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Physicochemical evaluation of sheep milk yogurts containing different levels of inulin

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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

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at a compound annual growth rate of 11.4% between 2012 and 2018; Europe is the global revenue leader in 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 ± 2°C for 5 min in a stainless steel double-jacketed container and cooled to 40 ± 2°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 ± 1°C for 6 h. The SMY with different inulin levels were stirred and stored under refrigeration at 4 ± 2°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°C ± 1°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°C ± 1°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 ± 2°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 × *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°C/min for 1 min, followed by a temperature increase of 10°C/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