

Viability of probiotics, physicochemical and microbiological characterization of beverage (smoothie) with symbiotic yogurt and berries pulp

Viabilidade de probióticos, caracterização físico-química e microbiológica de bebida (smoothie) com iogurte simbiótico e polpa de frutas vermelhas

Viabilidad de probióticos, caracterización fisicoquímica y microbiológica de bebida (batido) con yogur simbiótico y pulpa de frutos rojos

Received: 01/20/2022 | Reviewed: 01/28/2022 | Accept: 02/13/2022 | Published: 02/18/2022

Darlila Aparecida Gallina

ORCID: <https://orcid.org/0000-0001-8787-5050>

Centro de Tecnologia de Carnes, Instituto de Tecnologia de Alimentos, Brazil

E-mail: darlila@ital.sp.gov.br

Paula de Paula Menezes Barbosa

ORCID: <https://orcid.org/0000-0002-8455-6991>

Colégio Técnico de Campinas, Universidade Estadual de Campinas, Brazil

E-mail: paulamenezesbarbosa@gmail.com

Abstract

The aim of this study was to evaluate the viability of commercial probiotic bacteria (bifidobacteria) in symbiotic beverages made with symbiotic yoghurts and berries pulp, after manufacturing and refrigerated storage. Six beverage formulations were prepared using symbiotic yogurt (60%) containing inulin or fructooligosaccharides (FOS) and commercial probiotic cultures (*Bifidobacterium* spp.) and berries pulp (40%), as follows: F1 (Howaru HN 019, inulin); F2 (Howaru HN 019, FOS); F3 (Lafti B 94, inulin); F4 (Lafti B 94, FOS); F5 (Kit Bifi, inulin); F6 (Kit Bifi, FOS). The beverages were evaluated for microbiological quality (total and thermotolerant coliforms, molds and yeasts), viability of probiotics, pH and titratable acidity. The pH, acidity and probiotic counts were investigated during 30 days of storage. The physicochemical characterization of the formulations with better performance regarding the maintenance of probiotic counts was carried out. The beverages elaborated showed appropriate sanitary hygienic quality, decreased pH and increased acidity, which is common in fermented dairy products. The probiotic Howaru HN019 exhibited better stability in the beverage than Lafti B4 and Kit Bifi. The highest level of bifidobacteria was found in F1 and F2 beverages containing *B. animalis* (Howaru HN019) and inulin or FOS, remained around 8 log CFU.mL⁻¹ for up to 30 days, whereas levels of 6 to 7 log CFU.mL⁻¹ were maintained in the other beverages. The beverages made with symbiotic yogurt and berries pulp can be considered an appropriate vehicle for the incorporation of probiotics and fibers, whereas, the probiotics remain at a satisfactory level throughout storage.

Keywords: *Bifidobacterium*; Prebiotics; Microbial viability; Functional beverage; Fermented dairy products.

Resumo

O objetivo deste estudo foi avaliar a viabilidade de bactérias probióticas comerciais (bifidobactérias) em bebidas simbióticas elaboradas com iogurtes simbióticos e polpa de frutas vermelhas, após a fabricação e durante o armazenamento refrigerado. Seis formulações de bebidas foram preparadas utilizando iogurte simbiótico (60%) contendo inulina ou fruto-oligossacarídeos (FOS) e culturas probióticas comerciais (*Bifidobacterium* spp.) e polpa de frutas vermelhas (40%), sendo: F1 (Howaru HN 019, inulina); F2 (Howaru HN 019, FOS); F3 (Lafti B 94, inulina); F4 (Lafti B 94, FOS); F5 (Kit Bifi, inulina); F6 (Kit Bifi, FOS). As bebidas foram avaliadas quanto a qualidade microbiológica (coliformes totais e termotolerantes, bolores e leveduras), viabilidade de probióticos, pH e acidez titulável. O pH, acidez e contagens de probióticos foram investigados durante 30 dias de estocagem. Realizou-se a caracterização físico-química das formulações com melhor desempenho quanto à manutenção das contagens de probióticos. As bebidas elaboradas apresentaram qualidade higiênico-sanitária apropriada, diminuição do pH e aumento da acidez, o que é comum em produtos lácteos fermentados. O probiótico Howaru HN019 apresentou melhor estabilidade na bebida do que o Lafti B4 e o Kit Bifi. O maior nível de bifidobactérias foi nas bebidas F1 e F2 contendo *B. animalis* (Howaru HN019) e inulina ou FOS, e permaneceu em torno de 8 log UFC.mL⁻¹ durante 30 dias, enquanto os níveis de 6 a 7 log CFU.mL⁻¹ foram mantidos nas outras bebidas. As bebidas elaboradas com iogurte simbiótico e polpa de frutas vermelhas podem ser consideradas um veículo adequado para a incorporação de probióticos e fibras, visto que os probióticos permaneceram em nível satisfatório durante o armazenamento.

Palavras-chave: *Bifidobacterium*; Prebióticos; Viabilidade microbiana; Bebida funcional; Produtos lácteos fermentados.

Resumen

El objetivo de este estudio fue evaluar la viabilidad de bacterias probióticas comerciales (bifidobacterias) en bebidas simbióticas elaboradas con yogures simbióticos y pulpa de frutos rojos, después de su fabricación y durante el almacenamiento refrigerado. Se prepararon seis formulaciones de bebidas utilizando yogur simbiótico (60%) que contenía inulina o fructooligosacáridos (FOS) y cultivos probióticos comerciales (*Bifidobacterium* spp.) y pulpa de frutos rojos (40%), como sigue: F1 (Howaru HN 019, inulina); F2 (Howaru HN 019, FOS); F3 (Lafti B 94, inulina); F4 (Lafti B 94, FOS); F5 (Kit Bifi, inulina); F6 (Kit Bifi, FOS). Las bebidas fueron evaluadas en calidad microbiológica (coliformes totales y termotolerantes, mohos y levaduras), viabilidad de probióticos, pH y acidez titulable. Se investigó el pH, acidez y conteo de probióticos hasta 30 días de almacenamiento. Se realizó la caracterización fisicoquímica de las formulaciones con mejor desempeño en cuanto al mantenimiento de los conteos de probióticos. Las bebidas elaboradas presentaron adecuada calidad higiénica sanitaria, disminución del pH y aumento de la acidez, lo cual es común en los productos lácteos fermentados. El probiótico Howaru HN019 exhibió mejor estabilidad en la bebida que Lafti B4 y Kit Bifi. El nivel más alto de bifidobacterias se encontró en las bebidas F1 y F2 que contenían *B. animalis* (Howaru HN019) e inulina o FOS, alrededor de 8 UFC.mL⁻¹ hasta 30 días, mientras que en las demás bebidas se mantuvieron niveles de 6 a 7 log CFU.mL⁻¹. Las bebidas elaboradas con yogur simbiótico y pulpa de frutos rojos pueden considerarse un vehículo adecuado para la incorporación de probióticos y fibras, mientras que los probióticos se mantuvieron en un nivel satisfactorio durante el almacenamiento.

Palabras clave: *Bifidobacterium*; Prebióticos; Viabilidad microbiana; Bebida funcional; Productos lácteos fermentados.

1. Introduction

In recent years, fermented dairy products have been considered as ideal vehicles for delivering probiotics to the human gut (Zielinska et al., 2021), being the yogurts and fermented milks the most widespread dairy probiotic products (Mani-López et al., 2014). There is an increasing demand for health-promoting beverages, which is prompting the dairy industry to develop functional probiotic yogurts to meet this demand (Nyanzi et al., 2021). Probiotic yoghurt may be a potential alternative to cater the expanding market of functional foods (Sarkar, 2019).

The main products for inserting probiotic foods in the diet of the population are milk-based, from lactic fermentation, with yogurts being preferred due to the inclusion of fruit flavoring in addition to the appeal in health promotion (Hussein et al., 2021; Barros et al., 2021). Yogurt is a fermented dairy product widely consumed for its therapeutical, nutritional and sensory properties (Nyanzi et al., 2021; Mudgil et al., 2016; Illupapalayam et al., 2014). Yogurts and fermented milk are the most usual vehicles for delivery of functional ingredients (Fabersani et al., 2018; Shori, 2016; Illupapalayam et al., 2014). Yogurt is obtained by the fermentation of whole, skimmed, or standardized milk, through the action of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, which can be accompanied by other lactic acid bacteria that confer specific characteristics to the final product (Brasil, 2007). High functionality of the yogurt is attributed to the presence of living microorganisms such as lactic acid bacteria (LAB), streptococci, bifidobacteria or their combinations (Cruz et al., 2013).

Probiotics have been defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Martín et al., 2019; Reid et al., 2019; Hill et al., 2014). Selection of a suitable base product for delivering probiotics is a key step toward the development of probiotic foods (Ranadheera et al., 2010). Fermented milks have been used for a long time as the main vehicles for probiotic strains. The main probiotics used commercially to be incorporated in yogurts are predominantly bacteria from the genera *Lactobacillus* and *Bifidobacterium* (Tripathi et al., 2014; Tamime et al., 2007).

Prebiotics were classified as substrates that are selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2016). The most important prebiotics are inulin and oligosaccharides, which are soluble and fermentable fibers. Inulin and other fructooligosaccharides are fibers that have been employed together with probiotics in dairy products. Ordinarily, the increase in the number of *Bifidobacterium* spp. in the intestinal microbiota has been the focus of prebiotic addition (Gibson et al., 2016; Oliveira et al., 2009; Akalin et al., 2004).

Yogurt drinks fortified with value-added ingredients such as prebiotics and probiotics can positively affect the consumer's perception of the product, as it has increased its sensory acceptability compared to products containing only

probiotics and conventional products (Allgeyer et al., 2010). In yogurts, prebiotics may act as a substrate for the growth of gut microbiota. Symbiotics, which are functional foods comprising mixtures of probiotics and prebiotics, refer to the combined use of a prebiotic compound that selectively favors a probiotic organism (Gonzalez et al., 2011). Most functional foods containing symbiotics are currently in dairy matrices.

Fruit and vegetables are an essential part of the human diet, and are rich in vitamins, minerals and other bioactive compounds, depending on the plant species. In recent decades, smoothies (a form of ready-to-drink beverage) have become one of consumers' preferred choices of drink, with suitable sensory properties in combination with nutritional benefits (Safefood, 2009). Smoothies are blended forms of beverages, which are typically semiliquid, presenting a smooth consistency. They contain fruit, fruit juice, and other complements such as yogurt, milk, and are prepared with different combinations of fruit and vegetables (Keenan et al., 2012; Teleszko et al., 2014). Pattaro et al. (2020) prepared smoothies using fruit pulps (apple and banana) and milk fermented by kefir of different species (cow, sheep and goat), being that the smoothie with goat milk fermented presented the most positive purchase intention.

The incorporation of fruits into the yogurt has contributed significantly to a healthy image and increased yogurt consumption (Hernández-Herrero et al., 2014). The effect of natural fruit juices on the growth of probiotics and yogurt starter culture has been reported to be species as well as strain specific. However, only a few studies have investigated the effect of added commercial fruit preparations on the growth and survivability of probiotic bacteria (Ranadheera et al., 2012).

The viability of bacteria is an important characteristic of the use of probiotics in beverages, once they should survive during the shelf life, with minimum viable cells of 10^6 - 10^7 CFU.mL⁻¹ (Komatsu et al., 2008; Shori, 2016). Thus, the use of probiotic cultures in different food matrices should be investigated. Moreover, it is necessary to maintain the viability of the probiotics above the therapeutic minimum ($>10^6$ CFU.mL⁻¹) to provide health benefits to the host, which is influenced by the composition of food matrix and processing conditions (Hussain et al., 2016). Besides a good sensory acceptability, the assessment of bacterial viability in a probiotic product is a critical parameter for quality evaluation.

The main objective of this study was to evaluate the viability of probiotic bacteria in six formulations of smoothie beverages prepared with symbiotic yogurt made using different prebiotics (inulin and FOS), and commercial probiotic cultures (Howaru Bifido HN 019, Lafti B94, Kit Bifi), in addition to berries pulp.

2. Methodology

2.1 Yogurt starter culture and probiotics cultures

The yogurt starter culture (Yo-Mix 863 LYO, 500 DCU; Danisco, Copenhagen, Denmark), a combination of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was employed. The culture was propagated in whole milk after thermal treated at 85 °C for 30 min. and transferred to sterile flasks, then stored at -20 °C for posteriorly use. The necessary inoculum was calculated to give approximately 8-9 log colony-forming units (CFU.mL⁻¹) in the yoghurt after inoculation.

The probiotics cultures used were Howaru Bifido HN 019 (Danisco, Copenhagen, Denmark) and Lafti B 94 (DSM, Moorebank, Australia) containing the probiotic strain *Bifidobacterium animalis* ssp. *lactis*, and the Kit Bifi (CSL, Zelo Buon Persico, Italy) containing the mixed probiotic culture (*Bifidobacterium longum*, *B. infantis* and *B. breve*). The cultures were propagated in whole milk after receiving thermal treatment at 85 °C for 30 min. and transferred to sterile flasks, then were stored at -20 °C for later use. The probiotic colony-forming units present in the aliquots, after one day of storage at -20°C were: 10.25; 8.9 and 9.36 log CFU.mL⁻¹ for Howaru Bifido HN 019, Lafti B94 e Kit Bifi, respectively.

2.2 Manufacture of symbiotic beverages

The materials used for the preparation of yogurt symbiotic were: yogurt starter culture, probiotics cultures, inulin

(Orafti® GR, Beneo, Mannheim, Germany), oligofructose (Orafti® P95, Beneo, Mannheim, Germany), skimmed powder milk (Molico, Nestlé, São Paulo, SP, Brazil); pasteurized fruit pulp (DeMarchi, Jundiaí, SP, Brazil); sucrose (refined sugar, União, Sertãozinho, SP, Brazil).

For the preparation of six formulations, yogurt was made using reconstituted skim milk (12%), sucrose (8%) and 4% of fibers (inulin or fructooligosaccharides (FOS)). This base mixture was heat-treated at 85°C for 20-30 minutes, cooled (42-44°C) and the starter culture Yo-mix 863 LYO and the probiotic cultures were inoculated. The fermentation was performed in a chamber at 42-43 °C until reaching pH 4.6 ± 0.1 . Then the mix was cooled and the pasteurized fruit pulp (berries is a blend containing blackberry, strawberry, and raspberry) and potassium sorbate (0.03%) were added to yogurt formulations at yogurt/pulp ratio of 60/40 (w/w). An additional amount of sucrose (heat-treated at 85°C/10 min.) was added to the yogurt to reach 10% sucrose in the final product. Six formulations were prepared as follow: F1 (Howaru HN 019, inulin); F2 (Howaru HN 019, FOS); F3 (Lafti B 94, inulin); F4 (Lafti B 94, FOS); F5 (Kit Bifi, inulin); F6 (Kit Bifi, FOS).

The six beverages were stored in a cold room at $8 \pm 2^\circ\text{C}$ and evaluated for microbiological characterization (total and thermotolerant coliforms, molds and yeasts), viability of probiotics, pH and titratable acidity, after 1 day of manufacture. After 10, 20 and 30 days of storage, the pH, titratable acidity and probiotic counts were verified. Then, the physicochemical composition (pH, titratable acidity, total dry matter, total lipids, total proteins, ashes and total carbohydrates) was evaluated for the formulations with better performance in maintenance of probiotic counts.

2.3 Physicochemical and Microbiological evaluations

The pH values of the pulp and beverages were measured in a digital pH meter (B-375, Micronal, São Paulo, Brazil). The titratable acidity of the pulp was determined by titration with 0.1 N sodium hydroxide to pH 8.3 (IAL, 2008), and the results were expressed as % citric acid per 100g sample. The total soluble solids content (°Brix) of the pulp was measured in a digital refractometer (HI96801, Hanna, Barueri, Brazil). The titratable acidity of the beverages was determined by titration with 0.1 N NaOH and the results were expressed as % lactic acid per 100 grams of the product, and the total dry matter and total lipids were determined according to Brasil (2006).

The pH, titratable acidity, dry matter total, and lipids were determined according to Brasil (2006). The total nitrogen content was determined by the official Kjeldahl method, according to the International Dairy Federation (1993). The total protein content was calculated by multiplying the total nitrogen content by 6.38. The fixed mineral residue content (ash) was determined according to Horwitz (2000). The total carbohydrates were calculated by difference, according to the formula: Total carbohydrates = $[100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ lipids})]$.

The methodology proposed by the International Dairy Federation (IDF, 2007) was used for the selective enumeration of the probiotic microorganisms (*Bifidobacterium* spp.), with modifications. For that, MRS agar medium was supplemented with lithium chloride (0.1%), L-cysteine (0.05%) and dicloxacillin ($0.5 \text{ mg}\cdot\text{L}^{-1}$), using pour plate and incubation under anaerobic conditions at $37 \pm 1^\circ\text{C}$ for $72 \pm 3 \text{ h}$. The total and thermotolerant coliforms counts were measured using the most probable number (MPN) method, using a series of three tubes of lauryl sulfate tryptose broth (LST, Difco) for sample dilution and incubating at $35 \pm 1^\circ\text{C}$ for $24 \pm 2 \text{ h}$ (ISO 4831/ The international organization for standardization, 2006). To confirm the presence of total coliforms, aliquots from the LST tubes exhibiting microbial growth and gas production were transferred to tubes containing 2% brilliant green broth (BGB, Difco) and EC broth (Difco). The tubes with BGB broth were incubated at $35 \pm 1^\circ\text{C}$ for $24 \pm 2 \text{ h}$ to confirm the presence of total coliforms and the EC tubes were maintained for up to $48 \pm 2 \text{ h}$ at $44 \pm 1^\circ\text{C}$ for confirmation of the presence of thermotolerant coliforms (ISO 7251/ The international organization for standardization, 2005). Molds and yeasts were enumerated using dichloran rose bengal chloramphenicol agar (DRBC, Difco), incubated for 5 days at $25 \pm 1^\circ\text{C}$ (Frank et al., 2004).

2.4 Statistical analysis

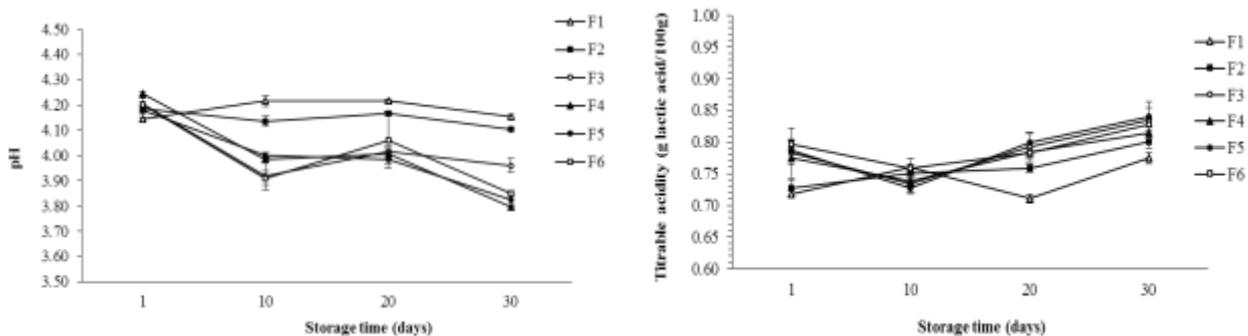
Results are presented as mean \pm standard deviation (SD) of duplicate samples. Significant differences between symbiotic beverages were assessed using one-way ANOVA followed by Tukey test ($P \leq 0.05$). T-test ($P \leq 0.05$) was used to compare the formulations physicochemical composition. Statistical analyses were performed using Minitab software, version 16.1.1.

3. Results and Discussion

The pH, °Brix, and titratable acidity of the berries pulp used in the preparation of the six beverages were respectively, 3.0; 8.0 and 0.8731 g citric acid/100g.

It is common that pH values of yogurts and fermented milks decrease during storage due to post acidification. *L. delbrueckii* ssp. *bulgaricus* is the main responsible for additional fermentation during yogurt storage (Mani-López et al., 2014). The Figure 1 shows the pH and acidity of the beverages throughout the storage time.

Figure 1. pH and titratable acidity of six beverages during refrigerated storage: F1Howaru/inulina (Δ), F2 Howaru/FOS(\blacksquare), F3 Lactis/inulina (\circ), F4 Lactis/FOS (\blacktriangle), F5 Kit Bifi/inulina (\bullet), F6 Kit Bifi/FOS (\square).



Source: Authors.

The initial and final pH of the beverages ranged from 4.25 to 4.15 and 4.16 to 3.8, respectively. A decline of pH of beverages occurred during refrigerated storage. The pH for all the beverages reduced during the storage except to the beverage F1, which remained constant. A decrease in pH is common during the storage of fermented dairy products, due to the production of organic acids by lactic acid bacteria. The post-acidification of yogurts during the refrigerated storage is due to the production of lactic acid, especially by *L. bulgaricus* (Korbekandi et al., 2015). Significant differences ($P < 0.05$) in the pH of yogurts during storage were detected, corroborating the residual acidification during storage. Declining pH can be attributed to the residual activity of microorganisms (Mani-López et al., 2014). Gallina et al. (2012) obtained initial pH value near 4.40 in a beverage formulation made from guava pulp and milk fermented (50/50%) with yogurt starter culture and bifidobacteria, with and without the addition of prebiotics (FOS). Dave & Shah (1997) also found similar decreases in pH values during storage of yoghurts containing *L. acidophilus* and bifidobacteria being that the initial pH values decreased from 4.33–4.41 at day 0 to 4.16–4.22 at the end of 35 days of storage. Kailasapathy et al. (2008) described a decline in yogurt pH with the addition of commercial fruit pulps, with a value of 4.45 on the first day of manufacture and 4.25 after 35 days at 4 °C.

Figure 1 also shows the values of titratable acidity of the beverages throughout the refrigerated storage. The acidity ranged from 0.72 to 0.80 g lactic acid/100 g sample after one day of manufacture and 0.77 to 0.84 g lactic acid/100 g at the end of 30 days of storage. A significant increase in the acidity ($P < 0.05$) was observed for the beverages. Acidity is an important

quality attribute for yoghurts and a factor that limits its acceptance, being a desirable value for yoghurts to have an acidity around of 0.70 - 0.72% of lactic acid (Moreira et al., 1999; Tamime et al., 2007). Hossain et al. (2012), Gallina et al. (2012) and Barbosa et al., (2017) also observed an increase in acidity and a decrease in pH of yogurts containing fruit pulp. The symbiotic beverages presented titratable acidity values similar to those observed for fermented milk, usually between 0.6 and 1.0 g of lactic acid per 100 g of product (Brasil, 2007). The pH parameters and acidity play an important role on the sensory characteristics and probiotics viability of the products (Barbosa et al., 2017).

The sensitivity of probiotic cultures is affected by low pH values and this sensitivity is extremely dependent on the strain, especially for Bifidobacteria (Almeida et al., 2008). The pH values can affect the probiotic viability in yoghurts. Bifidobacteria are sensitive to pH of fruit juices (pH 3-4) (Barbosa et al., 2017). However, *B. animalis* strains are more resistant to acids when compared to strains of other species. The addition of vegetables and fruit juices or pulps to dairy beverages may be deleterious to the viability of some probiotic species, due to the acidity and presence of antimicrobial compounds such as organic acids (benzoic acid) and flavor compounds (Shori, 2016).

For the success of probiotic dairy beverages, it is important that the probiotic strains employed maintain their viability and functional activity during the entire shelf-life of the product. Many factors may affect the viability of *Bifidobacterium* spp. in dairy beverages including the probiotic strains used, pH, the presence of hydrogen peroxide and dissolved oxygen, the concentration of metabolites such as lactic and acetic acids, the medium buffering capacity, storage temperature and the nature of the added ingredients, among others (Shori, 2016; Donkor, 2006). Further, the viability of probiotic bacteria during storage is inversely related to storage temperature. Highest viability of *B. lactis* BB-12 in yogurt was observed when the optimum storage temperature was 8 °C. This is attributed to the low resistance of Bifidobacteria cells to low refrigeration temperatures (Tripathi et al., 2014).

The Table 1 shows the viability of the probiotic microorganisms on symbiotic beverages during 30 days of cold storage.

Table 1. Viable counts of *Bifidobacterium* spp. (log CFU.mL⁻¹) on beverages during 30 days storage at 8 ± 2 °C.

Formulations	Time of storage (days)			
	1	10	20	30
F1 (HOWARU/INU)	8.68 ± 0.02 ^{a, A}	8.68 ± 0.18 ^{a, A}	8.70 ± 0.04 ^{a, A}	8.18 ± 0.14 ^{b, A}
F2 (HOWARU/FOS)	8.66 ± 0.13 ^{a, A}	8.58 ± 0.26 ^{ab, A}	8.33 ± 0.11 ^{ab, A}	8.09 ± 0.23 ^{b, A}
F3 (LACTIS/INU)	7.65 ± 0.03 ^{a, B}	7.39 ± 0.13 ^{a, B}	7.30 ± 0.09 ^{a, B}	7.49 ± 0.69 ^{a, AB}
F4 (LACTIS/FOS)	7.22 ± 0.17 ^{a, B}	7.22 ± 0.10 ^{a, B}	6.82 ± 0.02 ^{a, C}	6.74 ± 0.04 ^{a, B}
F5 (KIT BIF/INU)	7.86 ± 0.11 ^{a, B}	7.57 ± 0.02 ^{ab, B}	7.44 ± 0.05 ^{ab, B}	7.24 ± 0.02 ^{b, AB}
F6 (KIT BIF/FOS)	7.41 ± 0.01 ^{a, B}	7.33 ± 0.10 ^{a, B}	7.25 ± 0.01 ^{a, B}	7.16 ± 0.02 ^{a, AB}

a Values (mean ± SD) within a line followed by different lowercase letters are significantly different (P≤0.05), tested by one-way ANOVA followed by post-hoc Tukey. A Values (mean ± SD) within a column followed by different uppercase letters are significantly different (P≤0.05), tested by one-way ANOVA followed by post-hoc Tukey. Source: Authors.

There was a significant difference (P≤0.05) in the viability of probiotics between the beverage formulations in all days of storage and over the storage time (Table 1). The *Bifidobacterium* counts in the symbiotic beverages ranged 8~7log CFU.mL⁻¹ during cold storage, except for the F4 formulation that after 20 days of storage presented lowered counts, about 6 log CFU.mL⁻¹. There was a decrease of one logarithmic cycle in the counts of bifidobacteria when comparing F3 with F4 within 30 days of storage, which suggests a trend towards a higher prebiotic effect of inulin in relation to FOS, what wasn't evidenced comparing F1 and F2 even as F5 and F6.

Several authors have reported the importance of the survival of the probiotic bacteria in populations high enough to confer health benefits to the consumer. Food products sold with any claim of probiotic benefits should meet the criteria of advised minimum number 10^6 CFU.mL⁻¹ at the time of consumption (Vasiljevic & Shah, 2008). Shori (2016) reported that the beverage must exhibit a minimum number of viable probiotic cells during the shelf life (10^6 - 10^7 CFU.mL⁻¹), while Kumar & Kumar (2016) reported that probiotics must reach the intestine in sufficient numbers between 6 and 7 log CFU.mL⁻¹ of product to confer health benefits. Thus probiotics should be consumed in sufficient amounts, to maintain the minimum levels of 10^6 to 10^8 CFU.mL⁻¹ needed for exerting beneficial effects (Tamime et al., 2005).

Barbosa et al., (2017) found probiotic counts around 6 log CFU.mL⁻¹ in beverages made with probiotic yogurt and mango pulp, and Gallina et al. (2012) observed counts of 10^6 - 10^7 CFU.mL⁻¹ in a beverage containing probiotic yogurt and guava pulp during 30 days of storage. Kumar et al., (2016) observed a significant reduction in the number of viable probiotic cells during storage in yogurts, probably due to the processing conditions and the low pH and high acidity of the products. Kailasapathy, Harmstorf, and Phillips (2008) reported a correlation between pH of yogurts and the probiotics viability during storage, which was affected by the addition of fruit pulp, indicating that fruit-based yogurts and the properties of fruit preparations, such as pH, can affect the viability of probiotic bacteria.

For the six beverages of the present study, probiotics counts remained in appropriate numbers during the storage. Almeida et al. (2008) also reported that the growth of probiotic cultures (*L. acidophilus* and *B. bifidum*) in yogurts made with açai pulp was not inhibited by the low pH values during the storage, which exhibited a log reduction after 21 days at 4 °C, with the probiotic counts still in accordance with the Brazilian legislation (Brasil, 2007), which recommends minimum bifidobacteria counts of 10^6 CFU.mL⁻¹. Sidhu et al. (2020) observed that the viability of both probiotic bacteria, *Lactobacillus acidophilus* LA5 and *Bifidobacterium* BB12, decreased significantly during refrigerated storage (4°C) for five weeks, however, the ultimate probiotic count in all probiotic yogurts exceeded the minimum therapeutic value of 6 log CFU/g.

Allgeyer et al. (2010) incorporated prebiotics and probiotics into a vanilla-flavored fat-free yogurt drink. The prebiotics tested included soluble corn fiber, polydextrose, and chicory inulin, and the probiotics used in the study were *B. lactis* Bb-12 and *L. acidophilus* LA-5. Though the polydextrose mildly improved shelf life, the viability study concluded that there was a 2 to 3-log reduction in viable cells in yogurt drinks with or without added prebiotics, after 30 d of refrigerated storage. This indicates that none of these prebiotics are good for extending the viability of *L. acidophilus* or *B. lactis* over shelf-life. However, Kariyawasam et al. (2021) reported that FOS positively influences probiotic viability, as evidenced by the high probiotic viability in synbiotic yoghurts (> 8 log CFU.mL⁻¹) during 21 days of refrigerated storage.

The viability of bifidobacteria (>7 log CFU.mL⁻¹) was maintained at a satisfactory level in all formulations in the current study. Therefore, all beverages formulations presented the recommended level until the end of storage in sufficient quantity to exert potential benefits to the consumer. Considering the results obtained, the probiotic that presented better performance or higher viability during the 30 days of cold storage period was Howaru Bifido (*lactis* HN 019) with a level of 7-8 log CFU.mL⁻¹. Thus, it was verified the physical chemical composition of the two beverages made with this probiotic, F1 and F2.

The physicochemical characterization (pH, titratable acidity, total dry matter, fat, protein, ash and carbohydrates) was determined for the formulations F1 and F2 beverages prepared from symbiotic yogurt containing the probiotic Howaru Bifido HN 019 and the fibers inulin and FOS with berries pulp. The results are described in Table 2.

Tabela 2. Physicochemical composition (average \pm standard deviation) of symbiotic beverages.

Parameter	F1 (HOWARU/INU)	F2 (HOWARU/FOS)
pH	4.32 ^a	4.25 ^a
Titrateable acidity (g/100g of lactic acid)	0.7642 \pm 0.0133 ^b	0.7875 \pm 0.0076 ^a
Total dry matter (%)	19.35 \pm 0.0129 ^a	19.35 \pm 0.2435 ^a
Fat (%)	0.1726 \pm 0.0050 ^b	0.1987 \pm 0.0003 ^a
Protein (%)	2.04 \pm 0.1429 ^a	2.09 \pm 0.0347 ^a
Ash (%)	0.6735 \pm 0.0027 ^a	0.6777 \pm 0.0039 ^a
Carbohydrates (%)	16.47	16.38

^a Values (mean \pm SD) within a line followed by different lowercase letters are significantly different ($P \leq 0.05$), tested by t-test. Samples evaluated (n= 2) and duplicate analysis. Source: Authors.

The initial pH of F1 and F2 beverages was similar, ranging from 4.2 to 4.3. Sah et al. (2016) obtained pH around 4.5 to yogurt samples manufactured using starter and probiotic cultures in absence or presence of inulin or pineapple peel powder after one day of storage at 4 ° C. Kailasapathy et al. (2008) reported that initial pH (day 0, before the addition of fruit) ranged between 4.35 and 4.54, and the final pH ranged from 4.13 to 4.35 when the effect of commercial fruit preparations (mango, mixed berry, passion fruit and strawberry) was evaluated on the viability of probiotic bacteria, *B. animalis* ssp. *lactis* - LAFTIS B94 in stirred yogurts during refrigerated storage. Kumar & Kumar (2016) conclude that yogurts supplemented with fruits rich in antioxidants have lower pH and higher titrateable acidity when compared to yogurts without the addition of fruits.

The fat content of F1 and F2 beverages is below 0.5g/100g of product, which can characterize the beverages as skimmed. This is due to the use of skim milk powder for the production of yogurts and also to the fact that the fruit pulp and other ingredients used do not affect this parameter.

Protein contents were lower than expected for fermented milks and yogurt, due to the addition of a considerable amount of fruit pulp (40%). However, the value of approximately 2.05% showed that the beverages still have a considerable protein content, which is even above what is allowed in the Brazilian legislation for fermented milk drinks, of at least 1.2g of protein per 100g of product. Thus, this product can be considered nutritionally adequate due to its protein content.

Ash contents (0.7%) are similar to those observed for fermented dairy products with approximate compositions, in terms of total solids and protein contents. Total carbohydrate contents are within expectations, considering the addition of 10% sucrose in the final product. At this value, the lactose content remaining in the yogurt after fermentation and the carbohydrate content from the fruit pulp must be considered.

Gallina et al. (2018) obtained similar composition when prepared probiotic yogurt with 18% of berries pulp, obtained the medium values of total dry matter of 17,67%; fat 0,22%; protein 3,51%; ash 1,05 % and total carbohydrates 12,90%. Gallina et al. (2019) also prepared two probiotic smoothie formulations made with probiotic yogurt (60%) and mango pulp (40%) being the formulation F1 containing 2.0 % chitoooligosaccharides modified with glucose (COS-Glc) and the formulation F2 without the addition of COS-Glc. The values (%) obtained were similar, with total dry matter 19.98 and 17.31, proteins 2.67 and 1.99, fat 0.18 and 0.58, ash 0.81 and 0.83, total fiber 0.59 and 0.36 and carbohydrates 15.74 and 13.88, to F1 and F2, respectively.

Variations in the composition of beverages are expected considering the ingredients used, variations in the composition of the skim milk powder; content and composition of the fruit pulp; carbohydrates content from the milk and fruit pulp, residual lactose and glucose after the fermentation and through the addition of sucrose.

The microbiological quality of six beverage samples was evaluated after one day of manufacture. Total and

thermotolerant coliforms counts were less than 0.3 MPN.mL⁻¹ for all the beverages. The yeasts and molds counts were less than 10 CFU.mL⁻¹ for the beverages F1, F2 e F4 while the formulations F3 e F5 presented 10 CFU.mL⁻¹, and the counts were higher in F6 (30 CFU.mL⁻¹). Because of its low pH, yogurt favors yeast growth. Yogurts with added sugar or fruit are especially susceptible to yeast growth (Moreira et al, 1999). However, the results observed are within the limits established by the Brazilian legislation for similar products, such as fermented milk and fermented milk beverages (Brasil, 2007). Therefore, the beverages elaborated in this study presented adequate microbiological quality, guaranteeing the safety of the product.

4. Conclusion

All six beverages formulations prepared with symbiotic yogurt (60%) containing inulin or oligofructose (FOS), commercial probiotic cultures (*Bifidobacterium* strains) and berries pulp (40%) presented sufficient probiotic viability during cold storage with levels of 10⁶ to 10⁸ CFU.mL⁻¹, needed for exerting beneficial effects. The fibers inulin and FOS presented similar effect on the beverages and the supplementation with these prebiotic fibers could have contributed for the probiotic stability during cold storage. The probiotic Howaru HN 019 showed better performance on the smoothie beverage with berries pulp. The beverage made with these commercial probiotics and the fibers inulin or FOS maintained the viable probiotic counts required for the health claim. The smoothie beverages made with symbiotic yogurt and berries pulp can be considered an appropriate vehicle for the incorporation of probiotics and fibers being considered a new functional product.

Acknowledgments

The authors would like to thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support to the Project (Process 481486/2011-9).

References

- Akalin, AS, Fenderya, S, Akbulut, N (2004). Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage. *International Journal of Food Science and Technology*, 39, 613-621. doi: <https://doi.org/10.1111/j.1365-2621.2004.00829.x>
- Allgeyer, LC, Miller, MJ, Lee, SY (2010). Drivers of liking for yogurt drinks with prebiotics and probiotics. *Journal of Food Science*, 75(4), S212-S219. doi: <https://doi.org/10.1111/j.1750-3841.2010.01579.x>
- Almeida, MHB, Zoellner, SS, Cruz, AG, Moura, MRL, Carvalho, LMJ, Freitas, MCJ, Sant'Ana, A (2008). Potentially probiotic açai yoghurt. *International Journal of Dairy Technology*, 61(2), 178-182. doi: <https://doi.org/10.1111/j.1471-0307.2008.00390.x>
- Barbosa, PPM & Gallina, DA (2017). Viabilidade de bactérias (starter e probióticas) em bebidas elaboradas com iogurte e polpa de manga. *Revista do Instituto de Laticínios Cândido Tostes*, 72(2), 85-95. doi: <https://doi.org/10.14295/2238-6416.v72i2.580>
- Barros, VC, Freitas, AC, Hunaldo, VKL, Souza, IHS (2021). Probiotic products consuming analysis with a university education institution students. *Research, Society and Development*, 10(7), e47710716728. doi: <https://doi.org/10.33448/rsd-v10i7.16728>
- Brasil (2006). Ministério da Agricultura, Pecuária e Abastecimento. Secretaria da Defesa Agropecuária. Laboratório Nacional de Referência Animal. Instrução Normativa nº 68, de 12 de dezembro de 2006. Oficializa os métodos analíticos oficiais físico-químicos para controle de leite e produtos lácteos. V – Métodos quantitativos. *Diário Oficial da União*, Brasília, DF.
- Brasil. (2007). Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº 46, de 23 de outubro de 2007. Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. *Diário Oficial da União*, seção 1, p.5, 24 de outubro de 2007. Brasília, DF.
- Cruz, AG, Cavalcanti, RN, Guerreiro, LMR, Sant'Ana, AS, Nogueira, LC, Oliveira, CAF, Deliza, R, Cunha, RL, Faria, JAF, Bolini, HMA (2013). Developing a prebiotic yogurt: Rheological, physico-chemical and microbiological aspects and adequacy of survival analysis methodology. *Journal of Food Engineering*, 114, 323-330. doi: <https://doi.org/10.1016/j.jfoodeng.2012.08.018>
- Dave, RI & Shah, NP (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal*, 7(1), 31-41. doi: [https://doi.org/10.1016/S0958-6946\(96\)00046-5](https://doi.org/10.1016/S0958-6946(96)00046-5)
- Donkor, ON, Henriksson, A, Vasiljevic, T, Shah, NP (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal*, 16(10), 1181-1189. doi: <https://doi.org/10.1016/j.idairyj.2005.10.008>
- Fabersani, E, Grande, MV, Araújo, MVC, Zannier, ML, Sánchez, SS, Grau, A, Honoré, SM (2018). Metabolic effects of goat milk yogurt supplemented with yacon flour in rats on a high-fat diet. *Journal of Functional Foods*, 49, 447-457. doi: <https://doi.org/10.1016/j.jff.2018.08.042>

- Frank, J. F. & Yousef, A. E. (2004). Tests for groups of microorganisms. In: Wehr, HM & Frank, JF (Ed.). *Standard Methods for the examination of dairy products* (17th ed.). American Public Health Association, Washington, D.C., 227-247. doi: <https://doi.org/10.2105/9780875530024>
- Gallina, D. A., Antunes, A. E. C., Azambuja-Ferreira, N. C., Mendonça, J. B., Norbona, R. A. (2012). Caracterização de bebida obtida a partir de leite fermentado simbiótico adicionado de polpa de goiaba e avaliação da viabilidade das bifidobactérias. *Revista do Instituto de Laticínios Cândido Tostes*, 67(386), 45-54. doi: <https://doi.org/10.5935/2238-6416.20120035>
- Gallina, D. A., Ormenese, R. C. S. C., Garcia, A. O. (2018). Iogurte probiótico com polpa de frutas vermelhas: caracterização físico química e microbiológica, aceitabilidade sensorial e viabilidade dos probióticos. *Revista do Instituto de Laticínios Cândido Tostes*, 73(4), 196-208. doi: <https://doi.org/10.14295/2238-6416.v73i4.681>
- Gallina, D. A., Pacheco, M. T. B., Barbosa, P. P. M., Amorim, M. M. F., Cobas, AC (2019). Effect of modified Chitooligosaccharides on the physicochemical and microbiological characteristics of a probiotic fermented beverage. *Revista do Instituto de Laticínios Cândido Tostes*, 74(4), 240-251. doi: <https://doi.org/10.14295/2238-6416.v74i4.753>
- Gibson, G, Hutkins, R, Sanders, M, Prescott, S. L, Reimer, R. A, Salminen, S. J, Scott, K, Stanton, C, Swanson, K. S, Cani, P. D., Verbeke, K, Reid, G (2016). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14, 491–502 (2017). doi: <https://doi.org/10.1038/nrgastro.2017.75>
- Gonzalez, N. J, Adhikari, K, Sancho-Madriz, M. F (2011). Sensory characteristics of peach-flavored yogurt drinks containing prebiotics and symbiotics. *LWT - Food Science and Technology*, 44(1), 158-163. doi: <https://doi.org/10.1016/j.lwt.2010.06.008>
- Hernández-Herrero, J. A; Frutos, M. J (2014). Effect of concentrated plum juice on physicochemical and sensory properties of yoghurt made at bench top scale. *International Journal of Dairy Technology*, 67(1), 123-128. doi: <https://doi.org/10.1111/1471-0307.12101>
- Hill, C, Guarner, F, Reid, G, Gibson, G. R, Merenstein, D. J, Pot, B, Morelli, L, Canani, R. B, Flint, H, Salminen, S, Calder, P. C, Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11, 506–514. doi: <https://doi.org/10.1038/nrgastro.2014.66>
- Horwitz, W (2000). Official Methods of Analysis of AOAC International. 17th Ed., Vol. II. *Food Composition; Additives; Natural Contaminants*, chap 33 p.10; 54; 61; 71. (Proc. 920.108; 930.30; 935.42 and 945.46).
- Hossain, M. N, Fakruddin, M, Islam, M. N (2012). Quality comparison and acceptability of yoghurt with different fruit juices. *Journal of Food Processing & Technology*, 3(8), 1-5. doi:10.4172/2157-7110.1000171
- Hussain, S. A, Patil, G R, Yadav, V, Singh, R. R. B, Singh, A. K (2016). Ingredient formulation effects on physico-chemical, sensory, textural properties and probiotic count of *Aloe vera* probiotic dahi. *LWT - Food Science and Technology*, 65, 371-380. doi: <https://doi.org/10.1016/j.lwt.2015.08.035>
- Hussein, Z. E. H, Silva, J. M, Alves, E. S, Castro, M. C, Ferreira, C. S. R, Chaves, M. L. C, Bruni, A. R. D. S, Santos, O. O (2021). Technological advances in probiotic stability in yogurt: a review. *Research, Society and Development*, 10 (12), e449101220646. doi: <https://doi.org/10.33448/rsd-v10i12.20646>
- Illupapalayam, V. V, Smith, S. C, Gamalath, S (2014). Consumer acceptability and antioxidant potential of probiotic-yogurt with spices. *LWT - Food Science and Technology*, 55, 255-262. doi: <https://doi.org/10.1016/j.lwt.2013.09.025>
- Instituto Adolfo Lutz (IAL) (2008). Métodos físico-químicos para análise de alimentos. *Normas Analíticas do Instituto Adolfo Lutz*. 4. ed. São Paulo: IMESP, 1020.
- International Dairy Federation (IDF) (1993). Determination of the total nitrogen content of milk by Kjeldahl method. Brussels: FIL/IDF, 1993. 11p.
- International Dairy Federation (IDF) (2007). Bulletin of the IDF N° 411/2007. Selective enumeration of bifidobacteria in dairy products: development of a standard method, 20 p.
- ISO 4831 (2006). The International Organization for Standardization. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms, Most probable number technique. 3rd ed.
- ISO 7251 (2005). The International Organization for Standardization. Microbiology of food and animal stuffs. Horizontal method for the detection and enumeration of presumptive *Escherichia coli*, Most probable number technique. 3rd ed.
- Kailasapathy, K, Harmstorf, I, Phillips, M (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. *Food Science and Technology*, 41(7), 1317-1322. doi: <https://doi.org/10.1016/j.lwt.2007.08.009>
- Kariyawasam, KMGMM, Lee, N-K, Paik, H-D (2021). Symbiotic yogurt supplemented with novel probiotic *Lactobacillus brevis* KU200019 and fructooligosaccharides. *Food Bioscience*, 39, 100835, 1-8. doi: <https://doi.org/10.1016/j.fbio.2020.100835>
- Keenan, DF, Röble, C, Gormley, R, Butler, F, Brunton, NP (2012). Effect of high hydrostatic pressure and thermal processing on the nutritional quality and enzyme activity of fruit smoothies. *LWT - Food Science and Technology*, 45(1), 50-57. doi: <https://doi.org/10.1016/j.lwt.2011.07.006>
- Komatsu, T, Burity, FCA, Saad, SMI (2008). Inovação, persistência e criatividade superando barreiras no desenvolvimento de alimentos probióticos. *Brazilian Journal of Pharmaceutical Sciences*, 44(3), 329-347. doi: <https://doi.org/10.1590/S1516-93322008000300003>
- Korbekandi, H, Abedi, D, Maracy, M, Jalali, M, Azarman, N, Iravani, S (2015). Evaluation of probiotic yoghurt produced by *Lactobacillus paracasei* ssp. *tolerans*. *Journal of Food Biosciences and Technology*, 5(1), 37-44.
- Kumar, A & Kumar, D (2016). Development of antioxidant-rich fruit supplemented probiotic yogurts using free and microencapsulated *Lactobacillus*. *Journal of Food Science and Technology*, 53(1), 667-675. doi: <https://doi.org/10.1007/s13197-015-1997-7>

- Mani-López, E, Palou, E, López-malo, A (2014). Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria. *Journal of Dairy Science*, 97(5), 2578–2590. doi: <https://doi.org/10.3168/jds.2013-7551>
- Martín, R, Langella, P (2019). Emerging health concepts in the probiotics field: streamlining the definitions. *Frontiers in Microbiology*, 10:1047. 1-5. doi: [10.3389/fmicb.2019.01047](https://doi.org/10.3389/fmicb.2019.01047).
- Moreira, S. R, Schwan, R. F, Carvalho, E. P, Ferreira, C (1999). Análise microbiológica e química de iogurtes comercializados em Lavras – MG. *Ciência e Tecnologia de Alimentos*, 19(1), 147-152. doi: <https://doi.org/10.1590/S0101-20611999000100027>
- Mudgil, D, Barak, S, Khatkar, BS (2016). Development of functional yoghurt via soluble fiber fortification utilizing enzymatically hydrolyzed guar gum. *Food Bioscience*, 14, 28–33. doi: <https://doi.org/10.1016/j.fbio.2016.02.003>
- Nyanzi, R, Jooste, PJ, Buys, EM (2021). Invited review: Probiotic yogurt quality criteria, regulatory framework, clinical evidence, and analytical aspects. *Journal of Dairy Science*, 104(1), 1–19. doi: <https://doi.org/10.3168/jds.2020-19116>
- Oliveira, R. P, Perego, P, Converti, A, Oliveira, MN (2009). The effect of inulin as a prebiotic on the production of probiotic fibre-enriched fermented milk. *International Journal of Dairy Technology*, 62(2), 195-203. doi: <https://doi.org/10.1111/j.1471-0307.2009.00471.x>
- Pattaro, L, Silva, JAG, FARIAS, LCB, Medeiros, JS, Teixeira, PC, Cunha, JVT, More, JCRS, Almeida, TV, Nicolau, ES, Silva, MAP (2020). Physico-chemical and sensory analyzes of milk smoothies of different species fermented by kefir, flavored with banana and apple. *Research, Society and Development*, 9 (5), p. e112953145. doi: <https://doi.org/10.33448/rsd-v9i5.3145>
- Ranadheera, R. D. C. S, Baines, S. K, Adams M. C (2010). Importance of food in probiotic efficacy. *Food Research International*, 43, 1–7. doi: <https://doi.org/10.1016/j.foodres.2009.09.009>
- Ranadheera, C. S, Evans, C. A, Adams, M. C, Baines, S. K (2012). Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. *Food Chemistry*, 135, 1411-1418. doi: <https://doi.org/10.1016/j.foodchem.2012.06.025>
- Reid, G, Gadir, A. A, Dhir, R (2019). Probiotics: reiterating what they are and what they are not. *Frontiers in Microbiology*, 10:424, 1-6. doi: [10.3389/fmicb.2019.00424](https://doi.org/10.3389/fmicb.2019.00424).
- Safefood, 2009. SMOOTHIES consumer knowledge, attitudes and beliefs around the nutritional content of smoothies. http://www.safefood.eu/SafeFood/media/SafeFoodLibrary/Documents/Publications/Research%20Reports/9354-Smoothies-AW_web-FINAL-030309.pdf
- Sah, B. N. P, Vasiljevic, T., McKechnie, S, Donkor, O. N. (2016). Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. *LWT - Food Science and Technology*, 65, 978-986. doi: <https://doi.org/10.1016/j.lwt.2015.09.027>
- Sarkar, S (2019). Potentiality of probiotic yoghurt as a functional food – a review. *Nutrition & Food Science*, 49 (2), 182-202. doi: <https://doi.org/10.1108/NFS-05-2018-0139>
- Shori, AB (2016). Influence of food matrix on the viability of probiotic bacteria: a review based on dairy and non-dairy beverages. *Food Bioscience*, 13, 1-8. doi: <https://doi.org/10.1016/j.fbio.2015.11.001>
- Sidhu, MK, Lyu, F, Sharkie, TP, Ajlouni, S, Ranadheera, CS (2020). Probiotic yogurt fortified with chickpea flour: Physico-chemical properties and probiotic survival during storage and simulated gastrointestinal transit. *Foods*, 9, 1144, 1-13. doi: <https://doi.org/10.3390/foods9091144>
- Tamime, A. Y., Robinson, R. K. (2007). *Tamime and Robinson's Yoghurt (Third Edition) - Science and Technology*, Woodhead Publishing Series in *Food Science, Technology and Nutrition*. Elsevier.
- Tamime, A. Y., Saarela, M., Korslund Sondergaard A., Mistry, V. V, Shah, N. P (2005). Production and maintenance of viability of probiotic microorganisms in dairy products. In: Tamime, A.Y. (ed.). *Probiotic Dairy Products*. Blackwell Publishing Ltd, p. 39-72. doi: <https://doi.org/10.1002/9780470995785.ch3>
- Tripathi, M. K., Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of functional foods*, 9, 225-241. doi: <https://doi.org/10.1016/j.jff.2014.04.030>
- Teleszko, M, Wojdylo, A. (2014). Bioactive compounds vs. organoleptic assessment of 'smoothies'-type products prepared from selected fruit species. *International Journal of Food Science & Technology*, 49, 98-106. doi: <https://doi.org/10.1111/ijfs.12280>
- Vasiljevic, T., Shah, N. P (2008). Probiotics- from Metchnikoff to bioactives. *International Dairy Journal*, 18 (7), 714-728. doi: <https://doi.org/10.1016/j.idairyj.2008.03.004>
- Zielinska, D., Marciniak-Lukasiak, K., Karbowski, M, Lukasiak, P. (2021). Effects of fructose and oligofructose addition on milk fermentation using novel *Lactobacillus* cultures to obtain high-quality yogurt-like products. *Molecules*, 26, 5730, 1-19. doi: <https://doi.org/10.3390/molecules26195730>