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Influence of commercial starter culture on fermentation dynamics and quality characteristics of yogurts obtained with different formulations

Running title: Influence of commercial starter on different yogurt characteristics

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Abstract

BACKGROUND: four commercial starter cultures containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (S1, S2, S3 and S4), and S3 also contained *Limosilactobacillus fermentum*, were compared for fermentation, volatile flavor compounds, physicochemical parameters and microbiology, in yogurt prepared from three milk base formulation increased in protein (B1, B2 and B3).

RESULTS: the fermentation patterns differed among starters, with Yoflex® Mild 1.0 (S4) and SLB95 (S2) showing the longest fermentation time, depending on the formulation. At

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21 days, *S. thermophilus* counts were similar among starters and higher than 8.52 log CFU mL^{-1}, for all yogurts. The highest counts (6.86 log CFU mL^{-1}) for *L. delbrueckii* subsp. *bulgaricus* was found for S2 yogurts made from whey protein hydrolysate (B3). Minor water-holding capacity was detected for YF-L811 (S1) yogurts. Yoflex® Harmony 1.0 (S3) starter containing *Lim. fermentum* produced a distinctive volatile profile characterized by aldehydes respect to yogurts prepared with S1, S2 and S4 which were characterized by ketones.

CONCLUSION: results indicate the usefulness of carrying out studies similar to the present one to select the most appropriate process conditions depending on the desired product.

Keywords: yogurt process optimization, commercial starter cultures, carbohydrates and organic acids, microbiology, physicochemical parameters, volatile compounds.

Introduction

Yogurt is the most popular fermented milk and it is perceived as a very healthy food; in the last decades, the production and consumption of yogurt has increased in the world and also in Argentine (www.magyp.gob).[1] Global yogurt market is projected to grow 4% towards 2023 (www.alliedmarketresearch.com/yogurt-market). The growing prospects in production and consumption are partly explained by an intense innovative diversification of formulations, ingredients and technologies giving rise to new and different yogurt varieties.[2] In particular, high-protein yogurts has gained popularity partly driven by improvements in taste and texture (thicker and creamier products), and also by increased scientific evidence claiming health benefits of dairy proteins.[3-5]

Different strategies can be employed to increase protein content during yogurt manufacture, which can be applied before fermentation (evaporation, ultrafiltration, and addition of

protein source ingredients) or to freshly fermented yogurt (membrane filtration, centrifugation).[4-6] Relative to the methodologies employed before fermentation, changes in composition, nature and relative proportions of the different proteins and buffer capacity of the milk base, can impact on metabolic activity of the starter culture, kinetic of biochemistry of fermentation, and quality of the final product. In fact, some flavor defects in increased protein yogurts are burnt/beefy, too acidic, bitter, and astringent mouthfeel. Particulary, the burnt/beefy flavor detected in some "Greek yogurts" containing whey protein concentrate or milk protein concentrate was associated with the sulphurous compounds.[4] Moineau-Jean *et al*.[7] detected higher counts of *Lactobacillus helveticus* and *S. thermophilus* in fresh Greek yogurts made by milk ultrafiltration compared to regular stirred yogurt. Chua *et al*. [8] found that altering the casein:whey protein ratio in milk for yogurt, by addition of whey protein isolate, sodium caseinate and low-heat skim milk powder, modified the textural, rheological and microstructural characteristics of yogurts but there was not change in volatile compound.

On the other hand, starter cultures for yogurt production must be composed of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus;* other strains may also be used for giving particular attributes to products. The specific strains and their levels contained in the commercial starter cultures, influence the fermentation process and characteristics (texture, taste and flavor) of the yogurt.[5]

The aim of this work was to study the influence of different commercial starter cultures of yogurts, on fermentation and volatile flavor compounds profiles, physicochemical parameters and microbiological counts, of products. Different formulations increased in proteins using whey protein products, were employed.

Materials and Methods

Materials

Partially skim milk (PSM; 3.0% protein, 4.8% lactose, 1.3% fat), skim milk powder (SMP; 35.5% protein, 50.0% lactose, 1% fat) and whey protein concentrate 40% (WPC40; 38-41% protein, 46% lactose, 4% fat) were kindly provided by Milkaut S. A. (Franck, Argentine); whey protein concentrate 80% (WPC80; 76%-80% protein, 9% lactose, 10% fat) and whey protein hydrolysate (WPH, lacprodan HYDRO365, 23%-29% degree of hydrolysis; 78% protein, 4% lactose, 0.2% fat) were supplied by Arla Food Ingredients (Porteña, Argentine). Four commercial starter cultures for direct vat set (DVS) addition: YF-L811 (S1), SLB95 (S2), Yoflex® Harmony 1.0 (S3) and Yoflex® Mild 1.0 (S4) were employed. S1, S3 and S4 were supplied by Chr. Hansen (Hoersholm, Denmark) and S2 by Diagramma S. A. (Santo Tomé, Argentine). S1, S2 and S4 were composed by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, and S3 also included *Lim. fermentum*.

Yogurt making process

Lab-scale experiments (500 mL) were performed according to Vénica *et al*.[9] Milk bases were formulated by mixing PSM and SMP (30 g L^{-1}), to which the following dairy ingredients were added individually (20 g L^{-1}): WPC40 (B1), WPC80 (B2), and WPH (B3). Each milk base was distributed in four flasks and they were kept overnight at 5±1 °C for hydration of powders. Then, milk bases were pasteurized at 85 °C for 20 min and cooled to 42 °C. Starter cultures were individually inoculated in the dosage suggested by the supplier. Fermentation was conducted at 42 ºC and stopped when the pH reached 4.75±0.05 by placing the yogurt in a cold-water bath. pH was monitored during fermentation. Yogurts

were stored at 5±1 ºC for up to 21 days. Physicochemical composition, water-holding capacity (WHC) and profiles of carbohydrates, organic acids and volatile compounds were analyzed at 21 days; carbohydrates and organic acids were also determined in milk bases. Microbiological counts were monitored immediately after inoculation and in the yogurt after 1 and 21 days of storage at 5 °C. Three separate replicate experiments were made.

Physicochemical determinations

pH was determined using a digital pH meter (Orion 3-Star Benchtop, Thermo Fisher Scientific Inc., USA), titratable acidity (TA) was accomplished by titration with 0.11 M NaOH until pH 8.3 (1 °D = 100 mg lactic acid L^{-1}), protein was determined via the Kjeldahl method, total solids (TS) was analyzed by oven-drying at 102 °C, fat content was measured by the Gerber method and WHC was evaluated by centrifugation according to Vénica *et al*.[9] Analyses were made in duplicate.

Carbohydrates and organic acids analyses

The concentrations of carbohydrates (lactose, glucose and galactose) and organic acids (lactic, citric, orotic and hippuric) were determined according to Vénica *et al*.[10], using a high performance liquid chromatography (HPLC) equipment with a column oven and UV–visible and refractive index (RI) detectors (Perkin Elmer, Norwalk, USA). Briefly, 2.5 g of milk or yogurt were diluted with 10 mM H_2SO_4 to 25 mL. The suspension was homogenized and centrifuged at 15,000×g/20 min/4 °C. The supernatant was filtered through a 0.45 μm membrane (Millex, Millipore, São Paulo, Brazil) and injected into the chromatograph, using a loop of 60 μL. Chromatographic separation was carried out isocratically at 65 °C with a mobile phase of 10 mM 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, USA). UV-Vis and RI detectors were set at 210 nm and 35 °C for the detection of organic acids and carbohydrates, respectively. Data were processed with the software Chromera® (Perkin Elmer). Quantification was achieved using the peak areas from external calibration with standard solutions (Sigma Aldrich, Saint Louis, USA) and results were expressed as mg kg^{-1} for organic acids and g kg^{-1} for carbohydrates. Analyses were made in duplicate.

Microbiological analyses

Cell counts from inoculated milk and yogurts were performed on M17 agar (Biokar, Beauvais, France; 37 ºC, 48 h, aerobiosis) for *S. thermophilus* and acidified (glacial acetic acid, Ciccarelli, San Lorenzo, Argentine) MRS agar (Biokar, Beauvais, France; pH 5.4, 37 ºC, 72 h, anaerobiosis) for *L. delbrueckii* subsp. *bulgaricus*.[11] In the case of S3 starter culture, cell counts of *Lim. fermentum* were performed on MRS agar (37 ºC, 48 h, aerobiosis). Determinations were done in duplicate.

Volatile compounds analysis

Volatile compounds were determined by headspace-solid phase microextraction (HS-SPME) coupled with gas chromatography-flame ionization detector/mass spectrometry (GC-FID/MS), according to Wolf *et al*.[12], with slight modifications. A mix of yogurt (5 g) and saturated NaCl solution (5 g) was transferred to screw-top glass vials (40 mL) sealed with a Teflon-lined silicone rubber septum. A microstirring bar was also introduced into the vials, which were placed on an aluminum block maintained at 45 °C and stirred at 250 rpm, using IKA heater/stirrer (Instrumentalia S. A., Buenos Aires, Argentina). Then, a SPME

fiber (DVB/Car/PDMS 50/30 µm, Supelco, Bellefonte, USA) was inserted into the headspace of the vial. After 10 min, it was exposed at 45 °C for 30 min.

Analytes retained in the fiber were thermally desorbed in splitless mode at 250 ºC during 5 min into the injector port of the GC (Perkin Elmer, Massachusetts, USA) equipped with a split/splitless injector and a flame ionization detector (FID). Separation was performed on a fused silica capillary column (60 m x 0.25 mm x 0.25 µm; HP-Innowax, Agilent J&W, USA). The peaks identified by GC-FID and confirmed by GC-MS were integrated from chromatograms and the relative areas were calculated and expressed as arbitrary units. Then, compounds were grouped by chemical families and percentage values of each group were calculated from the total area of volatile compounds. Analysis was made in duplicate.

Data analysis

Tukey´s test of one-way analysis of variance (ANOVA) with a 95% confidence level was used to determine significant differences among starter cultures for each milk base. SPSS 10.0 software (Chicago, USA) was used and the results were expressed as the mean value ± standard deviation.

Results and discussion

Evolution of pH during fermentation

The pH changes during fermentation with the different commercial starters for each milk base are shown in **Fig. 1**. For B1 (**Fig. 1A**), the decrease in pH was more pronounced ($p<0.05$) at 90, 150 and 240 min for S1; then, the values were similar to those of S2 and S3. S4 presented the highest pH values ($p<0.05$) at 150, 210 and 240 min, which was reflected

in a delay in reaching the target pH. In fact, the end of fermentation occurred at 285 min for S4, 270 min for S3 and 255 min for S1 and S2.

B2 (**Fig. 1B**) had a similar pattern to that observed for B1. The lowest values ($p<0.05$) of pH were detected at 90 and 150 min for S1 and the highest ($p<0.05$) from 150 min up to the end of fermentation for S4. The fermentations lasted 285 min for S4 and 255 min for S1, S2 and S3.

Greater differences were observed for fermentations made from B3 (**Fig. 1C**). S1 had the lowest pH values ($p<0.05$) at 90 and 150 min, then, the values were similar to those observed for S3 and S4. The highest pH values ($p<0.05$) were found for S2 from 150 min up to the end, resulting in a delay in fermentation; S2 ended at 360 min; S3 and S4 at 300 min and S1 at 270 min.

A minor modification in the ratio of either bacterial strains or activity of the single strains affect the pH decrease.[13] In fact, our results demonstrated differences in the ratio of both strains in the starters employed (see Microbiological analyses section). Regardless of this, the evolutions of pH and fermentation times obtained were consistent with reported data (fermentation times between 3.5 and 6.0 h) for commercial yogurt.[14] Körzendörfer *et al*.[15] found that the final pH was reached faster in yogurt made with the YC-471 than with YF-L 901 (both from Chr. Hansen).

In general, the performance of the four starters was similar for B1 and B2 milk bases and differed from B3. B1 and B2 were prepared from whey protein concentrates (WPC40 and WPC80, respectively), in comparison to B3 made from WPH which contain di- and tripeptides, according to the manufacturer´s data. Our results differed from those reported by Lucas *et al*.[16] in which the incorporation of hydrolyzed whey protein accelerated the fermentation. They prepared probiotic yogurts from milk supplemented with WPH (0.25 to

4.0 g L^{-1}) containing different degree of hydrolysis: 8%, 12% and 27% and using different commercial strains: *S. thermophilus* ST-7 plus *Lactobacillus acidophilus* LA-5 and *S. thermophilus* ST-7 plus *Lacticaseibacillus rhamnosus* LR-35 (Chr. Hansen). Lower fermentation times was obtained for ST-7/LA-5 (from 8.4 to 10.7 h) than for ST-7/LR-35 (from 12.6 to 19.1 h) which was attributed to the higher acidifying activity of *L. acidophilus* compared to *Lc. rhamnosus*. In addition, the higher degree of hydrolysis of WPH produced a shorter fermentation time.

Physicochemical composition and water-holding capacity of yogurts

As expected, the global composition (protein, total solids and fat) of the yogurts was not dependent of the starter cultures. The mean values for proteins were 48.9±2.1, 51.4±2.8 and 55.3±1.7 g kg^{-1}, and for total solids were 144.0±0.5, 143.1±0.7 and 142.3±0.8 g kg^{-1}, for B1, B2 and B3, respectively; fat content was similar for all bases (15.0±1.0 g kg^{-1}). The products are classified as partially skim yogurts according to Food Argentinean Legislation.[17] In relation to protein content, Food Argentinean Legislation only establishes a minimum content (29 g kg^{-1}) for yogurt, and does not define the high-protein yogurt.

Values of pH, TA and WHC of the yogurts with 21 days, are shown in **Table 1**.

In yogurts made from B1, no differences were found among starter cultures for pH and TA. Lower values ($p<0.05$) of WHC were detected for S1 yogurts compared to the rest. In the B2 yogurts, S1 had the lowest pH values ($p<0.05$) and S4 the highest values ($p<0.05$). However, this fact was not reflected in the AT values. Yogurts made with S1 had the lowest ($p<0.05$) values of WHC. In the B3 yogurts, differences ($p<0.05$) were observed for the three parameters. S1 and S2 yogurts had the lowest and the highest pH values, respectively,

which was correlated with the TA values (S1~S4>S2). For WHC, the behavior was similar to those found for B1 and B2 yogurts (S2~S3~S4>S1).

Brodziak *et al*.[18] made yogurts with the addition of WPC80 (1% and 2%) and employing commercial starter cultures (Mild 1.0 Yo-Flex and YC-X11 Yo-Flex, Chr. Hansen). The effect of the starters was only found for yogurts with 2% WPC at 21 days of storage; pH values were 4.55 and 4.37, and TA were 97.2 and 126.9 °D for yogurts made with Mild 1.0 and YC-X11, respectively. WHC of yogurt is an indicator of the ability of gel to retain whey, thus reflecting the degree of syneresis. This parameter depends on several factors including the composition of the milk base and the starter culture employed. Brodziak *et al*.[18] found higher WHC for yogurts with both levels of WPC80 addition compared to yogurts without this ingredient, regardless of the starter cultures used. Dabija *et al*.[19] reported differences in WHC of yogurts made with starter cultures (S.C. TUDIA S.R.L., Romania) that had different proportions of *L. delbruekii* subsp. *bulgaricus* and *S. thermophilus*.

Microbiological analyses

Cell counts in milk bases after inoculation for *S. thermophilus* ranged from 6.83 to 7.19 log CFU mL^{-1} and were similar ($p>0.05$) among starters. *L. delbrueckii* subsp. *bulgaricus* counts varied according to the starter used; values for S1, S2 and S4 were similar ($p>0.05$) and ranged from 2.63 to 3.44 log CFU mL^{-1} and for S3 was minor than 2 log CFU mL^{-1}. Counts for both strains were similar in the three bases. Cell counts in yogurts at 1 and 21 days are shown in **Table 2**. In all yogurts, *S. thermophilus* reached levels ranging from 8.44 to 9.49 log CFU mL^{-1} at 1 day and they maintained their viability till the end of storage; differences ($p<0.05$) among starters were only found for B2 yogurts at 1 day. Counts of *L.*

delbrueckii subsp. *bulgaricus* differed among yogurts; at 1 day, values ranged from not detected (< 2 log CFU mL^{-1}) to 5.90 log CFU mL^{-1}, and at 21 days, values ranged from not detected to 6.86 log CFU mL^{-1}. The higher values corresponded to S2 yogurts prepared from B3. In particular for S3 yogurts, counts of *Lim. fermentum* were approximately 5.55±0.12 log CFU mL^{-1} in the three milk bases, and ranged from 5.70 to 6.90 log CFU mL^{-1} and from 5.48 to 6.27 log CFU mL^{-1} after 1 and 21 days, respectively.

Similar results were found by Asensio-Vegas *et al*.[20], for yogurts prepared with 6 different commercial starters. The values of *S. thermophilus* ranged from 7.68 to 9.29 log CFU mL^{-1}, and for *L delbrueckii* subsp. *bulgaricus*, cell counts were between 4.22 and 7.86 log CFU mL^{-1} at the end of storage (28 days).

Carbohydrates and organic acids profiles

In milk bases, lactose concentrations were 63.6, 56.6 and 56.0 g kg^{-1}, for B1, B2 and B3, respectively; glucose and galactose were very low in all cases (3.1 and 1.2 g kg^{-1}, respectively). Citric acid was majority; values were 2815, 2464 and 3413 mg kg^{-1} for B1, B2 and B3, respectively. The lactic acid values were 169, 64 and 399 mg kg^{-1} for B1, B2 and B3, respectively. The values of orotic and hippuric acids were low and located in the range of 83-94 and 31-33 mg kg^{-1}, respectively (data not shown).

Concentrations of carbohydrates and organic acids of the yogurts at 21 days are shown in **Table 3**. In general, the effect of the starter cultures was similar for all milk bases.

In yogurts from B1, differences ($p<0.05$) were found for citric, hippuric, glucose and galactose. For citric concentration, the highest values were for S1 and the lowest values for S3. For hippuric concentration, the highest values were for S2 and S4 and the lowest values

for S1. The highest values of glucose and galactose were detected for S1 and the lowest values for S4 yogurts.

For B2 yogurts, the hippuric and monosaccharides differed ($p<0.05$) and they followed a similar pattern than that observed for B1 yogurts.

For B3 yogurts, differences ($p<0.05$) were observed for lactic, hippuric and monosaccharides. The highest values of lactic was for S1. Yogurts made with S4 had the highest concentration of hippuric, and differed from S1, which presented the lowest values. The highest content of glucose was for S2 and the lowest for S4. The galactose contents were similar for S1, S2 and S3 yogurts and higher than S4 counterpart.

To our knowledge, there are no reports to date about the effect of different commercial starter cultures used in the yogurt making, on the organic acids and carbohydrates profiles. The levels of carbohydrates and organic acids in yogurt depend not only on the type of starter culture and the metabolic activities of the strains that compose them, but also on the composition of the milk base formulation which is related to ingredients employed.

Lactic acid values were lower than those reported by Ekinci and Gurel[21] (22470 mg kg^{-1} at 15 days), but the citric and orotic are in agreement (2560 and 120 mg kg^{-1}, respectively) to our results, for yogurt made with UHT whole milk and SMP (14% total solids and 3.8% of protein) and with the commercial starter YC-380 (Chr. Hansen). Cutrim *et al*.[22] also found higher values of lactic acid (33700 mg L^{-1}) at 24 h, for yogurt prepared with UHT whole milk and a commercial starter YF-L812 (Chr. Hansen); they reported 31 g L^{-1} of lactose, 0.6 g L^{-1} of galactose and the absence of glucose. Contrary, Cruz *et al*. [23] found lower levels of lactic (2880 mg L^{-1} at 21 days) for probiotic yogurt (*S. thermophilus* TA 040, *L. delbrueckii* ssp. *bulgaricu*s LB 340, *L. acidophilus* La14 and *B. longum* Bl05, Danisco) made from raw milk fortified with 3.5% of SMP.

Volatile compounds profile

In the present study, twenty-four volatile compounds identified in the yogurts at 21 days were grouped in four categories: aldehydes (acetaldehyde, 2-methyl butanal, 3-methyl butanal, benzaldehyde), alcohols (ethanol, 1-propanol, 1-hexanol, 2-nonanol), ketones (propanone, butanone, 2,3-butanedione or diacetyl, 2,3-pentanedione, 3-hydroxy-2-butanone or acetoin, 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone, 2-undecanone) and acids (acetic acid, butyric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid) (**Fig. 2**), which have been reported as constituents of the volatile profile of yogurts.[24] In particular, carbonyl compounds such as aldehydes, ketones, acids and esters are considered the main aromatic substances of yogurt.[25] Most of the volatiles identified in our samples belonged to this category of compounds.

As can be seen in **Fig. 2A**, yogurts made from B1 and using S1, S2 and S4 starters had a similar pattern among them, which were characterized mainly by ketones and followed by acids and aldehydes. By contrast, in yogurts made with S3, the aldehydes were the majority group followed by ketones and acids.

The values of ketones ranged from 50% to 58% of the total area of compounds for S1, S2 and S4 yogurts; in S3 yogurt a fraction of 31% was recorded. Differences were detected among samples; the highest values were obtained in yogurts prepared with S2 and the lowest in S3 yogurts ($p<0.05$). In all samples, 2-heptanone was the most abundant (taking into account the peak area value), followed by 2-propanone, 2-butanone, diacetyl and acetoin.

Acids group represented around 27% of the total area of compounds in yogurts made using S1, S2 and S4 starters ($p>0.05$), while those yogurts fermented by S3 had a percentage

value of 13% ($p<0.05$). Acidic fraction contained only *n*-acids. Hexanoic was the main fatty acid, followed by butanoic and octanoic acids.

Yogurts made with S3 had the highest percentage of aldehydes (54%), being benzaldehyde the most abundant. This chemical group represented between 12% and 17% of the total area of volatile compounds in S1, S2 and S4 yogurts. Differences ($p<0.05$) were detected among samples (S3>S1=S4>S2).

Alcohols were minor compounds (percentage around 2% of the total area), being ethanol and 1-hexanol the most representatives. Regardless of starter culture used, the yogurts showed a similar pattern ($p>0.05$) of alcohols both quantitative and qualitative.

The volatile fraction of yogurts made from B2 and with the four starter cultures studied (**Fig. 2B**) was similar to those observed for B1 milk base.

The percentages of ketones varied from 30% to 62%, and differences among yogurts ($p<0.05$) were found (S2>S4>S1>S3). Similar to those observed for B1, the highest values were obtained in yogurts prepared with S2 and the lowest in S3 yogurts. The percentages values of acidic compounds were similar among S1, S2 and S4 yogurts (about 25%), but different ($p<0.05$) from those for S3 yogurt (approx. 13%). Aldehydes were the group with major quantitative differences among samples. They ranged from 8% to 52% of the total area of compounds. Benzaldehyde was the most abundant aldehyde. Differences ($p<0.05$) were detected among yogurts (S3>S1>S4>S2). The alcohol group constituted a minority fraction (around 3%) and no differences ($p>0.05$) were detected among samples.

Volatile fraction of yogurts made from B3 and with the four starter cultures is presented in **Fig. 2C**. As can be see, the profiles were again similar to those obtained for B1 and B2. Ketones was the most abundant group in yogurts prepared with S1, S2 and S4, but it not

happened for S3 yogurt; percentages varied between 27% and 56% and differences were observed (S2>S4>S1>S3; $p<0.05$). Acidic fraction ranged from 15% to 28%; differences ($p<0.05$) were detected among yogurts (S1~S2~S4>S3). Aldehydes represented between 15% and 57% of the total compounds (S3>S1=S4>S2). Benzaldehyde was the majority aldehyde in all cases. The starter S2 also produced 2-and 3-methyl butanal. Alcohols constituted a minority fraction and differences were not detected among samples ($p>0.05$).

Our results demonstrated that the volatile compound profiles depend on the starter employed. S3 containing *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *Lim. fermentum* produced a distinctive volatile profile, clearly different to those found in yogurts made with starter composed only by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (S1, S2 and S4). Regardless of the milk base formulation, yogurts made with S3 showed a predominance of aldehydes, with a preferential biosynthesis of benzaldehyde. Concerning volatile compound production in fermented milk by *Lim. fermentum*, no previous reports are available to date. Some researchers have shown the production of benzaldehyde by strains of *S. thermophilus*,[26, 27] although the levels found in our study were low. Benzaldehyde can impart a unique flavor to fermented milk, giving aromatic notes of bitter almonds or maraschino cherries depending on the levels.[27]

Conversely, the type of strain of *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus,* or the relation between them, appeared to have less effect on the overall volatile profile. Although statistical differences in some chemical families were detected for yogurts fermented by S1, S2 and S4, the profiles were always characterized by ketones as main group of compounds followed by acids and aldehydes. Scarce data about chemical groups of volatile compounds produced by typical commercial starter cultures of yogurts are provided in the literature.

Conclusions

To develop new yogurt varieties, a comprehensive assessment of the changes that occur during fermentation and refrigerated storage of the products, is crucial. The findings of this study demonstrate the considerable differences among commercial starter cultures inoculated on different milk base formulations. The combination of starter-milk base for yogurt making had different impact on the length of fermentation process, physicochemical parameters of products such as acidity, water-holding capacity as a measure of syneresis, microbial counts, carbohydrate and organic acid profiles, and volatile flavor compounds profile. In the industrial practice, the shortest fermentation times are preferred, without compromising product quality. For example, of the four products obtained for each of the three milk base formulations, the most suitable ones could be: S2-B1, S2-B2, and S4/S3-B3. Results highlight the importance of carrying out these studies in order to select the most suitable conditions depending on the product sought.

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Data Availability Statement

Research data are not shared.

Ethical approval

Ethics approval was not required for this research.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the research, authorship or publication of this article.

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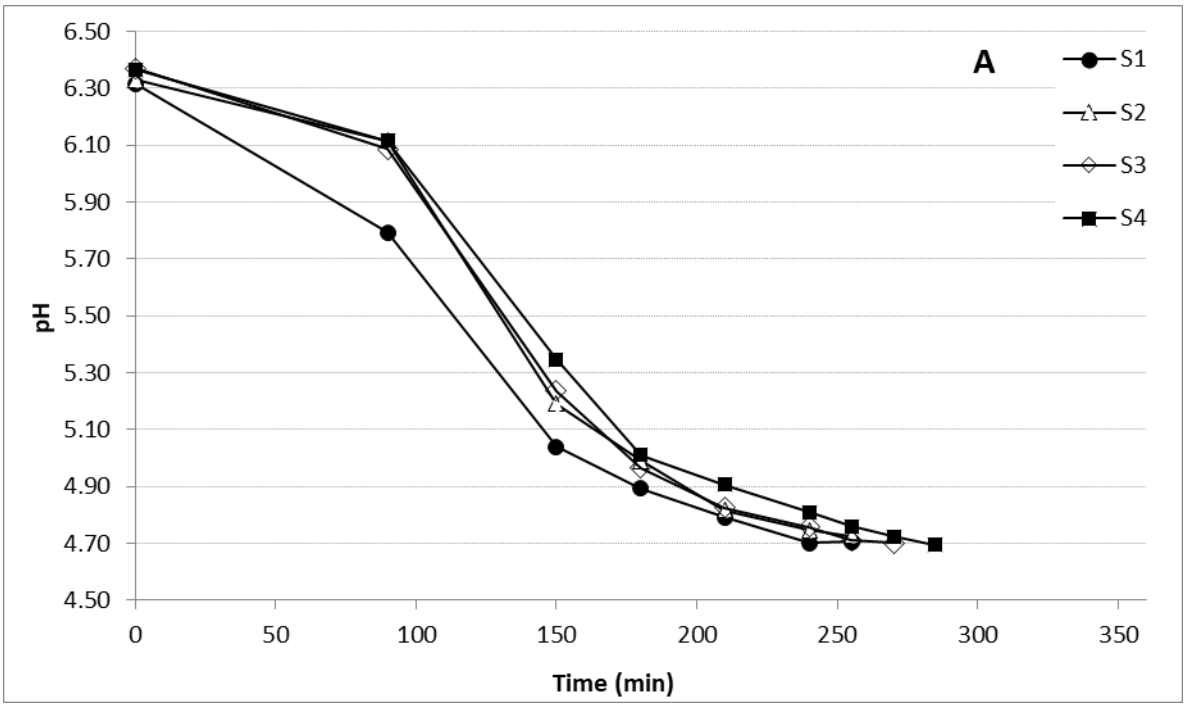
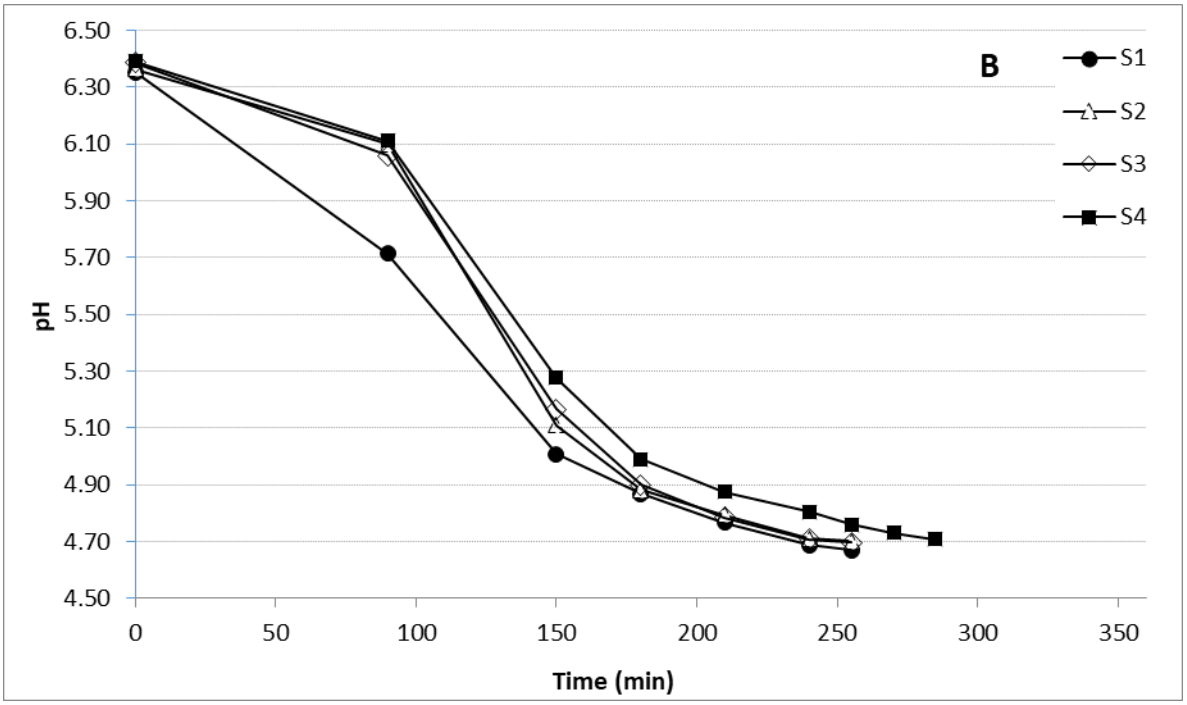
Legends to figures

Figure 1. Evolution of pH during yogurt fermentation with different commercial starter cultures: S1, S2, S3 and S4, for different milk base formulations: B1, B2 and B3 (A, B and C, respectively) (mean; $n = 3$).

Figure 2. Volatile profiles of yogurts made with different commercial starter cultures: S1, S2, S3 and S4, for different milk base formulations: B1, B2 and B3 (A, B and C, respectively) after 21 days of storage (mean ± standard deviation; $n = 3$).

Figure 1. Evolution of pH during yogurt fermentation with different commercial starter cultures: S1, S2, S3 and S4, for different milk base formulations: B1, B2 and B3 (A, B and C, respectively) (mean; $n = 3$).





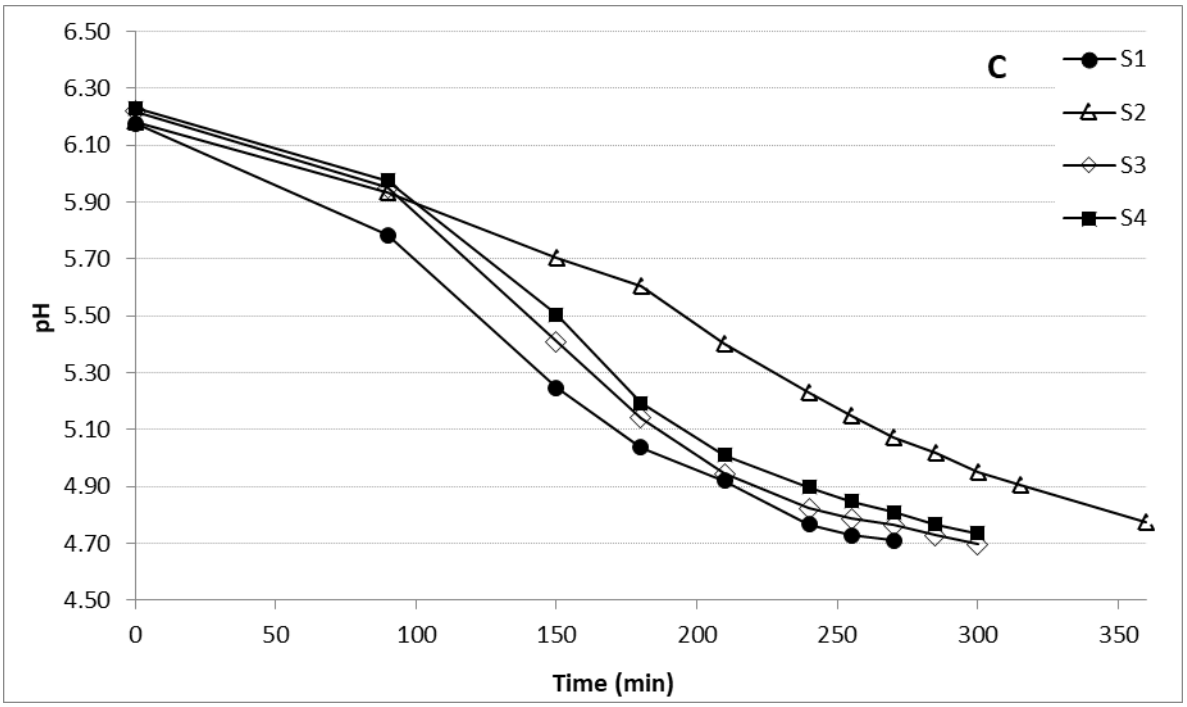


Figure 2. Volatile profiles of yogurts made with different commercial starter cultures: S1, S2, S3 and S4, for different milk base formulations: B1, B2 and B3 (A, B and C, respectively) after 21 days of storage (mean ± standard deviation; $n = 3$).

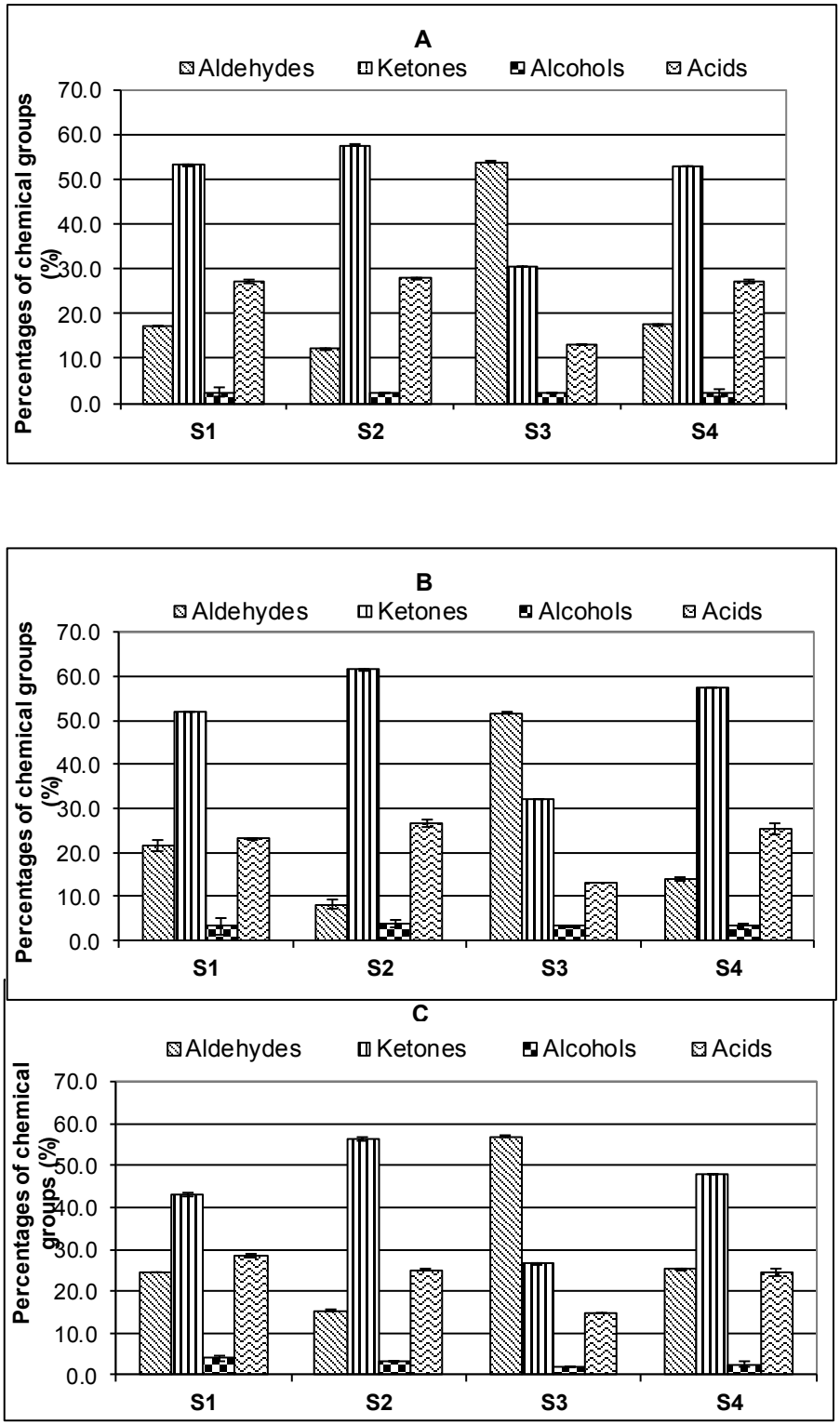


Table 1. pH, titratable acidity (TA) and water-holding capacity (WHC) of yogurts after 21 days of storage (mean ± standard deviation; $n = 3$).

	S1	S2	S3	S4
Yogurts made from B1				
pH	4.40±0.05^{a}	4.47±0.02^{a}	4.49±0.06^{a}	4.48±0.02^{a}
TA (°D)	109.83±5.04^{a}	105.90±1.54^{a}	100.33±6.83^{a}	102.89±1.33^{a}
WHC (%)	43.19±0.67^{b}	46.89±0.93^{a}	47.61±0.97^{a}	48.08±1.01^{a}
Yogurts made from B2				
pH	4.39±0.06^{b}	4.46±0.04^{a,b}	4.48±0.02^{a,b}	4.51±0.04^{a}
TA (°D)	110.51±2.36^{a}	111.43±0.83^{a}	107.34±4.53^{a}	105.29±2.98^{a}
WHC (%)	47.05±1.71^{b}	52.35±1.23^{a}	51.69±1.95^{a}	52.72±1.98^{a}
Yogurts made from B3				
pH	4.41±0.03^{c}	4.58±0.04^{a}	4.56±0.04^{a,b}	4.48±0.02^{b,c}
TA (°D)	133.69±1.30^{a}	124.83±2.47^{b}	127.87±0.90^{a,b}	130.69±2.69^{a}
WHC (%)	31.05±0.81^{b}	36.13±1.15^{a}	36.32±0.30^{a}	37.82±1.68^{a}

S1, S2, S3 and S4, commercial starter cultures. B1, B2 and B3, milk base formulations.

Values with different superscripts within a same row are significantly different (Tukey´s test, $p<0.05$).

Table 2. Cell counts for *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *Lim. fermentum* in yogurts, in M17, acidified MRS agar and MRS agar, respectively (average log CFU mL^{-1} ± standard deviation; $n = 3$).

	S. thermophilus				*L. delbrueckii* subsp. *bulgaricus*				*Lim. fermentum*
	S1	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S3**
Yogurts made with B1									
1 day	9.28±0.12[a]	9.32±0.06[a]	8.99±0.09[a]	9.26±0.10[a]	5.12±0.01[a]	4.52±0.17[b]	<LD	2.30±0.01[c]	5.70±0.37
21 days	9.04±0.28[a]	9.24±0.09[a]	9.21±0.16[a]	9.03±0.03[a]	4.07±0.01[a]	4.28±0.05[a]	<LD	<LD	5.48±0.40
Yogurts made with B2									
1 da	9.49±0.01[a]	9.39±0.04[a]	8.66±0.01[b]	9.20±0.03[c]	5.11±0.02[a]	3.85±0.08[b]	<LD	3.00±0.01[b]	6.30±0.01

y									
21 da ys	8.99±0.01[a]	9.29±0.07[a]	9.15±0.12[a]	9.09±0.08[a]	4.06±0.20[a]	3.60±0.12[a]	<LD	3.28±0.01[a]	5.73±0.54
Yogurts made with B3									
1 day	9.37±0.22[a]	9.01±0.01[a]	8.44±0.17[a]	8.92±0.12[a]	4.74±0.03[a]	5.90±0.20[b]	<LD	4.21±0.64[a]	6.90±0.29
21 da ys	9.17±0.11[a]	8.52±0.25[a]	8.79±0.40[a]	8.66±0.30[a]	4.29±0.19[a]	6.86±0.10[b]	<LD	4.16±0.68[a]	6.27±0.07

S1, S2, S3 and S4, commercial starter cultures. B1, B2 and B3, milk base formulations. LD, limit detection (2 log CFU mL^{-1}).

Values with different superscripts within a same row and the same microorganism, are significantly different (Tukey´s test, $p<0.05$).

Table 3. Concentrations of carbohydrates (g kg^{-1}) and organic acids (mg kg^{-1}) of yogurts after 21 days of storage (mean ± standard deviation; $n = 3$).

	S1	**S2**	**S3**	**S4**
Yogurts made with B1				
Lactic	11976.8±51.3[a]	11956.1±457.0[a]	11376.5±459.1[a]	11321.4±79.7[a]
Citric	2804.3±5.9[a]	2765.0±7.2[a,b]	2626.3±23.8[c]	2672.7±55.9[b,c]
Orotic	88.5±1.1[a]	89.1±2.8[a]	86.4±4.4[a]	85.5±0.1[a]

Hipuric	10.7±4.6^{b}	30.6±0.7^{a}	21.9±2.5^{a,b}	28.6±1.6^{a}
Lactose	44.4±0.8^{a}	45.7±0.5^{a}	45.0±1.6^{a}	46.1±0.2^{a}
Glucose	4.8±0.1^{a}	3.7±0.1^{a,b}	3.3±0.1^{b}	2.8±0.5^{b}
Galactose	11.3±0.1^{a}	10.5±0.2^{a,b}	9.7±0.4^{b}	8.0±0.4^{c}
Yogurts made with B2				
Lactic	12054.5±755.1^{a}	11759.1±452.5^{a}	11635.8±130.2^{a}	11309.3±759.6^{a}
Citric	2536.7±122.7^{a}	2442.2±54.9^{a}	2423.1±9.2^{a}	2398.2±57.4^{a}
Orotic	80.9±1.5^{a}	79.0±1.3^{a}	78.4±2.5^{a}	78.0±3.5^{a}
Hipuric	16.3±3.7^{b}	27.0±0.8^{a}	21.6±2.5^{a,b}	27.4±0.8^{a}
Lactose	36.2±0.2^{a}	37.1±0.6^{a}	38.5±0.9^{a}	39.7±2.0^{a}
Glucose	4.6±0.3^{a}	3.5±0.1^{b}	3.2±0.1^{b,c}	2.5±0.3^{c}
Galactose	11.4±0.3^{a}	10.4±0.3^{b}	10.0±0.1^{b}	7.8±0.1^{c}
Yogurts made with B3				
Lactic	13443.0±265.1^{a}	12115.4±59.3^{b}	12327.3±139.0^{b}	12460.3±124.5^{b}
Citric	3202.7±111.1^{a}	3246.7±87.2^{a}	3205.9±134.8^{a}	3186.4±41.5^{a}
Orotic	91.4±3.4^{a}	90.1±3.2^{a}	89.9±4.2^{a}	85.5±2.0^{a}
Hipuric	2.9±2.3^{c}	9.8±3.4^{b,c}	17.0±3.7^{a,b}	27.8±1.6^{a}
Lactose	35.2±0.7^{a}	33.6±1.6^{a}	37.5±1.0^{a}	36.8±0.2^{a}
Glucose	4.6±0.2^{a,b}	5.5±0.2^{a}	4.0±0.1^{b,c}	3.1±0.4^{c}
Galactose	11.9±0.1^{a}	11.1±0.1^{a}	10.7±0.1^{a}	8.5±0.6^{b}

S1, S2, S3 and S4, commercial starter cultures. B1, B2 and B3, milk base formulations.

Values with different superscripts within a same row are significantly different (Tukey´s test, $p<0.05$).