Universidade Estadual Paulista
"Júlio de Mesquita Filho"

Faculdade de Ciências Farmaceuticas

Desenvolvimento de iogurte *skyr* simbiótico por diferentes processos de produção e avaliação na microbiota intestinal

Fernanda Campos Freire

Tese apresentada ao Programa de Pós-graduação em Alimentos e Nutrição para obtenção do título de Doutora em Alimentos e Nutrição.

Área de concentração: Ciência Nutricionais

Orientadora: Profa. Dra. Katia Sivieri

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RESUMO

Durante a última década, o interesse por alimentos nutritivos e funcionais aumentou significativamente, tanto pelos consumidores quanto pelas indústrias alimentícias. Dessa forma, pesquisas que comprovem os efeitos benéficos para a saúde advindos do consumo destes produtos tem se tornado essenciais. Atualmente, os produtos lácteos probióticos constituem os principais alimentos funcionais comercialmente disponíveis, particularmente os iogurtes. O consumo de iogurte tornou-se mundialmente popular devido aos seus efeitos benéficos à saúde. Existem diversos tipos de iogurtes no mercado mundial que divergem quanto a composição, sabor, consistência e valor calórico. Essas características variam de acordo com a matéria-prima e cultura utilizada, bem como com o método empregado no processo de produção. Neste sentido, o iogurte tipo *skyr* é um produto tradicional da Islândia e, atualmente, é reconhecido pelo alto teor de proteínas e reduzido teor de gorduras, quando comparado aos outros tipos de iogurtes. A utilização da tecnologia de ultrafiltração no processo de produção de iogurtes tem se tornado uma alternativa bastante interessante e atrativa para a indústria de laticínios, uma vez que proporciona uma alta qualidade ao produto, do ponto de vista microbiológico e nutricional, como também otimiza algumas etapas do processo de produção. O objetivo deste trabalho foi desenvolver iogurtes tipo *skyr* probiótico e simbiótico (adicionado do prebiótico frutooligossacarídeo - FOS) por meio da filtração em tecido, bem como pela tecnologia de ultrafiltração, e investigar seus efeitos sobre a composição e metabolismo da microbiota intestinal de indivíduos saudáveis, utilizando o modelo *in vitro* Simulador do Ecossistema Microbiano Humano (SEMH®). O capítulo 1 apresenta os resultados referentes ao processo de produção dos iogurtes. Já o capítulo 2 apresenta os resultados referentes ao efeito da administração dos iogurtes na composição e metabolismo da microbiota intestinal no SEMH®. Nossos resultados sugerem que ambos os processos foram eficientes para produzir iogurtes com alto teor de proteína. Os iogurtes tipo *skyr* probiótico e simbiótico permaneceram estáveis durante os 28 dias de armazenamento refrigerado e mostraram ser uma boa matriz para carrear o microrganismo

probiótico e o ingrediente prebiótico. Ainda, a administração dos iogurtes probiótico e simbiótico proporcionou um efeito positivo na microbiota intestinal. Ambos os iogurtes alteraram a composição e melhoraram o metabolismo bacteriano no SEMH®. Dessa forma, os iogurtes tipo *skyr* probiótico e simbiótico podem ser considerados alimentos funcionais e podem oferecer uma nova perspectiva para a produção de alimentos com potenciais efeitos benéficos à saúde.

Palavras-chave: *skyr*, iogurte hiperproteico, probiótico, simbiótico, ultrafiltração, filtração em tecido, microbiota intestinal.

ABSTRACT

During the last decade, interest in nutritious and functional foods has increased significantly, both by consumers and by the food industry. Thus, research to prove the health benefits of consuming these products has become essential. Currently, probiotic dairy products are the main commercially available functional foods, particularly yogurts. Yogurt consumption has become popular worldwide for its health benefits. There are several types of yogurts on the world market that differ in composition, taste, consistency, and caloric value. These characteristics vary according to the raw material and culture used, as well as the method employed in the production process. In this sense, skyr yogurt is a traditional Icelandic product and is now recognized for its high protein and low-fat content when compared to other types of yogurts. The use of ultrafiltration technology in the yogurt production process has become a very interesting and attractive alternative for the dairy industry, since it provides a high-quality product, from the microbiological and nutritional point of view, and optimizes some steps of the production process. The objective of this study was to develop probiotic and synbiotic skyr yogurts (with the addition of the prebiotic fructooligosaccharide - FOS) by cloth filtration, as well as by ultrafiltration, and to investigate their effects on the composition and metabolism of the intestinal microbiota of healthy individuals, using the *in vitro* Simulator of Human Intestinal Microbial Ecosystem (SHIME®) model. Chapter 1 presents the results concerning the skyr-type yogurts production process. Chapter 2 presents the results concerning the effect of skyr-type yogurts on the composition and metabolism of the gut microbiota in the SHIME®. Our results suggest that both processes were efficient to produce skyr-types yogurts with high-protein content. All formulations remained stable during the 28 days of refrigerated storage and showed to be a good matrix for carrying the probiotic microorganism and prebiotic ingredient. Also, the administration of high-protein yogurts (probiotic and synbiotic skyr) provided a positive effect on the intestinal microbiota. Probiotic and synbiotic skyr yogurts altered the composition and improved bacterial metabolism in SHIME® model. Probiotic

and synbiotic skyr yogurts can be considered functional foods and may offer a new perspective to produce foods with potential beneficial effects on health.

Keywords: skyr, high-protein yogurt, probiotic, synbiotic, ultrafiltration, cloth filtration, gut microbiota.

SUMÁRIO

INTRODUÇÃO

Atualmente, os produtos lácteos constituem os principais alimentos funcionais comercialmente disponíveis, em especial os iogurtes. O iogurte é produzido através da fermentação do leite pasteurizado por bactérias lácteas específicas, como *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus* (1). O consumo de iogurte tornou-se mundialmente popular devido aos seus diversos efeitos benéficos à saúde. É reconhecido pelos consumidores como um alimento saudável, uma vez que contém microrganismos vivos e ativos que promovem a saúde intestinal. Ainda, é rico em nutrientes, possui um excelente perfil de proteínas, carboidratos, cálcio, fósforo e vitaminas (2).

Os iogurtes com alto teor de proteínas são considerados uma nova tendência no mercado, não só pela palatabilidade, como também pelo alto valor nutritivo (3). A procura por produtos deste segmento está em constante crescimento devido a seus diversos efeitos benéficos, como ganho de massa magra, ação imunomoduladora e ação anti-hipertensiva (4,5).

As proteínas do leite, além dos benefícios nutricionais, também são bastante utilizadas pela indústria alimentícia na busca por melhorias nos produtos processados, nos quesitos palatabilidade, textura e rendimento (6,7). O teor de proteínas nesses produtos pode ser aumentado antes do processo de fermentação através da adição de leite em pó, ou após, por meio da filtração, separação mecânica ou separação por membranas (8,9). O iogurte tipo *skyr* é um produto tradicional da Islândia e, atualmente, é reconhecido pelo alto teor de proteínas e reduzido teor de gorduras, quando comparado a

outros tipos de iogurtes disponíveis (10). Essa categoria de iogurtes está disponível em todo o mundo e a nomenclatura dos produtos varia de acordo com a origem: *Labneh* (Mediterrâneo Oriental), *Torba* (Turquia), *Stragisto* (Grécia), *Chakka*, (Índia) e *Ymer* (Dinamarca) (11).

Tradicionalmente, o *skyr* era obtido através da fermentação do leite desnatado por bactérias produtoras de ácido lático, seguido pela dessoragem do coalho através de um tecido (11). A produção do *skyr* sofreu intensas modificações nos últimos anos, especialmente na etapa de dessoragem, e atualmente esta etapa vem sendo substituída pelo processo de centrifugação (centrífuga tipo Quark) ou pela tecnologia de ultrafiltração (UF) (3,8,12). A UF tem se tornado bastante interessante e atrativa quando comparada a outros tipos de processos convencionais (decantação, filtração e centrifugação), uma vez que proporciona alta qualidade ao alimento processado, do ponto de vista microbiológico e nutricional, e atua na simplificação do fluxo de processo, otimizando algumas etapas da produção (3,9,13). A UF vem sendo utilizada a fim melhorar a composição nutricional do produto, já que proporciona o aproveitamento das propriedades técnicas das proteínas (solubilidade, emulsificação, cremosidade e viscosidade) e minimiza a perda da caseína no soro (14). Ainda, quando aplicada antes do processo de fermentação evita a produção de soro ácido, um resíduo problemático para a indústria de laticínios (15).

A adição de ingredientes funcionais em iogurtes também tem sido adotada pelas indústrias alimentícias, já que a matriz tecnológica desses produtos permite o enriquecimento com diversos compostos, diversificando assim as

apresentações comerciais e ampliando o público consumidor (4,16–18). A incorporação de ingredientes funcionais é considerada uma abordagem terapêutica interessante para a promoção da saúde.

Um dos recursos mais utilizados pela indústria alimentícia é a adição de bactérias probióticas (19). Já é bem fundamentado que a utilização dessas bactérias contribui a favor da modulação das funções fisiológicas dos indivíduos, uma vez que promove resistência gastrintestinal à colonização por patógenos, estimula o sistema imune, sintetiza vitaminas, aumenta a absorção de minerais e previne o risco de desenvolvimento de câncer de cólon (20–24). As principais cepas probióticas empregadas no desenvolvimento de iogurtes são as pertencentes aos gêneros *Lactobacillus* spp e *Bifidobacterium* spp (19) .

Ainda, a adição de fibras prebióticas também é considerada uma inovação nos produtos deste segmento. Os prebióticos são classificados como ingredientes alimentares não digeríveis e são fermentados de maneira seletiva no cólon. São responsáveis por contribuir com a proliferação de bactérias benéficas, regularizar a função intestinal (volume, consistência e frequência das fezes), aumentar a absorção de minerais e melhorar o sistema imune (25,26).

Uma das principais terapias utilizadas para modular o ecossistema intestinal é a utilização de microrganismos probióticos e fibras prebióticas (26,27). Atualmente, diversos estudos abordam o papel da microbiota no estado de saúde e como alterações na diversidade microbiana (disbiose) podem afetar diretamente o metabolismo e a resposta imune do hospedeiro,

contribuindo para a patogênese de doenças inflamatórias, autoimunes e comportamentais, como obesidade, diabetes, doenças inflamatórias intestinais, asma e distúrbios do sistema nervoso central (26,28–33).

Diante da importância da disbiose na gênese de diferentes doenças e o seu impacto no estado de saúde, houve um crescente interesse em estratégias alimentares que contribuam para a modulação do ecossistema intestinal (34–36). Neste sentido, estudos sobre a microbiota intestinal podem ser realizados utilizando modelos *in vivo* e *in vitro*. Em modelos *in vivo*, as abordagens são mais representativas, uma vez que os parâmetros e as interações fisiológicas com o organismo hospedeiro são levados em conta. Contudo, a composição da população microbiana nas diferentes regiões do cólon não pode ser observada, visto que somente a microbiota fecal é analisada (37,38).

Já os modelos *in vitro* são capazes de fornecer informações sobre as etapas do processo de fermentação nas diferentes regiões do cólon humano. São úteis para investigar a microbiota intestinal, bem como seus metabólitos, além de proporcionar resultados com elevada reprodutibilidade (39–42). Dessa forma, o Simulador do Ecossistema Microbiano Humano (SEMH®) consiste em um modelo dinâmico do trato gastrointestinal humano, composto por cinco reatores conectados que representam o estômago, o duodeno e os cólons ascendente, transverso e descendente, com seus respectivos valores de pH, tempo de residência, temperatura e capacidade volumétrica (43). Atualmente este modelo tem sido bastante utilizado em estudos

experimentais, uma vez que permite analisar a composição e a atividade da população microbiana do intestino (39,40,44–46).

Dessa forma, o desenvolvimento de iogurtes tipo *skyr* probiótico e simbiótico (adicionado do prebiótico frutooligossacarídeo) pode contribuir para o surgimento de um novo produto funcional. A proposta torna-se também interessante em função da inexistência no mercado brasileiro de um iogurte tipo *skyr* adicionado de prebiótico e que utilize em seu processo de produção a tecnologia de ultrafiltração. A figura 1 apresenta o protocolo experimental realizado neste estudo.

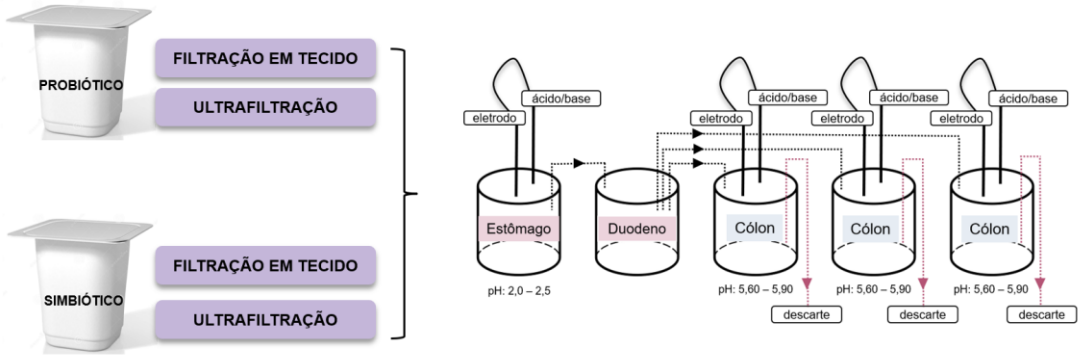

Figura 1. Representação esquemática do experimento realizado neste estudo.

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Capítulo 1.

Synbiotic skyr: an alternative of functional product

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SYNBIOTIC SKYR: AN ALTERNATIVE OF FUNCTIONAL PRODUCT

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Abstract

The aim of this study was to develop a synbiotic skyr-type yogurt using two manufacturing processes, cloth filtration and ultrafiltration, and to evaluate the physicochemical, microbiological, and sensorial characteristics during 28 days of refrigerated storage. Four formulations were produced: probiotic skyr (SP and SP-UF) and synbiotic skyr (SS and SS-UF). Fructooligosaccharide (5%) was added only in SS and SS-UF skyr-types. The results demonstrated that both processes were efficient to produce skyr-types yogurts with high-protein content. The addition of FOS increased the total solid contents in SS and SS-UF skyr-types. The skyr-type yogurts stability regarding pH, acidity and syneresis indicated a suitability of the skyr matrix and the storage for 28 days. B. lactis Bb-12 reached viable counts of at least 7 log CFU g^{-1} in all skyr-types during the storage, which indicates the good stability of this probiotic strain in skyr matrix. In *in vitro* digestion assay, B. lactis Bb-12 showed a decrease in survival during the intestinal phase. In the sensory evaluation, CATA data pointed that SP and SS presented favorable attributes expected in fermented products. All formulations remained stable during the 28 days of refrigerated storage and showed to be a good matrix for carrying the probiotic microorganism and prebiotic ingredient.

Keywords: functional food, skyr, synbiotic, ultrafiltration, cloth filtration.

1. Introduction

High-protein yogurt has acquired increased consumer interest over last years, partly driven by improvements in organoleptic characteristics but also on dairy protein health benefits, such as satiety and as a trigger for muscle protein synthesis (Jørgensen et al., 2019).This category of yogurts is available around the world, which are named differently according to their origin, such as Labneh (Eastern Mediterranean), Torba (Turkey), Stragisto (Greece), Chakka, (India), Ymer (Denmark), and Skyr (Iceland) (Tamime et al., 2014).

Nowadays, skyr has been recognized for its smooth taste, high-protein content, and reduced fat. It's considered a versatile food, since it can be consumed by itself or used as a base for sweet or salty preparations. The differential of skyr yogurt is the texture, which is more consistent and creamier when compared to the traditional yogurts. Skyr is produced by coagulation of skim milk proteins through a combined action of lactic acid bacteria, with a small quantity of rennet. Traditionally, skyr is produced from an earlier batch as a starter and separation of curds and whey is made by cloth filtration (Litopoulou-Tzanetaki & Tzanetakis, 2014). In recent years, skyr production has undergone several modifications. At present, the process is accurately controlled using standardized raw material, including pure starter cultures, and by others drainage processes. Among these processes, quarg-separators and ultrafiltration (UF) have raised the interest in dairy industry for obtaining products with distinct nutritional and sensorial characteristics (Jørgensen et al., 2019). Quarg-separators process promoted a higher protein yield of the final product, however a large part of the whey proteins was still lost with the whey (Moineau-Jean et al., 2020). Quarg-separators are still in use, but today most of the production is performed by UF. This technique utilizes a crossflow membrane in which the feed solution is forced through the membrane under pressure. The solution flows over the membrane and solids are retained (retentate) while the removed materials are present in the permeate. The use of membrane processing in the cultured dairy products area is restricted to concentration of skim milk for fat-free yogurt manufacture (Chandan et al., 2017). The UF process results in a higher protein yield, since the loss of casein

is minimized, and provides the use of the technical properties of proteins such as solubility, emulsification, and gelation (Leu et al., 2017). In additional, concentration of milk by UF prior to fermentation prevents the production of acid whey, a major problem for the dairy industry (Smithers, 2015).

As the popularity of yoghurts in this segment continues to grow, researchers are increasingly investigating the added value of ingredients such as probiotics and prebiotics. Commercial interest in functional foods containing pro and prebiotics has increased due of the benefits for gut health and disease prevention (Chapman et al., 2011; Cimperman et al., 2011; Rook & Brunet, 2005). Probiotics are living microorganisms, which when administered in adequate amounts, confer health benefits to their host (Hill et al., 2014). In this context, bifidobacteria, especially *Bifidobacterium animalis* subsp*. lactis* Bb-12®, are commonly used strains in yogurts manufacture because of their well-documented health-promoting properties (Vinderola et al., 2011). Bb-12® improve bowel function, have a protective effect against diarrhea, reduce side effects of antibiotic treatment, and increases the body's resistance to common respiratory infections (Jungersen et al., 2014). One method to enrich bifidobacteria in food matrix (increase the number of viable cells and enhance their metabolic activity) is through the addition of prebiotic ingredients (Barrangou et al., 2006; Van der Meulen et al., 2006). Prebiotics are food components that confer health benefits on the host associated with the modulation of the gut microbiota (Gibson et al., 2017). In this sense, fructooligosaccharide (FOS) is non-digestible fructan frequently used as a functional food ingredient that offers interesting nutritional properties and important technological benefits (Tabasco et al., 2014). The synergistic combination of prebiotics and probiotics is known as synbiotic. Various combinations of synbiotics showed therapeutic effects against diseases, like gastrointestinal diseases, respiratory infections, hypercholesterolemia, atopic dermatitis, allergy, diabetes, liver diseases and cancer (Markowiak & Ślizewska, 2017; Vitali et al., 2010). No prior studies reported the development of a synbiotic skyr-type yogurt. Therefore, the present study aimed to develop a synbiotic skyr-type yogurt using two alternative manufacturing processes:

cloth filtration and ultrafiltration, and to evaluate the physicochemical, microbiological, and sensorial characteristics during 28 days of refrigerated storage.

2. Materials and methods

2.1. Skyr-type yogurts preparation

Skyr-type yogurts were produced at Department of Food and Nutrition in São Paulo State University (UNESP), Brazil. Two independent batches were performed using the processes described below.

2.1.1. Cloth filtration

Two formulations were produced: probiotic skyr (SP) and synbiotic skyr (SS). Skim milk was used for the preparation of yogurts. Fructooligosaccharide (5%) (Focus Flora FOS®, Brazil) was added only in SS formulation. Next, the milk was heated to 65 °C for 30 minutes and then cooled to 43 °C for the inoculation of the Icelandic probiotic culture SKYR 1.0 (0,03%) (Chr. Hansen®), which contain *Bifidobacterium animalis* subsp*. lactis* Bb-12®, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* StCth-52. The formulations were fermented in a BOD incubation chamber (MA1415/780, Marconi®) at 43 ºC until a pH of approximately 4.8 was reached. Then, the yogurts were cooled to 5 °C for 24 hours. Whey drainage was carried out using a cloth bag for a period of 6 hours under refrigeration. The final products were packed in high-density polyethylene bottles and stored at 5 ± 1 °C for 28 days.

2.1.2. Ultrafiltration process (UF)

Two formulations were produced: probiotic skyr (SP-UF) and synbiotic skyr (SS-UF). Skim milk was submitted to UF process. The process was carried out in a pilot scale UF-system (T.I.A Brazil, Brazil) with a single membrane cartridge. The filter used was a polyethersulfone UF membrane (Koch Membrane System, USA) with a molecular mass cut-off of 10 kDa and 0.32 m^2 of membrane area. The UF was performed at transmembrane

pressure of 5 bar and 18 ◦C. The milk was concentrated in batch mode, i.e., the retentate was recycled back and permeate was continuously removed until a theoretical concentration factor of 3.0 was reached. Next, FOS (5%) (Focus Flora FOS®, Brazil) was added only in SS-UF formulation. The fermentation and packaging occurred under the same conditions previously described in item 2.1.1.

2.1.2.1 Permeation flow during UF

Permeation flow rate was measured every 15 minutes by weighing permeate collected during 1 minute until the desired concentration factor was achieved. Flow values were calculated as in equation: J= Fp/ A x t, where Fp is the permeate flow rate in L, A is the membrane area in m^2 and t is the time in hours.

2.1.2.2 Rejection coefficient

The rejection coefficients (σ) of the membrane for protein was calculated as in equation: σ = 1−Cp/Cr, where Cp and Cr are the component's concentration in the permeate and retentate, respectively.

2.1.3. Centesimal composition

The centesimal composition was performed for all skyr-type yogurts. Moisture, ash, protein, fat and dietary fiber were determined using the methods proposed by the AOAC (2005). All analyses were run in triplicate.

2.2. Evaluation of skyr-type yogurts during refrigerated storage

All analyses were performed after processing (day 1), 14 and 28 days of refrigerated storage at 5 ±1 °C.

2.2.1. pH and titratable acidity

pH values of the skyr-type yogurts were measured using a digital pH meter. The pH was determined by inserting an electrode directly into a homogenized sample. Titratable acidity was determined by titration with 0.1 N

NaOH, until reaching the pale pink endpoint with phenolphthalein indicator. The results were expressed in lactic acid g 100 g^{-1}. The analyses were run in triplicate.

2.2.2. Viability of strains

One mL of skyr-type yogurts samples was suspended in 9 mL of sterile peptone water and serial dilutions were carried out. *B. animalis* subsp*. lactis* Bb-12® was plated in MRS agar with sodium propionate (3 g L^{-1}) (Sigma-Aldrich®, USA) and lithium chloride (2 g L^{-1}) (Merck®, Germany). The plates were incubated in anaerobiose (Probac, Brazil) at 37 ºC for 72 h (Cardarelli et al., 2008). *L. delbrueckii* subsp. *bulgaricus* was plated in acidified MRS agar (Merck®, Germany) and incubated in anaerobiose (Probac, Brazil) at 37 ºC for 72 h (Cardarelli et al., 2008). *S. thermophilus* was plated in M17 agar (Oxoid®, United Kingdom) containing lactose (5.0 g L^{-1}) and incubated in aerobioses at 37 °C for 48 h (Oliveira et al., 2001). The results were expressed as log CFU g^{-1}.

2.2.3. Syneresis

Syneresis of the skyr-type yogurts was evaluated according to the methodology proposed by (Keogh, 1998). 30 g of the skyr-type yogurts was centrifuged at 222 x g for 10 minutes at 4 °C. The supernatant was weighed and recorded as syneresis (%).

2.2.4. Sensorial analysis: Check-all-that-apply (CATA)

One hundred consumers were recruited for completing the CATA, both men and women, all being consumers of dairy products. The detection of *Salmonella* spp. and Coliforms at 45 ºC were performed immediately after the preparation of all skyr-type yogurts. All skyr-type yogurts were used in the test. Before the start of the analysis, a simplified explanation was given to each consumer about the method. The CATA evaluation was performed according to the methodology described by (Ares et al., 2014) The samples were presented monadically in disposable cups coded with three digits, at 10 °C.

The consumers were asked to indicate all the terms that they considered appropriate to describe the sample from a list containing 50 words.

2.2.5. *In vitro* digestion

In vitro digestion was carried out according to the standardized INFOGEST protocol (Minekus et al., 2014). For the oral phase, 5 g of the skyr-type yogurts was mixed with 3.5 mL of simulated salivary fluid, 25 µL of 0.3 M $CaCl_2$, and 975 µL of distilled water. Then, the mixture was incubated for 2 minutes at 37 °C in a water bath under constant stirring at 100 rpm. For the gastric phase, the mixture was added with 7.5 mL of simulated gastric fluid (SGF), 5 µL of 0.3 M $CaCl_2$, 200 µL of 1 M HCl (to adjust the pH to 3), 695 µL of distilled water, and 1.6 mL of porcine pepsin solution (Sigma P6887) made up in SGF. The mixture was incubated for 2 h at 37 °C under stirring at 100 rpm. In the intestinal phase, the mixture was added with 11 mL of simulated intestinal fluid (SIF), 40 µL of 0.3 M $CaCl_2$, 0,15 µL of 1 M NaOH (to adjust the pH to 7), 1,31 mL of distilled water, 2,5 mL of fresh bile (160 mM) (Sigma B3883), and 5.0 mL of a pancreatin solution 800 U mL^{-1} made up in SIF. The solution was incubated for 2 h at 37 °C under stirring at 100 rpm. Samples were taken during each stage of digestion for microbiological analysis. Strains were counted by pour-plating method previously described in item 2.2.2.

2.3. Statistical analysis

The results of physicochemical and microbiological analysis of all skyr-types yogurts were evaluated by analysis of variance (ANOVA) and Tukey test with 5% of significance. CATA data were tabulated as 50 columns (49 corresponding to the attributes and 1 of the global acceptance) with 4 data lines for each assessor, corresponding to each of the four samples. The data related to the sensorial attributes were tabulated in a binary form, being assigned 1 when the attribute was mentioned and 0 when it was absent. When the effects were significant, the differences were calculated by ANOVA using the Cochran Q test. It was also performed analysis of multiple comparisons

paired with the Marascuilo test, analysis of correspondence to the attributes through the chisquare distances, besides a principal component analysis (PCA) applied to the correlation coefficients relative to acceptance.

3. Results and discussion

3.1. Cloth filtration x UF process

UF process

Two independent batches were performed for SP-UF and SS-UF skyr-types production. Skim milk permeate flow showed a decrease in function of time (at 5 bar pressure and 18 ± 1 °C). The process lasted ~ 4 hours for SP-UF and SS-UF production (Figure 1), being the initial permeation rate 9.74/ 10.04 and 9.25/ 9.50 L m^{-2} h^{-1} and final permeation rate 3.52/ 3.43 and 3.45/ 3.47 L m^{-2} h^{-1}, respectively. The permeate flow decrease during 4 hours of the process, however the values remained similar in both processes. This performance can be attributed to the protein molecules deposited on the membrane surface, which cause concentration polarization and fouling layer formation. Concentration polarization is defined as the accumulation of particles at a membrane surface, while fouling results from protein adsorption and protein-protein interactions on the membrane surface and in membrane pores. It is known that an increase on milk concentration influences density, viscosity, and mass diffusivity of the solution, leading to a decrease in permeate flow (Ho & Zydney, 2002). However, it is important to highlight that the flow reduction was expected and not very intense, since all processes were carried out at 18 °C. According to Ng et al., (2018), the high flow decline can be associated with the low temperature process.

One way to evaluate the performance of the UF process, in addition to evaluating the protein content in retentate and permeate samples, is through the membrane rejection rate used (Asbi & Cheryan, 1992). In this study, the membrane showed a good rate of protein rejection for SP-UF and SS-UF UF milks, 98.3% and 97.3%, respectively. Table 1 shows the protein content found in milk samples, permeate and retentate. According to the results, it can be

observed that the UF process has concentrated approximately 3.5 x the milk proteins, which is close to the theoretical target concentration factor of 3.0.

Fermentation

pH values decreased ($p \leq 0.05$) after 2 hours of fermentation for all skyr-type yogurts (Figure 2). The fermentation time required to achieve pH values close to 4.8 was 4 hours for SP and SS. However, for SP-UF and SS-UF the time required was 6 hours. This time extension may be attributed to buffering capacity of the micellar casein, which was concentrated by UF. The casein micelle releases colloidal calcium phosphate upon acidification counteracting the pH change. Studies suggest that physical properties of casein micelles are affected when concentrating milk protein by UF, especially at high volume reductions (Ferrer et al., 2011; Valencia et al., 2018). In milk, large part of the calcium content is linked to the casein micelle as calcium phosphate and the remaining portion is dissolved in the whey (Li & Corredig, 2014). During UF process, this soluble calcium passes through the membrane into permeate. In fermentation, the pH level starts to decline, which leads to the solubilization of colloidal calcium phosphate and greater proportion of ionic forms in the soluble phase. The maximum dissociation of colloidal calcium phosphate occurs between pH 5.6 and 5.1 (Anema, 2008). In additional, studies suggest that the addition of prebiotic ingredient can stimulate fermentative process and the development of lactic acid bacteria (Akalin et al., 2004, 2007; Martín-Diana et al., 2003). Bifidobacteria produce enzymes that, unlike animal digestive enzymes, hydrolyze FOS and obtain carbon and energy through the fermentation of this substrate (McKellar & Modler, 1989; Mitsuoka, 1990). However, in this study the addition of FOS had no significant influence on the fermentation of SS and SS-UF skyr-types since the time of fermentation was the same when compared to the skyr-type controls. These results are in accordance with Albuquerque et al. (2019) which related that FOS did not affect the fermentation kinetic parameters for fermented soy products. The titratable acidity values significantly increased in all skyr-types yogurts throughout the process (Figure 3).

Yield

Yogurt's yield is affected by many factors including milk composition, quality, amount and genetic variants of casein, somatic cell count in milk, pasteurization, coagulant type, and manufacturing parameters. Yogurt's yield is vital in an economic sense for dairy industry since small differences in yield translate into big differences in profits (El-gawad & Ahmed, 2011).Table 2 shows the results of skyr-type yogurts yield (kg 100 kg^{-1}). UF process increased the SP-UF and SS-UF skyr-types yield by approximately 65% when compared to SP and SS skyr-types. UF process changes the milk composition increasing the protein and colloidal mineral contents in the concentrate, reducing the water, mineral, and lactose contents (Uduwerella et al., 2017; Valencia et al., 2018). In addition, cloth filtration method is slow, unhygienic, labor-intensive and has a low yield. In this study, these results suggest the use of UF process for greater-yielded yogurt elaboration.

3.2. Centesimal composition

Table 3 shows the centesimal composition of the skyr-type yogurts. Statistical differences between the formulations were detected for all parameters. All skyr-types showed a high protein content. In this study, both processes resulted in products with similar protein content (~ 9 g 100 g^{-1}). According to Brazilian laws, a product is considered a high protein content when it has 12 g of protein per portion. All skyr-types produced in this study adhere to current Brazilian laws and are classified as foods with high protein content in a daily portion of fermented milk intake. High-protein yogurts could be beneficial in infant, elderly, or sports nutrition due to the ability of whey proteins to increase plasma amino acids (Hall et al., 2003) and trigger muscle protein synthesis (Garlick Peter, 2005; Tipton et al., 2007). Furthermore, these yogurts could be beneficial in calorie-restricted diets because the energy intake from protein seems to have a greater effect on satiety compared to intake of fat or carbohydrate.

All skyr-types produced in this study are fat-free. Nowadays, consumer's demand for a low-fat product has significantly increased aiming at

health promotion. Studies have already proven the relationship between the excessive consumption of fats and several diseases, such as obesity, cancer, and cardiovascular diseases (Howard et al., 2006; White et al., 1992). Thus, the low-fat market forced the dairy industry to create products that meet the specific needs of consumers and maintained the sensory characteristics of this products. According to Food and Drug Administration (FDA), all skyr-types can be classified as “healthy” because they presented a low-fat content and more than 10% of proteins.

The enrichment of yogurts with prebiotics is a trend. Prebiotics can improve the microbiological and physicochemical properties of this products and add health benefits. In this context, FOS can exert numerous positive effects in the gastrointestinal tract, including modulation of gut microbiota, regulation of appetite, stimulation of intestinal peristalsis, increase of metabolites production (for example, short-chain fatty acids), stimulation of immune system, provision of energy for colonic epithelial cells, control of colonic pH, and promotion of mucus production (Koh et al., 2016; Musso et al., 2011). In the current study, FOS contributed to a higher dietary fiber content in SS and SS-UF skyr-types. Both products adhere to current Brazilian laws and are classified as foods with high dietary fiber content in a daily portion of fermented milk (SS: 10,78 g 200 g^{-1} and SS-UF: 11,68 g 200 g^{-1}). Thus, developing foods with higher content of prebiotic fibers may increase daily fiber intake and promote a health gut, benefitting human health.

As expected, the addition of 5% of FOS increased the total solid contents in SS and SS-UF formulations. FOS can affect the nutritional value and yogurt structure development, especially improving texture, viscosity and flavor (Tamime & Robinson, 2007). SS and SS-UF skyr-types showed values for total solids of 21.63 and 22.62 g 100^{-1}, respectively. According to Tamime & Robinson (1999), increase in total solid contents to greater than 22 g 100 g^{-1} hinders the lactic acid bacteria growth due to the development of higher osmotic pressure in yoghurt base (Tamime & Robinson, 1999). However, in this study, this effect was not observed.

3.3. Evaluation of skyr-type yogurts during refrigerated storage

3.3.1. pH and titratable acidity

Table 4 shows the pH and titratable acidity values during the storage time. The pH values varied for SS, SP-UF and SS-UF skyr-types ($p \leq 0.05$), suggesting the occurrence of post-acidification, a phenomenon normally found in fermented dairy products. SP-UF and SS-UF skyr-types showed pH values higher than SP and SS formulations ($p \leq 0.05$). This result probably may be attributed to buffering capacity of the micellar casein, which was concentrated by UF, as previously explained. The titratable acidity of all skyr-types yogurts during storage was between 1.40 and 1.80 g expressed in lactic acid 100 g^{-1}, respectively. According to the Brazilian Technical Regulations for the Identity and Quality of Fermented Milks, which establishes acidity values at 0.6 to 2.0 g 100 g^{-1} for these products, all the formulations produced herein adhered to Brazilian legislation.

3.3.2. Viability of strains

Table 5 shows the viability of strains during the storage. *B. lactis* Bb-12® reached viable counts of at least 7 log CFU g^{-1} in all skyr-type yogurts, which indicates the good stability of this probiotic strain in skyr matrix. However, a small but significant decrease in *B. lactis* Bb-12® counts along the storage was observed only in SP-UF skyr-type. *B. lactis* Bb-12® is a common commercial probiotic used in fermented dairy production that may convey health-promoting properties in hosts (Henrique-Bana et al., 2020; Jungersen et al., 2014). Bb-12® ingestion promotes innumerable health benefits, such as maintenance the intestinal microbial balance, diarrhea prevention, stimulation of the phagocytic activity in peripheral blood samples, and treatment of atopic eczema in infants (Palaria et al., 2012). Moreover, the addition of prebiotics, such as FOS, can positively affect the probiotic viability during the storage. Bifidobacteria are saccharolytic microorganisms. They obtain carbon and energy through fermentation of dietary carbohydrates. These strains preferably ferment the fructans to other carbohydrate sources such as starch,

pectin or polydextrose (Jungersen et al., 2014). The high specificity of FOS as substrates for bifidobacteria results from the activity of enzymes β-fructosidases (inulinases) associated with specific cells, which hydrolyze fructose monomers from the non-reducing end of the inulin molecule or from certain sugars where the fructose residue occurs at position β (2-1) (Rossi et al., 2005; Saad, 2006; Valdés-Varela et al., 2017). However, in this study, the addition of FOS did not significantly affect viability of *B. lactis* Bb-12® strains.

L. bulgaricus and *S. thermophilus* reached viable counts of approximately 7 and 8 log CFU g^{-1}, respectively in all skyr-types samples until the 28th day of storage. *L. bulgaricus* and *S. thermophilus* are strains commonly used as a starter culture in the production of fermented milks, such as yogurts and cheeses. These microorganisms are responsible for accelerating of the fermentation process through the production of lactic acid and secondary metabolites, which contribute to the sensory properties of fermented products (Irigoyen et al., 2012; Uriot et al., 2017).

3.3.3. Syneresis

Syneresis is defined as the expulsion of whey from the yogurt's protein matrix, and it is considered as one of the major technological defects in yogurts production. This phenomenon can adversely affect the consumer acceptability of product, as consumers may consider that there is a microbiological problem (Purwandari et al., 2007). Table 6 shows the syneresis for all skyr-type yogurts during storage. Syneresis values were stable and low (less than 1.50 %) for all samples throughout storage ($p > 0.05$). To reduce or eliminate this problem, dairy industry usually uses stabilizers such as starch, gelatin and vegetable gum or increase the level of milk solids and especially of the protein percentage. In this study, the addition of FOS and high protein content in all yogurts positively influenced matrix density and decreased syneresis. Thus, the results obtained in this study are not in accordance with previous studies that have documented the common increased in syneresis in yogurts throughout refrigerated storage (Witschinski et al, 2018).

3.3.4. CATA

Cochran's Q test showed differences in most attributes among the samples, suggesting that the CATA method was able to detect differences in participants perceptions (Table 7). Correspondence analysis of the CATA data showed that the first and second dimensions explain 98.49% of the experiment variation (Figure 4). The skyr-type yogurts were positioned into three different groups and obtained good separation of attributes distributed among the four quadrants. SP and SS samples were characterized by the terms nice taste, appropriate texture, firm, consistent, curd flavor, velvety texture, nice appearance, sour flavor, agreeable texture, lightly acidic flavor, cream cheese flavor and matte appearance. SP-UF sample was associated to the term's fluid, unpleasant taste, homogeneous, delicate texture, shiny and smooth appearance. SS-UF skyr-type was associated to the term's inexistent aroma, neutral flavor, very soft taste, nasty appearance, watery flavor and yellow color. That way, we can observe that the production processes and the addition of FOS, especially in SS-UF skyr-type, influenced the final characteristics of yogurts. The sensory profile of yogurts is directly influenced by the metabolic activity of the lactic acid bacteria, which interact strongly with the components of the matrix to convert certain metabolic products during the growth, particularly organic acids (Serra et al., 2009). In this case, the differences found especially in SP-UF and SS-UF skyr-types may be attributed to buffering capacity of the micellar casein, which has hindered pH decline and made it impossible to develop specific characteristics of fermented products, such as flavor, aroma and texture (Casarotti et al., 2014).

It is noteworthy that the formulations tested in this work was not added with sweetening or flavoring agents, and thus the sensory characteristics of skyr obtained by ultrafiltration can be optimized from these additions and future formulation adjustments. The better standardization of the product and the better hygienic control of the process for obtaining skyr by ultrafiltration justify the use of this technology to replace the use of cloth filtration.

In PCA (Figure 5) the position of attributes cited by most tasters, such as velvety texture, lightly acidic flavor, curd flavor, cream appearance, nice

taste, white color, consistent, firm, and homogeneous suggests that although they are attributes that the evaluators consider to be characteristics of an ideal skyr-type, they are not attributes with great discriminative power or of positive or negative relevance for yogurts of this type.

3.3.5. *In vitro* digestion

The survival of *B. lactis* Bb-12®, *L. bulgaricus* and *S. thermophilus* after each simulated gastrointestinal digestion steps are shown in Table 8. No significant decreases in *B. lactis* Bb-12®, *L. bulgaricus* and *S. thermophilus* counts were detected after exposure to simulated mouth conditions (data not shown). This behavior may be attributed to the short time of contact with the oral conditions (Verruck et al., 2020). In the gastric phase, the population of *B. lactis* Bb-12® remained stable for SP and SP-UF skyr-types. A small reduction in counts of *B. lactis* Bb-12® was observed in SS and SS-UF formulations, suggesting your sensitivity towards simulated gastric juice containing HCl and pepsin. (Matsumoto et al., 2004) evaluated the acid tolerance of Bb-12® strains when exposed to a pH range of 2-5. The authors observed that BB-12® showed high survival rates. This characteristic was shown to be due in part to the low pH induction of H^+-ATPase activity, an enzyme complex involved in maintaining intracellular pH homeostasis in bacteria. Resistance to gastrointestinal conditions is a key requirement for probiotic strains. Several factors can affect the viability of these strains such as an acidic pH value, presence of digestive enzymes and bile salts (Vinderola & Reinheimer, 2003). It was observed that the addition of FOS in SS and SS-UF skyr-types had no protective effect on viability of *B. lactis* Bb-12® in this phase, as SP and SP-UF skyr-types presented same counts ($p > 0.05$). The population of *B. lactis* BB-12® decreased ($p \leq 0.05$) after exposure to intestinal conditions for all skyr-types samples. However, the presence of FOS in SS-UF skyr-type may have a protective effect on the viability of *B. lactis* BB-12®. According to (Vernazza et al., 2006), BB-12® did not grow well at bile-containing medium (1%) but demonstrated high survival rates by optical density. The significant decreased in probiotics survival during digestion is one of the reasons for the

recommendation for constant consumption of probiotics to promote health effects.

The same pattern was observed for *L. bulgaricus*. However, SP and SS skyr-types exhibited higher survival rates when compared to SP-UF and SS-UF samples. *S. thermophilus* counts was significantly reduced during all phases that simulated gastrointestinal digestion steps. However, at the end of digestion this strain showed counts above 7 log CFU mL^{-1}.

4. Conclusion

Both process, cloth filtration and UF, were efficient to produce skyr-types yogurts with high-protein content. The addition of FOS increased the total solid contents in SS and SS-UF skyr-types. The skyr-type yogurts stability regarding pH, acidity and syneresis indicated a suitability of the skyr matrix and the storage for 28 days. *B. lactis* Bb-12® reached viable counts of at least 7 log CFU g^{-1} in all skyr-types during the storage, which indicates the good stability of this probiotic strain in skyr matrix. However, in *in vitro* digestion assay, *B. lactis* Bb-12® showed a decrease in survival during the intestinal phase. This finding justifies one of the reasons for recommending constant consumption of probiotics to promote health effects. In the sensory evaluation, CATA data pointed that SP and SS presented favorable attributes expected in fermented products. However, SS-UF and SP-UF may have been adversely affected by buffering capacity of the micellar casein, which has hindered pH decline and made it impossible to develop specific characteristics of fermented products, such as flavor, aroma and texture. Finally, this study showed that skyr is a good carrier matrix for probiotics, in addition to being a product with a high concentration of proteins, being a market trend. Ultrafiltration proved to be an efficient process, but some adjustments are needed in the formulation so that the skyr produced can achieve greater sensory acceptance.

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Table 1. Protein content in samples of milk, permeate and retentate.

Skyr-type yogurts		Protein (g 100 g^{-1})
SP-UF	Milk	2.97 ± 0.09 $^{b, A}$
	Retentate	8.64 ± 0.44 $^{a, A}$
	Permeate	0.15 ± 0.03 $^{c, B}$
SS-UF	Milk	2.56 ± 0.09 $^{b, B}$
	Retentate	8.33 ± 0.59 $^{a, A}$
	Permeate	0.24 ± 0.03 $^{c, A}$

Average ± standard deviation (n=6). Different lower-case letters in the columns indicate statistically significant differences ($p < 0.05$) in milk, retentate and permeate for each skyr-type. Different capital letters in the columns indicate statistically significant differences ($p < 0.05$) in milk, retentate and permeate between probiotic skyr (SP-UF) and synbiotic skyr (SS-UF).

Table 2. Skyr-type yogurts yield (kg 100 kg^{-1}) according to different processes of production.

Skyr-type yogurts	Yield (kg 100 kg^{-1})
SP	22.33 ± 1.07
SS	22.08 ± 0.29
SP-UF	87.43 ± 0.68
SS-UF	88.73 ± 0.48

Average ± standard deviation (n=3). SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

Table 3. Centesimal composition of the skyr-types yogurts.

Parameters	**SP**	**SS**	**SP-UF**	**SS-UF**
Total solid (g 100 g^{-1})	17.13 ± 0.31 $^{b, B}$	21.63 ± 1.66 $^{a, A}$	19.93 ± 0.49 $^{b, A}$	22.62 ± 1.97 $^{a, A}$
Moisture (g 100 g^{-1})	82.87 ± 0.31 $^{a, A}$	78.37 ± 1.66 $^{b, A}$	80.07 ± 0.49 $^{a, B}$	77.38 ± 1.97 $^{b, A}$
Ash (g 100 g^{-1})	0.97 ± 0.03 $^{a, B}$	0.44 ± 0.06 $^{b, B}$	1.49 ± 0.04 $^{a, A}$	1.09 ± 0.05 $^{b, A}$
Proteins (g 100 g^{-1})	9.67 ± 0.20 $^{a, A}$	8.28 ± 0.42 $^{b, B}$	8.64 ± 0.44 $^{a, B}$	9.11 ± 0.47 $^{a, A}$
Fat (g 100 g^{-1})	nd	nd	nd	nd
Fiber (g 100 g^{-1})	nd	5.39 ± 0.30 ^{A}	nd	5.84 ± 0.32 ^{A}

Average ± standard deviation (n=3). nd: not detectable. SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF. Different lower-case letters in the rows indicate statistically significant differences ($p < 0.05$) between products (SP and SS/SP-UF and SS-UF). Different capital letters in the rows indicate statistically significant differences ($p < 0.05$) between process (SP and SP-UF/SS and SS-UF).

Table 4. pH and titratable acidity values during refrigerated storage.

			Storage (days)		
			1	14	28
pH	Products	SP	4.30 ± 0.01^{a,A}	4.26 ± 0.06^{a,A}	4.25 ± 0.03 ^{a,A}
		SS	4.27 ± 0.01^{a,B}	4.25 ± 0.02^{a,b,A}	4.24 ± 0.01^{a,A}
		SP-UF	4.85 ± 0.01^{a,B}	4.70 ± 0.01^{b,A}	4.69 ± 0.01^{b,B}
		SS-UF	4.93 ± 0.01^{a,A}	4.71 ± 0.01^{b,A}	4.71 ± 0.01^{b,A}
Titratable acidity (g 100 g^{-1})	Products	SP	1.79 ± 0.01^{b,A}	1.83 ± 0.03^{a,A}	1.75 ± 0.02^{a,A}
		SS	1.64 ± 0.04^{a,B}	1.49 ± 0.06^{b,B}	1.63 ± 0.01^{a,B}
		SP-UF	1.49 ± 0.02^{b,B}	1.68 ± 0.04^{a,A}	1.69 ± 0.03^{a,B}
		SS-UF	1.55 ± 0.03^{c,A}	1.70 ± 0.01^{b,A}	1.77 ± 0.02^{a,A}

Average ± standard deviation (n=3). SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF. Different lower-case letters in the rows indicate statistically significant differences ($p < 0.05$) in each skyr-type yogurt during storage. In products, different capital letters in the columns indicate statistically significant differences ($p < 0.05$) between SP/SS and SP-UF/SS-UF.

Table 5. Viable counts of *B. lactis* Bb-12®, *L. bulgaricus* and *S. thermophilus* during refrigerated storage.

		Storage (days)		
		1	**14**	**28**
B. animalis subsp. *lactis* Bb-12® (log UFC g $^{-1}$) Products				
	SP	7.77 ± 0.05 $^{a, A}$	7.86 ± 0.13 $^{a, A}$	7.81 ± 0.15 $^{a, A}$
	SS	8.16 ± 0.42 $^{a, A}$	7.93 ± 0.12 $^{a, A}$	7.83 ± 0.10 $^{a, A}$
	SP-UF	8.93 ± 0.19 $^{a, A}$	7.97 ± 0.21 $^{b, A}$	7.96 ± 0.08 $^{b, A}$
	SS-UF	8.08 ± 0.27 $^{a, B}$	8.11 ± 0.33 $^{a, A}$	7.86 ± 0.18 $^{a, A}$
L. delbrueckii subsp. *bulgaricus* (log UFC g $^{-1}$) Products				
	SP	7.94 ± 0.09 $^{a, B}$	7.81 ± 0.14 $^{a, B}$	7.86 ± 0.07 $^{a, A}$
	SS	8.92 ± 0.14 $^{a, A}$	8.34 ± 0.47 $^{b, A}$	7.73 ± 0.14 $^{c, A}$
	SP-UF	8.91 ± 0.32 $^{a, A}$	8.04 ± 0.10 $^{b, A}$	7.97 ± 0.02 $^{b, A}$
	SS-UF	8.39 ± 0.19 $^{a, B}$	7.69 ± 0.24 $^{b, B}$	7.73 ± 0.16 $^{b, B}$
S. thermophilus (log UFC g $^{-1}$) Products				
	SP	9.57 ± 0.08 $^{a, B}$	9.71 ± 0.18 $^{a, A}$	9.77 ± 0.14 $^{a, A}$
	SS	9.93 ± 0.04 $^{a, A}$	8.78 ± 0.09 $^{b, B}$	8.15 ± 0.44 $^{c, B}$
	SP-UF	9.58 ± 0.09 $^{a, A}$	9.75 ± 0.05 $^{b, B}$	9.32 ± 0.08 $^{c, A}$
	SS-UF	9.21 ± 0.56 ^{a,} A	8.56 ± 0.25 $^{b, A}$	7.84 ± 0.36 $^{c, B}$

Average ± standard deviation (n=3). SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF. Different lower-case letters in the rows indicate statistically significant differences ($p < 0.05$) in each skyr-type yogurt during storage. In products, different capital letters in the columns indicate statistically significant differences ($p < 0.05$) between SP/SS and SP-UF/SS-UF.

Table 6. Syneresis values during refrigerated storage.

Skyr-type yogurts		Storage (days) 1	14	28
Products	SP	0.38 ± 0.03 $^{a, A}$	0.39 ± 0.02 $^{a, A}$	0.38 ± 0.01 $^{a, A}$
	SS	0.13 ± 0.03 $^{a, B}$	0.14 ± 0.02 $^{a, B}$	0.14 ± 0.01 $^{a, B}$
	SP-UF	1.39 ± 0.02 $^{a, A}$	1.40 ± 0.02 $^{a, A}$	1.39 ± 0.02 $^{a, A}$
	SS-UF	0.98 ± 0.02 $^{a, B}$	1.02 ± 0.07 $^{a, B}$	1.01 ± 0.08 $^{a, B}$

Average ± standard deviation (n=3). SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF. Different lower-case letters in the rows indicate statistically significant differences ($p < 0.05$) in each skyr-type yogurt during storage. In products, different capital letters in the columns indicate statistically significant differences ($p < 0.05$) between SP/SS and SP-UF/SS-UF.

Table 7. Frequency mention of sensory attributes associated with each sample of skyr-type yogurts by consumers.

	Skyr-type yogurts				
Attributes	**SP**	**SS**	**SP-UF**	**SS-UF**	**p-value**
Shiny appearance	31 [a]	38 [a]	81 [b]	71 [b]	< 0.0001
Matte appearance	48 [b]	38 [b]	3 [a]	7 [a]	< 0.0001
Nice appearance	53 [b]	50 [b]	36 [ab]	30 [a]	0.001
Cream appearance	53 [b]	51 [b]	19 [a]	45 [b]	< 0.0001
Smooth appearance	27 [ab]	20 [a]	71 [c]	37 [b]	< 0.0001
Nasty appearance	2 [a]	4 [ab]	10 [ab]	13 [b]	0.007
Crude appearance	11 [b]	15 [b]	0 [a]	7 [ab]	0.000
Consistent	68 [b]	71 [b]	9 [a]	23 [a]	< 0.0001
Firm	68 [b]	61 [b]	3 [a]	12 [a]	< 0.0001
Pasty	40 [b]	37[b]	15 [a]	47 [b]	< 0.0001
Cream	34 [ab]	25[a]	33 [ab]	43 [b]	0.058
Homogeneous	73 [b]	71 [b]	81 [b]	44 [a]	< 0.0001
Non homogeneous	3 [a]	4 [a]	3 [a]	26 [b]	< 0.0001
Whey detachment	0 [a]	3 [a]	7 [a]	29 [b]	< 0.0001
Granules	3 [a]	3 [a]	0 [a]	27 [b]	< 0.0001
Sandy texture	14 [b]	13 [b]	0 [a]	8 [ab]	0.001
Velvety texture	35 [a]	39 [a]	27 [a]	24 [a]	0.034
Agreeable texture	57 [c]	49 [bc]	31 [a]	35 [ab]	< 0.0001
Delicate texture	6 [a]	6 [a]	38 [b]	13 [a]	< 0,0001
Appropriate texture	26 [b]	25 [b]	7 [a]	18 [ab]	0.000
Elastic	9 [a]	10 [a]	24 [b]	11 [a]	0.002
Viscous	27 [a]	19 [a]	26 [a]	23 [a]	0.426
Fluid	0 [a]	1 [a]	56 [c]	20 [b]	< 0.0001
Robust	67 [c]	69 [c]	5 [a]	25 [b]	< 0.0001
Mild aroma	49 [a]	60 [a]	48 [a]	47 [a]	0.136
Inexistent aroma	15 [a]	13 [a]	18 [ab]	31 [b]	0.002
Discreet fermented aroma	32 [a]	36 [a]	28 [a]	24 [a]	0.229
Moderate fermented aroma	11 [a]	7 [a]	12 [a]	8 [a]	0.547
Strong fermented aroma	3 [a]	2 [a]	5 [a]	3 [a]	0.653
Milk aroma	8 [a]	1 [a]	6 [a]	6 [a]	0.120
White color	83 [ab]	88 [b]	90 [b]	75 [a]	0.004
Yellow color	7 [a]	5 [a]	5 [a]	16 [b]	0.000
Sweet flavor	2 [a]	5 [a]	2 [a]	3 [a]	0.494
Soft taste	39 [a]	39 [a]	32 [a]	29 [a]	0.285
Very soft taste	3 [a]	9 [ab]	8 [ab]	15 [b]	0.025
Sour flavor	46 [b]	39 [ab]	35 [ab]	28 [a]	0.030
Watery flavor	1 [a]	1 [a]	18 [b]	17 [b]	< 0.0001
Neutral flavor	11 [a]	13 [a]	19 [ab]	28 [b]	0.005
Nice taste	32 [bc]	42 [c]	14 [a]	21 [ab]	< 0.0001
Unpleasant taste	14 [ab]	9 [a]	23 [b]	17 [ab]	0.020
Fermented flavor	29 [a]	20 [a]	26 [a]	18 [a]	0.114
Lightly acidic flavor	36 [ab]	46 [b]	29 [a]	23 [a]	0.001

Moderate acid flavor	14 [a]	11 [a]	11 [a]	6 [a]	0.308
Strong acid flavor	4 [a]	3 [a]	3 [a]	0 [a]	0.308
No residual flavor	16 [a]	28 [a]	23 [a]	26 [a]	0.118
Low residual flavor	35 [a]	27 [a]	27 [a]	26 [a]	0.383
Moderate residual flavor	10 [a]	7 [a]	12 [a]	4 [a]	0.183
Strong residual flavor	5 [a]	1 [a]	3 [a]	6 [a]	0.107
Cream cheese flavor	23 [b]	22 [b]	9 [a]	8 [a]	0.000
Curd flavor	34 [c]	30 [bc]	18 [ab]	14 [a]	0.000

n=100. p-value ≤ 0.05 indicates significant difference on Cochran's Q test. SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

Table 8. Viability of the strains during the simulated gastrointestinal digestion steps.

	Oral phase	Gastric phase	Intestinal phase	Survival (%)
B. animalis subsp. *lactis* Bb-12® (log CFU mL^{-1})				
SP	8.19 ± 0.06 ^{a,B}	8.34 ± 0.13 ^{a,A}	6.46 ± 0.11 ^{b,A}	78.88
SS	9.23 ± 0.11 ^{a,A}	7.68 ± 0.16 ^{b,B}	6.57 ± 0.07 ^{c,A}	71.80
SP-UF	7.59 ± 0.06 ^{a,A}	7.30 ± 0.12 ^{a,A}	5.45 ± 0.45 ^{b,B}	71.80
SS-UF	7.59 ± 0.05 ^{a,A}	6.60 ± 0.08 ^{b,B}	6.25 ± 0.07 ^{c,A}	82.34
L. delbrueckii subsp. *bulgaricus* (log CFU mL^{-1})				
SP	7.08 ± 0.11 ^{a,B}	7.19 ± 0.18 ^{a,A}	5.84 ± 0.06 ^{b,B}	82.48
SS	7.38 ± 0.07 ^{a,A}	5.45 ± 0.28 ^{b,B}	6.41 ± 0.13 ^{c,A}	86.85
SP-UF	7.65 ± 0.06 ^{a,A}	7.36 ± 0.11 ^{b,A}	3.82 ± 0.13 ^{c,A}	49.93
SS-UF	7.56 ± 0.11 ^{a,A}	4.40 ± 0.12 ^{b,B}	4.06 ± 0.14 ^{c,A}	53.70
S. thermophilus (log CFU mL^{-1})				
SP	9.86 ± 0.57 ^{a,A}	8.55 ± 0.01 ^{b,A}	7.37 ± 0.19 ^{c,A}	74.74
SS	9.34 ± 0.04 ^{a,A}	8.46 ± 0.01 ^{b,B}	7.79 ± 0.01 ^{c,A}	83.40
SP-UF	8.31 ± 0.08 ^{a,A}	8.07 ± 0.03 ^{b,B}	7.33 ± 0.18 ^{c,A}	88.20
SS-UF	8.44 ± 0.14 ^{a,A}	8.32 ± 0.07 ^{a,A}	7.50 ± 0.06 ^{b,A}	88.86

Average ± standard deviation (n=3). SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF. Different lower-case letters in the rows indicate statistically significant differences ($p < 0.05$) between the digestion phase of the same product, by comparing each digestion phase with the previously phase. Different capital letters in the columns indicate statistically significant differences ($p < 0.05$) between products (SP-SS and SP-UF/SS-UF).

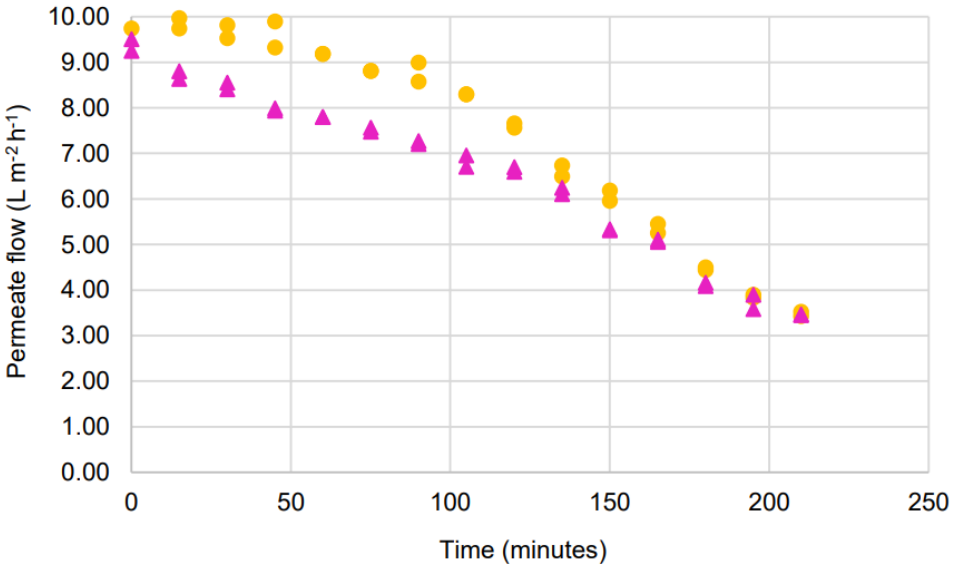

Fig. 1. Permeate flow for SP-UF and SS-UF UF milks over time (L m^{-2} h^{-1}). ● SP-UF; ▲ SS-UF. SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

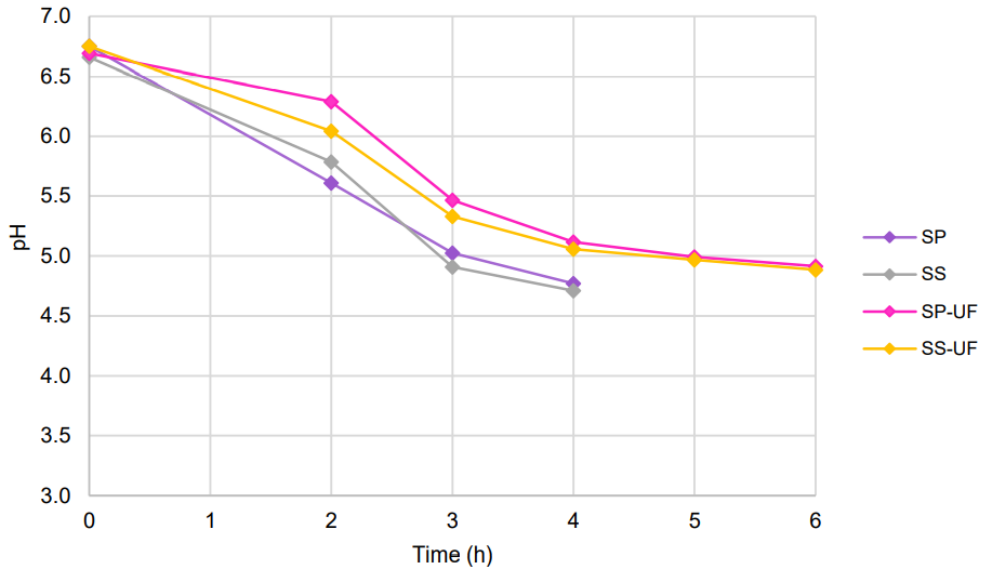

Fig. 2. pH values for all skyr-types during the process of fermentations. SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

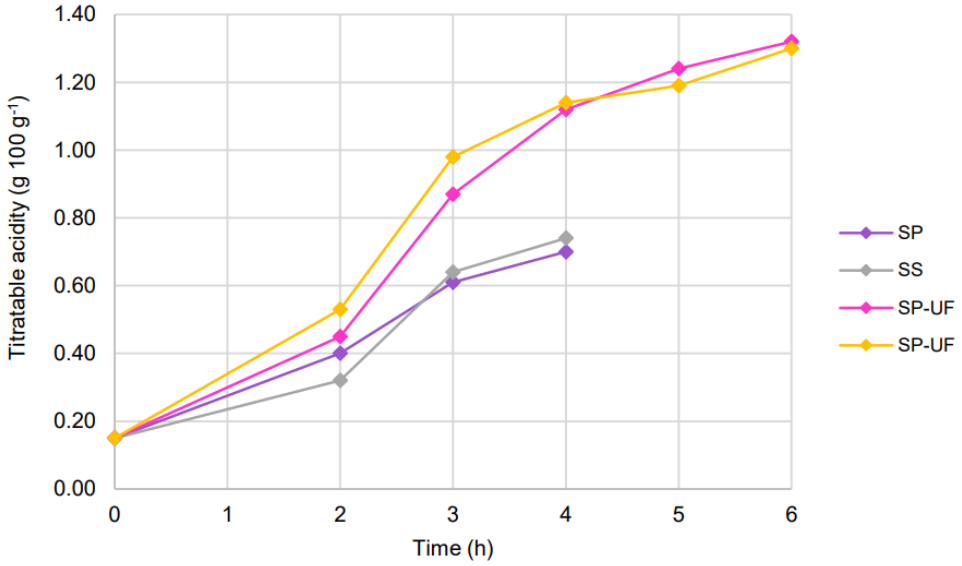

Fig. 3. Titratable acidity for all skyr-types during the process of fermentation. SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

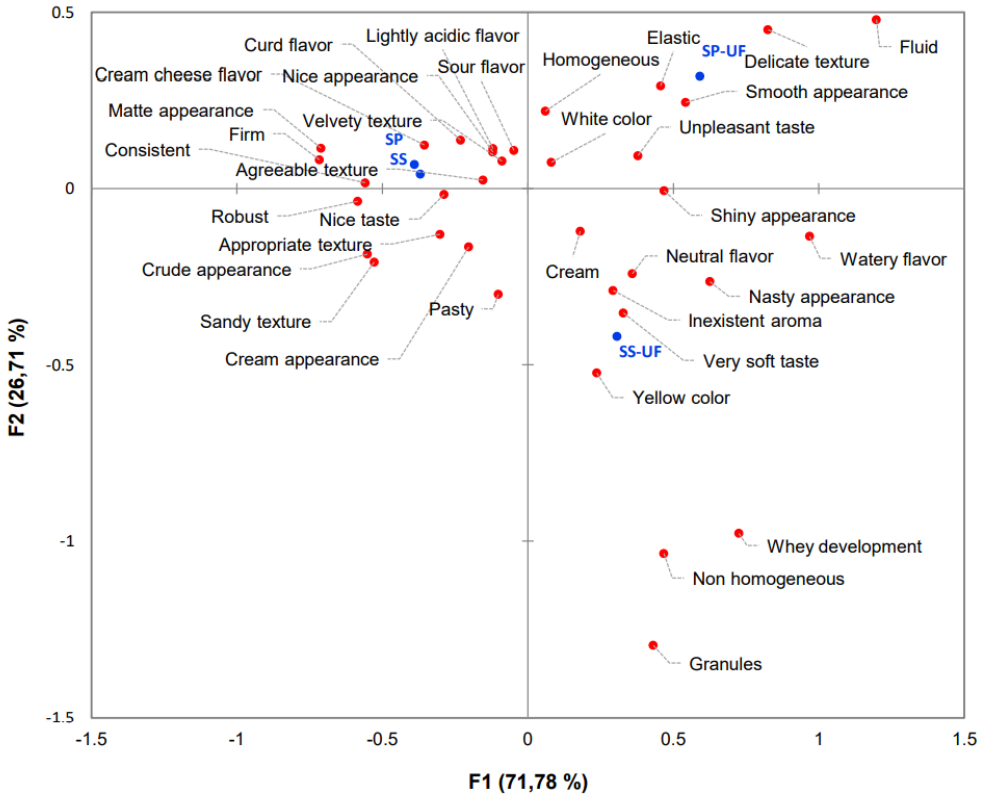

Fig. 4. Symmetric plot of sensorial descriptors in CATA analysis. SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

Fig. 5. Principal Coordinates Analysis (PCA) plot of acceptance. SP: probiotic skyr by cloth filtration; SS: synbiotic skyr by cloth filtration; SP-UF: probiotic skyr by UF; SS-UF: synbiotic skyr by UF.

Capítulo 2.

Beneficial effects of skyr yogurts on gut microbiota

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BENEFICIAL EFFECTS OF SKYR YOGURTS ON GUT MICROBIOTA

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Abstract

The aim of this study was to evaluate the functional effect of probiotic and synbiotic skyr yogurts on gut microbiota in a Simulator of Human Intestinal Microbial Ecosystem (SHIME®). Samples from the ascending colon vessels were collected in each experimental period (controls 1 and 2, and treatments). The microbiota composition and microbial community structure were analyzed using 16S rRNA gene sequencing. Changes in microbial metabolism were evaluated through the production of short chain fatty acids (SCFA) and ammonia. The experimental protocol was performed in the SHIME® for 6 weeks, in triplicate. The results demonstrated that probiotic and synbiotic skyr yogurts kept *B. lactis* - Bb 12 viable during their passage through the gastrointestinal tract. The administration of probiotic skyr increased the abundance of *Bacteroides*, *Coriobacteriaceae* UCG-003 and *Lactobacillus*. On the other hand, the administration of synbiotic skyr increased the abundance of *Bifidobacterium*, *Coriobacteriaceae* UCG-003 and *Lactobacillus*. Skyr yogurts increased the production of all SCFA. Probiotic skyr increased ammonia concentrations. The increase of these ions was probably associated with the presence of proteins in its composition (~19 g/200 g of yogurt). However, ammonia concentrations remained stable during treatment with the synbiotic skyr. This found may be attributed to the increase in *Bifidobacterium* abundance. Finally, probiotic and synbiotic skyr yogurts showed a positive modulation of the gut microbiota and metabolic activity.

Keywords: Gut microbiota; High-protein yogurts; Skyr, Probiotic; Synbiotic; Gut model; SHIME®.

1. Introduction

The human intestine is a complex ecosystem and is inhabited by approximately 10^{14} cells/mL, including some 1000 bacterial species (Sender et al., 2016). Gut microbiota is mainly composed of *Bacteroidetes* and *Firmicutes*, that represent more than 90% of the entire phylogenetic type (Eckburg et al., 2005). Intestinal bacteria play an essential role in maintaining the homeostasis of the human body and its balance and stability have direct implications on the physiological functions of the host (Uchiyama et al., 2019).

Gut microbiota is responsible for protecting the intestinal membrane against pathogenic bacteria, digestion of complex polysaccharides, regulation of bile acid metabolism, production of vitamins, maintenance of intestinal barrier integrity and regulation of the immune system (Sánchez-Tapia et al., 2019). Diet has a pivotal role on the host health status by modulating the composition of the intestinal microbiota (Leeming et al., 2019). Therefore, the incorporation into the diet of probiotics and prebiotics, as foods or dietary supplements, is part of a comprehensive nutritional strategy to improve the function of the gut microbiota (Antunes et al., 2020).

Fermented dairy products, such as yogurts and fermented milks, are the major vehicles of probiotics delivery (Ranadheera et al., 2017). Probiotics can prevent diseases and treat some health disorders such as insulin resistance, high blood pressure, lactose intolerance, high serum cholesterol, liver inflammation, diabetes, and inflammatory bowel diseases (Cheng et al., 2019). Also, probiotics have anti-carcinogenic effects and enhance the immune system (George Kerry et al., 2018). In addition, the incorporation of prebiotics adds functionality to these products and can be used as a marketing strategy for health promotion. Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit. Health effects of prebiotics have been linked to modulating microbiota homeostasis, gut–brain axis, immune system crosstalk, and the intestine barrier function (Gibson et al., 2017).

High-protein yogurts are considered the newest trend in the dairy products market, as they are an excellent source of protein and essential

amino acids (Gómez-Gallego et al., 2018). The rise in consumer demand for yogurts of this segment is related to the beneficial effects of proteins on health, as well as the sensory characteristics of these products, such as texture and flavor (Jørgensen et al., 2019). High protein-yogurts are commonly used in the nutrition of children, exercise practitioners, athletes, and the elderly, as they contribute to muscle synthesis and bone health (Keršienė et al., 2020). Furthermore, they can be used as a food strategy for calorie-restricted diets, as they promote greater satiety (Russell et al., 2011). This category of yogurt is available worldwide and its nomenclature varies by country of origin, such as Labneh (Eastern Mediterranean), Torba (Turkey), Stragisto (Greece), Chakka, (India), Ymer (Denmark), and Skyr (Iceland) (Tamime et al., 2014).

Skyr is a traditional Icelandic dairy product. Currently, it is gaining popularity for its nutritional composition, creamy and thick texture and exemption of additives and preservatives. It is low in calories, fat, and carbohydrates and high in protein, vitamins, and minerals content. Skyr is made with four times more milk than conventional yogurt (Narvhus & Abrahamsen, 2022). It is obtained by fermentation with dairy cultures, but the drainage step provides differentiated sensory characteristics (Jørgensen et al., 2017).

High-protein yogurts reached the dairy market to meet the several nutritional requirements and sensory characteristics of consumers. We know that dairy products, including yogurts, can beneficially alter the gut microbiota. However, how high-protein yogurts affects the gut microbiota is still unknown. Therefore, the aim of this study was to evaluate the functional effect of probiotic and synbiotic skyr yogurts on gut microbiota using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®).

2. Material and methods

2.1. Preparation of probiotic and synbiotic skyr yogurts

Skim milk was used for the preparation of skyr yogurts. Fructooligosaccharide (5%) (Focus Flora FOS®, Brazil) was added only in synbiotic formulation. Next, the milk was heated to 65°C for 30 minutes and

then cooled to 43°C for the inoculation of the Icelandic probiotic culture SKYR 1.0 (0,03%) (Chr. Hansen®, Denmark), which contain *Bifidobacterium animalis* subsp. *lactis* Bb-12*, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* StCth-52. The formulations were fermented in a BOD incubation chamber (MA1415/780, Marconi®, Brazil) at 43ºC until a pH of approximately 4.8 was reached. Then, the skyr yogurts were cooled to 5°C for 24 hours. Whey drainage was carried out using a cloth bag for a period of 6 hours under refrigeration. The final products were packed in high-density polyethylene bottles and stored at 5 ± 1°C for 28 days. Centesimal composition was evaluated on the first week after the production. Moisture, ash, protein, fat, and dietary fiber were determined using the methods proposed by the AOAC (2005). All analyses were run in triplicate. Table 1 shows the centesimal composition of probiotic and synbiotic skyr yogurts.

2.2. Simulator of Human Intestinal Microbial Ecosystem (SHIME®)

SHIME® is an *in vitro* dynamic model of the human gastrointestinal tract. It's composed of five double-jacketed vessels representing the stomach, the duodenum, the ascending colon, the transverse colon, and the descending colon, with their respective pH values, retention time, temperature, and volumetric capacity (Molly et al., 1994). In this study, SHIME® was adapted to triplicate the ascending colon vessel, as shown in Fig. 1.

During the experimental period, all vessels were kept at 37ºC under magnetic stirring and anaerobic conditions through daily 30-minute injections of nitrogen. The pH of each vessel was controlled by automatically adding of NaOH 1 N or HCl 1 N (Possemiers et al., 2004). At the beginning of the experiment, the ascending colon vessels were inoculated with stool samples from three health adult volunteers who had not taken antibiotics, probiotics, and prebiotics in the last six months. The stool inoculum was prepared using the method previously described by Possemiers et al. (2010). The fecal inoculum was stabilized for two weeks in a carbohydrate-based medium for the bacterial community could adapt to specific conditions of the ascending colon vessels (Possemiers et al., 2004).

2.2.1. Experimental protocol

The experimental protocol was performed in the SHIME® for 6 weeks, as shown in Fig. 2. After fecal inoculation, a two-week stabilization period was performed. In this period, 240 mL of the carbohydrate-based medium was added to the system twice a day for 14 days. Then, the control period 1 was started and 240 mL of the carbohydrate-based medium + 60 mL of artificial pancreatic juice (12.5 g/L of $NaHCO_3$, 6 g/L of Oxgall and 1.9 g/L of pancreatin) were added to the system twice a day for 7 days. After this period, one-week of treatment with probiotic skyr was added to the system once a day for 7 days (240 mL of carbohydrate-based medium + 200 mL of probiotic skyr + 60 mL of artificial pancreatic juice). Washout period (control period 2) was carried for one-week, where only 240 mL of carbohydrate-based medium + 60 mL of artificial pancreatic juice were added to the system. Finally, the treatment period with synbiotic skyr was started. During this period, 240 mL of carbohydrate-based medium + 200 mL of synbiotic skyr + 60 mL of artificial pancreatic juice were added to the SHIME® once a day for 7 days.

2.2.2. Survival of *Bifidobacterium animalis* subsp. *lactis* - Bb 12 under the simulated conditions of the stomach and duodenum in the SHIME®

During the treatments, samples were collected from the reactors corresponding to the stomach and duodenum to verify the survival of *B. lactis* - Bb 12. One mL of samples from each reactor were suspended in 9 mL of sterile peptone water and serial dilutions were carried. *B. lactis* - Bb 12 was plated in BIM-25 agar (Difco®, France) and incubated under anaerobiose (Probac®, Brazil) at 37 °C for 72 h (Vinderola & Reinheimer, 1999).

2.2.3. Microbiota composition by 16S rRNA gene sequencing

Samples from the ascending colon vessels were collected in each experimental period (controls 1 and 2, and treatments), and were then lyophilized and stored at −80°C until prepared for sequencing. Bacterial DNA was extracted using the DNeasy PowerLyzer PowerSoil kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. The microbiological

analysis employing 16S rRNA gene sequencing (n = 2) was performed by Next-generation Sequencing (Neoprospecta Microbiome Technologies, Brazil) with specific primers amplified the V3–V4 region of the 16S rRNA, 341F and 806R of 1 ng of DNA (Caporaso et al., 2011). The analysis of the sequences and the identification of the taxonomic units were based on the methodology of Giongo et al. (2010) and Hong et al. (2005). The PCR was carried out in triplicate using a Platinum Taq (Invitrogen, USA) under the following conditions: initial denaturation at 94°C for 3 min, 20 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 30 s, extension at 65°C for 90 s and final extension at 65°C for 10 min. The PCR products were purified using AMPure beads (Beckman coulter) and quantified by fluorometry using Qubit® 2.0 Fluorometer (Invitrogen, USA) (Fagen et al., 2012).

2.2.4. Ammonia (NH_4^+) and short chain fatty acids (SCFA) analysis

Samples from ascending colon vessels were collected in each experimental period (control 1 and 2, and treatments), and stored at −80°C. The production of NH_4^+ was determined using an ion-selective electrode (Model No. 95-12, Orion) according to Bianchi et al. (2014).

The SCFA were analyzed using a Trace GC Ultra gas chromatograph (Thermo Scientific, Italy) equipped with a flame ionization detector. The SCFA were separated using a DB-WAX capillary column (30m x 0.25mm x 0.25µm) under the following conditions: injector temperature: 220°C; column: 35°C; 2°C/min; 38°C; 10°C/min; 75°C; 35°C/min; 120°C (1min); 10°C/min; 170°C (2min); 40°C/min; 190°C (2min) and detector: 250°C. Helium was used as a carrier gas at a flow rate of 1 mL/min (Medeiros et al., 2021). The analysis was performed in triplicate.

2.3. Statistical analysis

The analyses were performed in triplicate in three independent experiments, and the results were expressed as means ± standard deviation. Student's t-test ($p \leq 0.05$) was applied for comparison between control and treatment periods in the ascending colon vessels, using a Statistica 10.0

software (StatSoft® Inc., USA). For the microbiota analysis using 16S rRNA gene sequencing data, the sequencing libraries were prepared according to Neoprospecta® Microbiome Technologies and the sequencing was carried out using the MiSeq platform (Illumina). Bioinformatics analyzes were performed using the QIIME program (Quantitative Insights into Microbial Ecology) (Caporaso et al., 2011). The taxonomic descriptions were generated based on the NCBI taxonomy database (http://www.ncbi.nl m.nih.gov) for the RDP database entries using the set of scripts listed in the TaxCollector pipeline (Giongo et al., 2010). Therefore, the strings were filtered and classified according to similarity. A minimum of 80% similarity was considered for domain and phylum identification, 90% for order and class identification, 90% for family, 95% for the genus, and 99% similarity for species identification (Savy et al., 2018). The 16S rRNA gene sequencing analyses were carried in RStudio, version 3.2.4 (R Core Team, 2018), utilizing the phyloseq package to import sample data and calculate alpha and beta diversity. The significance of categorical variables was determined using the non-parametric Wilcoxon test for two category comparisons or the Kruskal-Wallis test when comparing three or more categories. Principal coordinate plots relied on the PERMANOVA test to estimate p values (Fierer et al., 2010).

3. Results

3.1 Survival of *Bifidobacterium animalis* subsp. *lactis* Bb-12 under the simulated conditions of the stomach and duodenum in the SHIME®

Fig. 3 shows the survival of *B. lactis* Bb-12 in probiotic and synbiotic skyr yogurts under the simulated conditions of the stomach and duodenum in the SHIME®. The passage of both yogurts through the stomach-simulating reactor did not affect ($p > 0.05$) *B. lactis* Bb-12 survival. However, during the passage through the duodenum-simulating reactor, a reduction ($p \leq 0.05$) in *B. lactis* Bb-12 survival was observed in both yogurts (7.42 CFU log mL^{-1} and 7.53 CFU log mL^{-1}, respectively).

3.2. Effects of probiotic and synbiotic skyr on gut microbiota

A total of 1,033,522 million high-quality reads were obtained from microbiota samples collected during control and treatment periods. After normalizing the data, 868,564 thousand sequences were produced.

Chao1 and Shannon indices were used to describe the alpha diversity. The alpha diversity analysis showed that the treatment with probiotic skyr had low richness and low diversity (mean Chao 1 of 27; mean Shannon of 1.9, respectively) when compared to control 1 (mean Chao 1 of 30.5; mean Shannon of 2.3, respectively). However, the treatment with synbiotic skyr showed high richness and low diversity (mean Chao 1 of 40.7; mean Shannon of 1.9, respectively) when compared to control 2 (mean Chao 1 of 29.2; mean Shannon of 2.2, respectively). No significant differences ($p > 0.05$) were found between control periods and respective treatment periods for the Chao1 and Shannon indices (Fig. 4).

The beta diversity analysis based on the Bray-Curtis dissimilarity (Fig. 5) did not show differences in bacterial communities between the probiotic and synbiotic skyr treatments ($p = 0.075$). However, weighted, and unweighted UniFrac distances (Fig. 6) revealed significant differences between the treatments ($p = 0.024$ and $p = 0.045$, respectively).

The effect of the treatments on the relative abundance of the four most relevant phyla present in the human microbiome obtained from healthy adult subjects was analyzed. Fig. 7 shows the relative abundance of phyla and genera in ascending colon vessels during the experimental protocol. Four main phyla were identified during the control and treatment periods: *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. The probiotic skyr increased the abundance of *Bacteroidetes* and decreased the abundance of *Firmicutes*, when compared to control 1. Conversely, synbiotic skyr increased the abundance of *Actinobacteria* and decreased the abundance of *Bacteroidetes* and *Firmicutes*, when compared to control 2. Fig. 8 shows the ratio of bacterial phyla *Firmicutes* to *Bacteroidetes* (F/B ratio).

At the genus level, the taxonomic assignment performed in ascending colon vessels in SHIME® model showed that probiotic skyr increased the

abundance of *Bacteroides*, *Coriobacteriaceae* UCG-003 and *Lactobacillus,* as well as decreased the abundance of *Bifidobacterium*, *Faecalibacterium* and *Veillonella*, when compared to control 1. Synbiotic skyr increased the abundance of *Bifidobacterium*, *Coriobacteriaceae* UCG-003 and *Lactobacillus* and decreased the abundance of *Bacteroidetes*, *Faecalibacterium* and *Parabacteroides*, when compared to control 2.

A more detailed OTU-level based pairwise comparison was performed between control period 1 vs. probiotic skyr and control period 2 vs. synbiotic skyr (DESeq analysis, $p < 0.05$; Fig. 9a and 9b). Signatures of the genera belonging to the phylum *Bacteriodetes* (*Bacteroides*), *Firmicutes* (*Acidaminococcus* and *Faecalibacterium*) and *Actinobacteria* (*Bifidobacterium* and *Coriobacteriaceae* UCG−003) were higher abundant in probiotic skyr. Whereas for the synbiotic skyr were *Bacteriodetes* (*Bacteroides*), *Firmicutes* (*Lactobacillus* and *Streptococcus*) and *Actinobacteria* (*Bifidobacterium* and *Coriobacteriaceae* UCG−003).

3.3. Metabolic activity: Ammonia (NH_4^+) and short chain fatty acids (SCFA) production

In this study, both treatments (probiotic and synbiotic skyr) increased ($p \leq 0.05$) all SCFA concentrations in the ascending colon vessels (Table 2). An increase in ammonium levels was observed during the treatment with probiotic skyr ($p < 0.0001$). Conversely, the treatment with synbiotic skyr had no significant effect ($p > 0.05$) on the ammonium concentrations.

4. Discussion

Currently, there is a growing interest in functional foods with high protein content, as a promising strategy for weight loss, providing the benefits of decreasing fat mass, increasing lean mass, and improving satiety (Johnston et al., 2017). Many studies have shown the health benefits of probiotics and prebiotics (Granato et al., 2020; Cunningham et al., 2021). However, there is little knowledge of how high-protein foods can affect the gut microbiota. In this

way, we evaluated the influence of probiotic and synbiotic skyr yogurts on the gut microbiota using dynamic fermentation microbiome model.

One of the premises of the probiotic microorganisms is to enrich the colon with beneficial bacteria, but these microorganisms must survive gastric, enzymatic and bile salts stress during their passage through the gastrointestinal tract (Liu et al., 2019). In this way, probiotic and synbiotic skyr yogurts reached the ascending colon vessels with approximately 7.5 log CFU mL^{-1}. According to Hill et al. (2014) a product can be considered probiotic when it offers a minimum viable count of sufficient strains to exert beneficial effects on health. In additional, *B. lactis* Bb-12 is one of the most used strains in the manufacture of probiotic dairy products (Jungersen et al., 2014). This strain has high tolerance to gastric acid and bile salts, inhibits pathogenic microorganisms through the production of antimicrobial compounds, improves constipation, reduces the incidence and duration of diarrheal episodes, and increases resistance to common respiratory infections (Prasanna et al., 2014). In this study, probiotic and synbiotic skyr yogurts showed to be a good matrix to carry *B. lactis* Bb-12.

To our knowledge, this is the first study to assess the impact of probiotic and synbiotic high-protein yogurts on the human gut microbiota. One of the key parameters that provides information about a microbiome is the alpha diversity index, as it describes the species richness based on the OTU count (Calle, 2019). Our study did not show significant changes in the alpha index between the control periods and after skyr yogurts treatments. However, our results showed significant positive relationships between the beta diversity of skyr yogurts treatments and gut microbiota. These results agree with Aslam et al. (2020) which showed that neither casein or whey nor quantity of dairy consumed modify the gut bacterial taxa from phylum to species level and overall diversity. However, beta diversity is the measure for differences between samples from different groups (Lkhagva et al., 2021). In this study, beta diversity showed ($p < 0.05$) difference between treatments and control periods.

In control periods we found the major bacterial groups which colonize the adult human gut: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* (Rinninella et al., 2020). However, it was observed after probiotic skyr treatment an increase of phylum *Bacteroidetes* and a decrease of *Firmicutes*.

The proteolytic activity in the large intestine of human has been mainly attributed to the genera *Bacteroides.* Members of the phylum *Bacteroidetes* are responsible for breaking down the nondigestible components of diet and producing metabolites, such as SCFA (Johnson et al., 2017). An increase in *Bacteroidetes* species is correlated with weight loss in overweight and obese individuals, increased sensitivity to insulin, reduced inflammation, and a higher proportion of regulatory T cells in peripheral blood (Gibiino et al., 2018). Studies showed that the microbiota of obese subjects are more enriched with bacteria of the phylum *Firmicutes* and less with *Bacteroidetes* (Ley et al., 2005; Ridaura et al., 2013). Species of *Firmicutes* species are more efficient at extracting energy from foods, thus promoting a greater absorption of calories and consequently an increase in body weight (Magne et al., 2020). When in greater proportion in the gut microbiota, they cause a reduction in the secretion of anorexigenic hormones such as glucagon like peptide-1 (GLP-1), peptide YY (PYY), and leptin. Also, decreased satiety, increased fat storage in adipose tissue, and damage to the intestinal barrier, contributing to lipopolysaccharide (LPS) translocation and inflammation (Perry et al., 2016). However, these findings are still conflicting in the literature, as studies have shown divergent results. The F/B ratio is multifactorial and depends on several factors, such as genetics, body composition and comorbidities (Abenavoli et al., 2019).

In this study, the main genera that represented the *Bacteroidetes* phylum was *Bacteroides*. This genus is usually associated with the consumption of diets rich in animal foods (high fat and high protein) and low in fiber (Wu et al., 2011). In additional, *Bacteroides* secrete proteases with presumed activity near the brush border of absorptive cells (Zhao et al., 2019). Probably, the increase in this genus may be related to the protein content of probiotic skyr yogurt. According to Wexler (2007), species of *Bacteroides*

genus maintain a complex and generally beneficial relationship with the host, but in cases of dysbiosis they can cause bacteremia and abscess formation at multiple sites in the body.

The administration of synbiotic skyr yogurt resulted in increase of *Actinobacteria* abundance. This phylum represents a small percentage of the four main phyla of the microbiota. However, they are essential for the maintenance of intestinal homeostasis (Binda et al., 2018). In this study, the main family represented by the *Actinobacteria* were *Coriobacteriaceae* UCG-003 and the main genera was *Bifidobacterium*. Interesting, *Coriobacteriaceae* family is recognized for its role in glucose homeostasis, bile salt metabolism, lipid metabolism, and conversion of polyphenols, steroids, and phytoestrogens. In this way, an increase in species of this family may contribute to health promotion (Liu et al., 2018).

Bifidobacterium genera has been associated with a reduction of serum levels of ammonium ions, production of B vitamins, modulation of the immune system and production of SCFA, especially butyrate (Fernandez-Julia et al., 2021). Probably, the addition of FOS in synbiotic skyr yogurt may have contributed to these findings since FOS is bifidogenic (Rezende et al., 2021). FOS is a complex carbohydrate highly resistant to the hydrolytic action of digestive enzymes, reaching the colon intact. It currently dominates the category of prebiotic ingredients, as it is recognized for modulating the composition and metabolism of the gut microbiota (Farias et al., 2019). In additional, *Bifidobacterium* exert an inhibitory effect on the growth of pathogenic species, such as *Escherichia coli*, *Salmonella* and *Clostridium perfringens* through the reduction of pH, acetate, butyrate, and lactate production (Zhang et al., 2019).

Our results showed a substantial increase ($p \leq 0.05$) in SCFA production during the probiotic and synbiotic skyr treatments. Several amino acids released from dietary protein in the large intestine are precursors for SCFA synthesis. For example, acetic acid can be produced by the gut bacteria from glycine, alanine, threonine, glutamate, lysine, and aspartate. Propionate can be formed from alanine and threonine. In additional, butyrate can be

produced from glutamate and lysine (Zhao et al., 2019). Therefore, SCFA play a major role in maintaining the healthy structure of the gut. SCFA production varies throughout the colon and the highest concentrations are found in the proximal portion (Holscher, 2017). They are used as an energy source for colonocytes, promote intestinal pH reduction, maintain intestinal homeostasis, suppress inflammation, and contribute to the immune system (Xu et al., 2022). In additional, SCFA play a key role in the inflammatory signaling cascade, lipid and glycemic metabolism, and appetite control (Chambers et al., 2015).

The administration of probiotic skyr yogurt significantly increased the concentration of ammonia ion. Gut microbiota produces ammonia through the fermentation of the proteins. Most of ammonia are absorbed in the colon and transported to the liver, where they are transformed into urea, and then excreted in the urine (Scott et al., 2013). This process is responsible for maintaining a low blood concentration of ammonia, as high concentrations can cause metabolic acidosis and dysbiosis (Adeva et al., 2012). Fecal ammonia concentrations increase with an increase in dietary protein intake (Scott et al., 2013). The accumulation of these ions in the colon can negatively impact intestinal cell morphology and may act as a tumor promoter in the gut. In addition, it may cause an increase in intestinal permeability, allowing LPS and uremic toxins to reach the circulatory system and cause inflammation (Davila et al., 2013). In humans, fecal ammonia concentrations range from 12 to 30 mmol (Scott et al., 2013).

The administration of probiotic skyr yogurt significantly increased the concentration of ammonia. Considering that ammonia is the main metabolite produced by the fermentation of proteins by microbiota, the increase of these ions in this treatment was probably associated with the presence of proteins in its composition (~19 g/200 g of yogurt). However, ammonia concentrations in synbiotic skyr yogurt remained stable when compared to control 2 period ($p > 0.05$). This found may be attributed to the increase in *Bifidobacterium* abundance observed during this treatment. According to Ghoddusi and Tamime (2014) species of *Bifidobacterium* are commonly associated with a decrease of putrefactive products, such as ammonia.

5. Conclusion

Our results suggest that the administration of high-protein yogurts (probiotic and synbiotic skyr) provided a positive effect on the intestinal microbiota. Probiotic and synbiotic skyr yogurts altered the composition and improved bacterial metabolism in SHIME® model. Probiotic and synbiotic skyr yogurts can be considered functional foods and may offer a new perspective to produce foods with potential beneficial effects on health. Finally, clinical studies will be necessary to elucidate the relationship between dietary protein and gut microbiota as well as the interaction of microbial function and host health.

Authors' contributions

Fernanda Campos Freire: Conceptualization, Methodology, Formal analysis and investigation, Writing – original draft preparation, review and editing. **Giulio Kai Saragiotto:** Conceptualization, Writing – review and editing. **Victoria Mesa:** Formal analysis, Methodology. **Adilson Sartoratto:** Formal analysis, Methodology. **Katia Sivieri:** Conceptualization, Writing – review and editing, Funding acquisition, Supervision.

Author statement

All authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Table 1. Centesimal composition of probiotic and synbiotic skyr yogurts.

Parameters	**Probiotic skyr**	**Synbiotic skyr**
Total solid (g 100 g^{-1})	17.13 ± 0.31	21.63 ± 1.66
Moisture (g 100 g^{-1})	82.87 ± 0.31	78.37 ± 1.66
Ash (g 100 g^{-1})	0.97 ± 0.03	0.44 ± 0.06
Protein (g 100 g^{-1})	9.67 ± 0.20	8.28 ± 0.42
Fat (g 100 g^{-1})	nd	nd
Dietary fiber (g 100 g^{-1})	nd	5.39 ± 0.30

Average ± standard deviation (n=6). nd: not detectable.

Table 2. SCFA (mmol/L) and NH_4^+ (mmol/L) concentrations in the ascending colon vessels during the experimental protocol.

	Acetic acid	t(p)	**Propionic acid**	t(p)	**Butyric acid**	t(p)	**NH_4^+**	t(p)
Control 1	9,37 ± 0,79	-16,52	2,34 ± 0.07	-18,02	2,46 ± 0,53	-16,51	11,73 ± 0,79	-14,68
Probiotic skyr	23,65 ± 2,31	(< 0,0001)	16,72 ± 1.94	(< 0,0001)	13,40 ± 1,67	(< 0,0001)	22,33 ± 1,07	(< 0,0001)
Control 2	3,36 ± 1,49	-14,02	2,31 ± 0,64	-6,57	2,49 ± 0,84	-4,98	15,99 ± 0,22	0,19
Synbiotic skyr	18,51 ± 2,14	(< 0,0001)	7,00 ± 1,87	(0,0012)	4,86 ± 0,83	(0,0041)	15,70 ± 3,52	(0,8540)

Averages ± standard deviation (n= 9); Student's t-test ($p \leq 0.05$). Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

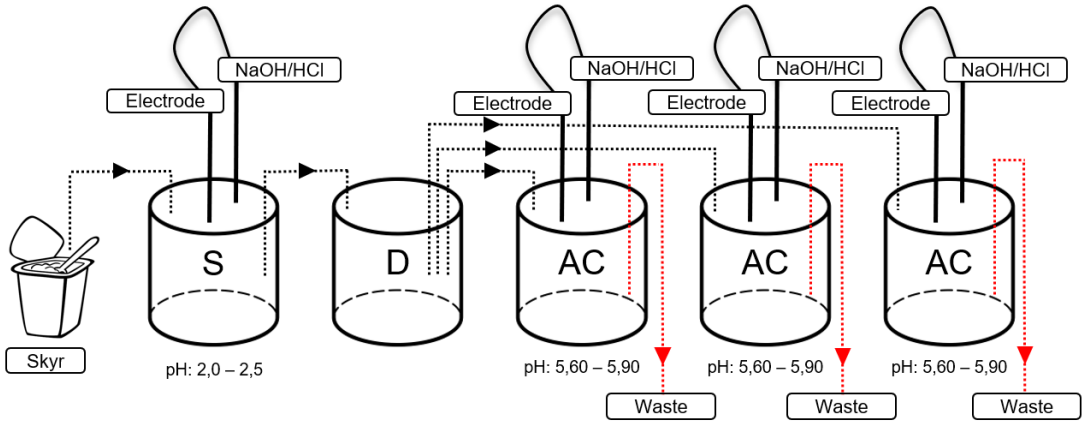

Fig. 1. Schematic representation of the SHIME®. S= stomach; D=duodenum; AC = ascending colon.

Fig. 2. Schematic representation of the experimental protocol used in SHIME®. Stabilization (carbohydrate-based medium); Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

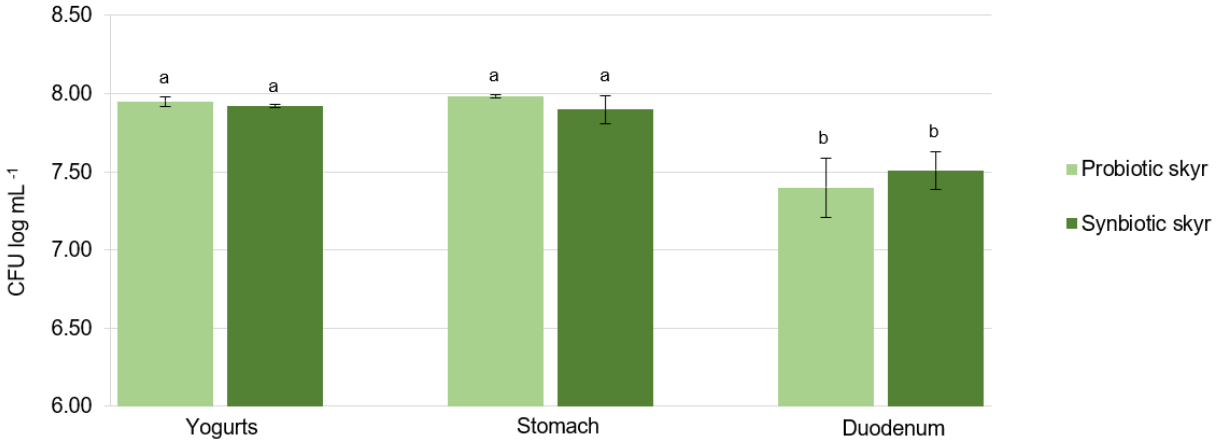

Fig. 3. Survival of *B. lactis* Bb-12 after the incubation in vessels representing the stomach and duodenum. Different letters presented significant differences in the Student's T-test for pairwise comparisons ($p \leq 0.05$).

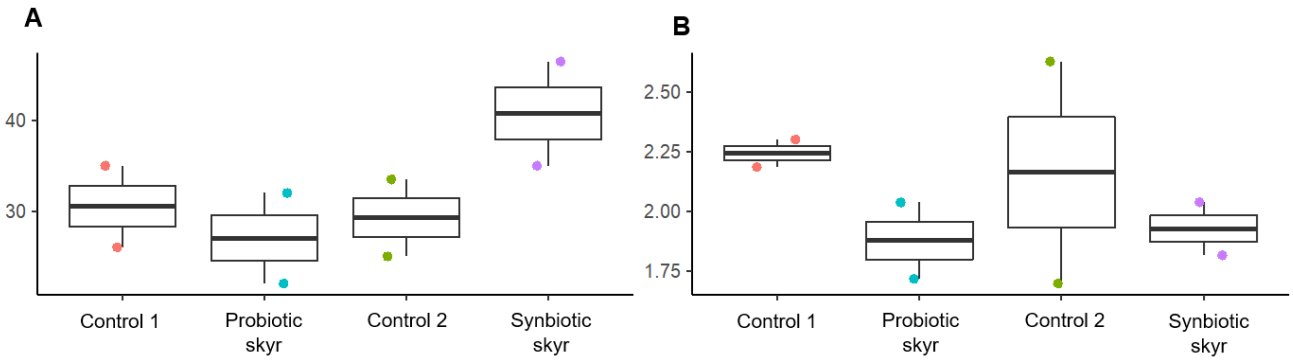

Fig. 4. Alpha diversity metrics of the gut microbiota using the SHIME® model. (A) Chao1 index (p = 0.2348) and (B) Shannon diversity index (p = 0.5385) over the course of the probiotic and synbiotic skyr treatments. Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

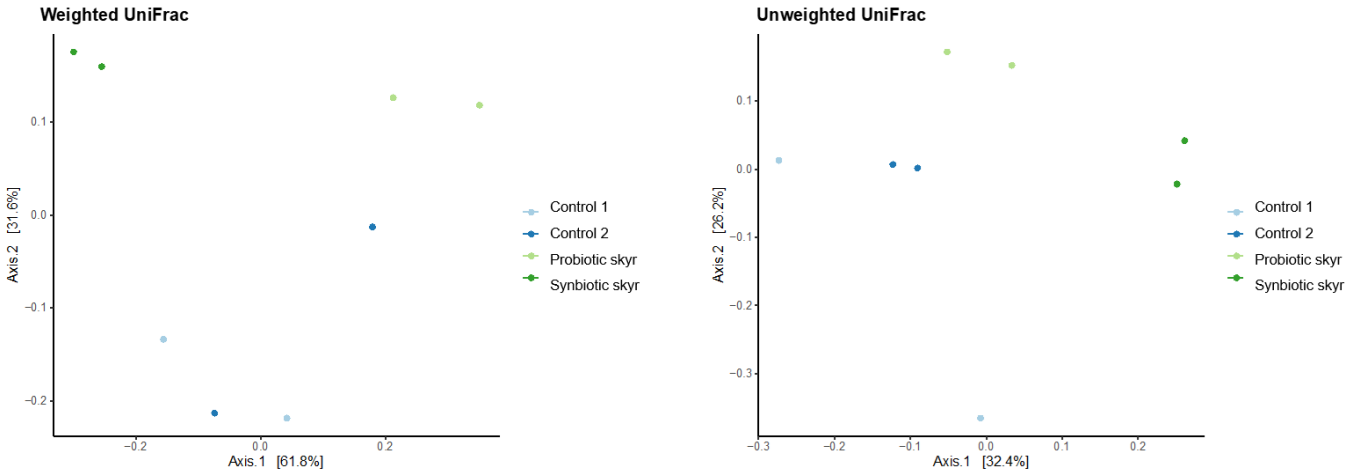

Fig. 5. Principal coordinates analysis (PCoA) of bacterial beta diversity based on weighted and unweighted UniFrac distances of the gut microbiota. Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

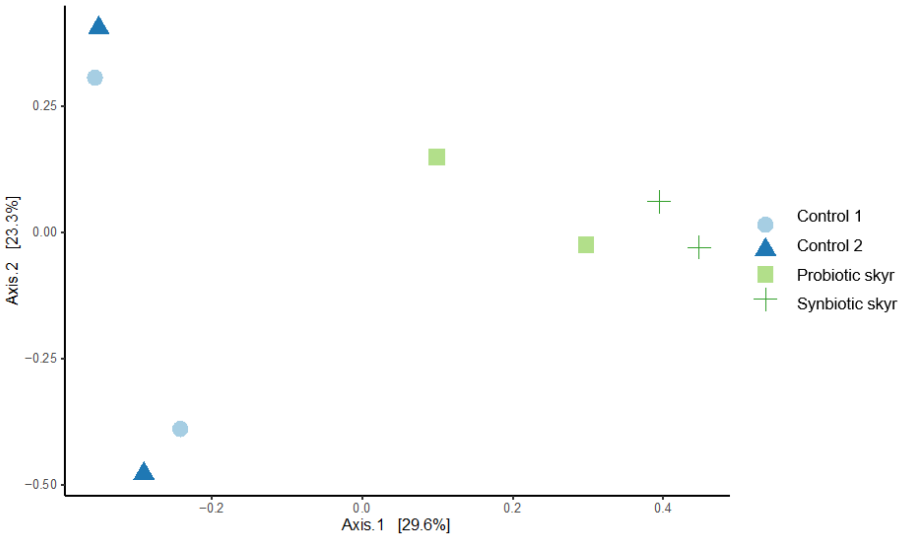

Fig. 6. Principal coordinates analysis (PCoA) of bacterial beta diversity based on Bray-Curtis dissimilarity of the gut microbiota. Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

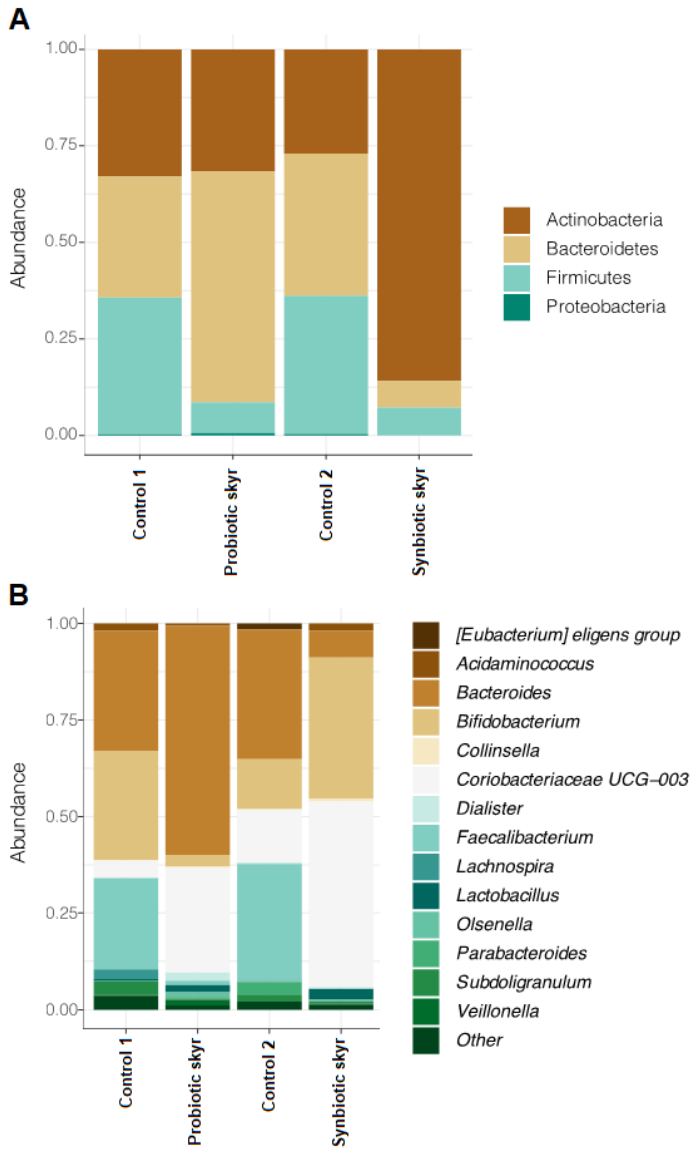

Fig. 7. Relative abundance of different phyla (A) and genera (B) in the microbiota during the experimental protocol in SHIME®. Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

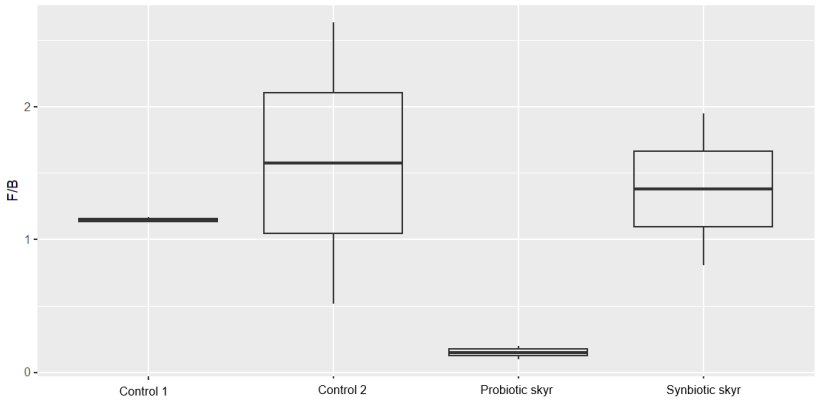

Fig. 8. The ratio of *Firmicutes* to *Bacteroidetes* (F/B) boxplot. Control 1 (carbohydrate-based medium + artificial pancreatic juice); Probiotic skyr (carbohydrate-based medium + probiotic skyr + artificial pancreatic juice); Control 2 (carbohydrate-based medium + artificial pancreatic juice); and Synbiotic skyr (carbohydrate-based medium + synbiotic skyr + artificial pancreatic juice).

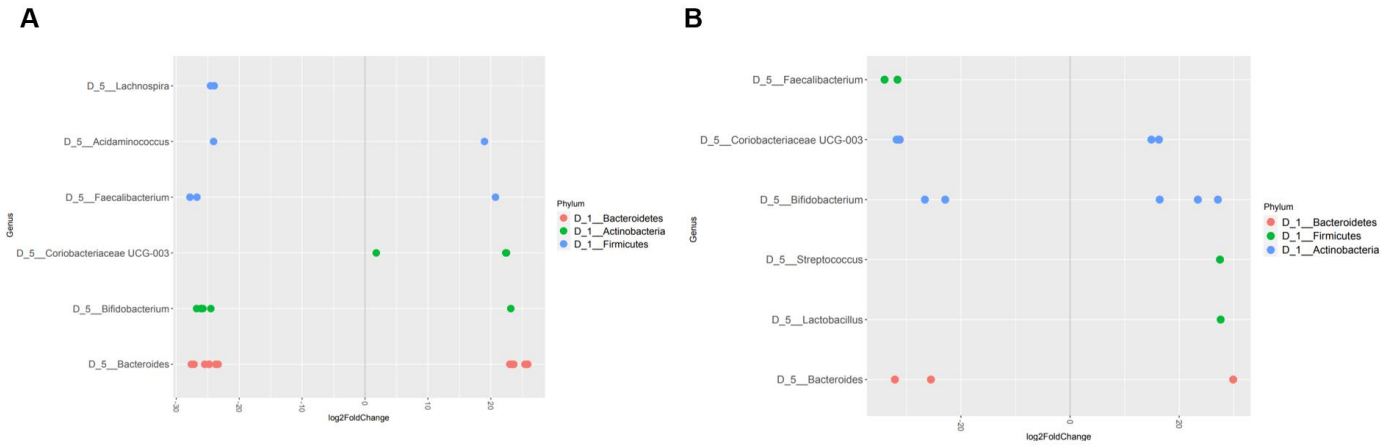

Fig. 9. Pairwise comparison (DeSeq analysis). Differential abundant OTUs ($p < 0.05$) in probiotic skyr (A) and synbiotic skyr (B) in AC vessels. OTUs were assigned to genus (y-axis) and amount of respective OTUs (when >1) are shown in brackets. Negative “log2 Fold Change” values (x-axis) indicate for higher abundance in Control period and Positive values indicate higher abundances in probiotic skyr

CONSIDERAÇÕES FINAIS

A partir dos resultados apresentados, conclui-se que:

1. Os iogurtes tipo *skyr* desenvolvidos apresentaram alto teor de proteínas e boas características sensoriais.
2. Em ambos os iogurtes, a viabilidade *do B. lactis* Bb-12 foi preservada durante a passagem pelo trato gastrointestinal simulado.
3. Ambos os iogurtes exerceram efeitos positivos sobre a microbiota intestinal, uma vez que alteraram a composição da comunidade microbiana e aumentaram a produção de ácidos graxos de cadeia curta.
4. A análise de sequenciamento do gene 16S rRNA demostrou que ambos os iogurtes exerceram um efeito positivo sobre a microbiota intestinal, em termos de composição.
5. Estudos *in vivo*, especialmente estudos clínicos, são necessários para uma comprovação dos resultados encontrados *in vitro*.