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Proteolysis in goat "coalho" cheese supplemented with probiotic lactic acid bacteria

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ABSTRACT

This study aimed to analyse the proteolytic effects of adding isolated and combined probiotic strains to goat "coalho" cheese. The cheeses were: QS – with culture Start, composed by *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R704); QLA – with *Lactobacillus acidophilus* (LA-5); QLP – with *Lactobacillus paracasei* subsp. *paracasei* (*L. casei* 01); QB – with *Bifidobacterium animalis* subsp. *lactis* (BB 12); and QC, co-culture with the three probiotic microorganisms. The cheeses were analysed during 28 days of storage at 10 °C. The probiotic cell count was higher than 6.5 and 7 log colony-forming units (CFU) g^{-1} of cheese at the 1st and 28th days of storage, respectively. The addition of co-culture influenced ($p < 0.01$) proteolysis in the cheese and resulted in a higher content of soluble protein and release of amino acids at the 1st day after processing. However, over all 28 days, the cheese supplemented with *Bifidobacterium lactis* in its isolated form showed the highest proteolytic activity, particularly in the hydrolysis of the alpha-s_2 and kappa-casein fractions.

1. Introduction

"Coalho" cheese is a highly appreciated product because of its sensory properties, including a slightly salty and acid flavour, mild aroma, and compact and soft texture. 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 with no access to industrial facilities for milk processing (Oliveira, Garcia, Queiroga, & Souza, 2012; Queiroga et al., 2013; Silva et al., 2012).

Goat milk presents some specificities related to its chemical properties, specially due to the characteristics of its proteins, which display reduced levels, or even a lack of, α_{s1}-casein, as well as structural differences in α-lactalbumin and beta (β) lactalbumin. These characteristics make it less allergenic when compared to bovine milk. Therefore, cheeses prepared using goat milk present a number of desirable properties to many consumers, especially those who are allergic to the type of protein present in bovine milk (Albenzio & Santillo, 2011).

Researchers have dedicated special attention to goat "coalho" cheese because it is considered a functional food, especially due to its peptide profile and antioxidant activity (Silva et al., 2012). Furthermore, cheeses such as "coalho" cheese, are products with peculiar characteristics which protect probiotic bacteria against oxygen, and also against low pH and bile salts, when going through the gastrointestinal tract. This group of characteristics, which also includes, amongst others, a pH close to neutral, a normally high level of water activity (which clearly depends on the amount of salt in the cheese and on the maturation conditions, in case the product is matured), a solid matrix (which facilitates the "insertion" of bacteria) and a relatively high fat concentration make these products more adequate as probiotic vehicles when compared to fermented milk and yoghurt (Bergamini, Hynes, Quiberoni, Sauárez, & Zalazar, 2005). In literature, some studies have already demonstrated the potential of "coalho" cheese as a carrier matrix for probiotic lactic bacteria, enabling the count of microorganisms such as *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei* and *Bifidobacterium animalis* subsp. *lactis* at the end of shelf life, in accordance with recommendations for this kind of product (at least 10^7 CFU) (Garcia, Oliveira, Queiroga, Machado, & Souza, 2012; Madureira et al., 2005; Oliveira et al., 2012; Santos et al., 2012). This quantity (10^7 CFU) is the minimum number of bacteria

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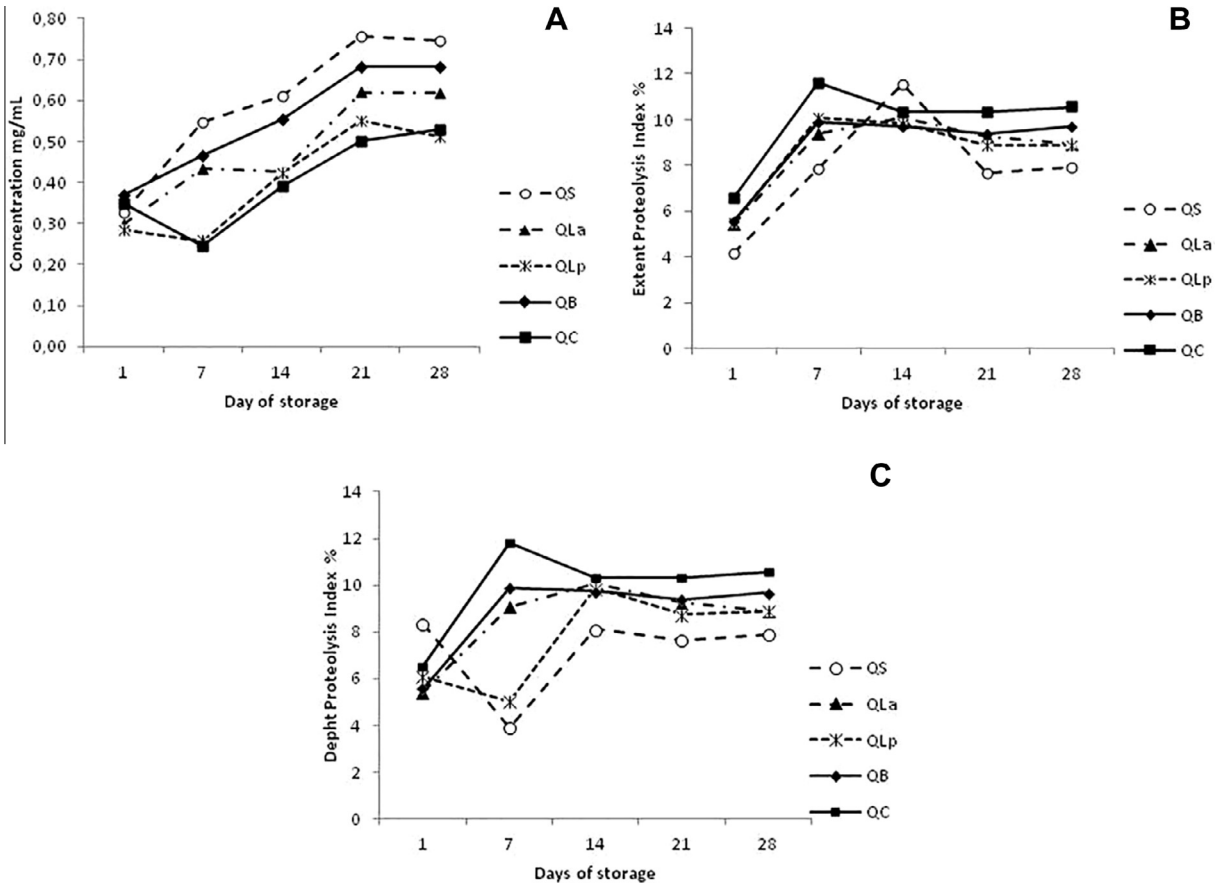

Fig. 1. (A) – Soluble protein (mg mL^{-1}); (B) – extent of proteolysis index (%); (C) – depth of proteolysis index (%) of goat "coalho" cheeses supplemented with probiotic lactic acid bacteria and the starter culture over 28 days of storage at 10 °C ± 2 °C. QS – with starter culture; QLA – with *Lactobacillus acidophilus*; QLP – with *Lactobacillus paracasei*; QB – with *Bifidobacterium lactis*; QC – with co-culture.

required at the moment of ingestion, in order to ensure a favourable impact on consumer health (De Vuyst, 2000; Talwalkar, Miller, Kailasapathy, & Nguyen, 2004).

The probiotic genera most frequently used in different traditional cheeses from Brazil, including "Minas" cheese, "Coalho" cheese, and cream cheese, are *Bifidobacterium* and *Lactobacillus* (Burns et al., 2012; Gomes et al., 2011; Rodrigues et al., 2011). The addition of the strains *L. acidophilus*, *L. paracasei* subsp. *paracasei* and *B. animalis* subsp. *lactis* in coalho" cheese, which already presents inherent advantages regarding the composition of its proteins, is an option in order to refine the final quality of the product, enhancing its technological, physicochemical and sensory profiles and thus making it more attractive to consumers (Escobar et al., 2012). In addition to these benefits to the nutritional matrix, probiotics also have beneficial effects on consumer health, when ingested in the correct amount. Amongst these benefits are an improvement in the immune system (Lollo et al., 2012) and the strengthening of intestinal immunity (Modzelewska-Kapituła, Kobukowski, & Kłebukowska, 2010).

The nutritional and sensory improvements in probiotic cheeses are related to the wide spectrum of enzymes that are contained in probiotics, catalyse biochemical reactions over the period of cheese storage, and lead to the production and release of different compounds that affect the quality of the final product, especially the texture and flavour (Albenzio et al., 2013; Randazzo, Pitino, Ribbera, & Caggia, 2010).

Such biochemical reactions include a set of protein-related events known as proteolysis. The proteolytic process involves the action of enzymes naturally found in the milk, coagulant agent and microbial enzymes produced by lactic acid bacteria intentionally added during cheese-making. Proteolysis involves the destabilisation of the casein micelle through the release of peptides and amino acids that undergo a catabolic process, thus forming other volatile compounds such as amines, acids (isobutyric, isovaleric and valeric), thiols, esters and others (Garcia et al., 2012; Steele, Broadbent, & Kok, 2013; Wolf, Perotti, Bernal, & Zalazar, 2010).

The potential of goat "coalho" cheese as a functional food, especially as a food matrix source of different probiotic bacteria (such as *L. acidophilus*, *L. paracasei*, and *Bifidobacterium lactis*), has been reported in literature (Oliveira et al., 2012; Santos et al., 2012; Silva et al., 2012).

However, extensive studies on the proteolytic changes caused by the activity of probiotic bacteria added during the processing and storage of goat "coalho" cheese (probiotic and conventional) have not been described. Thus, this study aimed to investigate the effects of proteolytic activity resulting from the addition of *L. acidophilus* (LA-5), *L. paracasei* subsp. *paracasei* (*L. casei* 01) and *B. animalis* subsp. *lactis* (BB 12), in isolated and combined form, to goat "coalho" cheese. The bacteria with probiotic effects were selected according to the optimal viability in the matrix according to literature (Oliveira et al., 2012; Santos et al., 2012).

2. Materials and methods

2.1. Cultures and reagents

Five goat "coalho" cheese formulations were processed in different batches in triplicate using lyophilised commercial cultures