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Volatile profile in goat coalho cheese supplemented with probiotic lactic acid bacteria

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ABSTRACT

The aim of this study was to evaluate the aromatic profiles of both traditional goat Coalho cheese and cheeses added with isolated and combined probiotics, during 28 days of storage. The cheeses were named as follows: C, with *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; L, with *Lactobacillus acidophilus*; P, with *Lactobacillus paracasei*; B, with *Bifidobacterium lactis*; and M, a "mixed" culture with the three probiotic microorganisms. Based on the results, it was verified that both the use of different cultures of probiotic lactic bacteria and the length of storage affect the volatile profile. The length of storage had the most influence on the number of volatiles produced. A total of twenty five aromatic compounds were identified in the goat cheese; of these twenty five, there were six alcohols, four hydrocarbons, four terpenes, three acids, three ketones, three aldehydes and two esters. Esters and ketones can have a positive influence on goat Coalho cheese, since their pleasant aroma can help minimize other undesirable odours.

1. Introduction

Researchers have dedicated special attention to goat Coalho cheese because it is considered a functional food, since it can be a potential "carrier" of lactic acid bacteria, acting as a "vehicle" for the proper transportation of probiotic microorganisms and CLAs – Conjugated Linoleic Acids (Garcia, Oliveira, Queiroga, Machado, & Souza, 2012; Santos et al., 2012; Silva et al., 2012). Additionally, this cheese has been produced and consumed for over 150 years and has great importance in the economy of goat milk-producing regions, especially for small producers who have no access to industrial facilities for milk processing (Bezerra et al., 2016; Oliveira, Garcia, Queiroga, & Souza, 2012; Queiroga et al., 2013; Silva et al., 2012).

Probiotics are living microorganisms considered able to improve the intestinal microbial balance, producing beneficial effects on consumer health when ingested in satisfactory quantities (10^6–10^7 CFU g^{-1}) (Oliveira et al., 2012; Santos et al., 2012). Several studies have found the benefits related to the intake of probiotics, including improvements in the immune and gastrointestinal systems, reduction on diarrhea, constipation and irritable bowel syndrome, also reduction on the propensity to lactose intolerance and diabetes, cholesterol (LDL) and triglycerides (Favretto, Pontin, & Moreira, 2013; Lollo et al., 2012, 2015; Rodriguez-Figueroa, Gonzalez-Cordova, Astiazaran-Garcia, Hernandez-Mendoza, & Vallejo-Cordoba, 2013).

Studies have already reported the addition of probiotic lactic bacteria of the *Lactobacillus* and *Bifidobacterium* genera to different types of cheese, such as "Minas" cheese added with *Lactobacillus acidophilus* (Coman et al., 2012); Coalho cheese prepared with *L. acidophilus*, *Bifidobacterium lactis* and *Lactobacillus paracasei* (Rodrigues et al., 2011); cream cheese added with *Lactobacillus plantarum* and *L. paracasei* (Burns et al., 2012), entretanto, em todos os estudos, ocorre a recomendação da utilização dos probióticos como alternativa para produção de queijos, impactando no seu valor nutricional e qualidade sensorial.

During cheese processing, various chemical and biochemical reactions take place simultaneously; these reactions have a crucial

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role in the final quality of the product, since they lead to changes in the aroma development, both of which are important criteria for cheese acceptance and consumption (González-Martín et al., 2014; Randazzo, Pitino, Ribbera, & Caggia, 2010; Santos et al., 2012).

The aromatic profile of cheeses also directly affects their acceptance or rejection. When researching literature, the authors found studies on the sensory and microbiological quality of goat Coalho cheese, and also on its physicochemical profile and texture (Medeiros et al., 2014; Oliveira et al., 2012; Queiroga et al., 2013). However, no studies were found on the profile of volatile compounds and the effects of storage on those compounds when using a goat Coalho cheese food matrix.

It must be emphasized that goat Coalho cheese has the potential to receive a protected designation of origin (PDO), since it has well defined characteristics (such as texture and flavour) which are the result of its traditional production methods (Delgado, González-Crespo, Cava, García-Parra, & Ramírez, 2010). This potential to receive a PDO label makes it necessary to carry out further detailed studies, especially on its volatile profile.

Considering the small amount of information in literature regarding the biochemical changes caused by the use of probiotic lactic bacteria in probiotic Coalho cheese, a more detailed study was needed, one which focused on the aspects of volatiles. Therefore, the aim of this research was to evaluate, during 28 days, the effects of the addition of probiotic lactic bacteria (in isolated and combined form) on the volatiles of goat Coalho cheese, comparing it to goat Coalho cheese prepared with a conventional mesophilic starter culture.

2. Material and methods

2.1. Experimental design

A 5 × 2 factorial completely randomized design (CDR) was used, generating five types of cheeses (control cheese inoculated with mesophilic starter, and four probiotic cheeses, inoculated with *L. acidophilus, L. paracasei, B. lactis,* or a mixture of the three probiotic strains). They were analysed at two storage times (1 and 28 days) performed in triplicate, totalling 30 goat Coalho cheese samples.

2.2. Cultures and reagents

Five goat Coalho cheese formulations were processed in individual batches, prepared in triplicate, using lyophilised commercial cultures (Chr. Hansen, Valinhos, São Paulo, Brazil). The cultures were added at 0.01% (100 mg of each culture per 1 L of milk) to the different formulations: "C" (*Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* – R704, batch 3128520); "L" (*L. acidophilus* LA-5, batch 3139352); P (*L. paracasei* – L. casei-01, batch 3089189); "B" (*B. lactis* BB 12, batch 3100870); and "M" (*L. acidophilus, L. paracasei* and *B. lactis* at a ratio of 1:1:1, totaling a mixture of 100 mg). The mesophilic starter culture was only added to the C formulation.

The reagents (lactic acid and Calcium chloride) and the standard n-alkanes C6 – C25, used for the analyses were obtained from laboratory suppliers (Sigma Aldrich Chemie GmbH, Steinheim, Germany).

2.3. Cheese manufacture

For the manufacture of each cheese formulation, 10 L of refrigerated and pasteurized (65 °C for 30 min) goat milk obtained from native breeds that belong to the Cooperative of Farmers of Monteiro, Paraíba ("Cooperativa de Produtores Rurais de Monteiro" – CAPRIBOM) were used. Initially, the milk was heated to 90 °C for 10 min, then cooled to 45 ± 1 °C and then treated by direct acidification with lactic acid (0.85 mL 100 mL^{-1}) at 0.25 mL L^{-1}. The lactic acid bacteria cultures were added in the concentration of 100 mg L^{-1}, being inoculated directly into the vat. Calcium chloride (0.5 mL L^{-1}) and a commercial coagulating agent containing chymosin (0.9 mL L^{-1}) (Chr. Hansen Brazil®, Valinhos, Minas Gerais, Brazil) were also added to the vat.

The vats were maintained at 36 °C until a firm "coalho" was obtained (approximately 40 min). The resulting gel was carefully sliced into cubes (1.5–2.0 cm), and half of the serum was removed for the preparation of the brine (12 g L^{-1} NaCl). The brine was added to the "coalho" and then homogenised. Next, the "coalho" was drained and placed in perforated rectangular moulds (approximately 250 g capacity), which were maintained at 36 °C under pressure for 4 h. The cheeses were then vacuum packaged and stored at 10 °C for 28 days.

2.4. Volatile profile

The grated goat "coalho" cheese, 20 ± 0.1 g was placed in a 60 mL glass vial, with a screw cap containing one centre hole of 3 mm radius and a Teflon-lined septum. Extractions were carried out with an SPME device (Supelco) containing a fused-silica fibre coated with a 50/30 μm layer of Divinylbenzene/Carboxen/Polydimethylsiloxane. The stainless steel needle, housing the fibre, penetrated the septum, and after equilibration at 45 °C for 20 min, the fibre was exposed to the headspace above the goat "coalho" cheese for 40 min. After extraction, the SPME device was removed from the cheese sample vial and inserted directly into the injection port of the GC–MS. Before the extraction the fibre was conditioned, by heating it in the gas chromatograph injection port at 250 °C.

The separation was carried out on a gas chromatograph Varian Saturn 3800 2000 R, coupled to a mass detector Varian Saturn 2000R 2000, coupled to a VF-5 ms fused-silica capillary column (60 m × 0.25 mm I.D., 0.25 μm film thickness, Varian). The temperature program employed was 10 min at 40 °C, a ramp of 5 °C min^{-1} to 240 °C, and held for 11 min. Helium was used as the carrier gas. The mass spectrometer was operated in electron impact mode with a source temperature of 200 °C, an ionising voltage of 70 eV, and a scan range from *m/z* 29 to *m/z* 400 at 3.33 scans s^{-1}.

Identification of the compounds was based on the comparison of their mass spectra with spectra from authentic compounds previously analysed, spectra from the NIST/EPA/NIH Mass Spectral Database (Version 2008), or spectra published elsewhere (Atasoy, Hayaloglu, Kirmaci, Levent, & Türkoğlu, 2013; Hayaloglu, Tolu, & Yasar, 2013; Madruga, Dantas, Queiroz, Brasil, & Ishihara, 2013). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C6 – C25 n-alkanes, and by comparing the LRI with those of authentic compounds analysed under similar conditions. Volatile compounds results isolated are expressed as unit area (area units, AU, × 10^4).

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was carried out on the quantitative data for each compound identified in the GC–MS analyses of volatiles, in order to determine the overall effects exerted by bacteria and storage time. Principal component analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used in order to visualize the variation between the analysed compounds and samples.

The data set consists of a 10 × 32 matrix, in which the lines represented the cheese (control cheese inoculated with mesophilic