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Galacto-oligosaccharides formation during manufacture of different varieties of yogurt. Stability through storage

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ABSTRACT

Galacto-oligosaccharides (GOS) have interest in the food industry due to their recognized functional properties. In this work, we studied the effect of a commercial β-galactosidase enzyme from *Kluyveromyces lactis* (YNL-2, GODO) and *Lactobacillus acidophilus* La-5, on GOS formation during the manufacture and storage of drinkable and stirred yogurts. In a preliminary step, GOS synthesis and lactose hydrolysis by β-galactosidase was evaluated at different initial lactose concentrations and doses of enzyme. The GOS formation was favored with increasing of lactose concentration and enzyme doses, while the hydrolysis dominated at lower level of lactose. In turn, the presence of GOS was already evident at 45 min of fermentation in yogurts with addition of β-galactosidase. Mean concentrations were 0.36 and 0.62 g/100 g for fresh drinkable and stirred yogurts, respectively. No changes in the GOS levels were observed through storage, indicating that they were stable in the products. The probiotic bacteria added were not able to produce GOS. The diminution of lactose was significant in yogurts with β-galactosidase; contents of residual lactose were around 1.3 g/100 g. We obtained different varieties of reduced-lactose yogurts enriched in galacto-oligosaccharides. The presence of probiotic and prebiotic would increase the functional properties of yogurts.

1. Introduction

Currently, galacto-oligosaccharides have attracted particular interest for research and applications in the field of food, due to their recognized functional properties. GOS are non-digestible and non-cariogenic carbohydrates that modulate the colonic microbiota, promoting the healthy balance (prebiotic effect), among other positive health effects (Caselato de Sousa, Freitas dos Santos, & Sgarbieri, 2011; Mussatto & Mancilha, 2007). These compounds are comprised of a variable number of galactose units and, in some cases, a terminal glucose unit, joined by glycosidic bonds. They are produced from lactose (or other galactoside) by enzymatic *via* with β-galactosidases. The first step involves the formation of the galactosyl–enzyme complex and release of the glucose unit. After that, two reactions can concomitantly occur, hydrolysis and transgalactosylation, depending on the galactosyl-moiety acceptor present in the reaction medium. When the acceptor is water, the hydrolysis takes place and lactose is split into glucose and galactose; while, when the acceptor is galactose (or potentially any sugar), the galactosyl transfer happens and a complex mixture of GOS is formed (Gosling, Stevens, Barber, Kentish, & Gras, 2010; Otieno, 2010). The predominance of the GOS synthesis over the hydrolysis, and the yield and composition of the GOS mixture obtained are significantly affected by the origin of β-galactosidase enzyme and the operating conditions (lactose concentration, dose of enzyme, temperature/time and pH) (Boon, Janssen, & van't Riet, 2000; Gosling et al., 2010).

GOS are used as functional food ingredients, alone or with fructo-oligosaccharides or inulin, into infant formulas to mimic the beneficial effects of human milk oligosaccharides (Bode, 2009). Other processed foods that are important for the inclusion of GOS are beverages, bakery and dairy products because their functional and technological aspects (high solubility, clean taste, stability, low glycemic index) (Torres, Gonçalves, Teixeira, & Rodrigues, 2010). However, GOS can also be formed *in situ* during the manufacture of fermented dairy foods as a result of the metabolic activity of strains (Gosling et al., 2010). The formation of oligosaccharides in yogurts prepared by using yogurt cultures combined with bifidobacteria strains has been reported (Lamoureux, Roy, & Gauthier, 2002). In turn, Martínez-Villaluenga, Cardelle-Cobas, Corzo, and Olano

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(2008) tested the GOS contents in commercial products: traditional yogurts, yogurts containing bifidobacteria and ready-to-drink yogurts with *Lactobacillus casei*. In both studies, it was found a wide variation among samples analyzed; probiotic yogurts showed higher amount of GOS compared to traditional ones. The stability of GOS in the dairy matrix is an important aspect to be considered. Mozaffar, Nakanishi, and Matsuno (1985) detected a disappearance almost complete of GOS at the latter stage of milk incubation with a commercial β-galactosidase enzyme. However, Lamoureux et al. (2002), Martínez-Villaluenga Cardelle-Cobas, Corzo, Olano, and Villamiel (2008) and Yadav, Jain, and Sinhá (2007) indicated that no hydrolysis of GOS occurred through storage. Hence, the results reveal that the amount of GOS produced depends on the strains and the processing parameters used in the preparation of different varieties of fermented milks.

On the other hand, the direct addition of β-galactosidase enzyme in the production of reduced-lactose products could lead to simultaneous production of GOS. Delactozed dairy foods are destined for individuals who are affected by lactose intolerance, because they are deficient of the lactase enzyme in the digestive tract needed to properly absorb the lactose. The problem of lactose intolerance is well-known and widespread in more than half of the Latin American population (Ruiz-Matute et al., 2012). Some studies evaluate different conditions in order to obtain low-lactose milks containing GOS (Chen, Hsu, & Chiang, 2002; Mahoney, 1998; Ruiz-Matute et al., 2012). However, according to our knowledge, there are scarce data about this topic in fermented milks. The yogurt market in Argentine has experienced steady growth in recent years and different varieties of products have been launched; nevertheless, reduced-lactose yogurts with increasing amounts of GOS are yet absent.

The aim of this work was to study the effect of the inclusion of commercial β-galactosidase from *Kluyveromyces lactis* and the probiotic bacteria *Lactobacillus acidophilus* La-5 on the GOS formation during the manufacture and storage of drinkable and stirred yogurts. In a preliminary step, GOS synthesis and lactose hydrolysis by the β-galactosidase enzyme was evaluated at different initial lactose concentrations and doses of enzyme.

2. Materials and methods

2.1. Enzymatic hydrolysis/transgalactosylation from lactose in buffer

Enzymatic hydrolysis and synthesis of GOS from lactose solution was studied at three different concentrations of initial lactose and three different doses of enzyme at laboratory trials. A commercial food grade β-galactosidase enzyme derived from *K. lactis*, YNL-2 GODO (50000 U ONPG/g) produced by Shusei Company Limited (Tokyo, Japan) and kindly donated by Milkaut S.A. (Santa Fe, Argentine), was employed. These preliminary experiences were performed to know the ability of this enzyme for GOS production, in order to apply it for the obtaining of different varieties of yogurts enriched in GOS.

Lactose monohydrate (Sigma–Aldrich, Saint Louise, USA) solutions (100 mL) of 5, 10 and 20 g/100 mL were prepared in 100 mmol/L potassium phosphate buffer (pH 6.8) (Sigma–Aldrich, Saint Louise, USA) containing 1 mmol/L $MgCl_2$ (Sigma–Aldrich, Saint Louise, USA). The enzyme was added at different doses, 0.16, 0.25 and 0.40 g/L (equivalent to 8,000, 12,500 and 20,000 units, respectively), and the reaction mixtures were incubated in a water bath at 42 ± 1 °C for 3 h. At different times (40, 60, 100, 140 and 180 min), aliquots (4 mL) were withdrawn and immediately immersed in a boiled water bath for 8 min to deactivate the enzyme. The samples were stored at −18 °C for carbohydrates analysis. The incubation experiences were carried out in duplicate.

The amounts of remaining lactose, and the amount of GOS, glucose and galactose produced were expressed as percentage by weight of the total carbohydrates content in the reaction mixtures.

2.2. Yogurt manufacture

Two varieties of sweetened yogurts, drinkable and stirred were made at laboratory scale; stainless steel vats of 5 L of capacity each were employed (Vénica, Perotti, & Bergamini, 2014).

The results obtained in preliminary experiences were taken into account to select the doses of enzyme for the production of yogurts with high levels of GOS. Therefore, for drinkable yogurts, whose milk base had approximately 5 g/100 mL of lactose, the lower dose of enzyme was used, while for the stirred yogurts, with levels of initial lactose around 7 g/100 mL, the intermediate level of enzyme was chosen.

A factorial design was used for each variety of yogurt. Two factors were studied, the addition of β-galactosidase enzyme, and the incorporation of *L. acidophilus* La-5 (Chr Hansen, Horsholm, Denmark) and inulin (Orafti®GR, Mannheim, Germany), at two levels each, with and without addition. Thus, four different types of yogurt were manufactured: unhydrolyzed (**C**); unhydrolyzed symbiotic (with probiotic and prebiotic) (**P**); hydrolyzed (**E**) and hydrolyzed symbiotic (**EP**). These yogurts were performed in triplicate resulting in a total of 12 experimental units for drinkable and stirred yogurts, respectively.

Bulk bovine milk 3 g/100 mL fat content (Milkaut S.A., Santa Fe, Argentine) with addition of 8 g/100 mL sucrose (Ingenio Ledesma S.A., Tucumán, Argentine) was tempered until it reached approximately 40 °C. At this moment, 2.25 g/100 mL skim milk powder (SMP) and 2.00 g/100 mL whey protein concentrate (WPC35) (Milkaut S.A., Santa Fe, Argentine), were added for stirred yogurts. In symbiotic yogurts, 1.00 g/100 mL inulin was also aggregated. The ingredients were dissolved by manual agitation for 15 min. Milk bases were heated at 90 ± 2 °C, stand for 5 min, immediately cooled to 42 ± 2 °C, and inoculated with freeze-dried direct vat set (DVS) YF-L811 (Chr. Hansen, Buenos Aires, Argentine) containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. β-galactosidase enzyme (0.16 and 0.25 g/L, for drinkable and stirred yogurts, respectively) was added together with the starter culture for hydrolyzed yogurts (**E** and **EP**). The incubation process was conducted at 42 ± 2 °C until pH 4.70 ± 0.10 was reached. At this point, freeze-dried DVS culture of *L. acidophilus* La-5 was added in order to give initial cell count of 10^7 CFU/g in symbiotic yogurts (**P** and **EP**). The yogurts were immediately cooled to 25 °C in an ice water bath, applying intermittent manual agitation, followed by placing in screw cap glass flasks (500 mL). Finally, the yogurts were stored at 5 ± 1 °C for 21 days.

Aliquots were removed at different times during fermentation and in freshly made yogurts to measure pH, concentration of GOS and lactose. In addition, throughout the entire refrigerated storage period, pH, titratable acidity, and concentrations of lactose, GOS and lactic acid were determined. Overall composition (total solids, protein and fat) and microbiological counts were also evaluated.

2.3. Carbohydrates and lactic acid analysis by HPLC

HPLC equipment for the analysis of carbohydrates and lactic acid consisted of a quaternary pump, an on-line degasser, UV–visible detector (Series 200), a refractive index detector and a column oven (Series Flexar) (Perkin Elmer, Norwalk, USA). Data were collected and processed on a computer with the software Chromera® (Perkin Elmer, Norwalk, USA).