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Effect of supplementation with probiotic lactic acid bacteria, separately or combined, on acid and sugar production in goat 'coalho' cheese

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ABSTRACT

This study analyzed the glycolytic effects of adding isolated and combined probiotic bacterial strains to goat 'coalho' cheese. The cheeses were: C-with added *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; L-with added *Lactobacillus acidophilus*; P-with added *Lactobacillus paracasei*; B-with added *Bifidobacterium lactis*; and M, added a "mixed" culture with the three probiotic. Based on the results, the different probiotic lactic acid and the length of storage were verified as factors affecting the organic and fatty acids profiles. Probioticlactic acid bacteria exhibited higher glycolytic potential throughout the 28-day storage, leading to high sugar consumption and organic acid production, with the consequent formation of a more intense cheese aroma. When evaluating the fatty acid profile, the addition of the *B. lactis* culture and of the mixed culture was observed to have a positive effect, providing a fatty acid profile with better nutritional value, i.e., with a higher of conjugated linoleic acids and unsaturated fatty acids.

1. Introduction

When preparedfromgoat milk, 'coalho' cheesestands out as a dairy product with high market value for a number of reasons. Its production process is not only simple, with a high yield and low cost, but also results in a product with an excellent nutritional value that provides low allergenic potential and high digestibilityas well as the capacity to act as an excellent carrier for probiotic lactic acid bacteria (Oliveira, Garcia, Queiroga, & Souza, 2012; Queiroga et al., 2013).

Probiotic lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* are often added to cheese because of their resistance to the milk matrix (Garcia, Oliveira, Queiroga, Machado, & Souza, 2012; Santos et al., 2012). During cheese processing, various chemical and biochemical reactions occur simultaneously. These reactions play a crucial role in the final quality of the product because they lead to changes in the organic acid and fatty acid profiles, 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). More specifically, the biochemical modifications associated with the presence and action of probiotic bacteria in cheeses can lead to the synthesis and the release of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively), which are considered health promoters and inhibitors of fatty liver (O'Shea, Cotter, Stanton, Ross, & Hill, 2012).

In the *Lactobacillus* genus, the species *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus acidophilus* stand out because of their ability to produce isomers of conjugated linoleic acid (CLA) (Andrade et al., 2012). CLAs have excellent bioactive potential, displaying anti-inflammatory, anticancer, anti-atherogenic, and antidiabetogenic properties, among others

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(Medeiros et al., 2014; O'Shea et al., 2012).

The glycolytic process promoted by bacteria involves various reactions leading to degradation of the lactose molecule, which is rapidly converted into lactic acid by the action of lactic acid bacteria. The acid produced can be used as a substrate for microbial growth and lead to the production of various aromatic substances such as acetic acid, diacetyl, acetone, acetaldehyde, ethanol and ethyl (Kaminarides, Stamou, & Massouras, 2007; Voigt, Chevalier, Qian, & Kelly, 2010). Some of these compounds provide specific odor notes to the aroma profile of the final product, especially in fresh cheese (Rehn et al., 2011; Souza, Costa Júnior, Perrone, Stephani, & Almeida, 2014). Organic acid analysis is extensively used by scientists to study the type of fermentation occurred and assess the cheese maturation time. However, the production of these acids can occur in cheeses because of bacterial metabolism and the degradation of proteins, lipid hydrolysis and oxidation into low molecular weight acids (such as acetic acid), lactose hydrolysis into glucose and galactose and their subsequent degradation (Buffa, Guamis, Saldo, & Trujillo, 2004; Ong & Shah, 2009; Tofalo et al., 2015).

Despite of the relevant consequences of these biochemical reactions on cheese quality and the potential impact of probiotic bacteria on these phenomena, there is sparse information in the literature regarding the biochemical changes caused by the use of probiotic lactic acid bacteria in probiotic 'coalho' cheese. As a result, a more detailed study focused on the sugar production and analysis of fatty and organic acids profile is needed. Therefore, the aim of the present research was to evaluate the effectsof adding probiotic lactic acid bacteria (in isolated and combined forms) to goat 'coalho' cheese on the production ofsugars, organic acids, and the fatty acid profileover 28 days and compare these effects by that displayed by a mesophilic starter culture.

2. Material and methods

2.1. Experimental design

A 5 × 2, factorial, completely randomized design was used, generating five types of cheeses (control cheese inoculated with starter, and four probiotic cheeses inoculated with either *L. acidophilus, L. paracasei, B. lactis,* or a mixture of the three probiotic strains). The cheeses were analyzed in triplicate at two storage times (1 and 28 days). According to a previous study that evaluated microbiological, physicochemical and sensory parameters in 'coalho' cheese (Oliveira et al., 2012) and general industrial production practices, 28 days covers the period of time required for the full processing of this type of cheese.

2.2. Cultures and reagents

Five goat 'coalho' cheese formulations were processed in individual batches, prepared in triplicate, using lyophilized 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, as follows:"C"-*Lactococcus lactis* subsp. *lactis* and *Lactococcus 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"-a mixture of *L. acidophilus, L. paracasei*, and *B. lactis* at a ratio of 1:1:1, totalling 100 mg. The mesophilic starter culture was only added to the C formulation.

The reagents and chemical products used for the analyses namely, sulfuric acid, standard sugars and organic acids, methanol, chloroform, sodium sulfate, hexane, ethyl ether, and fatty acid methyl esters (FAMEs) standard mixture, were obtained from Sigma Aldrich (Chemie GmbH, Steinheim, Germany).

2.3. Cheese manufacture

Cheeses were manufactured in a laboratory scale following the procedure as proposed by Oliveira et al. (2012). After processing, the cheeses were then vacuum packaged and stored at 10 °C for 28 days.

2.4. Soluble extract

The sugar and organic acid profiles were determined using a soluble extract of the evaluated cheeses. These extracts were obtained using the method described by Zeppa, Conterno, and Gerbi (2001) and were collected after first adding 10 mL of Ultrapure water to 2 g of the 'coalho' cheese. Soon after, the sample was homogenizedwith an ULTRA-TURRAX tube disperser (IKA, Staufen, Germany) for 3 min at 18,000 rpm. Next, the sample was centrifuged at 2060g for 10 min, passed through filter paper, and then transferred to a syringe containing a filter with 0.45 μm diameter pores.

2.5. Sugar profile

The sugars were determined in the soluble extracts of 'coalho' goat cheese samples through High Performance Liquid Chromatography (HPLC) (Varian, Waters, California, USA) using an isocratic solvent system. The separation of sugars was performed in Agilent Hi-Plex Ca column (7.7 × 300 mm, 8 μm) at 85 °C, with ultra pure water as mobile phase, flow 0.6 mL/minute. They were injected 20 μL of the soluble extract and the total run time totaled 30 min. Detection was performed using refractive index detector (Varian, Waters, California, USA) and the data processed in GALAXIE Chromatography Data System software. The quantification of the sugars profile was obtained by injection of standard curve sugars and expressed as g/100 g of cheese.

2.6. Organic acid profile

The organic acids were determined in the soluble extracts of 'coalho' goat cheese samples through High Performance Liquid Chromatography (HPLC) (Varian, Waters, California, USA) coupled to a binary solvent system. The separation of organic acids was performed in Agilent Hi-Plex H column (7.7 × 300 mm, 8 μm) at 65 °C, with sulfuric acid (0.009 M) as mobile phase, flow 0.7 mL/minute. The detection of the separated organic acids was performed using a diode array detector (Varian 330, Waters, California, USA) with 220–275 nm wavelengths. The data processed in GALAXIE Chromatography Data System software and the quantification of the organic acids profile was obtained by injection of standard curve acids and expressed as g/100 g of cheese.

2.7. Fatty acid analysis

Lipid extraction was performed in accordance with the methodology described by Folch, Lees and Sloane Stanley (1957). The extraction was performed on 2 g of each cheese using cold solvents (chloroform: methanol 2: 1). After extraction, the fatty acids underwent transesterification using the methodology proposed by Hartman and Lago (1973). The transesterification was performed in 5 mL aliquot of the lipid extract using potassium hydroxide and then methanolic solution acidified with sulfuric acid. The esters were separated using a gas chromatograph (Varian GC-430, California, USA) coupled to a flame ionization detector and a 60 m × 0.25 mm x 0.25 μm fused-silica capillary column (Varian, CP