Physicochemical evaluation of sheep milk yogurts containing different levels of inulin

C. F. Balthazar,* C. A. Conte Júnior,* J. Moraes,† M. P. Costa,* R. S. L. Raices,† R. M. Franco,* A. G. Cruz,† and A. C. O. Silva*¹

*Department of Food Technology, Veterinary College, Fluminense Federal University, 24230-340 Niterói, RJ, Brazil

†Federal Institute of Education, Science and Technology of Rio de Janeiro, 20270-021 Rio de Janeiro, RJ, Brazil

ABSTRACT

The present study aimed to evaluate the physicochemical parameters of sheep milk yogurt smoothies (SMY) containing inulin at different levels (0, 2, 4, and 6%). Titratable acidity and pH, yogurt bacteria counts, fatty acids profile, and healthy lipid indices were evaluated during 28 d of refrigerated storage. As expected for yogurts, *Streptococcus thermophilus* counts decreased 1 to 3 log cycles and *Lactobacillus delbrueckii* ssp. *bulgaricus* counts decreased 1 to 2 cycles from d 1 to 28. The protective effect of inulin on bacteria survival and viability in the food matrix was not verified in the prebiotic SMY during storage, regardless of inulin level. Although lower post-acidification was observed in prebiotic SMY due to inulin addition, no changes were verified in short-chain fatty acids (SCFA) or polyunsaturated fatty acids (PUFA). In contrast, an increase in medium- and long-chain fatty acids (MCFA and LCFA) and monounsaturated fatty acids (MUFA) was observed during storage in all SMY. The most significant levels of fatty acids in SMY were oleic acid, followed by palmitic and myristic acids. A high positive correlation between conjugated linoleic acid (CLA) and oleic acid ($r = 0.978$) was observed. The *cis*-9,*trans*-11 CLA isomer represented approximately 78% of total PUFA and 2% of total fatty acids, whereas α -linoleic acid comprised about 22% PUFA and 1% of total fatty acids in SMY. The fatty acid changes during storage were associated with the metabolic activity of the starter bacteria, especially for oleic acid and *cis*-9,*trans*-11 CLA isomer. Thus, the SMY represented a great source of these compounds. We observed that inulin levels did not affect fatty acids. A nonsignificant decrease in atherogenic index was observed during storage in all SMY, and a positive correlation ($r = 0.973$) was found between atherogenic index and thrombogenic index of SMY. High correlations were observed between

lauric and myristic acids and saturated fatty acids ($r = 0.907$ and $r = 0.894$, respectively), providing evidence of their atherogenic and thrombogenic potential. A negative correlation was observed between stearic acid and atherogenic index ($r = -0.612$) and between oleic acid and atherogenic index. Sheep milk yogurt could be characterized as a food with low atherogenic and thrombogenic risk because of its healthy lipid composition. Therefore, addition of inulin to SMY could be a good option to improve functionality of this food matrix for dairy companies wishing to enter the functional food market.

Key words: acidity, atherogenic index, fatty acids, thrombogenic index, prebiotic

INTRODUCTION

Ovine milk has higher contents of protein, lipids, minerals, and vitamins essential to human health compared with caprine and bovine milk (Park et al., 2007). Yogurt manufacture using sheep milk (SM) is an interesting approach because of the improvement in nutritional value compared with cow and goat milks (Dixit et al., 2012). In addition, among the fatty acids in SM, the higher level of CLA can be an advantage, as it may have beneficial effects in the body, such as prevention of cardiovascular diseases, anticarcinogenic and lipase activity, increased muscle mass, and decreased blood glucose levels due to hyperinsulinemia (Yuan et al., 2014; Khosravi et al., 2015; Wang and Lee, 2015).

Functional foods, in particular the prebiotic category, play an important sector in modern food industry, with the dairy industry having a fundamental role (Buriti and Saad, 2014; Morais et al., 2014; Balthazar et al., 2015b). Prebiotic ingredients are important because they produce microbial biomass, increasing the number of defecations, similar to the effects attributed to dietary fiber (Rolim, 2015). The worldwide market demand for prebiotics was \$2.3 billion in 2012, and it is estimated to reach \$4.5 billion in 2018, growing at a compound annual growth rate of 11.4% between 2012 and 2018; Europe is the global revenue leader in

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¹Corresponding author: adrianasilva@id.uff.br

prebiotics, and the market was dominated by inulin, accounting for over 40% overall in 2011 (Transparency Market Research, 2015).

Studies on the manufacture of dairy foods using sheep milk as a raw material containing prebiotic ingredients and inulin are scarce in the literature. The aim of this study was to evaluate the physicochemical parameters of sheep yogurt containing inulin at different concentrations. Post-acidification was assessed by pH values and titratable acidity, yogurt bacterial counts, and fatty acid profile during 28 d of refrigerated storage, as well as the atherogenic and thrombogenic indices, which are linked to cardiovascular diseases promoted by fat intake.

MATERIALS AND METHODS

Yogurt Manufacture

To prepare a prebiotic sheep milk yogurt (SMY) smoothie, whole raw milk containing 5% fat (around 10% solids nonfat) was obtained from a Lacaune dairy ewe flock located in a mountainous region of Rio de Janeiro State, Brazil. Inulin prebiotic fiber was added (Ingredients and Systems Biotechnology, São Paulo, SP, Brazil) at the following concentrations: 0% (SMY0), 2% (SMY2), 4% (SMY4), and 6% (SMY6). The batches were heated at $93 \pm 2^\circ\text{C}$ for 5 min in a stainless steel double-jacketed container and cooled to $40 \pm 2^\circ\text{C}$ for 5 min. Then, 1% (wt/wt) thermophilic cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, Direct Vat Set YF-L903, Yo-Flex, Chr. Hansen, Valinhos, SP, Brazil) were added to each batch. After homogenization, the treatments were incubated at $43 \pm 1^\circ\text{C}$ for 6 h. The SMY with different inulin levels were stirred and stored under refrigeration at $4 \pm 2^\circ\text{C}$ for 28 d.

Yogurt Bacterial Count

Enumeration of *Streptococcus thermophilus* was performed using M17 agar (Agar M17 Base, M929, HiMedia Laboratories, Mumbai, India) incubated at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 48 h under aerobic conditions; and enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus* was performed using de Man, Rogosa, and Sharpe agar (Lactobacillus MRS Agar, M641, HiMedia Laboratories) incubated at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 72 h under anaerobic conditions, according to the procedures outlined by the International Organization of Standardization Standard for yogurt enumeration of characteristic microorganisms colony-count technique at 37°C (ISO-IDF, 2003). All bacterial counts were performed in triplicate.

Physicochemical Analyses

Titratable acidity (TA) was measured by titration with sodium hydroxide using phenolphthalein as indicator according to AOAC International (1999), and pH value was determined using a digital pH-meter (model PG1800, Cap Lab, São Paulo, Brazil) by direct insertion of the electrode into a 10-g sample. The analyses were performed in triplicate once a week for 28 d. The yogurts were kept under refrigerated storage ($4 \pm 2^\circ\text{C}$) for 28 d.

Fatty Acid Profile

Lipid extraction and methylation procedures were performed according to Conte-Junior et al. (2007) with modifications. Briefly, 5 g of sample and 50 μL of heptadecanoic standard were mixed, and lipids were extracted in a separatory funnel using 4 mL of methanol, 2 mL of chloroform, and a pinch of butylated hydroxytoluene. The mixture was stirred vigorously for 3 min, 2 mL of chloroform and 2 mL of distilled water were added, and the mixture was stirred again for 20 s, followed by centrifugation at $1,000 \times g$ for 15 min. Then, the solvents were evaporated under a stream of nitrogen.

For the methylation procedure, 6 mL of 10% HCl in methanol was added to the tube and placed in a water bath at 60°C for 20 min. Then, the solution was kept in an ice bath for 5 min. After 5 min, 1 mL of water and 1 mL of hexane were added and stirred in a vortex for 1 min. The top layer was removed to a vial and refrigerated until analysis.

The FAME of the methylated fat aliquots were determined by GC (7890A, Network GC System, Agilent Technologies, Santa Clara, CA), equipped with a flame-ionization mass spectrometry detector (5975C, Network GC System, Agilent Technologies) and a CP Wax 52 CB capillary column (60 m length, 0.25 mm internal diameter, 0.25 μm film; J&W Scientific/Agilent Technologies). The operation conditions were as follows: carrier gas (helium) at a flow rate of 1 mL/min; injection temperature of 250°C ; detector temperature of 270°C ; injection volume of 1 μL (split 1:10). The temperature was programmed as follows: initial temperature of 70°C for 10 min, from 70 to 80°C at $5^\circ\text{C}/\text{min}$ for 1 min, followed by a temperature increase of $10^\circ\text{C}/\text{min}$ up to 240°C for 30 min. Identification of the FAME peaks was performed by comparing the retention times of the FAME standards. A FAME mix (18919-1AMP Supelco FAME Mix, C4-C24 Unsaturates) and heptadecanoic acid (C17:0; 51633 Fluka methyl heptadecanoate) were used as internal standards and purchased from Sig-

maAldrich (St. Louis, MO). Agilent Technologies software (G2070 Chemstation for GC Systems) was used for integration of peaks. Fatty acid levels in SMY were expressed as milligrams per milliliter of sample. The fatty acid profile was performed in triplicate. Short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), MUFA, and PUFA were also determined. Atherogenic and thrombogenic indices of lipids quality were calculated according to Albenzio et al. (2013).

Statistical Analyses

Statistical analyses were performed using the statistical package SAEG 8.1 (System for Statistical Analysis and Genetic; UFV, 2003). Descriptive statistics of the data were performed to obtain the average and deviation patterns, and normality was assessed by Lilliefors test. Normal parametric variables were subjected to ANOVA for linear models and comparison of means by Tukey test at 5% significance. The Pearson correlation index was also used to find significant interactions ($P < 0.05$) between data.

RESULTS AND DISCUSSION

Yogurt Bacterial Counts

The bacterial counts throughout the refrigerated storage are shown in Table 1. Significant differences were observed in SMY for *Streptococcus thermophilus* (9 to 11 log cfu/mL) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (8 to 9 log cfu/mL) counts on d 1 of storage, decreasing at the end of storage (d 28) to values of 8 to 9 log cfu/mL and 6 to 7 log cfu/mL for *S. thermophilus* and *L. bulgaricus*, respectively ($P < 0.05$). As expected for yogurts, *S. thermophilus* counts decreased 1 to 3 log cycles ($P < 0.05$) from d 1 to 28, whereas *L. bulgaricus* counts decreased 1 to 2 cycles in the same period ($P < 0.05$). Thus, the protective effect of inulin on bacterial survival and viability in the food matrix (Buriti et al., 2010; Hernandez-Hernandez et al., 2012) was not verified in the prebiotic SMY during storage, regardless of inulin level. This behavior may be due to interactions among inulin, bacteria, and the food matrix, leading to gel formation and thus, reducing the water availability in the matrix for microbial growth (Karimi et al., 2015). All SMY confirmed the adequacy of SM as a food matrix for yogurt bacteria growth, and were in accordance with Codex Alimentarius (2010), which has established 7 log cfu/g as a sum of *S. thermophilus* and *L. bulgaricus* bacteria in yogurt, suggesting that SMY containing different inulin levels have fulfilled the regulatory requirements.

Physicochemical Analyses

Figures 1 and 2 show the pH and TA values. The pH (Figure 1) of all SMY decreased significantly, from 4.54 in d 1 SMY2 to 4.37 in d 28 SMY0 measurements, whereas increasing ($P < 0.05$) TA values (Figure 2) were observed during storage, from 1.00% (% lactic acid) in d 1 SMY6 to 1.35% in d 28 SMY measurements. A high negative correlation ($r = -0.835$) between pH and TA was obtained when data were subjected to correlation analysis. Significant differences were also observed between the SMY during storage ($P < 0.05$), and SMY0 was the most acidic; the pH of SMY0 ranged from 4.52 to 4.37 and TA from 1.15 to 1.35% lactic acid. In contrast, SMY2 and SMY6 were the least acidic yogurts: pH ranged from 4.54 to 4.39 and TA from 1.00 to 1.26% lactic acid, respectively. The pH values were not significantly affected by the inulin addition, which is in accordance with other authors (Guggisbert et al., 2009; Mazloomi et al., 2011).

High TA values are due to lactose fermentation by yogurt lactic acid bacteria; thus, TA increases and pH decreases during storage due to ongoing metabolic and enzymatic activities of the mixed cultures during low-temperature storage (Paseephol et al., 2008). The present study showed that inulin levels contributed to a lower increase of lactic acid in the prebiotic SMY, which is an advantage from the technological point of view, suggesting less acidic products. Balthazar et al. (2015a) investigated the sensory acceptance of prebiotic SMY with different inulin contents, and found that consumers classified the inulin-free SMY as more acidic than the ideal, resulting in lower acceptance by consumers. In addition, the lower acidic environment is an ideal condition to add probiotic bacteria in the food matrix, which are sensitive to post-acidification of yogurt (Granato et al., 2010). Thus, inulin may be a

Table 1. Enumeration (log cfu/mL) of synbiotic cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in sheep milk yogurt with different inulin levels throughout refrigerated storage

Yogurt culture	Treatment ¹	Storage (d)	
		1	28
<i>S. thermophilus</i>	SMY0	9 ^a	8 ^b
	SMY2	10 ^a	8 ^b
	SMY4	11 ^a	8 ^b
	SMY6	11 ^a	9 ^b
<i>L. bulgaricus</i>	SMY0	8 ^a	6 ^b
	SMY2	8 ^a	7 ^b
	SMY4	8 ^a	7 ^b
	SMY6	9 ^a	7 ^b

^{a,b}Mean values in the same row with different letters differ ($P < 0.05$).

¹SMY0 to SMY6 = sheep milk yogurt with 0, 2, 4, and 6% inulin, respectively.

Figure 1. pH values of sheep milk yogurt with different inulin levels during 28 d of storage. SMY0 to SMY6 = sheep milk yogurt with 0, 2, 4, and 6% inulin, respectively. Color version available online.

viable alternative to reduce post-acidification in SMY, in addition to the health benefits of prebiotics to consumers, who normally reject acidic dairy foods (Cruz et al., 2013; Perina et al., 2015).

Fatty Acid Profile

The fatty acid profile in sheep milk yogurt with different inulin levels is shown in Tables 2 and 3. Although the CLA isomer *trans*-10,*cis*-12 was detected, the levels were below the quantification limit, thus suggesting low levels of this compound in SMY. With respect to the fatty acid groups (Table 2), no significant differences were observed for the SCFA in all SMY, with values of 0.14 mg/mL (SMY0 and SMY6) at d 1, and 0.14 to 0.19 mg/mL at the end of refrigerated storage (d 28). Similar behavior was observed for MCFA and LCFA in the different treatments, which ranged from 4.54 mg/

mL (SMY6) to 4.97 mg/mL (SMY0) on d 1 and from 13.11 to 13.94 mg/mL on d 28 ($P > 0.05$). In contrast, an increase in MCFA and LCFA was observed during storage in all SMY, with MCFA values ranging from 4.97 mg/mL at d 1 to 8.98 mg/mL at d 28 (SMY0), and LCFA ranging from 9.65 mg/mL at d 1 to 13.25 mg/mL at d 28 (SMY2) ($P < 0.05$). No significant differences were found for MUFA levels ranging from 4.67 mg/mL (SMY2) to 5.33 mg/mL (SMY0), despite a significant increase during storage, ranging from 4.67 mg/mL at d 1 to 8.77 mg/mL at d 28 (SMY2). Finally, no changes were observed for PUFA levels in all SMY at the end of storage, with values ranging from 0.48 and 0.75 mg/mL at d 1 and 28 of storage (SMY4) and from 0.47 mg/mL (SMY2) to 0.55 mg/mL (SMY0) between treatments ($P > 0.05$).

With respect to individual fatty acids (Table 3), the most significant level in SMY was oleic acid (C18:1n-9) ranging from 4.28 mg/mL (SMY2) to 10.01 mg/mL (SMY6), followed by palmitic acid (C16:0) ranging from 5.28 mg/mL (SMY2) to 6.80 mg/mL (SMY4), and myristic acid (C14:0) ranging from 2.69 mg/mL (SMY2) to 5.39 mg/mL (SMY6) ($P < 0.05$). A high positive correlation ($r = +0.978$) was observed between CLA and oleic acid (C18:1n-9). In addition, *cis*-9,*trans*-11 CLA increased significantly during storage for all SMY, ranging from 0.38 mg/mL at d 1 to 0.68 mg/mL at d 28 (SMY2). However, there were no significant differences ($P > 0.05$) in CLA levels with respect to inulin content in different SMY treatment.. α -Linoleic acid decreased in the first week of storage in all SMY, ranging from 0.92 mg/mL at d 1 to 0.19 mg/mL at d 7 (SMY4; $P < 0.05$). This behavior changed at d 7, remaining constant until the last day of storage, ranging from 0.16 mg/mL at d 7 to 0.19 mg/mL at d 28 (SMY; $P > 0.05$). Therefore, inulin did not change the fatty acids concentration in the SMY.

In animal tissues, the most abundant fatty acids are palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9), and linoleic acids, whereas fatty acids with fewer than 14 carbon atoms are present only in minor concentrations (Tvrzicka et al., 2011). In the present study, the SMY with different inulin levels had less palmitic acid than Greek sheep milk yogurt in studies performed by Serafeimidou et al. (2012), who reported that palmitic acid (C16:0) is the major SFA responsible for hypercholesterolemia. Human studies in the United States identified palmitic (C16:0) and stearic (C18:0) acids as fatty acids associated with a higher risk of obesity, a high level of serum triglycerides, inflammation biomarkers, and insulin resistance (Ma et al., 2015). On the other hand, oleic acid can improve the immune response associated with a more successful elimination of pathogenic bacteria and fungi by interfering with

Figure 2. Titratable acidity values of sheep milk yogurt with different inulin levels during 28 d of storage. SMY0 to SMY6 = sheep milk yogurt with 0, 2, 4, and 6% inulin, respectively. Color version available online.

the different system components such as macrophages, lymphocytes, and neutrophils (Sales-Campos et al., 2013). Although SFA have been involved in atherogenic and thrombogenic processes, not all of them express the same behavior as regards the increase of serum cholesterol (Garaffo et al., 2011). The LCFA mead acid arises from desaturation and elongation of oleic acid (C18:1n-9), and is produced in humans only when dietary intake of essential fatty acids is not sufficient (Tvrzicka et al., 2011). Diets rich in MUFA are very effective in reducing the risk of coronary diseases. Indeed, MUFA, in particular docosahexaenoic acid, have been recognized as beneficial as the PUFA n-3 class for human health because of their effect in lowering blood cholesterol (Garaffo et al., 2011). In the present study, oleic acid (C18:1n-9) demonstrated high negative correlations with atherogenic index ($r = -0.845$) and thrombogenic index ($r = -0.897$), indicating antiatherogenic and antithrombotic properties, respectively, due to the increase in ratio of high-density lipoprotein:low-density

lipoprotein cholesterol and lower aggregation of thrombocytes (Tvrzicka et al., 2011).

The *cis-9,trans-11* CLA represented approximately 78% of total PUFA and 2% of total FA; thus, the SMY represented a good source of CLA. Ordinarily, CLA in bovine dairy products constitutes about 1% of the fatty acids (Moreno et al., 2012). In dairy products, *cis-9,trans-11* CLA with 90% of the conjugated alkadienes was also shown to be the major CLA with the most biologically active form (Ye et al., 2013). This explains the higher *cis-9,trans-11* CLA concentration in SMY, as found by Serafeimidou et al. (2012). Moreover, this isomer exhibits interesting specific physiological activities such as antiobesity, anticarcinogenic, antiatherogenic, antidiabetic, antioxidant, apoptotic, immunomodulatory, and osteosynthetic effects (Rodríguez-Alcalá et al., 2011; Yuan et al., 2014; Wang and Lee, 2015). According to Li et al. (2013) and Yang et al. (2014), some lactobacilli strains are sensitive to unsaturated fatty acids such as oleic acid, with the ability to pro-

Table 2. Grouped fatty acid profile (means $\pm$ SD, $n = 3$; mg/mL) of sheep milk yogurt with different inulin levels during 28 d of storage

Fatty acid ¹	Treatment ²	Storage period (d)				
		1	7	14	21	28
SCFA	SMY0	0.14 ^{a,A} $\pm$ 0.92	0.20 ^{a,A} $\pm$ 0.23	0.14 ^{a,AB} $\pm$ 0.14	0.15 ^{a,A} $\pm$ 0.57	0.32 ^{a,A} $\pm$ 0.58
	SMY2	0.14 ^{a,A} $\pm$ 0.64	0.18 ^{a,A} $\pm$ 0.16	0.12 ^{a,B} $\pm$ 0.01	0.16 ^{a,A} $\pm$ 0.21	0.22 ^{a,A} $\pm$ 0.10
	SMY4	0.14 ^{a,A} $\pm$ 0.01	0.17 ^{a,A} $\pm$ 0.01	0.17 ^{a,B} $\pm$ 0.14	0.17 ^{a,A} $\pm$ 0.96	0.20 ^{a,A} $\pm$ 0.81
	SMY6	0.14 ^{b,A} $\pm$ 0.18	0.20 ^{b,A} $\pm$ 0.70	0.37 ^{a,A} $\pm$ 0.29	0.14 ^{b,A} $\pm$ 0.71	0.19 ^{b,A} $\pm$ 0.43
MCFA	SMY0	4.97 ^{c,A} $\pm$ 0.16	7.07 ^{b,A} $\pm$ 0.20	4.88 ^{c,A} $\pm$ 0.77	6.37 ^{b,A} $\pm$ 0.15	8.98 ^{a,A} $\pm$ 0.21
	SMY2	4.57 ^{c,A} $\pm$ 0.01	6.04 ^{b,A} $\pm$ 0.34	4.66 ^{c,A} $\pm$ 0.85	6.27 ^{b,A} $\pm$ 0.14	8.05 ^{a,A} $\pm$ 0.91
	SMY4	4.74 ^{c,A} $\pm$ 0.81	6.91 ^{b,A} $\pm$ 0.26	5.75 ^{c,A} $\pm$ 0.14	6.48 ^{b,A} $\pm$ 0.86	7.57 ^{a,A} $\pm$ 0.18
	SMY6	4.54 ^{b,A} $\pm$ 0.10	7.19 ^{a,A} $\pm$ 0.34	4.77 ^{b,A} $\pm$ 0.73	5.20 ^{b,A} $\pm$ 0.91	7.53 ^{a,A} $\pm$ 0.27
LCFA	SMY0	10.67 ^{b,A} $\pm$ 0.24	12.81 ^{a,A} $\pm$ 0.31	11.16 ^{b,A} $\pm$ 0.23	12.49 ^{a,A} $\pm$ 0.01	13.11 ^{a,A} $\pm$ 0.32
	SMY2	9.65 ^{b,A} $\pm$ 0.67	13.71 ^{a,A} $\pm$ 0.52	10.91 ^{b,A} $\pm$ 0.11	12.43 ^{a,A} $\pm$ 0.37	13.25 ^{a,A} $\pm$ 0.11
	SMY4	9.93 ^{c,A} $\pm$ 0.51	12.09 ^{b,A} $\pm$ 0.44	12.04 ^{b,A} $\pm$ 0.48	12.95 ^{a,A} $\pm$ 0.45	13.43 ^{a,A} $\pm$ 0.41
	SMY6	10.14 ^{b,A} $\pm$ 0.12	13.99 ^{a,A} $\pm$ 0.29	11.12 ^{b,A} $\pm$ 0.13	10.69 ^{b,B} $\pm$ 0.74	13.94 ^{a,A} $\pm$ 0.58
MUFA	SMY0	5.33 ^{b,A} $\pm$ 0.39	9.41 ^{a,A} $\pm$ 1.32	5.50 ^{b,A} $\pm$ 0.81	5.86 ^{b,A} $\pm$ 0.23	9.83 ^{a,A} $\pm$ 1.08
	SMY2	4.67 ^{c,A} $\pm$ 0.21	7.97 ^{a,A} $\pm$ 1.36	4.48 ^{c,A} $\pm$ 0.31	6.17 ^{b,A} $\pm$ 0.30	8.77 ^{a,A} $\pm$ 0.34
	SMY4	4.80 ^{c,A} $\pm$ 0.84	10.81 ^{a,A} $\pm$ 0.01	6.27 ^{b,A} $\pm$ 0.54	6.34 ^{b,A} $\pm$ 0.65	7.67 ^{b,A} $\pm$ 0.78
	SMY6	4.96 ^{c,A} $\pm$ 0.01	10.86 ^{a,A} $\pm$ 0.19	5.86 ^{c,A} $\pm$ 1.15	5.24 ^{c,A} $\pm$ 0.29	8.97 ^{b,A} $\pm$ 0.18
PUFA	SMY0	0.55 ^{a,A} $\pm$ 0.54	0.89 ^{a,A} $\pm$ 0.12	0.52 ^{a,B} $\pm$ 0.36	0.68 ^{a,A} $\pm$ 0.10	0.96 ^{a,A} $\pm$ 0.10
	SMY2	0.47 ^{a,A} $\pm$ 0.15	0.79 ^{a,A} $\pm$ 0.14	0.50 ^{a,B} $\pm$ 0.01	0.59 ^{a,A} $\pm$ 0.11	0.86 ^{a,A} $\pm$ 0.29
	SMY4	0.48 ^{a,A} $\pm$ 0.10	1.04 ^{a,A} $\pm$ 0.01	0.63 ^{a,AB} $\pm$ 0.29	0.67 ^{a,A} $\pm$ 0.10	0.75 ^{a,A} $\pm$ 0.70
	SMY6	0.51 ^{a,A} $\pm$ 0.01	1.05 ^{a,A} $\pm$ 0.31	0.74 ^{a,A} $\pm$ 0.20	0.49 ^{a,A} $\pm$ 0.13	0.88 ^{a,A} $\pm$ 0.39
Atherogenic index	SMY0	3.23 ^{a,A} $\pm$ 0.31	2.55 ^{a,A} $\pm$ 0.34	3.27 ^{a,A} $\pm$ 0.45	3.65 ^{a,A} $\pm$ 0.53	2.41 ^{a,A} $\pm$ 0.40
	SMY2	3.39 ^{a,A} $\pm$ 0.12	2.87 ^{a,A} $\pm$ 0.80	4.43 ^{a,A} $\pm$ 1.03	3.51 ^{a,A} $\pm$ 0.20	2.71 ^{a,A} $\pm$ 0.30
	SMY4	3.42 ^{a,A} $\pm$ 0.01	1.94 ^{b,A} $\pm$ 0.01	3.15 ^{a,A} $\pm$ 0.37	3.54 ^{a,A} $\pm$ 0.58	3.13 ^{a,A} $\pm$ 0.10
	SMY6	3.30 ^{a,A} $\pm$ 0.01	2.56 ^{a,A} $\pm$ 0.30	4.05 ^{a,A} $\pm$ 0.86	3.42 ^{a,A} $\pm$ 0.66	2.93 ^{a,A} $\pm$ 0.88
Thrombogenic index	SMY0	4.28 ^{a,A} $\pm$ 0.11	3.19 ^{a,A} $\pm$ 0.25	4.31 ^{a,B} $\pm$ 0.65	4.61 ^{a,A} $\pm$ 0.16	3.18 ^{a,A} $\pm$ 0.53
	SMY2	4.38 ^{b,A} $\pm$ 0.97	3.85 ^{b,A} $\pm$ 0.15	5.16 ^{a,A} $\pm$ 0.71	4.33 ^{b,A} $\pm$ 0.24	3.45 ^{b,A} $\pm$ 0.10
	SMY4	4.38 ^{a,A} $\pm$ 0.01	2.75 ^{b,A} $\pm$ 0.01	4.15 ^{a,B} $\pm$ 0.11	4.46 ^{a,A} $\pm$ 0.47	3.88 ^{a,A} $\pm$ 0.16
	SMY6	4.35 ^{a,A} $\pm$ 0.59	3.10 ^{a,A} $\pm$ 0.54	5.28 ^{a,A} $\pm$ 1.48	4.33 ^{a,A} $\pm$ 0.24	3.55 ^{a,A} $\pm$ 0.22

^{a-c}Mean values in the same row with different lowercase letters differ ($P < 0.05$) between days of storage.

^{A,B}Mean values in the same column with different uppercase letters differ ($P < 0.05$) between treatments.

¹SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; atherogenic index = $C12:0 + 4 \times C14:0 + C16:0 / [\Sigma \text{ MUFA} + \Sigma \text{ PUFA n-6 and n-3}]$; thrombogenic index = $C14:0 + C16:0 + C18:0 / [0.5 \times \Sigma \text{ MUFA} + 0.5 \times \Sigma \text{ PUFA n-6}]$. The fatty acid composition was classified as reported by Florence et al. (2009), who described SCFA as C2:0 to C4:0, MCFA as C6:0 to C12:0, and LCFA as C14:0 to C24:0.

²SMY0 to SMY6 = sheep milk yogurt with 0, 2, 4, and 6% inulin, respectively.

duce CLA due to their mechanism for detoxifying free fatty acids. In addition, Rodríguez-Alcalá et al. (2011) have reported that lactic acid bacteria may produce CLA from linoleic acid in milk. Other authors have found that certain *Streptococcus* spp. strains have the ability to produce *cis*-9,*trans*-11 CLA (Jiang et al., 1998). α -Linoleic acid has been reported to exert an antiarrhythmic effect on the heart, with a positive ef-

fect on neurological function by limiting injury to the central nervous system and protecting the heart against coronary disease (Lee and Pan, 2012; Walczewska et al., 2014). It has a nutritional importance due to its antithrombogenic effect, and the main metabolic products are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Calder, 2010). ALA comprises about 22% PUFA and 1% total fatty acids in SMY. In

Table 3. Individual fatty acid profile (means $\pm$ SD, n = 3; mg/mL) of sheep milk yogurt with different inulin levels during 28 d of storage

Fatty acid	Treatment ¹	Storage period (d)				
		1	7	14	21	28
C4:0	SMY0	0.14 ^{a,A} $\pm$ 0.92	0.20 ^{a,A} $\pm$ 0.23	0.14 ^{a,AB} $\pm$ 0.14	0.15 ^{a,A} $\pm$ 0.57	0.32 ^{a,A} $\pm$ 0.58
	SMY2	0.14 ^{a,A} $\pm$ 0.64	0.18 ^{a,A} $\pm$ 0.16	0.12 ^{a,B} $\pm$ 0.01	0.16 ^{a,A} $\pm$ 0.21	0.22 ^{a,A} $\pm$ 0.10
	SMY4	0.14 ^{a,A} $\pm$ 0.01	0.17 ^{a,A} $\pm$ 0.01	0.17 ^{a,B} $\pm$ 0.14	0.17 ^{a,A} $\pm$ 0.96	0.20 ^{a,A} $\pm$ 0.81
	SMY6	0.14 ^{b,A} $\pm$ 0.18	0.20 ^{b,A} $\pm$ 0.70	0.37 ^{a,A} $\pm$ 0.29	0.14 ^{b,A} $\pm$ 0.71	0.19 ^{b,A} $\pm$ 0.43
C6:0	SMY0	0.33 ^{b,A} $\pm$ 0.12	0.44 ^{b,A} $\pm$ 0.40	0.32 ^{b,A} $\pm$ 0.35	0.38 ^{a,A} $\pm$ 0.98	0.76 ^{a,A} $\pm$ 0.12
	SMY2	0.32 ^{b,A} $\pm$ 0.81	0.41 ^{b,A} $\pm$ 0.34	0.29 ^{b,A} $\pm$ 0.01	0.40 ^{a,A} $\pm$ 0.51	0.55 ^{a,B} $\pm$ 0.18
	SMY4	0.32 ^{a,A} $\pm$ 0.01	0.39 ^{a,A} $\pm$ 0.01	0.41 ^{a,A} $\pm$ 0.33	0.41 ^{a,A} $\pm$ 0.19	0.49 ^{a,B} $\pm$ 0.61
	SMY6	0.32 ^{b,A} $\pm$ 0.01	0.43 ^{ab,A} $\pm$ 0.16	0.39 ^{a,A} $\pm$ 0.15	0.34 ^{b,A} $\pm$ 0.19	0.48 ^{a,B} $\pm$ 0.74
C8:0	SMY0	0.74 ^{c,A} $\pm$ 0.50	0.94 ^{b,A} $\pm$ 0.69	0.69 ^{c,AB} $\pm$ 0.48	0.88 ^{b,A} $\pm$ 0.94	1.61 ^{a,A} $\pm$ 0.16
	SMY2	0.66 ^{c,A} $\pm$ 0.21	0.83 ^{b,A} $\pm$ 0.71	0.62 ^{c,B} $\pm$ 0.20	0.88 ^{b,A} $\pm$ 0.16	1.24 ^{a,B} $\pm$ 0.33
	SMY4	0.68 ^{c,A} $\pm$ 0.01	0.83 ^{b,A} $\pm$ 0.01	0.85 ^{b,A} $\pm$ 0.77	0.91 ^{a,A} $\pm$ 0.35	1.07 ^{a,C} $\pm$ 0.72
	SMY6	0.65 ^{c,A} $\pm$ 0.01	0.91 ^{b,A} $\pm$ 0.29	0.76 ^{c,A} $\pm$ 0.44	0.75 ^{c,B} $\pm$ 0.38	1.11 ^{a,C} $\pm$ 0.42
C10:0	SMY0	2.46 ^{b,A} $\pm$ 0.21	3.45 ^{a,A} $\pm$ 0.31	2.37 ^{b,A} $\pm$ 0.86	3.13 ^{a,A} $\pm$ 0.46	3.43 ^{a,A} $\pm$ 0.18
	SMY2	2.23 ^{b,A} $\pm$ 0.40	2.95 ^{a,A} $\pm$ 0.35	2.41 ^{b,A} $\pm$ 0.15	3.07 ^{a,A} $\pm$ 0.86	3.52 ^{a,A} $\pm$ 0.10
	SMY4	2.27 ^{c,A} $\pm$ 0.01	3.32 ^{ab,A} $\pm$ 0.01	2.82 ^{b,A} $\pm$ 0.28	3.24 ^{ab,A} $\pm$ 0.17	3.74 ^{a,A} $\pm$ 0.11
	SMY6	2.21 ^{b,A} $\pm$ 0.29	3.46 ^{a,A} $\pm$ 0.10	2.20 ^{b,A} $\pm$ 0.30	2.53 ^{b,B} $\pm$ 0.16	3.50 ^{a,A} $\pm$ 0.45
C12:0	SMY0	1.44 ^{c,A} $\pm$ 0.34	2.24 ^{b,A} $\pm$ 0.27	1.50 ^{c,A} $\pm$ 0.85	1.98 ^{b,A} $\pm$ 0.13	3.18 ^{a,A} $\pm$ 0.59
	SMY2	1.36 ^{c,A} $\pm$ 0.26	1.85 ^{b,B} $\pm$ 0.28	1.34 ^{c,A} $\pm$ 0.40	1.92 ^{b,A} $\pm$ 0.34	2.74 ^{a,B} $\pm$ 0.95
	SMY4	1.47 ^{b,A} $\pm$ 0.01	2.37 ^{a,A} $\pm$ 0.01	1.67 ^{b,A} $\pm$ 0.19	1.92 ^{b,A} $\pm$ 0.14	2.27 ^{a,C} $\pm$ 0.91
	SMY6	1.36 ^{b,A} $\pm$ 0.00	2.39 ^{a,A} $\pm$ 0.21	1.42 ^{b,A} $\pm$ 0.34	1.58 ^{b,B} $\pm$ 0.60	2.44 ^{a,C} $\pm$ 0.25
C14:0	SMY0	2.94 ^{a,A} $\pm$ 0.25	4.46 ^{a,A} $\pm$ 0.24	3.03 ^{a,A} $\pm$ 0.67	3.77 ^{a,A} $\pm$ 0.14	4.30 ^{a,A} $\pm$ 1.24
	SMY2	2.69 ^{b,A} $\pm$ 0.31	4.18 ^{a,A} $\pm$ 0.73	3.77 ^{a,A} $\pm$ 0.96	3.80 ^{a,A} $\pm$ 0.35	4.36 ^{a,A} $\pm$ 0.13
	SMY4	2.80 ^{a,A} $\pm$ 0.01	3.54 ^{a,A} $\pm$ 0.01	3.43 ^{a,A} $\pm$ 0.40	3.96 ^{a,A} $\pm$ 0.32	4.39 ^{a,A} $\pm$ 0.64
	SMY6	2.78 ^{b,A} $\pm$ 0.42	5.39 ^{a,A} $\pm$ 0.18	3.62 ^{b,A} $\pm$ 0.54	3.04 ^{b,A} $\pm$ 0.24	5.02 ^{a,A} $\pm$ 0.20
C16:0	SMY0	5.81 ^{a,A} $\pm$ 0.38	5.86 ^{a,A} $\pm$ 0.81	6.10 ^{a,A} $\pm$ 0.18	6.76 ^{a,A} $\pm$ 0.18	5.58 ^{a,A} $\pm$ 0.52
	SMY2	5.28 ^{a,A} $\pm$ 0.66	6.53 ^{a,A} $\pm$ 0.76	5.45 ^{a,A} $\pm$ 0.13	6.56 ^{a,A} $\pm$ 0.27	5.96 ^{a,A} $\pm$ 0.66
	SMY4	5.34 ^{b,A} $\pm$ 0.01	6.49 ^{a,A} $\pm$ 0.01	6.33 ^{a,A} $\pm$ 0.44	6.80 ^{a,A} $\pm$ 0.24	6.49 ^{a,A} $\pm$ 0.13
	SMY6	5.55 ^{a,A} $\pm$ 0.01	6.49 ^{a,A} $\pm$ 0.27	5.83 ^{a,A} $\pm$ 0.94	5.84 ^{a,A} $\pm$ 0.35	6.34 ^{a,A} $\pm$ 0.38
C16:1n-9	SMY0	0.36 ^{c,A} $\pm$ 0.22	0.75 ^{b,A} $\pm$ 0.12	0.39 ^{c,A} $\pm$ 0.49	0.52 ^{c,A} $\pm$ 0.12	0.90 ^{a,A} $\pm$ 0.57
	SMY2	0.33 ^{c,A} $\pm$ 0.30	0.57 ^{b,B} $\pm$ 0.13	0.20 ^{c,B} $\pm$ 0.12	0.56 ^{b,A} $\pm$ 0.24	0.76 ^{a,B} $\pm$ 0.28
	SMY4	0.33 ^{c,A} $\pm$ 0.00	0.85 ^{a,A} $\pm$ 0.01	0.51 ^{b,A} $\pm$ 0.58	0.56 ^{b,A} $\pm$ 0.38	0.67 ^{b,B} $\pm$ 0.99
	SMY6	0.35 ^{b,A} $\pm$ 0.52	0.84 ^{a,A} $\pm$ 0.36	0.38 ^{b,A} $\pm$ 0.12	0.45 ^{b,A} $\pm$ 0.48	0.73 ^{a,B} $\pm$ 0.58
C18:0	SMY0	1.92 ^{b,A} $\pm$ 0.11	2.49 ^{b,A} $\pm$ 0.33	2.03 ^{b,A} $\pm$ 0.42	1.96 ^{b,A} $\pm$ 0.79	3.23 ^{a,A} $\pm$ 0.59
	SMY2	1.68 ^{b,A} $\pm$ 0.10	3.00 ^{a,A} $\pm$ 0.51	1.69 ^{b,A} $\pm$ 0.10	2.07 ^{b,A} $\pm$ 0.10	2.93 ^{a,A} $\pm$ 0.12
	SMY4	1.79 ^{a,A} $\pm$ 0.01	2.06 ^{a,A} $\pm$ 0.01	2.28 ^{a,A} $\pm$ 0.27	2.19 ^{a,A} $\pm$ 0.24	2.55 ^{a,A} $\pm$ 0.10
	SMY6	1.81 ^{a,A} $\pm$ 0.01	2.11 ^{a,A} $\pm$ 0.71	1.67 ^{a,A} $\pm$ 0.30	1.81 ^{a,A} $\pm$ 0.56	2.58 ^{a,A} $\pm$ 0.36
C18:1n-9	SMY0	4.97 ^{b,A} $\pm$ 0.37	8.66 ^{a,B} $\pm$ 1.44	5.11 ^{b,A} $\pm$ 0.31	5.34 ^{b,A} $\pm$ 0.22	8.93 ^{a,A} $\pm$ 1.14
	SMY2	4.34 ^{b,A} $\pm$ 0.21	7.40 ^{a,C} $\pm$ 1.23	4.28 ^{b,A} $\pm$ 0.18	5.61 ^{b,A} $\pm$ 0.28	8.01 ^{a,A} $\pm$ 0.31
	SMY4	4.47 ^{b,A} $\pm$ 0.00	9.96 ^{a,A} $\pm$ 0.01	5.76 ^{b,A} $\pm$ 0.49	5.79 ^{b,A} $\pm$ 0.61	7.00 ^{b,A} $\pm$ 0.88
	SMY6	4.62 ^{c,A} $\pm$ 0.00	10.01 ^{a,A} $\pm$ 0.18	5.48 ^{c,A} $\pm$ 0.58	4.79 ^{c,A} $\pm$ 0.24	8.24 ^{b,A} $\pm$ 0.19
α -Linoleic acid	SMY0	0.81 ^{a,A} $\pm$ 0.14	0.16 ^{b,A} $\pm$ 0.23	0.10 ^{b,B} $\pm$ 0.22	0.14 ^{b,A} $\pm$ 0.27	0.19 ^{b,A} $\pm$ 0.99
	SMY2	0.93 ^{a,A} $\pm$ 0.79	0.14 ^{b,A} $\pm$ 0.24	0.14 ^{b,B} $\pm$ 0.01	0.11 ^{b,A} $\pm$ 0.75	0.18 ^{b,A} $\pm$ 0.13
	SMY4	0.92 ^{a,A} $\pm$ 0.01	0.19 ^{b,A} $\pm$ 0.01	0.13 ^{b,B} $\pm$ 0.18	0.13 ^{b,A} $\pm$ 0.11	0.14 ^{b,A} $\pm$ 0.30
	SMY6	0.86 ^{a,A} $\pm$ 0.01	0.21 ^{b,A} $\pm$ 0.17	0.36 ^{b,A} $\pm$ 0.24	0.30 ^{b,A} $\pm$ 0.34	0.17 ^{b,A} $\pm$ 0.28
CLA	SMY0	0.44 ^{b,A} $\pm$ 0.40	0.73 ^{a,A} $\pm$ 0.99	0.42 ^{b,A} $\pm$ 0.59	0.54 ^{b,A} $\pm$ 0.74	0.77 ^{a,A} $\pm$ 0.97
	SMY2	0.38 ^{b,A} $\pm$ 0.14	0.65 ^{a,A} $\pm$ 0.12	0.37 ^{b,A} $\pm$ 0.00	0.48 ^{b,A} $\pm$ 0.42	0.68 ^{a,A} $\pm$ 0.16
	SMY4	0.38 ^{b,A} $\pm$ 0.01	0.85 ^{a,A} $\pm$ 0.01	0.50 ^{b,A} $\pm$ 0.47	0.55 ^{b,A} $\pm$ 0.90	0.61 ^{a,A} $\pm$ 0.10
	SMY6	0.42 ^{b,A} $\pm$ 0.74	0.84 ^{a,A} $\pm$ 0.13	0.59 ^{b,A} $\pm$ 0.15	0.39 ^{b,A} $\pm$ 0.17	0.71 ^{a,A} $\pm$ 0.68

^{a-c}Mean values in the same row with different lowercase letters differ ($P < 0.05$) between days of storage.

^{A-C}Mean values in the same column with different uppercase letters differ ($P < 0.05$) between treatments.

¹SMY0 to SMY6 = sheep milk yogurt with 0, 2, 4, and 6% inulin, respectively.

the present study, fatty acid changes during storage may have been due to the metabolic activity of the starter yogurt bacteria in SMY. Even though changes in fatty acid levels as a function of bacterial metabolic activity in SMY during storage have been reported by other authors (Regula, 2007; Güler and Gürsoy-Balcı, 2011), further studies are required.

The atherogenic and thrombogenic indices are interesting parameters by which to measure FA profile (Table 2). These indices take into account the different effects that a single fatty acid might have on human health and in particular on the probability of increasing the incidence of pathogenic phenomena, such as atheroma or thrombus formation (Garaffo et al., 2011). In addition, some fatty acids can prevent or cause atherosclerosis and coronary thrombosis in humans (Vargas-Bello-Pérez et al., 2015). The consumption of SFA increases cholesterol levels, which is associated with increased mortality due to coronary heart disease (Dias et al., 2014; Nettleton et al., 2015). In contrast, Mensink (2005) reported that stearic acid (C18:0) decreases low-density lipoprotein cholesterol and increases high-density lipoprotein cholesterol, thus suggesting antiatherogenic properties. The atherogenic index is linked to the possibility of blockage of arteries, and a high atherogenic index can lead to cell adhesion of the immune and circulatory systems. In contrast, a lower atherogenic index prevents the occurrence of micro- and macro-coronary disease (Matheus et al., 2008) and is related to healthy lipid indices (Ross et al., 2012). In the SMY, we observed a nonsignificant decrease in atherogenic index during storage, ranging from 3.30 mg/mL at d 1 to 2.93 mg/mL at d 28 (SMY6), with no differences among SMY ranging from 3.42 mg/mL (SMY6) to 3.65 mg/mL (SMY0) at d 21 ($P > 0.05$). Similar behavior was observed for thrombogenic index in all SMY during storage, with values ranging from 4.38 mg/mL at d 1 to 3.45 mg/mL at d 28 (SMY2), and from 4.33 mg/mL (SMY2) to 4.61 mg/mL (SMY0) at d 21 ($P > 0.05$). A high positive correlation ($r = 0.973$) was found between atherogenic index and thrombogenic index of SMY. High correlations were observed between lauric (C12:0) and myristic (C14:0) acids and SFA ($r = 0.907$ and $r = +0.894$, respectively), evidencing their atherogenic and thrombogenic potential. A negative correlation was observed between stearic acid (C18:0) and atherogenic index ($r = -0.612$) and between oleic acid (C18:1n-9) and atherogenic index ($r = -0.845$).

Based on our results, SMY can be characterized as a food with low atherogenic and thrombogenic risk, due to its healthy lipid composition (Ross et al., 2012). Further studies are needed to evaluate sensory properties (Gaze et al., 2015), as well as clinical performance

(Lollo et al., 2013, 2015; Morato et al., 2015) to verify the existence of a benefit for consumer health.

CONCLUSIONS

Sheep milk is an interesting food matrix for lactic acid bacteria growth. Our study showed that inulin delayed the post-acidification of SMY during storage, which is an advantage from sensory and technological points of view. In addition, the lower acidic environment is an ideal condition under which to add probiotic bacteria in the food matrix, which are sensitive to post-acidification of yogurt. Several medium and long-chain FA levels increased significantly during SMY storage, especially oleic acid (C18:1n-9) and *cis*-9, *trans*-11 CLA isomer, which play an important role in human health, suggesting an interesting characteristic of the metabolic activity of lactic acid bacteria. Moreover, MUFA and PUFA may contribute to the prevention of cardiovascular diseases, as verified by the atherogenic and thrombogenic indices. The supplementation of inulin to sheep milk dairy products is interesting for the functional foods market because of the beneficial effects of this prebiotic fiber.

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