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Research Article

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Effect of Lactose Hydrolysis during Manufacture and Storage of Drinkable Yogurt

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Abstract

Reduced-lactose yogurts are still not available in the Argentinean market despite the fact in Latin America the incidence of deficient-lactose individual is very high. In this work, variables to hydrolyze the lactose (three levels of β-galactosidase enzyme and the time of its addition) were evaluated during manufacture of natural and sweetened (8% p/v) drinkable yogurts. Experimental yogurts (with enzyme) were compared with controls (without enzyme). The evolution of lactose content, pH, titratable acidity and syneresis were analyzed during 28 days of storage at 5ºC. Results showed feasibility in the simultaneous addition of enzyme and starter. The hydrolysis percentages in experimental yogurts ranged from 74 to 93% at the end of manufacture and stayed without changes during storage. For control yogurts, the lactose content decreased approximately 15%, which was doubled at 14 days. The inclusion of sucrose did not substantially affect the hydrolysis process. After 14 days, the pH and acidity in experimental yogurts were slightly higher and lower, respectively, in comparison to controls. On the other hand, the sensory panel found that the reduced-lactose and traditional yogurt where similar.

Keywords

Lactose intolerance; Enzymes; Yogurt; Lactose

Introduction

Lactose is a unique disaccharide in mammal's milk, which is about 4.5 to 5% in cow's milk [1]. In the digestion process, lactose is largely broken down into its monosaccharides, galactose and glucose, by the lactase enzyme (β-galactosidase, EC 3.2.1.23.), which is bound to the mucosal membrane of the small intestine. The lack of lactase activity observed in some populations can lead to gastrointestinal disorders known as lactose intolerance. The symptoms of this problem are bloating, diarrhoea, flatulence, abdominal pain and cramps, loss of appetite, nausea, etc. [2], and a loss of calcium and other minerals can also occur [3]. The problem of lactose intolerance is quite widespread in most part of the world. It has been estimated that over 70% of the world population suffers from the inability to use lactose [4,5].

The consumption of lactose-modified dairy products constitutes an attractive way to get nutritional rich milk with a lower level of lactose than the regular dairy foods [1,6]. In the last years, an increasing interest in the development of reduced-lactose or lactose-free products has emerged from dairy companies, due to the knowledge acquired in relation to the lactose intolerance problem [5,7]. Various technologies are employed for their production, such as enzymatic hydrolysis of lactose with β-galactosidase (soluble or immobilized) and physical separation processes (ultrafiltration and chromatography) [7-11].

In Latin America, which records a very high incidence of deficient-lactase individuals, several reduced-lactose products such as fluid and powder milks and some fermented milks, are available in the market. Nevertheless, this type of products is very limited in Argentine. Only one dairy company offers reduced-lactose UHT and powder milks. Therefore, in Argentina where the dairy products consumption is high there are still large possibilities of developed this area.

Yogurt is made by the symbiotic action of the bacteria *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, which must be active and at significative level in the product (higher than 10^7 cfu g^{-1}) [12]. It has been recognized as an excellent source of vitamins (B1, B2, B9 and B12), minerals (calcium, magnesium, phosphorus and zinc) and high-quality protein [13,14]. In addition, different types of yogurts with the addition of probiotics and prebiotics have been developed in order to increase their functional characteristics [15,16]. Several studies have shown that lactose digestion improves with yogurt ingestion compared to milk ingestion. Although the mechanisms involved are not yet clear, it is believed that this greater absorption is due in part to the β-galactosidase activity of starter culture [3,17]. However, some authors suggested that yogurt and fermented milks have a lactose content still too high (only about 20% of lactose is fermented) for consumption by individuals with an elevated degree of intolerance [5,18,19].

The procedure of lactose hydrolysis in milk with β-galactosidase to prepare yogurt could be carried out before (at different conditions of time/temperature) or during fermentation process [20,21]. In this regard, there are few publications about different aspect of the lactose-hydrolyzed yogurts. Variable results have been obtained regarding the lactose concentration and the physical and organoleptic characteristics of the final product. This fact depends on the type and level of the enzyme employed the moment of its addition (before fermentation or together with the starter) and the storage condition of yogurt [22-25].

In the last years, the yogurt consumption has undergone a notable increase in Argentine, rising from 6.6 Kg per person in 2000 to 11.9 Kg in 2010 [26]. Different yogurt varieties are available; however, reduced-lactose yogurts are still not present in the market.

In this context, we purpose to study different variables of lactose hydrolysis with a soluble β-galactosidase from *Kluyveromyces lactis* for yogurt making, and to analyze the evolution of lactose content, pH, titratable acidity and syneresis during storage. Besides, a sensory preference test was performed in order to compare the reduced lactose yogurt with the traditional one.

Materials and Methods

Raw bulk bovine milk (30 L), pH 6.70 ± 0.05, titratable acidity 18 ± 1ºD, lactose concentration 48.0 ± 0.5 g kg^{-1} was supplied by a nearby

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dairy plant, located in the main milk production area of Argentina (Milkaut SA, Santa Fe, Argentina).

Preliminary experiments at small scale were made in order to study the process of lactose enzymatic hydrolysis in milk, with or without the addition of starter culture. The most favourable conditions were applied for the preparation of yogurt at larger scale.

A soluble commercial preparation of β-galactosidase enzyme YNL-2 (from *Kluyveromyces lactis*) with a declared activity of 50000 GU g^{-1} (GODO Shusei Company Limited, Tokyo, Japan) was employed. The optimum conditions of enzyme activity are 40-45°C and pH 6-8 (as per supplier's product sheet).

Preliminary experiments

Assays of lactose hydrolysis with β-galactosidase enzyme (levels: 0.15, 0.25 and 0.40 g L^{-1}) were performed on milk (pH=6.7-6.8) at 42 ± 2°C during 4 h, with and without the incorporation of DVS lyophilized yogurt starter culture composed by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Chr. Hansen, Buenos Aires, Argentina) in the dosage suggested by the supplier. In the experiments in which the starter was included, it was added to milk simultaneously with the enzyme (at the three level of enzyme assayed) and after a pre-incubation period of 30 min for the intermediate level of enzyme (0.25 g L^{-1}). Controls without enzyme addition were made.

For this purpose, the enzyme and starter were added to 250 mL in the conditions previously mentioned, and incubated in a water bath at 42 ± 2°C for 4 h. The lactose concentration was measured at different times (0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min in the case where the starter was absent and 0, 30, 45, 60, 90, 120, 180, 210 and 240 min in the case where the starter was included) and the percentage of hydrolysis was calculated. All experiments were performed by duplicate.

Yogurt manufacture

Drinkable type yogurts with or without sucrose (S) in the formulation were made applying the traditional method adapted to laboratory scale [21]. For this purpose, five vats (5L capacity), were operated simultaneously. In experimental yogurts (E and ES) β-galactosidase enzyme was added at three levels, which were compared with control yogurts (C and CS) without enzyme addition (Table 1). All treatments were performed in triplicate resulting in 24 experimental units (yogurts).

Milk was standardized to 3% w/v fat content and sucrose 8% w/v (Ingenio Ledesma SA, Tucumán, Argentina) was added in sweetened yogurts. The milk base was heated to 90 ± 2°C for 5 min, cooled to 42 ± 2°C and inoculated with yogurt starter culture. At this moment, the β-galactosidase enzyme was added in the experimental yogurts. The incubation process was carried out at 42 ± 2°C until the pH reached a value of 4.70 ± 0.10, which was achieved in about 3.75 to 4.15 hours. The yogurts were immediately cooled in an ice water bath to 25°C.

Table 1: Experimental design of yogurt manufacturing.

Drinkable type yogurt		Level of enzyme (g.L^{-1} of milk)
Sweetened	Natural	
CS	C	0
ES1	E1	0.15
ES2	E2	0.25
ES3	E3	0.40

C: control yogurt; E: experimental yogurt; S: sucrose.

The samples were transferred to glass containers with screw cap (3 L) and stored at 5 ± 1°C for 28 days. At each sampling time, approx. 100 mL of sample was homogenized and small aliquots were weighed according to each analysis.

Carbohydrates analysis by HPLC

The lactose concentration was determined in samples during manufacture at 0, 45, 150 min and at the end of the fermentation process. In addition, lactose, glucose and galactose were determined during storage at 14 and 28 days. Regarding glucose and galactose, they were only quantified in the natural yogurts, because the presence of sucrose in the formulation of sweetened yogurts did not allow this quantification. In the chromatographic conditions used: acid medium and high temperature, the sucrose undergoes the reaction of inversion and the two constituent monosaccharides are formed: fructose and glucose. The peak of the fructose is overlapping with the galactose, and the peak of the glucose is the result of the sum of the glucose produced during manufacture of yogurts and those providing from the inversion of sucrose during the chromatographic analysis.

The analysis of these compounds was performed by HPLC method according to Zeppa et al. [27], with some modifications. Chromatographic separation was performed isocratically at 65°C with a mobile phase of 0.01 mol L^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1} on an Aminex HPX-87H column (300×7.8 mm) equipped with a cation H^+ microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). HPLC equipment consisted of a quaternary pump, an on-line degasser (Series 200) and a refractive index detector (Series Flexar) (Perkin Elmer, Norwalk, CT, USA). Data were collected and processed on a computer with the software Chromera® (Perkin Elmer).

For the sample preparation, 5 g of milk or yogurt were dispersed in 0.01 mol L^{-1} H_2SO_4 and adjusted to a final volume of 50 mL. The suspension was homogenized and centrifuged at 15000 g/20 min/4°C. The supernatant was filtered through 0.45 µm membranes (Millex, Millipore, São Paulo, Brazil) and injected into the chromatograph, using a loop of 60 µL. Quantification was based on the external method using lactose, glucose and galactose (Sigma Aldrich, USA) as standard to obtain the calibration curve. Analyses were performed in duplicate.

pH and Titratable acidity

pH was measured on freshly made yogurts and at 7, 14, 21 and 28 days of storage, using a digital pHmeter (Orion 3 star benchtop, Thermo Fisher Scientific Inc., USA).

Titratable acidity (TA) was determined in yogurts at 1, 7, 14, 21 and 28 days of storage, with 0.1 N NaOH and detecting the end-point of the titration at pH 8.3 with the pH meter [28]. Results were expressed as Dornic degree (1°D=100 mg lactic acid L^{-1}).

Analyses were performed in duplicate.

Syneresis

Syneresis was measured by the procedure described by Dello Staffolo et al. [29] which is based on spontaneous release of whey under the force of gravity from undisturbed yogurt samples. The yogurt samples were placed in a 100 mL glass graduated cylinder, maintained at 5°C and the whey level expelled was measured at 0, 7, 14, 21 and 28 days. The percentage of syneresis was expressed as millilitres of whey per 100 mL of yogurt. Analyses were performed in duplicate.

Sensory evaluation

For sensory evaluation, the products tested were manufactured at industrial scale of 4000 L in Milkaut S.A.; the operating procedures cannot be described due to confidentiality reasons. The reduced-lactose yogurt was made employing the intermediate level of enzyme (0.25 gL^{-1}) and 8% w/v of sucrose, and it was compared with the traditional yogurt made with 10% w/v of sucrose (level usually employed). The sucrose concentrations were chosen in order to achieve similar sweetness in the products, as the hydrolyzed and traditional yogurts have different proportions of lactose, glucose and galactose which differ in their sweetness. In effect, the relative sweetness of lactose, glucose and galactose is 0.4, 0.6 and 0.7, respectively, compared to that of sucrose, which is equal to 1 [21].

The yogurts were assessed for overall preference by the panelists at 7 days of storage, employing the paired preference test [30]. Panelists were also asked about the sweetness of the products. The preference test was conducted in the dairy industry with a total of 30 panelists (10 female and 20 male, that have between 30 and 50 years old), who were trained in sensory analysis of yogurt.

Data analysis

The SPSS software (version 10.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. One-way ANOVA was carried out on data of pH, titratable acidity, syneresis, glucose, galactose and lactose percentage hydrolysis, using a general linear model procedure with least significant difference pairwise comparison at 95% confidence level.

Results and Discussion

Preliminary experiments

Results of lactose hydrolysis percentages (%) obtained in the assays without starter addition during 4 h of incubation are showed in Figure 1A. As expected, the percentages increased with the increasing of β-galactosidase enzyme levels, the values were above of 80% at the end of incubation period for the three levels of enzyme tested, reaching this value at approx. 90 min for the highest level.

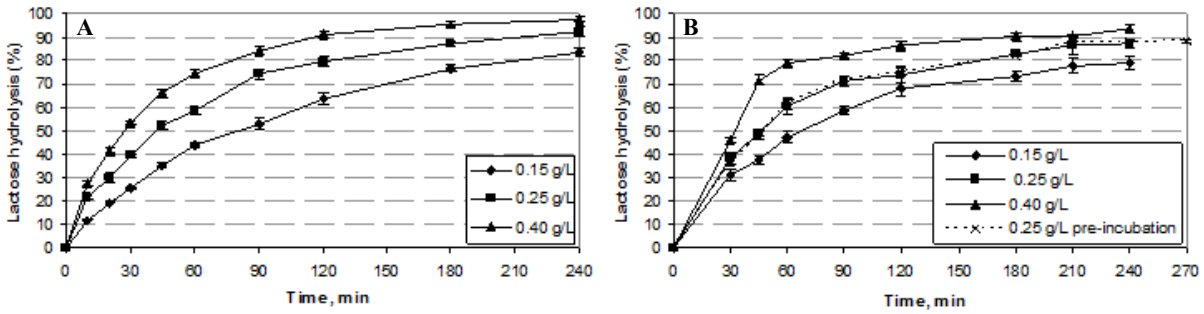
Figure 1B shows the lactose hydrolysis (%) obtained in the assays in which the starter was included. The values were slightly lower than those in which the starter was absent (Figure 1A); probably the acidification produced by the starter culture gradually reduces the β-galactosidase activity, achieving its total inactivation at below of pH 5.0 (as per supplier's product sheet).

In addition, the percentage values found in the assays including a pre-incubation step were similar (about of 90% at 4 h) to those obtained when the enzyme and starter were added together.

From an industrial viewpoint, the manufacture of reduced lactose yogurt in which the hydrolysis step is developed at the same time that the fermentation, is more advantaged due to the usual production time of yogurt is not increased. On other hand, the use of any of the three levels of enzymes tested are suitable to make reduced lactose yogurt according to the Argentinean Legislation, which establishes that this type of product must have at least 70% of lactose hydrolysis [31]. Thus, in the following experiences, we added the enzyme at the three levels together with the yogurt starter.

Lactose hydrolysis percentage during manufacture and storage of yogurts

Results of lactose hydrolysis percentages for natural and sweetened yogurts (experimental and control) during manufacture and storage are showed in Figure 2A and Figure 2B, respectively. In experimental yogurts, the values increased as the process was carried out and with the level of enzyme. As can be seen, the values at 45 min of incubation were higher than 40% in all cases. The greatest percentages were reached approximately at 150 min, which were maintained without significant changes ($P>0.05$) until the end of the manufacture. At this point, the percentages in natural yogurts (E1, E2 and E3) ranged from 80 to 93%, being the values for E3 significantly higher ($P<0.05$) than those obtained for E1. In sweetened yogurts (ES1, ES2 and ES3), the percentages ranged from 74 to 91%; the values for ES3 were significantly higher ($P<0.05$) than those obtained for ES1. During storage for 28 days at 5°C, no significant changes ($P>0.05$) were observed for each type of yogurt. These results are probably due to the inactivation of enzyme at the yogurt pH value. However, the significant differences between E1 and E3, and ES1 and ES3 were remained. The residual lactose content in experimental yogurts ranged from 4 to 12 g kg^{-1}. In



A: without addition of yogurt starter
B: with addition of yogurt starter together with the enzyme and after 30 min of pre-incubation with the enzyme.

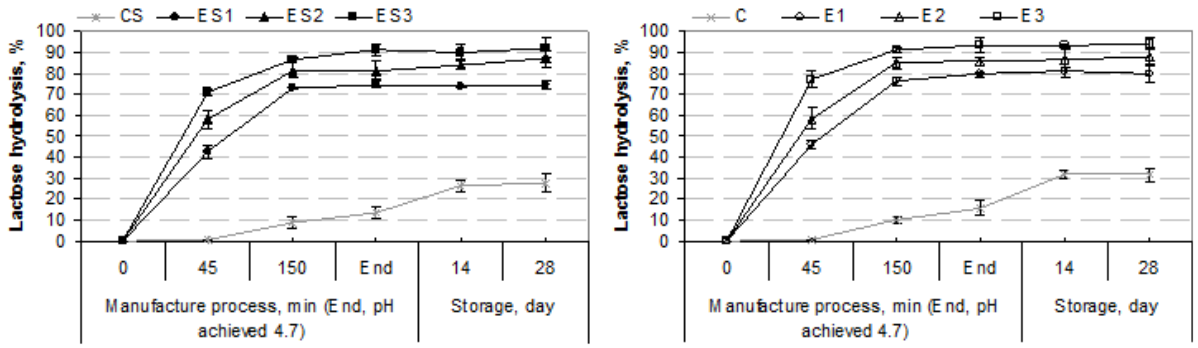
Figure 1: Percentages of lactose hydrolysis of milk with ß-galactosidase enzyme at three levels at 42°C: (A) without addition of yogurt starter (B) with addition of yogurt starter together with the enzyme and after 30 min of pre-incubation with the enzyme.

all cases, the results obtained were within the parameters established by the Argentinean Legislation for reduced lactose products [31].

In control yogurts, the percentage values at the end of yogurt manufacturing were 16 and 13% for natural and sweetened respectively

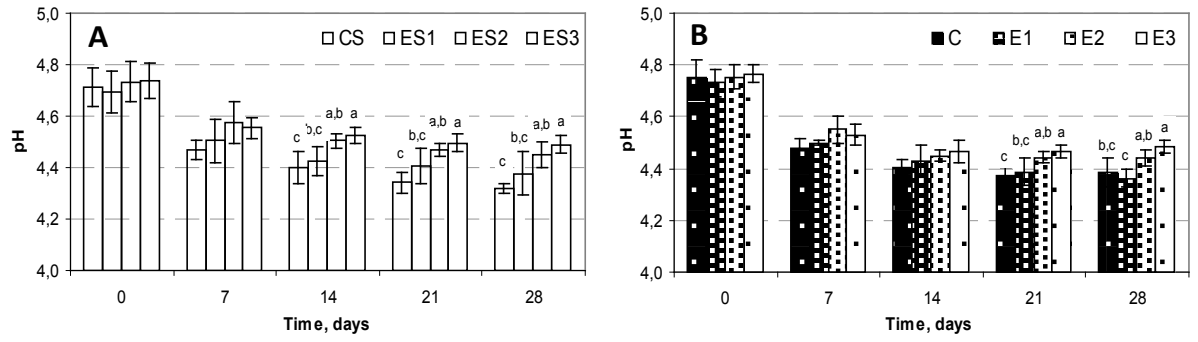
(C and CS), which were significantly lower ($P<0.05$) than those found for all experimental yogurts (E and ES). As well known, the decrease in the lactose content was produced only by the activity of starter. At 14 days of storage, the percentages were approximately the double that those obtained in freshly made yogurts. After that, the values remained almost constant up to 28 days. This behaviour observed in control yogurts during storage was different to experimental ones. The incorporation of the β-galactosidase enzyme, that splits the lactose into glucose and galactose, produced modifications in the carbohydrate profile, which could affect the metabolic activities of starter. In effect, control yogurts had higher level of lactose but lower levels of glucose and galactose than the experimental yogurts. The values of glucose and galactose in control yogurts at 14 days of storage ranged from 1 to 2 g kg^{-1} and 7 to 9 g kg^{-1}, respectively, while in experimental yogurts the values ranged from 15 to 17 g kg^{-1} and 19 to 21 g kg^{-1}, respectively. These values remained almost constant until the end of storage time (28 days). Several works reported that the effect of various carbohydrates (glucose, lactose, galactose, sucrose) on the viability of microorganisms is strain-dependant [32-35]. O´Leary and Woychik [36] and Amoroso et al. [37] found significant differences in the viability of pure and mixed cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* in a medium with different carbohydrates (glucose, galactose, lactose and sucrose).

As mentioned, the information available on reduced-lactose yogurts obtained by enzymatic hydrolysis is scarce. Indeed, Ismail et al. [22] prepared yogurt adding the enzyme (Maxilact and Lactozym) in different levels together with starter. They obtained a concentration of lactose of 19 – 29 g L^{-1} in freshly made products, which decreased to 13 – 24 g L^{-1} at 6 days. Also, employing similar manufacturing conditions, Toba et al. [23] made yogurts adding β-galactosidase from *Aspergillus oryzae*. The lactose content at the end of manufacture (approx. 8 h) ranged from 0.2 to 2.5% for the highest and lowest level of enzyme employed, respectively. However, contrary to our results, the lactose hydrolysis continued throughout storage, reaching lactose levels of 0.1 to 0.7%. This fact was due to the enzyme used by Toba et al. [23] maintain its activity at pH value of yogurt. Recently, Martins et al. [25] obtained a lactose reduction ranged from 97 to 99% employing the commercial enzyme Lactomax



C: control yogurt; E: experimental yogurt; S: sucrose.1, 2, 3: 0.15; 0.25; 0.4 g L^{-1} of enzyme, respectively.

Figure 2: Percentages of lactose hydrolysis during manufacture and over 28 days of storage at 5ºC in sweetened (A) and natural yogurts (B).



C: control yogurt; E: experimental yogurt; S: sucrose.1, 2, 3: 0.15; 0.25; 0.4 g L^{-1} of enzyme, respectively.

Figure 3: Evolution of pH over 28 days of storage at 5ºC in sweetened (A) and natural yogurts (B).

Flex composed of β-galactosidases produced by *Kluyveromyces lactis* and *Aspergillus niger*. They used elevated levels of enzyme higher than 0.5 g L^{-1}. Likewise, Cruz et al. [38] detected lactose values higher than 1% for probiotic yogurts. Similar data were found by Ibarra et al. [39], who included a pre-incubation step with the enzyme for 2.3 h to obtain a probiotic reduced-lactose yogurt. On the other hand, the lactose concentration in traditional yogurts is very variable. Martins et al. [25] reported values from 4.73 to 7.84%. Meanwhile, Batista et al. [19], who analyzed the lactose content of 110 yogurts belonging to 22 commercial brands, found values from 3.01 to 4.95%.

pH and Titratable acidity during storage

The evolution of pH and titratable acidity of yogurts during 28 days of storage are shown in Figures 3 and 4, respectively. The most important changes in pH and acidity were produced until 14 days, and then remained almost constant towards the end of the period.

Significant differences in pH values between control and experimental yogurts were only found after 14 days for sweetened yogurts and after 21 days for the natural ones. The values for control (C and CS) and experimental yogurts with the lowest level of enzyme (E1 and ES1) were significantly lower (P<0.05) than those obtained with the highest level of enzyme (E3 and ES3). Similarly, Toba et al. [23] reported slight decrease in post-acidification for experimental yogurts in comparison to controls during storage depending on the enzyme employed. On the other hand, Rodriguez et al. [40] prepared reduced-lactose yogurts with goat milk, and they did not found significantly differences in pH between control and experimental freshly yogurts.

The decrease in pH during storage (post-acidification) ranged from 0.25 to 0.39 units (Figure 3A) and 0.28 to 0.37 units (Figure 3B) for sweetened and natural yogurts, respectively. The post-acidification values were similar to those reported by other authors. Indeed, Birollo et al. [41] found a post-acidification of approx. 0.3 units of pH for regular drinkable yogurts stored for 60 days. Higher values, up to 0.6 units of pH were obtained by Cruz et al. [42] and up to 0.48 by Cruz et al. [43], in probiotic yogurts stored for 28 days. Meanwhile, Ismail et al. [22], Toba et al. [23] and Cruz et al. [38] found post-acidification values of 0.1, 0.3 (at 10 days) and 0.5 (at 15 days) units of pH for hydrolyzed yogurts, respectively.

As it was expected, the increase in the titratable acidity for all samples followed an opposite behaviour to pH. The values were in the range of 61 to 68 ºD (Figure 4A) and 65 to 75 ºD (Figure 4B) for sweetened and natural yogurts, respectively. These values were in accordance with those established by Argentinean Legislation [12].

The yogurts with addition of sucrose had less development of acidity compared to natural yogurts. This result was similar to that found by Slocum et al. [32] who reported that the acidity values in sweetened yogurts were significantly lower than in natural products. The authors suggested that the presence of sucrose could inhibit the bacterial growth probably due to the osmotic pressure [21,33].

The acidity values for C yogurts were significantly higher (P<0.05) than those obtained for E2 and E3 yogurts after 21 days of storage, and CS was significantly higher (P<0.05) than ES3, after 14 days.

Syneresis during storage

The syneresis percentages gradually increased over storage in all products and fluctuated within the range of 0 – 8.2% and 0 – 10.8% from day 0 to 28, for sweetened and natural yogurts, respectively. The values at 28 days are shown in Table 2. As can be seen, the sweetened yogurts had a lower syneresis than those prepared without sucrose, which could be due to the increase in the total solids [44]. The values obtained in this study for natural control yogurts were similar to those reported by Fly et al. [45]. On the other hand, no significant differences (P>0.05) were found between experimental and control yogurts (E and C; ES and CS, respectively), for each time of storage.

To the best of our knowledge, there are few works that summarize results about syneresis values in hydrolyzed yogurts during storage. Nagaraj et al. [24] reported increased syneresis in freshly yogurt with 90% of lactose hydrolysis compared with unhydrolyzed yogurts. The authors attributed these results to the higher amount of more soluble sugar in the mixture, which imparts a softer body and creamier texture.

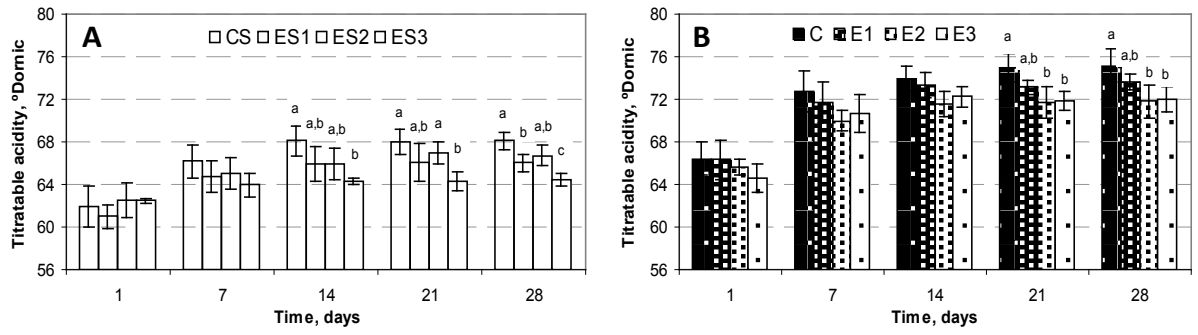
Sensory evaluation

Pursuant to the sensorial preference test, the reduced-lactose yogurt was preferred by 50% of panelists in comparison to traditional yogurt, indicating that the reduced-lactose yogurt did not differ from the traditional one. These results are very important because the consumer perception is a relevant parameter in order to have a chance of success on a competitive market [16].

In addition, 53% of panelists found that the reduced-lactose yogurt was sweeter than the traditional; even when the sucrose content was 2% lower. These results are due to the higher levels of glucose and galactose present in the reduced-lactose yogurt, as mentioned above. Thus, it was possible to obtain a reduced-lactose yogurt with similar sweetness to traditional product.

Conclusions

The lactose intolerance is a problem with high impact in the Latin America population. In the argentinean market, dairy products



C: control yogurt; E: experimental yogurt; S: sucrose.1, 2, 3: 0.15; 0.25; 0.4 g L^{-1} of enzyme, respectively.

Figure 4: Evolution of titratable acidity (ºD) over 28 d of storage at 5ºC in sweetened (A) and natural yogurts (B).

Table 2: Syneresis percentage in sweetened and natural yogurts at 28 days of storage at 5ºC.

Sweetened yogurt	Whey released (%)	Natural yogurt	Whey released (%)
CS	7.0 ± 0.5	C	10.0 ± 2.0
ES1	6.7 ± 0.6	E1	9.3 ± 1.3
ES2	7.3 ± 0.8	E2	9.8 ± 1.9
ES3	8.2 ± 1.0	E3	10.8 ± 1.3

C: control yogurt; E: experimental yogurt; S: sucrose.
1, 2, 3: 0.15; 0.25; 0.4 g.L^{-1} of enzyme, respectively.

modified in their lactose content are very scarce; in particular, low- or reduced-lactose fermented milks are absent. For this reason, there are still large possibilities for growth in this area, with the aim to provide this nutritive food to persons that suffer of lactase deficiency.

In the present work, the conditions of lactose hydrolysis with a soluble β-galactosidase enzyme for the preparation of reduced-lactose yogurt were studied. The addition of enzyme (at three levels) together with the yogurt starter culture demonstrated to be a satisfactory strategy. This is an important aspect from the industrial viewpoint since it would not increase the usual production time of yogurt.

The lactose hydrolysis percentages were above 80%, depending on the amount of β-galactosidase added. The residual levels of lactose in the final product ranged from 4 to 12 g kg^{-1}, which are in agreement with the Argentinean Legislation for modified foods in their carbohydrate composition. However, from an economic viewpoint, it is more convenient to use the lowest enzyme concentration assayed. In addition, the pH, titratable acidity and syneresis of products during storage at refrigeration temperature were adequate.

In terms of sensorial preference and sweetness, no differences were noted between reduced-lactose and traditional yogurts containing different levels of sucrose, manufactured in a local dairy industry.

Further research is ongoing to study the effect of other ingredients and probiotics, usually employed in the yogurt formulations (such as dairy powders, starch, prebiotics, etc.), on lactose hydrolysis and yogurt quality. Likewise, the formation of galacto-oligosacharides during yogurt making, by the transgalactosidase activity of the β-galactosidase enzyme, is being evaluated.

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