ELISÂNGELA RAMIERES GOMES

DESENVOLVIMENTO E CARACTERIZAÇÃO FÍSICO-QUÍMICA E MICROESTRUTURAL DE IOGURTES

Tese apresentada a Universidade Federal de Viçosa como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos para obtenção do título de *Doctor Scientiae.*

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RESUMO

GOMES, Elisângela Ramieres, D.Sc, Universidade Federal de Viçosa, janeiro de 2022. **Desenvolvimento e caracterização físico-química e microestrutural de iogurtes.** Orientador: Ítalo Tuler Perrone. Coorientadores: Antônio Fernandes de Carvalho e Rodrigo Stephani.

Produtos lácteos fermentados são amplamente consumidos em todo mundo devido as suas características sensoriais e nutricionais. Nos últimos anos houve um aumento da demanda por parte dos consumidores e também por órgãos governamentais para o desenvolvimento de produtos com menores teores de açúcares e gorduras. No entanto, é um desafio para a indústria de alimentos reduzir a gordura e açúcar de produtos fermentados como o iogurte, porque esses componentes influenciam no sabor, textura e viscosidade desses produtos. Portanto, o objetivo desse trabalho foi o desenvolvimento e caracterização de iogurtes (i) iogurtes adicionados de leitelho, com consequente redução de gordura; (ii) adição de fibras solúveis como substituto parcial do açúcar e (iii) adição de fibras solúveis em iogurte com alta proteína sem adição de gordura e sem açúcar. Foram realizadas análises de composição, acompanhamento da curva de fermentação, análise do perfil de textura, viscosidade aparente, capacidade de retenção de água, sinérese, distribuição do tamanho de partículas por difração à laser, microscopia eletrônica de varredura e tamanho médio dos poros dos diferentes géis formados. Como resultados pode-se destacar no artigo 1 o iogurte adicionado de 3,34% $m \cdot m^{-1}$ de leitelho (T3) no final da fermentação apresentou o menor tamanho de partícula quando comparado ao controle. A adição do leitelho promoveu à formação de uma microestrutura de rede menos compactada, apresentando menor elasticidade após 21 dias e menor dureza após 21 e 42 dias. No artigo 2, são apresentados os resultados que indicaram que as fibras solúveis (fibra de milho e polidextrose) empregadas no iogurte como substitutas parciais do açúcar, impactaram a microestrutura (tamanho dos poros) na e sinérese. A redução do nível de sacarose do iogurte de 9% para 0% levou a uma diminuição no tamanho dos poros da matriz e aumento da sinérese. Além disso, a adição de 2% de fibra de milho ou polidextrose no iogurte com redução de 50% do teor de sacarose quando comparado ao controle, aumentou o tamanho dos poros da matriz e diminuiu a sinérese. E no artigo 3, os dados mostraram que os iogurtes com alta proteína suplementados com

10% de fibra de milho 70%, fibra de milho 85% ou polidextrose proporcionaram uma maior queda de pH entre 3 e 6 horas de fermentação. Além disso, foi possível constatar que todos os iogurtes apresentaram uma alta capacidade de retenção de água. Os valores de d_{90} diminuíram conforme aumentou as concentrações das fibras solúveis adicionadas. Os resultados obtidos dos diferentes estudos abrem perspectivas para produção de iogurtes contendo diferentes ingredientes como leitelho e as fibras solúveis.

Palavras-chave: Iogurte. Textura. Microestrutura. Fibras solúveis.

ABSTRACT

GOMES, Elisângela Ramieres, D.Sc, Universidade Federal de Viçosa, January 2022. **Development, physical-chemical and microstructural characterization of yogurts.** Advisor: Ítalo Tuler Perrone. Co-advisors: Antônio Fernandes de Carvalho and Rodrigo Stephani.

Fermented dairy products are widely consumed worldwide due to their sensory and nutritional characteristics. In recent years there has been an increase in demand from consumers and also from government agencies for the development of products with lower levels of sugars and fats. However, it is a challenge for the food industry to reduce fat and sugar in fermented products such as yogurt, because these components influence the flavor, texture and viscosity of these products. Therefore, the objective of this work was the development and characterization of yogurts (i) yogurts added with buttermilk, with consequent fat reduction; (ii) addition of soluble fiber as a partial substitute for sugar and (iii) addition of soluble fiber in high-protein, fat-free and sugar-free yogurt. Composition analysis, fermentation curve monitoring, texture profile analysis, apparent viscosity, water holding capacity, syneresis, particle size distribution by laser diffraction, scanning electron microscopy and average pore size of the different formed gels. As results, it can be highlighted in article 1, the yogurt added with 3.34% $p \cdot p^{-1}$ of buttermilk (T3) at the end of fermentation showed the smallest particle size when compared to the control. The addition of buttermilk promoted the formation of a less compacted network microstructure, showing less elasticity after 21 days and less hardness after 21 and 42 days. In article 2, the results are presented that indicated that the soluble fibers (corn fiber and polydextrose) used in yogurt as partial substitutes for sugar, impacted the microstructure (pore size) in and syneresis. Reducing the sucrose level of yogurt from 9% to 0% led to a decrease in the pore size of the matrix and an increase in syneresis. In addition, the addition of 2% corn fiber or polydextrose in yogurt with a 50% reduction in sucrose content when compared to the control, increased the pore size of the matrix and decreased syneresis. And in article 3, the data showed that high protein yogurts supplemented with 10% 70% corn fiber, 85% corn fiber or polydextrose provided the greatest pH drop between 3 and 6 hours of fermentation. In addition, it was possible to verify that all yogurts had a high-water retention capacity. The d90 values decreased as the concentrations of added soluble fibers increased. The results obtained from the different studies open perspectives for the production of yogurts containing different ingredients such as buttermilk and soluble fibers.

Keywords: Yogurt. Texture. Microstructure. Soluble fibers.

SUMÁRIO

1- INTRODUÇÃO GERAL

Iogurte é definido de acordo com a IN 46 (2007), como o produto obtido da fermentação realizada com os cultivos protosimbióticos de *Streptococcus salivarius* subsp. *thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus*, aos quais podem acompanhar, de forma complementar, outras bactérias ácido-lácticas que, por sua atividade, contribuem para a determinação das características do produto final. Sendo que, essas bactérias devem estar viáveis, ativas e abundantes no produto final e durante seu prazo de validade (BRASIL, 2007).

O consumo médio de iogurte no Brasil deverá chegar a 8,87 kg por pessoa em até o final desse ano de 2022 segundo dados do STATISTA MARKET FORECAST, (2022). O iogurte é um produto versátil que pode ser consumido desde sobremesa, café da manhã ou como alimento nutricional (SILVA; ALBERTO, 2020). De acordo com dados da Embrapa, 59% dos consumidores manteve o consumo de iogurte durante a pandemia de COVID-19 (SIQUEIRA, 2020). Esses dados reforçam a relevância desse produto tanto do ponto de vista mercadológico, como nutricional para os consumidores.

Produtos fermentados como o iogurte são consumidos em todo mundo por proporcionar benefícios à saúde além de suas características sensoriais. Esses alimentos possuem em sua composição proteínas de alta qualidade nutricional, gorduras de fácil digestão e abundância de cálcio e fósforo biodisponíveis (MIR; RASTOGI; HARIPRIYA, 2021). O aumento da conscientização da população para o consumo de alimentos mais saudáveis, tem feito com que a indústria de alimentos desenvolva novas formulações de iogurtes como: sem ou reduzido em açúcar/gordura, adicionados de probióticos e/ou prebióticos, fibras dietéticas entre outros (MUDGIL et al., 2016).

O leitelho é o produto obtido da fabricação da manteiga, que possui em sua composição proteínas, glicoproteínas e fosfolipídeos, com potencial funcional (MORIN; POULIOT; JIMÉNEZ-FLORES, 2006). O leitelho em pó pode ser adicionado em vários produtos como substituto parcial da gordura do leite no desenvolvimento de produtos (ZHAO et al., 2018).

A adição de fibras ou leitelho (produto obtido a partir da fabricação da manteiga) ao iogurte é uma forma inovadora de contornar os problemas causados pela redução do açúcar/gordura, uma vez que esses ingredientes podem melhorar muitas propriedades do iogurte, como sinérese, microestrutura, reologia, características sensoriais, viabilidade de bactérias de ácido láctico, tempo de fermentação e propriedades funcionais (APORTELA-PALACIOS; SOSA-MORALES; VÉLEZ-RUIZ, 2005; DELLO STAFFOLO et al., 2004; ESPÍRITO-SANTO et al., 2013; ESPÍRITO SANTO et al., 2012; IZYDORCZYK et al., 2008; MUDGIL; BARAK; KHATKAR, 2016; PASEEPHOL; SMALL; SHERKAT, 2008; PERRIGUE; MONSIVAIS; DREWNOWSKI, 2009; RAMIREZ-SANTIAGO et al., 2010).

Diversas fibras são utilizadas como agentes de volume para melhorar a textura de alimentos com baixo teor de açúcar, devido à sua alta solubilidade e baixo teor calórico (AUERBACH et al., 2007). Vários estudos relatam o efeito positivo da adição de polidextrose ao leite fermentado, iogurte e sobremesas congeladas como um substituto parcial de gordura ou açúcar; as pesquisas mostraram uma redução na sinérese e pontuações mais altas na aparência, cor e textura (BISAR et al., 2015; SPECTER; SETSER, 1994; SRISUVOR et al., 2013). Já a fibra solúvel do milho auxilia na redução das respostas glicêmicas e insulinêmicas e aumenta a absorção de cálcio nos ossos (KENDALL et al., 2008; PALACIOS et al., 2020). Por ser uma fibra solúvel contribui para o aumento da viscosidade da fase contínua diminuindo a sinérese em iogurte.

Considerando as informações expostas anteriormente, o objetivo do presente trabalho é analisar as características microestruturais, distribuição do tamanho de partículas, textura de diferentes formulações de iogurte, reduzido em açúcar, gordura, e adicionados de leitelho ou adicionado das fibras de milho e polidextrose, conforme descrito nos diferentes artigos:

- **Artigo 1:** Addition of buttermilk powder to yogurt: effects on particle size, microstructure and texture
- **Artigo 2:** Effect of sugar reduction and addition of corn fibre and polydextrose on pore size and syneresis of yoghurt

- **Artigo 3:** Efeito da adição de diferentes fibras solúveis em iogurtes com alta proteína na capacidade de retenção de água, distribuição do tamanho de partículas, viscosidade aparente e microestrutura

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Abstract
The addition of buttermilk powder as partial fat replacer in yogurt formulations with constant dry matter was investigated. Three formulations of yogurt were produced containing 0% (T1), 1.36% $w \cdot w^{-1}$ (T2) and 3.34% $w \cdot w^{-1}$ (T3) of buttermilk powder in the final product. Particle size and pH variation were monitored during fermentation; scanning electron microscopy and texture profile analysis were performed in the final product. The control sample showed larger particle size on the day after production and at the end of fermentation, as well as a more compact network microstructure with a smaller average pore size. Compared to the prototypes with added buttermilk the control sample showed greater higher firmness. Buttermilk powder could act as fat replacer for yogurt but favors the formation of a less compacted network microstructure, with large pores, less springiness after 21 days, and less hardness in the two evaluated times (21 and 42 days).
Keywords: Buttermilk; Particle size; Phospholipids; Fermentation.

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Keywords: Buttermilk; Particle Size; Phospholipids; Fermentation.

Resumo

A adição de leitelho em pó como substituto parcial da gordura em formulações de iogurte com matéria seca constante foi investigada. Foram produzidas três formulações de iogurte contendo 0% (T1), 1,36% $p \cdot p^{-1}$ (T2) e 3,34% $p \cdot p^{-1}$ (T3) de leitelho em pó no produto final. O tamanho das partículas e a variação do pH foram monitorados durante a fermentação; microscopia eletrônica de varredura e análise do perfil de textura foram realizadas no produto final. A amostra controle apresentou maior tamanho de partícula no dia seguinte à produção e no final da fermentação, bem como uma microestrutura de rede mais compacta com menor tamanho médio de poro. Em comparação com os protótipos com leitelho adicionado, a amostra de controle apresentou maior firmeza. O leitelho em pó pode atuar como substituto da gordura do iogurte, mas favorece a formação de uma microestrutura de rede menos compactada, com poros dilatados, menor elasticidade após 21 dias e menor dureza nos dois tempos avaliados (21 e 42 dias).
Palavras-chave: Leitelho; Tamanho da Partícula; Fosfolipídios; Fermentação.

Resumen

Se investigó la adición de suero de leche en polvo como sustituto de grasa parcial en formulaciones de yogur con materia seca constante. Se produjeron tres formulaciones de yogur que contenían 0% (T1), 1,36% $p \cdot p^{-1}$ (T2) y 3,34% $p \cdot p^{-1}$ (T3) de suero de leche en polvo en el producto final. El tamaño de las partículas y la variación del pH se controlaron durante la fermentación; Se realizaron microscopía electrónica de barrido y análisis del perfil de textura en el producto final. La muestra de control mostró un tamaño de partícula más grande el día después de la producción y al final de la fermentación, así como una microestructura de red más compacta con un tamaño de poro promedio más pequeño. En comparación con los prototipos con suero de leche añadido, la muestra de control mostró una mayor firmeza. El suero de leche en polvo podría actuar como sustituto graso del yogur pero favorece la formación de una microestructura de red menos compactada, con poros dilatados, menor elasticidad a los 21 días y menor dureza en los dos tiempos evaluados (21 y 42 días).
Palabras clave: Suero de Leche; Tamaño de Partícula; Fosfolípidos; Fermentación.

1. Introduction

Butter manufacture consists of churning cream with high fat content at high shear rates, destabilizing the oil/water emulsion with subsequent phase separation (Hickey *et al.,* 2017). One phase is rich in triglycerides that coalesce due to their hydrophobic nature, forming grains that are subsequently conjoined to produce butter (> 80% fat) (Govindasamy-Lucey *et al.,* 2006). The other phase resembling skim milk is called buttermilk and contains the components of the fat globule membrane; the latter is excluded from the lipid matrix during the butter-making process and recovered in the aqueous phase together with most proteins, lactose, and minerals

Buttermilk is rich in several proteins, glycoproteins, and phospholipids that have the potential for functional and nutraceutical applications (Morin *et al.,* 2006). Water-holding and emulsifying capacity stands out among its important functional properties, which means that buttermilk can be used in the production of foods such as dulce de leche, cheese, and yogurt (Le *et al.,* 2011; Morin *et al.,* 2008; Munck *et al.,* 1983; Roesch *et al.,* 2004). Due to its nutraceutical properties, buttermilk is considered a beneficial ingredient for infant formulas, as it is related to many health benefits such as prevention of colon cancer (Dewettinck *et al.,* 2008; Fuller *et al.,* 2013; Spitsberg, 2005; Sprong *et al.,* 2002; Ward *et al.,* 2009).

Buttermilk used as raw material in the food industry undergoes processes such as homogenization, pasteurization, dehydration by evaporation and/or spray drying (Ferreira *et al.,* 1999; Vanderghem *et al.,* 2010). Buttermilk powder can be used as a partial substitute for solids in various products as baked goods and yogurt (Hickey *et al.,* 2018; Scalbert *et al.,* 2005). The evaluation of sensory properties and volatile compounds of whole (fat) yogurt, skimmed yogurt, and skimmed yogurt added with different concentrations of buttermilk showed that buttermilk provided sensory improvements in low-fat yogurts, is therefore recommended as a partial substitute for milk fat in product development (Zhao *et al.,* 2018).

The aim of this work was to evaluate the impact of adding different concentrations of buttermilk powder and water in yogurts (keeping the solids content constant) in the texture profile, particle size, and the microstructure of the yogurt.

2. Methodology

The present work was conducted in a partnership between the Milk and Dairy Product laboratory (INOVALEITE) of the Federal University of Viçosa (UFV) and the laboratories of spectroscopy, microstructure and microanalysis of the Spectroscopy and Molecular Structure Nucleus of the Federal University of Juiz de Fora (UFJF). The experiment consisted of yogurts production supplemented with buttermilk and posterior analysis for microstructure characterization. Two independent productions were made (n=2).

2.1 Yogurt production

Yogurts were prepared from pasteurized whole milk and were standardized to 3.5% fat ($w \cdot w^{-1}$). The formulations used to produce yogurts are exposed in Table 1. The treatments consisted of standardized milk (T1), standardized milk added with 1.36% $w \cdot w^{-1}$ buttermilk (CONFEPAR®, Londrina, Brazil) and 8.64% $w \cdot w^{-1}$ of water in the final product (T2) and standardized milk added with 3.34% $w \cdot w^{-1}$ buttermilk and 21.26% $w \cdot w^{-1}$ of water in the final product (T3) (Figure 1). These buttermilk and water concentrations were defined so that the total solids remained constant at 12.5% w·w^{-1}. After sample preparation, each treatment (T1, T2, and T3) were heated to 40 °C and homogenized (APV model 1000-2000, Bydgoszcz, Poland) at 20 MPa, in a two-stages homogenizer, whereby p1 ~ 15 MPa (first stage) and (p2) ~ 5 MPa (second stage). Afterward, the mixes were heat treated (90 °C for 5 min) in a water bath, then cooled to 42 °C in an ice bath and 1% of the starter culture (composed of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*, (Delvo-YogTM, CY-340, DSL, DSM®, Liverpool, Australia) added. The fermentation was monitored until pH 4.6 ± 0.1 was reached. Then, the yogurt was cooled and the gel was broken with the aid of a plastic rod with circular movements for 30 seconds. The yogurts were placed in 130 mL packages with approximately 130 g of the yogurt, and the packages were sealed with aluminum foils and closed with plastic lids and subsequently stored at 4 ± 1 °C for 42 days.

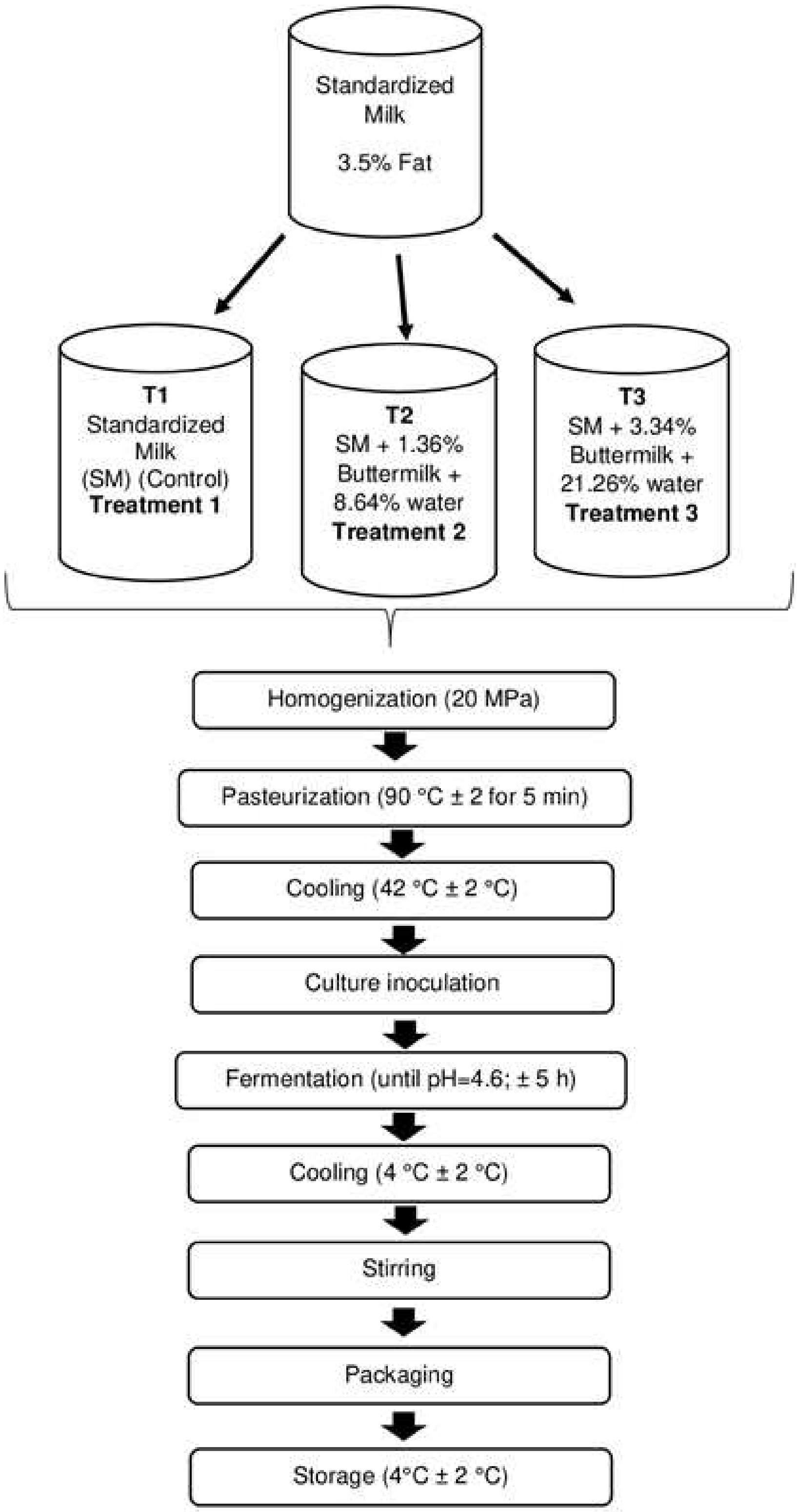
Table 1: Yogurt's formulation

	Treatments		
Ingredients	**T1 (%w·w^{-1})**	**T2 (%w·w^{-1})**	**T3 (%w·w^{-1})**
Milk	100	90	75.4
Buttermilk	-	1.36	3.34
Water	-	8.64	21.26

T1 = No added buttermilk; T2 = 1.36% buttermilk added; T3 = 3.34% buttermilk added.

Source: Research data (2021).

Figure 1. Flowchart of yogurt production



Source: Research data (2021).

2.2 Physico-chemical analysis

Samples T1, T2, and T3 were analyzed for total solids by the standard gravimetric method, total protein by the micro Kjeldahl method, fat by the Gerber (butyrometer) method, ashes by incineration at 550 °C, and lactose by the Fehling reduction method, following to the official methodology described by the Ministry of Agriculture, Livestock and Supply (MAPA) (Brasil, 2017).

2.3 Fermentation curve

Beakers with a 1000 mL capacity received 900 g of the formulations of each treatment. Fermentation was monitored using a Consort D130 system (Turnhout, Belgium), a multichannel pH meter. An electrode was inserted in each beaker and the pH was measured every minute during the whole fermentation time until the final pH of 4.6 with the assistance of a software (Data Acquisition System). Thus, it was possible to monitor the entire fermentation and evaluate the acidification rate and the time required to arrive at a pH of 4.6.

2.4 Scanning electron microscopy

A thin layer of each yogurt sample was applied individually in a double-sided adhesive carbon tape, mounted on a stub, and then frozen and lyophilized. The microstructures of the yogurt were evaluated by Scanning Electron Microscopy (SEM) (Hitachi TM 3000, Hitachi Ltd., Tokyo, Japan) as described by Mudgil *et al.* (2018). The images were recorded at different magnifications for better evaluation. The average pore sizes of the images were calculated using Image J 1.47v freeware (National Institutes of Health, USA). The images obtained from the SEM are added in the Image J, the scale is defined after the image is transformed into a black and white binary, so the pores are highlighted, so with particle analysis the average pore size is calculated. Image analyzes were performed in triplicate.

2.5 Particle size analysis

Particle size distribution was determined every hour from the dairy culture inoculation until the end of fermentation. This analysis was also carried out a day after the production of the yogurt. The Beckman Coulter LS 13 320 laser diffraction analyzer (Beckman Coulter, Miami, FL, USA) coupled to the liquid analysis module (Aqueous liquid module, Beckman Coulter, Miami, FL, USA) was used. In the equipment compartment containing water at 25 °C standard equipment, the yogurt sample was added until reaching a "Differential Intensity Scattering Polarization (PIDS)" of approximately 45% (recommendation by the manufacturer of the laser diffraction analyzer). The PIDS comprises three wavelengths 450 nm, 600 nm and 900 nm that illuminate the sample with polarized light vertically and horizontally. The calculation of the diameters was based on the theory of Mie, using the refractive indices of 1.33 for water 1.57 for protein and 1.47 for fat. The particle size diameter value below those of which 90% of the particle volume (d_{90}) found was used to characterize the formation of the gel. The analyses were performed in duplicate.

2.6 Texture

Texture analysis was performed using a texture-meter (Amatek Brookfield CT3, Middleborough, United States). All treatments were analyzed after 21 and 42 days. The yogurt samples were stored in plastic containers with a diameter of 60 mm and a height of 50 mm. A probe with a diameter of 25 mm (TA 25/1000) was used and the test was performed at a speed of 1 $mm \cdot s^{-1}$ to a depth of 10 mm and contact force was 2.0 g. Data were collected using the Texture Pro CT V1.4 Build software. Texture Profile Analysis (TPA) was performed to evaluate the parameters firmness (maximum compression force), cohesiveness (resistance to removing the sample from the probe when raised), and elasticity (extent and speed at which the sample returns to the original state after deformation force is removed).

2.7 Statistical analysis

The experiment followed a completely randomized design (CRD). Two independent yogurt productions were carried out for all treatments. Statistical analyses were performed at 5% probability using Tukey test and SPSS® statistical software.

3. Results and Discussion

3.1 Proximate composition of formulations

The composition and mass balance of the bases used for yogurt production are shown in Table 2. The results show that the added amount of buttermilk to the formulations had no significant impact on total solids, protein, and ash content ($p > 0.05$). Total solids were targeted at about 12.5 % ($w \cdot w^{-1}$). However, Table 2 shows that fat content decreased with an increase in the amount of buttermilk and water added.

Table 2- Composition and mass balance of the base mixtures for yogurt production

Mass balance	Treatment	Dry matter (% $w \cdot w^{-1}$)	Fat (% $w \cdot w^{-1}$)	Lactose (% $w \cdot w^{-1}$)	Protein (% $w \cdot w^{-1}$)	Ash (% $w \cdot w^{-1}$)
Theoretic	T1	12.53	3.5	4.9	3.5	1.1
	T2	12.59	3.2	5.0	3.5	1.3
	T3	12.66	2.8	5.1	3.6	1.5
Composition	T1	12.53 ± 0.25^a	3.5 ± 0.0^a	4.9 ± 0.0^a	3.5 ± 0.0^a	1.1 ± 0.3^a
	T2	12.40 ± 0.26^a	3.3 ± 0.0^b	5.2 ± 0.0^b	3.5 ± 0.1^a	1.4 ± 0.3^a
	T3	12.83 ± 0.05^a	3.0 ± 0.0^c	4.9 ± 0.1^a	3.6 ± 0.1^a	0.9 ± 0.1^a

Within a column, different superscripts indicate significant differences ($p < 0.05$). Data are expressed as means ± standard deviations of triplicate determinations.

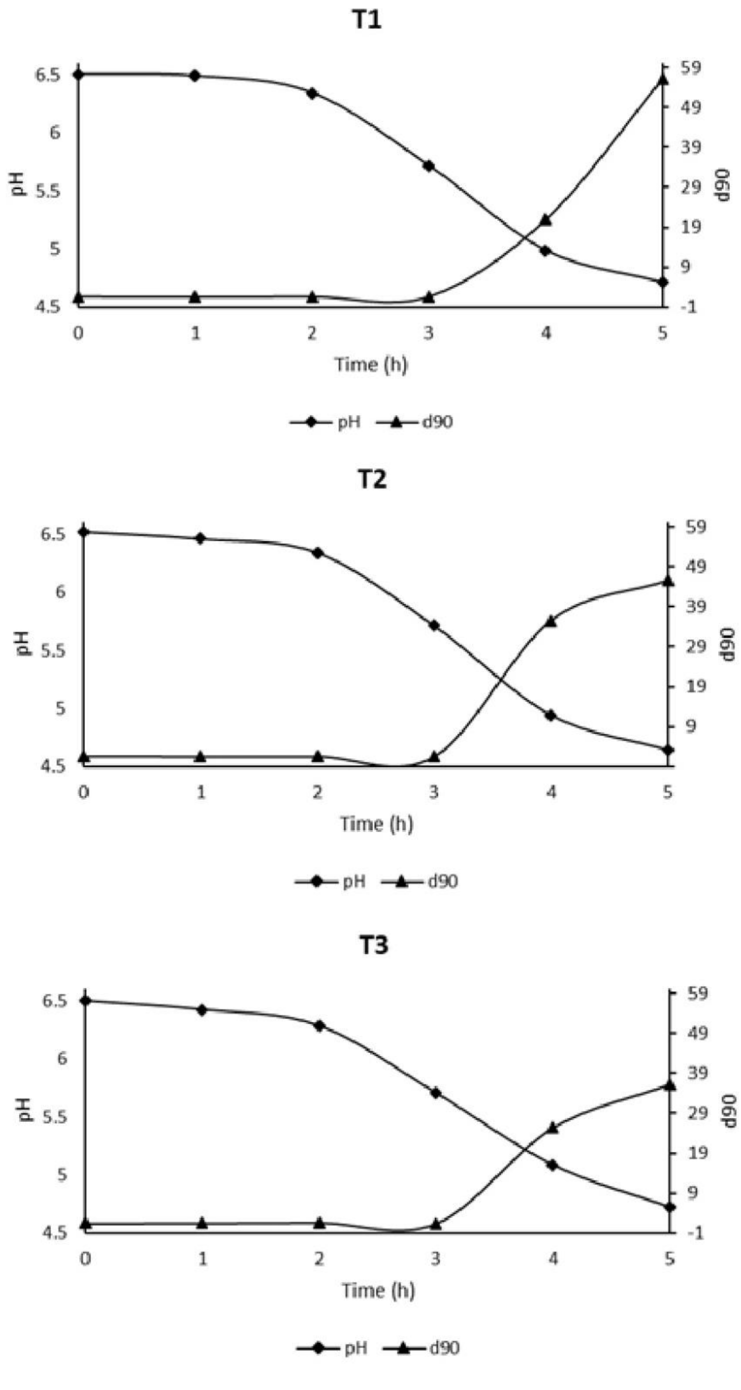
T1 = No added buttermilk; T2 = 1.36% buttermilk added; T3 = 3.34% buttermilk added.

Source: Research data (2021).

3.2 Characterization of the fermentation curve and the particle size distribution

Figure 2 shows the pH evolution and particle size distribution during the fermentation step. The fermentation curves were similar among treatments, suggesting that buttermilk's addition did not influence the fermentation time. During the first three hours of fermentation, there was a slight variation in the pH and the size distribution of particles, considering that d_{90} had sizes ranging from 1.60 ± 0.05 to 1.58 ± 0.09 µm for T1, from 1.62 ± 0.10 to 1.55 ± 0.22 µm for T2 and 1.47 ± 0.09 to 1.44 ± 0.26 µm for T3 (Table 3). After 3 hours, at pH below 5.7, the dissociation of the casein micelles begins to occur (Tamime & Robinson, 2003); this was observed in the increase in particle size, in which 90% of the particles are smaller than 20.96 ± 2.99; 35.54 ± 7.44 and 25.36 ± 4.16 µm for treatments T1, T2 and T3 respectively. With 4 hours of fermentation, T2 presented a larger particle size when compared to T1 and T3 ($p < 0.05$). This may have occurred due to the pH variation among treatments since T2 had a lower pH than the other treatments (Table 3). Already at the end of fermentation, treatment T2 with d_{90} value of 45.55 ± 6.43 µm showed no statistical difference compared to the T1 control (d_{90} = 56.22 ± 4.20 µm) and T3 (d_{90} = 36.23 ± 6.29 µm). There was a significant difference between treatments T1 and T3, with T3 showing the smallest particle size ($p < 0.05$).

Figure 2. Relationship between particle size distribution and pH of the yogurt during fermentation.



T1 = No added buttermilk; T2 = 1.36% buttermilk added; T3 = 3.34% buttermilk added.

Source: Research data (2021).

Table 3: Particle size distribution (d_{90}) and pH during fermentation of yogurt added buttermilk

Time (h)	T1		T2		T3	
	pH	d_{90} (µm)	pH	d_{90} (µm)	pH	d_{90} (µm)
0	6.51±0.01	1.60± 0.05[a]	6.53±0.04	1.62±0.10[a]	6.50±0.01	1.47±0.09[a]
1	6.49±0.11	1.52±0.08[a]	6.47±0.04	1.57±0.01[a]	6.43±0.04	1.59±0.12[a]
2	6.35±0.22	1.62±0.08[a]	6.35±0.11	1.59±0.07[a]	6.29±0.13	1.65±0.35[a]
3	5.72±0.81	1.58±0.09[a]	5.72±0.66	1.55±0.22[a]	5.71±0.57	1.44±0.26[a]
4	4.99±0.40	20.96±2.99[a]	4.95±0.23	35.54±7.44[b]	5.09±0.45	25.36±4.16[a]
5	4.72±0.16	56.22±4.20[a]	4.65±0.02	45.55±6.43[ab]	4.72±0.14	36.23±6.29[bc]

Within a column, different superscripts indicate significant differences ($p<0.05$). Data are expressed as means ± standard deviations of triplicate determinations.

T1 = No added buttermilk; T2 = 1.36% buttermilk added; T3 = 3.34% buttermilk added.

Source: Research data (2021).

The development of the gel structure is influenced by heat treatment and fat content (Xu *et al.,* 2008), which might explain the reason why the yogurts with the highest fat content (T1 and T2) presented the largest particle size. The fat globules are bound to the protein after the homogenization and heat treatment, act as pseudoprotein particles and thus are trapped in the protein matrix (Obeid *et al.,* 2020; Ciron *et al.,* 2010), causing the particles to be larger in size. The denaturation of β-lactoglobulin and α-lactalbumin during heat treatment and their association with the fat globule membrane (Ye *et al.,* 2004) also influences the size of the particles, but this alone cannot explain why yogurts without the addition of buttermilk (3.5% $w \cdot w^{-1}$ fat - T1) and yogurt with 1.36% $w \cdot w^{-1}$ buttermilk in the final product (3.3% $w \cdot w^{-1}$ fat - T2) did not differ in the size of its particles in solution, but differed from T3 (with lower fat content ~ 3.0% $w \cdot w^{-1}$).

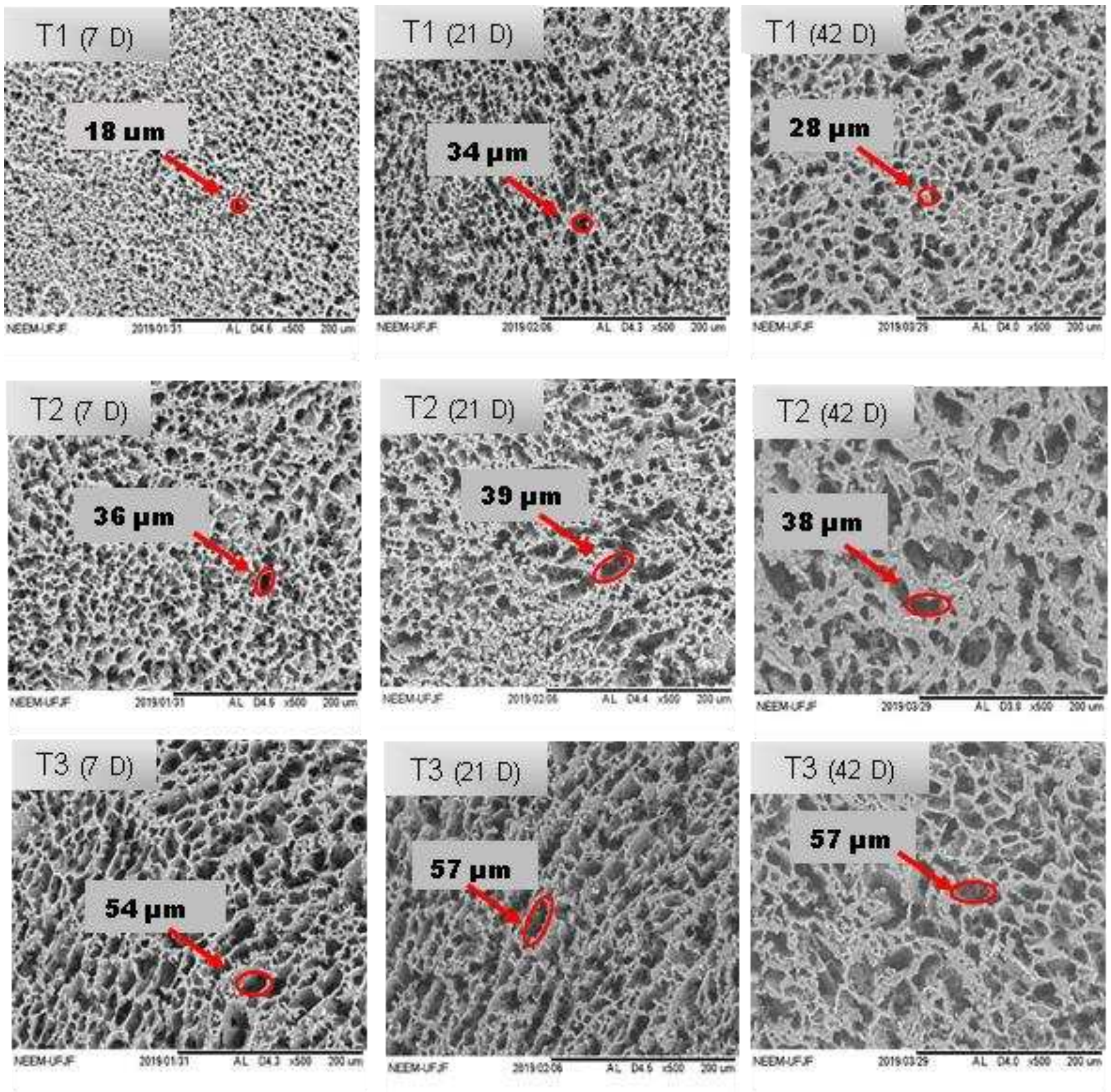
One factor that can interfere with the denaturation of whey proteins is the presence of phospholipids in the formulation (Kasinos *et al.,* 2014). The addition of phospholipid rich products such as sweet buttermilk powder and residue of cream powder in evaporated milk improves the thermal stability of this product (Kasinos *et al.,* 2014). Phospholipids can modify the secondary structure of whey proteins due to hydrophobic interactions (Kasinos *et al.,* 2014; Kristensen *et al.,* 1997), promoting an increase in heat stability (Kasinos *et al.,* 2014; Saffon *et al.,* 2014). As a greater amount of buttermilk was added to T3, the phospholipids may have interfered in the whey protein denaturation and consequently in the process of gel formation. This can explain why the particle size of treatment T3 was smaller when compared to T1 in the time of 5 h of fermentation (Table 3).

However, it is interesting to note that the structure of the gel formed showed that the yogurt without buttermilk added (T1) had the largest particle size, while treatments T2 and T3 had no statistical difference ($p>0.05$). These results show that a reduction of 5.7% (T2) or 14.3% (T3) of fat in these yogurts in relation to the control did not interfere in the particle size of the gel between treatments T2 and T3.

3.3 The microstructure of the yogurts

The microstructures of yogurts without (T1) and with (T2 and T3) added buttermilk after 7, 21, and 42 days of storage are shown in Figure 3. After 7 days of storage, the yogurt without added buttermilk (T1) presented a more compact protein network with a medium pore size of 18 µm, whereas T2 and T3 with the addition of buttermilk, showed larger pores with average sizes of 36 µm and 54 µm respectively. Romeih, Hamid & Awad, (2014) also observed in the micrographs a structure more compact, dense with less empty spaces in whole milk buffalo yogurt when compared to fat-free yogurt added 1% or 2% powdered buttermilk, and concluded that the fat globules were dispersed in the protein matrix. Another study showed that yogurt made with skimmed milk had larger pores when compared to yogurts added with buttermilk powder, in addition, when the concentrations of 2% and 4% buttermilk were added, almost all pores of the gels were filled with phospholipids (Zhao, Feng & Mao, 2020). After 21 days, the average pore size obtained by SEM was T1 = 34 µm, T2 = 39 µm, that is, the difference between the average pore size from each treatment was smaller when compared to the 7-day time, generating an attribute of hardness with the same magnitude for these treatments in 21 days. In addition, the average pore size for T3 was 57 µm, which provided a hardness attribute equal to that of the T2 treatment. However, after 42 days, T1 presented an average pore size of 28 µm, smaller than T2 with 38 µm and T3 with 57 µm, making T2 and T3 less firm than T1 (Table 3). These results indicate that the structure of the protein network, considering the attribute average pore size determined by electron microscopy, is related to the texture of the analyzed yogurts, which is apparently influenced by the levels of fat and phospholipids present.

Figure 3- Microstructure of yogurt added with buttermilk.



Magnification: 500x. Microscopy after 7, 21, and 42 days of production. T1 = No added buttermilk; T2 = 1.36% buttermilk added; T3 = 3.34% buttermilk added. The values presented by the arrows indicate the average pore size.

Source: Research data (2021).

It was observed that the average pore sizes of T1 yogurt showed great variation during the shelf life (7 days = 18 µm; 21 days = 34 µm and 42 days = 28 µm), which might be attributed to rearrangements in the protein network. Meanwhile, after 21 and 42 days, treatments T2 (21 days = 39 µm; 42 days = 38 µm) and T3 (21 days = 57 µm; 42 days = 57 µm) presented the average pore size with little or no variation (Figure 3). The latter findings suggest a role of the added buttermilk in the stabilization of the formed network.

A more compact protein network structure was observed in the scanning electron microscopy of the yogurt without the addition of buttermilk (T1), which is the treatment with higher fat content (Table 2). This was probably due to the fat globules fusing with the casein, forming aggregates with smaller empty spaces (pores) (Torres *et al.,* 2018). Another factor that may have influenced the microstructure of the yogurt gel is the denaturation of β-lactoglobulin during pasteurization, which makes it possible to form complexes between this protein and the κ-casein and the fat globules that are incorporated into the protein network (Obeid *et al*., 2020; Tamime, 2006). Possible reactions of denatured β-Lg include interaction with other β-Lg, interaction with κ-casein on the surface of casein micelles and interaction with fat globule membrane, which results in an approximate doubling of the amount of fat-bound protein. The lipoprotein particles from the added buttermilk might have enhanced the thickness of the formed secondary membrane after the homogenization treatment, which might be involved in the formation of a different network as in sample T1 and might also account for the differences in the rheological and microstructural properties.

The microstructures of the yogurt gels in this study (Figure 3) presented a branched structure with empty spaces as observed by Vital *et al.* (2020) in their research with yogurts added with whey protein or soy flour. This branched network is also described as three-dimensional networks of protein chains and aggregation of casein micelles (Aichinger *et al.,* 2003; Jaya, 2009). Further, yogurts with lower fat content (T2 and T3) presented microstructures with larger pores, as reported by Trachoo & Mistry (1998).

3.4 Texture profile of yogurts

The results from the texture profile analysis of the yogurt samples are shown in Table 4. There were significant differences ($p>0.05$) on hardness/firmness between the sample T1, and the samples T2 and T3 with added buttermilk after 21 days of storage. The results showed that the greater the amount of buttermilk added, less hardness was presented by the final products T2 and T3. The decrease in firmness in yogurt with the increase in buttermilk added concentration was also reported by Le *et al.* (2011) who evaluated the addition of skimmed-milk powder (at concentrations of 12, 11, 10, 9 and 8% of total solids), buttermilk or fat globule membrane at concentrations of 1, 2, 3 and 4% of solids in yogurts. Saffon *et al.* (2013) also reported that the aggregates of buttermilk protein added to yogurt decreased firmness as the level of substitution increased (replacement levels were 0%, 20%, 40%, 60%, 80% or 100%). However, Zhao (2020) showed that the addition of 1 or 2% powdered buttermilk increased the firmness of skimmed yogurt, while the addition of 4% powdered buttermilk decreased the firmness of skimmed yogurt when compared to skimmed yogurt without added buttermilk.

Table 4: Analysis of yogurt texture after 21 and 42 days of production

Treatment	D+21			D+42		
	Hardness (g)	**Springiness (mm)**	**Cohesiveness**	**Hardness (g)**	**Springiness (mm)**	**Cohesiveness**
T1	167.00±1.50[a]	9.90±0.12[a]	0,72±0,00[a]	173.00±1.41[a]	9.83±1.08[a]	0,72±0,09[a]
T2	133.00±8.48[b]	8.94±0.19[b]	0,73±0,05[a]	127.00±10.60[b]	8.80±0.31[a]	0,74±0,04[a]
T3	98.00±3.75[c]	8.86±0.12[b]	0,77±0,01[a]	110.00±0.70[b]	8.93±0.24[a]	0,79±0,01[a]

Within a column, different superscripts indicate significant differences ($p<0.05$). Data are expressed as means ± standard deviations of triplicate determinations.
T1 = No added buttermilk; T2 = 1.36% buttermilk added; T3 = 3.34% buttermilk added.
Source: Research data (2021).

The differences in hardness between T1 (always showing the highest hardness values) and T2, T3 were significant ($p>0.05$) and remained about the same after 21 and 42 days. A lower firmness of yogurt with less fat content can be attributed to the formation of a protein network composed mainly of chains of casein micelles, since yogurt with higher fat content, casein micelles are extensively fused, since the fat globules act as a promoter of the protein network, as shown by a study by Sandoval-Castilla *et al.* (2004), with control yogurt with 2.99% $w \cdot w^{-1}$ fat and reduced yogurt with 1.38% $w \cdot w^{-1}$ fat. Another study revealed that the addition of buttermilk performed poorly during coagulation in cheese making (Morin, Pouliot & Britten, 2008). These authors suggested that the interaction between fragments of the fat globule membrane and casein resulting from homogenization may prevent the formation of the gel and create a weak clot. These data reinforce the results obtained in the present study, in which yogurts added with buttermilk presented less firmness (T2 and T3) when compared to yogurt without added buttermilk (T1).

Yogurts with a smooth consistency were considered closer to ideal when compared to yogurts with a firmer consistency, and the information contained in the labels influenced the decision of consumers (Ogliare & Novelho, 2021). A fermented product with added buttermilk and whey had good acceptance with a high purchase intention (Pereira *et al.*, 2021). Therefore, the yogurts added with buttermilk produced in the present study may present a good sensory evaluation by consumers, mainly because this product has a claim to reduce fat and bring health benefits due to the addition of buttermilk.

Moreover, a larger pore size of the T2 and T3 treatments may explain changes in the structure of the yogurt added with buttermilk, which impacts on firmness of the product. These results suggest that the amount of buttermilk added to the yogurt in order to standardize the total solids, while the fat content was diminished, was not enough to form a gel with a stronger and similar structure as the sample T1 without added buttermilk, which consequently impacted the texture of the yogurt. However, this also suggests that formulation can be a way to modify the structure of acidified, fat-reduced products such as yogurt.

Other texture parameters evaluated were springiness, which can be defined as how much a material can be deformed before breaking, and cohesiveness, which is the material's ability to return to its initial shape after being compressed (Mudgil, Barak & Khatkar, 2017). After 21 days, the springiness of yogurt without the addition of buttermilk (T1) was greater than the other treatments (T2 and T3) and after 42 days there was no significant difference between treatments. It is worth mentioning that cohesiveness showed no significant difference between treatments at any of the evaluated times. A summary of the main results of the experiment is presented in Table 5.

Table 5- The main results of fermentation, average pore size and hardness of the yogurts produced with different concentrations of fat levels and added buttermilk

Treatment	Fermentation				D+7	D+21		D+42	
	Time (h)	*pH*	*Particle size in solution d_{90} (µm)*		*Average pore size (µm)*	*Average pore size (µm)*	*Hardness (g)*	*Average pore size (µm)*	*Hardness (g)*
			After 4 h	After 5 h					
0% **3.5% Fat**	5	4.7	20.96[b]	56.23[a]	18	34	167[a]	28	173[a]
+1.3% **3.3% Fat** -5.7%	5	4.6	35.54[a]	45.55[ab]	36	39	127[ab]	38	127[b]
+3.34% **3.0% Fat** -14.3%	5	4.7	25.36[b]	36.23[b]	54	57	98[b]	57	110[b]

Within a column, different superscripts indicate significant differences ($p<0.05$).

Blue arrow indicates percentage ($w \cdot w^{-1}$) of buttermilk in the yogurt.

Green arrow indicates fat reduction.

Source: Research data (2021).

4. Conclusion

Buttermilk was added to yogurt formulations in a way to not interfere with the total solids content (12.5%). Consequently, the fermentation time was not affected by the addition of buttermilk in the yogurt nor the particle size during the first hours of fermentation. However, at the end of the fermentation, the yogurt with no buttermilk added (T1) presented the largest particle size, and the yogurt with 3.34% $w \cdot w^{-1}$ of buttermilk added (T3) presented the smallest particle size. The products had different fat content in their composition, which affected the structure. Buttermilk can be used as a fat replacer to produce fermented dairy products with reduced fat content, without affecting the fermentation time, but affecting the particle size distribution and network pore size. However, it leads to the formation of a less compacted network microstructure, less springiness after 21 days and less hardness after 21 and 42 days. Further experiments focused on how much milk fat can be replaced by the fat contributed by the phospholipids from buttermilk, are suggested, especially considering the variables total solids and fat content and sensory evaluation.

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2- ARTIGO 2

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Effect of sugar reduction and addition of corn fibre and polydextrose on pore size and syneresis of yoghurt

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Effect of sugar reduction and addition of corn fiber and polydextrose on pore size and syneresis of yogurt

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ABSTRACT

This study investigated the effect of sugar reduction and replacement by corn fiber and polydextrose on yogurt gel pore size and syneresis. Five yogurt formulations were produced with 9, 4.5 and 0% sucrose, and also 4.5% with added 2% corn fiber or polydextrose. The increasing demand for sugar reduction in dairy products justifies studies on new formulations such as fiber addition. Sucrose reduction from 9% to 0% led to a decrease in average matrix pore size and increased syneresis. Fiber or polydextrose addition effectively reduced syneresis, despite bigger matrix pore size. Fermentation profile, acidity and pH values were not significantly affected by compositional changes.

1. Introduction

The World Health Organization guidelines recommend that the consumption of sugars should not exceed 10% of the daily calorie intake (WHO, 2016). Sugar reduction in foods (e.g., yogurts) is an industrial challenge because it contributes not only to the flavor but also to texture, viscosity, and product shelf life (Spillane, 2006; McCain, Kaliappan, & Drake, 2018). This demand still is a challenge for the Brazilian dairy industry as well as around the globe, whereby replacement with prebiotics have been in focus (Ramirez-Santiago et al., 2010; Espírito-Santo et al., 2013; Mudgil, Barak, & Khatkar, 2016; Kieserling et al., 2019; Huang et al., 2020).

Belyakova et al. (2014) demonstrated the effect of sucrose on the functional properties of caseins, favoring hydrophobic protein-protein interactions at concentrations of 10-60% sucrose. Another study showed that yogurts produced with sugar had a matrix of associated caseins in the form of agglomerates, which allows the matrix to form empty spaces that trap water (Haque & Aryana, 2002). Sucrose at concentrations of 0-40% in 1% bovine serum albumin (BSA) solution at pH 6.9 was able to increase the thermal stability of BSA, in addition to increasing the gelation temperature due to thermal stabilization of the native state of the protein (Baier & McClements, 2011). In addition, soluble polysaccharides are capable of forming hydrogen bonds with water, which helps to reduce syneresis by acting as thickeners (Bisar et al., 2015). Polysaccharide-protein interactions can stabilize the emulsion system and yogurt gels (Dickinson, 2009).

In the literature, we could not find any study on simultaneous sugar reduction in yogurt and replacement with soluble corn fiber or polydextrose, specifically on the effect on gel pore size and syneresis. These fibers are interesting because they have a high digestive tolerance, are stable under low pH conditions, and do not decompose into sugars, as occurs with other soluble fibers during storage. As mentioned earlier, most Brazilian yogurt products contain a high level of carbohydrates (approximately 5.7% to 15.9%) and its reduction is a challenge (Dantas et al., 2021).

Based on the arguments presented above, the hypothesis of this study was to partially replace the sucrose content by soluble corn fibers or polydextrose. The fibers acting as thickeners in the continuous phase, represented in the microstructure by the different pore sizes, which stabilizes the network protein by immobilization of water and possibly steric stabilization with a consequent reduction in syneresis. The present study aims to demonstrate the substitution effect on pore size and syneresis over 21 days shelf-life.

2. Material e Methods

2.1 Yogurt manufacturing process

Cream (Laticínios Coalhadas, Juiz de Fora, Brazil) was added to pasteurized whole milk (Benfica®, Juiz de Fora, Brazil) to standardize the fat content to 3.5% ww^{-1}. Hereafter, five formulations (F) were prepared (n = 3): F1 = 9% ww^{-1} sucrose added (control); F2 = 4.5% ww^{-1} sucrose added; F3 = yogurt 0% sucrose added; F4 = 4.5% ww^{-1} sucrose + 2% ww^{-1} of soluble corn fiber addition (Promitor®, Soluble Corn Fiber 70; Tate & Lyle, Decatur, USA); F5 = 4.5% ww^{-1} sucrose added + 2% ww^{-1} polydextrose (STA-Lite® 90 NG R EXP; Tate & Lyle, Shanghai, China) (Table A1). Dry matter content was not standardized in the formulations, which can be considered a limitation of the study, since dry matter plays an important role in the microstructure and syneresis of the gel. However, the aim of the study was to add soluble fiber as a substitute for added sucrose.

The formulations were homogenized at 20 MPa (APV-1000, Bydgoszcz, Poland). Afterwards, the samples were heat treated at 90 ºC for 5 minutes, followed by cooling until 42 ºC; and added with 1% ww^{-1} starter culture (*Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* Delvo-Yog™, CY-340, DSL, DSM®, Liverpool, Australia). The samples were incubated (42 ± 1 ºC for 5.5 h, Appendix Fig. A1) and the acidification was monitored using a Consort D130 system (Turnhout, Belgium) until a pH of 4.6 was achieved. Hereafter, the samples were transferred to a refrigerator (from 42 ± 1 ºC until 20 ± 1 ºC) for approximately 3 h and manually stirred with circular movements for 20 seconds to break the curd. The samples were manually placed into plastic containers, stored (7 ± 1 ºC) and analyzed (at 1, 7, 14, and 21 days) during storage.

2.2 Composition Analysis

Yogurts were analyzed for proximate composition. Moisture content was quantified in a moisture analyzer (Sartorius, MA 150, Goettingen, Germany); total protein was determined using the Kjeldahl method (AOAC, 2000); fat content was determined by a Gerber butyrometer method (AOAC, 1995), and ashes were calculated by weighting the residues after incineration in a muffle furnace at 550 ºC for three hours.

2.3 Determination of pH and titratable Acidity

The acidity was determined by titration with NaOH solution 0.1 $mol{\cdot}L^{-1}$ and expressed as % lactic acid. pH was measured with a calibrated pH meter (PG 1400, GEHAKA®, São Paulo, Brazil) (AOAC, 2012).

2.4 Scanning electron microscope

A thin layer of yogurt sample was applied in a double-sided adhesive carbon tape, mounted on a *stub* and lyophilized at -84 °C in a laboratory freeze dryer (Labconco-FreeZone 2.5 Plus, Canada, USA). The microstructure of the lyophilized samples was analyzed by scanning electron microscope (Hitachi TM 3000, Hitachi Ltd., Tokyo, Japan) according to Mudgil et al. (2018). The average pore size of the yogurt samples was automatically calculated after the images were processed (500x magnification, 200 µm size) with Image J 1.47v freeware (National Institutes of Health, USA).

2.5 Syneresis

Syneresis was estimated according to Kieserling et al. (2019) with modifications. At the moment of packaging, approximately 15 g of samples were weighted and placed in a 15 mL falcon tube. The samples were centrifuged at 512.2 x *g* for 20 minutes and the level of syneresis calculated using Equation 1.

$$\text{Syneresis [\%]} = (\text{mass of supernatant (g)} / \text{mass of yogurt sample}) \times 100 \tag{1}$$

2.6 Statistical Analysis

Three independent productions were made (n = 3). A completely randomized design was applied to this experiment. Statistical analyses were conducted at a significance level of 0.05 by a Tukey test with the assistance of R® Software (R Core Team, 2019).

More details of the materials and methods are in the supplementary material.

3. Results and discussion

3.1 Yogurt fermentation curves and composition

The decrease in pH over time during fermentation was similar in all formulations, according to Fig. 1, which suggests that the addition of fibers and the sugar concentration did not interfere in the development of the lactic acid. The decrease in pH is caused by the production of lactic acid from lactose through the dairy culture; thus, the presence of sucrose and fibers did not configure fermentative substrates nor did they act to inhibit the microbiota. Neither the initial pH (6.40 – 6.52) nor the pH after 5 h incubation (4.60 - 4.80) was statistically significant ($p>0.05$) between formulations. A pH of 4.60 was achieved between 5 h and 5.5 h of incubation. The pH at 1d was even lower (4.40 – 4.54, $p>0.05$) for all samples, this rapid post-acidification occurred due to not having an immediate cooling of the yogurts. This was due to the type of yogurt produced, as the stirred yogurt, after fermentation, is cooled inside the vat before breaking the curd and then bottled, it takes longer to cool, when compared, for example, to set yogurt, where at the end of fermentation, the packages with the yogurts are placed directly in cold chambers or cooled quickly in cooling tunnels (Weerathilake et al., 2014, Mokoonlall, Nobel & Hinrichs, 2016).

The variation in pH and acidity between formulations did not show significant differences ($p>0.05$) during storage; however, the pH and acidity changes from 1d to 21d was significant ($p<0.05$): pH (4.40 – 4.54 (1d) → 4.12 – 4.21 (21d)) and acidity (0.80% – 0.84% (1d) → 0.93% – 0.97% (21d)) (Fig. 2). These results indicate that the different sugar levels tested and the fibers added did not affect the acidity and pH development of the yogurts during the shelf life. These results are consistent with those obtained during fermentation, in which the presence of fiber and sugar do not interfere in the bacterial metabolic activity that consumes lactose and consequently increases levels of lactic acid and galactose.

The average composition of yogurts is shown in Table 1. Yogurts F1, F2, F4 and F5 had the highest dry matter and ash content compared to yogurt without additions (F3). These results were expected, since different types and amount of carbohydrates (9% and 4.5% sucrose and 4.5% + 2% polysaccharides), were added to the formulations, which means addition of solids, and in F3 yogurt there was no addition of other ingredients. Similar differences of dry matter content in yogurts with added polysaccharides and/or sucrose when compared to the control have also been reported in other studies (Debon et al., 2010; Villegas et al., 2010; Srisuvor et al., 2013). The difference in solids did not affect the fermentation; however, it could affect syneresis (Amatayakul et al., 2006) and microstructure.

3.2 Microstructure of yogurt

Fig. 3 presents the images of the microstructures of the yogurts with the calculated values for the matrix pores size after 7 and 21 days. After 7 days, the mean pore size was smallest in sample F3 (25.8 µm, $p<0.05$), then followed F2 < F5 ≈ F1 and < F4 (57.5 µm, the biggest). The pore size increased slightly in samples F2 and F4, and significantly ($p<0.05$) in F5, after 21 days of storage. These results suggest that sucrose addition leads to an increase in pore size (F1, F2, F4, F5 with added sucrose) compared to F3 (no sugar added). F3 had slightly higher protein + fat contents and lower dry matter, and no interfering components (sugar, fibers) in the matrix formation, resulting in more contact points between the matrix elements. Presumably, this gives origin to the smaller pores, which also prevailed at 21d. The higher average pore size in F4 compared to the other samples suggests a role for both the sucrose and corn fiber added, interfering with the network formation. The effect of polydextrose (F5) on pore size, compared to F2 (with the same sucrose level) seems to be evident at 7d and especially at 21d. Considering only the sucrose level, sample (F1 with 9% sucrose) showed a higher mean pore size than F2 (4.5% sucrose) and the latter, in turn, higher than F3 (0% sucrose). The latter samples (F1, F2 and F3) also have lower decreasing dry matter content (22.1%, 17.3% and 13.3%, respectively).

During acidification, soluble fibers can adsorb on casein micelles, which hinder the visualization of fibers in the protein network. Similar results were obtained by Tamime et al. (1996) and Kieserling et al. (2019). The adsorption of fiber on casein micelles may also indicate a good fiber integration into the yogurt structure, as demonstrated by Krzeminski et al. (2014). Furthermore, as sucrose and fiber are soluble, these two ingredients were probably dissolved in the serum phase, which, after lyophilization, are represented in the pores of the samples. So, these two ingredients were stabilizing the protein network, as shown by the syneresis results.

The types of interactions between polysaccharides and proteins depend on the type and concentration of polysaccharide used. These interactions can be of non-covalent, attraction or repulsion nature, including electrostatic, hydrophobic, hydrogen and Van der Waals forces (Wusigale, Liang and Luo, 2020; Khubber et al., 2021; Gilbet & Turgeon, 2021). The fibers used in this study are highly soluble when added to yogurt formulations because they have long branched chains containing -OH groups, which allow the molecules to bind to water. The hydrocolloids can retain large amounts of water and keep it immobile. In addition, they react with the constituents of milk, mainly protein, and stabilize the protein network preventing the free movement of water (Tamine & Robinson, 2007).

3.3 Syneresis

Structural organization of the matrix elements at different levels (molecular, particle, clusters, aggregate) leads to distinct interactions and pores/cavities formation (filled with serum) during yogurt making (Table 2). During the shelf life, sample F1 (9% sucrose) showed lower syneresis than all other samples. Then followed F5≈F4 < F2 < F3. The highest syneresis showed sample F3 (0% sucrose) with the smaller average pore size and lowest dry matter (13.3%). Sucrose reduction (F1, F2 and F3) led to increased significantly ($p<0.05$) syneresis (concomitant with solids reduction from 22.1% to 13.3%), and changed only slightly over time (1d to 21d, Table 2).

Moreover, in yogurts with added fiber and polydextrose and higher dry matter (~20%), the syneresis at 1d (F4, F5) was significantly lower (1.5% and 1.3% respectively, $p<0.05$) compared to F2 (13.4% syneresis, 4.5% sucrose), highlighting the effect of additives, and increased significantly ($p<0.05$) at 21d to 4.4% and 4.0%, respectively. Huang et al. (2020) observed that yogurt with added 3% polydextrose showed less water-holding capacity and sensory acceptance after 14 and 21 days of manufacturing. The water-holding capacity is inversely proportional to the yogurt syneresis (with correlation values ranging from -1.0, - 0.97, - 0.92 and - 0.95 to for the times of 1, 7, 14 and 21 days respectively). Polydextrose is structured mainly by glucose in its highly branched polymer and with small quantities randomly distributed of sorbitol and citric acid. Polydextrose has a small chain compared to other polymers such as inulin, and can extend its branched structure more evenly into casein aggregates resulting in more extensive protein-carbohydrate interactions. Those interactions would give the gel better stability, as reflected by lower syneresis (Bisar et al., 2015).

Soluble corn fibers are polymers made of maltotriose units with an average weight degree of polymerization of approximately 3,000 and with a high capacity to link water molecules (Kendall et al., 2008). Therefore, it is possible that the presence of sugar and fiber in the yogurt formulation holds more water, leading these yogurts (F1, F2, F4, F5) to show less syneresis. Hence, yogurt without sugar (F3) tends to release more water (albeit not significant over time 1d to 21d), presumably because the interaction between water and the protein network, in this case, was less than in the presence of sugar and fibers. Intense shearing (avoided in the present work) of yogurt gels and high incubation temperature (42 ºC) leads to a looser heterogeneous matrix with fewer pores and higher syneresis (Gilbert et al., 2020).

Considering the results shown in the present study, the addition of polydextrose (F5) contributed to the production of low-sugar yogurt with properties closer to the control yogurt (F1) for syneresis.

4. Conclusion

The present study demonstrated that the presence and type of carbohydrates impacted the yogurt microstructure in different forms (pore size) and syneresis. Reducing the yogurt sucrose level from 9% to 0% led to a decrease in matrix pore size and increased syneresis. Moreover, the addition of corn fiber or polydextrose to a sucrose-reduced yogurt increased the pore size of the matrix and decreased syneresis. The reduction or removal of sugar with or without the addition of fiber in a yogurt formulation did not influence the fermentation time, neither acidity nor pH after 1d or 21d storage; however, sugar reduction and added fibers lead to lower syneresis at 1d, which significantly increase during shelf-life. In summary, sugar influences the physical-chemical properties of yogurt and the addition of soluble fibers such as polydextrose can be a viable alternative for sugar replacement as fibers can modulate the characteristics of yogurt reduced in sugar making it closest to traditional yogurt. There are some sensory studies in the literature concerning corn fibers and polydextrose (Allegeryer; Miller and Lee, 2010 a; Allegeryer; Miller and Lee, 2010 b), however, future studies can be carried out focusing on sensory analysis using these fibers as a sugar substitute. In conclusion, the addition of soluble corn fiber and polydextrose in yogurts as partial sucrose substitutes can be an alternative for the food industry, and thus meet a market demand from health-conscious consumers.

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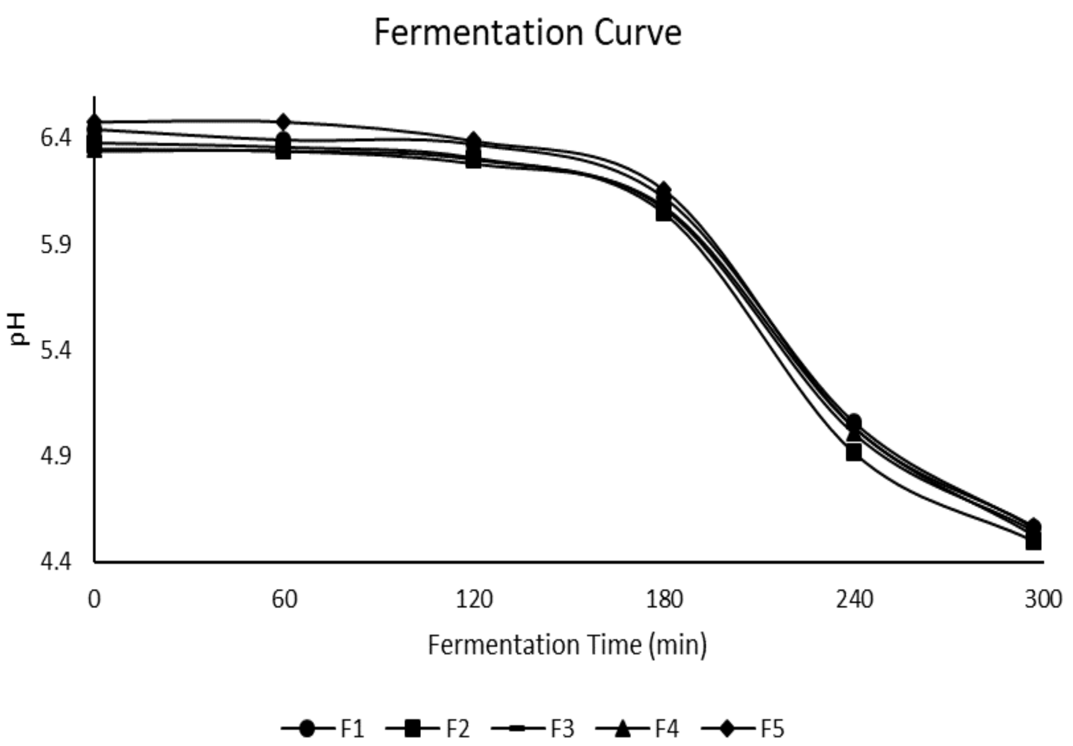


Fig. 1 - Yogurt fermentation curve. —●— F1 = yogurt with 9% ww^{-1}; —■— F2 = yogurt with 4.5% ww^{-1} sugar; —▬— = yogurt without added sugar; —▲— = yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} corn fiber and F5 = yo—◆—with 4.5% ww^{-1} added sugar and 2% ww^{-1} polydextrose.

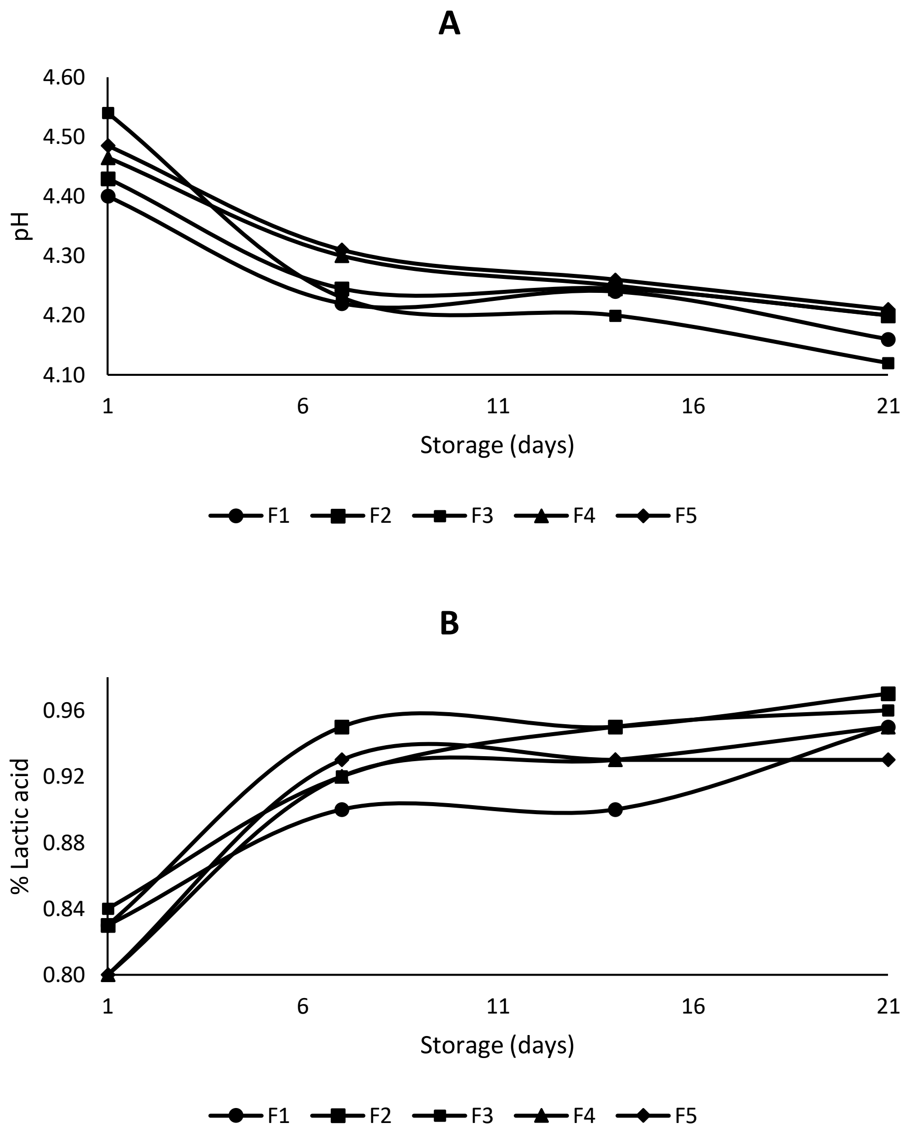


Fig. 2 - pH (A) and acidity (B) development of the yogurts during the storage tim F1 = yogurt with 9% ww^{-1} sugar; F2 = yogurt with 4.5% ww^{-1} sugar; 3 = yogurt without added sugar; F4 = irt with 4.5% ww^{-1} added sugar and 2% ww^{-1} corn fiber and F5= yogurt wit % ww^{-1} added sugar and 2% ww^{-1} polydextrose.

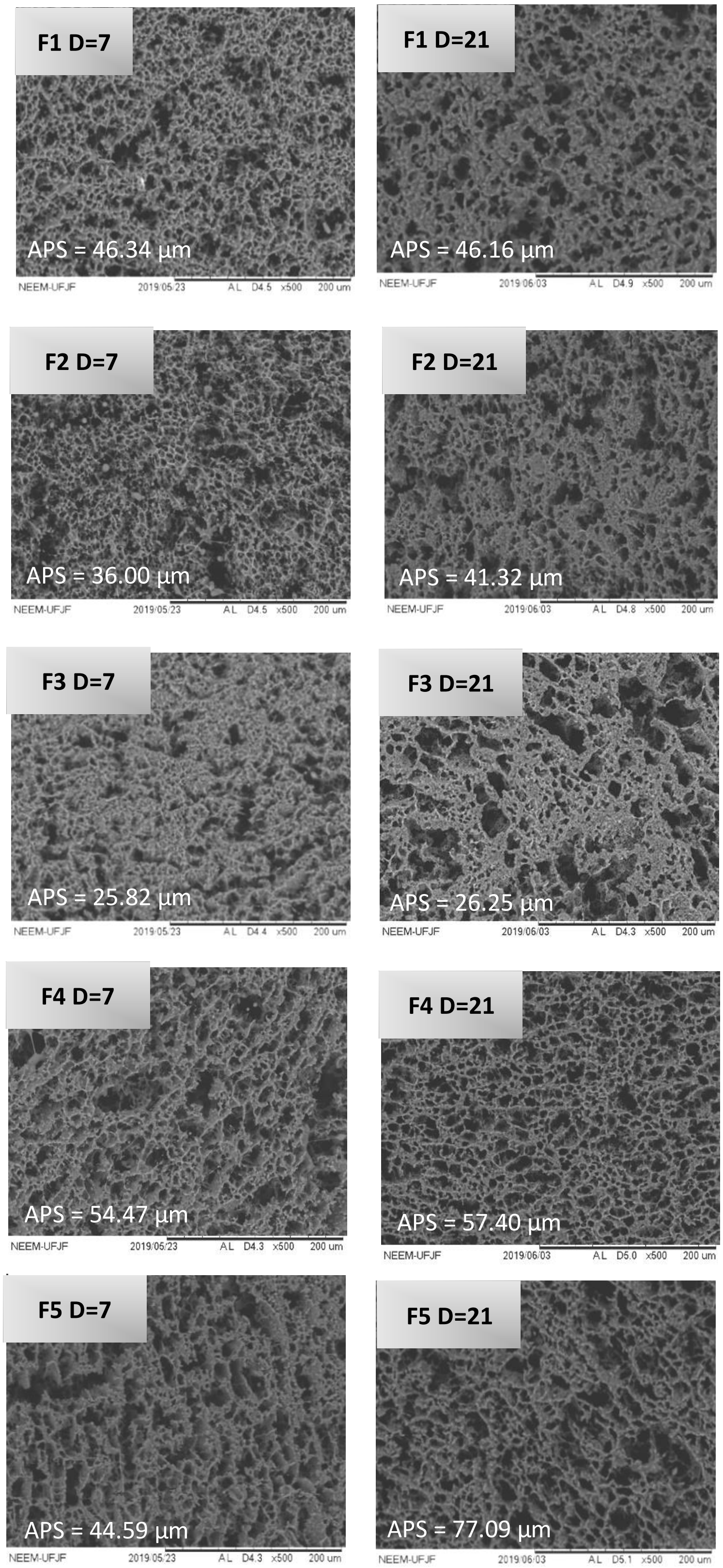


Fig. 3 - Scanning electron microscopy of yogurts. Magnification: 500x. F1 = yogurt with 9% ww^{-1} sugar; F2 = yogurt with 4.5% ww^{-1} sugar; F3 = yogurt without added sugar; F4 = yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} corn fiber and F5= yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} polydextrose. D = days and APS = Average Pore Size

Table 1

Composition of the yogurts (n=3)

Formulations	Fat (% ww^{-1})	Dry matter (% ww^{-1})	Ash (% ww^{-1})	Protein (% ww^{-1})
F1	3.00 ± 0.00^{a}	22.06 ± 1.14^{a}	0.87 ± 0.02^{a}	3.02 ± 0.23^{b}
F2	3.33 ± 0.25^{a}	17.29 ± 0.82^{b}	0.89 ± 0.15^{a}	3.44 ± 0.17^{a}
F3	3.50 ± 0.00^{a}	13.31 ± 0.40^{c}	0.80 ± 0.02^{a}	3.43 ± 0.05^{a}
F4	3.00 ± 0.00^{a}	19.87 ± 1.05^{ab}	1.02 ± 0.30^{a}	3.16 ± 0.06^{ab}
F5	3.33 ± 0.25^{a}	20.44 ± 1.95^{ab}	1.15 ± 0.56^{a}	3.25 ± 0.02^{ab}

Values are means ± SD of 3 independent runs. Within a column, different superscripts indicate significant differences ($p<0.05$).

F1 = yogurt with 9% ww^{-1} sugar; F2 = yogurt with 4.5% ww^{-1} sugar; F3 = yogurt without added sugar; F4 = yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} corn fiber and F5= yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} polydextrose.

Table 2

Syneresis of yogurts produced with different sugar contents and the addition of fiber

Formulation	Syneresis (%)			
	D+1	D+7	D+14	D+21
F1	0.07 ± 0.12^{c}	0.07 ± 0.14^{c}	0.13 ± 0.23^{c}	0.20 ± 0.35^{c}
F2	5.40 ± 1.64^{b}	7.20 ± 2.47^{ab}	4.96 ± 0.61^{b}	4.67 ± 0.23^{b}
F3	13.4 ± 0.14^{a}	12.25 ± 1.76^{a}	12.8 ± 2.69^{a}	13.2 ± 3.25^{a}
F4	1.50 ± 0.36^{c}	2.46 ± 0.71^{bc}	2.80 ± 0.60^{bc}	4.44 ± 0.28^{bc}
F5	1.33 ± 0.25^{c}	2.83 ± 0.92^{bc}	2.75 ± 0.06^{bc}	4.03 ± 0.65^{bc}

* Values are means ± SD of 3 independent runs. Within a column, different superscripts indicate significant differences ($p<0.05$) by Tukey test.

Data are expressed as means ± standard deviations of triplicate determinations.

D=days

Where F1 = yogurt with 9% ww^{-1} sugar; F2 = yogurt with 4.5% ww^{-1} sugar; F3 = yogurt without added sugar; F4 = yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} corn fiber; F5 = yogurt with 4.5% ww^{-1} added sugar and 2% ww^{-1} polydextrose.

SUPPLEMENTARY MATERIAL AND METHODS

Homogenization: The formulations were homogenized at 20 MPa, with an inlet temperature of 40 °C, and the flow rate of 22 L·h^{-1} (APV-1000, Bydgoszcz, Poland).

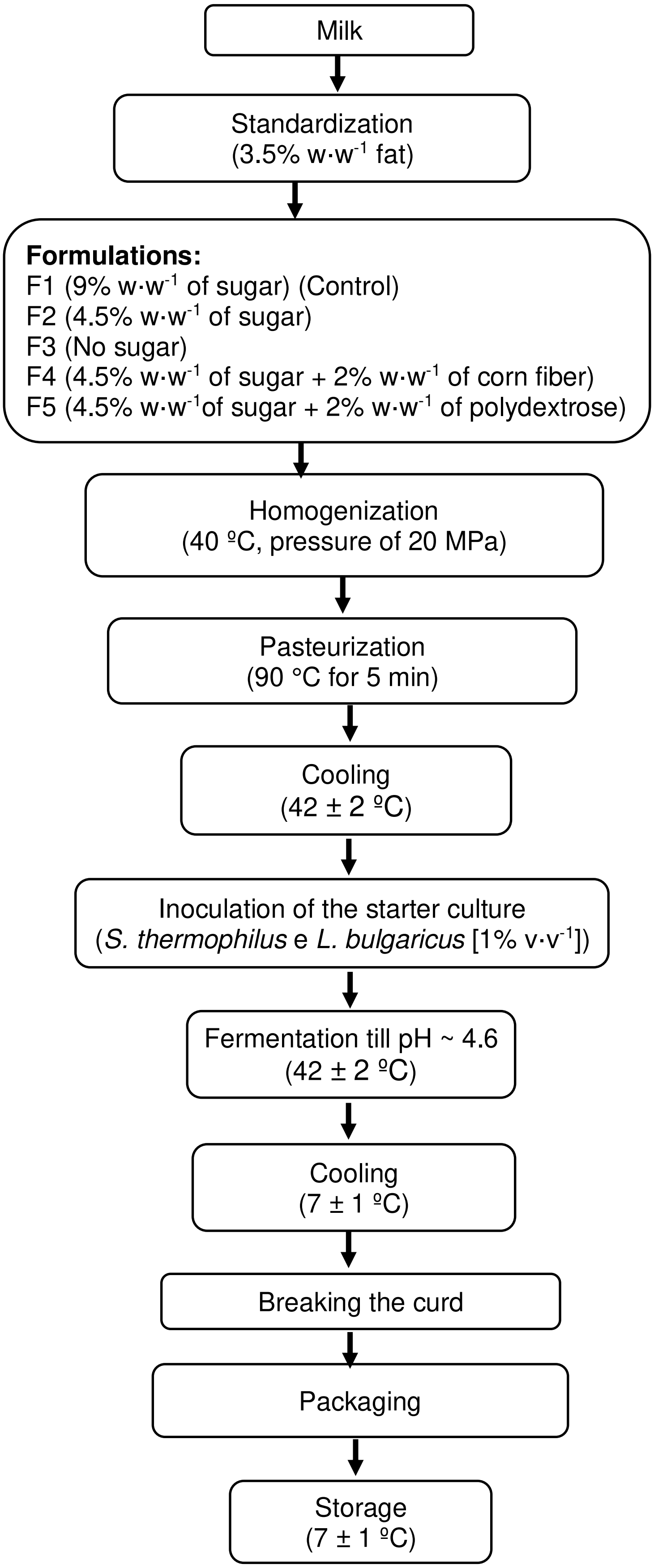
Yogurt cooling and filling: The culture had been previously prepared using the contents of a package that was diluted in one liter of UHT milk; then the content was kept frozen until, the day of the production of the yogurts when the culture was thawed and added in the proportion of 1% (v·v^{-1}) to the volume of milk.

Preparation of starter culture: “The culture had been previously prepared using the contents of a package that was diluted in one liter of UHT milk; then the content was kept frozen until, the day of the production of the yogurts when the culture was thawed and added in the proportion of 1% ($v \cdot v^{-1}$) to the volume of milk.”

Yogurt cooling and filling: Then the samples were transferred into a cooler (7 ± 1 °C) for approximately 3 h until reached a temperature of 20 ± 1 °C and manually stirred with circular movements for 20 seconds to break the curd, using a plastic spatula. Approximately 130 g of the yogurt samples were manually placed into 130 ml plastic containers and stored for 21 days at 7 ± 1 °C. The entire procedure was performed in a temperature-controlled environment at 23 ± 1 °C.

Fermentation curve: Beakers with a 1000 mL capacity received 900 g of the formulated and treated milk samples and were kept in a water bath at 42 ± 1 °C. The kinetics of acidification was monitored using a Consort D130 system (Turnhout, Belgium), which is a multichannel pH meter.

Scanning electron microscope: The images were recorded in the magnification of 100, 500, 1000, 1500, and 2000x. The 500x magnification with 200 µm size was chosen because they were the best images that characterized the yogurt gel.


Milk
Standardization
(3.5% w·w-1 fat)
Formulations:
F1 (9% w·w-1 of sugar) (Control)
F2 (4.5% w·w-1 of sugar)
F3 (No sugar)
F4 (4.5% w·w-1 of sugar + 2% w·w-1 of corn fiber)
F5 (4.5% w·w-1of sugar + 2% w·w-1 of polydextrose)
Homogenization
(40 ºC, pressure of 20 MPa)
Pasteurization
(90 °C for 5 min)
Cooling
(42 ± 2 ºC)
Inoculation of the starter culture
(S. thermophilus e L. bulgaricus [1% v·v-1])
Fermentation till pH ~ 4.6
(42 ± 2 ºC)
Cooling
(7 ± 1 ºC)
Breaking the curd
Packaging
Storage
(7 ± 1 ºC)

Appendix Fig. A1 - Flowchart of yogurt production.

Table A1

Formulation of the yogurts

Ingredients	Formulation				
	F1	F2	F3	F4	F5
Milk (% ww^{-1})	91.0	95.5	100	93.3	93.3
Fiber (% ww^{-1})	-	-	-	2.0[a]	2.0[b]
Sucrose (% ww^{-1})	9.0	4.5	-	4.5	4.5
Total	100	100	100	100	100

[a]Corn fiber (Promitor®, Soluble Corn Fiber 70; Tate & Lyle);
[b]Polydextrose (STA-Lite® 90 NG R EXP; Tate & Lyle).

3- ARTIGO 3

Efeito da adição de diferentes fibras solúveis em iogurtes com alta proteína na capacidade de retenção de água, distribuição do tamanho de partículas, viscosidade aparente e microestrutura

Resumo

Atendendo a demanda de consumidores preocupados com sua saúde o desenvolvimento de iogurte com alta proteína e adicionados de fibras, sem adição de açúcar, sem gordura, podem ser uma alternativa promissora. O objetivo desse estudo foi avaliar o efeito de diferentes tipos de fibras (Fibra solúvel de milho 70%; Fibra solúvel de milho 85%; Polidextrose) e concentrações (2,5%; 5% e 10%) em iogurte com alta teor de proteína, sem gordura, sem açúcar. Foram avaliados os parâmetros: composição, curva de fermentação, e nos tempos de 0, 30 e 45 dias foram avaliadas a capacidade de retenção de água, distribuição do tamanho de partículas, viscosidade aparente e microestrutura. Durante a fermentação os iogurtes com 10% de qualquer uma das fibras testadas, reduziram o pH mais rapidamente que os demais tratamentos. Os resultados de composição confirmaram que os iogurtes são de alta proteína, conforme determina a legislação brasileira. Todos os iogurtes e nos tempos avaliados apresentam alta capacidade de retenção de água. Os iogurtes com maior concentração das fibras apresentaram uma menor distribuição do tamanho de partícula (d_{90}). Além disso a viscosidade aparente no final da vida de prateleira foi igual para todas as formulações. E com relação a microestrutura pode-se observar que o teor de proteína foi mais determinante que a concentração das fibras, sendo que o iogurte com 10% de polidextrose apresentou poros maiores, o que pode ter ocorrido devido ao aumento da concentração da fibra. Esses resultados são promissores para as indústrias que desejam produzir iogurte com características diferenciadas, como alta proteína e adicionados de fibras.

Introdução

Iogurte é um produto amplamente consumido no mundo, com crescente demanda por iogurtes sem adição de açúcar e gordura é uma tendência, principalmente por pessoas que buscam um estilo de vida saudável (LESME et al., 2020). No entanto, é um desafio para indústria produzir iogurte *clean label*, ou seja, produtos com menos ingredientes, com propriedades de textura e sensoriais exigidas pelos consumidores. Algumas opções para melhorar as características do iogurte é a adição de proteína e fibras. Iogurtes com alta proteína tem melhor sabor e textura, além de proporcionar benefícios a saúde comprovados cientificamente (JORGENSEN et al., 2019). Enquanto que, as fibras podem ser empregadas como agente de volume, apresentam funcionalidade semelhante à sacarose, aumentam a viscosidade e podem ser usadas como espessantes devido a sua capacidade de retenção de água (AIDOO; AFOAKWA; DEWETTINCK, 2014; HESPELL, 1998; MUDGIL; BARAK, 2013). Além disso, as fibras modulam a microbiota intestinal, promovem maior sensação de saciedade e o seu consumo está associado a prevenção de doenças como câncer de colon, doenças coronárianas e diabetes tipo 2 (CUI et al., 2019; KACZMARCZYK; MILLER; FREUND, 2012; MUDGIL; BARAK, 2013; ROBERTS; HEYMAN, 2000).

A Organização Mundial da Saúde recomenda a ingestão de 25 g de fibras por dia, o que geralmente não é alcançado pela maioria das pessoas, portanto a adição de fibras em iogurte é uma alternativa promissora para alcançar essa recomendação. As fibras polidextrose e fibras solúvel de milho são escolhas interessantes para atingir o recomendado, já que essas apresentam alta tolerância digestiva, podendo ser ingeridas até 90 g/dia de polidextrose e 65 g/dia de fibra solúvel de milho (FAO/WHO, 1985; FLOOD; AUERBACH; CRAIG, 2004; STEWART et al., 2010).

A polidextrose é um polissacarídeos altamente ramificado de alto peso molecular, formado por moléculas de glicose ligada aleatoriamente por ligações glicosídicas α-1,6, com grau de polimerização médio de 12 DP e um peso médio molecular de 2000 (BURDOCK; FLAMM, 1999; CRAIG et al., 1988; FALLOURD; VISCIONE, 2009). A fibra solúvel de milho contém oligossacarídeo com ligações glicosil aleatórias (HOODA et al., 2012; LAHTINEN et al., 2016; STEWART et al., 2010; VESTER BOLER et al., 2011).

O esquema fatorial com fator adicional (grupo controle), ainda é pouco utilizado nos experimentos com alimentos. No entanto, essa análise estatística é interessante porque permite estudar tanto os efeitos do esquema fatorial, como comparar estatisticamente todos os níveis em relação ao controle. O esquema fatorial permite medir os efeitos dos fatores individuais (variáveis independentes) sobre a variável resposta (variáveis dependentes), assim como, esses fatores interagem uns com os outros impactando na resposta (CAVALCANTI; FERREIRA; STORTI, 2015; CHENG, 2016).

Na literatura são encontrados vários estudos mostrando o comportamento reológico, microestrutura e tamanho de partículas de iogurtes reduzido em gordura, açúcar, adicionado de fibras ou proteína do soro de leite. No entanto, não foram encontrados estudos que avaliassem essas características em iogurte adicionado das fibras de milho e polidextrose, assim como o impacto das diferentes concentrações dessas fibras em iogurte com alta proteínas. Portanto, a hipótese desse estudo é que as fibras (solúveis de milho nas concentrações de 70% (Fibra 1) e 85% (Fibra 2) e polidextrose (Fibra 3)) são capazes de modular as características de capacidade de retenção de água, distribuição do tamanho de partículas, viscosidade aparente e microestrutura de iogurte com alta proteína. O presente estudo tem como objetivo avaliar o efeito das fibras solúveis de milho nas concentrações de 70% (Fibra 1) e 85% (Fibra 2) e polidextrose (Fibra 3) nas características de capacidade de retenção de água, distribuição do tamanho de partículas, viscosidade e microestrutura do iogurte com alta proteína. Esses resultados podem fornecer informações importantes no potencial uso dessas fibras como componente funcional em iogurte com alta proteína.

2. Material e Métodos

2.1 Material

Leite pasteurizado desnatado (Benfica®, Juiz de Fora, Brazil), polidextrose (STA-Lite® 90 NG R EXP; Tate & Lyle, Shanghai, China), fibra solúvel de milho 70% (Promitor®, Soluble Corn Fiber 70; Tate & Lyle, Decatur, USA), fibra solúvel de milho 85% (PROMITOR® Soluble Corn Fiber 85, Tate & Lyle, Decatur, USA) e concentrado proteico de leite 80% (MPC 80; Vitalus Nutrition, Abbotsford, Canada), pectina (Tecgem®,Tate & Lyle Gemacom Tech, Juiz de Fora, Brasil) e sorbato de potássio (Tate & Lyle, Gemacom Tech, Juiz de Fora, Brasil). A cultura composta por *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Delvo-YogTM, CY-340, DSL, DSM®, Liverpool, Australia), foi preparada conforme recomendação do fabricante, em que o conteúdo de uma embalagem foi diluído em um litro de leite UHT; fracionado em frascos menores, então o conteúdo foi mantido congelado em um freezer (13 ºC ± 2 ºC) até o dia da produção dos iogurtes.

2.2 Preparação das formulações

Foram realizadas 3 produções independentes (n=3). Os tratamentos consistiram em 10 formulações de *stirred* iogurte com alta proteína, os quais continham: leite desnatado, MPC 80 nas proporções descritas na Tabela 1 de modo a obter aproximadamente 6,25% a 7% de proteína no produto final. Os tratamentos foram divididos entre controle sem adição de fibra (T1) e os demais tratamentos que continham aproximadamente 5,50% a 7% de proteína mais fibra solúvel de milho 70% (Fibra 1) ou fibra solúvel de milho 85% (Fibra 2) ou polidextrose (Fibra 3), todas as fibras foram testadas nas concentrações de 2,5%; 5%; e 10% (Tabela 1).

Tabela 1 – Design experimental de formulações de iogurte com alto teor de proteína com diferentes teores de fibra com um total de 10 tratamentos (concentrações g · 100 g^{-1})

Ingredientes		**Fibra solúvel de milho 70% (Fibra 1)**			**Fibra solúvel de milho 85% (Fibra 2)**			**Polidextrose 90% (Fibra 3)**		
Tratamentos	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Leite desnatado	94,83	92,13	89,51	84,31	92,13	89,51	84,31	92,13	89,51	84,31
MPC 80	4,88	5,08	5,20	5,40	5,08	5,20	5,40	5,08	5,20	5,40
Fibra	0,00	2,50	5,00	10,00	2,50	5,00	10,00	2,50	5,00	10,00
Pectina	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25
Sorbato de potássio	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Total	100	100	100	100	100	100	100	100	100	100

MPC 80 = Milk protein concentrate (concentrado proteico do leite) 80%.

As formulações dos iogurtes foram preparadas adicionando ao leite desnatado, o MPC, as fibras, a pectina e o sorbato de potássio. Essas ficaram hidratando por 30 min, à temperatura ambiente (23 ºC ± 2 ºC) sobre agitação com um agitador mecânico com rotação 250 rpm. Em seguida as misturas foram aquecidas à 50 ºC ± 2 ºC em banho-maria e homogeneizada (APV model 1000-2000, Bydgoszcz, Poland) a 5 MPa em um estágio. Após a homogeneização as formulações ficaram em geladeira (7 ºC ± 2 ºC) *overnight,* posteriormente aplicou-se o tratamento térmico de 90 ºC ± 2 ºC por

3 min em banho-maria, então foram resfriadas em banho de gelo até a temperatura de 42 ºC ± 1 ºC e foi inoculada 1% v/v da cultura iniciadora, que havia sido descongelada 1 h antes do uso. A fermentação foi acompanhada até atingir o pH de aproximadamente 4,6, em seguida os iogurtes foram resfriados em geladeira (7 ºC ± 2ºC) por aproximadamente 3 h e então realizou-se o procedimento padrão de quebra da massa com auxílio de haste de plástico com movimentos circulares por 30 segundos. Depois os iogurtes foram envasados em embalagens plásticas previamente higienizadas, seladas com folhas de alumínio e colocadas em geladeira (7 ºC ± 2 ºC) por 0, 30 e 45 dias.

2.3 Análises físico-químicas

Foram realizadas análise de gordura, proteína, lactose, extrato seco do leite utilizado nas formulações empregando a técnica de espectrometria de absorção no infravermelho médio (ISO 9622 | IDF 141). Os iogurtes foram analisados quanto ao teor proteína pelo método de Kjeldahl (AOAC, 2000); sólidos totais usando a balança termogravimétrica (Sartorius, MA 150, Goettingen, Germany); determinação do teor de açúcares redutores expressos em lactose do leite pelo método de Fehling (INSTITUTO ADOLFO LUTZ, 2008) e as cinzas foram calculadas pelo peso dos resíduos após a incineração em mufla por 3 horas a uma temperatura de 550 ºC (INSTITUTO ADOLFO LUTZ, 2008).

2.4 Capacidade de retenção de água

A capacidade de retenção de água (CRA) dos iogurtes foram determinadas conforme descrito por BAKRY; CHEN; LIANG, (2019) com adaptações. Tubos Falcon contendo aproximadamente 15 g do iogurte a uma temperatura de 7 ºC ± 2 ºC foram centrifugados (MARCA, MODELO) 512,2 x g por 20 min. A (CRA) foi calculada usando a Equação 1:

$$CRA\ (\%) = (P - S / P) \times 100 \qquad (1)$$

em que P é o peso da amostra e S e o peso do soro expelido. Todas as análises foram realizadas em triplicata.

2.5 Distribuição do tamanho de partículas

As distribuições do tamanho das partículas foram determinadas nos tempos 0, 30 e 45 dias após a produção dos iogurtes. Foi utilizado o analisador de difração a laser Beckman Coulter LS 13 320 (Beckman Coulter, Miami, FL, EUA) acoplado ao módulo de análise de líquido (módulo de líquido aquoso, Beckman Coulter, Miami, FL, EUA). No compartimento do equipamento contendo água a 25 ° C as amostras de iogurte foram adicionadas até atingir uma "Polarização de Espalhamento de Intensidade Diferencial (PIDS)" de aproximadamente 45% (recomendação do fabricante do analisador de difração a laser). O PIDS compreende três comprimentos de onda de 450 nm, 600 nm e 900 nm que iluminam a amostra com luz polarizada verticalmente e depois horizontalmente. O cálculo dos diâmetros foi baseado na teoria de Mie, utilizando os índices de refração de 1,33 para água 1,57 para proteína e 1,47 para gordura. O valor do diâmetro da partícula abaixo daqueles em que se encontra 90% do volume da partícula (d_{90}) foi utilizado para caracterizar o gel (GOMES et al., 2021b). As análises foram realizadas em duplicata

2.6 Viscosidade aparente

As amostras do iogurte foram retiradas da geladeira (7 ºC ± 2 ºC) e realizada a análise de viscosidade aparente usando um viscosímetro (Quimis, Q860M21, Diadema, Brasil) com o spindle L3 e rotação de 10 rpm. O valor de viscosidade aparente em $mPa \cdot s^{-1}$ foi registrado após 60 segundos do início dos testes.

2.7 Microscopia eletrônica de varredura

Uma fina camada de cada amostra de iogurte foi aplicada individualmente em uma fita adesiva dupla-face de carbono, montada em um stub em seguida liofilizada. As microestruturas das amostras foram avaliadas por Microscopia Eletrônica de Varredura (SEM) (Hitachi TM 3000, Hitachi Ltd., Tóquio, Japão) conforme descrito por MUDGIL et al. (2018). As imagens foram gravadas em diferentes ampliações para melhor avaliação.

2.8 Análise estatística

O experimento consistiu em três produções independentes (n=3), utilizando um Delineamento Inteiramente Casualizado (DIC). A médias da composição foram comparadas com o teste de Tukey com o nível de significância de 0,05.

As médias obtidas dos iogurtes foram analisadas por um esquema fatorial (3x3) com fator adicional (controle). Inicialmente verificou-se diferença significativa entre o controle com os demais tratamentos adicionados de fibras. Em seguida foi realizado o teste de Dunnet, em que se compara o controle com cada um dos tratamentos (tipos de fibras e concentrações). E por último foi avaliado o esquema fatorial, o qual consistiu em três níveis de tipos de fibras (Fibra 1, Fibra 2 e Fibra 3) e três concentrações (2,5%, 5% e 10%). Então verificou se houve interação significativa entre os tipos de fibras e as concentrações, ou seja, se os fatores são dependentes. Se a interação foi significativa então, realizou a análise de todos os dados juntos (tipos de fibras e concentrações). Se a interação foi não significativa então foi avaliado separadamente se houve diferença entre os tipos de fibras, e se houve diferenças entre as concentrações. Todas as análises foram realizadas utilizando o software R (R Core Team, 2019).

3 Resultados e Discussão

3.1 Composição dos iogurtes com alta proteína

As composições dos iogurtes adicionados de diferentes tipos e concentrações de fibras são apresentadas na Tabela 1. Não foram encontradas diferenças significativas entre os teores de lactose e de cinzas ($p>0,05$). Enquanto para os teores de proteína e de sólidos totais houve diferenças. O teor de proteína variou de 6,25 para o tratamento T10 a 7,04 para o tratamento T8, portanto, todos os iogurtes podem ser classificados como alta proteína de acordo com a legislação brasileira (BRASIL, 2012). Os teores de sólidos totais variaram de acordo com nível de adição da fibra, sendo que quanto maior o teor da fibra adicionada menor o teor de sólidos totais, apesar dessas diferenças não serem significativas. Uma variação dos sólidos totais de acordo com o nível de substituição dos prebióticos polidextrose e inulina também foi observado SRISUVOR et al. (2013) em iogurte com baixo teor de gordura. Os iogurtes desse estudo também podem ser classificados como sem gordura, por obter um teor de gordura menor que 0,05%. Em relação aos teores de lactose e de cinzas não foram encontradas diferenças significativas (p>0,05).

Tabela 2- Composição (g.100 g $^{-1}$) do leite e do iogurte (n=3)

	Gordura	Proteína	Lactose	Sólidos Totais	Cinzas
Leite	0,21 ± 0,05	3,23 ± 0,06	4,62 ± 0,11	9,11 ± 0,02	-
T1	< 0,05	6,67 ± 0,24^{ab}	4,59 ± 0,40^{a}	16,27 ± 3,17^{b}	1,20 ± 0,01^{a}
T2	< 0,05	7,02 ± 0,14^{a}	4,28 ± 0,29^{a}	16,32 ± 0,67^{b}	1,19 ± 0,03^{a}
T3	< 0,05	6,55 ± 0,34^{ab}	5,64 ± 0,12^{a}	18,58 ± 3,28^{ab}	1,18 ± 0,06^{a}
T4	< 0,05	7,00 ± 0,25^{a}	5,07 ± 0,83^{a}	16,62 ± 0,15^{b}	1,12 ± 0,09^{a}
T5	< 0,05	6,82 ± 0,05^{ab}	5,37 ± 0,40^{a}	19,27 ± 0,05^{ab}	1,17 ± 0,03^{a}
T6	< 0,05	6,47 ± 0,32^{ab}	5,40 ± 0,67^{a}	19,83 ± 0,04^{ab}	1,16 ± 0,02^{a}
T7	< 0,05	6,34 ± 0,48^{ab}	5,04 ± 0,37^{a}	19,81 ± 0,06^{ab}	1,20 ± 0,03^{a}
T8	< 0,05	7,04 ± 0,12^{a}	5,23 ± 0,55^{a}	22,89 ± 0,51^{a}	1,11 ± 0,03^{a}
T9	< 0,05	6,54 ± 0.15^{ab}	5,02 ± 0,25^{a}	21,14 ± 1,61^{ab}	1,11 ± 0,02^{a}
T10	< 0,05	6,25 ± 0,02^{b}	4,83 ± 0,32^{a}	21,96 ± 0,22^{a}	1,09 ± 0,09^{a}

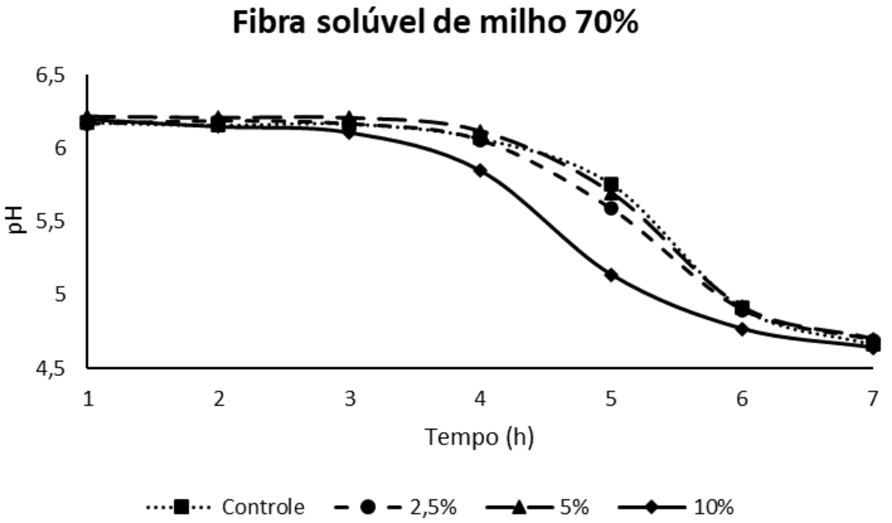
Valores médios ± desvio padrão de três repetições independentes. Dentro da coluna, diferentes sobrescritos indicam diferenças significativas (p<0,05).
T1=Controle; T2 = 2.5% Fibra 1; T3 = 5.0% Fibra 1; T4= 10.0% Fibra 1; T5= 2.5% Fibra 2; T6= 5.0% Fibra 2; T7= 10.0% Fibra 2; T8= 2.5% Fibra 3; T9= 5.0% Fibra 3; T10= 10.0% Fibra 3.

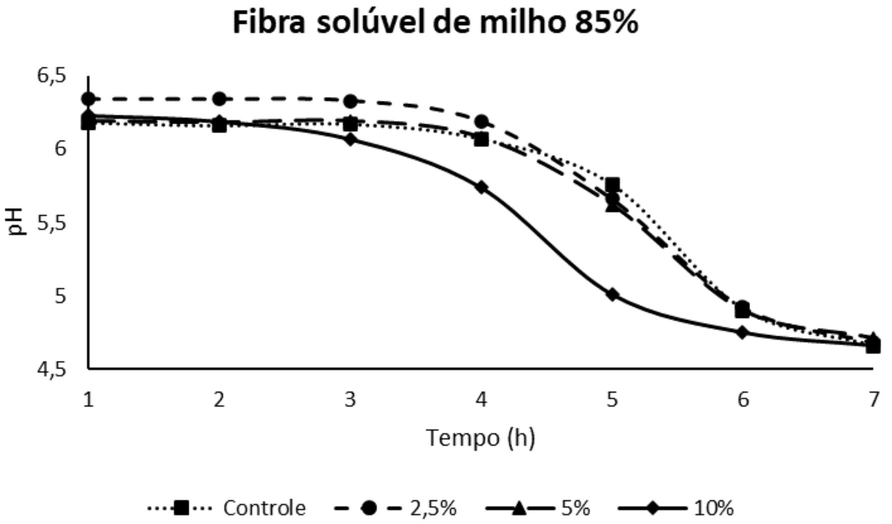
3.2 Curvas de Fermentação

A adição das fibras ao iogurte com alta proteína não modificaram o tempo de fermentação (Figura 1). Durante a fermentação, entre os tempos 3 h e 6 h, observou-se menores valores de pH para os tratamentos adicionados de 10% de fibra, independentemente do tipo da mesma. Considerando o trabalho de GOMES et al. (2021a) não esperado que a presença das fibras afete a capacidade de produção de ácido lático pela microbiota fermentadora. Desta forma pode-se inferir que, entre os tempos de fermentação de 3 h e 6 h para uma mesma quantidade de ácido lático produzida menores valores de pH foram encontrados para os tratamentos adicionados de 10% de fibras, indicando, portanto, menor capacidade tamponante destes tratamentos.

As proteínas do leite, caseína e as proteínas do soro tem capacidade tamponante (SALAÜN; MIETTON; GAUCHERON, 2005), todos os iogurtes produzidos no presente estudo possuem em sua composição alta concentração de proteína. Considerando que quanto maior a quantidade de fibra adicionada, houve um efeito de diluição dos demais componente como nas proteínas que compõe a rede do gel, portanto, pode-se sugerir que uma maior mudança de pH nos iogurtes com 10 % de fibras, se dá devido a um menor efeito tampão.

Uma maior diminuição do pH com o aumento dos sólidos solúveis em iogurtes também foi observado por KHUBBER et al. (2021). O efeito da adição de prebiótico/fibras na taxa de acidificação e diminuição do pH durante a fermentação apresentaram resultados distintos de acordo com o tipo de prebiótico e probiótico adicionado assim com os níveis de prebiótico avaliados conforme relatado por HEYDARI et al. (2011).





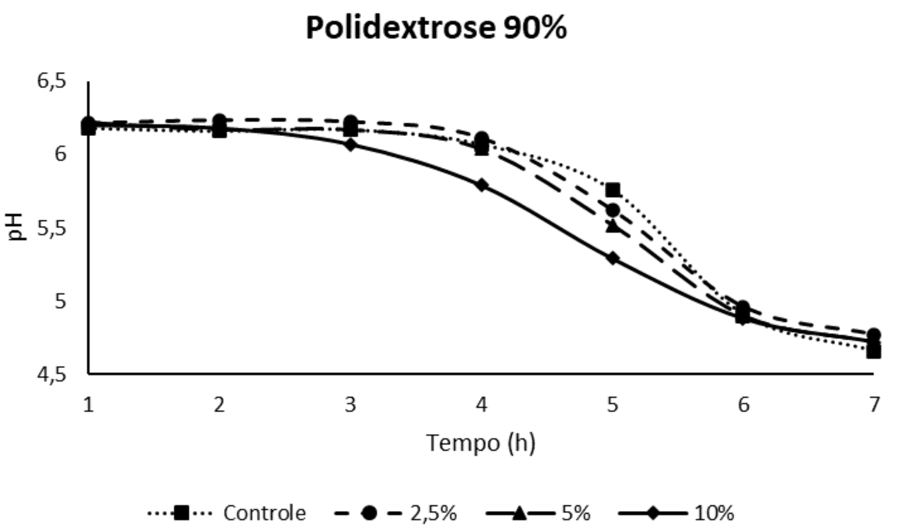


Figura 1- Curvas de fermentação do iogurte com alto teor proteico adicionado com diferentes concentrações de fibras.

3.3 Capacidade de Retenção de Água (CRA)

A CRA dos iogurtes adicionados de fibras considerando todas as concentrações e tipos de fibras avaliadas (uma média geral para todos os tratamentos) em relação ao iogurte controle (sem adição de fibras) foram iguais em todos os tempos avaliados (0 e 45 dias). Também não foram encontradas diferenças significativas quando comparadas as médias para cada tipo de fibras (Fibra 1, Fibra 2 e Fibra 3) e suas diferentes concentrações (2,5%; 5% e 10%) em relação ao controle nos mesmos tempos pelo teste de Dunnet (Figura 2).

Os iogurtes adicionados de diferentes tipos e concentrações de fibras apresentaram uma interação não significativa para CRA, nos tempos 0, 30 e 45 dias (Tabela 3). A interação não significativa indica que os fatores: diferentes tipos de fibras e as diferentes concentrações, atuam de forma independentes, ou seja, independente da fibra que for utilizada na concentração de 2,5% sempre haverá um mesmo resultado para CRA. Essa análise pode ser usada para as demais concentrações e tipos de fibras. Portanto, nos tempos 0 e 30 dias independentemente do tipo de fibra utilizado, as concentrações de 5% e 10% apresentam uma maior capacidade de retenção de água. Esses resultados mostraram que a adição das fibras aumentou a estabilidade da coalhada nesses tempos. Quanto maior a estabilidade da coalhada maior a capacidade de retenção do soro na estrutura do gel (GYAWALI; IBRAHIM, 2016). Além disso, polissacarídeos solúveis, como os utilizados no presente estudo, são capazes de formar ligação de hidrogênio com a água, o que ajuda a reduzir a sinérese, que é uma propriedade inversa a CRA, portanto os polissacarídeos solúveis agem como espessante (BISAR et al., 2015). No tempo de 45 dias não houveram diferenças tanto para o tipo de fibras nem para as concentrações testadas, com valores de CRA variando de 96% a 99%, o que indica uma alta CRA, resultados semelhantes foram obtidos por SRISUVOR et al. (2013), que avaliaram diferentes níveis de polidextrose e inulina em iogurte. A adição de hidrocoloides como as fibras e as proteínas, melhoram a textura e a qualidade dos iogurtes, pois esses dois aditivos tem a capacidade de reter água ou soro na estrutura e formar uma rede similar ao um gel (GYAWALI; IBRAHIM, 2016). Dessa forma, os resultados obtidos com 45 dias mostraram o efeito da proteína adicionada, já que todos os iogurtes são de alta proteína, essa característica é interessante principalmente para iogurtes sem adição de açúcar e gordura como os produzidos no presente estudo.

3.4 Distribuição de tamanho de partículas (d_{90})

A distribuição do tamanho de partículas (d_{90}) quando comparado o controle com os demais tratamentos nos tempos 0 e 45 dias não apresentaram diferenças significativas (Dados não mostrados). As estimativas para a distribuição do tamanho de partículas mostraram que quanto maior a quantidade de fibras adicionadas maiores as estimativas do d_{90}. No tempo 0, todos os tratamentos adicionados de 2,5% e 5% de fibras foram iguais ao controle, já na concentração de 10% foram diferentes do controle com estimativas de d_{90} menores. No tempo de 45 dias os resultados seguiram a mesma tendência dos obtidos no tempo 0, diferenciando apenas nas estimativas d_{90} para a concentração 2,5% com estimativas iguais ao controle (Figura 2).

Os resultados obtidos da análise de distribuição do tamanho de partículas em solução (d_{90}) apresentaram uma interação não significativa em todos os tempos avaliados (Tabela 3). Quando analisamos os dados com relação as concentrações das fibras empregadas, independente do tipo da fibra utilizada (Fibra 1, Fibra 2 e Fibra 3) quanto maior a concentração da fibra (2,5%; 5% e 10%) menor o d_{90}, sendo que nos tempos 0 e 30 dias, com relação aos tipos de fibras utilizados também não houve diferença significativa. Após 45 dias, a Fibra 1 apresentou maior d_{90} e Fibra 3 o menor d_{90}. CAYOT et al. (2008) relacionaram a cremosidade e consistência do iogurte "*stirred*" sem gordura com o tamanho de partículas, esses autores relataram que quanto maior o tamanho de partículas menor a percepção visual e oral de cremosidade do iogurte. Portanto, como os valores de distribuição do tamanho de partículas obtidos no presente estudo variaram de 154-133 µm para a menor concentração das fibras de 2.5% e de 79-89 µm para a maior concentração das fibras testadas de 10%, baseados nesses resultados podemos sugerir que os iogurtes com maiores concentrações de fibras poderiam apresentar maior cremosidade.

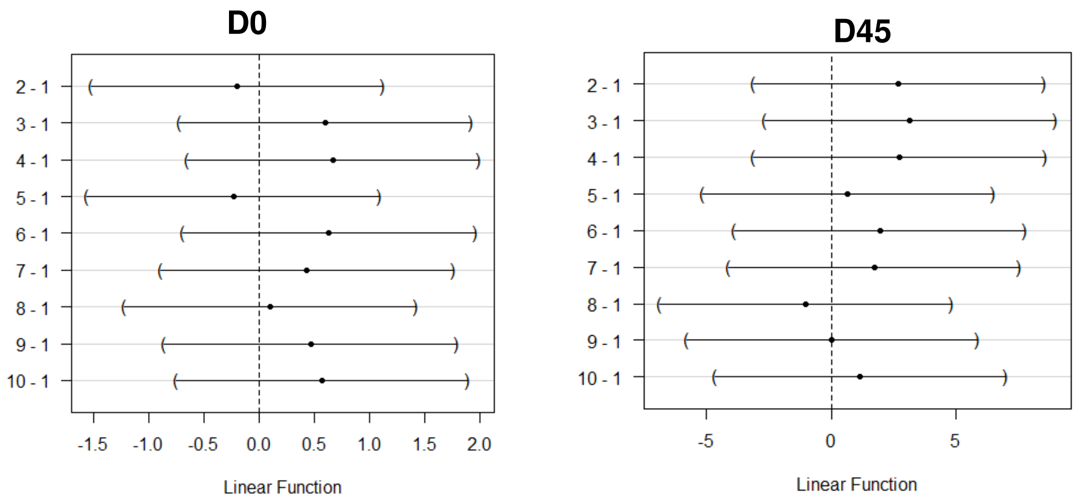
3.5. Viscosidade Aparente

Os iogurtes adicionados de fibras apresentaram uma viscosidade maior que o controle no tempo 0, no entanto essas diferenças não permaneceram no tempo de 45 dias, em que o controle foi significativamente igual aos iogurtes adicionados de fibras (Dados não mostrados).

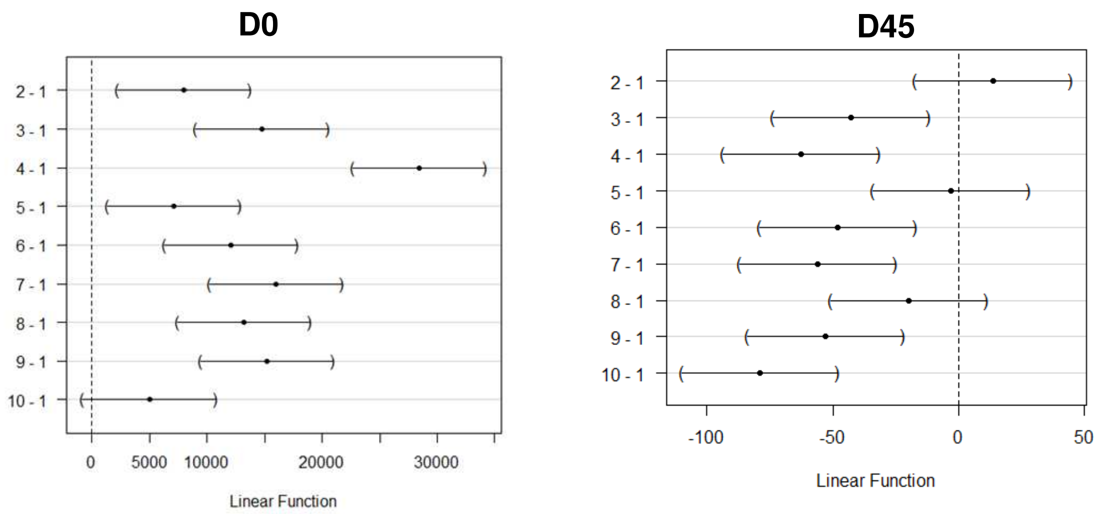
A viscosidade aparente no tempo 0 mostrou que quanto maior a concentração da fibra, maior a viscosidade em relação ao controle para as Fibras 1 e 2. A Fibra 3 apresentou um comportamento similar quando observadas as concentrações 2,5% e 5% e diferente na concentração de 10% em que foi igual ao controle (Figura 2). Uma menor viscosidade aparente na maior concentração de polidextrose (Fibra 3) testada pode ter ocorrido devido a uma estabilização estérica entre as caseínas e o polissacarídeo, resultados similares aos reportados em outros estudos (EVERETT; MCLEOD, 2005; SRISUVOR et al., 2013). No tempo de 45 dias todos os tratamentos foram iguais ao controle para a viscosidade aparente. Esse resultado ocorreu devido aos rearranjos da rede proteica do iogurte durante a vida de prateleira, no qual as interações entre as proteínas foram mais determinantes para a viscosidade do que as fibras adicionadas.

A viscosidade aparente dos iogurtes com alta proteína apresentaram uma interação significativa, ou seja, os fatores tipos de fibras e as concentrações das fibras são dependentes para um determinado resultado de viscosidade aparente no tempo 0 (Tabela 3). Quanto maior a concentração adicionada de polidextrose ao iogurte, maior a viscosidade observada, com valor máximo de 56085 $mPa \cdot s^{-1}$ para a concentração de 10% desta fibra. Já nos tempos de 30 e 45 dias foram constatadas uma interação não significativa. Sendo que, com 30 dias não houve diferenças entre os tipos de fibras e as concentrações, o que indica que independente da fibra empregada e da concentração não houve modificação na viscosidade aparente. Enquanto no último tempo avaliado (45 dias), todas as concentrações das fibras adicionadas ao iogurte proporcionam um mesmo impacto na viscosidade, já com relação ao tipo da fibra, a Fibra 1 apresentou maior viscosidade e a polidextrose menor viscosidade.

Capacidade de Retenção de Água (CRA)



Distribuição do Tamanho de Partículas (d_{90})



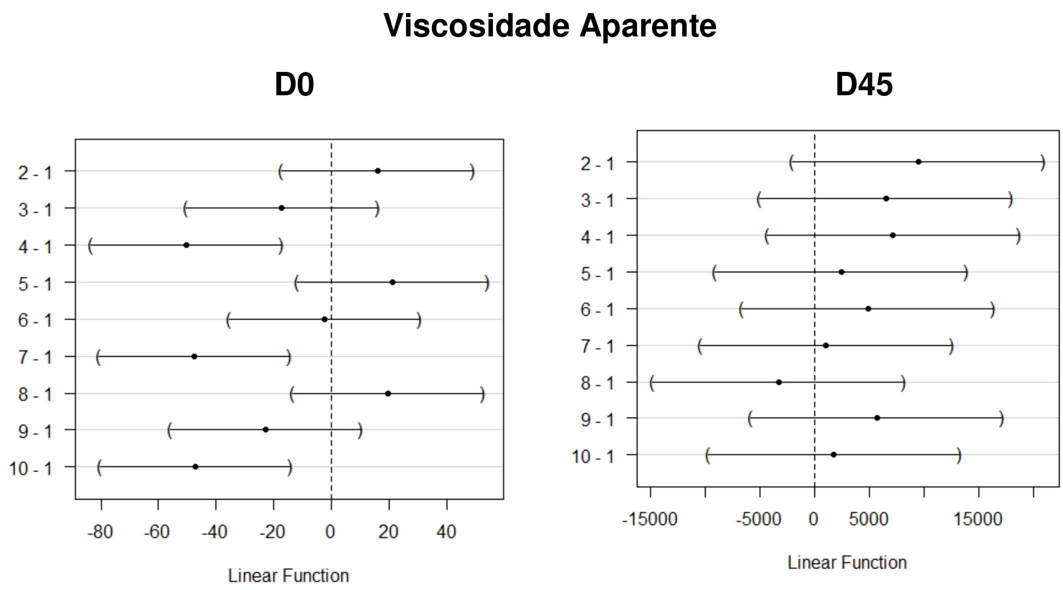


Figura 2- Comparação dos tratamentos T2 → T10 com o controle T1 “0” (----), usando o teste de Dunnet. T2 = 2.5% Fibra 1; T3 = 5% Fibra 1; T4 = 10% Fibra 1; T5 = 2.5% Fibra 2; T6 = 5% Fibra 2; T7 = 10% Fibra 2; T8 = 2.5% Fibra 3; T9 = 5% Fibra 3; T10 = 10% Fibra 3.

Tabela 3 – Efeito do tipo e concentração das fibras na Capacidade de Retenção de Água (CRA), distribuição do tamanho de partículas (d_{90}) e viscosidade aparente dos iogurtes com alta proteína

Tempo	Parâmetros	Tipos de fibras	Concentrações das fibras			Médias
D0	CRA (%)	F1	99,00	99,8	99,87	99,55±0,50^{A}
		F2	98,97	99,83	99,63	99,47±0,70^{A}
		F3	99,30	99,67	99,77	99,47±0,51^{A}
		médias	99,08±0,77^{b}	99,76±0,19^{a}	99,75±0,25^{a}	
	d_{90} (µm)	F1	151,43	118,33	85,28	118,34±27,67^{A}
		F2	156,73	133,13	87,77	125,87±30,65^{A}
		F3	155,17	112,77	88,27	118,73± 30,19^{A}
		médias	154,44±11,11^{a}	121,41±13,20^{b}	87,10±9,34^{c}	
	Viscosidade aparente (mPa·s^{-1})	F1	34.766±1261^{Bb}	39.724±1533^{Aa}	43.668±3297^{Ba}	n.a.
		F2	40.864±1189^{Aa}	42.884±1377^{Aa}	32.657±2477^{Bb}	n.a.
		F3	35.657±676^{Bc}	42.424±108^{Ab}	56.085±3536^{Aa}	n.a.
		médias	n.a.	n.a.	n.a.	n.a.
D30	CRA (%)	F1	96,93	99,13	99,13	98,42±1,30^{A}
		F2	95,67	98,50	98,00	97,34±1,61^{A}
		F3	95,10	96,23	97,30	96,27±2,31^{A}
			95,81±1,51^{b}	98,00±2,28^{a}	98,23±1,21^{a}	
	d_{90} (µm)	F1	149,82	116,95	81,21	115,92±03,96^{A}
		F2	127,88	123,55	80,29	110,57±28,19^{A}
		F3	122,72	93,00	78,08	97,97±22,08^{A}
			133,47±17,58^{a}	111,19±23,52^{b}	79,86±10,88^{c}	
	Viscosidade aparente (mPa·s^{-1})	F1	58.767	64.769	64.653	52.647±16.242^{A}
		F2	59.591	60.238	66.909	51.641±16.630^{A}
		F3	46.108	62.263	54.962	47.549±18.140^{A}
			47.102±15.138^{a}	52.790±16.506^{a}	51.943±19.333^{a}	
D45	CRA (%)	F1	99,03	99,50	99,06	99,19±0,50^{A}
		F2	97,00	98,27	98,06	97,78±0,70^{A}
		F3	95,30	96,36	97,49	96,38±0,51^{A}
			97,11±0,77^{a}	98,04±0,19^{a}	98,20±0,25^{a}	
	d_{90} (µm)	F1	168,83	112,40	92,53	124,60±33,42^{A}
		F2	152,05	107,00	99,06	119,37±25,28^{AB}
		F3	135,10	102,10	76,14	104,45±24,74^{B}
			152,10±17,17^{a}	107,17±7,02^{b}	89,24±12,37^{c}	
	Viscosidade aparente (mPa·s^{-1})	F1	51.300	48.324	48.999	49.541±2.849^{A}
		F2	44.235	46.707	42.879	44.607±3.921^{AB}
		F3	38.521	47.530	43.564	43.205±5.661^{B}
			44.685±6.206^{a}	47.520±3.795^{a}	45.147±4.832^{a}	

Para tipo de fibra para cada parâmetro, médias com diferentes letras maiúsculas nas colunas apresentam diferença significativas pelo teste Tukey ($p < 0,05$). Para concentração das fibras para cada parâmetro letras minúsculas nas linhas apresentam diferenças significativa pelo teste de Tukey ($p < 0,05$). F1 = Fibra 1; F2 = Fibra 2 and F3 = Fibra 3. n.a. = não se aplica.

3.4 Microestrutura do iogurte com alta proteína adicionado de fibras solúveis

As imagens das microestruturas dos iogurtes são apresentadas na Figura 3. Foram obtidas imagens com diferentes magnificações, sendo escolhido o aumento de 500x no tempo 0. Pode-se observar que o iogurte controle apresentou poros maiores distribuídos na rede proteica quando comparados aos iogurtes adicionados de fibras. A presença das diferentes fibras e concentrações na microestrutura dos iogurtes não pode ser claramente notada. TAMIME; BARRANTES; SWORD, (1996) também observaram resultados similares em iogurtes adicionados de hidrocoloide como substitutos da gordura, em que atribuíram esse resultado ao fato dos hidrocoloides serem altamente solúveis. Além disso, esses mesmos autores ressaltaram que a única diferença notável foi obtida no iogurte adicionado de polidextrose, em que a matriz era menos compacta e a microestrutura possuía maior tamanho de poro no produto fresco, assim como apresentado na Figura 3 (F3 [10%]).

KHUBBER et al. (2021), relataram que o aumento da adição de pectina com baixo teor metoxil em iogurte promoveu maior reticulação com caseína e preenchimento das cavidades, no entanto, houve uma ruptura da rede de caseína em níveis máximos da pectina. O que esses autores atribuíram a uma interação eletrostática de caseína e a pectina. Na Figura 3, pode-se notar que F3[10%] apresentou poros maiores quando comparados as demais imagens, o que pode ter ocorrido devido a maior concentração da polidextrose nessa formulação do iogurte.

A rede proteica do iogurte é formada por micelas de caseínas e os espaços vazios, eram ocupados pelo soro antes da liofilização (RAMIREZ-SANTIAGO et al., 2010). Sendo que, iogurtes com sólidos totais mais elevados, como os iogurtes do presente estudo, formam redes de gel mais densas e ramificadas com maior sobreposição de fitas individuais e menores tamanhos de poros (HECK et al., 2021). Segundo LI et al. (2021), quanto mais uniforme for a agregação das caseínas no iogurte melhores serão as propriedade gelatinosa, como por exemplo, maior viscosidade, o mesmo foi observado para os iogurtes adicionados de fibra no tempo 0 do presente estudo.

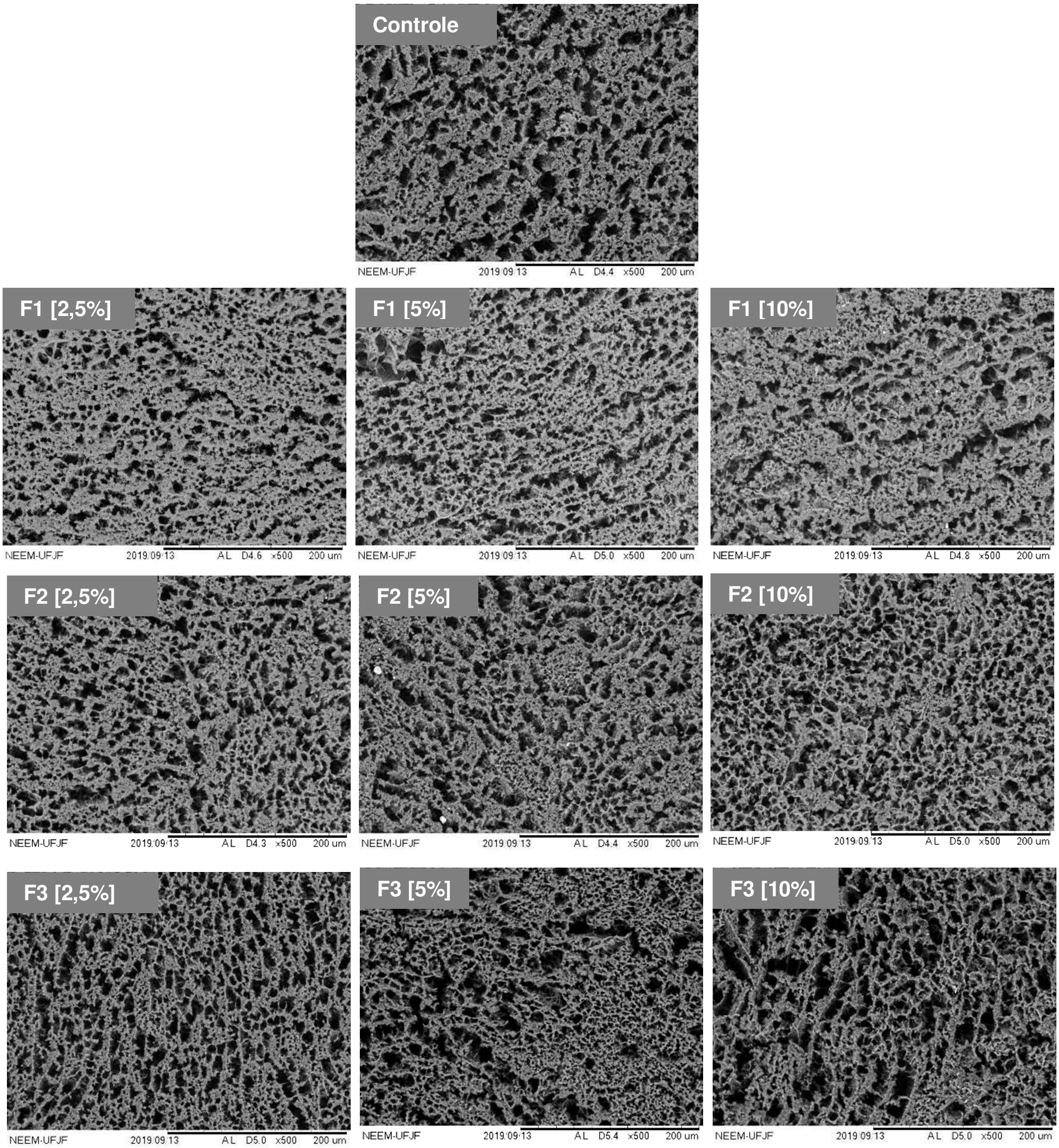


Figura 3- Microscopia eletrônica de varredura dos iogurtes no tempo 0. Magnificação 500x. F1=Fibra 1; F2 =Fibra 2 and F3 = Fibra 3.

4. Considerações finais

Pode-se destacar que os iogurtes com alta proteína quando adicionados da concentração de 10% de qualquer uma das fibras aplicadas nas formulações promoveram uma maior queda de pH após três horas de fermentação. Além disso, foi possível constatar que todos os iogurtes apresentam uma alta CRA, e a viscosidade aparente variou de acordo com a fibra adicionada e o tempo avaliado, em que no final da vida de prateleira as viscosidades dos iogurtes adicionados de fibras foram iguais ao controle. Os valores de d_{90} diminuíram conforme aumentou as concentrações das fibras solúveis adicionadas. As imagens das microestruturas dos iogurtes foram semelhantes, o que mostra que o efeito de uma alta concentração de proteína foi mais determinante que a adição das fibras. Esses resultados trazem informações importantes para a formulação de iogurtes com alta proteína, sem açúcar e gordura e com alta quantidade de fibras, uma vez que esse produto possui mais um atributo (adição de fibras) que contribui para uma dieta mais saudável dos consumidores.

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5- OUTRAS PUBLICAÇÕES DURANTE O DOUTORADO

5.1- ARTIGOS PUBLICADOS

1. SILVA, Fernanda Lopes et al. Monitoramento da distribuição do tamanho das partículas do leite integral e desnatado durante os processos de coagulação ácida ou enzimática **Research, Society and Development**, v. 11, n.1, e7011124438, 2022.

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5.2- CAPÍTULO DE LIVRO PUBLICADO

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6- CONCLUSÕES GERAIS

A gordura e o açúcar contribuem para várias características do iogurte, como textura, sinérese e sensorial, portanto, ao reduzir ou retirar um desses constituintes impacta nas características do produto. A adição de diferentes ingredientes em iogurte pode ser uma alternativa inovadora para o desenvolvimento de produtos com características mais próximas ao iogurte tradicional. No artigo 1, a adição de leitelho em pó como substituto de gordura, resultou em produtos com uma rede da microestrutura menos compacta, com poros maiores, e menor elasticidade e dureza que o iogurte controle. Esses resultados podem contribuir para o direcionamento de desenvolvimento de iogurtes adicionados de leitelho. No artigo 2, mostrou que a adição de 2% da fibra de milho ou polidextrose ao iogurte reduzido em 50% de açúcar em relação ao controle (9% de sacarose) aumentou o tamanho dos poros da rede proteica e diminuiu a sinérese, o que revela o potencial destas fibras como substitutas do açúcar. Estudos futuros podem avaliar a aceitação sensorial dos iogurtes reduzido em açúcar e adicionados de fibras. E no artigo 3, os iogurtes com alta proteína adicionados de diferentes tipos de fibras (fibra de milho 70%, fibra de milho 85% ou polidextrose) e diferentes concentrações (2,5%, 5% e 10%) apresentaram alta capacidade e retenção de água, a distribuição do tamanho de partículas representado pelo d_{90} diminuiu com o aumento do teor das fibras, todos os iogurtes apresentaram altos valores de viscosidade aparente. Os resultados obtidos dos diferentes estudos abrem perspectivas para produção de iogurtes contendo diferentes ingredientes como leitelho e as fibras solúveis.