Small Ruminant Research 149 (2017) 154–162

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Development of new probiotic yoghurt with a mixture of cow and sheep milk: effects on physicochemical, textural and sensory analysis

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ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form 9 February 2017
Accepted 12 February 2017
Available online 16 February 2017

Keywords:
Mixed yoghurt
Probiotic
Firmness
Sheep milk
Small ruminant
Scanning electron microscopy

ABSTRACT

The purpose of the present study is to develop a new probiotic yoghurt with a mixture of cow and sheep milk and evaluate on physicochemical, textural and sensory parameters of these products. For each trial (n = 3), cow (CM) and ewe (EM) milk were used to manufacture yoghurts with either cow (Y1), ewe (Y5) or mixtures of cow to ewe milk ratios of 3:1 (Y2), 1:1 (Y3), and 1:3 (Y4). Physicochemical (pH, titratable acidity, contents of total solids, protein, lipid, ash, water-holding capacity – WHC, and spontaneous syneresis), textural (firmness and apparent viscosity), microstructural and sensory (appearance, aroma, flavour, and consistency) properties were evaluated. In general, Y5 and Y4 exhibited increased ($P < 0.05$) proximate composition values, with greater ($P < 0.05$) firmness, WHC, apparent viscosity and lower ($P < 0.05$) spontaneous syneresis values than Y1 and Y2; Y3 demonstrated intermediate values in textural parameters. Yoghurt micrographic structures supported that the greater ewe milk content the denser is the gel structure (Y5) as a result of more interconnected protein clusters. No apparent differences among mixed probiotic yoghurt microstructures were observed. While Y1 demonstrated the lowest ($P < 0.05$) consistency, overall impression, appearance and purchase intention scores; Y3 was the only treatment exhibiting ideal consistency. Therefore, the probiotic yoghurt mixture containing equal proportion of cow and ewe milks represents an advisable technological alternative for dairy industries.

1. Introduction

Cow milk is the main milk type from commercial and industrial perspectives due to lower price and greater production when compared to ewe milk (Pandya and Ghodke, 2007; Renobales et al., 2012). Nonetheless, ewe milk production system represents an alternative in countries with no favorable climate and terrain features for cow farming (Pandya and Ghodke, 2007; Park et al., 2007). In addition, ewe milk demonstrates greater nutritional value than cow counterpart (Renobales et al., 2012) containing increased amounts of total solids and nutrients such as linolenic acid, essential amino acids, vitamins, casein, fat and calcium. The aforementioned differences between ewe and cow milk composition can affect the processing properties of dairy products favoring faster clotting and formation of firmer curds in ewe milk based products (Park et al., 2007; Kaminarides et al., 2007). Thus, the advantageous chemical composition and technological properties (no fortification step required) lead to the production of more viscous and firmer yoghurt (Boyazoglu and Morand-Fehr, 2001). Furthermore, ewe milk yoghurts exhibit desirable organoleptic characteristics that include a pleasant creamy-sour flavour demanded by yoghurt consumers (Kaminarides et al., 2007).

Yoghurt is obtained through acid fermentation of milk by specific lactic acid bacteria (Serafeimidou et al., 2012), and can be used as a vehicle for probiotic cultures (Lourens-Hattingh and Viljoen, 2001; Costa et al., 2013). The consumption of sufficient amounts of these live microorganisms promotes health benefits (WHO, 2001) and can positively influence the stabilization of the gut mucosal barrier (Kailasapathy and Chin, 2000). Previous studies reported the beneficial effect of *Lactobacillus acidophilus* as probiotic (Wang et al., 2012; Ng et al., 2011) acting on the gastrointestinal tract to promote the therapeutic effects (Kailasapathy and Chin, 2000).

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http://dx.doi.org/10.1016/j.smallrumres.2017.02.013
0921-4488/

Furthermore, textural and microstructural characteristics in yoghurt are important parameters influencing consumer market acceptability (Park, 2007). The commercial success of a food on the consumer market is related with sensory characteristics well accepted by the consumer, safety guarantees for consumption, and nutritional qualities (Cruz et al., 2010). These parameters are governed by a three-dimensional milk proteins network formed with casein micelles aggregation (Tamime and Robinson, 2007; Paseephol et al., 2008) in conjunction with denatured whey proteins through hydrophobic and electrostatic bonds (Paseephol et al., 2008). Thus, the protein content of milk is the most important component influencing yoghurt textural and chemical properties. In addition, the increase in protein content improves the amount of bound water, and consequently the gel firmness (Saxelin et al., 2003). Scanning electron microscopy (SEM) is a powerful tool to understand how chemical and textural properties are associated, and is useful to visualize structural elements such as microorganisms, casein micelle structure, and matrix changes that occur during manufacture and ripening processes of dairy products (Everett and Auty, 2008).

In terms of organoleptic characteristics of yoghurt, consumers expect an adequate firmness and smooth surface without evidence of syneresis (Amatayakul et al., 2006; Park, 2007; Unal and Akalin, 2013). Furthermore, although previous researches studied milk mixtures utilizing different species for yoghurt production such as goat and cow (Vargas et al., 2008; Kuçukçetin et al., 2011), and goat and ewe (Guler and Gursoy-Balci, 2011; Matos et al., 2003), there is limited information regarding about textural and microstructural properties of yoghurts manufactured with mixtures of milk from different species. Therefore, the aim of the present research was to develop a new probiotic yoghurt by mixture different ratios of sheep and cow milk and evaluate the influence of the formulation on physicochemical, textural, microstructural, and sensory characteristics of this dairy product.

2. Materials and methods

2.1. Yoghurt preparation

A volume of 7.5 L of raw milk from each species (cow milk, CM; and ewe milk, EM) were obtained from dairy farms located in Miracema (Rio de Janeiro, Brazil) and Vassouras (Rio de Janeiro, Brazil), respectively. Raw milk samples were pasteurized separately at 85 °C for 5 min in a stainless steel double jacket container (GCA Corporation, Greensboro, North Carolina, United States) and cooled to 40 °C. After cooling, starter (Yo-Flex®, Chr Hansen, Valinhos, SP, Brazil) and *Lactobacillus acidophilus* (*Lb. acidophilus*) LA-5 cultures (Chr. Hansen) were inoculated at concentration of 1% (v/v) and 5% (v/v), respectively to produce probiotic yoghurts as described by Costa et al. (2014). A total of five probiotic yoghurt treatments were prepared using either solely cow (Y1), ewe (Y5) milk, and their mixtures of cow to ewe milk ratios of 3:1 (Y2), 1:1 (Y3), and 1:3 (Y4). Each milk mixture (Y1–Y5), containing starter and probiotic cultures, was transferred to 200 mL sterile flasks, and incubated at 43 °C until the pH reached 4.5 (AOAC, 2012). The fermentation step was interrupted with a rapid cooling in two steps, first during 30 min until 18 °C and the second step during 30 min until 10 °C. Then, the yoghurts were stored for 28 days under refrigeration at 4 °C. The yoghurt manufacture was repeated three times for each treatment (n = 3).

2.2. Bacteriological analysis

Streptococcus thermophilus (*S. thermophilus*) was evaluated using M17 agar (Difco Company, KS, USA) incubated under aerobic condition at 37 °C for 48 h whereas for *Lactobacillus* (*Lb.*) *delbrueckii* ssp. *bulgaricus*, MRS agar (Difco Company, KS, USA) was utilized and incubated under anaerobic condition at 37 °C for 72 h (Codex Alimentarius, 2010). As for *L. acidophilus* LA-5 content, MRS agar (Difco Company, KS, USA) supplemented with 0.15% (w/v) bile salts was used followed by incubation at 37 °C for 72 h under aerobic conditions (Lima et al., 2009). In each trial, bacteriological evaluation of the yoghurts was performed in triplicate at the 1st and the 28th days of storage (4 °C).

2.3. Physicochemical analysis

2.3.1. Chemical composition

Total solids content was determined by gravimetric method, Kjeldahl technique was utilized to estimate the protein content, Gerber method was employed to determine the lipid content whereas, ash content was quantified using muffle furnace at 550 °C (AOAC, 2012). The aforementioned analyses were performed in triplicate on the final products.

2.3.2. Evaluation of pH and titratable acidity

A digital pH meter (Digimed® Model DM-32, São Paulo, Brazil) was utilized to determine pH values (Nguyen et al., 2014c), whereas the acid concentration was estimated according to Tamjidi et al. (2012). Yoghurt samples (10 g) were added with 0.5 mL pH indicator (phenolphthalein at 5% w/v), and then titrated with 0.1 M NaOH solution to an end point of stable faint pink color for 1 min. The titratable acidity (TA) was expressed as grams of lactic acid in 100 g of samples. These analyses were carried out in triplicate at the 1st, 14th and 28th days of storage (4 °C).

2.4. Texture evaluation

2.4.1. Gel strength as firmness

Instrumental Texture Analyzer (TA-XT plus®, Stable Micro System Ltd., Godalming, Waverley District, United Kingdom) equipped with a 5 kg load cell and cylindrical probe (36R) was utilized to estimate the yoghurt firmness (Paseephol et al., 2008). Yoghurt samples were compressed up to 10 mm depth at a constant speed (1 mm/s); gel firmness was characterized as the maximum force (N) on time curve force compression and was evaluated in triplicate at the 1st, 14th and 28th days of storage (4 °C).

2.4.2. Apparent viscosity

Apparent viscosity was measured using a rotational Viscometer Microprocessor (Q860M21, Quimis®, Sao Paulo, SP, Brazil) with a spindle number 4 (Balthazar et al., 2015). The spindle was rotated at 20 rpm, and the readings were recorded and expressed as millipascal seconds (mPa-s). Apparent viscosity was carried out in triplicate at the 1st, 14th and 28th days of storage (4 °C).

2.4.3. Water-holding capacity (WHC)

Yoghurt samples were centrifuged (Hermle Z 360 K, Wehingen, Germany) for 20 min at 4500 × *g* (4 °C) according to Remeuf et al. (2003) with slight modifications. The decanted whey (DW) was weighed, and the WHC was calculated as:

$$\mathrm{WHC}(\%) = 100(\mathrm{Y} - \mathrm{DW})/\mathrm{Y}$$

where Y means weight of yoghurt and DW means weight of decanted whey. This parameter was determined in triplicate at the 1st, 14th and 28th days of storage (4 °C).

3.0.4. Spontaneous syneresis

Spontaneous syneresis was evaluated according to Dannenberg and Kessler (1988) with slight modifications, and expressed as the