



Effect of the carbohydrates composition on physicochemical parameters and metabolic activity of starter culture in yogurts



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ABSTRACT

The carbohydrate composition of the milk base plays a crucial role in the fermentation process of yogurt. In this study we investigate the effect of the traditional (TRAD) and delactosed (DEL) milks, added or not sucrose (S) in yogurt making, on the physicochemical parameters, growth of cultures and the formation of organic acids and volatile compounds during fermentation and storage. Yogurts made with delactosed milk had a delay in the fermentation up to 30 min in reaching the final pH and a slight minor postacidification, in comparison to yogurts prepared from traditional milk. Sucrose addition decreased the fermentation time in delactosed products. These observations were not accompanied by a significant change in the counts of thermophilic streptococci and thermophilic lactobacilli bacteria. The most notable differences in volatile compounds profiles were recorded in DEL + S respect to the remaining yogurts. The highest levels of diacetyl, 2,3-pentanedione, ethanol and ethyl esters found in DEL + S yogurts could be correlated with the higher glucose content, the main precursor of these compounds.

1. Introduction

Yogurt is the most popular fermented milk resulting of milk acidification and gelification linked to a co-culture of two species of lactic acid bacteria (LAB), *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. They ferment the carbohydrates present in milk producing lactic acid and other organic acids, and flavor compounds which contribute to characteristic and unique flavor and texture of yogurt (Tamime & Robinson, 2007). Worldwide yogurt consumption has increased greatly in recent years given its positive health image, sensory property and its moderate cost (Shiby & Mishra, 2013). This fact has motivated the dairy industry to develop new yogurt varieties to meet consumer demand for foods with increasingly properties (Tamime & Robinson, 2007). Despite the high prevalence among the Latin American population of lactose intolerance, yogurts with reduced content of lactose are absent in the Argentinean market. Therefore, the development of different varieties of reduced lactose yogurts constitutes a huge niche of market to Argentinean dairy industry. Reduced lactose yogurt can be made from reduced lactose milk, also called delactosed milk, which can be obtained by enzymatic process with β -galactosidase enzyme. In fact, the β -galactosidase enzyme hydrolyzes the lactose and can also produce galactooligosaccharides (GOS) by

glycosyltransferase activity (Ruiz-Matute et al., 2012).

Milk base composition plays a determinative role in the fermentation process affecting the microbial viability and metabolic activity of starter culture, fermentation time and thus impacting on the characteristics of the final product (Moreira et al., 2017; Sodini, Lucas, Oliveira, Remeuf, & Corrieu, 2002). In particular, the type and content of carbohydrates depend on the variety of yogurt and their effects on the aspects mentioned above are variable and are strain-dependant. In addition, the presence of GOS in delactosed milk for preparing reduced lactose yogurt could enhance the growth and activity of the starter cultures as they are recognized as prebiotic carbohydrates (Prasad, Sherkat, & Shah, 2013). According to our knowledge, there is limited data regarding to the influence of these factors on the fermentation and volatile profiles during yogurt manufacture, in particular for delactosed yogurts.

Birollo, Reinheimer, and Vinderola (2000) studied the viability of a commercial mixed culture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (70 and 30) in five different formulations of yogurts during storage. They reported a slight inhibitory effect on lactobacilli and streptococci viability only in one type of yogurt, which was attributed to the high sugar concentration. Cutrim et al. (2016) and Moreira et al. (2017) found variable fermentation time according to the lactose

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content of the milk bases; an acceleration of the process was observed using delactosed milk with 1.5 g/100 mL of lactose whereas a delay was produced with delactosed milk containing < 0.6 g/100 mL of lactose, in comparison with yogurt made with traditional milk (approximately 4.5 g/100 mL of lactose). The low levels of lactose of the milk bases affected the metabolic activity of starter cultures as a minor lactic acid production was found (Cutrim et al., 2016); while this behavior was not revealed by Moreira et al. (2017). Wolf, Vénica, and Perotti (2015) detected minor differences in the volatile compounds among delactosed and traditional yogurts, at the end of fermentation. During storage, acetaldehyde and diketone levels were higher in delactosed yogurts than traditional products.

In this study we proposed to investigate the effect of the carbohydrate composition of the milk base on the physicochemical parameters, growth of cultures and the profiles of carbohydrates, organic acids and volatile compounds during fermentation and storage of yogurt.

2. Materials and methods

2.1. Preparation of yogurts

Delactosed and traditional drinkable yogurts (450 mL) were prepared in the laboratory from commercial lactose-hydrolyzed (fat: 3.0 g/100 mL; protein: 3.2 g/100 mL; lactose: 0.9 g/100 mL; glucose: 1.9 g/100 mL; galactose: 1.9 g/100 mL) and traditional (fat: 3.0 g/100 mL; protein: 3.2 g/100 mL; lactose: 4.5 g/100 mL; glucose and galactose: not declared in the nutritional information) ultra-high-temperature (UHT) milks (La Serenisima, Argentina), respectively. Besides, for each case, sweetened and natural yogurts were made. For sweetened yogurts, sucrose (Ingenio Ledesma S.A., Argentina) was added to the milk at a level of 8 g/100 mL, while in natural ones, sucrose was not included in the formulation. Therefore, four yogurt varieties with different carbohydrate composition of the milk bases were manufactured each day: yogurts from traditional milk and without sucrose addition (TRAD); yogurts from traditional milk and with sucrose addition (TRAD + S); yogurts from delactosed milk and without sucrose addition (DEL), and yogurts from delactosed milk and with sucrose addition (DEL + S).

Milk bases contained in screw-cap flasks were heat treated at 85 °C for 15 min and then cooled at 43 °C. At this point commercial lyophilized starter culture (YF-L811) containing *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* provided by Chr. Hansen (Argentina) was inoculated following the recommendation prescribed by the suppliers (density cells inoculated were approximately 7.0 log CFU/g for *S. thermophilus* and 2.0 log CFU/g for *L. delbrueckii* subsp. *bulgaricus*). During the incubation at 43 ± 1 °C in a water bath, the acidity was monitored periodically recording the pH; fermentation was arrested at pH ~4.70. Yogurts were immediately cooled in an ice water bath, transferred to 5 °C (0 day) and then stored for 28 days. Samples were removed from the refrigerator at different storage time and subjected to analysis.

2.2. Physicochemical determinations

pH was analyzed during fermentation (0, 150, 240 min and in freshly made yogurts (0 day) and at weekly intervals during storage for 28 days, using a digital pH meter (Orion 3 star benchtop, Thermo Fisher Scientific Inc., USA). Titratable acidity (TA), expressed as Dornic degree (1 °D = 100 mg lactic acid/L), was analyzed at weekly intervals for 28 days, by titration with 0.11 M NaOH (IDF, 2012). Protein (IDF, 2001), total solid (IDF, 2005), fat (Bradley et al., 1992) and ash (AOAC, 2005) contents of the yogurts with 7 days of storage, were determined. All assays were performed by duplicate.

2.3. Microbiological counts

Differential cells counts of starter strains (thermophilic streptococci

and thermophilic lactobacilli) were performed on fresh yogurt and after weekly during storage (28 d). Thermophilic streptococci were determined on skim milk agar and thermophilic lactobacilli were determined on acidified MRS agar (IDF, 1988). Additionally, moulds and yeast counts were performed at the end of storage in yeast extract-glucose-chloranphenicol agar (IDF, 1985). Analyses were performed by duplicate.

2.4. Chromatographic determination of carbohydrates and organic acids

The concentrations of organic acids (lactic, citric, acetic and piruvic) and carbohydrates (lactose, glucose, galactose and GOS) were determined in milk bases, in freshly made yogurts and at 21 days. Simultaneous analysis of these compounds was performed by high performance liquid chromatography (HPLC) coupled to UV and IR detectors and using Aminex HPX-87H and HPX-87N columns, according to Vénica, Perotti, and Bergamini (2014) and Vénica, Bergamini, Rebechi, and Perotti (2015). Quantification was performed by external calibration using suitable standards (Sigma Aldrich, Saint Louise, USA). Regarding the quantification of GOS, the trisaccharide raffinose was used as standard. Analyses were performed by duplicate.

2.5. Volatile compounds analysis

Volatile compounds were determined in milk bases, in freshly made yogurts and at 21 days. Sample preparation, isolation of volatile compounds from matrix, chromatographic separation, identification of compounds and semi-quantitative analysis (peak area values) were performed according to Wolf et al. (2015). Analyses were made in triplicate.

2.6. Statistical analysis

The experiment was replicated on three different days; each making day was considered as a blocking variable. To identify significant effect of carbohydrate milk composition and storage time on different parameters, one-way blocked analysis of variance (ANOVA) were conducted using SPSS software version 10.0 (SPSS Inc., Chicago, USA). Significant difference was determined with mean values comparison by Tukey test at 5% confidence level ($P < 0.05$). According to the Argentinean Legislation, total lactic acid bacteria in the final product must be active and at a high number ($> 10^7$ CFU/g) and the acidity not higher than 150°D during its shelf-life (CAA 2014). The shelf-life estimated by the Argentinean dairy industry is between 28 to 35 days. For that, we decided to analyze these parameters during refrigerated period. On the other hand, the determinations of carbohydrates and organic acids, and volatile profiles, which are not considered by the Argentinean Legislation, were made only at 21 days of storage, in order to characterize the products and describe the differences among them; in previous works, we concluded that in general the changes in these parameters are negligible toward the end of storage.

3. Results and discussion

3.1. Physicochemical parameters

Total solids were higher and fat and protein were lower ($P < 0.05$) in DEL + S and TRAD + S yogurts in comparison to DEL and TRAD products (Table 1). The mean values were 17.84, 2.6 and 2.81 g/100 g for the sweetened yogurts, and 11.08, 3.0 and 3.00 g/100 g for the natural ones, respectively. The ash contents were similar among yogurt varieties; the mean value was 0.69 g/100 g.

Initial pH of the different milk bases was similar; the mean value was 6.52 ± 0.05. The reduction of pH during fermentation was significantly lower ($P < 0.05$) in delactosed yogurts with and without sucrose in comparison with traditional ones and impacted in the

Table 1Total solids, protein, fat and ash contents in yogurts at 7 days (mean \pm standard deviation; n = 3).

Parameters (g/100 g)	Yogurt varieties			
	TRAD	TRAD + S	DEL	DEL + S
Total solids	11.34 \pm 0.28 ^b	17.81 \pm 0.35 ^a	10.83 \pm 0.22 ^b	17.88 \pm 0.21 ^a
Fat	3.0 \pm 0.1 ^a	2.6 \pm 0.1 ^b	3.0 \pm 0.1 ^a	2.6 \pm 0.1 ^b
Protein	2.99 \pm 0.02 ^a	2.88 \pm 0.04 ^b	3.01 \pm 0.01 ^a	2.75 \pm 0.05 ^b
Ash	0.71 \pm 0.02 ^a	0.68 \pm 0.01 ^a	0.67 \pm 0.01 ^a	0.68 \pm 0.01 ^a

TRAD: traditional yogurt; TRAD + S: traditional yogurt with sucrose; DEL: delactosed yogurt; DEL + S: delactosed yogurt with sucrose. Different lowercase letters superscripts depict the statistical difference ($P < 0.05$) within a row.

fermentation time. Mean values of pH for delactosed (DEL and DEL + S) and traditional (TRAD and TRAD + S) yogurts were 5.64 ± 0.05 and 5.33 ± 0.02 at 150 min and 4.82 ± 0.02 and 4.72 ± 0.01 at 240 min, respectively. The fermentation took more time in DEL samples (270 ± 5 min), intermediate times were seen in DEL + S samples (255 ± 5 min) and was faster in TRAD and TRAD + S yogurts (240 ± 5 min). Similar to our findings, [Moreira et al. \(2017\)](#) detected a shorter fermentation process in traditional natural yogurts compared to delactosed (< 0.2 g lactose/100 mL) natural yogurts, and the presence of sugar led to an increase in the fermentation time. According to [Cutrim et al. \(2016\)](#), traditional yogurt with 4.46 g of lactose/100 mL took 5.5 h and delactosed yogurts with 1.53 and 0.60 g of lactose/100 mL took 4.5 h and 6.5 h, respectively. The lower fermentation time observed in delactosed yogurts with intermediate levels of lactose could be explained by the presence of free glucose and galactose, which are more readily used by LAB, while the delay obtained in delactosed yogurt with low levels of lactose could be due to the partial inhibition of the lactose transport system into cells ([Cutrim et al., 2016](#)).

The values of pH and TA during storage of yogurts are shown in [Table 2](#) and [Fig. 1](#), respectively. As expected, pH values followed an opposite trend to that observed for TA measurements. pH values were similar among yogurt varieties from the onset up to 21 d, but DEL yogurts had higher values ($P < 0.05$) than TRAD and TRAD + S yogurts at 28 d. TA values were similar for milk bases, higher values ($P < 0.05$) for TRAD than DEL + S yogurts were recorded from 7 until 28 d. The differences in the TA values between TRAD and DEL + S yogurts were approximately 11 °D. This finding might suggest an effect of the different formulations employed on the metabolic and acidifying activity of starter culture.

On the other hand, the evolution over time followed a similar trend for all yogurts varieties, gradual decrease of pH and increase of TA ([Table 2](#) and [Fig. 1](#), respectively). In general, the most noticeable changes ($P < 0.05$) were observed in the first week of refrigerated storage and then minor modifications were recorded.

Table 2pH values of yogurts during storage at 5 °C (mean \pm standard deviation; n = 3).

Storage period (day)	Yogurt varieties			
	TRAD	TRAD + S	DEL	DEL + S
0	4.73 \pm 0.01 ^{aA}	4.72 \pm 0.01 ^{aA}	4.74 \pm 0.01 ^{aA}	4.74 \pm 0.01 ^{aA}
7	4.50 \pm 0.01 ^{aB}	4.52 \pm 0.02 ^{aAB}	4.57 \pm 0.01 ^{aB}	4.57 \pm 0.03 ^{aAB}
14	4.41 \pm 0.03 ^{aBC}	4.42 \pm 0.06 ^{aB}	4.49 \pm 0.01 ^{aB}	4.49 \pm 0.02 ^{aB}
21	4.38 \pm 0.05 ^{aC}	4.40 \pm 0.10 ^{aB}	4.48 \pm 0.05 ^{aB}	4.47 \pm 0.06 ^{aB}
28	4.40 \pm 0.05 ^{bBC}	4.41 \pm 0.09 ^{bB}	4.52 \pm 0.06 ^{aB}	4.49 \pm 0.09 ^{aB}

TRAD: traditional yogurt; TRAD + S: traditional yogurt with sucrose; DEL: delactosed yogurt; DEL + S: delactosed yogurt with sucrose.

Different lowercase letter superscripts depict the statistical difference ($P < 0.05$) within a row.

Different capital letter superscripts depict the statistical difference ($P < 0.05$) between means for each type of yogurt at different time.

3.2. Microbiological counts

Microbiological counts of thermophilic streptococci and thermophilic lactobacilli in the yogurts are shown in [Table 3](#). The different carbohydrate composition of the milk bases did not affect bacterial counts at each sampling time. For all samples, thermophilic streptococci and lactobacilli counts ranged from 9.1 to 9.3 log CFU/g and from 2.2 to 2.8 log CFU/g, respectively. Counts were not affected by cold storage during 28 d, indicating a good stability of the starter culture in the different matrices.

These results were similar to those found by [Vinderola and Reinheimer \(2000\)](#), who reported concentrations of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* ranging from 8 to 9 log CFU/g and from 2 to 7 log CFU/g, respectively, in Argentinean fermented dairy products. The use of industrial starter with dominant *S. thermophilus* is common in the local market, and allows obtaining yogurts with a reduced acidity and with lesser risks of postacidification. Moulds and yeasts counts at the end of the storage were always lower than 20 CFU/g.

3.3. Carbohydrates and organic acids

The contents of carbohydrates and organic acids were monitored during fermentation and storage of yogurts ([Table 4](#)). As expected, significant differences ($P < 0.05$) in the carbohydrate composition among samples were observed at each sampling time. In milk bases, lactose was higher and glucose and galactose were lower in TRAD and TRAD + S in comparison to DEL and DEL + S; GOS was only detected in DEL and DEL + S samples. Towards the end of fermentation, the carbohydrates concentration changed in all cases as a result of starter culture activity. Lactose contents were different ($P < 0.05$) among yogurts at 0 and 21 d; the highest value was for TRAD + S, followed by TRAD, DEL + S and DEL yogurts. The amounts of glucose and galactose were higher ($P < 0.05$) in both varieties of delactosed yogurts than traditional ones, at 0 and 21 d. GOS were higher in DEL + S and DEL samples while were absent in TRAD and TRAD + S samples both in the fresh products and at 21 d. The concentration of glucose was lower than galactose in TRAD, TRAD + S and DEL yogurts at 0 and 21 d, while the glucose was higher than galactose in DEL + S samples.

The evolution of carbohydrates over time was different among yogurt varieties ([Table 4](#)). The lactose decline was less pronounced in sweetened yogurts than in natural ones. In fact, lactose decreased ($P < 0.05$) 24% and 44% in freshly made yogurt (0 d) in comparison with milk base for TRAD and DEL yogurts, respectively, and no change was produced toward 21 d. For TRAD + S, the diminution (17%) was only evident during storage ($P < 0.05$) (from 0 to 21 d). For DEL + S, no change was detected. Glucose content decreased significantly ($P < 0.05$) in DEL yogurts from milk base to 21 d (22%). For the rest of yogurts no changes were observed. Regarding galactose content, significant increases ($P < 0.05$) were produced during fermentation and storage in TRAD and TRAD + S yogurts, whereas the accumulation of galactose was negligible in DEL and DEL + S products. The content of GOS remained unchanged in delactosed yogurts.

These results demonstrate that the carbohydrates composition of the

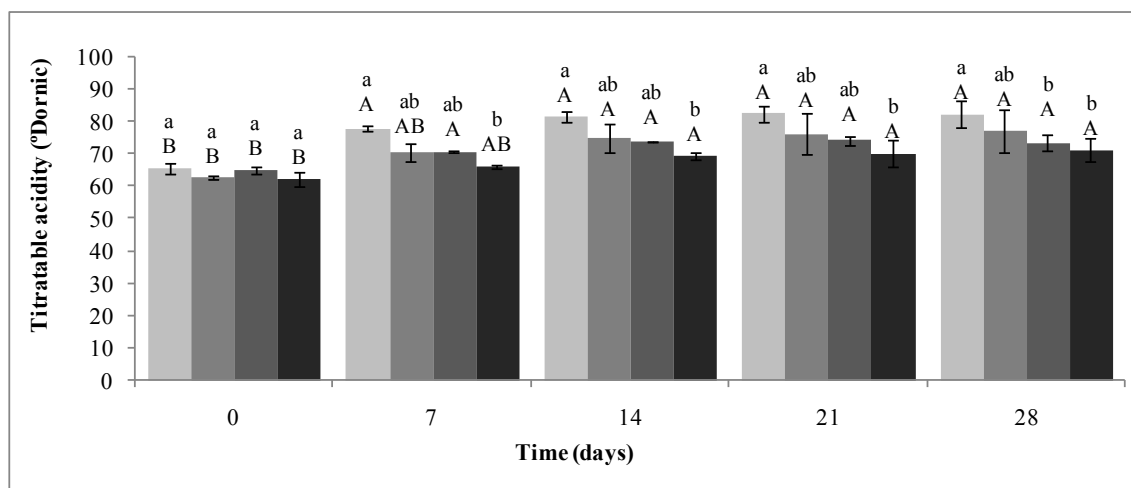


Fig. 1. Titratable acidity of yogurts during storage at 5 °C (mean \pm standard deviation; n = 3).

TRAD: traditional yogurt (■); TRAD + S: traditional yogurt with sucrose (■); DEL: delactosed yogurt (■); DEL + S: delactosed yogurt with sucrose (■). Mean values with different lowercase letter within the same time are significantly different ($P < 0.05$). Mean values with different capital letter in the same yogurt variety are significantly different ($P < 0.05$).

Table 3

Differential starter strains counts performed on yogurts during storage at 5 °C (mean \pm standard deviation; n = 3).

Storage time (days)	Microbiological counts (log CFU/g) ^a							
	Thermophilic streptococci				Thermophilic lactobacilli			
	TRAD	TRAD + S	DEL	DEL + S	TRAD	TRAD + S	DEL	DEL + S
0	9.25 \pm 0.06	9.34 \pm 0.02	9.27 \pm 0.04	9.13 \pm 0.04	2.27 \pm 0.25	2.75 \pm 0.47	2.70 \pm 0.14	2.81 \pm 0.46
7	9.18 \pm 0.03	9.22 \pm 0.07	9.17 \pm 0.04	9.23 \pm 0.20	2.70 \pm 0.25	2.56 \pm 0.28	2.79 \pm 0.04	2.63 \pm 0.30
14	9.25 \pm 0.06	9.19 \pm 0.14	9.27 \pm 0.04	9.26 \pm 0.04	2.39 \pm 0.50	2.27 \pm 0.57	2.55 \pm 0.84	2.68 \pm 0.42
21	9.24 \pm 0.12	9.25 \pm 0.05	9.26 \pm 0.03	9.26 \pm 0.03	2.29 \pm 0.46	2.10 \pm 0.66	2.59 \pm 0.43	2.58 \pm 0.40
28	9.28 \pm 0.02	9.30 \pm 0.16	9.33 \pm 0.10	9.20 \pm 0.05	2.39 \pm 0.12	2.21 \pm 0.09	2.52 \pm 0.25	2.29 \pm 0.36

TRAD: traditional yogurt; TRAD + S: traditional yogurt with sucrose; DEL: delactosed yogurt; DEL + S: delactosed yogurt with sucrose.

^a No statistical differences ($P > 0.05$) were observed between treatment and through time for thermophilic streptococci and lactobacilli according to one-way blocked analysis of variance.

milk bases affected the metabolic activity of starter culture during yogurt making by different ways. In particular, in DEL + S yogurts no changes in the level of lactose, glucose, galactose and GOS were observed, which could suggest that the starter employed the sucrose as a carbon source. This fact could also have occurred in TRAD + S yogurts, since a minor use of lactose was observed in this type of yogurt compared with TRAD yogurts. Comparable results in relation to the evolution of the carbohydrates profiles and the effect of sucrose in reduced lactose yogurt were obtained by Moreira et al. (2017) and Cutrim et al. (2016). Regarding GOS, its absence in the traditional yogurts and the lack of changes in the concentrations in the delactosed yogurts revealed the incapacity of starter culture used in our study to form and consume them, respectively, during fermentation and storage. Our results are disagree with the findings of Prasad et al. (2013) who found an improvement of the growth of starter culture, a shorter fermentation time and higher concentration of lactic and acetic acids by the addition of GOS (2%) in yogurt making.

The performance of the different reactions involved in the carbohydrate metabolism of LAB depends essentially on the nature of the carbon source and its concentration, and impact in the metabolite production (Bennama, Ladero, Alvarez, Fernández, & Bensoltane, 2012). Lactose fermentation by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* begins with the transport of lactose across the cell membrane. Lactose is split to glucose and galactose by a cytoplasmic β -galactosidase present in both species. Glucose is phosphorylated by glucokinase to glucose-6-phosphate, and is further metabolized by glycolysis (Tamime & Robinson, 2007). Most strains of *S. thermophilus* and *L.*

delbrueckii subsp. *bulgaricus* are unable to ferment galactose, excreting it into the medium (Anbukkarasi et al., 2014). However, according to Sobowale, Efuntoy, and Adesetan (2011) the rate of uptake of one sugar in solution by LAB generally depends on presence or absence of another sugar. In effect, they showed that four strains of *S. thermophilus* and one strain of *L. bulgaricus* consumed galactose in the presence of sucrose, suggesting that sucrose could act as an enhancer for galactose uptake by galactose non-fermentative yogurts strains. In addition, Bennama et al. (2012) showed that three strains of *S. thermophilus* displayed a high level of growth in M17 medium added with sucrose and a decrease in the pH was observed. Nevertheless, *S. thermophilus* growth on sucrose is less efficient and an inhibitory effect at high content (10–12%) due to an adverse osmotic effect and a low water activity has often been reported (Bennama et al., 2012; Zourari, Accolas, & Desmazeaud, 1992).

On the other hand, the concentration of organic acids was generally similar for the four varieties of yogurts during incubation and storage. Differences were only detected for lactic acid in the milk bases and in the yogurts at 21 d. In particular, TRAD and TRAD + S milk bases had higher ($P < 0.05$) contents than DEL and DEL + S milk bases. At 21 d, both traditional yogurts had higher content of lactic acid ($P < 0.05$) than DEL + S yogurts. Citric was the second important acid found in all yogurt varieties, followed by pyruvic and acetic.

With regard to the evolution of organic acids the deeper changes were seen for lactic. The concentration was increased ($P < 0.05$) from the milk bases until the end of fermentation and then the values increased in a lesser extend ($P > 0.05$) at 21 d, in all yogurt varieties. In

Table 4

Carbohydrates (g/100 g) and organic acids (mg/100 g) in milk bases (MB), freshly made yogurts (0 d) and yogurts at 21 days (21 d) of storage (mean \pm standard deviation; n = 3).

Parameter	Time	Yogurt varieties			
		TRAD	TRAD + S	DEL	DEL + S
Lactose	MB	4.42 \pm 0.02 ^{2A}	4.27 \pm 0.11 ^{1A}	0.78 \pm 0.01 ^{1B}	0.75 \pm 0.02 ^{1B}
	0 d	3.36 \pm 0.27 ^{2B}	4.00 \pm 0.05 ^{1A}	0.44 \pm 0.02 ^{2B}	0.74 \pm 0.07 ^{1A}
	21 d	2.93 \pm 0.06 ^{2B}	3.53 \pm 0.03 ^{2B}	0.42 \pm 0.02 ^{2B}	0.66 \pm 0.04 ^{1A}
Glucose	MB	0.18 \pm 0.02 ^{2B}	0.19 \pm 0.01 ^{1A}	2.09 \pm 0.02 ^{1A}	1.96 \pm 0.01 ^{1A}
	0 d	0.17 \pm 0.02 ^{2B}	0.16 \pm 0.03 ^{1A}	1.75 \pm 0.01 ^{1B}	1.97 \pm 0.11 ^{1A}
	21 d	0.26 \pm 0.02 ^{1A}	0.23 \pm 0.01 ^{1A}	1.62 \pm 0.04 ^{1B}	1.93 \pm 0.06 ^{1A}
Galactose	MB	0.02 \pm 0.01 ^{1C}	0.17 \pm 0.01 ^{1B}	1.73 \pm 0.01 ^{1A}	1.68 \pm 0.02 ^{1A}
	0 d	0.52 \pm 0.06 ^{2B}	0.31 \pm 0.05 ^{2B}	1.91 \pm 0.05 ^{1A}	1.81 \pm 0.09 ^{1A}
	21 d	0.78 \pm 0.07 ^{1A}	0.51 \pm 0.02 ^{1A}	1.89 \pm 0.12 ^{1A}	1.82 \pm 0.01 ^{1A}
GOS	MB	Nd ^{1A}	Nd ^{1A}	0.25 \pm 0.02 ^{1A}	0.23 \pm 0.01 ^{1A}
	0 d	Nd ^{1A}	Nd ^{1A}	0.24 \pm 0.01 ^{1A}	0.22 \pm 0.01 ^{1A}
	21 d	Nd ^{1A}	Nd ^{1A}	0.24 \pm 0.01 ^{1A}	0.23 \pm 0.02 ^{1A}
Lactic	MB	56.46 \pm 12.26 ^{1B}	53.41 \pm 1.38 ^{1B}	10.30 \pm 0.43 ^{2B}	15.96 \pm 4.75 ^{2B}
	0 d	682.62 \pm 75.90 ^{1A}	693.13 \pm 0.65 ^{1A}	653.60 \pm 50.96 ^{1A}	540.84 \pm 41.60 ^{1A}
	21 d	939.95 \pm 107.70 ^{1A}	866.37 \pm 129.84 ^{1A}	799.35 \pm 90.20 ^{1A}	661.17 \pm 41.62 ^{1A}
Citric	MB	197.84 \pm 8.43 ^{1A}	187.99 \pm 1.17 ^{1A}	223.95 \pm 0.25 ^{1A}	186.12 \pm 2.84 ^{1A}
	0 d	213.43 \pm 27.71 ^{1A}	192.30 \pm 0.81 ^{1A}	214.72 \pm 25.48 ^{1A}	232.20 \pm 18.04 ^{1A}
	21 d	204.40 \pm 10.32 ^{1A}	216.10 \pm 28.24 ^{1A}	207.46 \pm 10.33 ^{1A}	231.18 \pm 14.38 ^{1A}
Pyruvic	MB	1.86 \pm 0.14 ^{1B}	1.48 \pm 0.06 ^{1A}	1.70 \pm 0.20 ^{1B}	0.95 \pm 0.83 ^{1B}
	0 d	6.85 \pm 1.50 ^{1A}	6.21 \pm 1.92 ^{1A}	6.75 \pm 1.31 ^{1A}	5.75 \pm 0.46 ^{1A}
	21 d	6.09 \pm 1.04 ^{1A}	5.47 \pm 0.68 ^{1A}	6.06 \pm 0.89 ^{1A}	4.41 \pm 0.47 ^{1A}
Acetic	MB	2.87 \pm 0.01 ^{1B}	3.30 \pm 0.61 ^{1A}	3.18 \pm 0.50 ^{1A}	2.86 \pm 0.01 ^{1B}
	0 d	4.51 \pm 0.26 ^{1A}	4.01 \pm 0.44 ^{1A}	4.80 \pm 0.28 ^{1A}	4.40 \pm 0.09 ^{1A}
	21 d	4.64 \pm 0.69 ^{1A}	4.25 \pm 0.81 ^{1A}	4.85 \pm 0.75 ^{1A}	4.34 \pm 0.42 ^{1A}

TRAD: traditional yogurt; TRAD + S: traditional yogurt with sucrose; DEL: delactosed yogurt; DEL + S: delactosed yogurt with sucrose. Nd: not detected.

Different lowercase letter superscripts depict the statistical difference ($P < 0.05$) within a row.

Different capital letter superscripts depict the statistical difference ($P < 0.05$) between means for each type of yogurt at different time.

particular, for TRAD and TRAD + S yogurts the value was 12 times higher at 0 days than those in the milk bases, while for DEL + S and DEL yogurts the content was 34 and 65 times higher, respectively. From 0 to 21 d, the increment was only 1.2 times for all yogurts varieties. Content of citric acid remained almost unchanged during fermentation and storage. Contents of pyruvic and acetic acids generally exhibited an increase throughout the fermentation period and then remain constant until the end of storage.

The minor content of lactic acid in delactosed yogurt compared to traditional ones we have already seen in a previous work (Vénica et al., 2014), in which we also reviewed the results of organic acids profiles in traditional and delactosed, natural and sweetened yogurts obtained by several authors. Similarly, Moreira et al. (2017) reported lower concentration of lactic acid in traditional and delactosed sweetened yogurts than traditional and delactosed natural ones. Likewise, Cutrim et al. (2016) found higher levels of lactic acid in traditional yogurts in comparison with delactosed yogurts.

3.4. Volatile compound analysis

The 21 volatile compounds identified in the yogurt samples are shown in Table 5. They belonged to the chemical families of ketones, alcohols, aldehydes, esters and acids. All products contained the major volatile compounds reported as responsible for imparting desirable flavor to yogurt, such as 2-propanone, 2-butanone, 2,3-butanedione or diacetyl, 2,3-pentanedione, 3-hydroxy-2-butanone or acetoin, ethanol, acetaldehyde, acetic acid, among others (Alonso & Fraga, 2001; Güler & Park, 2011; Laye, Karleskind, & Morr, 1993; Ott, Fay, & Chaintreau, 1997).

Ketones: The content of some ketones such as 2-propanone, 2-butanone, 3-hydroxy-2-butanone and 2-pentanone was similar among the different varieties at each sampling time. The diketones production was the most affected by the medium composition. 2,3-butanedione was found at the highest level in TRAD + S milk bases, but its content was higher in DEL + S yogurts than the rest of the samples at the end of fermentation and during storage. 2,3-pentanedione was not detected in any of the milk bases. DEL + S yogurts contained the highest level at the end of fermentation, similarly to that observed for diacetyl. At 21 d, the content of 2,3-pentanedione was statistically different among the different samples, which followed the decreasing order: DEL + S > TRAD + S > DEL > TRAD. Statistical differences ($P < 0.05$) in 2-heptanone and 2-nonanone were only detected in the milk bases; DEL yogurts had the higher level than the rest of products. 2-hexanone content varied among samples at the end of fermentation; DEL + S yogurts exhibited the highest values.

Ketones play an important role in the aroma of fermented dairy products. In particular, 2,3-butanedione and 2,3-pentanedione have been reported as impact compounds of yogurt (Schlichtherle-Cerny, Oberholzer, & Zehnter, 2008). Different metabolic pathways have been proposed for diketones. Some authors suggested that they are produced during conversion of citrate to pyruvate by specific bacteria citrate (+) and then pyruvate is metabolized to α -acetolactate, the precursor of diacetyl. However, some studies show that this route is unlikely in yogurt. In fact, de Leonardis, Lopez, Nag, and Macciola (2013) reported that the added citrate in delactosed UHT did not improve the diacetyl production. Similarly, Monnet and Corrieu (2007) and Ott, Germond, and Chaintreau (2000b) proposed that biosynthesis of diacetyl come only from pyruvate derived from glucose since thermophilic starter

Table 5

Volatile compounds and peak area values ($\times 10^3$) in milk bases (MB), freshly made yogurts (0 d) and yogurts at 21 days (21 d) of storage (mean \pm standard deviation; n = 3).

Volatile compounds	Time	Yogurt varieties			
		TRAD	TRAD + S	DEL	DEL + S
KETONES					
2-propanone	MB	198.4 \pm 5.7 ^{aA}	186.0 \pm 18.1 ^{aA}	186.7 \pm 6.8 ^{aA}	191.7 \pm 5.4 ^{aA}
	0 d	202.9 \pm 11.8 ^{aA}	169.9 \pm 13.5 ^{aA}	179.4 \pm 13.2 ^{aA}	190.7 \pm 14.7 ^{aA}
	21 d	202.1 \pm 32.1 ^{aA}	200.0 \pm 19.6 ^{aA}	191.8 \pm 16.6 ^{aA}	197.7 \pm 23.9 ^{aA}
2-butanone	MB	93.9 \pm 4.2 ^{aA}	96.2 \pm 8.8 ^{aA}	95.4 \pm 8.0 ^{aA}	97.8 \pm 4.6 ^{aA}
	0 d	113.0 \pm 10.6 ^{aA}	89.4 \pm 9.0 ^{aA}	91.2 \pm 13.0 ^{aA}	109.1 \pm 6.8 ^{aA}
	21 d	103.1 \pm 11.1 ^{aA}	103.0 \pm 9.0 ^{aA}	89.4 \pm 10.0 ^{aA}	94.1 \pm 8.6 ^{aA}
2-pentanone	MB	29.0 \pm 2.8 ^{aB}	25.3 \pm 1.8 ^{aB}	32.3 \pm 1.5 ^{aA}	28.9 \pm 2.4 ^{aA}
	0 d	47.6 \pm 3.3 ^{aA}	41.8 \pm 3.5 ^{aA}	43.8 \pm 6.6 ^{aA}	44.1 \pm 5.8 ^{aA}
	21 d	46.9 \pm 5.4 ^{aA}	47.5 \pm 4.8 ^{aA}	36.5 \pm 3.0 ^{aA}	41.9 \pm 5.0 ^{aA}
2,3-butanedione	MB	124.2 \pm 10.7 ^{bB}	177.2 \pm 14.2 ^{aB}	107.8 \pm 4.5 ^{bB}	138.8 \pm 12.7 ^{bA}
	0 d	119.3 \pm 5.3 ^{bB}	156.7 \pm 16.7 ^{bB}	117.8 \pm 10.9 ^{bB}	241.8 \pm 9.7 ^{aB}
	21 d	394.7 \pm 16.7 ^{bA}	408.6 \pm 24.2 ^{bA}	319.5 \pm 20.2 ^{cA}	448.5 \pm 11.5 ^{aC}
2,3-pentanedione	MB	Nd ^{aA}	Nd ^{aA}	Nd ^{aA}	Nd ^{aA}
	0 d	103.3 \pm 11.6 ^{bB}	118.2 \pm 3.9 ^{bB}	112.6 \pm 9.3 ^{bB}	177.2 \pm 10.9 ^{aB}
	21 d	190.4 \pm 16.4 ^{dC}	356.3 \pm 4.2 ^{bC}	280.1 \pm 19.0 ^{cC}	434.8 \pm 15.3 ^{aC}
2-hexanone	MB	36.0 \pm 3.7 ^{aA}	40.2 \pm 3.6 ^{aA}	28.1 \pm 0.7 ^{aA}	32.2 \pm 6.5 ^{aA}
	0 d	17.2 \pm 2.3 ^{bB}	19.9 \pm 2.2 ^{bB}	28.7 \pm 2.3 ^{abA}	39.7 \pm 6.3 ^{aA}
	21 d	28.2 \pm 3.2 ^{aAB}	31.7 \pm 2.6 ^{aAB}	37.8 \pm 6.1 ^{aA}	40.2 \pm 7.4 ^{aA}
2-heptanone	MB	232.6 \pm 19.1 ^{bB}	262.3 \pm 17.2 ^{bB}	335.4 \pm 7.6 ^{aB}	248.0 \pm 3.1 ^{bB}
	0 d	409.1 \pm 26.9 ^{aA}	385.1 \pm 26.4 ^{aA}	409.6 \pm 12.4 ^{aA}	396.8 \pm 30.0 ^{aA}
	21 d	414.3 \pm 23.4 ^{aA}	381.4 \pm 18.9 ^{aA}	378.7 \pm 15.9 ^{aA}	359.6 \pm 22.9 ^{aA}
3-hydroxy-2-butanone	MB	Nd ^{aB}	Nd ^{aB}	Nd ^{aB}	Nd ^{aB}
	End	238.8 \pm 23.0 ^{aA}	242.1 \pm 24.8 ^{aA}	224.2 \pm 9.0 ^{aA}	259.8 \pm 12.8 ^{aA}
	21 d	247.6 \pm 19.0 ^{aA}	256.8 \pm 20.3 ^{aA}	223.5 \pm 15.4 ^{aA}	256.6 \pm 6.0 ^{aA}
2-nonanone	MB	34.5 \pm 4.2 ^{bB}	41.7 \pm 4.0 ^{bB}	60.6 \pm 1.9 ^{aB}	42.2 \pm 1.4 ^{bB}
	0 d	67.7 \pm 7.7 ^{aA}	65.0 \pm 1.7 ^{aA}	77.8 \pm 2.5 ^{aA}	69.6 \pm 5.3 ^{aA}
	21 d	72.0 \pm 5.3 ^{aA}	60.2 \pm 1.7 ^{aA}	75.9 \pm 5.1 ^{aA}	65.7 \pm 5.2 ^{aA}
ALCOHOLS					
ethanol	MB	28.0 \pm 2.7 ^{aA}	35.5 \pm 1.0 ^{aA}	39.3 \pm 3.7 ^{aA}	34.3 \pm 3.4 ^{aB}
	End	19.5 \pm 1.5 ^{aB}	21.2 \pm 3.6 ^{aB}	21.9 \pm 2.7 ^{aB}	26.8 \pm 2.9 ^{aB}
	21 d	33.5 \pm 2.3 ^{bA}	36.6 \pm 2.7 ^{bA}	38.2 \pm 2.9 ^{bA}	52.4 \pm 3.4 ^{aA}
1-propanol	MB	43.3 \pm 1.7 ^{aA}	40.9 \pm 2.9 ^{aA}	41.2 \pm 3.7 ^{aA}	38.8 \pm 2.6 ^{aA}
	0 d	17.2 \pm 2.3 ^{aB}	15.2 \pm 2.2 ^{aB}	16.3 \pm 2.2 ^{aB}	19.0 \pm 2.3 ^{aB}
	21 d	18.2 \pm 3.8 ^{aB}	13.6 \pm 2.1 ^{aB}	19.1 \pm 2.1 ^{aB}	21.7 \pm 2.1 ^{aB}
1-pentanol	MB	Nd ^{aC}	Nd ^{aB}	Nd ^{aB}	Nd ^{aC}
	0 d	44.8 \pm 4.5 ^{abB}	55.8 \pm 3.7 ^{aA}	53.1 \pm 6.7 ^{abA}	36.3 \pm 3.2 ^{bB}
	21 d	66.7 \pm 5.8 ^{aA}	60.1 \pm 2.5 ^{aA}	53.9 \pm 1.5 ^{aA}	58.4 \pm 3.0 ^{aA}
1-hexanol	MB	Nd ^{aB}	Nd ^{aB}	Nd ^{aB}	Nd ^{aB}
	0 d	11.3 \pm 1.5 ^{aA}	17.8 \pm 1.7 ^{aA}	12.9 \pm 1.7 ^{aA}	16.4 \pm 1.7 ^{aA}
	21 d	13.8 \pm 1.4 ^{aA}	19.7 \pm 3.6 ^{aA}	18.1 \pm 2.9 ^{aA}	21.0 \pm 1.7 ^{aA}
ALDEHYDES					
acetaldehyde	MB	Nd ^{aB}	Nd ^{aC}	Nd ^{aB}	Nd ^{aB}
	0 d	146.4 \pm 20.8 ^{bA}	259.6 \pm 23.8 ^{aA}	97.5 \pm 14.7 ^{bA}	149.5 \pm 17.6 ^{bA}
	21 d	128.0 \pm 9.6 ^{aA}	150.3 \pm 22.8 ^{aB}	104.3 \pm 16.0 ^{aA}	156.3 \pm 19.6 ^{aA}
ESTERS					
Ethyl butanoate	MB	59.5 \pm 12.8 ^{aA}	55.3 \pm 6.0 ^{aA}	56.5 \pm 4.7 ^{aA}	51.3 \pm 4.2 ^{aA}
	0 d	55.5 \pm 5.5 ^{aA}	49.7 \pm 4.9 ^{aA}	45.8 \pm 5.1 ^{aA}	43.4 \pm 6.6 ^{aA}
	21 d	50.4 \pm 2.0 ^{bA}	32.2 \pm 3.2 ^{bB}	52.6 \pm 6.3 ^{bA}	69.5 \pm 3.4 ^{aA}

(continued on next page)

Table 5 (continued)

Volatile compounds	Time	Yogurt varieties			
		TRAD	TRAD + S	DEL	DEL + S
Ethyl hexanoate	MB	6.2 ± 0.9 ^{aA}	7.3 ± 1.0 ^{aA}	7.5 ± 1.4 ^{aB}	6.4 ± 0.5 ^{aB}
	0 d	11.9 ± 2.9 ^{aA}	9.1 ± 1.5 ^{aA}	9.2 ± 0.6 ^{aAB}	8.4 ± 1.1 ^{aB}
	21 d	8.8 ± 0.6 ^{cA}	7.9 ± 0.8 ^{cA}	15.2 ± 1.9 ^{bA}	29.8 ± 3.6 ^{aA}
ACIDS					
Acetic acid	MB	Nd ^{aC}	Nd ^{aC}	Nd ^{aC}	Nd ^{aC}
	0 d	44.1 ± 2.2 ^{aB}	41.3 ± 2.1 ^{aB}	51.2 ± 6.6 ^{aB}	35.8 ± 3.6 ^{aB}
	21 d	74.8 ± 2.9 ^{aA}	63.7 ± 2.9 ^{aA}	72.5 ± 5.5 ^{aA}	48.9 ± 2.1 ^{bA}
Butanoic acid	MB	Nd ^{aC}	Nd ^{aC}	Nd ^{aC}	Nd ^{aC}
	End	91.3 ± 3.5 ^{aB}	74.2 ± 1.7 ^{bB}	68.3 ± 3.1 ^{bB}	55.3 ± 2.8 ^{cB}
	21 d	163.2 ± 4.7 ^{aA}	135.0 ± 4.2 ^{bA}	107.4 ± 2.6 ^{cA}	99.9 ± 5.1 ^{cA}
Hexanoic acid	MB	11.1 ± 2.0 ^{aC}	8.1 ± 0.7 ^{aC}	7.5 ± 1.2 ^{aC}	8.1 ± 0.5 ^{aC}
	0 d	300.3 ± 10.4 ^{aB}	244.4 ± 8.4 ^{bB}	225.0 ± 10.7 ^{bB}	178.8 ± 4.8 ^{cB}
	21 d	472.0 ± 7.3 ^{aA}	413.1 ± 14.6 ^{bA}	329.1 ± 5.1 ^{cA}	301.2 ± 6.7 ^{cA}
Octanoic acid	MB	23.7 ± 1.6 ^{aC}	18.1 ± 1.1 ^{aC}	19.4 ± 1.0 ^{aC}	17.5 ± 3.0 ^{aC}
	0 d	203.0 ± 2.8 ^{aB}	158.8 ± 4.5 ^{bB}	151.7 ± 5.6 ^{bB}	126.5 ± 5.5 ^{cB}
	21 d	242.5 ± 10.9 ^{aA}	210.8 ± 8.3 ^{bA}	185.3 ± 6.4 ^{cA}	179.9 ± 6.1 ^{cA}
Decanoic acid	MB	25.8 ± 4.2 ^{aB}	26.1 ± 3.6 ^{aB}	20.2 ± 2.7 ^{aB}	26.2 ± 1.1 ^{aB}
	0 d	78.9 ± 2.1 ^{aA}	64.1 ± 4.2 ^{bA}	61.0 ± 2.6 ^{bA}	46.3 ± 2.6 ^{cA}
	21 d	76.0 ± 4.9 ^{aA}	65.0 ± 2.2 ^{bA}	64.1 ± 1.6 ^{bA}	56.0 ± 3.3 ^{cA}

TRAD: traditional yogurt; TRAD + S: traditional yogurt with sucrose; DEL: delactosed yogurt; DEL + S: delactosed yogurt with sucrose. Nd: no detected.

Different lowercase letter superscripts depict the statistical difference ($P < 0.05$) within a row.

Different capital letter superscripts depict the statistical difference ($P < 0.05$) between means for each type of yogurt at different time.

cultures are not able to use citrate. Taking into account that the glucose is the main precursor of diketones and that the carbohydrate profiles change in different way during fermentation and storage depending on the yogurt variety, differences in diketones content for the yogurts assayed are expected. In the present work, higher levels of 2,3-butanedione and 2,3-pentanedione were detected in DEL + S yogurts at 21 d, which was in accordance with the higher concentration of glucose. Similar results were obtained in previous studies (Wolf et al., 2015). Unlike diketones, the presence of methyl ketones in yogurt is related to lipolytic activity of starter or it can come from milk (Güler & Park, 2011). It is well known that the lipolytic activity as well as the ability to metabolize the fatty acids by yogurt culture is low. This finding was in agreement with our results, which revealed only minor differences in the levels of all methyl ketones at each sampling time.

During fermentation and storage, the behavior of ketones showed a different trend depending on the type of ketone and the type of yogurt. 2-pentanone increased ($P < 0.05$) in traditional yogurts at the end of fermentation and remained unchanged toward 21 d, whereas in delactosed products the levels were similar from the onset up to the end of storage. The content of 2-heptanone and 2-nonanone in all yogurts showed a statistical increase at the end of fermentation, then the values remained unchanged until 21 d. The evolution of diacetyl showed a similar trend for TRAD, TRAD + S and DEL yogurts; they had a significant increase at 21 d. By contrast, DEL + S yogurts exhibited a marked increase ($P < 0.05$) in diacetyl content during the studied period.

The variations in the levels of 3-hydroxy-2-butanone and 2,3-pentanedione throughout the fermentation and storage was found to be independent of the yogurt type; for acetoin a marked increase was recorded only at the end of fermentation, whereas 2,3-pentanedione level increased from milk bases to 21 d. The production of diketones during storage of yogurts has been previously observed (Wolf et al., 2015).

Alcohols: Among this group, linear-chain primary alcohols such as ethanol, 1-propanol, 1-pentanol and 1-hexanol, were mainly detected. The area values of the majority of alcohols did not differ among yogurts at each sampling time, with exception of ethanol and 1-pentanol. In

particular, the highest value of ethanol ($P < 0.05$) was found in DEL + S yogurts at 21 d.

Primary alcohols ($C > 2$) can be originate from the corresponding aldehydes derived from fatty acid metabolism or can be provided by milk (Imhof, Glättli, & Bosset, 1994). By contrast, ethanol may be formed by the reduction of acetaldehyde, a typical compound derived from glucose metabolism (Cheng, 2010). The higher level of glucose found in DEL + S yogurts could explain the higher production of ethanol.

Regarding to the effect of fermentation and storage on alcohols levels, some similarities were observed among yogurts. Overall, ethanol and 1-propanol decreased toward the end of fermentation and, increased (ethanol) or remained unchanged (1-propanol) at 21 d. In the case of 1-pentanol and 1-hexanol, their levels were only increased at 0 d. Studies about the evolution of alcohols in yogurts during shelf-life have reported different trends (Georgala, Tsakalidou, Kandarakis, & Kalantzopoulos, 1995; Güler, 2007; Kaminarides, Stamou, & Massouras, 2007; Laye et al., 1993).

Aldehydes: only acetaldehyde was identified in yogurt samples and its presence was not evidenced in the milk bases. Differences in acetaldehyde content among yogurts were only detected at 0 d. At this time, TRAD + S yogurts had the highest values. The evolution of acetaldehyde showed a general trend to increase during incubation in all yogurts. At 21 d, a marked decrease ($P < 0.05$) was detected in TRAD + S yogurts, whereas for the rest of products the changes were not significant. This behavior of acetaldehyde during processing or shelf-life of yogurts is not surprising. Other authors have reported that its concentration decreased (Cruz et al., 2012; Gaafar, 1992; Guven, Yasar, Karaca, & Hayaloglu, 2005; Güler & Gürsoy-Balci, 2011; Laye et al., 1993), increased (Pourahmad & Assadi, 2007) or remained unchanged (Conurso, Verzera, Romeo, Ziino, & Conte, 2008).

Acetaldehyde is a predominant volatile compound produced by yogurt starter cultures (Beshkova, Simova, Frengova, & Simov, 1998) and it is considered to have an impact on the aroma of fermented milks (Imhof et al., 1994). Today, several pathways for acetaldehyde production in yogurts by LAB have been reported and it is possible that

more than one metabolic route operate simultaneously (Chaves et al., 2002), although some authors point out that glucose is its main precursor during milk fermentation (Ott, Germond, & Chaintreau, 2000a). According to this, differences in acetaldehyde levels between traditional and delactosed products could be expected. In fact, higher levels of acetaldehyde in delactosed than in traditional yogurts both at the end of fermentation and during storage were previously detected (Wolf et al., 2015). Nevertheless, the results of the current study did not demonstrate such dependence. The reasons for this discrepancy may be diverse; the acetaldehyde concentration in yogurt is affected by several factors such as the thermal treatment of milk, fat and carbohydrate contents, presence of protein fortifiers, hydrocolloids, etc. (Soukoulis, Panagiotidis, Koureli, & Tzia, 2007). Besides, the acetaldehyde accumulation in the growth medium is dependent on the enzymatic activities of starter culture which convert this compound to other metabolites, mainly ethanol, and the redox potential of medium (Cruz et al., 2012; Lees & Jago, 1978; Martin et al., 2011).

Esters: two ethyl esters were detected in yogurt samples: ethyl butanoate and ethyl hexanoate. Their levels were similar in the different milk bases and yogurts at the end of fermentation. At 21 d, DEL + S yogurts reached the highest values. At 0 d, the levels of ethyl esters were similar to milk bases for all yogurts. During storage, ethyl butanoate decreased only in TRAD + S yogurts, while ethyl hexanoate increased in both delactosed products ($P < 0.05$).

Due to the ethyl esters are originated from the enzymatic or chemical esterification of acids with ethanol (Cheng, 2010), their contents in fermented products are related to the precursors (acids and ethanol) levels and esterases activities of starter. The bioavailability of ethanol is regarded as the limiting factor of ester synthesis (Thierry, Maillard, Richoux, & Lortal, 2006). In our study a relationship between level of ethanol and ethyl ester was verified. In fact, the highest levels of ethyl esters detected at 21 d in DEL + S yogurts were correlated with their higher levels of ethanol.

Acids: among acid group, linear chain fatty acids of even number of carbons from C₂ to C₁₀, were identified. Acetic acid was not detected in any of the milk bases and its content was similar in all yogurt samples at the end of fermentation. At 21 d, the level reached in DEL + S products was the lowest. Acetic acid is considered a common end product in the breakdown of glucose and it is metabolically related to acetaldehyde and ethanol. This fact may explain the higher ethanol content and lower acetic acid level detected in DEL + S yogurts at 21 d.

Butanoic, hexanoic and octanoic acids had the highest peak area values in TRAD and TRAD + S yogurts and the lowest values in DEL + S yogurts at the end of fermentation ($P < 0.05$). At 21 d, the highest levels were again found in traditional yogurts, whereas DEL and DEL + S yogurts had the lowest levels. With the exception of decanoic acid, the fatty acids increased ($P < 0.05$) substantially, regardless of the yogurt variety, from the onset up to 21 d. These acids are produced from milk fat hydrolysis by lipolytic activity of starter culture. The increase observed during fermentation and storage was in accordance with our previous study (Wolf et al., 2015) and with those reported by other researchers (Gaafar, 1992; Guven et al., 2005; Güler & Gürsoy-Balci, 2011).

4. Conclusions

The composition of milk base employed for yogurt making plays an important role in the fermentation process which could impact on the metabolic activity of the starter culture and thus on the characteristics of the product.

The results of this study showed that the proportion of carbohydrates consumed by microorganisms during fermentation and storage was dependent of the carbohydrates composition of the milk base. A delay in the fermentation time and a slight minor postacidification (pH, TA and lactic acid content) in delactosed yogurts compared to yogurts made with traditional milk, were observed. These findings were not

accompanied by a significant change in the counts of thermophilic streptococci and thermophilic lactobacilli bacteria. In relation to the volatile compounds profiles, some differences were detected in the different milk bases and yogurts during fermentation and storage. The most notable differences were recorded during storage in delactosed yogurt added with sucrose, respect to the other yogurt varieties. A relationship between the levels of volatile compounds related to glucose metabolism, such as diacetyl, 2,3-pentanedione, ethanol and ethyl esters, and glucose content of yogurts was verified.

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