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Probiotic milk with sweetener: Development, characterization, and *in vitro* gastrointestinal resistance of *Bifidobacterium lactis* HN019

Leite probiótico com adoçante: desenvolvimento, caracterização e resistência gastrointestinal *in vitro* de *Bifidobacterium lactis* HN019

Leche probiótica con edulcorante: desarrollo, caracterización y resistencia gastrointestinal *in vitro* de *Bifidobacterium lactis* HN019

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Abstract
The consumption of functional foods, in particular those containing bioactive ingredients and low calories, has increased in line with greater concerns regarding healthy eating habits. In this study, skimmed milk with added probiotic *Bifidobacterium animalis* subsp. Lactis HN019™ and sweetener was developed and characterised. Probiotic viability during cold storage (5 °C for 60 days) and resistance to simulated gastric and enteric conditions were also evaluated. The product was evaluated by untrained panellists who undertook preference and intent to purchase tests, comparing it to a probiotic milk with added stevia and a non-sweetened probiotic milk. The ready-to-eat product contained 0.38% lactic acid, had a pH of 5.34, protein content of 3.92%, carbohydrate of 4.08%, total dry extract of 8.81%, and ash content of 0.81%. The probiotic *B. lactis* HN019® grew and remained in the product at high concentrations (9.04 log UFC/mL). During cold storage, there was a decrease of only one cycle log of viability. A subtle reduction in the pH value and increase in the titratable acidity ($p < 0.05$) was found. During simulation of GIT conditions, the HN019 strain showed a survival rate of 93.72% and 83% in probiotic milk that was newly produced and stored for 60 days, respectively. Related to sensory acceptance, the panellists showed a preference for the sucrose sweetened milk, followed by the milk with sweetener while the no sugar fermented milk had a lower preference. Applying the scale of intent to purchase, the panellists would maybe buy the product with a sweetener if it were available on the market. The product was defined as a light probiotic skim milk, since the caloric value was reduced by 53.04% compared to commercial cultured milks. Its mild taste, due to low acidity, high viability, and resistance to the probiotic in the gastrointestinal tract *in vitro*, and the low caloric content differentiated it from the commercial fermented milks that are currently available. Therefore, this low-calorie sweetened milk may fill a poorly explored

market niche for consumers that require caloric restrictions and who value the consumption of functional foods such as probiotics.

Keywords: Functional food; Bifidobacteria; Fermentation; Sensory analysis; Stevia.

Resumo

O consumo de alimentos funcionais, principalmente aqueles que contêm ingredientes bioativos e baixas calorias, tem aumentado em sincronia com a maior preocupação com hábitos alimentares saudáveis. Neste estudo, um leite desnatado com adição do probiótico *Bifidobacterium animalis* subsp. Lactis HN019™ e edulcorante foi desenvolvido e caracterizado. A viabilidade do probiótico durante o armazenamento refrigerado (5 ° C/60 dias) e a resistência às condições gástricas e entéricas simuladas também foram avaliadas. O produto foi avaliado por provadores não treinados que realizaram testes de preferência e intenção de compra, comparando-o a um leite probiótico com açúcar e a um leite probiótico não adoçado. O produto pronto para consumo apresentou 0,38% de ácido lático, pH 5,34, teor de proteína 3,92%, carboidrato 4,08%, extrato seco total 8,81% e teor de cinzas 0,81%. O probiótico *B. lactis* HN019® multiplicou e permaneceu no produto em altas concentrações (9,04 log UFC / mL). Durante o armazenamento refrigerado, houve redução de apenas um ciclo logarítmico na viabilidade do probiótico. Foi observada uma redução sutil no valor do pH e aumento na acidez titulável (p <0,05). Durante a simulação das condições do trato gastrointestinal (TGI), a linhagem HN019 apresentou uma taxa de sobrevivência de 93,72% e 83% no leite probiótico recém-produzido e armazenado por 60 dias, respectivamente. Em relação à aceitação sensorial, os provadores demonstraram preferência pelo leite adoçado com açúcar, seguido pelo leite com edulcorante, enquanto o fermentado não adoçado teve menor preferência. Aplicando a escala de intenção de compra, os painelistas indicaram que talvez comprassem o produto com adoçante caso ele estivesse disponível no mercado. O produto foi definido como um leite desnatado probiótico light, uma vez que o valor calórico foi reduzido 53.04% se comparado a leites cultivado comerciais. Seu sabor suave, devido à baixa acidez, alta viabilidade e resistência do probiótico ao TG *in vitro*, e o baixo teor calórico, o diferenciam dos leites fermentados comerciais atualmente disponíveis. Portanto, esse produto com baixas calorias pode preencher um nicho de mercado pouco explorado por consumidores com restrições calóricas e que valorizam o consumo de alimentos funcionais como os probióticos.

Palavras-chave: Alimento funcional; Bifidobactérias; Fermentação; Análise sensorial; Stevia.

Resumen

El consumo de alimentos funcionales, especialmente los que contienen ingredientes bioactivos y bajas calorías, se ha incrementado en línea con la mayor preocupación por los hábitos alimentarios saludables. En este estudio, la leche desnatada con la adición de probiótico *Bifidobacterium animalis* subsp. *Lactis* HN019 ™ y edulcorante se desarrolló y caracterizó. También se evaluó la viabilidad del probiótico durante el almacenamiento refrigerado (5 ° C durante 60 días) y la resistencia a condiciones gástricas y entéricas simuladas. El producto fue evaluado por catadores no entrenados que realizaron pruebas de preferencia e intención de compra, comparándolo con una leche probiótica con stevia agregada y una leche probiótica sin azúcar. El producto listo para consumir contenía 0,38% de ácido láctico, pH 5,34, contenido de proteínas 3,92%, contenido de carbohidratos 4,08%, extracto seco total 8,81% y contenido de cenizas 0,81%. El probiótico *B. lactis* HN019® multiplicado y permaneció en el producto en altas concentraciones (9,04 log UFC / mL). Durante el almacenamiento refrigerado, hubo una reducción de solo un ciclo logarítmico en la viabilidad del probiótico. Se encontró una reducción sutil en el valor del pH y un aumento en la acidez titulable (p <0.05). Durante la simulación de las condiciones del tracto gastrointestinal (TGI), la cepa HN019 mostró una tasa de supervivencia de 93,72% y 83% en leche probiótica recién producida y almacenada durante 60 días, respectivamente. Acerca de la aceptación sensorial, los evaluadores mostraron preferencia por la leche endulzada con azúcar, seguida de la leche con edulcorante, y finalmente a la leche sin azúcar. Aplicando la escala de intención de compra, los panelistas indicaron que podrían comprar el producto con edulcorante si estuviera disponible en el mercado. El producto se definió como una leche probiótica, desnatada y bajo en calorías, ya que el valor calórico se redujo en un 53,04% en comparación con las leches cultivadas comerciales. Su sabor suave, debido a la baja acidez, alta viabilidad y resistencia a los probióticos en el tracto gastrointestinal *in vitro*, y bajo contenido calórico lo diferencian de las leches fermentadas comerciales actualmente disponibles. Así, esta leche azucarada baja en calorías puede llenar un nicho de mercado poco explorado por los consumidores que demandan restricciones calóricas y valoran el consumo de alimentos funcionales como los probióticos.

Palabras clave: Alimento funcional; Bifidobacterias; Fermentación; Análisis sensorial; Stevia.

1. Introduction

The increasing demand for a high quality of life and disease prevention has favoured the market for functional foods, such as probiotics and/or low-calorie foods, whose characteristics are well exploited by well-defined market niches. The functional food and beverage market component have grown the most, accounting for more than 85% of this sector. Dairy products are highly representative, with this trend continuing until 2024 (Transparency Market Research, 2018). Thus, foods containing probiotics are highly valued. Once the benefits are assigned to specific strains, they can leave the research scene and become a reality in the food industry, routine food, and even clinical practice. The concept of this term has undergone subtle changes over time (FAO/WHO, 2002; Fuller, 1989; Hill et al., 2014). Although the consensus of regulatory agencies and experts have not determined a specific amount of probiotics for beneficial effects (Brasil, 2016; FAO/WHO, 2002; Hill et al., 2014), most studies indicate the ingestion of 106–107 CFU/mL or g of product (Bernini et al., 2016; Dapoigny et al., 2012; Granato et al., 2010; Meng et al., 2016; Shah, 2000). Studies have also shown beneficial effects even in inactivated cells or cellular components (Salminen et al, 2002; Havenaar et al., 1992; Adams, 2010).

Concerning the viability of probiotics, they must be tolerant to the production process and the shelf life, as well as surviving gastrointestinal conditions to maintain a high number of active and viable cells (Vandenplaset al., 2015; Shori, 2016).

In addition, the effects attributed to probiotics are for specific strains; therefore, one species or strain cannot be extrapolated to others, even those that are closely related (Bertazzoni et al., 2013; FAO/WHO, 2002; Gueimonde & Salminen, 2006; Hill et al., 2014).

Microorganisms of the genera *Lactobacillus* sp. and *Bifidobacterium* sp. are the most important commercially (Charteris et al., 1998; Soccol et al., 2010). In particular, *Bifidobacterium* sp. is considered beneficial among the diverse microbiota of the human gastrointestinal tract (GIT) (Briczinski et al., 2009). *B. lactis* HN019™ strain has a long history of resistance to GIT conditions, modulation of the intestinal microbiota, inhibition of enteropathogens, and beneficial effects on health human and animals (Bernini et al., 2016; Liu et al., 2010; Meile et al., 1997; Nagpal et al., 2012; Prasad et al., 1999; Ricoldi, et al, 2017).

There are a few market supplies of dairy products containing probiotics and natural sweeteners such as steviol glycosides from Stevia rebaudiana (Bertoni). Stevia glycosides are low-energy natural sweeteners that can be used by people with diabetes and phenylketonuric patients, as well as by overweight and obese people (Christaki et al., 2013). These compounds present a sweetness that is 250–300 times greater than that of sucrose (Soejarto et al., 1983) and they are stable, which allows application in a variety of food products (Boileau, Fry; Murray, 2012).

In this context, we aimed to develop and characterise a probiotic milk containing *B. lactis* HN019 and sweetener stevia, evaluate the capacity of multiplication, the maintenance of the probiotic in the product, and its resistance in the gastrointestinal simulations *in vitro*. Moreover, probiotic milk was evaluated by preference sensory test and intention to purchase, comparing it to two formulations, a probiotic with added sucrose and the same product non-sweetened.

2. Methodology

2.1 Probiotic milk

The commercial freeze-dried Bifido HOWARU®, *B. lactis* HN019 (Dupont™, Copenhagen, Denmark), stored at -18 °C was used. A total of 10% (w/v) reconstituted skimmed milk powder (Confepar, Londrina, Brazil), was sterilised at 121 °C for 15 min. Subsequently, the milk was cooled to 37 °C. The *B. lactis* HN019™ (Direct Vat Set) was added directly to the milk at a concentration of 0.1% (w/v) at 37 °C for 14 h. The development of *B. lactis* HN019 was monitored every 2 h during 14 h by pH analyse (Tecnal, Tec-5, Piracicaba, Brazil) and titratable acidity expressed as % lactic acid (AOAC, 2016).

For *B. lactis* counts, serial dilutions in 0.1% (w/v) buffered peptone water (Oxoid, Cambridge, UK) were created and plated in de Man, Rogosa, and Sharpe agar (Man, Rogosa and Sharpe-Kasvi, São José dos Pinhais, Brazil) enriched with L-cysteine (Labsynth, Diadema, Brazil), according to the methodology described by Casteele et al. (2006) with modifications (Henrique-Bana et al., 2019). Petri dishes were incubated at 37 °C for 72 h under anaerobiosis (Anaerobac, Probac, Brazil).

Post fermentation, the probiotic milk was cooled (5 °C) and added to 0.05% (w/v) of vanilla flavour (Duas Rodas

Industrial, Jaraguá do Sul, Brazil) and 0.01% (w/v) of stevia sweetener (Linea, Anápolis, Brazil) and packaged aseptically in 80 mL bottles (Inplavel, Joinville, Brazil). The probiotic milk was produced according to the current Brazilian legislation (Brazil, 2001, 2007). The viability of *B. lactis* HN019, pH, and acidity was monitored during 1, 7, 14, 21, 30, 45, and 60 days of cold storage.

2.2 Physical-chemical analyses

The ash, protein and total solid content was determined according to the methodology of AOAC (2016). Lipids were analysed by the gerber method (Case, Bradley, Williams, 1985) and carbohydrates by the difference (Brazil, 2003). All analyses were performed in triplicate from the three independent samples.

2.3 Survival of *Bifidobacterium lactis* HN019 under *in vitro* simulated gastrointestinal conditions

The survival of the probiotic in the fresh product and at 60 days of cold storage (5 °C) was submitted to gastric and enteric simulated conditions (Buriti, Castro, And Saad; 2010). To enumerate the probiotic during the *in vitro* assays, samples were collected at time zero and sequentially after 2, 4, and 6 h from the initial test and the results were presented as log CFU/mL. Additionally, the survival rate was calculated based on the methodology described by Guo et al. (2009).

2.4 Sensory analyses

The study was approved by the Ethical Committee on Human Research of the Universidade Pitágoras Unopar, Brazil (process number 67602217.1.0000.0108)

All were packaged in 50 mL bottles, refrigerated at 5 ± 1 °C, and labelled with random three-digit codes. All the formulations were characterised by microbiological afety as well as the probiotic viability (log CFU/ mL), pH and acidity (% lactic acid) and showed no significant differences between them ($p > 0.05$).

The untrained panellists ($n = 101$) evaluated the three products simultaneously during the preference sorting test. They were instructed to indicate the samples in order of preference as to the most preferred, intermediate and least preferred formulation in relation to the sweetness, acidity and general preference attributes. Already, the intent to purchase test was conducted using the following five-point scale− 5: “definitely buy,” 4: “probably buy,” 3: “may or may not buy,” 2: “probably not buy,” and 1: “definitely not buy.”

2.5 Statistical analysis

Statistical analysis was carried out using SAS 13.0 (Statsoft, Tulsa, OK, USA). The comparisons of differences between the means of the shelf life were tested using analysis of variance at a significance level of $P < 0.05$.

3. Results and Discussion

B. lactis HN019 changed the pH of milk from 6.03 to 5.34 after 14 h of fermentation, whereas the titratable acidity increased from 0.26% to 0.38% (lactic acid). The probiotic milk that was ready for consumption showed high viability of *B. lactis* HN019, with counts of 1.25 × 109 CFU/mL. A titratable acidity lower than 0.6% does not classify it as fermented milk based on current Brazilian and international legislation (Brasil, 2007; IDF, 1991). However, the product can be characterised as a probiotic cultured milk because it contains > 106 CFU/mL of bifidobacteria (Brasil, 2007; IDF, 1988).

Bifidobacteria are used for milk fermentation in a limited way because, although milk is a medium containing essential nutrients, amino acids, and small peptides, it is not a suitable matrix for the growth of this genus, which has no essential proteolytic activity (Gomes et al., 1998; Prasanna; Grandison; Charalampopoulos, 2014). Bifidobacteria are usually used in combination with other lactic acid bacteria for the production of fermented dairy products (Shah, 2000). However, the development of a product containing bacteria that has slow acidification can provide a smooth and delicate product, but with high cellular viability, something that our results revealed. Moreover, the high viability in the product and potential therapeutic value of strains of this genus for human health justify the formulation of such products that have a unique culture.

The time of fermentation was superior to the production of traditional dairy fermentation, whose processes occur in 3.5 to 6 h (Dave & Shah, 1997; Thamer & Penna, 2006). However, 14 h was determined as adequate for the production of the probiotic milk because at this time the product presented a mild and typical flavour of fermented dairy, with these characteristics improving consumer acceptance of the product.

Inefficient lactic acid production causes a prolonged fermentation time, and this can be explained by the typical metabolism of bifidobacteria, which can ferment hexoses with the formation of acetic and lactic acid in the molar ratio of 3:2 (De Vries & Stouthamer, 1967; Scardovi et al., 1971; Sidarenka et al., 2008).

The temperature of industrial fermentation for bifidobacteria is up to 42 °C; however, when the incubation occurs at 37 °C associated with long fermentation times and/or if the pH post-fermentation is greater than 5.0, there is better growth and survival of the bifidobacteria during storage of dairy products (Kailasapathy & Chin, 2000). However, long fermentation times culminate with the appearance of acetic acids and a reduction in sensory acceptance (Henrique-Bana et al., 2019; Rodrigues et al., 2011).

B. lactis HN019® exhibited higher acetate production than that of other bifidobacteria strains under the same temperature (Chick et al., 2001; Østlie et al., 2003). Our research group have observed the metabolism of this strain by the quantification of organic acids by nuclear magnetic resonance spectrometry (Henrique-Bana, et al, 2019).

All the characteristics of the probiotic milk, apart from acidity, were adequate for the current technical regulations (32.0 Kcalories, Table 1) (Brasil, 2000, 2007). The low acidity and mild taste of the probiotic milk differentiated it from the fermented milks that are commercially available, with these features being an attraction for this product. In addition, the non-addition of conventional sugars appeals to a niche market, i.e., people with diabetes, whose diet is restrictive to this ingredient.

Table 1 - Physico-chemical and microbiological composition of probiotic milk.

Composition	Data
Log CFU/mL	9.09 ± 0.11
pH	5.34 ± 0.02
Acidity (% Lactic acid)	0.38 ± 0.01
Protein (%)	3.92 ± 0.07
Fat (%)	0.00 ± 0.00
Carbohydrates (%)	4.08 ± 0.05
Total Solids (%)	8.81 ± 0.11
Ash (%)	0.81 ± 0.01
Energy (Kcal)	32.00± 0.02

Note: The data are expressed as mean ± standard deviation (n = 9).
Source: Authors.

Probiotic milk sweetened with stevia had a lower carbohydrate content (4.08%) compared to sugar-sweetened probiotic milk (7.54%) that was produced by Bernini et al. (2016) using the same strain. The protein content and total solids were directly related to the composition of the raw material to the probiotic milk production. According to current legislation, a minimum of 2.9 g of protein/100 g is recommended; therefore, the product is suitable. The energy content was reduced by 53.04 and 32.21% compared to commercial fermented milk and sugar-sweetened probiotic milk used in this work, respectively. Thus, the product is suitable for the light category, as the energy value has been reduced by at least 25% as required by law (Brasil, 2012). Related to the composition, the product presented conformity with Brazilian and Mercosul legislation (Brasil, 2000; 2007). Pathogens were not detected (Brasil, 2019), thus the product was characterised as suitable for consumption.

Over its shelf life, the product presented high viability and survival rate of the HN019® strain, as well as low post-acidification during cold storage (Table 2). These are positive characteristics. The probiotic viability was reduced in only one cycle log during the 60 days of storage. High probiotic counts during the shelf life of a product are required to maintain viable cells at the end of the digestive process.

Although Brazilian and/or international legislation does not determine the number of probiotics in foods to promote beneficial health (Brasil, 2018; FAO/WHO, 2001; Hill, et al., 2014), scientific studies have revealed a consensus that the beneficial effects observed are strain-dependent. Many published data indicate a minimum number of viable probiotic should be in the range of 10^6 to 10^8 CFU/mL or g of product (Bernini et al., 2016; Dapoigny et al., 2012; Hill, et al., 2014; Meng et al. 2016; Shah, 2000).

The pH decreased from 5.3 to 4.98 after 60 days, whereas the titratable acidity ranged from 0.38% to 0.44% (Table 2). The post-acidification was quite discreet during storage. Although lactic acid production by bifidobacteria is lower than that of traditional lactic acid bacteria, they can also use rebaudiosides and steviol glycosides of *S. rebaudiana* as a source of carbon, yet, in a minor extent in relation to glucose metabolism (Gardana et al., 2003; Kunová et al., 2014; Lopes et al., 2017).

We evaluated the samples stored in glass bottles under refrigeration (5 °C) for 120 days, that showed viability of 8.39 log CFU/mL, pH of 4.98, and acidity of 0.51% (v/v). Therefore, there was little difference when compared to the product stored in the plastic bottles at 60 days. The product in the glass containers or thicker plastic containers might increase the survival and viability of bifidobacteria due to oxygen impermeablility (Klaver et al., 1993; Shah, 2000). In addition, the high survival rate of the strain in the product after a long shelf life is an important result, regardless of the type of packaging used.

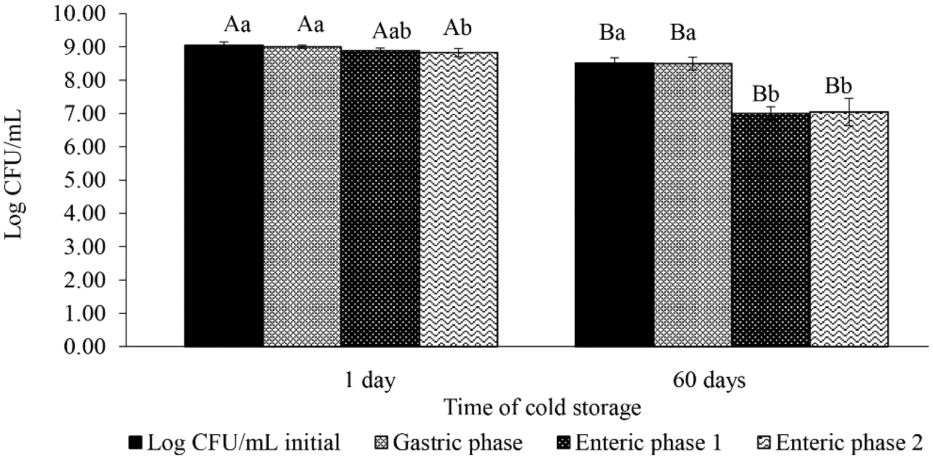
Table 2 - Viability of *B. lactis* HN019, pH and titrable acidity of the probiotic milk during the shelf life refrigerated (5±1°C).

Storage (days)	**Viability (Log CFU/mL)**	**pH**	**Acidity (% lactic acid)**
1	9.04 ± 0.13^{a}	5.4 ± 0.02^{a}	0.38 ± 0.02^{b}
7	8.99 ± 0.06^{a}	5.23 ± 0.02^{b}	0.35 ± 0.01^{c}
14	8.87 ± 0.11^{ab}	5.19 ± 0.01^{cd}	0.39 ± 0.02^{b}
21	8.82 ± 0.17^{ab}	5.21 ± 0.01^{bc}	0.35 ± 0.02^{c}
30	8.79 ± 0.19^{ab}	5.17 ± 0.01^{de}	0.35 ± 0.01^{c}
45	8.83 ± 0.09^{ab}	5.15 ± 0.02^{e}	0.32 ± 0.01^{d}
60	8.51 ± 0.17^{b}	4.98 ± 0.02^{f}	0.44 ± 0.02^{a}

Note: The data are expressed as mean ± standard deviation (n = 9). Values followed by different letters on the same columns, indicates significant differences over shelf life, by the Tukey test ($p<0.05$). Source: Authors.

Related to the survival of *B. lactis* HN019 in the probiotic milk (Figure 1), there was no significant reduction in HN019® populations ($p > 0.05$) in any of the three phases of the GIT simulatio. In the product stored for 60 days, the survival of HN019 remained in the product without variation until the gastric phase ($p > 0.05$). However, there was a significant reduction ($p \leq 0.05$) in enteric phases 1 and 2, with the level of reduction being 1.89 log CFU/mL and 1.79 log CFU/mL after 4 and 6 h of simulation, respectively.

Figure 1 – Survival of *B. lactis* HN019 (Log CFU / mL) subjected to *in vitro* simulated gastrointestinal conditions, evaluated in probiotic milk with 1 and 60 days of storage at 5°C.



Means with different capital letters (A, B) in the same bars denote a significant difference between different storage periods in the same phases of the *in vitro* assay by the Tukey test (p<0.05). Means with different small letters (a, b) in the different bars at the same storage day are significantly different between the phases of the *in vitro* assay by the Tukey test (p<0.05). Source: Authors.

There were no significant changes in the HN019® populations after the end of the gastric phase (2 h) during storage for 60 days (p > 0.05). Therefore, there was high resistance of the HN019 strain to the GIT conditions. Some bifidobacteria strains demonstrate reduced viability when exposed to low pH (Presti et al., 2015).

The maintenance at viability of *B. lactis* higher than 10^7 CFU/mL in the probiotic milk strain produced and stored for a long shelf life can be mediated by the protective effect of pepsin during exposure to low pH, by decreasing the hyperpolarisation of cells (Mattö et al., 2006), which is associated with the enzymatic ability of *F0F1-ATPase* regulating intracellular pH in bacteria facilitating anaerobes under acidic conditions (Ferrandiz, et al, 2002; Mattö et al., 2006; Sanchez et al., 2007).

Although there was a significant decrease in the viability of *B. lactis* HN019 (p < 0.05) when comparing gastric conditions to enteric phases, the viable cell count remained above 7 log CFU/mL for the evaluated storage periods. The survival of *Bifidobacterium* sp. in the bile salts of the intestine has been associated with exopolysaccharides production (Leivers et al., 2011), as has the ability of the genus to improve intrinsic tolerance via strategies of adaptation throughout the GIT (Collado; Sanz, 2007; Sanchez et al., 2013). However, tolerance to bile is strain-dependent (Liu et al., 2007; Mehdi et al., 2015).

At the end of the *in vitro* (6 h) simulation, the viability of the HN019® strain was 8.86 and 7.4 log CFU/mL in the probiotic milk with 1 and 60 days of cold storage, respectively. Our results differed from those of Bogsan et al. (2013) in fermented milk using the same strain and stored for 7 days, whose data was approximately 9 and 5 log CFU/mL at initial and final *in vitro* GIT simulation.

The probiotic showed high resistance to gastric acids and bile salts and the survival rate of the product refrigerated for 1 day after production, after the gastric phase, was 98%. Refrigerated milk stored for 60 days presented a survival rate of 83% compared to the initial count (black column). The choice of food matrix is very important because of the protection that its components exert on probiotic microorganisms along the GIT (Ranadheera et al, 2010).

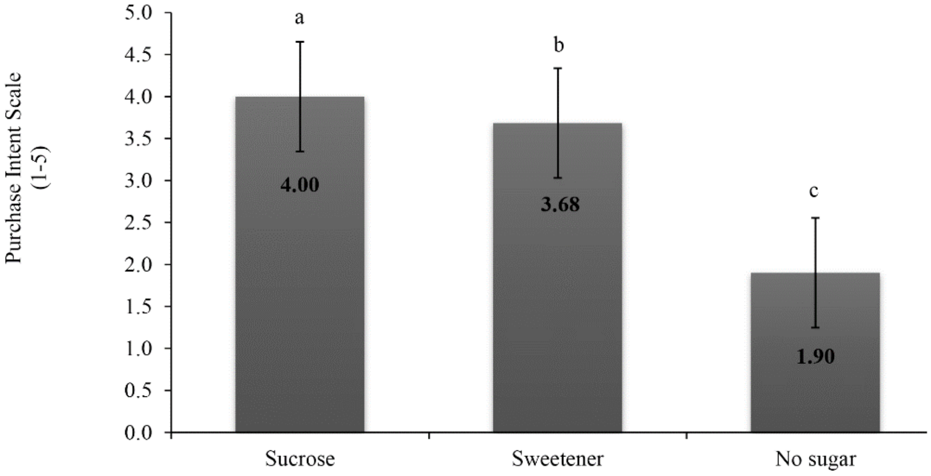
Although milk is not a suitable matrix for the growth of genus *Bifidobacteria* because of the lack of essential proteolytic activity (Prasanna et al., 2014), here, we observed high multiplication and maintenance of the strain used. The presence of a sweetener from *S. rebaudiana* might have contributed to the high survival rate because it can be metabolised by the beneficial microbiota (Lopes et al., 2017; Oliveira et al., 2011). In contrast, the ability to hydrolyse stevioside and rebaudioside A is unusual for the genus (Gardana et al., 2003; Kunová et al., 2014). Lastly, the maintenance of viability can be attributed to the protective effect of the milk protein and stevia compounds (Charteris et al., 1998; Capela et al., 2006).

During the production of probiotic beverages, one of the main requirements is the ability of the microorganisms to maintain their viability during product storage. However, sensorial evaluation is very important because it establishes a direct association with product quality, processing characteristics, and acceptability for consumption (Shori, 2016).

We evaluated the perception of the sensory panellists regarding the consumption of fermented milk containing sugar, sweetener, or without sugar. The three formulations did not present significant differences in pH and titratable acidity ($p > 0.05$), evidencing that the metabolic activity of the strain was similar and stable in the milk matrix. For the preference sorting test, the sample with 4% (m/v) sucrose was considered less acidic and the preferred option of the assessors in terms of sweetness and general preference ($p < 0.05$) followed by the probiotic milk with sweetener and no sugar. Most of the panellists indicated a preference for a sweeter and less acidic taste, demonstrating that sweetness masks the sensorial acidity, since the percentage of lactic acid in the formulations did not show any difference among them ($p > 0.05$). Therefore, the low stevia concentration (0.01%) could be responsible for the intermediate preference. Agarwal, Kochhar and Sachdeva (2010) demonstrated that people with diabetes attributed a high acceptance of dairy products fermented with 0.25% stevia. Additionally, the panellists were people whose diet is not restricted to sugars, that is, they are not used to consuming sweeteners.

Related to the intent to purchase (Figure 2), the scores (1 to 5) indicate that the panellists would definitely buy the product with sugar, maybe purchase the sweetener product, whereas they would definitely not purchase the no sugar formulation.

Figura 2 – Intention to purchase probiotic milk with sugar, sweetener and no sugar based on the results from the sensory evaluation.



Means with different letters denote a significant difference between the fermented milks by the Tukey test ($p<0.05$). Source: Authors.

4. Conclusion

The product can be characterised as a light probiotic skim milk. The HN019 strain had a low acid production, however, it multiplied and remained at high levels in the product for more than 60 days of shelf life under refrigerated storage.

The sensorial acceptance of the probiotic milk was well accepted, thus being an alternative to the dairy market, because it is free from fats, has a low caloric value, and has added probiotic. The *B. lactis* HN019 presented satisfactory cell viability in storage and capacity to overcome, at least *in vitro*, the physiological barriers found during digestion. Thus, the probiotic milk can be targeted at those consumers whose calorie-restricted diets, as well as consumers who value food that may have beneficial effects on their health.

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