink with equal volume of whey and soy milk. Starter culture: *B. infantis* and *S. thermophilus*, (4) fermented drink with equal volume of whey and soy milk. Starter culture: *B. animalis* and *L. plantarum*, and (5) fermented drink with equal volume of whey and soy milk. Starter culture: *B. breve*, *L. plantarum*, and *S. thermophilus.*

They performed the sensory evaluation using the Quantitative Description Analysis (QDA) method [61]. They selected descriptors or attributes to be evaluated and used a 10 cm unstructured linear scale, shown in monitors, converting the results on a numerical scale (from 0 to 10 units) expressing them in conventional units. They employed a panel of six people (four women and two men) trained according to the International Standards (ISO 1993), with at least 1 year of experience in descriptive tests of different foods, who received varied samples of 20 mL, each in triplicate. The results showed highly significant differences in attributes such as soy milk odor, cereal odor, fermented taste, strawberry odor, sweet taste, and after taste. They concluded that the addition of processed fruits helps to improve the characteristics of the beverages substantially, and therefore, the qualification as organoleptic quality. Soy milk has been used to prepare products such as yoghurt, but its poor organoleptic characteristics have been responsible for a very low acceptance by consumers.

2.5. Whey-based fermented beverages shelf-life

It is a fact that there are physico-chemical factors that can influence micro-organism survival in fermented beverages, being the most important acidity, temperature, oxygen concentration, type of inoculum and storage conditions. Theoretically, it is expected that for a reasonable time, the product will maintain the characteristics that define it as probiotic, so that quality can be guaranteed to the consumer. For this reason, many researches have evaluated the beverages shelf-life.

In this order of ideas, Fiorentini et al. [37] performed a viable lactic bacteria count in fermented beverages prepared from whey and soybean addition, after 7, 14, and 21 days storage under refrigeration at 7°C. They performed a selective count of *L. acidophilus* on modified MRS agar, with aerobic incubation and a count of *B. bifidum* on MRS agar modified with anaerobic incubation, both at 37°C for 72 h, finding that the number of alive micro-organisms in the fermented drink was 10^7 UFC/ml. Londoño et al. [18] evaluated the viability of the beverage based on fresh whey inoculated with *L. casei*. They performed counts on two types of agar, MRS and M17, both at acidic pH (2.4) and neutral pH (7.2), finding that shelf-life of the product was 21 days. In addition, they did not observe marked variability in the results of the physico-chemical tests.

Similarly, Hernández et al. [46] performed a viable micro-organisms count to a beverage made from whey inoculated with *L. reuteri* 2% and *Bifidobacterium bifidum* 0.5%. Count was during storage at 4°C for 30 days. They counted *L. reuteri* on a modified medium of LBS agar, *B. bifidum* on MRS agar and total population of viable micro-organisms in MRS medium (pH = 5.5), finding that the beverage fulfilled the criterion of probiotic foods in an acceptable manner.

In other research, Sepúlveda et al. [20] carried out an evaluation of the physicochemical characteristics during the storage of a beverage prepared with whey with addition of Maracuyá pulp. As the days went by, a decrease in pH and an increase in acidity were observed, suggesting a shelf-life of no more than 21 days. Oliveira et al. [48] performed a microbiological analysis of four whey-based drinks inoculated with probiotic cultures at 1, 7, 14, 21, and 28 days of storage at 4°C. They used MRS-bile agar for counting *L. acidophilus*, MRS agar for *L. delbrueckii* ssp. *bulgaricus* and *L. rhamnosus* and agar for *S. thermophilus*. Although the probiotic count decreased during storage (28 days), they found that the beverages contained, on average, 5.3×10^6 CFU/mL of probiotics after 28 days of storage. In addition, they observed that, on average, pH remained at 4.5 after the first day of storage, showing a decrease between 0.14 and 0.32 units during the first week, decreasing slightly (less than 0.12 pH units) up to 28 days of storage, considering it stable.

Cóndor et al. [56] performed a microbiological evaluation of a whey-based beverage using immobilized *Kluyveromyces Marxianus* cells, during storage at room temperature and under refrigeration. They counted viable aerobic mesophiles, numbering of total coliforms and count of molds and yeasts, according to Peruvian Technical Standard 202.083 (1988). After storage viable aerobic mesophilic micro-organisms count and molds and yeasts (CFU/ml) were less than 10, while total coliforms (NMP/ml) were less than 3.0, indicating that the beverage was microbiologically acceptable. They also observed acidity and pH profile up to the fifth month of evaluation, both at room temperature (22°C) and under refrigeration (4°C).

3. Conclusions

Whey has been used for probiotic fermented beverages development significantly. It has excellent physico-chemical characteristics that make it become an excellent substrate, allowing probiotic bacteria growth in such a way that they reach high concentrations, achieving the probiotic effect and all of its health benefits. On the other hand, whey-based probiotic beverages have extraordinary organoleptic characteristic and they are widely accepted by consumers. An average shelf-life of these beverages is 21 days under refrigeration conditions, ensuring probiotics benefits during this period of time. Throughout this review, it is shown that probiotic consumption can be increased through the use of whey not only due to its excellent physico-chemical characteristics, but also due to its ability to develop beverages with high sensorial characteristics and due to its excellent acceptance.

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