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Influence of lactose hydrolysis on galacto-oligosaccharides, lactose, volatile profile and physicochemical parameters of different yogurt varieties

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Key Words:	compositional parameters, volatile compound profile, hydrolyzed-lactose yogurts, galacto-oligosaccharides

Influence of lactose hydrolysis on galacto-oligosaccharides, lactose, volatile profile and physicochemical parameters of different yogurt varieties

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Short title: **Influence of lactose hydrolysis on some properties of reduced-lactose yogurts**

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Summary

BACKGROUND: Different types of reduced-lactose yogurt, obtained by lactose hydrolysis using β-galactosidase enzyme, are commercially available. The breakdown of lactose modifies the carbohydrate profile, including the production of prebiotic galacto-oligosaccharides (GOS), which could affect the survival and activity of starter and probiotic cultures and the parameters of yogurt quality. The extension of these changes is dependent on the yogurt matrix composition. This study aimed to evaluate the influence of lactose hydrolysis on GOS, lactose, volatile profile and physicochemical parameters of different yogurt varieties during storage.

RESULTS: The presence of β-galactosidase enzyme did not affect neither the global composition nor the survival of cultures. Overall, the hydrolyzed products had lower acidity than traditional ones. GOS were found at similar levels in fresh hydrolyzed yogurts, whereas in traditional yogurts were not detected. The proportion of ketones, acids and aldehydes seems to be more dependent on yogurt variety than the addition of the enzyme. Likewise, the storage period affected the volatile fraction in different degree; the increase in acid compounds was more pronounced in hydrolyzed than traditional yogurts.

CONCLUSION: This work shows that it is possible to obtain different varieties of reduced-lactose yogurt, some of them with additional benefits to health such as reduced-fat, reduced-calories, added with probiotic/inulin and enriched in GOS, with similar characteristics to traditional products.

Keywords: hydrolyzed-lactose yogurts, galacto-oligosaccharides, compositional parameters, volatile compound profile.

INTRODUCTION

In recent years, the consumption of yogurt has increased markedly in Argentine (between 7.2 Kg per capita in 2003 to 12.4 Kg in 2012) [1]. This trend is in accordance with those observed in other parts of the world [2]. In fact, the increase of consumption and popularity of yogurt has been attributed to several reasons such as sensory aspects, nutritional properties and a higher awareness from consumer of their healthy effects [3, 4]. These beneficial characteristics are mainly attributable to lactic acid bacteria and can be improved by applying different approaches such as the reduction of fat content, the use of non-nutritive sweeteners, the addition of probiotics and prebiotics and the reduction of lactose content, among others. In particular, yogurt and fermented milks are the most popular food carriers for probiotic bacteria[5]. Several studies have reported the health benefits related to their regular consumption, being the stimulation of the immune system the principal effect [6].

Among sensory attributes of yogurt, aroma is considered one of the most important. It is basically the result of a delicate balance of both components initially present in milk and volatiles synthesized during fermentation process[7]. Several key odorant components such as lactic, acetic and formic acids, ethanol, acetaldehyde, acetoin, diacetyl and 2,3-butanediol, etc., are produced as by-product from fermentation of lactose [8, 9]. The levels of volatile compounds are related primarily to the metabolic activities of starter bacteria, and probiotic bacteria if present, which are strain-dependent. Likewise, other factors such as chemical composition of milk base (type of milk, fat and dry matter contents, type and quantity of added ingredients), processing parameters (heat treatment intensity, time and temperature of incubation, oxido-reduction potential of medium), storage conditions, etc., may affect the volatile profiles of final product [2, 10-14].

On the other hand, hydrolyzed yogurt are mainly consumed by lactose-intolerant persons, as they are not able to completely digest lactose due to insufficient amount or low activity of the lactase enzyme [3, 15]. Even though this variety of yogurts have became a popular drink in several parts of the world, they are still not available in Argentinian market despite the fact that in Latin America the incidence of intolerant people is very high (> 70%). The strategy more applied to obtain reduced-lactose yogurts is the lactose hydrolysis with β-galactosidase enzymes [16]. Additionally, this enzyme has transgalactosilase activity and can simultaneously produce galacto-oligosaccharides (GOS), a group of compounds widely recognized as bioactives. Among the most important effects, the prebiotic role, the stool improvement, enhances the mineral absorption, weight management, anticarcinogenesis, and allergy alleviation, can be mentioned [17]. So, they are employed as functional ingredient in a variety of food such as infant formulas [18], lactose-free UHT milks and dairy drinks [19], ice creams [20], and fermented milks [21].

Summarizing, the inclusion of the β-galactosidase enzyme in yogurt making changes the carbohydrate pattern in the matrix; hence, different relative proportions of lactose, glucose, galactose and GOS are found during process. This different sugar profile could affect the metabolic activity of cultures and consequently the production of derived compounds as well as some physicochemical characteristics of the products [22-24]. Besides, the extension of these changes can be influence by the matrix composition.

Although data about physicochemical parameters of different varieties of hydrolyzed and traditional yogurts are available in the literature [2, 9, 12, 23, 25, 26], to the best of our knowledge the study of the GOS content and volatile compound profiles of yogurts made from different milk bases is limited. In this context, the aim of the present work was to assess the influence of lactose hydrolysis on galacto-oligosaccharides and lactose contents, volatile profile and physicochemical parameters of different yogurt varieties.

MATERIALS AND METHODS

Yogurt manufacturing protocol

Lactose-hydrolyzed yogurts were manufactured by β-galactosidase enzyme (EC 3.2.1.23.) treatment, which were compared with traditional yogurts (without enzyme). Four different varieties were obtained: whole-fat yogurts sweetened with sucrose and with the addition of *L. acidophilus*/inulin; whole-fat yogurts sweetened with sucrose and without the addition of *L. acidophilus*/inulin, reduced-fat yogurts sweetened with aspartame and with the addition of *L. acidophilus*/inulin; reduced-fat yogurts sweetened with aspartame and without the addition of *L. acidophilus*/inulin. Two independent trials for each yogurt variety were made.

Whole-fat yogurts were prepared by the following protocol: cow milk with 3% w/w fat content (20 L) was obtained directly from a dairy plant (Milkaut S.A., Santa Fe, Argentina), refrigerated and transported at 4 ºC to the pilot plant of Instituto de Lactología Industrial (INLAIN, Santa Fe, Argentina). The milk was divided into 4 equal lots and tempered until it reached approximately 40 °C. At this moment, 22.5 g L^{-1} skim milk powder (SMP) and 20.0 g L^{-1} whey protein concentrate (WPC35) (Milkaut S.A., Santa Fe, Argentina), and 80.0 g L^{-1} sucrose (Ing. Ledesma, Tucumán, Argentina) were added. Inulin at level of 10.0 g L^{-1} (Orafti®GR, Mannheim, Germany) was also aggregated in appropriate cases. The ingredients were blended by manual agitation for 15 min. Milk bases were submitted to heat treatment at 90 ºC/5 min, cooled to 42 ºC, and inoculated with freeze-dried starter culture YF-L811 (Chr. Hansen, Buenos Aires, Argentina) comprising of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in the dosage recommended by manufacturer. β-galactosidase enzyme YNL-2 GODO (Shusei Company Limited, Tokyo, Japan) at level of 0.25 g L^{-1} was added for lactose-hydrolyzed yogurts. The incubation at 42 ºC was monitored and performed until pH values of 4.70 ± 0.05. At this point, freeze-dried culture *L. acidophilus* La-5 (Chr Hansen, Horsholm, Denmark) was added in order to reach an initial cell count of 10^7 CFU g^{-1} for yogurts containing inulin. Then, the yogurts were cooled down in an ice water bath, and the gel was broken manually by stirring for approximately 20 min. Finally, yogurts were packaged in screw cap glass flasks (0.5 L) and stored at 5 ºC during 21 days.

Reduced-fat yogurts were prepared applying the protocol mentioned above, with the following modifications. The milk employed had fat content of 1.50% w/w, and 0.30 g L^{-1} aspartame (Ajinomoto Group, Japan) was incorporated immediately after incubation in replacement of sucrose.

Samples were taken at different points during storage. In particular, microbial counts, pH and titratable acidity (TA) were tested at 1 and 21 days immediately after sampling. GOS and lactose contents, and volatile profiles were measured in freshly made yogurts and at 21 days, and global composition (total solids, protein and fat) at 7 days; the samples were kept frozen (-18 ºC) in 0.1 L glass containers previously to analyses.

Analytical determinations

- pH measurements were accomplished with a digital pHmeter (Orion 3 star benchtop, Thermo Fisher Scientific Inc., USA).
- Titratable acidity (TA) by titration with 0.11 M NaOH until pH 8.3 using a pHmeter [27]. The results were expressed as Dornic degree (1 ºD = 100 mg lactic acid L^{-1}).

- Total solids were analyzed according to the standard method [28].
- Total protein was determined via Kjeldahl method as nitrogen (%) x 6.25 [29].
- Fat content by Gerber method [30].
- Lactose and galacto-oligosaccharides analysis by HPLC using an Aminex HPX-87H column (300 x 7.8 mm) protected by a cation H^+ microguard cartridge (Bio-Rad Laboratories, Hercules, USA). Equipment consisted of a quaternary pump, an on-line degasser, 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). The chromatographic separation and sample preparation was performed according to Vénica *et al.*[23]. Lactose and GOS quantification was carried out by external calibration using lactose and raffinose standards, respectively (Sigma-Aldrich, St. Louis, USA) [31].
- Volatile compound analysis by SPME-GC/FID/MS according to method described by Wolf *et al.*[24].
- Total lactic acid bacteria were analyzed according to Vénica *et al.*[23]. The counts of *L. acidophilus* were determined on MRS agar by Vinderola and Reinheimer [32].

The samples were analyzed in duplicate, and the analyses were repeated if necessary.

Statistical analysis

The results were submitted to a one-way analysis of variance (ANOVA) to identified contrasts in pH, TA, lactose, GOS and volatile profile between hydrolyzed (H) and traditional (T) yogurts for each yogurt making trial (1 and 2) and for each sampling time (0, 1 and 21 days, as correspond). In order to avoid an excess of information, volatile compounds were grouped by chemical families and area values were used to calculate percentual values. One-way ANOVA was also used to detect the effect of storage period on these parameters for each yogurt. These analyses were carried out using SPSS 10.0 software (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Composition, acidity and microbiological counts

The overall composition was similar between both trials for each yogurt variety; therefore, the mean values are shown in **Table 1**. No effect of the addition of β-galactosidase enzyme on protein and fat contents and total solids was observed (*P*>0.05). Fat contents for reduced-fat yogurts were approximately 14.0 g Kg^{-1}, total solids ranged between 134.0 and 146.8 g Kg^{-1}, and protein ranged between 45.1 and 46.8 g Kg^{-1}. In particular, the higher total solids values and the lower protein values are related to inulin inclusion in the formulation. For whole-fat yogurts, fat values were approximately 28.0 g Kg^{-1}. As expected, the inclusion of sucrose produced an increase of total solids (205.4 – 214.0 g Kg^{-1}) and a decrease of protein content (41.5 – 42.7 g Kg^{-1}) compared with reduced-fat yogurts. Values of overall composition were in accordance with those established by Argentinian Legislation[33].

The values of pH and titratable acidity for both trials of each yogurt variety are presented in **Figure 1**. **Tables 2**, show the significance of enzyme treatment and storage time. Generally, the hydrolyzed yogurts had lower TA than traditional ones; significant differences were detected for some varieties between hydrolyzed and traditional freshly yogurts samples belonging to trial 2, while differences were observed for both trials and all varieties of yogurt at 21 days. In particular, it was observed that reduced-fat yogurts had higher TA values compared to whole-fat yogurts, which could be due to the presence of slightly higher amounts of milk proteins. The TA values ranged from 95.2

to 97.9 ºD and 97.9 to 99.5 ºD for reduced-fat hydrolyzed and traditional yogurts, respectively. For whole-fat yogurts, TA ranged from 84.1 to 92.9 ºD and 87.7 to 95.8 ºD for hydrolyzed and traditional products, respectively. Results were suitable as established by Argentinian Legislation (60 - 150 °D)[33]. The pH values were usually lower in hydrolyzed yogurts compared to traditional ones but significant differences were only detected in some trials of certain varieties. Particularly, the values ranged between 4.60 and 4.33 for hydrolyzed yogurts and between 4.50 and 4.29 for traditional yogurts, at 21 days. As expected, the postacidification was significant in all yogurts (**Table 2**).

With regards to the microbiological analyses, no differences among varieties of yogurt were found. Average counts between 8.92 and 9.40 and 8.86 and 9.28 log orders were observed for total LAB at day 1 and 21, respectively. In the case of yogurts with probiotic, counts between 7.08 and 7.53 and 7.30 and 7.53 log orders were found for *L. acidophilus* La-5 at day 1 and 21, respectively. All counts are in accordance with the Argentinian Legislation (minimum of 7 and 6 log orders for LAB and probiotic, respectively)[33]. These results suggest that the microbial viability was not influenced by the composition of carbohydrates for different varieties of yogurt. Contrary to our results, Ibarra *et al.*[6] reported that the growth rate of probiotic (*L. rhamnosus* HN001) correlated positively with the level of lactose hydrolysis. However, this fact is dependent to the fermentation conditions and strain employed. Likewise, Matijević *et al.*[34] found that viable cell count of *L. acidophilus* La-5 was higher in lactose hydrolyzed whey when compared with the control (not hydrolyzed), indicating that the higher disponibility of glucose enhancement the probiotic activity. Similar viable cell counts of *L. acidophilus* that our work, have been reported in other milk matrices (as cheese and fermented beverages), which also have proven to be good probiotic carrier [35, 36].

Lactose and galacto-oligosaccharides

GOS concentrations for both trials of hydrolyzed fresh yogurts and yogurts stored for 21 days are presented in **Figure 2**. **Figure 3** shows the contents of lactose obtained in trials 1 and 2 for hydrolyzed and traditional fresh yogurts and at 21 days. The significance of the statistical analysis is show in **Tables 2**.

GOS contents for H yogurts ranged from 6 to 7 g Kg^{-1} in fresh products (**Figure 2**); as can be seen, the values were similar for all hydrolyzed yogurt varieties. These compounds were produced by transgalactosylation activity of the added β-galactosidase enzyme on lactose. In T yogurts, GOS were not detected (**Table 2**, statistical significance of treatment effect) demonstrating that the cultures used in yogurt making were not able to generate them. In particular, the probiotic culture was added after incubation process. During yogurts storage, only in one case a decrease in the content of GOS was observed ($P<0.05$) (reduced-fat yogurts without La-5/inulin belonging trial 2); in the other cases, GOS concentration remained unchanged for 21 days indicating that these compounds were not hydrolyzed (**Table 2**, statistical significance of storage time).

Scarce and variable information about GOS synthesis during reduced-lactose yogurt manufacture employing enzymatic process is reported. The most recent work has been carried out by Martins *et al.*[22, 37]. They reported GOS values between 0.9 and 4.9 g L^{-1} for fresh hydrolyzed yogurts. On the other hand, some studies reported GOS synthesis in the preparation of traditional yogurts. The authors attributed this finding to the LAB and bifidobacteria cultures employed, reaching concentrations from 0.08 to 1.35 g L^{-1} [21, 38-40]. Likewise, Song *et al.*[41] detected β-galactosidase hydrolytic activities by LAB strains currently used in yogurt making (*L. acidophilus*, *Lc. Lactis*, *L. helveticus* and *L. paracasei*); besides, they found that *L. paracasei* had the highest hydrolytic activity and a good ability to synthesize GOS in reconstituted whey powder.

Concerning the GOS stability in the yogurt matrix during storage, our results are in agreement with Lamoureux *et al.*[38] and Yadav *et al.*[39], who found that GOS formed during fermentation did not undergo degradation.

It is not surprising that the lactose contents in H yogurts were lower (P<0.01) than T ones, for both trials of each yogurt variety and at two sampling time analyzed. The percentages of diminution for fresh products were in average 78% in H yogurts compared with 18% in T products. During storage, the decline of lactose was more marked in T than in H yogurts as can been seen in **Figure 2** and in **Table 2**. Martins *et al.*[37, 40, 22] employed a similar approach as that used in this work (simultaneous enzymatic hydrolysis and fermentation) and the addition of enzyme before the start of fermentation. The lactose values obtained ranged from 0.2 to 68.1 g L^{-1} for hydrolyzed yogurts depending on the β-galactosidase doses, initial lactose concentration in the milk and the time of the enzyme added. Nagaraj *et al.*[42] and Ibarra *et al.*[6] prepared yogurts with hydrolyzed milk and found levels of lactose reduction from 46 to 90% and 81 to 97%, respectively. Cruz *et al.*[43] reported lactose contents in probiotic yogurts ranged between 10.6 and 22.8 g L^{-1} due to an improvement in metabolic activity by the addition of glucose oxidase which cause a decrease the oxidative stress.

Finally, the lactose reduction rate and the GOS content obtained during manufacture of yogurts were not affected by the fat content in the milk base. Similar results was observed by Horner *et al.*[44]; they found that different fat contents of the milk base did not interfere the hydrolytic activity of added β-galactosidase enzyme.

Volatile profiles

A total of 22 volatile compounds were detected in some or all varieties of yogurts, belonging to different chemical classes (9 ketones, 5 acids, 4 alcohols, 2 aldehydes and 2 esters).

Among ketones group, methylketones (propanone, butanone, 2-hexanone, 2-heptanone, 2-nonanone), diketones (2,3-butanedione or diacetyl, 2,3-pentanodione), hydroxyketones (3-hydroxy 2-butanone or acetoin) and aromatic ketones (acetophenone) were identified. In the alcohols group, linear-chain primary alcohols such as ethanol, 1-pentanol, 1-hexanol and 2-ethyl 1-hexanol were mostly found in the volatile profiles of yogurts. The acidic fraction was represented by short- and medium-chain volatile acids: ethanoic or acetic, butanoic or butyric, hexanoic, octanoic and decanoic acids. Within the chemical families of aldehydes and esters, only acetaldehyde and benzaldehyde and ethyl butanoate and ethyl hexanoate, respectively, were detected. All the volatile compounds identified in samples have been reported as typical constituents of yogurt aroma [7, 25, 45-48].

Whole-fat yogurts without La-5/inulin: Figure 4a shows the volatile fraction of traditional and hydrolyzed yogurts made from whole-fat milk during storage.

Regardless to the type of yogurt (H or T) and storage time, acetoin, acetaldehyde, propanone, diacetyl, hexanoic acid, butanoic acid and acetic acid were the most abundant compounds (taking into account the area values) identified in all samples. They represented around 80% of the total area of compounds.

At the end of fermentation, statistical differences in percentual values of some chemical groups were detected between traditional and hydrolyzed yogurts. The volatile fraction of traditional freshly made yogurts from both trials was characterized by a predominance of ketones, which constituted around 63% of the total area of compounds. Aldehydes and acids accounted for approximately 16% each, while alcohols and esters reached 4% and <1%, respectively. In hydrolyzed yogurts from both trials, ketones group was found at similar levels of their respective T yogurts (P>0.05). For both trials, the percentages of aldehydes were significantly higher in H than T yogurts while the proportion of acids was higher in T yogurts (P<0.05).

The comparison of area values for each volatile compound between both types of yogurt revealed statistical differences for 13 compounds: acetaldehyde had higher levels in H yogurts; diacetyl was significantly higher in H2 than T2; 2,3-pentanedione and 2-hexanone were detected at higher levels in T yogurts; esters, benzaldehyde and acetophenone presented higher quantities in T1 than H1. With exception of acetic acid, all remaining acids reached higher levels in T yogurts, although these differences were statistically significant (P<0.05) for butanoic, hexanoic and octanoic from T1 and hexanoic, octanoic and decanoic from T2.

Some changes in the volatile profile of these yogurts were detected during refrigerated storage. The percentual values of ketones, alcohols and esters both in T and H yogurts did not change significantly (P>0.05) in comparison to their respective freshly made products. However, a decrease in the percentages of aldehydes and an increase in the percentages of acids were detected at 21 days for both types of yogurts. Similarly to the results obtained at the end of fermentation, aldehydes were found at higher levels in H yogurts than their counterparts made without enzyme, whereas acids reached higher levels in T yogurts.

A total of 12 compounds presented statistical differences between both types of yogurt at 21 days: acetaldehyde, ethanol and esters had higher levels in H than their respective T yogurts (P<0.05); 2,3-pentanedione and acetic, butanoic and hexanoic acids presented higher area values in T yogurts; diacetyl showed an opposite behavior in both trials; benzaldehyde, acetophenone and 1-pentanol were higher in T1 than H1 yogurts. On the other hand, the evolution of single volatile compounds during storage was similar in both types of yogurt. Overall, compounds belonging to chemical families of ketones, alcohols and acids showed a trend to increase whereas acetaldehyde level decreased at 21 days.

Whole-fat yogurts with La-5/inulin: The volatile profile of yogurts made from whole-fat milk and with added La-5/inulin is represented in **Figure 4b.**

Major volatile components in all samples were acetoin, hexanoic acid, octanoic acid, diacetyl, 2,3-butanedione and propanone. They constituted around 68% of the area total of compounds.

There were not statistically significant differences between H and their respective T yogurts for all groups of compounds at the end of fermentation. Accordingly, the area values of the majority of compounds were similar between both types of yogurts. The volatile fraction was mainly composed by volatiles belonging to ketones and acids families. Ketones ranged from 59 to 63% and acids from 28 to 32% in all samples. The proportion of aldehydes, alcohols and esters reached mean values of 5.3%; 3.0% and 1.8%, respectively.

At 21 days of storage, changes in the percentual values of ketones and acids were only detected in hydrolyzed yogurts in comparison to freshly made products. Consequently, the proportions of ketones were higher in T than H yogurts whereas acids had higher values in H products. The analysis of individual volatile compounds revealed that 1-hexanol and the majority of acids (butanoic, hexanoic, octanoic and decanoic) had higher levels in H than T yogurts. On the other hand, diketones such as diacetyl and 2,3-pentanodione reached higher levels in T1 than H1.

The evolution of volatile compounds during storage was similar for all yogurts. In general, ketones, alcohols and acids increased whereas levels of acetaldehyde remained constant until 21 days.

Reduced-fat yogurts without La-5/inulin: The volatile composition of yogurts made from reduced-fat milk with and without β-galactosidase enzyme at 0 and 21 days are presented in **Figure 4c**.

The profile of traditional yogurts analyzed at the end of fermentation was characterized by the presence of ketones and acids, which reached mean percentages of 50% and 37%, respectively. The main ketone found in these yogurts was acetoin, by representing more than 50% of the total ketones. Among acids group, the most representative were hexanoic and octanoic acids. Aldehydes, alcohols and esters represented 5.0%, 4.0% and 2.5%, respectively. The composition of hydrolyzed yogurts differed from traditional products in the percentual values of acids and aldehydes. However, the preponderance in the volatile profile of acetoin, hexanoic and octanoic acids was also observed in these yogurts. Acids group had higher values in T than their respective H yogurts whereas aldehydes reached higher percentages in H than T yogurts ($P<0.05$). This finding was in accordance with those observed in whole-fat without La-5/inulin samples.

For the majority of identified compounds, statistical differences were not detected between T and their respective H yogurts; only acetaldehyde, acetoin, 2,3-pentanodione and some acids reached higher values in T yogurts.

The volatile profile of T yogurts was not significantly influenced by the refrigerated storage since the percentual values of chemical groups of compounds were similar to those of freshly made products ($P>0.05$). By contrast, H yogurts showed a decrease in the percentual values of ketones and aldehydes, whereas acids increased. As consequence, statistical differences were found in ketones and acids at the end of storage (*P*<0.05). Ketones had higher levels in T than H yogurts whereas acids reached higher values in H yogurts. On the other hand, the comparison of peak area values for individual volatile compounds between T and their respective H yogurts at 21 days showed higher levels of acetoin and 2,3-pentanedione in T yogurts and higher levels of acids in H yogurts.

The behavior of volatile compounds during storage was similar for both types of yogurts. In general, compounds belonging to ketones, alcohols and acids groups showed a trend to increase whereas the levels of acetaldehyde and ethanol remained constants in all samples.

Reduced-fat yogurt with La-5/inulin: The typical volatile profile of yogurts made from reduced-fat milk and with added La-5/inulin is shown in F**igure 4d.**

All samples of freshly made yogurts were characterized by the majority presence of acetoin, propanone, diacetyl, hexanoic acid, octanoic acid, acetaldehyde and butanone. These compounds accounted for 70% of the total volatile compounds.

No statistical differences were found in the percentual values of chemical groups between H and their respective T yogurts. Ketones and acids were the most representative groups in H and T yogurts; they ranged from 51% to 55% for ketones and from 29% to 34% for acids. Aldehydes represented the third group of compounds (mean value of 8.5%) whereas alcohols (5.0%) and esters (1.7%) constituted a minority fraction. By analyzing the peak area values of individual compounds, differences were detected for 6 volatile compounds: acetaldehyde, butanone and 2,3-pentanedione had higher levels in T than H yogurts; propanone and 2-ethyl 1-hexanol reached higher levels in T2 and T1, respectively. On the other hand, 1-hexanol had higher levels in H than T yogurts.

At 21 days, the volatile profiles of T yogurts did not show important variations. A slight decrease in aldehydes and a slight increase of acids was observed in relation to freshly made products. By contrast, H yogurts underwent more profound changes. Percentages of ketones and aldehydes decreased and the proportions of acidic compounds increased markedly in comparison to the same yogurts analyzed at the end of fermentation. The evolution of individual compounds in the course of storage showed the same trend in both type of yogurts: acetaldehyde, propanone and butanone decreased whereas diketones, primary alcohols and acids increased. The comparison of the volatile composition between H and their respective T products at 21 days revealed that ketones were significantly higher in T products whereas acids reached the highest percentual values in H yogurts (P<0.05). Several carbonyl compounds such as acetaldehyde, propanone, diacetyl and 2,3-pentanodione had higher values in T than H products. By contrast, 1-hexanol and acidic compounds reached higher levels in H products.

As can be seen, regardless of the matrix composition, the volatile profiles of freshly made yogurts were characterized for a preponderance of ketones. Taking into account the peak area values, acetoin was the most abundant volatile compound in all yogurts analyzed, ranging from 30 to 65% of total ketones and from 18 to 32% of total area of volatile compounds. Diacetyl is another important flavour compound in fermented dairy products. Both diacetyl and acetoin are produced by the same mechanisms, being acetoin the reduction product of diacetyl. It has been proposed that glucose is the major precursor via pyruvate and activated acetaldehyde for diacetyl in yogurt [49].

The highest percentages of ketones were found in fresh yogurts made from whole-fat milk. In these products, the addition of both La-5/inulin and β-galactosidase enzyme did not modify substantially the percentages of this group of compounds. The lower preponderance of ketones in the volatile profile in yogurts made from reduced-fat milk revealed a relationship between fat content and ketones production. Similarly to those yogurts made from whole-fat milk, this fact was not dependent on the presence or absence of β-galactosidase enzyme and La-5/inulin. Ketones are common metabolites found in yogurts [46, 47, 50]. Among them, methyl ketones biosynthesis is related to milk fat hydrolysis and then, a β-oxidation step of saturated fatty acids followed by decarboxylation of β-ketoacids [46]. Accordingly, their presence in yogurt could be related to the milk fat content. Milk fat is recognized as a key factor for the texture and flavour of dairy products, as well as an important source of volatile compounds. Data available about the influence of milk fat content on volatile compound profile of yogurts are limited. Kaminarides *et al.*[46] studied the characteristics of set type yogurts made from ovine milk of different fat content but they did not observed differences in ketones content among yogurt samples.

At 21 days of storage, the proportion of ketones remained constant in traditional products and it decreased in some types of hydrolyzed yogurts, being this diminution more pronounced in those yogurts with added La-5/inulin.

Aldehydes were a prominent group only in the volatile profile of yogurts made from whole-fat milk and without La-5/inulin at the end of fermentation (16% of total area of compounds). In the remaining samples, this group in no case exceeded 10%.

Regardless of milk fat content, the proportions of aldehydes were statistically higher ($P<0.05$) in H yogurts made without La-5/inulin when compared with their counterpart traditional, whereas in those with added La-5/inulin, no differences were detected ($P>0.05$) between yogurts with and without β-galactosidase addition.

Overall, a decrease in the percentual values of aldehydes was observed in all samples during storage, being slightly more pronounced in hydrolyzed products. At 21 days, the proportions of aldehydes were always higher in H yogurts made from whole-fat milk without La-5/inulin in comparison to T yogurts, whereas in reduced-fat with La-5/inulin yogurts the percentages were higher in T yogurts than their counterpart hydrolyzed.

Acetaldehyde is the most representative compound belonging to this group. In addition, acetaldehyde is considered be the main volatile responsible for imparting desirable aroma to yogurt [45]. It can be produced by lactic acid bacteria from different metabolic pathways including threonine, pyruvate or nucleic acid as potential precursors [13, 51, 52]. As mentioned, β-galactosidase enzyme changes the sugar profile of yogurts and this fact could affect, at least partially, the acetaldehyde biosynthesis. The results showed that acetaldehyde had always a higher level in H yogurts made from whole-fat milk and without La-5/inulin than their respective T yogurts. By contrast, in yogurts made from reduced-fat milk and with added La-5/inulin the content of acetaldehyde was always higher in T than H yogurts. However, for both matrix compositions, a decrease in acetaldehyde level was observed during storage. For whole-fat with La-5/inulin and reduced-fat without La-5/inulin yogurts, the level of acetaldehyde was similar between H and T yogurts and no changes were detected during storage.

These results indicated that the production of acetaldehyde in yogurts and its evolution during storage was strongly affected by the matrix composition. Several studies have underlined the effect of compositional characteristics of milk bases (milk fat content, milk solids-not-fat fortifiers and hydrocolloids) as well as the oxido-reduction potential of the medium on acetaldehyde concentration [12, 53, 54]. In addition, the production of acetaldehyde by strains used as probiotics in fermented milk, including *L. acidophilus* has been investigated [55, 56]. From these studies has been demonstrated the ability of *L. acidophilus* strains to produce this compound from different sources. However, acidophilus milk products are commonly characterized by lack of flavour due to the fact that some of these strains possess alcohol dehydrogenase activity, which decrease the acetaldehyde content in yogurt. Gardini *et al.*[53] observed that the presence of *L. acidophilus* did not affect the acetaldehyde level of fermented milks. Guven *et al.*[10] observed that the presence of inulin decreased the levels of acetaldehyde in comparison to inulin-free yogurts. Besides, decreases of the acetaldehyde contents during storage were found to be statistically significant. Other authors have reported both increase and decrease in acetaldehyde levels throughout the storage period [11, 57]. The accumulation of acetaldehyde in yogurts seems to depend on whether the microorganisms have enzymes which convert it to other metabolites, mainly ethanol [52].

Acids have been reported as main components in the volatile profile of yogurts [2]. They contribute to maintain a balanced flavour. The most representative components of this group were acetic, butanoic and hexanoic acids for yogurts made from whole-fat and without La-5/inulin. For the remaining yogurts, the most abundant acids were hexanoic and octanoic. Acetic acid is related to lactose metabolism, and therefore, the different carbohydrate profile in hydrolyzed in comparison to traditional products can affect its production. Butanoic, hexanoic and octanoic acids are associated with lipolytic activities of LAB [51].

With exception of whole-fat without La-5/inulin yogurts, acids were the second group of compounds in the majority of freshly made yogurts, with percentages around 30% of total area of compounds.

Regardless of milk fat content, the proportions of acids were statistically higher in T yogurts made without La-5/inulin whereas in yogurts made with added La-5/inulin, no differences were detected between yogurts with and without β-galactosidase enzyme.

The refrigerated storage had an important impact in the acidic profile. This effect was dependent on the matrix composition and the yogurt type (H or T). Whole-fat without La-5/inulin yogurts made with and without β-galactosidase enzyme showed an increase in the percentages of acids during storage; however, a higher proportion of acids was detected in T than H yogurts. In whole-fat with La-5/inulin yogurts and in yogurts made from reduced-fat milk with and without La-5/inulin an increase in acid proportion was only detected for hydrolyzed yogurts, and the incidence of acids in volatile profile was significantly higher in H than T yogurts. For all yogurts, the majority of individual fatty acids increased during storage. Other authors have also observed an increase of acidic compounds [10, 46, 58], which was attributed to the increase in activity of *L. delbrueckii* subsp. *bulgaricus* during the storage [57]. *L. acidophilus* could also contribute to the production of acetic acid during refrigerated storage [53]. Besides, in some cases, this fact was closely related to the decrease in product acceptability [47]. It has also been reported that the addition of inulin influenced the volatile fatty acid content of fat-free yogurts [10].

Alcohols were a minority group of compounds in all yogurts. Their percentages ranged from 3 to 5%. Differences in the proportions of alcohols between T and H products belonging to each variety of yogurts were not detected either in freshly made products or in stored products. Overall, a trend to increase during storage was observed for almost all alcohols identified. Among alcohols, 1-hexanol was detected at higher levels in all samples. Ethanol, one of the most important alcohols found in fermented dairy products, was the most abundant only in those yogurts made from whole-fat without La-5/inulin throughout the storage period.

As can be seen, the enzyme inclusion in different matrix composition modified some characteristics of yogurts such as, acidity, carbohydrates composition and volatile profile. In addition, sensory attributes such as sweetness, creaminess, firmness, among others, could be affected and modify the consumer satisfaction [59]. Therefore, further studies are necessary to compare the sensory profile and acceptance by the consumer of the different varieties of hydrolyzed yogurts with their respective traditional products.

CONCLUSIONS

The process of hydrolysis of lactose by β-galactosidase activity that occurred during the manufacture of reduced-lactose yogurts affected slightly some parameters of yogurt quality such as acidity. Simultaneously to hydrolysis process, the enzyme had also the capability to GOS synthesis by transgalactosilase activity. In fact, GOS were only detected in hydrolyzed yogurts and the levels achieved were similar in all varieties of yogurt analyzed, regardless of yogurt matrix composition. The starter and probiotic cultures used had no ability to produce GOS during fermentation. On the other hand, the yogurt matrix was adequate to maintain the GOS levels unchanged during storage. Therefore, it is important to note that reduced-lactose yogurts have the potential to be GOS carrier.

The volatile profiles of yogurts were affected by milk base composition, presence or absence of β-galactosidase enzyme and storage time. For certain milk base compositions, differences between traditional and hydrolyzed products were detected for some chemical groups of compounds at each time sampling. During storage, the levels of individual volatiles and groups of compounds varied in yogurts depending on type of matrix. However, the same behavior of volatile constituents was observed throughout storage in all yogurts. Regardless of the yogurt composition a trend to increase acids was the most peculiar characteristic. This tendency was more pronounced in hydrolyzed yogurts of all varieties in comparison to traditional products.

This work shows that is possible to obtain different varieties of reduced-lactose yogurts, some of them with additional benefits to health such as reduced-fat, reduced-calories, added with probiotic/inulin and enriched naturally in GOS, with similar characteristics to traditional products. However, for certain varieties of yogurts special attention must be paid during storage since the acids group increases markedly. This fact is very important since the increase of acids can shorten the shelf-life of products.

This interesting work could represent a useful tool for dairies to assess the feasibility of launching new products to Argentinean market considering the limited supply of hydrolyzed lactose foods. Future studies should comprise the sensory analysis with trained and consumers panels in order to evaluate the impact of the enzyme addition on organoleptic characteristics.

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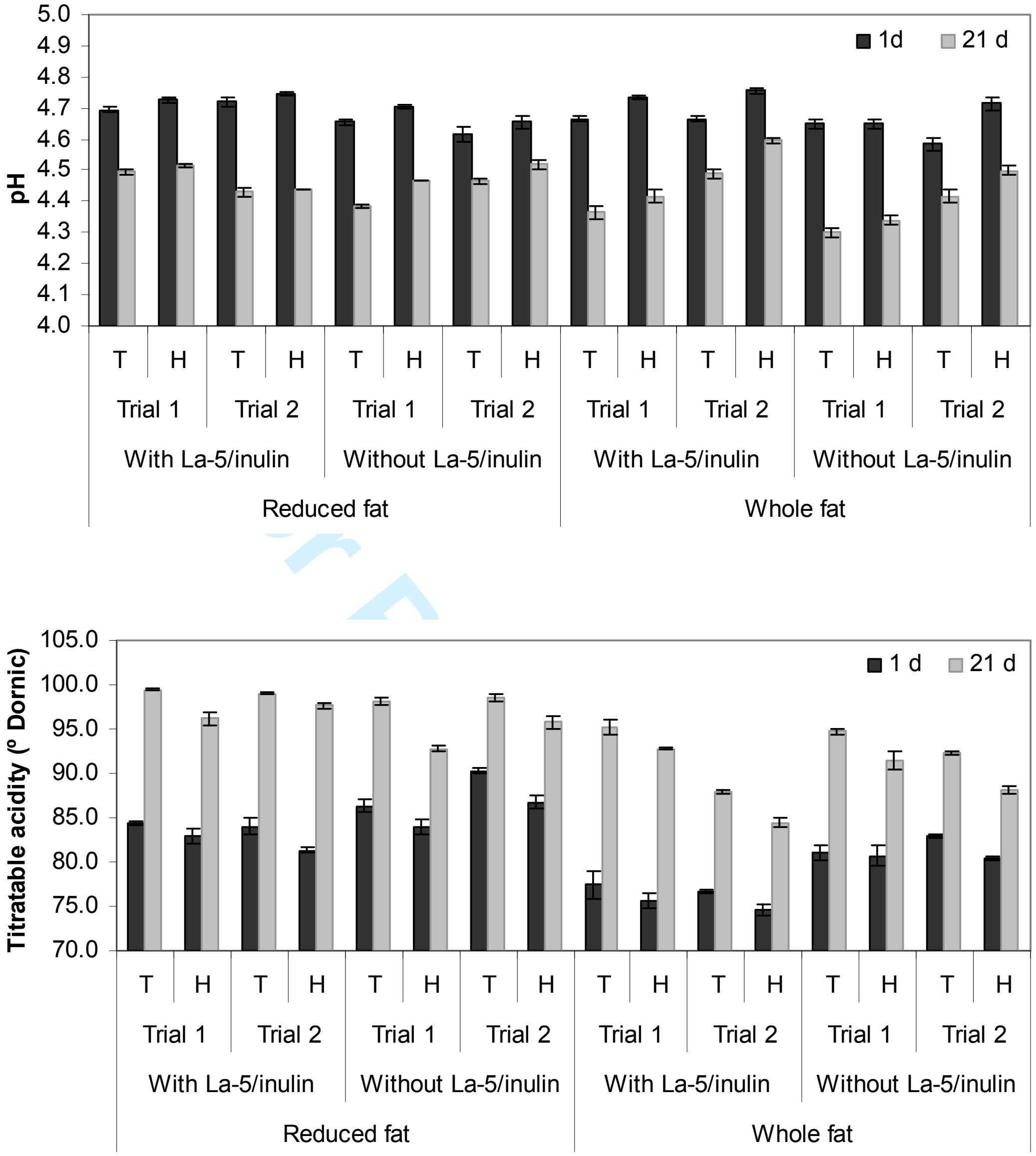

Figure 1: Acidity of yogurt. Values are means of duplicate analysis. Error bars denote standard deviation. For statistical analysis see Table 2. H: hydrolyzed yogurts; T: traditional yogurts.

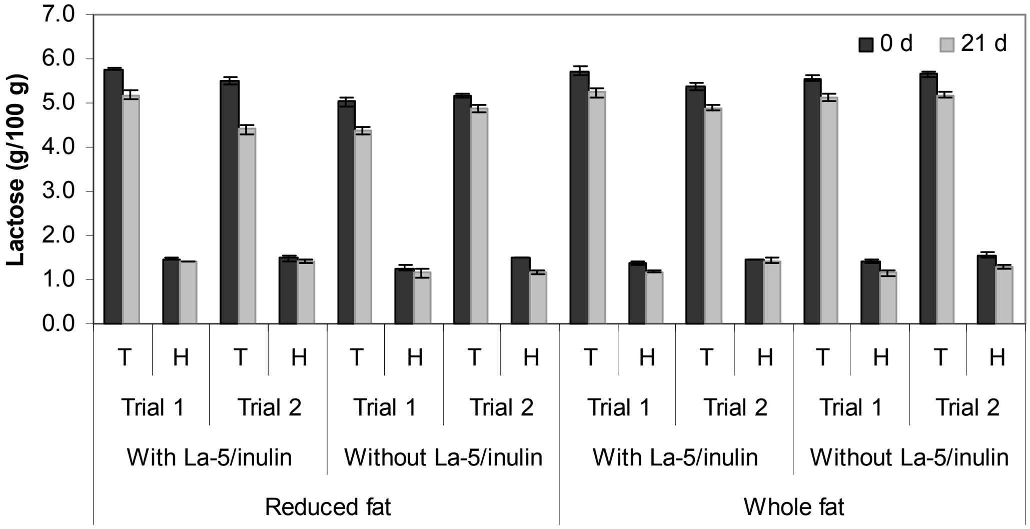

Figure 2: Lactose concentration in freshly made yogurts and at 21 days. Values are means of duplicate analysis. Error bars denote standard deviation. For statistical analysis see Table 2. H**:** hydrolyzed yogurts; T: traditional yogurts.

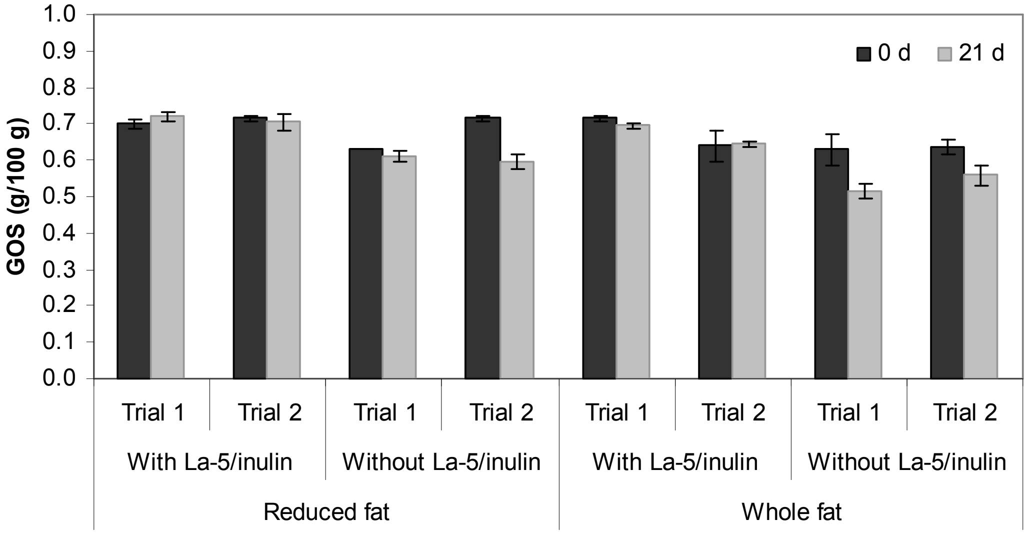

Figure 3: GOS concentration in freshly made yogurts and at 21 days. Values are means of duplicate analysis. Error bars denote standard deviation. For statistical analysis see Table 2. H**:** hydrolyzed yogurts; T: traditional yogurts.

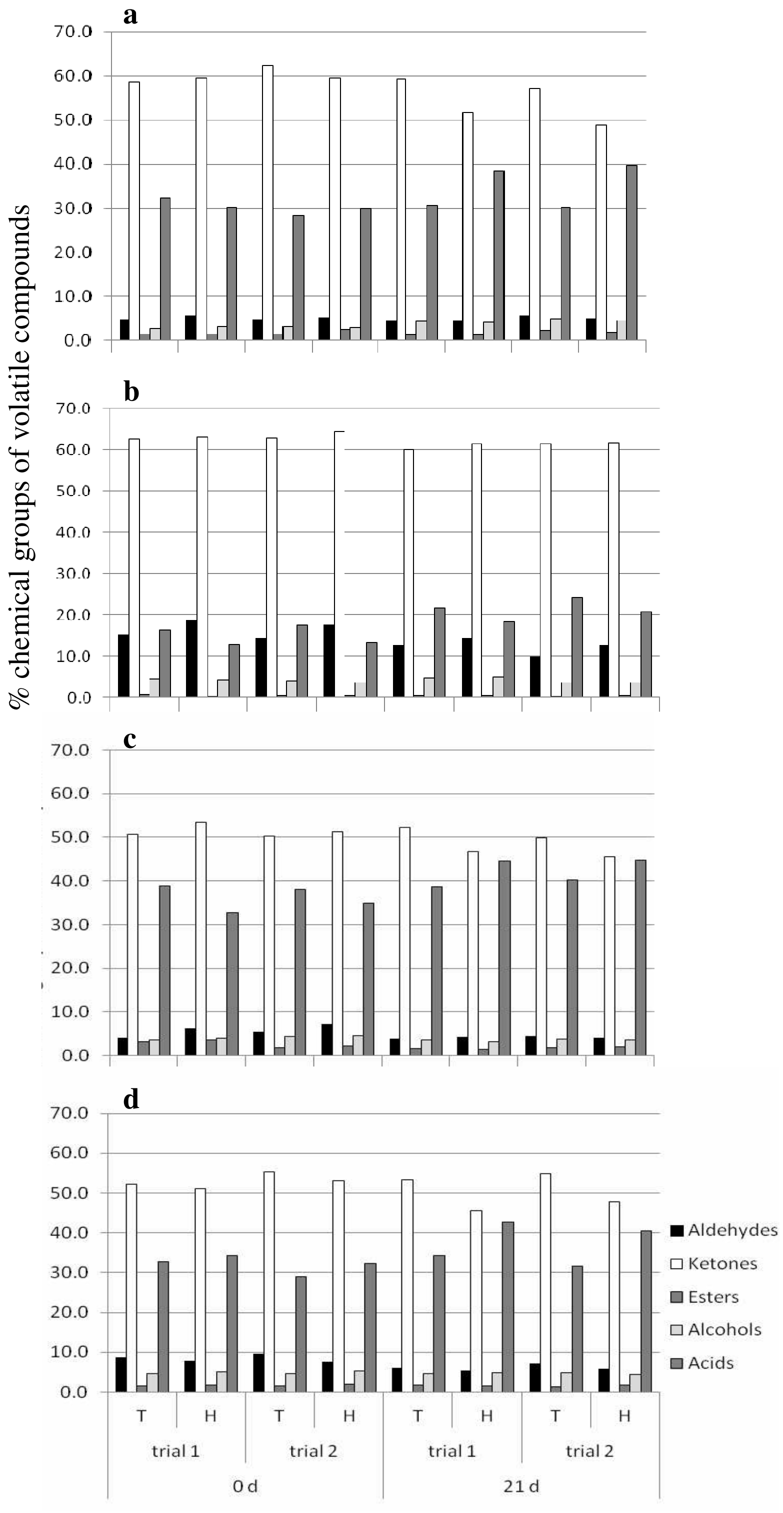

Figure 4: Volatile composition of yogurt during storage a) whole-fat without La-5/inulin, b) whole-fat with La-5/inulin, c) reduced-fat without La-5/inulin, d) reduced-fat with La-5/inulin. H: hydrolyzed yogurt; T: traditional yogurt.

Table 1

Composition (g Kg^{-1}) of yogurts at 7 days (mean ± standard deviation; *n* = 2).

			Fat	Total solids	Proteins
Reduced-fat	With La-5/inulin	T	15 ± 1	145.9 ± 0.9	45.4 ± 0.6
		H	14 ± 2	145.1 ± 1.1	45.9 ± 0.9
	Without La-5/inulin	T	14 ± 2	135.0 ± 1.4	46.0 ± 0.6
		H	14 ± 1	133.5 ± 0.7	46.5 ± 0.4
Whole-fat	With La-5/inulin	T	28 ± 1	213.7 ± 1.1	42.2 ± 0.7
		H	29 ± 2	213.8 ± 0.4	42.3 ± 0.2
	Without La-5/inulin	T	28 ± 2	205.9 ± 0.9	42.1 ± 0.4
		H	28 ± 1	205.7 ± 0.8	41.5 ± 0.4

T: traditional yogurt; **H**: hydrolyzed yogurt

Table 2

Statistical significance of treatment effect

	Reduced-fat				Whole-fat			
	With La-5/inulin		Without La-5/inulin		With La-5/inulin		Without La-5/inulin	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
pH								
1 day	ns	ns	*	ns	*	**	ns	*
21 days	ns	ns	**	*	ns	*	ns	*
TA								
1 day	ns	ns	ns	*	ns	*	ns	**
21 days	*	*	**	*	*	*	*	**
Lactose								
0 day	**	**	**	**	**	**	**	**
21 days	**	**	**	**	**	**	**	**
Galacto-oligosaccharides								
0 day	**	**	**	**	**	*	**	*
21 days	**	**	**	*	**	**	**	**

Statistical significance of storage time

	Reduced-fat				Whole-fat			
	With La-5/inulin		Without La-5/inulin		With La-5/inulin		Without La-5/inulin	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
pH								
T	**	**	**	**	**	*	**	*
H	**	**	**	*	**	**	**	**
TA								
T	**	**	**	**	**	**	**	**
H	**	**	**	*	**	**	*	**
Lactose								
T	*	**	*	*	*	*	*	*
H	*	ns	ns	**	*	ns	*	*
Galacto-oligosaccharides								
T	ns	ns	ns	ns	ns	ns	ns	ns
H	ns	ns	ns	*	ns	ns	ns	ns

T: traditional yogurt; H: hydrolyzed yogurt ; TA: titratable acidity

ns: not significant. *: $P < 0.05$; **: $P < 0.01$