

Development of yogurts with mixture of sheep and bovine milk: effect on chemical physical characteristics, protein profile and antioxidant activity

Desenvolvimento de iogurtes com mistura de leite ovino e bovino: efeito nas características físico química, perfil de proteínas e atividade antioxidante

Desarrollo de yogures con una mezcla de leche de oveja y bovina: efecto sobre las características fisicoquímicas, perfil proteico y actividad antioxidante

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Micheli Mayara Trentin

ORCID: <https://orcid.org/0000-0001-6208-8552>
Santa Catarina State University, Brazil
E-mail: mixeli@unochapeco.edu.br

Alexandra Fabiola Becker

ORCID: <https://orcid.org/0000-0001-9423-9108>
Santa Catarina State University, Brazil
E-mail: alexandraf.becker@gmail.com

Dalana Hanauer

ORCID: <https://orcid.org/0000-0001-6333-2983>
Santa Catarina State University, Brazil
E-mail: dalanahanauer@hotmail.com

Andressa Bianchi

ORCID: <https://orcid.org/0000-0001-9236-3949>
Santa Catarina State University, Brazil
E-mail: andressabianchi@gmail.com

Ligia Borges

ORCID: <https://orcid.org/0000-0002-1800-2843>
Santa Catarina State University, Brazil
E-mail: ligia_karen_borges@hotmail.com

Ana Luiza Bachmann Schogor

ORCID: <https://orcid.org/0000-0002-8952-8869>
Santa Catarina State University, Brazil
E-mail: ana.schogor@udesc.br

Elisandra Rigo

ORCID: <https://orcid.org/0000-0002-5405-5168>
Santa Catarina State University, Brazil
E-mail: elisandra.rigo@udesc.com.br

Abstract

Yogurt is one of the most popular fermented dairy products and an excellent medium for new ingredients/food source. This study sought to evaluate and characterize bovine milk (BM) and sheep milk (SM) and yogurt made from bovine (BM) and sheep (SM) milk, and BM:SM mixture with the respective 100:0 fractions: 75:25, 50:50, 25:75 and 0:100, and evaluate their influence on physicochemical parameters, antioxidant, protein profile, microstructure and color analysis for yogurts. Yogurts with higher levels of acidity, protein, fat, total solids, ash and calcium were the ones made from SM. Regarding the color attributes, all yogurts showed yellow-green characteristics after 21 days of storage by evaluating parameters a^* and b^* , with a predominance of bovine milk. The antioxidant profile showed a higher concentration at 100:0 and 0:100, but bovine milk has a higher DPPH (2,2-diphenyl-1-picrylhydrazyl) value, while yogurts maintained constant DPPH values during storage, being higher at 0:100. Scanning electron microscopy (SEM) showed that BM (100:0) has a more compact structure, possibly due to the larger size of the fat particles and the lower protein concentration compared to SM (0:100). Sheep milk presented better nutritional value, showing its potential in the manufacture of products.

Keywords: Antioxidants; Physical-chemical composition; Yogurts.

Resumo

O iogurte é um dos produtos lácteos fermentados mais populares e um excelente meio para novos ingredientes / fonte alimentar. Este estudo buscou avaliar e caracterizar o leite bovino (BM) e o leite ovino (SM) e o iogurte elaborado com leite bovino (BM) e de ovelha (SM), e mistura de BM: SM e as respectivas frações de 100:0, 75:25, 50:50, 25:75 e 0:100, e avaliar a influência destas nos parâmetros físico-químicos, antioxidante, perfil de proteínas, microestrutura e

análise de cor para os iogurtes. Os iogurtes com maiores teores de acidez, proteína, gordura, sólidos totais, cinzas e cálcio foram os elaborados com SM. Com relação aos atributos de cor, todos os iogurtes apresentaram característica verde-amarela após o armazenamento de 21 dias pela avaliação dos parâmetros a^* e b^* , com predominância para os de leite bovino. O perfil de antioxidantes apresentou maior concentração para 100:0 e 0:100, porém o leite bovino apresentou maior valor de DPPH (2,2-difenil-1-picrilhidrazil), e, durante o armazenamento os iogurtes mantiveram valores constantes de DPPH, sendo maior para o 0:100. Na microscopia eletrônica de varredura (MEV) observou-se que o BM (100:0) possui uma estrutura mais compacta, possivelmente devido ao maior tamanho das partículas de gordura e a menor concentração de proteínas em comparação ao SM (0:100). A eletroforese indicou alterações O leite ovino apresentou melhor valor nutricional, mostrando o potencial na fabricação de produtos com este leite.

Palavras-chave: Antioxidantes; Composição físico-química; Iogurtes.

Resumen

El yogur es uno de los productos lácteos fermentados más populares y un excelente medio para nuevos ingredientes/fuente de alimentos. Este estudio buscó evaluar y caracterizar la leche bovina (BM) y ovina (SM) y el yogur elaborado a partir de leche bovina (BM) y ovina (SM), y la mezcla BM:SM y las respectivas fracciones 100:0, 75:25, 50:50, 25:75 y 0:100, y evaluar su influencia en parámetros físicoquímicos, antioxidantes, perfil proteico, microestructura y análisis de color para yogures. Los yogures con mayores niveles de acidez, proteína, grasa, sólidos totales, cenizas y calcio se elaborados a la leche de oveja. En cuanto a los atributos de color, todos los yogures presentaron características de color amarillo verdoso después de 21 días de almacenamiento al evaluar los parámetros a^* y b^* , con predominio de la leche bovina. El perfil de antioxidantes mostró una mayor concentración a 100:0 y 0:100, pero la leche bovina presenta un mayor valor de DPPH (1,1-difenil-2-picrilhidrazilo), mientras que los yogures mantuvieron valores constantes de DPPH durante el almacenamiento, siendo mayores a 0:100. En SEM (Microestructura de Escaneo Electrónico) se observó que la BM (100:0) tiene una estructura más compacta, posiblemente debido al mayor tamaño de las partículas de grasa y la menor concentración de proteína en comparación con la SM (0:100). La leche de oveja presentó mayor valor nutricional, mostrando el potencial en la elaboración de productos con esta leche.

Palabras clave: Composición físicoquímica; Antioxidantes; Yogures.

1. Introduction

Yogurt is a fermented product produced from milk by the action of lactic acid bacteria as *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* et al. (Karnopp *et al.*, 2017; Jørgensen *et al.*, 2019; Fazilah *et al.*, 2018; Costa *et al.*, 2015; Costa *et al.*, 2017). This product presents high protein content and convenience of consumption (Mohammadi-Gouraji, Soleimani-Sad, & Ghiaci, 2019), with exponential growth in the world when compared to other dairy products, as well as the research development (Aryana & Olson, 2017). This can be attributed to health benefits, considering their regular intake (Mior, Novello, & Dinon, 2016). Thus, the use of differentiated raw materials, such as sheep milk (SM), may result in particular characteristics to yogurt, in relation to the source of high-quality proteins, calcium and lipids (Abreu *et al.*, 2018; Tribst *et al.*, 2018) and adding value to the product, which comes in line with the current scenario of the consumer market.

Sheep milk products highlight the nutritional and organoleptic traits due to the higher protein and solids content, linolenic acid, conjugated linoleic acid (CLA) (between 0.405 and 1,250 g CLA/100 g fat), essential amino acids, vitamins and minerals, easy digestibility and hypo allergenicity, immunoregulatory effect and activity such as antiobesity, antioxidant and antidiabetic when compared to bovine dairy products (Feng *et al.*, 2019; Kaminarides, Stamou, & Massouras, 2007; Yuan, Chen, & Li, 2014; Wang & Lee, 2015; Tribst *et al.*, 2020a). Yogurt and other dairy products using SM have higher creaminess and stability compared to bovine milk (BM) (Tribst *et al.*, 2018). Due to these properties, the interest is growing in SM yogurt, considering its nutritional value and sensory characteristics (Zamberlin & Samaržija, 2017; Tribst *et al.*, 2020a).

However, the amount of sheep dairy products is limited due to low and seasonal milk production throughout the year and a short lactation period (Tribst, Falcade, & Oliveira, 2019; Tribst *et al.*, 2020b). Studies with use of milk of different species, as well as mixtures of milk types with nearby physical-chemical characteristics may result in products with interesting characteristics such as protein, mineral and fat content, since it has easy digestion, containing small fat globules that provide greater contact area for digestive enzymes, making their use more efficient compared to other milks (Revers *et al.*, 2016; Roy *et*

al., 2020). These different flavor and nutritional profiles can stimulate the consumption of these yogurts (Rettedal *et al.*, 2019; Abreu *et al.*, 2016).

2. Methodology

2.1 Raw Material

Lacaune sheep milk (SM) ($5.71 \pm 0.09\%$ fat, $0.83 \pm 0.11\%$ ash, $4.73 \pm 0.06\%$ lactose, $15.72 \pm 0.33\%$ total solids, $10.03 \pm 0.33\%$ milk solids-not-fat), and bovine milk (BM) ($4.42 \pm 0.24\%$ fat, $0.65 \pm 0.05\%$ ash, $4.23 \pm 0.15\%$ lactose, $12.82 \pm 0.26\%$ total solids, $8.53 \pm 0.08\%$ milk solids-not-fat) was obtained in the municipality of Lajeado Grande, Santa Catarina state, Brazil.

Regarding the BM, it was obtained from crossbreed Holstein and Jersey (Jersolando), reared in pasture system with supplementation of corn silage concentrate, soybean meal and vitamins and minerals complex. The Lacaune ewes were reared in confinement system, with balanced feeding composed of hay and pre-dried grass, corn silage concentrate, soybean meal and vitamin and mineral complex.

Following the collection, the milks were transported in a box of isothermal material containing ice to keep under a temperature of 4 °C until the moment of analysis. Both milks and mixtures performed according to the following proportions of (BM): (SM): 100:0, 75:25, 50:50, 25:75, 0:100, were submitted to filtration and slow pasteurization (65°C for 30 minutes) in Thermomix™ (Vorwerk, Thermomix ® model 5 (TM5)).

All milks and their proportions were pasteurized and analyzed for pH (method 943.02) (mPA 210 - MS Tecnoyon), acidity (method 947.05), lactose by Lane - Eynon (by redox titration using alkaline CuSO₄), in agreement with the Association of Official Analytical Chemistry – AOAC (2016).

2.2 Yogurt Production

Were added to the mixtures 100:0, 75:25, 50:50, 25:75, 0:100, 3 % (w/v) of the culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, CHR-HANSEN) activated for 2 hours to 43 °C, later monitored the fermentation of yogurts through pH, where at pH 4.7 the yogurts followed for cooling in an air-conditioned chamber (SL 200-334 - Solab) at 4 °C. The yogurts were prepared in replica and analyzed after 24 hours.

2.3 Physicochemical characterization of yogurts

The physicochemical analyses of yogurts 100:0, 75:25, 50:50, 25:75, 0:100, were performed during the storage period (01, 07, 14 and 21 days) in triplicate. The pH by method 943.02 (AOAC, 2016), acidity (method 493/IV) according to Adolfo Lutz Institute (2008) and color by colorimetric method (Mini Scan EZ Hunterlab 4500L) according to methodology 14-22 (AACC, 2000) by the CIELAB system, where luminosity L*: which has a scale from zero (black) to 100 (white), the chromaticity coordinate a*: ranges from a* positive (color trend to red tint) to a* negative (color trend for green tint) and chromaticity coordinate b*: ranges from b* positive (color trend to yellow tint) to b* negative (color trend for blue tint).

Protein analyzes factor 6.38 (method 928.08), ash (method 920,153), total solids content (method 990.20), mineral content (method 990.08) and fat content (method 932.06) were performed in accordance with AOAC (2016).

2.4 Determination of antioxidant activity and phenolic content

The determination of the antioxidant activity of the yogurt samples and their fractions 100:0, 75:25, 50:50, 25:75, 0:100, was performed by inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl), as described by Feng *et al.* (2019). The extraction of the supernatant was used to determine the free radical elimination activity of the DPPH and the fractionation of nphenolic

compounds. The percentage of inhibition of DPPH-free radical was calculated from the absorbance value of 517 nm (Feng *et al.*, 2019).

The phenolic content of the yogurt samples 100:0, 75:25, 50:50, 25:75, 0:100 were determined according to the modified Folin-Ciocalteu method procedure (Wang *et al.*, 2011). Data were expressed as equivalents of gallic acid in mg per 100 g of weight (mg GAE.100g⁻¹). Both activities were determined in the yogurts on the 1st and 21st days of storage.

2.5 Scanning Electron Microscopy (SEM)

The microstructure was determined by a field emission scanning electron microscope (FEG) (JSM6701F, JEOL, Brazil), an equipment with x-ray spectrometry microanalysis (EDS) system. The samples for SEM analysis were rapidly frozen in liquid nitrogen at -196° C for 10 minutes, and lyophilized (ilShin® America, TFD series), comprising milks and their respective fractions after pasteurization (100:0, 75:25, 50:50, 25:75, 0:100) and also yogurts and their fractions (100:0, 75:25, 50:50, 25:75, 0:100) at 21 days of storage. Then added in sputter coater (SDC 050, Baltech, Brazil) and deposited on a circular aluminum stump, coated with golden conductive tape. The analyses were conducted at 15 kV of electron acceleration and 1000 times for sample enlargement and visualization (Yuliarti *et al.*, 2019).

2.6 Electrophoresis

The partial characterization of pasteurized milk proteins and their mixtures (100:0, 75:25, 50:50, 25:75, 0:100), and yogurt at 21 days of storage (100:0, 75:25, 50:50, 25:75, 0:100), was performed using electrophoresis in dodecyl sulfate-polyacrylamide gel (SDS PAGE), with modifications (Laemmli, 1970; Magenis *et al.*, 2014), with 15% separation gel and 4% stacking gel. The gels were colored with Coomassie Brilliant Blue R-250 (0.3%) in 40% of methanol and 10% of acetic acid and the molecular weights estimated from the bands of standard proteins in relation to the samples.

2.7 Statistical analysis

The results obtained from the analyses were statistically evaluated by the Tukey Test, through variance analysis (ANOVA), using statistic 10.0 software to detect the significant difference between samples, at a 95% confidence level.

3. Results and Discussion

3.1 Physicochemical analyses of milk

The results of the physicochemical analyses of milk and their mixtures (Table 1) indicate a relationship between the addition of sheep milk in the mixtures.

Table 1 – Analysis of pH, titratable acidity and lactose of bovine milk (BM) and sheep milk (SM) and their respective proportions (n = 6).

BM:SM	pH	Titratable acidity (% lactic acid)	Lactose (%)
100:0	6.63 + 0.01 ^{ab}	0.14 + 0.01 ^c	4.00 + 0.14 ^c
75:25	6.66 + 0.02 ^a	0.15 + 0.02 ^c	4.10 + 0.04 ^{bc}
50:50	6.61 + 0.04 ^b	0.16 + 0.01 ^{bc}	4.13 + 0.04 ^{bc}
25:75	6.58 + 0.04 ^c	0.18 + 0.01 ^{ab}	4.33 + 0.12 ^{ab}
0:100	6.56 + 0.01 ^c	0.20 + 0.02 ^a	4,44 + 0.10 ^a

* Different letters in the same line indicate a significant difference (p < 0.05). Source: Authors.

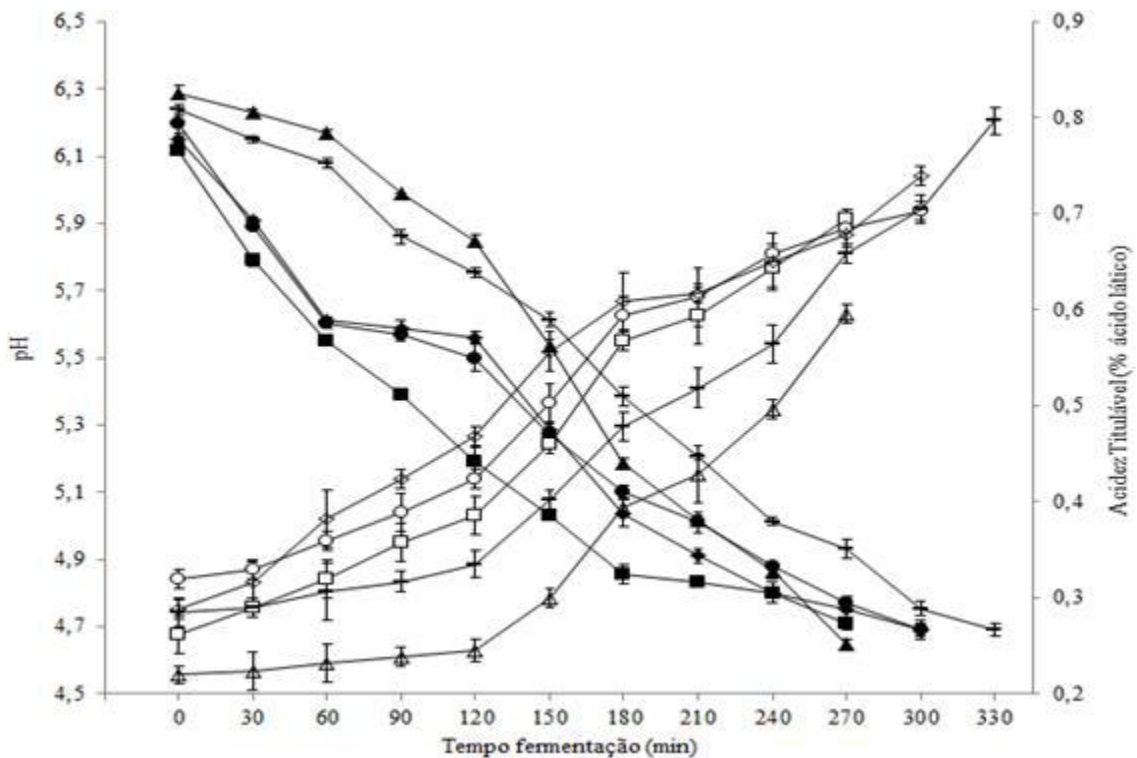
In relation to pH, BM presented values higher than SM ($p < 0.05$) and consequently lower acidity ($p < 0.05$). The same behavior can be observed in mixtures where the proportion of BM in SM was lower (Table 1). The pH and acidity values of sheep milk can be influenced by production in different seasons, with pH ~ 6,58 at the winter (Malta *et al.*, 2021), value similar to 0:100 (BM:SM). But the 100:0 was the even to 75:25 and 50:50 ($p < 0.05$). Similar behavior was observed for milk mixtures BM:SM (100:0) pH 6,77 and 6,41 (0:100) (Lima *et al.*, 2020).

The lactose content showed statistical difference ($p < 0.05$), from the mixtures of major proportion of sheep milk (25:75 and 0:100). Lactose content is one of the compounds that has lower sensitivity for variation due to seasonality. However Fava *et al.* (2014) observed quantitative changes in SM lactose throughout the year ranging from 4.17 ± 0.4 to 4.60 ± 0.16 %. Lactose also varies throughout the lactation stage for all ruminants, presenting lower levels at the beginning and end of lactation (Park *et al.*, 2007). Lactose showed no significant difference ($P < 0.05$) between the seasons of the year ~ 5 % (Malta *et al.*, 2021) and similar tendency was observed for milk mixtures BM:SM (100:0) 3,74 and 4,26 (0:100) (Lima *et al.*, 2020). The values found in this study were close to those obtained by Nguyen *et al.* (2013) to lactose content, 4.40%, similar to 0:100 (BM:SM).

3.2 Fermentation time

The fermentation time of yogurt was affected in relation to the type of milk, with the longest time of 330 min for SM compared to 240 min for BM (Figure 1), following the same behavior for yogurt with milk mixtures, and the highest concentrations of SM required longer periods to reach pH 4.7.

Figure 1 - Values of titratable acidity and pH during fermentation up to pH 4.7 of milk and their respective mixtures of bovine milk: sheep milk (BM:SM) (n = 6).



*100% bovine milk (100:0) (\square) acidity and (\blacktriangle) pH; 75% bovine milk and 25% sheep milk (75:25) (\square) acidity and (\blacksquare) pH; 50% bovine milk and 50% sheep milk (50:50) (\circ) acidity and (\bullet) pH; 25% bovine milk and 75% sheep milk (25:75) (\diamond) acidity and (\blacklozenge) pH; 100% sheep milk (0:100) (\circ) acidity and (\square) pH. Source: Authors.

The yogurt that had the highest concentration of SM (0:100) presented the highest fermentation time (330 min) and the highest titratable acidity value (0.79 % lactic acid), being the lowest value of acidity obtained for yogurt with BM (100:0) (0.59

% lactic acid / 265 min). These differences are possibly associated with higher levels of total solids, especially sheep milk protein (Asensio-Vegas *et al.*, 2018), which has a direct effect on milk structure and the mechanism of action of milk culture.

During yogurt fermentation, the living microorganisms of the initial culture added perform metabolic activities that modify the physical and nutritional characteristics, decomposing complex components and forming subproducts (Rettedal *et al.*, 2019), acting mainly on the lactose present in milk, breaking its structures and producing organic acid molecules, such as lactic acid (Pereira da Costa & Conte-Junior, 2015; Sultan *et al.*, 2016), stage in which texture and flavor characteristics are developed (Bett *et al.*, 2017).

3.3 Physicochemical analyses of yogurts

Table 2 presents the results of physical-chemical composition of yogurts produced from SM and BM and their mixtures.

Table 2 - Physical chemical composition of yogurts (means followed by standard deviations) from bovine milk (BM) and sheep milk (SM) and their respective mixtures (BM:SM) (n=6).

Yogurt BM:SM	Protein (%)	Fat (%)	Total solids (%)	Ashes (%)	Calcium (mg.L ⁻¹)
Yogurt 100:0	3.51 ± 0.19 ^d	3.85 ± 0.08 ^c	11.40 ± 0.34 ^e	0.71 ± 0.01 ^c	483.77 ± 0.45 ^e
Yogurt 75:25	3.50 ± 0.02 ^d	5.00 ± 0.23 ^d	12.61 ± 0.41 ^d	0.70 ± 0.05 ^c	502.5 ± 4.25 ^d
Yogurt 50:50	4.35 ± 0.05 ^c	5.72 ± 0.48 ^c	13.96 ± 0.68 ^c	0.83 ± 0.01 ^b	506.47 ± 5.40 ^c
Yogurt 25:75	5.39 ± 0.05 ^b	6.17 ± 0.91 ^b	14.86 ± 0.63 ^b	0.86 ± 0.01 ^a	573.06 ± 11.02 ^b
Yogurt 0:100	5.83 ± 0.04 ^a	6.47 ± 0.67 ^a	16.98 ± 0.64 ^a	0.88 ± 0.01 ^a	585.91 ± 17.30 ^a

* Different letters in the same line indicate a significant difference ($p < 0.05$), (Tukey, ANOVA). Source: Authors.

Yogurts differed statistically for the variables protein, fat, total solids, ash and calcium ($p < 0.05$), increasing values as the proportion of sheep milk increased (Table 2).

A study conducted by Vianna *et al.* (2017), showed this same trend, i.e. bovine milk yogurts had lower protein content (3.39 ± 0.00 %) when compared to sheep yogurt (5.25 ± 0.00 %). The higher protein content of sheep yogurt in relation to bovine yogurt is due to the compositions of both milks, being characteristic with higher protein contents in sheep milk (Pandya & Ghodke, 2007; De La Vara *et al.*, 2018). Serra *et al.* (2009), followed the shelf life of bovine milk yogurts, noting the hydrolysis of casein and the increase of soluble nitrogen, which consequently means a decrease in the total protein content after 28 days of storage.

The lipid compositions of yogurt mixtures showed statistical difference ($p < 0.05$), demonstrating an increase in the fat content caused by the addition of sheep milk (Table 2). The characteristics of sheep milk fat globules have a smaller diameter, around 3.5 μm , while bovine milk has a diameter of 4 μm (Balthazar *et al.*, 2017). In the manufacture of yogurt, the small uniform fat globules of sheep milk ensure in the processing homogenization and dispersion, avoiding the separation of the cream into yogurt (Kalyankar *et al.*, 2016). The diameter of fat globules also influences digestive capacity, i.e., in milks with a larger globular diameter, lipid metabolism acts less efficiently (Gantner *et al.*, 2015), being slowly digestible.

The total solids parameter showed statistical difference ($p < 0.05$), with an increase in the values in the mixtures along the addition of sheep milk, directly related to the higher protein and fat content (Table 2). The mixtures yogurt 25:75 and yogurt 0:100 (BM:SM) resulted in higher total solids contents 14.86 ± 0.63 % and 16.98 ± 0.64 %, respectively. The lower solids content in 100:0 yogurt (11.40 ± 0.34 %) is evident by the original composition of milk. The values for this parameter were similar to the study conducted by Eissa, Babiker and Yagoub (2011). The difference between the chemical compositions between milks is related to several factors, as described by Park *et al.* (2007), such as lactation period, age and breed of animals, milking intervals, season, temperature, nutrients from the diet and diseases in the udder.

The ash content is related to the mineral composition of the mixtures, which presented statistical difference ($p < 0.05$). The mixtures of yogurt 50:50, yogurt 25:75 and yogurt 0:100 showed the highest ash contents, $0.83 \pm 0.01\%$, $0.86 \pm 0.01\%$ and $0.88 \pm 0.01\%$, respectively (Table 2). Regarding mineral content, Vianna *et al.* (2017), obtained lower ash contents for bovine milk yogurts ($0.77 \pm 0.00\%$) compared to sheep yogurt ($0.99 \pm 0.00\%$), as well as studies presented by Gomes *et al.* (2013).

The mineral content available in yogurt is directly related to the content contained mineral in the raw material. In this study, only the analysis of calcium content was for its predominance, where according to studies (Park *et al.*, 2007; Payandeh *et al.*, 2015), the mineral calcium content in bovine milk can range from 107 to 133 mg.100 mL⁻¹ and 136 to 218.44 mg.100 mL⁻¹ for sheep milk.

The highest calcium contents were found in yogurt samples (25:75) with 573.06 ± 11.02 mg.L⁻¹ and yogurt (0:100) with 585.91 ± 17.30 mg.L⁻¹, with the predominance of sheep milk (Table 2). Comparing the values of mixtures with predominance of bovine milk in yogurt (100:0) and yogurt (0:100), the values present significant difference ($p < 0.05$). The results obtained are in line with the literature, where sheep's milk compared to other milks has higher concentrations of nutrients, including calcium (Yuksel *et al.*, 2012).

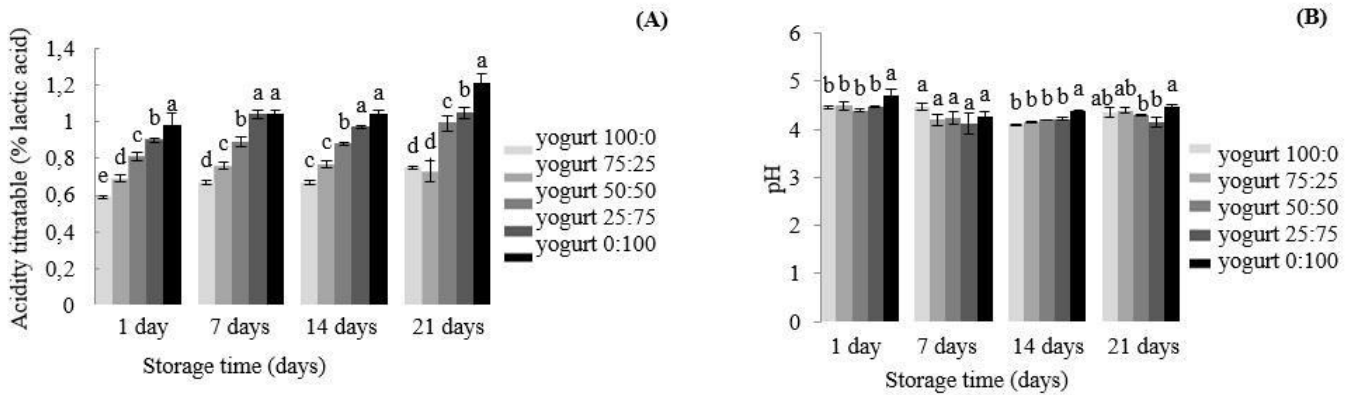
The content total solids, ash, minerals and other nutrients the sheep's milk may undergo greater variations when compared to bovine milk. These changes are influenced by lactation stage, animal nutritional status, environmental and genetic factors caused by feeding and seasonal variations (Kalyankar *et al.*, 2016).

3.4 Titratable acidity and pH of yogurts

Titrate acidity increased gradually (Figure 2A) while pH values showed a slight decreasing trend (Figure 2B) for all yogurts during the storage period of 21 days at 4 ± 1 °C. This behavior is related to milk cultures added to yogurt, which can develop on a smaller scale in adverse conditions such as storage temperature, producing organic acid and altering chemical characteristics of the product, such as pH and acidity.

Yogurt 100:0 prepared only by BM resulted in an increase in significant acidity ($p < 0.05$) between the 1st day and the 21st day of storage from $0.59 \pm 0.01\%$ lactic acid to $0.75 \pm 0.01\%$ lactic acid, respectively. Sheep yogurt (0:100) showed differences in more expressive acidity values ($p < 0.05$), $0.98 \pm 0.07\%$ lactic acid and $1.21 \pm 0.05\%$ lactic acid for 1st day and 21st days of storage, respectively. Significant differences in acidity between formulations during the storage period ($p < 0.05$) are observed, since milk from different species has different compositions of constituents that have effects on the process (Rettedal *et al.*, 2019). As in the raw material, the milk mixtures with the presence of sheep milk in yogurts (75:25, 50:50 and 25:75) present higher acidity when compared to yogurt (100:0) in different storage times. Similar results are observed in the study by Sultan *et al.*, (2016) to obtain yogurts with milk of different species (cow, sheep, goat and buffalo) for 10 days of storage.

Figure 2 - Acidity titratable and pH values of yogurt mixtures in the following proportions: bovine milk (BM): sheep's milk (SM) during the period of 21 days of storage at 4°C (n=2).



* Different letters in the same line indicate a significant difference ($p < 0.05$), (Tukey, ANOVA). Source: Authors.

The pH change after fermentation processing (Figure 2A), where the standard pH was 4.7 can be evidenced by its difference compared to the first day of storage (Figure 2B). It is observed that the pH values did not have great variations between analyzed times ($p < 0.05$) between the samples, but it is worth mentioning that the pH of yogurt can influence the syneresis process, which increases when the values are below 4.7, resulting from ferroprotein that are soluble, and milk caseins that do not remain at the isoelectric point (PI) (Farrell *et al.*, 2006).

3.5 Color Analysis

Table 3 - Variations in the color index for yogurt from the mixtures of bovine and sheep milk (BM:SM) in the following proportions, during 21 days of storage at 4°C (n=6).

Storage Days	Yogurt 100:0	Yogurt 75:25	Yogurt 50:50	Yogurt 25:75	Yogurt 0:100
L*	01 71.59± 0.69 ^c B	71.42± 0.54 ^c A	79.41± 0.41 ^b A	85.89± 0.57 ^a A	66.33± 0.76 ^d B
	07 66.55± 0.83 ^b C	70.29± 1.06 ^a AB	64.86± 0.69 ^b C	71.39± 0.97 ^a B	67.67± 0.96 ^b B
	14 66.76± 0.92 ^{abc} C	71.71± 1.01 ^a A	69.77± 1.13 ^{ab} B	67.90± 0.95 ^{bc} C	65.44± 0.37 ^c B
	21 78.30± 0.41 ^a A	72.81± 1.13 ^b A	69.45± 1.17 ^d B	71.62± 0.27 ^c B	71.78± 0.70 ^c A
a*	01 -6.17± 0.04 ^a A	-6.65± 0.15 ^a A	-6.38± 0.07 ^a A	-6.06± 0.09 ^a A	-6.32± 0.10 ^a A
	07 -6.51± 0.24 ^a A	-6.93± 0.71 ^a A	-7.07± 0.52 ^a A	-7.09± 0.72 ^a A	-6.68± 0.39 ^a A
	14 -6.25± 0.27 ^a A	-6.71± 0.07 ^a A	-6.45± 0.14 ^a A	-6.10± 0.18 ^a A	-6.36± 0.08 ^a A
	21 -6.76± 0.10 ^b A	-6.83± 0.09 ^b A	-6.76± 0.10 ^b A	-6.86± 0.02 ^b A	-8.56± 0.33 ^a A
b*	01 12.27± 0.11 ^a A	12.18± 0.27 ^a AB	11.83± 0.18 ^{ab} A	9.97± 0.21 ^b A	9.17± 0.29 ^b B
	07 11.60± 0.47 ^a AB	10.41± 0.29 ^b B	12.36± 0.90 ^a A	9.5± 0.32 ^{bc} A	9.37± 0.45 ^c B
	14 11.92± 0.37 ^a AB	12.04± 0.12 ^a AB	11.06± 0.23 ^b A	11.58± 0.58 ^{ab} A	9.52± 0.14 ^c B
	21 12.80± 0.45 ^a A	12.72± 0.43 ^a A	11.26± 0.17 ^b A	10.99± 0.04 ^{bc} A	10.80± 0.45 ^c A

* Different letters in the same line indicate a significant difference ($p < 0.05$) (Tukey, ANOVA). Source: Authors.

After 24 hours of the preparation of yogurts, the lowest value of *L, related to the yogurt sample (0:100) (Table 3), suggests that the greater amount of total solids found for this sample (Table 2) may favor the absorption and reduction of free water, with a lower reflection of light (García-Pérez *et al.*, 2005).

L* values elevated after 21 days of storage are correlated with yogurt whiteness that is associated with yogurt refrigeration after fermentation (Peixoto *et al.*, 2016; García-Pérez *et al.*, 2005).

The values of a* did not differ significantly ($p > 0.05$) between the samples during storage time and showed negative values which the yogurt is shown as greenish (Table 3).

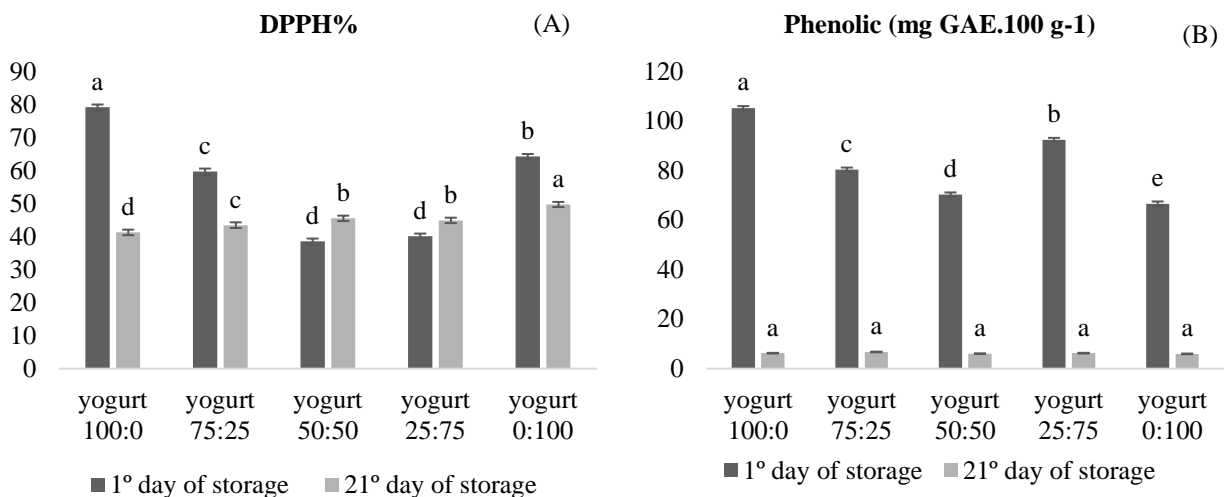
For parameter b* there was a significant difference between the evaluated samples ($p < 0.05$), (Table 3). The yogurt prepared with only bovine milk (100:0) presented the highest value of b*, statistically equivalent to the values obtained for the mixtures and consequently greater tendency to yellowing. Sheep milk has higher amounts of vitamin A than bovine milk (Park *et al.*, 2007). Sheep and goats convert all β -carotene into retinol in milk, resulting in whiter milks (Mohammadi-Gouraji *et al.*, 2019) opposing the more yellowish color caused by the carotenoids of bovine milk (Balthazar *et al.*, 2017).

3.6 Antioxidant activity

Studies have shown that casein and whey proteins have antioxidant properties, presumably based on their ability to bind transition metals and eliminate free radicals (Tong *et al.*, 2000).

Figure 3 shows that the samples 100:0 and 0:100 showed the highest antioxidant activity (DPPH), before the storage period, followed by the corresponding mixtures 75:25 and the 50:50 and 25:75 that not showed statistical difference ($p < 0.05$) on the 1st day.

Figure 3 - Antioxidant activity in DPPH (%) (Figure A) and phenolic content (gallic acid in mg.100g-1 sample) (Figure B) in relation to yogurts on the 1st day and yogurt on the 21st day from the mixtures of bovine and sheep milk (BM:SM) and their proportions stored at 4°C (n=6).



* Different letters in the same line indicate a significant difference ($p < 0.05$), (Tukey, ANOVA). Source: Authors.

The different behaviors of antioxidant compounds are defined according to the environment in which they are found (Cottica *et al.*, 2018), either in pure milk or in mixtures. According to Rashidi *et al.* (2016), antioxidants mostly work synergistically in the presence of other antioxidants.

Many factors, such as antioxidant concentration, temperature, pH and processing treatment, can influence the antioxidant activity of the products (Gazzani *et al.*, 1998). The yogurts 50:50 and 25:75 did not differ significantly ($p < 0.05$) for the DPPH values, since the concentrations of sheep and bovine milk resulted in less variation of the antioxidant factor during storage.

The DPPH radical elimination activity expresses the potential of yogurt substances to act as electron or hydrogen donors in the stable DPPH radical. According to studies by Brand-Williams, Cuvelier and Berset (1995), yogurts manufactured from sheep milk, cow or goat milk presented high levels in all yogurts for up to 21 days (58-70 %), similar to the results of present study.

According to Farvin *et al.* (2010) the oxidative stability of yogurt may be due to antioxidant peptides released during milk fermentation by lactic acid bacteria, we can consider the formation of new substances, with antioxidant activity. During storage of yogurts Lima *et al.*, (2014) also found an increase in antioxidant activity. This behavior may explain the similarity and stability in the percentage of radical elimination activity for the yogurt samples after storage for 21 days. Furthermore, nutrients can have their total amount changed due to chemical degradation caused by storage and can promote the release of new compounds, causing effects on the availability of active compounds (Caleja *et al.*, 2016).

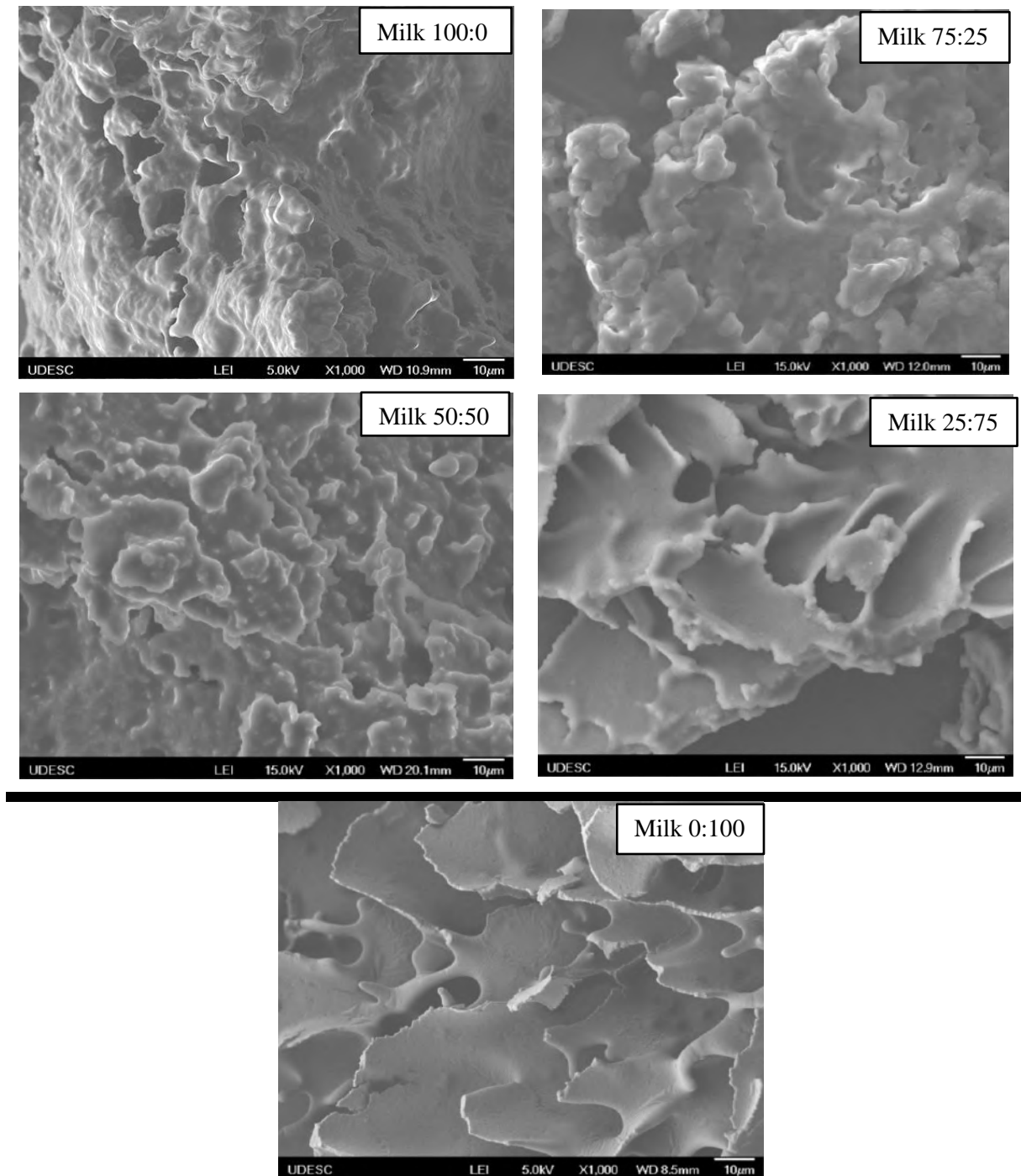
Polyphenols are part of the antioxidant group. Ruminants have a mechanism of detoxification of phenolic compounds by microorganisms in the rumen (Gagnon *et al.*, 2009), and this indicates that concentrations of polyphenols secreted in milk are very low. BM (100:0) exhibited the highest value in phenolics, which cooperated for the total percentage of radical elimination activity. All samples showed a significant difference ($p < 0.05$) from each other.

Storage time significantly affects the maintenance of phenolic compounds in all yogurts. A decrease in phenolic content was observed in all yogurt samples tested. Similar results were found in studies by Karaaslan *et al.* (2011), where they tested the addition of four varieties of grapes in yogurt by analyzing contents of phenolic compounds in storage. Phenolic compounds are easily degradable and form high reactivity compounds, in which the reduction in phenolic content is related to the decline in the antioxidant capacity of yogurts. The samples did not differ significantly differences ($p < 0.05$) in the 21 days of storage.

3.7 Scanning Electron Microscopy (SEM)

Sheep milk has smaller fat globules and also due to the higher protein content, it remains a less compact structure than bovine milk (Figure 4). Bovine milk with rounded forms, lower fat and protein content leave the structure with small imperfections and more compact, as shown by 100:0) and 75:25 that has higher bovine milk content in its composition.

Figure 4 - Scanning Electron Microscopy (SEM) in relation to milk on the 1st day from the mixtures of bovine and sheep milk (BM:SM) and its following proportions.



Scanning Electron Microscopy with magnification of 1000x. Source: Authors.

The pasteurization and fermentation process act on the milk structure thus generating modifications that are easily observed in the final product. Slow pasteurization uses lower temperatures for a longer period of time, which results in less interference on the conformity of proteins and fats. Milk fat content has an important effect on gel structure especially if a homogenization step is applied to reduce fat globule size. The homogenized fat globule interface consequently includes caseins and may then participate actively to the gel network and impact their properties (Gregersen *et al.*, 2021). In the micrographs, a differentiated microstructure is observed that may be related to the fat globules and the protein network of BM and SM milk and

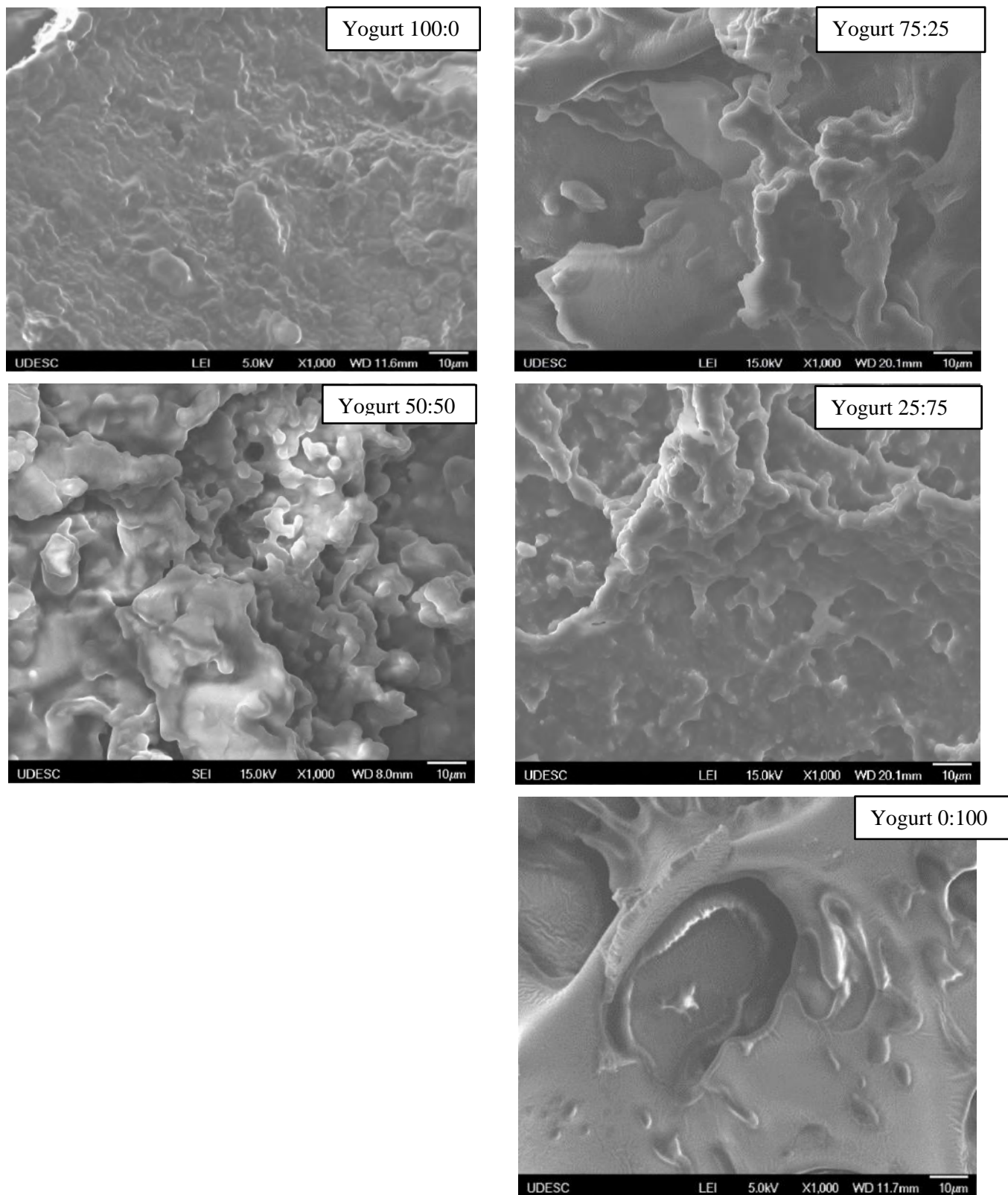
their mixtures. The differences between 100:0 and 0:100 were remarkable (Figure 4), in addition, the lower protein, fat and total solids content in BM may have influenced its microstructure.

In all yogurt gels, protein nets were formed after acidification of the medium (Figure 5). The microstructures of yogurts (100:0, 75:25, 50:50, 25:75 and 0:100), which consist of a three-dimensional network of chain and aggregates of casein micelles in which the 100:0 yogurt gel exhibited a more porous microstructure and less dense protein network, while the yogurt gel 0:100 has a more homogeneous structure (Nguyen, Afsar, & Day, 2018), with the most defined crevices, being observable in yogurts 25:75 and 0:100 with greater proportion of SM, with globular shape, interspersed with empty zones (Nguyen, Afsar & Day, 2018; Bramanti *et al.*, 2003).

The structural difference displayed in yogurts is the result of the different physical-chemical properties of dairy materials used in yogurt production (Nguyen, Afsar & Day, 2018), considering what water retention capacity, is dependent increase dry matter, protein and fat contents (Gregersen *et al.*, 2021), in addition in which acidification was induced by the fermentation of lactic acid bacteria, may be justification.

The similarity between 100:0 and 75:25 is remarkable, as well as between 25:75 and 0:100, demonstrating that the organization of the yogurt gel was influenced by the proportion of the type of milk used.

Figure 5 - Scanning Electron Microscopy (SEM) in relation to yogurts in the 21 days of storage, derived from the mixtures of bovine and sheep milk (BM:SM) and its proportions.

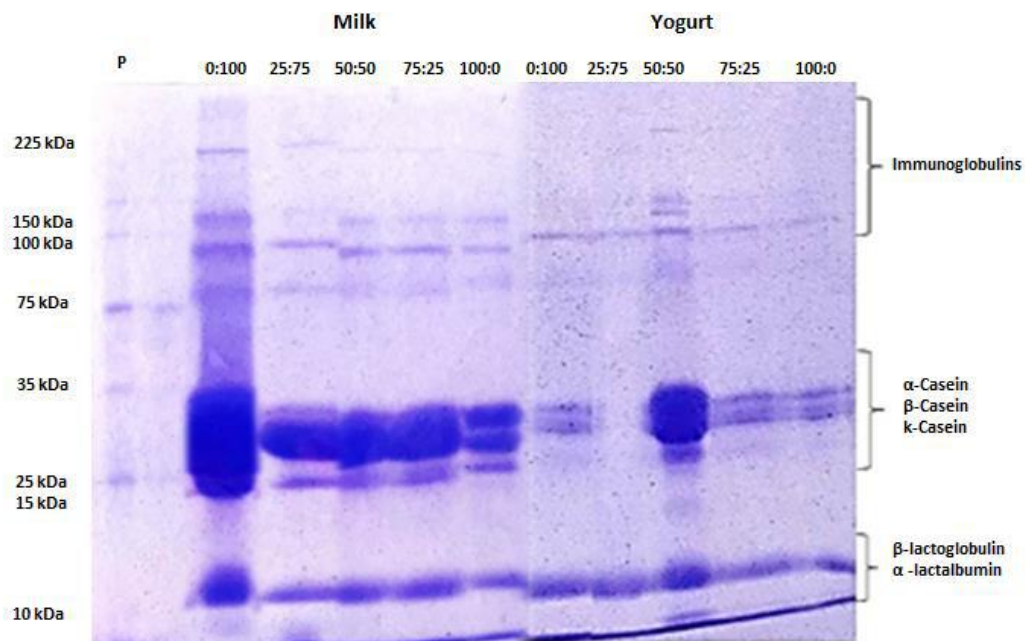


Scanning Electron Microscopy with 1000x magnification. Source: Authors.

3.8 Electrophoresis

The behavior of proteins in BM, SM and their mixtures and also in yogurts with respective mixtures, by the relationship of electrophoretic profiles, obtained with the addition of SDS (sodium dodecyl sulfate, anionic detergent). The relationship between standard molecular weight (P) and protein bands are identified as immunoglobulins include the range of 150 – 1,000 kDa, lactoperoxidase (75 kDa), α -s-casein (35 kDa), β -casein (25 kDa), β -lactoglobulin (15 kDa), α -lactalbumin (10 kDa) (Figure 6), fractions vary in molecular weight (PM), isoelectric point and phosphorylation level.

Figure 6 - Representation of proteins by the Electrophoresis technique in SDS-PAGE gel in bovine milk and yogurt (BM): sheep milk (SM) and its proportions.



P: Standard, BM, bovine milk, SM: sheep milk. Source: Authors.

Immunoglobulins (150 – 1,000 kDa) are at higher concentration in 0:100. During fermentation there is a decrease in pH, facilitating the hydrolysis of proteins in smaller peptides with bioactive properties in which yogurt prepared from BM the bands decreased in thickness indicating the lower presence.

The division between α and β -casein is hardly visible in electrophoresis gel due to the greater amount of these proteins in milk (Balthazar *et al.*, 2017), the darker intensity band is considered by the largest fraction of caseins (α s-casein and β -casein) observed in whole milks and their respective mixtures. The possible differences in the fraction of the main casein proteins and molecular structures of the SM proteins (Figure 5), result in the different structures of the yogurts, and there may be interference in the gelation and firmness of the yogurt.

The largest fraction of caseins of milk due to the transformations that occur during fermentation and yogurt production, were possibly transformed into peptides of molecular weight less than 10kD (Preci *et al.*, 2021).

β -lactoglobulin is present in greater amounts in SM than other whey proteins (immunoglobulin, lactoferrin, α -lactalbumin) (Selvaggi *et al.*, 2014; Balthazar *et al.*, 2018), which may have contributed to the stability of the gel and the lower syneresis observed especially for yogurt 0:100. The, camel milk no β -lactoglobulin, camel yoghurt has difficulty forming coagulum during fermentation compared with the cow yoghurt. Lack of β -lactoglobulin makes it more difficult to make camel milk yoghurt (Konuspayeva, 2020).

Other characteristics of SM proteins are smaller in size (193 nm) than BM (260 nm) (Park *et al.*, 2007). Therefore, SM has lower allergenicity (Dias *et al.*, 2022; Gantner *et al.*, 2015). According to Masoodi and Shafi (2010), α_{S1} and α_{S2} protein sequences of SM differ sharply from α_{S1} and protein sequence α_{S2} of BM. This fact suggests that SM promotes lower allergic sensitization. In addition, it has 99% similarity between the protein sequences of casein α_{S1} and α_{S2} of goat's milk, being less allergenic than bovine milk (Masoodi & Shafi, 2010).

4. Conclusion

The parameters analyzed demonstrated the individuality and distinct characteristics of each type of milk and mixtures. It was observed that sheep milk presented values for physical-chemical characteristics, in almost all parameters higher than those of bovine milk, and the same behavior was observed for yogurts. It was verified that the antioxidant profile presented higher concentration for 100:0 and 0:100, but bovine milk has higher DPPH value, while during storage yogurts maintained constant values of DPPH, being higher for 0:100. In SEM and electrophoresis, it was observed that sheep milk has a more compact structure and smaller fat globules, higher concentration of proteins and their fractions than BM, with the mixture being 50:50 which kept during storage fractions of more compact caseins. We showed that yogurts containing a higher amount of sheep milk have potential higher nutritional value, showing the potential for application of this milk in the manufacture of yogurts and other dairy products.

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