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FOOD TECHNOLOGY

Milk from different species on physicochemical and microstructural yoghurt properties

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ABSTRACT: *The aim of the present research was to evaluate differences in chemical properties and physical structure of yoghurt produced with milk from different species (sheep, cow, and goat). For each trial, whole raw sheep (S), cow (C), and goat (G) milk were used to manufacture 4L of yoghurts (Y) from each species (SY, CY, and GY, respectively). The SY demonstrated the greatest (P<0.05) values of total solids, protein, and lipid contents, reflecting on greater (P<0.05) firmness, apparent viscosity and water-holding capacity, and lower (P<0.05) syneresis index than CY and GY. Consequently, SY exhibited a more compact microstructure and homogeneous matrix with fewer pores. Furthermore, CY and GY microstructure contained a greater number of pores, which exhibited greater size heterogeneity than SY. Therefore, based on the evaluated physicochemical and microstructural properties of yoghurt, SY demonstrated the most desirable parameter values for dairy industry representing an alternative substitution for cow's milk yogurt.*
Key words: *fermented products, texture, microstructure, sheep milk, goat milk.*

Influência do leite de diferentes espécies nas propriedades físico-químicas e micro estruturais do iogurte

RESUMO: *O objetivo da presente pesquisa foi avaliar as diferenças nas propriedades químicas e na estrutura física do iogurte produzido com leite de diferentes espécies (ovinos, bovinos e caprinos). Para cada experimento, leite de ovelha cru (S), vaca (C) e cabra (G), foram usados para fabricar 4L de iogurtes (Y) de cada espécie (SY, CY e GY, respectivamente). O SY demonstrou os maiores (P<0,05) valores de sólidos totais, proteína e conteúdo lipídico, refletindo em maior (P<0,05) firmeza, viscosidade aparente e capacidade de retenção de água e menor (P<0,05) índice de sinérese do que CY e GY. Consequentemente, o SY exibiu uma microestrutura mais compacta e uma matriz homogênea com menos poros. Além disso, a microestrutura CY e GY continha um maior número de poros, que exibiam maior heterogeneidade de tamanho do que o SY. Portanto, com base nas propriedades físico-químicas e micro estruturais avaliadas do iogurte, o SY demonstrou os valores de parâmetros mais desejáveis para a indústria de laticínios, representando uma alternativa de produto adequada aos iogurtes à base de leite de vaca.*
Palavras-chave: *leite fermentado, textura, microestrutura, leite de ovelha, leite de cabra.*

INTRODUCTION

Yoghurt represents a great source of health-promoting substances due to high levels of viable lactic acid bacteria content, which compete with several opportunistic microorganisms and provide desirable metabolites (MCKINLEY, 2005; IRKIN & VAPUR EREN, 2008; ERKAYA & ŞENGÜL, 2012; SETTACHAIMONGKON et al., 2014; COSTA et al., 2015a; COSTA et al., 2017) that contribute to consumer health and the development of yoghurt aroma and flavour (CHENG, 2010). Yoghurt can be produced utilizing milk from different species (TAMIME & ROBINSON, 2007). Furthermore, yoghurt characteristics such as convenience, price and flavour are important attributes determinant to consumer final product acceptability (POHJANHEIMO & SANDELL, 2009; COSTA et al., 2017).

Previous studies in dairy science commonly utilized cow milk potentially due to their large volume and economic importance (AL-SHERAJI

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et al., 2012; MAYER et al., 2012; LEE et al., 2013; WEN et al., 2014; ZHANG et al., 2014). Nonetheless, in many countries specific climatic and geographical features favor dairy goat and sheep farming, making such activity essential for the national and regional economy (PANDYA & GHODKE, 2007; PARK et al., 2007). In addition, goat milk products have essential characteristics for human nutrition as higher digestibility (small fat globules) and less allergenicity (low α_{s1}-casein content) (UYSAL-PALA et al., 2006). Moreover, sheep milk products have high nutritive and organoleptic traits due to greater contents of proteins, linolenic acid, essential amino acids, vitamins, and minerals when compared to cow milk products (KAMINARIDES et al., 2007; PARK et al., 2007). Furthermore, previous studies have demonstrated that the aforementioned milks can be used for the production of fermented dairy products (KATSIARI et al., 2002; PAPADIMITRIOU et al., 2007; SANAL et al., 2011; ŞENEL et al., 2011; DE RENOBALES et al., 2012; DOMAGAŁA et al., 2013; SILVA et al., 2017; VIEIRA et al., 2017).

Yoghurt microstructure is composed by a protein network of casein micelle aggregates entrapping fat globules and serum which directly influences this type of fermented product texture (HERRERO & REQUENA, 2006; NGUYEN et al., 2015). This yoghurt microstructure and textural properties are directly associated with desirable functional and sensory parameters (LEE & LUCEY, 2003; RAO & SILVA, 2007). In addition, previous studies have documented relation between yoghurt textural properties and microstructure (DOMAGAŁA, 2009; WANG et al., 2012; NGUYEN et al., 2014a; NGUYEN et al., 2014b; YANG et al., 2014; NGUYEN et al., 2015). Electron scanning microscopy is a reliable tool to visualize yoghurt structure facilitating the characterization of protein chains, microorganisms, fat globules, carbohydrates, and clusters (NAKTHONG, 2012). Moreover, differences in physicochemical characteristics among milk from different species (PARK et al., 2007) directly influence yoghurt textural properties (SHAKEEL HANIF et al., 2012). These properties represent important sensory parameters for product acceptability (COSTA et al., 2016a). Thus, typical defects in yoghurts as low viscosity, reduced firmness (TAMIME & ROBINSON, 2007), and high level of syneresis can lead to consumer product rejection (AMATAYAKUL et al., 2006). In this context, the present study aimed to compare chemical, textural and microstructural properties of yoghurts produced utilizing sheep, cow and goat milk.

MATERIALS AND METHODS

Yoghurt processing

Freeze dried direct vat set starter culture YF-L903 containing 9.37 log CFU/g of *Streptococcus thermophilus* and 12.13 log CFU/g *Lactobacillus delbrueckii* subsp. *bulgaricus* (Yo-Flex®, Chr Hansen, Valinhos, São Paulo, Brazil) were prepared according to BALTHAZAR et al. (2015). For each trial, a total of 12 L whole raw sheep (SM), cow (CM) and goat (GM) milks were obtained from farms located in Vassouras (Rio de Janeiro, Brazil), Miracema (Rio de Janeiro, Brazil) and São Gonçalo (Rio de Janeiro, Brazil), respectively, to produce 4 L of yoghurts from each species. The (SM), (CM) and (GM) were collected from animals' flock belonging to the breed Lacaune, Brazilian Girolando and Saanen, respectively. At pilot plant of Dairy Laboratory of Universidade Federal Fluminense, each type of milk was pasteurized at 85 ºC for 30 min in a stainless-steel double jacket container (GCA Corporation, Greensboro, North Carolina, United States) and cooled to 40 ºC. Aliquots from starter culture were inoculated at a concentration of 1% (v/v) into whole pasteurized milk. After homogenization aliquots of milk (200 mL) from each species were transferred to flasks and fermented at 43 ºC until the pH reached approximately 4.5 according to AOAC 981.12 for pH measurement of Acidified Foods (AOAC, 2012), followed by rapid cooling. Yoghurts from different species were stored during 28 days under refrigeration (4 ºC), and bacteriological, chemical, textural, and microstructural parameters were evaluated. When all the analysis were being evaluated, we certified that temperature was kept under 7 ºC to avoid any detriment in the results. Two trials of yoghurt processing were performed for each species.

Bacteriological analysis

Streptococcus thermophilus count was performed utilizing M17 agar (Difco Company, Kansas, United States) and incubation at 37 ºC for 48 h under aerobic condition whereas, *Lactobacillus delbrueckii* ssp. *bulgaricus* was counted on MRS agar (Difco Company, Kansas, United States) at 37 ºC for 72 h under anaerobic condition utilizing anaerobic jar (Probac do Brasil, Rio de Janeiro, Brazil), both according to Codex Alimentarius standard for fermented milk in order to characterize the fermented product as yoghurt (Codex Alimentarius 2010). The bacteriological counts were analyzed in triplicate after yoghurt production on 1st and 28th days of storage (4 °C) for each trial.

Proximate composition

Proximate composition was determined on the final product (day 1 of storage) in triplicate according to AOAC (2012) procedures for each trial. Yoghurt total solids content was determined by gravimetric method using a drying oven until constant weight (AOAC 925.23), protein content was estimated by the Kjeldahl technique (AOAC 991.22), lipid content was obtained by the Gerber method (AOAC 2000.18), and ash content was determined after incineration at 550 °C in muffle furnace (AOAC 945.46).

Carbohydrates and organic acids

Carbohydrates (lactose, galactose, and glucose) and organic acids (lactic, citric, and formic acids) were determined by high-performance liquid chromatography (HPLC). Extraction of these molecules was carried out as described by González de Llano et al. (1996) with slight modifications (COSTA et al., 2016b). Briefly, 5 mL of H_2SO_4 (45 mmol/L) was added to 1 g of yoghurt samples and homogenized for 1 min in the vortex. Then, the solution was stirred for 1 h in a shaker table and centrifuged at 5,000 × *g* for 30 min at 4º C. The decanted was collected and filtered through Whatman no.1 filter paper. Filtered samples were injected (20 µL) in triplicate into HPLC system (Shimadzu® Kyoto, Japan) integrated with CBM-20A, connected to SPD-M20A diode array and refractive index RID-10A detectors. An Aminex HPX-87H column (300 x 7.8 mm) (Bio-Rad, Hercules, CA, USA), maintained at 60 ºC by oven column, was used. Carbohydrates identification was performed by a refractive index detector, whereas organic acids with a diode array detector at 210 nm. The chromatographic separation was achieved using 3 mM sulfuric acid solution at the isocratic condition and a flow rate of 0.5 $mL.min^{-1}$. Standard solutions of carbohydrates and organic acids (Sigma, St. Louis, MO) were utilized in order to plot external standard curves and carry out the quantitative analysis of the aforementioned molecules. All carbohydrates and organic acids were well separated in a 30-min total run time with good peak resolution, sharpness and symmetry. These analyses were evaluated in triplicate during the storage period (4 °C) on days 1, 7, 14, 21 and 28 for each trial.

Analysis of pH and titratable acidity

The pH values were measured according to NGUYEN et al. (2014a) with a digital pH meter (Digimed® Model DM-32, São Paulo, Brazil). Yoghurts titratable acidity (TA) were determined by a volume of 0.5 mL of phenolphthalein (5% w/v), as an indicator added to 10 g of yoghurt following titration with 0.1 M sodium hydroxide (NaOH) solution to an end point of stable faint pink color for 1 min; TA was expressed as lactic acid percentage (TAMJIDI et al., 2012). These analyses were performed in triplicate during fermentation and storage period (4 °C) on days 1, 7, 14, 21 and 28 for each trial.

Firmness

Firmness was determined at 5 °C by the Instrumental Texture Analyzer (TA-XT plus®, Stable Micro System Ltd., Godalming, Waverley District, United Kingdom) equipped with a 5 kg load cell according to PASEEPHOL et al. (2008). Yoghurts were compressed with a 20 mm diameter cylindrical probe (36R) up to 10mm depth at a constant speed (1 mm/s). Gel firmness is characterized as maximum force (N) on time force curve compression. This parameter was evaluated in triplicate for each trial on 1^{st}, 14^{th} and 28^{th} days of storage period.

Apparent viscosity

Apparent viscosity was evaluated at 5 °C utilizing a Rotational Viscometer Microprocessor (Q860M21, Quimis®, Sao Paulo, SP, Brazil) with spindle number 4. The spindle was rotated at 20 rpm. Readings were recorded at the 30^{th} second of the measurement period and expressed as millipascal seconds (mPa.s), as described by BALTHAZAR et al. (2015). Apparent viscosity was performed in triplicate on 1^{st}, 14^{th} and 28^{th} days of the storage period for each trial.

Water-holding capacity

Water-holding capacity (WHC) was analyzed as described by REMEUF et al. (2003) with slight modifications. Yoghurt (Y) samples (20 g) were centrifuged (Hermle Z 360K, Wehingen, Germany) for 20 min at 4,500 × *g* and 4 °C. Expelled whey (EW) was removed and weighed. WHC was obtained in triplicate for each trial on 1^{st}, 14^{th} and 28^{th} days of the storage period. The WHC was calculated as: WHC (%)=100(Y – WE)/Y.

Syneresis index

Yoghurts syneresis index were performed according to DANNENBERG and KESSLER H (1988) with slight modifications and expressed as whey percentage weight separated from the gel. A yoghurt portion was removed with an ice cream scoop (d=45 mm), in order to obtain approximately 29 g of hemispherical sample, and the flat side was placed onto a test sieve (mesh width 0.5 mm). The whey volume (mL) drained off was measured at 10 °C after 2 h. Syneresis index was evaluated in triplicate for each trial on 1st, 14th and 28th days of the storage period.

Microstructural analysis

Microstructural analysis was performed according to the method described by DOMAGAŁA et al. (2013) with slight modifications. Yoghurt samples microstructural characteristics were investigated using the scanning electron microscopy (SEM). Samples of 1 cm^3 of each yoghurt were fixed overnight in 2.5% glutaraldehyde and 0.1 M potassium cacodylate buffer at 4 ºC, rinsed (three times) with ultrafiltered water for 10 min, post-fixed overnight in 2% osmium tetroxide. Further, the fixed and post-fixed yoghurt samples were dehydrated with ethanol solutions (30 %, 50 %, 70 % and 95 %) for 10 min each, and three times on 100% ethanol. An additional drying step using the critical point method with liquid carbon dioxide (Bal-Tec SCD 050, Balzers, Liechtenstein) was executed. Dry yoghurt samples were stuck on aluminum stubs with silver epoxy and gold-coated under vacuum using a sputter coater (Bal-Tec SCD 050, Balzers, Liechtenstein). Yoghurt microstructures were examined by SEM (Zeiss evo ma10, Oberkochen, Germany). Six fields were observed for each sample.

Statistical analysis

Analysis of variance (ANOVA) was used in order to assess the influence of species on yoghurt chemical and textural properties. Tukey's test was utilized to determine differences among means at 0.05 of significance level. Data were analyzed using XLSTAT version 2012.6.08 (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Bacteriological analysis

The bacteria count of yoghurts manufactured in the 1st and 28th day of the storage period utilizing milk from different species are presented in table 1. Bacteriological counts during storage period after fermentation characterized the fermented milk as yoghurt, according to Codex Alimentarius (2010), in which the minimum count required is 7.00 log CFU/g. The viability of great amount of lactic acid bacteria in yoghurts has been correlated with several benefits for consumer's health, such as high lactose tolerance, intestinal microflora benefic balance, antimicrobial activity and immune system stimulation (BIROLLO et al., 2000). A decrease on bacterial counts was observed in the present study at the end of the storage period, by results reported by BEAL et al. (1999), BIROLLO et al. (2000), AKALIN et al. (2004).

Table 1 - Bacteria count in the 1th and 28th day of storage period of yoghurts manufactured utilizing milk from different species

	------------Bacteria count (log CFU/g)---------			
	Streptococcus thermophilus		*Lb. delbrueckii* ssp. bulgaricus	
Treatments*	1th day	28th day	1th day	28th day
SY	13.78	9.22	10.53	7.00
CY	13.83	10.02	12.22	8.15
GY	12.99	9.78	11.78	7.00

*Treatments: sheep milk yoghurt (SY); cow milk yoghurt (CY); goat milk yoghurt (GY).

Proximate composition

The proximate composition of the different raw milk species, as well as the breed of animals, substantially determines the final composition of the manufactured yogurts. Despite we have not detailed described different species raw milk composition in this research, the yogurt physical-chemical composition had already been determined and can be accessed in our research group previous publication (BALTHAZAR et al., 2015, VIANNA et al., 2017). In the present study, SY demonstrated greater (P<0.05) total solids, protein, and lipid contents than CY and GY, whereas these differences were not detected (P>0.05) between CY and GY (Table 2). Moreover, the three types of yoghurt exhibited similar (P>0.05) ash content. In general, sheep milk contains greater total solids, protein and lipid content than goat and cow milk (PARK, 2007), which potentially reflected on the differences above.

In accordance with the present research results, (ERKAYA & ŞENGÜL, 2012) studied yoghurts produced utilizing milk from different species and observed greater total solids, protein and lipid content in sheep milk yoghurt when compared to cow and goat milk yoghurts. GÜLER & SANAL (2009) also documented greater protein and lipid content in sheep milk yoghurt than cow and goat milk yoghurts; moreover, chemical composition of yoghurt produced with cow milk was similar to goat counterpart. In addition, GÜLER & GÜRSOY-BALCI

Table 2 - Proximate composition of yoghurts manufactured utilizing milk from different species.

Treatments*	----------Proximate composition (%)----------			
	Total solids	Protein	Lipid	Ash
SY	19.48 ± 0.00[a]	5.32 ± 0.00[a]	6.08 ± 0.00[a]	1.03 ± 0.00[a]
CY	14.49 ± 0.04[b]	3.89 ± 0.01[b]	4.39 ± 0.01[b]	0.78 ± 0.00[a]
GY	12.51 ± 0.00[b]	3.16 ± 0.00[b]	3.44 ± 0.00[b]	0.77 ± 0.00[a]

*Treatments: sheep milk yoghurt (SY); cow milk yoghurt (CY); goat milk yoghurt (GY).
Results (n=6) are expressed as the mean ± standard deviation.
Means without common superscripts (a–b) in a column are different (*P*<0.05).

(2011) produced yoghurts using sheep and goat milk and their mixture. These authors also observed that sheep milk yoghurt obtained greater values of total solids, protein and lipid than goat milk yoghurt.

Carbohydrates profile

Carbohydrates contents values from SY, CY and GY are presented in table 3. On the first day (day 1) of storage while CY and GY demonstrated greater (*P*<0.05) lactose content than SY whereas, SY exhibited the greatest (*P*<0.05) galactose content; after day 7 of storage, no difference (*P*>0.05) on the aforementioned molecule's contents was observed among all yoghurts. Furthermore, during the storage period, the lactose and galactose content values in SY remained similar (*P*>0.05) whereas, on CY and GY the lactose content decreased (*P*<0.05), and the galactose values increased (*P*<0.05). In contrast, the SY and CY glucose content were detected just at the beginning of storage, while GY demonstrated fluctuations (*P*<0.05) during the yoghurt storage period.

Lactose is the major carbohydrate in goat, sheep and cow milk and is composed by glucose and galactose monomers (PEREIRA DA COSTA & CONTE-JUNIOR, 2015). In general, sheep and cow milk types contain similar lactose levels (Park et al. 2007), which are greater than goat milk (MAYER & FIECHTER, 2012). Greater lactose content in milk can positively influence the yoghurt texture as due to the ability of the starter microorganisms to produce an exopolysaccharide (EPS) from this carbohydrate (TAMIME & ROBINSON, 2007). In this context, the lower values of lactose with greater content of galactose on SY than on CY and GY, at the beginning of storage, is potentially due to the hydrolysis of lactose into galactose by starter microorganisms during the fermentation period. Conversely, CY and GY demonstrated a slight decrease, which can be explained by this molecule consumption during storage with the release of galactose from lactose hydrolysis (FARNWORTH, 2008). The GY glucose content fluctuations during storage period can be attributed to glucose consumption (KAMINARIDES et al., 2007) and through lactose conversion by yoghurt microorganisms (VÉNICA et al., 2014). Similar to our results, (DOMAGAŁA, 2009) documented that lactose content values in cow and goat milk yoghurts were similar. Moreover, in partial agreement with the present research, (VÉNICA et al., 2014) reported an increase in galactose and lactic acid content, with a simultaneous decline in lactose content in fermented products manufactured with sheep and cow milk.

Organic acids profile

Organic acids values from yoghurts manufactured utilizing SM, CM and GM are exhibited in table 3. CY exhibited the lowest (*P*<0.05) lactic acid content, and GY the greatest (*P*<0.05) values from day 21 of storage. In addition, all yoghurt treatments demonstrated a lactic acid content increase (*P*<0.05) during the yoghurt storage period. In general, SY exhibited greater (*P*<0.05) citric and formic acid contents than CY and GY. Moreover; although, SY and CY citric acid content values exhibited an increase (*P*<0.05), GY did not exhibit (*P*>0.05) difference during the storage period. Furthermore, formic acid content generally remained similar (*P*>0.05) in all yoghurt treatments during the storage period.

Lactic acid is the major final product of lactic acid bacteria fermentative energy metabolism and its increase in yoghurts is common after refrigerated storage (DE ANCOS et al., 2000) due to the lactose utilization by starter cultures (TAMIME & ROBINSON, 2007), which explains the slight lactose decrease and lactic acid increase observed in the present research. According to (GRANATA & MORR, 1996), the adequate lactic acid amount can positively contribute with yoghurt texture leading to a minimum

Table 3 - Carbohydrates and organic acids content values of yoghurts manufactured utilizing milk from different species.

Molecules (mg/mL)		------Storage days------				
	*(T)	1	7	14	21	28
Lactose	SY	48.66 ± 0.78^{bA}	46.90 ± 0.55^{aA}	45.39 ± 0.87^{aA}	44.84 ± 0.93^{aA}	44.16 ± 1.85^{aA}
	CY	54.20 ± 0.79^{aA}	48.50 ± 0.37^{aAB}	44.34 ± 1.82^{aB}	43.12 ± 3.02^{aB}	43.08 ± 0.14^{aB}
	GY	52.73 ± 0.49^{aA}	50.62 ± 0.46^{aA}	42.84 ± 0.20^{aB}	42.88 ± 2.46^{aB}	43.10 ± 0.41^{aB}
Galactose	SY	6.17 ± 0.15^{aA}	6.40 ± 0.69^{aA}	6.72 ± 1.16^{aA}	6.45 ± 0.08^{aA}	6.66 ± 0.43^{aA}
	CY	5.05 ± 0.59^{bB}	5.79 ± 0.17^{aAB}	5.81 ± 0.14^{aAB}	5.70 ± 0.38^{aAB}	5.87 ± 0.09^{aA}
	GY	4.82 ± 0.20^{bB}	5.27 ± 0.46^{aAB}	5.67 ± 0.08^{aAB}	5.81 ± 0.81^{aAB}	6.69 ± 0.43^{aA}
Glucose	SY	0.23 ± 0.00^{bA}	0.13 ± 0.03^{aA}	ND	ND	ND
	CY	0.54 ± 0.00^{a}	ND	ND	ND	ND
	GY	0.09 ± 0.00^{cAB}	0.20 ± 0.03^{aA}	ND	0.05 ± 0.02^{B}	ND
Lactic acid	SY	17.00 ± 0.16^{aC}	17.41 ± 0.01^{abC}	18.20 ± 0.13^{aB}	20.50 ± 0.00^{bA}	20.54 ± 0.04^{bA}
	CY	14.85 ± 0.23^{bC}	16.21 ± 0.97^{bBC}	16.25 ± 0.03^{bBC}	16.86 ± 0.02^{cAB}	18.38 ± 0.18^{cA}
	GY	17.26 ± 0.06^{aD}	18.74 ± 1.84^{aC}	18.83± 0.23^{aC}	21.45 ± 0.13^{aB}	22.95 ± 0.04^{aA}
Citric acid	SY	7.02 ± 0.06^{aC}	8.10 ± 0.25^{aAB}	7.78 ± 0.17^{aB}	8.15 ± 0.03^{aAB}	8.42 ± 0.04^{aA}
	CY	5.57 ± 0.06^{bB}	6.63 ± 0.08^{bAB}	7.14 ± 0.42^{aA}	6.89 ± 0.58^{abA}	7.10 ± 0.01^{bA}
	GY	5.16 ± 0.00^{cA}	5.55 ± 0.10^{cA}	6.05 ± 0.86^{aA}	6.51 ± 0.33^{bA}	6.66 ± 0.07^{cA}
Formic acid	SY	5.75 ± 0.08^{aA}	7.09 ± 0.00^{aA}	6.58 ± 0.27^{aA}	6.77 ± 0.73^{aA}	6.93 ± 0.00^{aA}
	CY	5.19 ± 0.08^{bB}	6.05 ± 0.09^{bA}	6.05 ± 0.01^{aA}	5.81 ± 0.19^{aA}	6.04 ± 0.18^{bA}
	GY	3.53 ± 0.02^{cA}	3.58 ± 0.17^{cA}	3.54 ± 0.05^{bA}	3.88 ± 0.18^{bA}	3.89 ± 0.15^{cA}

*(T) = Treatments: sheep milk yoghurt (SY); cow milk yoghurt (CY); goat milk yoghurt (GY).
#ND = not detected.
Results (n = 6) are expressed as the mean ± standard deviation.
Means without common superscripts (A–D) in a row are different (*P*<0.05).
Means without common superscripts (a–c) in a column within a molecule are different (*P*<0.05).

syneresis index during the storage period. In the present research, GY obtained the greatest acid lactic content at the end of yoghurt storage (21th and 28th storage days) (Table 3), which potentially lead to an effect on the aforementioned yoghurt texture. Although, SY lactic acid content was similar to GY between the 1st and 14th storage day, SY obtained the greatest total solids and protein content (Table 2). Therefore the lactic acid content alone may not affect SY textural properties. Citric acid is the most abundant organic acid present in raw milk (TORMO & IZCO, 2004) thus, the observed citric acid content values increase indicates negligible citric acid utilization by starter cultures during storage (ADHIKARI et al., 2002).

In agreement with results reported in the present study, KAMINARIDES et al. (2007) concluded that sheep milk yoghurt with elevated fat content obtained greater texture scores such as firmness and lower syneresis index than other low- regular-fat treatments. The concentration of lactic acid in the aforementioned yoghurt was lower than those produced with different fat content. Thus the absence of textural defects in that study can be due to the sheep milk high total solids and low lactic acid content. In agreement with the present research, HERRERO & REQUENA (2006) evaluated the effect of supplementing goat milk with whey protein concentrate (WPC) on set-type yoghurt textural properties and reported that WPC increases the lactic acid content.

Analysis of pH and titratable acidity

Yoghurt pH and TA values from different species during storage period are exhibited in table 4. The SY, CY, and GY did not demonstrate (*P*>0.05) difference among pH values. When compared day 1 and 28 of the storage period CY exhibited a decrease (*P*<0.05) on the pH values. In addition, milk from different species affected (*P*<0.05) yoghurt TA; SY values were greater (*P*<0.05) than CY and GY. Also, during the storage period while the TA values of SY increased (*P*<0.05) CY and GY were not affected (*P*>0.05).

The observed pH results were expected based on the usual yoghurt pH decrease during storage period (LUCEY, 2004). The slight TA increase and pH decrease observed during storage period could

Table 4 - Values of pH and titratable acidity of yoghurts manufactured utilizing milk from different species.

		Storage days				
Parameters	[*](T)	1	7	14	21	28
pH						
	SY	4.66 ± 0.03[aA]	4.59 ± 0.08[aA]	4.54 ± 0.08[aA]	4.51 ± 0.07[aA]	4.50 ± 0.06[aA]
	CY	4.56 ± 0.09[aA]	4.49 ± 0.09[aAB]	4.46 ± 0.08[aAB]	4.45 ± 0.07[aAB]	4.42 ± 0.07[aB]
	GY	4.62 ± 0.08[aA]	4.59 ± 0.09[aA]	4.54 ± 0.06[aA]	4.51 ± 0.06[aA]	4.48 ± 0.08[aA]
Titratable acidity (%)						
	SY	0.92 ± 0.00[aC]	0.96 ± 0.00[aBC]	0.99 ± 0.00[aB]	1.01 ± 0.00[aAB]	1.05 ± 0.00[aA]
	CY	0.70 ± 0.00[bA]	0.73 ± 0.00[bA]	0.74 ± 0.00[bA]	0.76 ± 0.00[bA]	0.78 ± 0.00[bA]
	GY	0.72 ± 0.00[bA]	0.74 ± 0.00[bA]	0.76 ± 0.00[bA]	0.77 ± 0.00[bA]	0.80 ± 0.00[bA]

[*](T) = Treatments: sheep milk yoghurt (SY); cow milk yoghurt (CY); goat milk yoghurt (GY).
Results (n = 6) are expressed as the mean ± standard deviation.
Means without common superscripts (A–C) in a row are different (P<0.05).
Means without common superscripts (a–b) in a column within a parameter are different (P<0.05).

be due to lactic acid production through lactose hydrolysis promoted by starter culture and lactic acid bacteria (LOURENS-HATTINGH & VILJOEN, 2001; KAILASAPATHY, 2006). Nevertheless, the observed pH decreases on CY samples at the 28th day of storage is potentially due to the lower buffering capacity of cow milk than goat (PARK et al., 2007) and sheep counterparts. Moreover, the observed increase of TA on SY can be due to greater buffering capacity associated with greater mineral, protein and dissolved CO_2 content than cow and goat milks (SALAÜN et al., 2005). Consequently, it leads to a greater alkali solution volume during titration step.

In agreement with the present research, DOMAGAŁA (2009), ERKAYA & ŞENGÜL (2012) reported that sheep dairy product (milk and yoghurt, respectively) exhibited greater TA values than goat and cow counterparts, demonstrating that the effect on this parameter is specie-specific. Furthermore, ERKAYA & ŞENGÜL (2012) documented similar pH values for goat and sheep milk yoghurts, and the pH decrease associated with an increase on TA values on yoghurt from different species during storage is in accordance with other studies using cow (MATARAGAS et al., 2011), buffalo (ERKAYA & ŞENGÜL, 2012), sheep and goat (GÜLER & GÜRSOY-BALCI, 2011; ERKAYA & ŞENGÜL, 2012) milk.

Firmness

Firmness, apparent viscosity, WHC, and syneresis from yoghurts produced utilizing milk from three different species (sheep, cow, and goat) are presented in table 5. The SY were firmer (P<0.05) than CY and GY during all storage period. Moreover, on day 14 and 28 of the storage period, CY firmness was greater (P<0.05) than GY. In addition, the storage period only affected (P<0.05) GY, demonstrating a decrease in textural quality in this type of yoghurt.

Firmness is considered one of the main textural parameters for yoghurt acceptability (HARTE et al., 2007). During coagulation step on yoghurt manufacturing, destabilized casein micelles and calcium-phosphate bonds form a network, which in turn entraps fat and other solids (COSTA et al., 2015b). The speed of casein network formation is directly influenced by protein amount, mainly casein content, resulting in greater aggregation rate with firmer curd development (DIMASSI et al., 2005). Differences on total solids content among milk from different species affect yoghurt curd firmness (PARK et al., 2007), which can be evidenced in SY firmness (Table 5) with greater (P<0.05) total solids and protein content (Table 2) when compared with the other two yoghurt types studied.

Furthermore, MARTÍN-DIANA et al. (2003) demonstrated that firmness can also be influenced by milk casein content and micelle structure from each species. According to these authors; although, total solids content for cow and goat milk were similar, yoghurts produced with goat milk tends to be less firm than cow milk yoghurt; in the present study; although, GY and CY demonstrated similar (P>0.05) proximate composition values (Table 2), the first one was firmer (P<0.05) than the latter (Table 5). In addition, AND & GUO (2006) reported that goat milk exhibits less

Table 5 - Textural properties of yoghurts manufactured utilizing milk from different species.

		Storage days		
Parameters	*(T)	1	14	28
Firmness (g)				
	SY	7.66 ± 0.80^{aA}	7.70 ± 0.27^{aA}	8.02 ± 0.46^{aA}
	CY	2.18 ± 0.23^{bA}	2.12 ± 0.00^{bA}	1.97 ± 0.22^{bA}
	GY	0.70 ± 0.03^{bA}	0.44 ± 0.00^{cB}	0.43 ± 0.03^{cB}
Apparent Viscosity (mPa·s)				
	SY	785.52 ± 3.46^{aA}	779.85 ± 1.63^{aA}	777.47 ± 2.58^{aA}
	CY	721.45 ± 23.36^{aA}	675.43 ± 13.97^{bA}	650.65 ± 45.00^{bA}
	GY	223.60 ± 15.70^{bA}	214.45 ± 17.18^{cA}	199.03 ± 10.15^{cA}
WHC # (%)				
	SY	94.51 ± 0.02^{aA}	94.56 ± 0.01^{aA}	93.66 ±0.00^{aA}
	CY	69.82 ± 0.02^{bA}	68.99 ± 0.04^{bA}	66.98 ±0.02^{bA}
	GY	56.71 ± 0.03^{cA}	54.03 ± 0.05^{cA}	53.45 ±0.01^{cA}
Syneresis (%)				
	SY	11.21 ± 0.00^{cA}	11.25 ± 0.00^{cA}	11.26 ± 0.00^{cA}
	CY	35.57 ± 0.02^{bA}	38.26 ± 0.01^{bA}	39.10 ± 0.00^{bA}
	GY	44.35 ± 0.01^{aA}	50.14 ± 0.03^{aA}	51.18 ± 0.02^{aA}

*(T) = Treatments: sheep milk yoghurt (SY); cow milk yoghurt (CY); goat milk yoghurt (GY).
Water-Holding Capacity.
Results (n = 6) are expressed as the mean ± standard deviation.
Means without common superscripts (A–B) in a row are different (*P*<0.05).
Means without common superscripts (a–c) in a column within a parameter are different (*P*<0.05).

αs1-casein content than cow milk which potentially explains the less consistency on GY.

In agreement with observed firmness results, DOMAGAŁA (2008), ZUBEIR et al. (2012) demonstrated that sheep milk yoghurt was firmer than goat milk yoghurt. Moreover, DOMAGAŁA (2009) evaluated different textural parameters, such as hardness, adhesiveness and extrusion force among sheep, cow and goat milk yoghurts and demonstrated that yoghurts produced from goat milk exhibited the lowest values of textural parameters whereas, sheep milk yoghurts, the greatest ones; indicating that total solids and protein content can be considered important parameters to determine final product textural characteristics. In partial agreement with the present study, AMATAYAKUL et al. (2006) investigated firmness of set-yoghurts produced with different casein to whey ratios, as well as total solids content, and observed that the firmness did not change during storage. Additionally, KATSIARI et al. (2002) evaluated the effects of long-term deep-frozen storage on yoghurt characteristics and demonstrated that during cold storage, sheep milk yoghurt firmness slightly increased.

Apparent viscosity

This research demonstrated that apparent viscosity parameter was affected (*P*<0.05) by type of milk (different species) used to produce yoghurts. During all the storage period analyzed, GY obtained the lowest (*P*<0.05) apparent viscosity, whereas SY exhibited the greatest (*P*<0.05) values at days 14 and 28 of storage. In addition, the storage period did not affect (*P*>0.05) this parameter's values during storage period for all yoghurt types.

Variations on apparent viscosity results observed in the present study can be attributed to differences in total solids and protein content among milk types (JUMAH et al., 2001; MARTÍN-DIANA et al., 2003). Usually, yoghurt produced with cow and goat milk require fortification in order to improve total solids content and consequently yoghurt viscosity (REMEUF et al., 2003; HERRERO & REQUENA, 2006) as sheep milk yoghurt already contains high total solids content, the fortification step is not required (BOYAZOGLU & MORAND-FEHR, 2001). In accordance with aforementioned results, (KÜÇÜKÇETIN et al., 2011; ERKAYA & ŞENGÜL, 2012; WANG et al., 2012) evaluated apparent viscosity among yoghurt samples produced from different types of milk (goat, cow, and their mixture), and observed that yoghurt viscosity obtained from goat milk was lower than cow milk yoghurt and their mixtures.

Water-holding capacity

During all storage period analyzed, SY exhibited the greatest ($P<0.05$) WHC values, whereas GY the lowest ($P<0.05$) ones. Furthermore, storage period did not influence ($P>0.05$) this parameter. Total solids and protein content directly affected WHC, potentially due to greater milk proteins content which increases yoghurt gel network density, and consequently the WHC (KRASAEKOOPT et al., 2004). SODINI et al. (2004) reported that an increase in casein concentration can favor its micelles interaction as well as, leading to decrease of matrix pore dimensions and an increase of its density. In addition; although, GY and CY demonstrated similar total solids and protein contents (Table 2), GY exhibited lower ($P<0.05$) WHC values, which can be explained by differences on micelle hydration between these aforementioned types of milk (goat milk is less hydrated than cow milk) (PARK et al., 2007).

LE et al. (2011) compared yoghurts with a similar amount of dry solids content containing an increased milk fat globule membrane material concentration and observed a WHC improvement due to an increase in total solids content. Moreover, KÜÇÜKÇETIN et al. (2011) observed that yoghurt produced with goat milk obtained lower WHC than yoghurts manufactured utilizing cow milk. In addition, MALEK et al. (2001) reported that; although, yoghurts produced with cow and goat milk demonstrated similar total solids content, the former one released less water than those obtained from goat milk.

Syneresis index

The yoghurt syneresis index was ($P<0.05$) affected by milk type but not by storage period ($P>0.05$). In contrast with WHC, the lowest ($P<0.05$) syneresis index value was observed in SY whereas, the greatest ($P<0.05$) one in GY during all the storage period analyzed. Syneresis represents an important concern in yoghurt commercial manufacturing, which can lead to accumulation of whey (serum) on yoghurt gel surface, decreasing the consumer acceptance (GHASEMPOUR et al., 2012). According to AMATAYAKUL et al. (2006), an increase in total solids content favors syneresis decrease potentially clarifying why yoghurt samples that demonstrated the greatest total solids content, obtained the lowest syneresis index. Casein and colloidal calcium content also affect syneresis index. Thus, the greater sheep milk micelles mineralization levels than cow and goat milk (PARK et al., 2007), may explain the SY lowest syneresis index. Furthermore, in agreement with this study, DOMAGAŁA (2009), ERKAYA & ŞENGÜL, (2012) demonstrated that yoghurts from goat milk exhibited greater syneresis index than yoghurts produced with sheep milk.

Microstructural analysis

The scanning electron micrographs obtained from yoghurts elaborated with sheep, cow and goat milk are depicted in figure 1 and 2. The figures illustrate the yoghurt protein network microstructure entrapping fat globules and void

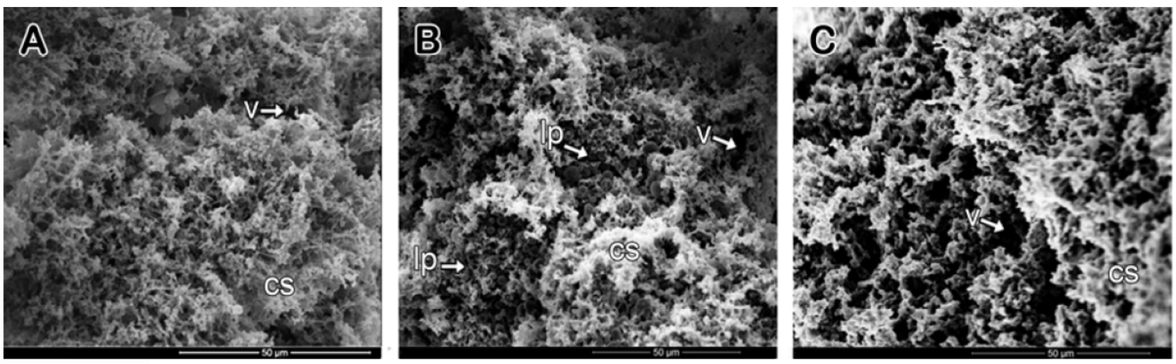

Figure 1 - Scanning electron microscopy (SEM) micrographs of yoghurts manufactured with sheep milk (A), cow milk (B) and goat milk (C). Bar = 50 µm. v, void space; cs, casein, lp, lipid.

spaces (Figure 1, 2) filled with bacterial cells (Figure 2). Yoghurt consists of a network composed by clusters or chains of casein particles forming a three-dimensional matrix (PENNA et al., 2007). Microstructural differences among the three yoghurt types were visualized. As depicted in figure 1, SY (Figure 1A) was characterized by a denser structure exhibiting fewer pores structure than CY (Figure 1B) and GY (Figure 1C), which in turn exhibited a more open structure. In addition, SY (Figure 2a) exhibited a more branched-structured gel and more interconnected clusters, demonstrating a very fine network composed by small and homogeneous void spaces, embedded with fat globules and bacterial cells. These observations support the strong link between yoghurt gel microstructure and textural properties. SY exhibited greater firmness, apparent viscosity and, water-holding capacity, and lower syneresis index values than CY and GY (Table 5); finer protein chains, smaller casein particles and pore sizes improves water immobilization (KRZEMINSKI et al., 2011). The GY (Figure 2c) exhibited plenty of void spaces with heterogeneous size and irregular microstructure, as well as CY; however, GY presented a coarser gel structure and large clusters, suggesting differences on micelle characteristics among the three studied yoghurt types. These GY microstructural differences can be explained due to differences in casein fraction relative proportions in goat milk when compared with cow and sheep milk.

TAMIME and ROBINSON (2007) also reported that the porosity of yoghurt gel was more compact or denser in sheep milk yoghurt than cow counterpart. As described by PARK et al. (2007), the casein micelle structure of cow milk differs from sheep milk in diameter, hydration and mineralization in addition to the smaller fat globule size on sheep milk than in cow counterpart potentially explain the CY weaker gel structure in comparison with SY. Furthermore, NGUYEN et al. (2014a) reported that other parameters in addition to total solids content, such as concentration of lactose, calcium, and fat globules as well as, fat globules surface area also influence yoghurt structure and textural properties. Thus, the observed differences among CY, SY and GY are potentially attributed to differences in milk physicochemical characteristics. Moreover, caprine casein micelles contain more calcium and inorganic phosphorus, and are less solvated, less heat stable, and release β-casein more rapidly than bovine casein micelles (PARK et al., 2007). During fermentation, the decrease in pH values closer to the casein micelles isoelectric point (pH 4.6) favors the colloidal calcium phosphate solubilization, increasing hydrophobic interaction. Ultimately, causing casein micelles aggregation into a three-dimensional chain network, LUCEY (2004) & (PHADUNGATH, (2005) demonstrated that casein micelles play an important role in milk acid coagulation. In agreement with this research, DOMAGAŁA (2009) reported that goat milk yoghurt microstructure was more delicate, less resistant to deformation and more susceptible to syneresis than cow and sheep counterparts; sheep milk yoghurt exhibited the strongest gel matrix. Additionally, VARGAS et al. (2008) demonstrated that yoghurt formulations with 100% of goat milk were characterized by a smaller number of junction points, which led to a more open structure with larger pores and a greater number of smaller fat globules than formulations with 100% cow milk.

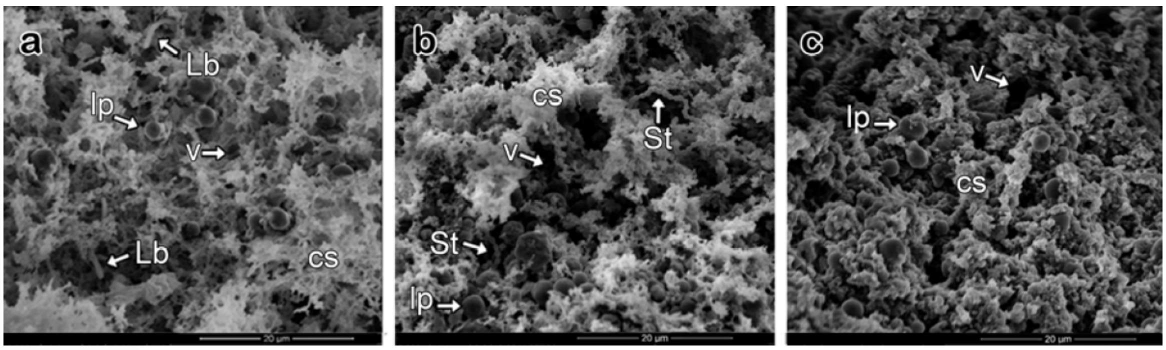

Figure 2 - Scanning electron microscopy (SEM) micrographs of yoghurts produced with sheep milk (a), cow milk (b) and goat milk (c). Bar=20 µm. St, Streptococcus thermophilus; Lb, Lactobacillus delbrueckii ssp. bulgaricus; v, void space; cs, casein, lp, lipid.

CONCLUSION

The chemical parameters of yoghurts, mainly total solids, protein, lipid and lactic acid content are specie-specific. These differences potentially affected textural and microstructural yoghurt properties. The present study demonstrated a clear correlation among chemical, textural, and microstructural yoghurt parameters. Sheep milk produced yoghurt with the most desirable textural characteristics for consumer market while goat milk yoghurt exhibited the lowest attractive textural properties. In Brazil, the extensive livestock area, the large cattle farming and the popular culture of the cow milk consume are reasons why sheep's milk yogurt is not produced on a commercial scale yet. Nevertheless, sheep milk yoghurt can be considered a suitable alternative to cow milk yoghurt, especially in states with a low level of cattle production like Rio de Janeiro.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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