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Effect of the Heat Exchanger Type on Stirred Yogurt Properties Formulated at Different Total Solids and Fat Contents

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Abstract: In this work stirred yogurts were produced using a technical scale pilot in which the cooling step was processed using either a tubular (THX; low shear) or a plate (PHX, high shear) heat exchanger. The aim was to determine how total solids (TS, adjusted using lactose) and fat contents (FC) impact stirred yogurt properties during storage, depending on the heat exchanger used. Using raw milk, cream, skim milk powder, and lactose, four yogurts were formulated at 16.5% TS and 4.2% proteins, with different FC (0.0, 1.3, 2.6, and 3.9%); one more control yogurt was formulated at 14% TS, 4.2% proteins, and 0.0% FC. Analyses of yogurts (firmness, viscosity, induced syneresis) were realized at days 1, 3, 7, 21, and 34 after production. The addition of lactose between the non-fat yogurt at 14 or 16.5% TS had little to no effect on stirred yogurt properties. Increasing FC reduced syneresis while increasing firmness and viscosity. The use of PHX reduced the syneresis compared to THX; however, it also tended to reduce the firmness of the yogurts with 3.9% FC.

Keywords: smoothing process; technical scale unit; rheology; syneresis

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1. Introduction

A variety of yogurt is commercially available to consumers nowadays. Yogurts can be categorized as non-, low-, or full-fat, representing under 0.05%, 1–3%, and 3–4% fat content (**FC**), respectively, but the latter cannot exceed 10% [1].

Yogurt processing includes different steps: standardization (formulation) of the yogurt mix, homogenization, thermal treatment (pasteurization), and fermentation [2]. For set-style yogurts, the milk is fermented directly into the packaging, then cooled, stored, and sold as such. For stirred yogurts, fermentation takes place in large vats [3], once the gel is obtained, it is stirred and pumped to be transported across pipes, smoothing and cooling devices, and finally it is packaged to be stored and sold [2,3]. This post-fermentation process breaks down the gel into small microgels resulting in firmness and viscosity loss compared to the set gel [4]. Then, during storage, a rebodying occurs during which the network is reorganized causing an increase in firmness and viscosity [5,6].

Yogurt mix fortification in protein, non-fat solids (**NFS**), total solids (**TS**), or FC using milk ingredients (whey protein concentrate (**WPC**), skim milk powder (**SMP**), calcium caseinate, cream, etc.) does improve rheological and textural properties while decreasing syneresis compared to unfortified yogurt [7–10]. At a lab-scale production (<1 L, manual stirring), various stirred yogurt formulations using SMP and WPC to change the casein (**CN**) content, the whey protein (**WP**) content, and the CN:WP ratio were studied [11]. It was found that the addition of WPC produced stirred yogurts with a more homogeneous network and higher viscosity. Few studies have looked at the effect of adjusting TS using lactose, but it was demonstrated that the addition of lactose has the potential to increase set acid dairy gel elasticity and water-holding capacity [12]. Excessive use of powdered dairy protein ingredients in yogurt formulation can lead to major defects such as a grainy texture and an over-acidification, sometimes generating more syneresis [10,13–15]. Powdered dairy ingredients can be partially replaced using milk fat or fat replacers to improve textural and rheological properties of stirred yogurts comparatively to non-fat stirred yogurts [15]. After homogenization and thermal treatment, the fat globules' diameter reduced from 5 to 1–2 μm, with an interface covered in the majority with CN, modifying their capacities to interact with the protein network during fermentation [16–19]. Therefore, increasing the FC of stirred yogurts contributes to the formation of a gel network with higher water-holding capacity, firmness and viscosity compared to non-fat stirred yogurt [20].

Reproducing the post-fermentation process at a lab scale is a challenge. Some authors have used standardized manual methods (spoons, perforated disks, etc.), syringes, or mechanical mixers [5,21,22]. Those studies demonstrated that the mechanical actions of stirring broke the protein strands of the network lowering firmness and viscosity compared to the firm yogurt [4,23]. However, this type of post-fermentation process does not represent adequately the stirring, smoothing and conditioning process of stirred yogurt at the industrial scale. Some authors have modeled individual processing operations. For instance, to study the effect of transportation through pipes, Abu-Jdayil et al. [24] used lab-scale pilot and semi-industrial systems composed of a pump and a straight pipe with lengths varying from 2 to 10 m long. They showed that the stirred yogurt undergoes more intensive shears when flowing close to the pipe walls destructuring further the protein network, especially when the stirred yogurt FC is reduced. Yogurt gel with increased FC showed higher resistance toward mechanical treatments and shear stress [24]. However, those studies did not integrate the effect of other unitary operations of the post-fermentation process. Afonso and Maia [25] and more recently Guénard-Lampron et al. [26] studied the sequential impact of post-fermentation processes by using an industrial scale and a complete technical scale (>30 L), respectively. Both authors found that the cooling step had a major impact on the final product. For instance, the use of a tubular heat exchanger (**THX**) rather than a plate heat exchanger (**PHX**) minimalized the loss of firmness and viscosity of the final product [26]. However, no change in yogurt formulation was tested to see how it may interact with the post-fermentation process on the evolution of rheological properties of yogurt. Recently, the interaction between the smoothing temperature and the addition of WP into formulation was investigated with a technical scale [14], but no study has been made concerning the FC or the TS adjusted using lactose.

The aim of this study was to determine the impact of TS adjusted using lactose and FC on the evolution of physicochemical, textural and rheological properties of different stirred yogurts during a storage of 34 days after production. A technical scale unit of production was used. The cooling operation was performed with either a THX (low shear intensity) or a PHX (high shear intensity).

2. Material and Methods

2.1. Dairy Ingredients

Raw milk was kindly provided by Chalifoux Inc. (Sorel-Tracy, QC, Canada). Lactose (grade 300) and SMP were supplied by Quadra Chemicals (Vaudreuil, QC, Canada), and WPC was provided by Agropur (Saint-Hyacinthe, QC, Canada). The dairy powders composition is presented in Table 1.

Table 1. Dairy powders composition.

Composition (w/w)	SMP [1]	WPC (34%) [2]	Lactose Powder
Total protein content (%)	35.00	34.12	0
Whey protein content (%)	5.73	ND [3]	0
Casein content (%)	28.93	ND	0
Total solids (%)	97.65	97.40	99.44
Fat contents (%)	0.68	1.44	0

[1] Skim milk powder; [2] Whey protein concentrate; [3] Not determined.

2.2. Yogurt Starter Culture Preparation

A non-ropy freeze-dried starter (Yo-Dolce) constituted of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* at a 95:5 ratio was kindly provided by Biena (St-Hyacinthe, QC, Canada). Starter cultures were always prepared the day before yogurt production according to Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26] procedure. Skim milk was reconstituted at 12% (w/w) using SMP and deionized water. After it was sterilized (110 °C; 6 min), it was quickly cooled down to 41 °C using an ice-bath and inoculated with the Yo-Dolce freeze-dried starter at 0.1% (w/v). It was then incubated, at 41 °C, for 4.5 ± 0.5 h until it reached a pH of 4.7. The starter culture was immediately stored, at 4 °C, until use the following day.

2.3. Yogurt Production

2.3.1. Yogurt Mix Preparation

The day before yogurt production, raw milk was heated-up to 63 °C and skimmed by centrifugation (DeLaval Company Limited, model ref. 618, Peterborough, ON, Canada). The skimming debit was adjusted to obtain a cream at 58 ± 3% (w/w) FC. The cream quantity needed for standardization was immediately mixed with 5 L of skim milk while both the cream and milk were still warm. This dairy mix was stored, at 4 °C, for production the next day.

On the day of production, yogurt mixes were standardized and prepared according to Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26]. The 5 L diluted cream, skim milk, and dairy powders were mixed, at room temperature, in a closed-loop system composed of a vat and a pump (FM-O/115; Alfa-Laval, Lund, Sweden). All yogurt mixes were standardized at 4.2% (w/w) protein content with a ratio CN:WP of 2.8:1. Two yogurt mixes stayed at 0%(w/w) FC, but one was standardized at 14% TS (**YC**, w/w). Four other yogurt mixes were standardized at 16.5% TS (w/w) using lactose addition and with 0 (**Y0.0**), 1.3 (**Y1.3**), 2.6 (**Y2.6**), and 3.9% (**Y3.9**) FC (w/w). Formulation targets (fat contents, total solids, lactose content) are summarized in Supplementary Table S1.

The impact of TS (or more specifically the impact of lactose addition) was studied using the comparison between the two non-fat yogurts YC and Y0.0, while YC and Y2.6 had similar milk solid non-fat content (and lactose content) but different FC. Moreover, Y2.6 could be compared to YC or Y0.0 to compare fat addition at equal non-fat solid content or the replacement of lactose by fat at equal TS.

Yogurt mixes were then homogenized, at 60 °C, in a 2-stage homogenizer (500–2000 L/h, Alfa-Laval, Lund, Sweden) at 13.80 MPa (first stage) and 3.45 MPa (second stage). Pasteurization took place, at 94.5 °C, for 5 min using a plate pasteurizer (2000 L/h, Alfa-Laval) and the milk was immediately cooled down to 40 ± 1 °C in the regeneration section of the plate pasteurizer to be transferred by batches of 25 kg into 30 L conical vats.

2.3.2. Yogurt Fermentation

Yogurt vats were placed into yogurt incubators (Magelis, Schneider Electric, Brossard, QC, Canada), at 40 °C, and inoculated at 1.5% (v/v) with the starter culture (see Section 2.2). Yogurt mixes acidification was followed using pH measurement (HI 99161, Hanna Instruments, Laval, QC, Canada), and yogurt vats were removed from incubators 3.50 ± 0.25 h later when pH reached 4.7 ± 0.1.

2.3.3. Technical Scale Unit and Stirring Operations

Stirring, cooling and smoothing operations, simulating industrial conditions, were performed in the technical scale unit developed by Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26] using the configuration schematized in Figure 1. Once the conical yogurt vat was plugged into the technical scale unit, a stainless-steel helical blade mixer [2] was inserted into the vat to stir the yogurt at 30 rpm for 5 min (Penta KB power drive, NEMA-4X/IP-65; Baldor, Fort Smith, Arkansas). The stirring speed was reduced to 15 rpm before starting the positive displacement pump (Seco DANA, model 210; Bronco Industries, Delta, BC, Canada), and was kept at 15 rpm for the rest of the process. Yogurt circulated at a constant 0.88 L/min through the system. Before cooling, the yogurt was smoothed using a 425 µm conical filter nozzle (#40 sieve, surface area of 31 cm^2). After smoothing, the in-line thermometer (thermocouple type K, Omega Engineering, Stamford, CT, USA) indicated that the yogurt temperature had already dropped to 37 °C. The product was then transported toward the cooling step using a bi-directional valve leading to either a THX (diameter = 3.4 cm, length = 4.3 m, PG7757/84, Sepak Industries Pty Ltd., Sydney, Australia) or a PHX (3.4 L, type A3-HBM, Alfa Laval). Both were counter-flow cooling devices able to drop the yogurt temperature from 37 to 20 °C using water at 10 °C for the THX and 20 °C for the PHX. THX cooled down the yogurts in 3 min, while the PHX systems did it in a few seconds. Stirred yogurts were then packaged at the end of the system using a restricting right-angled pipe (diameter = 2.3 cm, length = 30.5 cm) delivering the product into 175 g packaging cups (Plastipac, GenPak, Boucherville, QC, Canada). Yogurts were immediately stored, at 4 °C, in a forced-air cold room (Bally engineered structure Inc., Bally, PA, USA) and were analyzed at days 1, 3, 7, 21, and 34 after production.

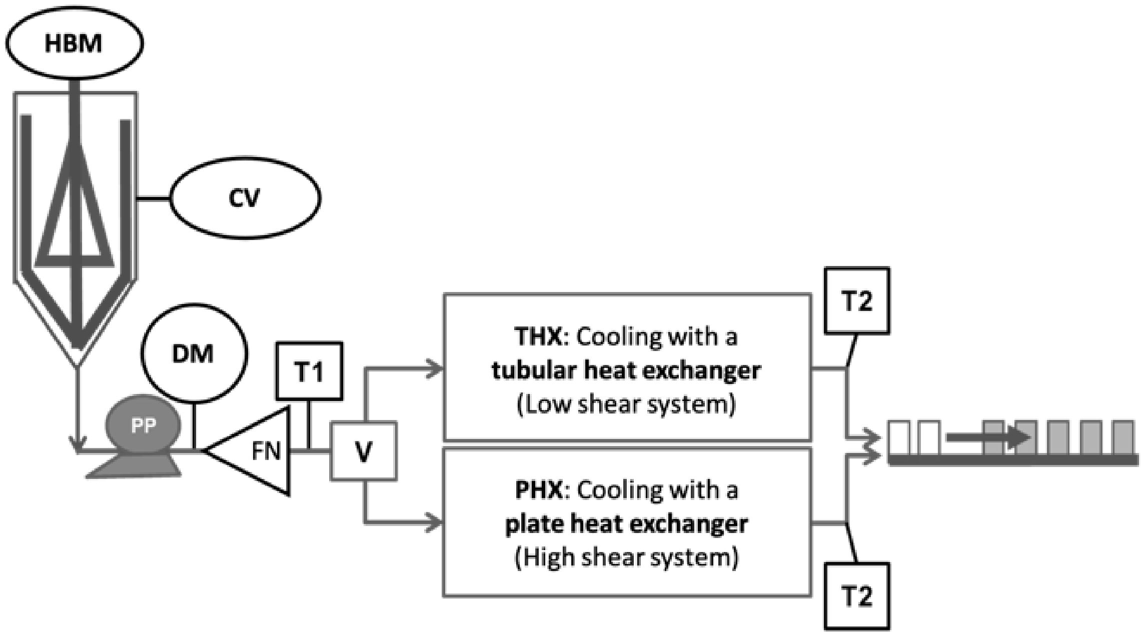

Figure 1. Scheme of the technical scale unit. HBM = helical blade mixer; CV = conical vat; PP = positive pump; DM = Digital manometer; FN = Filter nozzle; T1 = temperature following smoothing; V = Bi-directional valve; T2 = temperature following cooling.

2.4. Analyses

Composition analyses, induced syneresis, firmness, apparent viscosity, and bacterial counts were measured according to Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26].

2.4.1. Composition Analyses

An infrared FT-120 (Foss North America, MN, USA) was used to measure the composition (FC, casein content, whey protein content, TS) of both the raw skim milk and the cream obtained after skimming for standardization purposes, and the composition of yogurt mixes after pasteurization.

The yogurt mixes before heat treatment and the dairy ingredients were analyzed using AOAC methods: Fat content was analyzed using the Röse-Gottlieb method with a Mojonnier extraction flask [27] (Method 989.05) in triplicate; the total nitrogen content, non-protein nitrogen content, and non-casein nitrogen fractions were obtained according to Gentès et al. [28] and measured by a macro-Kjeldahl method [27] (Method 991.20) in duplicate; the total solid content was determined using the AOAC International [29] standard method 990.20 in duplicate.

2.4.2. pH and Titratable Acidity

Two aliquots of yogurt (10 mL) were sampled. The pH was measured under stirring (DL15 Mettler-Toledo, Anachemia/VWR, Anjou, QC, Canada). Deionized water (10 mL) was then added to the yogurt sample under stirring, and NaOH 1/9 (0.1 N) was added progressively; the titratable acidity (expressed in % of lactic acid w/v) was measured when pH 8.6 was reached (DL15 Mettler-Toledo, Anachemia/VWR).

2.4.3. Induced Syneresis

The day of analysis, yogurts were sampled (25 g) using an in-house sampler and introduced into 50 mL conical tubes [30]. After centrifugation (210× *g*, 20 min, 4 °C; Sorvall ST40R centrifuge, TX-750 rotor; Thermo Scientific, ON, Canada), the supernatant was collected and weighed. Induced syneresis was expressed as the ratio of the collected serum mass to the yogurt mass expressed in percentages. Induced syneresis was measured in duplicates.

2.4.4. Firmness

Firmness was measured directly on the yogurt in cups, at 4 °C, using a texturometer TA-XT2 equipped with the 5 kg load cell (Texture Technologies Corporation, Scarsdale, NY). The cylindrical probe (25 × 35 mm) lowered down (1 mm/s speed) to a depth of 10 mm into the product, and the firmness was defined as the maximum penetration force (N) divided by the probe area (4.91×10^{-4} m^{-2}). Firmness was measured using the mean of 5 measurements.

2.4.5. Apparent Viscosity and Hysteresis Loop

One aliquot of yogurt (21 g) was transferred using an in-house sampler [30] into the concentric cylinder cup and bob geometry (CC-27 27, 28.92 mm diameter; Anton Paar Gmbh, Ostfildern, Germany) of a rheometer (Physica MCR 301, Anton-Paar Gmbh, Ostfildern, Germany). The probe was lowered, and the yogurt temperature was maintained, at 4 °C, using a Peltier system (C-PTD 200, Anton-Paar Gmbh). After probe insertion, a 5 min soaking time took place before a hysteresis loop was performed from 0 to 100 s^{-1} [31]. Shear stress was recorded over 20 points (one every 5,0 ± 0.3 s^{-1} interval) on both the upward and downward flow curves of the hysteresis loop using the rheometer software (Rheoplus 3.40, Anton-Paar Gmbh). The apparent viscosity at 10.5 s^{-1} on the upward flow curve, and the thixotropic area defined as the area between the upward and downward flow curve (arbitrary units), were calculated by the software.

2.4.6. Microbiological Counts

The official methods ISO-IDF [32] (ISO 7889/IDF 117) was followed for microbiological counting. Peptone (Bacto™ no 211677), granulated agar (Difco™ no 214530), M17 broth (Difco™ no 218561), MRS Broth (Difco™ Lactobacilli no 288130), and lactose (Difco™ no 215620) were all supplied by Becton, Dickinson and Company (Spark, MD, USA). Streptococci were counted on M17 agar medium supplemented with 0.5% (v/v) lactose. Lactobacilli were counted on MRS Agar medium acidified with 0.2% (v/v) glacial acetic acid (99.7%; Laboratoire MAT, Montreal, QC., Canada). Plates were incubated at 37 °C for 48 h.

2.5. Experimental Plan and Statistics

The experimental plan was a factorial randomized “split-plot” with repetitive measurement analyzed as a “split-split-plot” with the yogurt milk standardization as the main factor, the cooling device as the secondary factor, and finally, the storage time was included as the tertiary factor. Yogurt milk standardization was fully randomized over the days of production and each factorial combination was repeated four times. Statistical analyses using ANOVA were run using the GLM procedure of the SAS software (SAS Server Interface, version 2.5.14; SAS Institute Inc., Cary, NC, USA). Factor or combination of factor significance was determined using a level of 5%. Multiple comparisons were carried out using the protected Fisher's least square significant test with a level of 5%, according to the significant factors. The ANOVA hypothesis was verified using the Shapiro–Wilk statistic at 5% for the normality assumption, and the plot of residuals for the variance homogeneity assumption.

3. Results and Discussion

3.1. Yogurt Milk Standardization

All targeted standardizations were met as required. For all yogurts, total protein, true protein, casein, and whey protein contents were, respectively, 4.49 $\pm$ 0.24%, 4.24 $\pm$ 0.05%, 3.11 $\pm$ 0.03%, 1.12 $\pm$ 0.02% (percentages are expressed in w/w), giving a casein to whey protein ratio of 2.79 $\pm$ 0.06. YC and Y0.0 had a FC under 0.1%; otherwise, the FC of Y1.3, Y2.6, and 3.9 were, respectively, 1.31 $\pm$ 0.12, 2.67 $\pm$ 0.13%, and 3.92 $\pm$ 0.10% (percentages are expressed in w/w).

In general, the TS content in yogurt (set or stirred) ranges between 11 and 15%, and according to the regulation, milk solid non-fat content must be at a minimum of 9.5% [33]. In the present study, the TS was increased using lactose up to 16.5% (w/w) for all yogurts except YC, which was at 14% (w/w).

3.2. Bacterial Counts

No effect of yogurt standardization, cooling devices, or even storage time was detected on streptococci populations that stayed at 7.6 $\pm$ 1.0 $\times$ 10^8 CFU/mL. However, a significant interaction between the yogurt standardization and the storage time was found for lactobacilli populations ($p < 0.05$). As shown in Figure 2, globally, lactobacilli populations decreased during storage and were always smaller than the streptococci populations. Y2.6 and Y3.9 tended to have smaller lactobacilli populations compared to the other yogurts.

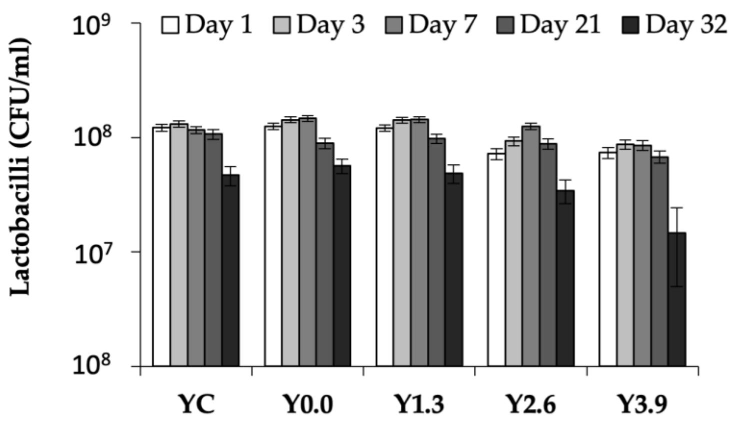

Figure 2. Lactobacilli population evolution during storage of non-fat stirred yogurt standardized at 14% (YC) or 16.5% (Y0.0) total solids and for yogurt standardized at 16.5% total solids and 1.3% (Y1.3), 2.6% (Y2.6), 3.9% (3.9) fat content (percentages are expressed in w/w). Statistical differences are presented in supplementary material Figure S1. Bars represent the standard error of the mean (n = 4).

According to regulations in many countries, yogurt living lactic acid bacteria concentration must be at a minimum of 10^7 CFU/g until the expiration date [1,31], which was the case in the present study of 34 days. Similarly to the present work, Damin et al. [34] showed that, over 35 storage days, in stirred yogurts, streptococci populations are constant, while lactobacilli populations can drop by 2 log. This behavior could be due to the strain sensitivity toward lactic acid or the a packaging material that is too porous toward oxygen increasing the content of dissolved oxygen during storage [34,35]. However, in the present study dissolved oxygen measurements have not been carried out.

YC and Y0.0 had the same bacterial growth, meaning that the difference in lactose content did not modify bacterial growth during fermentation and storage. However, the results from Y2.6 showed that, compared to YC at equal non-fat solid content, the addition of fat limited lactobacilli growth during fermentation and the first two days of storage.

3.3. Physico-Chemical Yogurt Properties

3.3.1. Titratable Acidity and pH

Yogurt pH was influenced by the storage time depending on the yogurt standardization ($p < 0.01$), while the titratable acidity was influenced by the individual effects of yogurt standardization, cooling devices, and storage time ($p < 0.05$). Globally, the pH decreased with storage (Figure 3a). The pH levels of YC and Y2.6 at day 1 were smaller than that of the other yogurts; however, only YC presented the lowest pH throughout the storage period. Titratable acidity tended to decrease with increasing fat content of yogurts and was similar between YC and Y0.0, indicating that titratable acidity was not impacted by the addition of lactose in non-fat stirred yogurts (Figure 3b). Titratable acidity was generally slightly higher in yogurts cooled down with the THX device (Figure 3c). It increased with storage time and was relatively stable after 21 days (Figure 3d).

The diminution of pH and increase in titratable acidity during storage, namely, post-acidification, was expected since lactic acid bacteria keep acidifying yogurts during storage, at 4 °C [35,36]. Post-acidification intensity is strain specific and can be modulated by yogurt formulation, process, or even packaging [35].

In the literature, the effect of fat content on post-acidification is not clear, but it seems that higher FC reduces post-acidification. Similarly to our results, Lorenzen et al. [37] found that titratable acidity tended to be lower in full-fat yogurts compared to non-fat yogurts. Güler-Akin et al. [38] observed opposite trends. While their results indicated a very slight diminution of pH when decreasing FC, the tendency of the titratable acidity to increase was not significant. In the present study, the effect of FC on titratable acidity may have to do with the slightly higher lactobacilli population in yogurts observed with decreasing fat content.

The difference in titratable acidity (0.03% w/v) found between THX and PHX yogurts could be too small to translate into a difference in pH between THX and PHX yogurts. However, this slightly higher titratable acidity in THX yogurts can be explained by the slower cooling rate in the THX system compared to the PHX system (see Section 2.3.3).

The two yogurts with equal lactose content, YC and Y2.6, presented a slightly lower pH than other yogurts, especially during the first week of storage. Previously, the lactose/protein ratio has been observed to impact fermentation kinetics [39]. While lactose is one of the main carbon sources for lactic acid bacteria, the ideal milk composition is strain specific [12,20]. In the present study, yogurt standardization did not impact the fermentation time, but similar mechanisms as in Alvarez, Argüello, Cabero, Riera, Alvarez, Iglesias and Granda [39] may have impacted post-acidification. However, no statistical impact of lactose content was found on stirred yogurt titratable acidity. In general, for both experimental and commercial yogurts, lactose concentration is rarely a limiting factor for bacterial growth. The starter cannot use all the lactose as it is in excess.

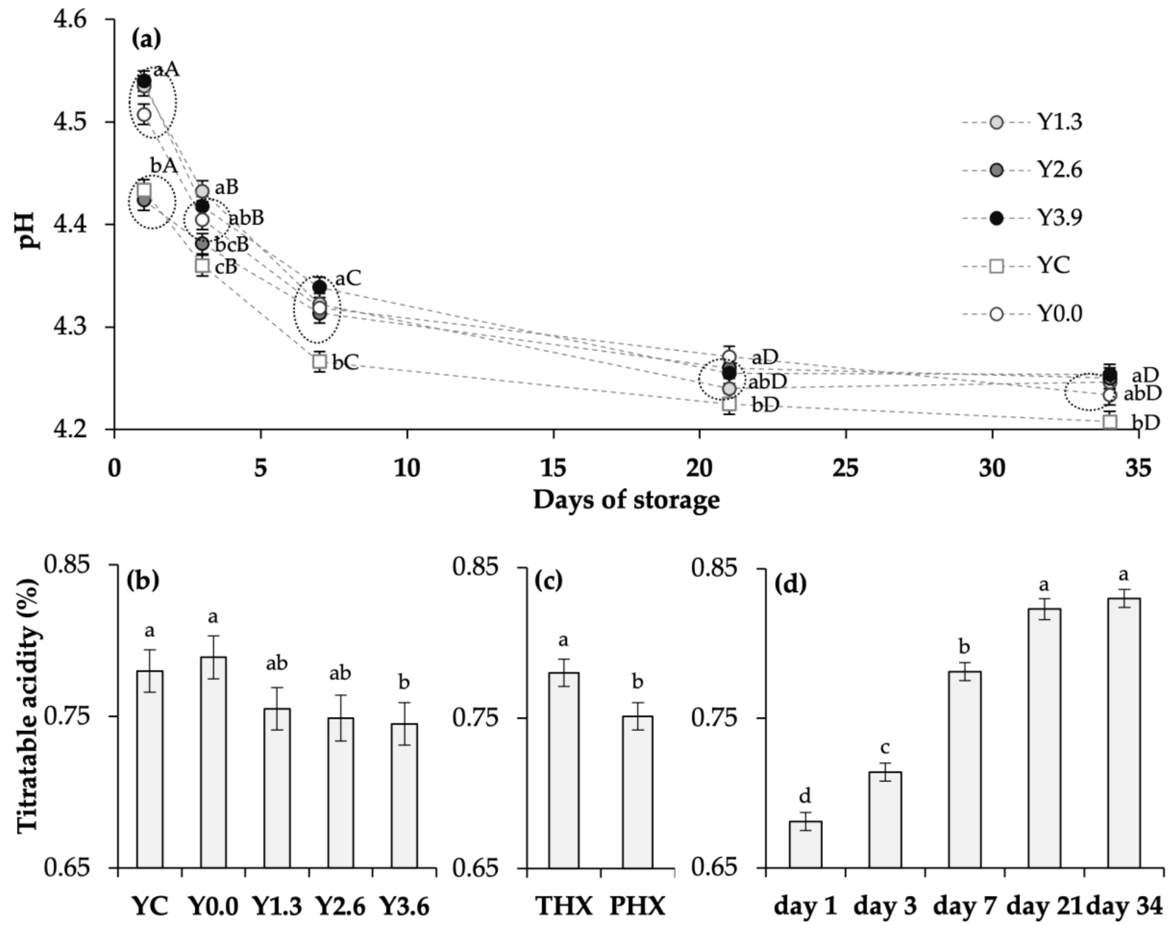

Figure 3. Stirred yogurt pH (**a**) and titratable acidity (% w/v) (**b**–**d**) evolution depending on storage time (days of storage) (**a**,**d**), standardization ((**a**,**b**): non-fat stirred yogurt standardized 14% [YC] or 16.5% [Y0.0] total solids; yogurt standardized at 16.5% total solids and 1.3% [Y1.3], 2.6% [Y2.6], 3.9% [3.9] fat content (w/w), and the cooling device used during stirring/smoothing process (**c**: tubular heat exchanger [THX], plate heat exchanger [PHX]). (**a**): Different letters (a, b, c) indicate significative differences between formulations ($p < 0.05$) for the same days, and different letters (A, . . . , D) indicate significative differences ($p < 0.05$) between days for the same formulation. (**b**–**d**): Different letters (a, . . . , d) indicate significative differences ($p < 0.05$). Bars represent the standard error of the mean ($n = 4$).

3.3.2. Firmness and Rheological Properties

A significant ($p < 0.001$) triple interaction (stirred yogurt standardization, cooling device types, and storage time) was observed for firmness. Figure 4 presents the firmness evolution during storage depending on the yogurt standardization and the cooling device used (statistically significant differences are presented in supplements Figure S2). Globally, firmness increased with the FC when it was higher than 1.3% (w/w). In the non-fat yogurts, lactose addition in Y0.0 did not increase firmness compared to YC. Stirred yogurt firmness increased between days 1 and 21 of storage, especially between days 7 and 21. Stirred yogurt cooled with the THX system tended to be slightly firmer than yogurts cooled with the PHX, apart from YC and Y3.9. No statistical difference in YC cooled down using THX or PHX was found, whatever the storage time. However, Y3.9 cooled down using the PHX system were less firm at day 1 than with the THX but became firmer from day 3 to day 34.

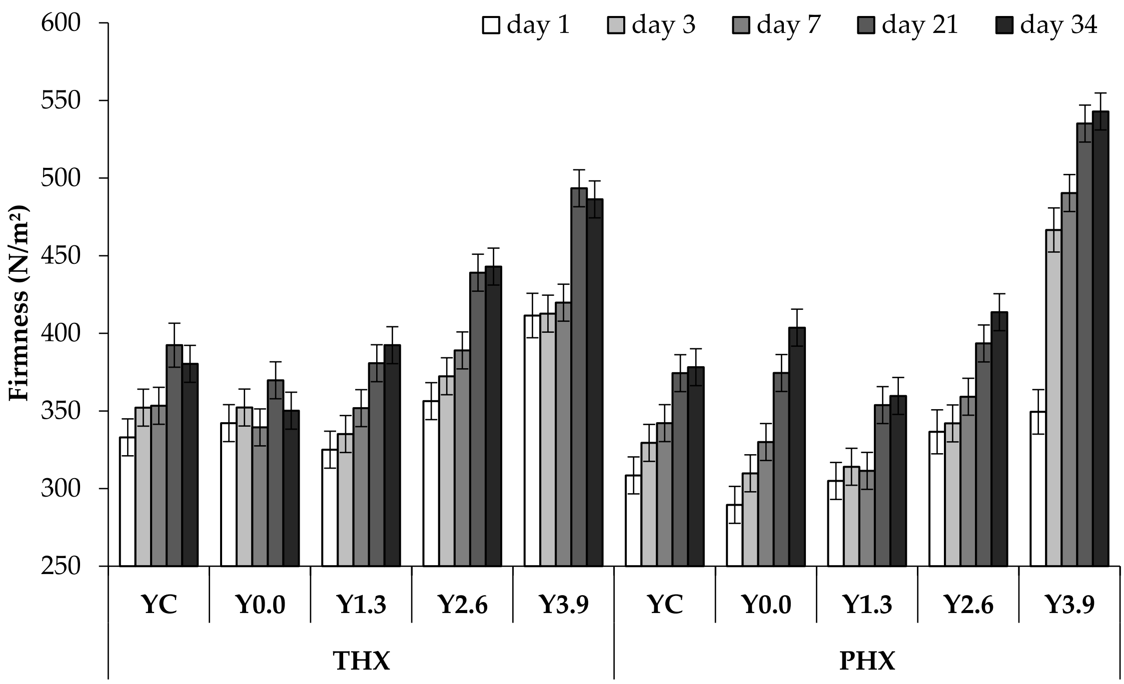

Figure 4. Stirred yogurt firmness evolution depending on storage time and standardization (non-fat stirred yogurt standardized at 14% [YC] or 16.5% [Y0.0] total solids; yogurt standardized at 16.5% total solids and 1.3% [Y1.3], 2.6% [Y2.6], 3.9% [3.9] fat content (w/w), and the cooling device used during stirring/smoothing process (tubular heat exchanger [THX], plate heat exchanger [PHX]). Statistical differences are presented in supplementary material Figure S2. Bars represent the standard error to the mean (n = 4). Yogurt standardization influenced both the apparent viscosity of yogurt at 10 s^{-1} and the thixotropic area of the hysteresis loop (p < 0.01).

Storage influenced only the apparent viscosity (p < 0.01), and the cooling device had no impact on the apparent viscosity or the hysteresis loop. Figure 5a shows the most representative hysteresis loop for each yogurt standardization. The upward curve amplitude globally increased with fat content, especially for Y2.6 and Y3.9. On Y3.9 upward flow curve, a sudden shear stress decline is observed between 5 and 15 s^{-1}. This stress overshoot could indicate the sudden breakage in one of the yogurt gel structure levels, inducing a response that is partially elastic [13,25,40]. The thixotropic area of the hysteresis loop is shown in Figure 5b for each yogurt standardization. The thixotropic area tended to increase with the FC content, Y0.0 and Y3.9 having, respectively, the smallest and the highest thixotropic area. Similarly to the thixotropic area, apparent viscosity at 10 s^{-1} increased with the fat content (Figure 5c), and during the first seven days of storage, after which it was stable (Figure 5d).

Many authors have found that TS increase has a major impact on yogurt properties [7,41]. However, usually, TS content is increased mainly by SMP addition during standardization, which also increases protein content impacting the protein network of yogurt. Very little if any difference in firmness, viscosity, or thixotropy was found between YC and Y0.0. In the literature, higher lactose content, depending on the protein/lactose ratio, has been shown to impact milk gelation and set gel maximum elasticity (G′) [12]. In the present study, lactose was increased from 9.8 (YC) to 12.3% (w/w) (Y0), which may have been insufficient to induce any change in stirred yogurt rheological properties. Additionally, it may be hypothesized that set gel improvement by lactose addition was lost during stirring.

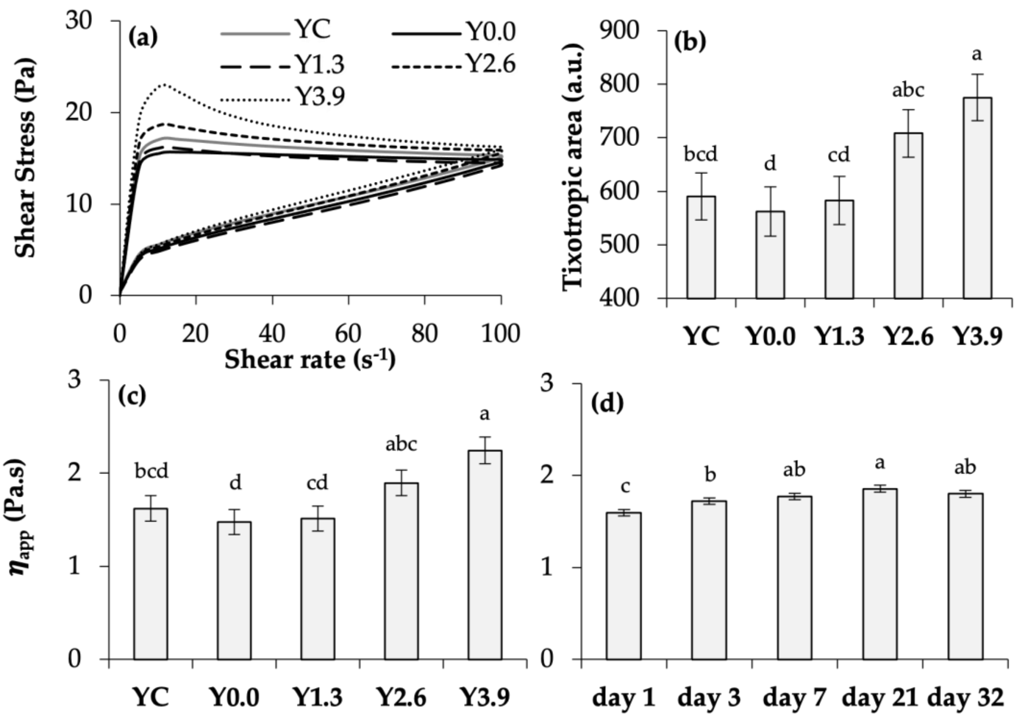

Figure 5. Hysteresis loop (**a**), thixotropic area (arbitrary unit) (**b**), and apparent viscosity at 10 s^{-1} (**c**) of stirred yogurts with different standardization and days of storage (**d**). Non-fat stirred yogurt standardized at 14% total solids [YC] or 16.5% total solids and 0% [Y0.0], 1.3% [Y1.3], 2.6% [Y2.6], 3.9% [3.9] fat content (w/w). Different letters (a, . . . , d) indicate significative statistical differences ($p < 0.05$). Bars represent the standard error of the mean ($n = 4$).

The effect of yogurt FC on viscosity and firmness was expected since higher fat content, in yogurts made from homogenized milk, has often been linked to firmer and more viscous yogurts [7,42]. Concerning the hysteresis experiment, yogurts presented a hysteresis loop when subjected to upward and downward shear flow experiments, which indicates a thixotropic behavior [40,41]. Some authors described the thixotropic area as being proportional to yogurt viscosity and gel destructuration: a higher thixotropic area would mean that a higher energy is required to destructure the yogurt gel [24]. Y3.9 had the highest ascending flow curve and thixotropic area, meaning that structural modifications during shearing have more impact compared to YC, Y0.0, and Y1.3. Moreover, the sudden shear decline observed in Y3.9 (Figure 5a) may reveal the sudden breakage of stronger interactions in the network of yogurts with higher fat content. After homogenization, fat globules are functionalized by the reorganization of the fat globule interface to incorporate caseins, allowing them to act as a pseudocasein particle during fermentation and adding a level of structuration into the gel in both set and stirred yogurt [20,43–45]. If the fat content increases in yogurt formulations, more fat globules will integrate and densify the yogurt gel network, thus increasing the rheological properties (viscosity, elasticity, firmness) in both set and stirred yogurt [8,42,44]. For instance, it has been reported that at a constant shear rate, the apparent viscosity of this denser network composed of protein and fat globules takes longer to decrease toward a stable value because there are more interactions to break down [24]. Comparing YC and Y2.6, while Y2.6 tend to have a higher thixotropy and viscosity than YC, no statistically significant differences were observed. Nevertheless, Y2.6 was significantly more viscous and presented a significantly higher thixotropy than Y0.0. These results are complex to understand, and the variability of results in the present study shows that the yogurt fat content should probably be higher than 2.6% (w/w) to clearly induce higher viscosity in the yogurt.

The increase in viscosity and firmness from day 1 to 21 of storage was also expected, as it is the rebodying of stirred yogurts [46]. Many authors have linked rebodying to the post-acidification phenomena [5,14,23,47,48]. However, not all authors agree on the underlying mechanisms relating to post-acidification and rebodying. Rebodying is a protein network reorganization after shearing induced by either post-acidification [5,23], or the weakening of hydrophobic interactions under cold storage leading to the network expansion [49], or a combination of both phenomena. Stirred yogurt rebodying intensity depends highly on the stirring post-fermentation process sequence [4,47].

At the end of fermentation in the vat, the set yogurt gel interactions structuring the network are broken under the mechanical shearing of the stirring/smoothing process [4,6]. It provokes a gel fragmentation into microgels of varying sizes and shapes depending on yogurt formulation, process temperature and shear intensity [6]. Those microgels can re-aggregate [50,51]. At different production scales (laboratory to industrial scale) many authors have shown that each unitary operation has its own impact on the final stirred yogurt physical properties [25,26]. Yogurt flow inside pipes also contributes to yogurt network destructuration depending on the pressure at each pipe extremities, the presence of right-angled sections, and yogurt density [52,53]. Yogurt network destructuration can thus be minimized by using a cooling system inducing minimal shear.

Using a lab-scale smoothing process, Renan et al. [54] found that shearing creates microgels with surface properties (hydrophobic and electrostatic) favoring inter-particle reorganization that increases stirred yogurt viscosity during storage (i.e., explaining rebodying). Stirred yogurt firmness and viscosity depended on microgel size, aggregation, and microgel inner properties (porosity, rigidity, etc.) [6,14]. As mentioned in Section 2.3.3, the THX system created less intense shearing of the gel than the PHX system and a slower cooldown from 37 to 20 °C. In the literature, post-fermentation shearing of fermented milk gel at higher temperature (close to fermentation temperature) and low shearing intensity has been associated with the formation of larger microgels [22,50]. In the present work, higher microgel sizes and microgel aggregation associated with slightly higher acidity, are probably what lead most THX yogurts to be slightly firmer than PHX yogurts. However, it was not true for YC and Y3.9. Further work is needed to confirm this hypothesis using microstructural analyses.

The results for YC partly contradict Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26], who manufactured YC yogurts using the same formulation and the same equipment as the present study. The results from apparent viscosity at 10 s^{-1} of YC were similar to the present study. However, Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26] found a significantly higher firmness (327 ± 8 vs. 311 ± 8 $N.m^{-2}$) when YC was cooled down using the THX system compared to the PHX system. Between the two studies, the only manufacturing difference was the water temperature in the THX system (14 °C versus 10 °C in the present study). It may have affected the pH of YC in the two studies at day 1 (4.36 ± 0.01 vs. 4.42 ± 0.01 in the present study). Additionally, a difference in the bacterial population is observable. The lactobacilli and streptococci populations were approximately 0.6 and 2.6 times the respective populations in the study of Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26]. However, concerning firmness, the present results on YC are very close to Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26] with values of 333 ± 12 and 308 ± 12 $N.m^{-2}$ when YC was cooled down, respectively, with the THX or the PHX system. It is more probably the slightly higher variability of results in the present study that is responsible for the different statistical outcomes of firmness.

After 2 days of storage, Y3.9 was the only yogurt that was firmer when cooled with PHX rather than THX. In the literature, it has been observed that increasing the yogurt fat content of stirred yogurts tends to reduce microgel size measured by laser diffraction [8,55], but it may also increase network interconnectivity, as observed by confocal laser microscopy [8,56]. The higher shearing process induced by the PHX system may have created smaller microgels in Y3.9-PHX compared to Y3.9-THX, but these may have a higher ability to reaggregate during storage, possibly impacting firmness. For instance, Rasmussen, Janhøj and Ipsen [51] showed that reducing the smoothing filter size from 100 to 50 μm, which increases shearing intensity, greatly increased microgel aggregation after 7 days of storage. This higher reaggregation could explain the higher firmness of Y3.9-THX compared to Y3.9-PHX in the present study. However, granulometric and microstructure characterization is necessary to confirm this effect.

3.3.3. Induced Syneresis

Induced syneresis depended on the individual effect of yogurt standardization and of the cooling device used in the stirring/smoothing process ($p < 0.05$) but did not change during storage ($p > 0.05$). Induced syneresis was not different between the two non-fat yogurts YC and Y0.0; however, it tends to decrease with increasing fat content (Figure 6a). Yogurts produced using the PHX system presented lower values for induced syneresis compared to the yogurt produced with the THX.

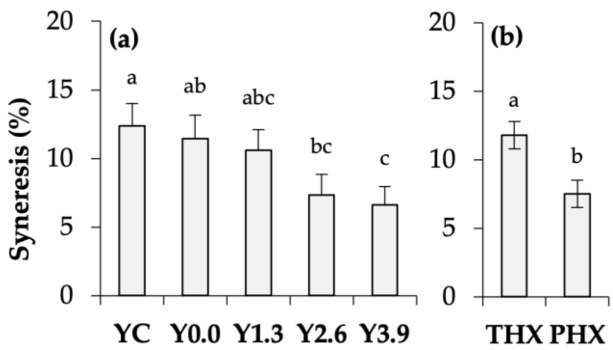

Figure 6. Stirred yogurt induced syneresis depending on standardization: (**a**) non-fat stirred yogurt standardized at 14% [YC] or 16.5% [Y0.0] total solids; yogurt standardized at 16.5% total solids and 1.3% [Y1.3], 2.6% [Y2.6], 3.9% [3.9] fat content (w/w), and the cooling device used during stirring/smoothing process; (**b**) tubular heat exchanger [THX], plate heat exchanger [PHX]). Different letters (a, b, c) indicate significant statistical differences ($p < 0.05$). Bars represent the standard error of the mean.

The impact of the FC content was expected. Yogurts (stirred or set) made from homogenized milk with higher FC have high water-holding capacity because of a denser network interconnection with small pores and microstructures characterized by high water binding capacity [44,45]. The effect of lactose content on syneresis, however, was reported to depend on many factors. Lactose has been found to enhance protein hydration and protein–protein interactions during gelation, which helps increase the water-holding capacity of the gel and thus reduce syneresis [12,57]. However, the lactose/protein ratio can also slow down or speed-up acidification during fermentation depending on the lactic acid bacteria strain used [39,58]. Acidification rates control gel structure and consequently water-holding capacity [12]. In the present study, no difference was found between YC and Y0.0 meaning that the amount of lactose added to equalize TS content was not sufficient to influence syneresis. Looking at Y2.6 it was significantly lower than YC and tended to be lower than Y0.0 but was not significantly different from Y0.0. Again, as observed with viscosity, the effect of FC on syneresis indisputably becomes different when FC is higher than 2.6% (w/w) due to results variability.

The induced syneresis results for YC were similar to Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [26], who worked with the same yogurt formulation. However, in their following work, Guénard-Lampron, St-Gelais, Villeneuve and Turgeon [47] found a slight increase in induced syneresis ($1.6 \pm 0.3\%$ w/w) during the first 13 days of storage of YC. The difference between the two studies may be explained by higher variability in the present study. As shown in Figure 6, the standard error was about 1.0 to 1.7% (w/w), depending on the significant factor. The effect of the cooling system on syneresis can be explained by both the shearing intensity and cooling rate in each system. For instance, Gilbert, Rioux, St-Gelais and Turgeon [50] showed that intense stirring of yogurt at 20 °C instead of 42 °C led to yogurt with more homogeneous microstructure and smaller microgels which reduced induced syneresis. Intense shearing has been found to drastically reduce microgels sizes [22,59]; however, the effect on induced syneresis is more complex, and it seems that an optimal shearing intensity has to be found. High-intensity shears would highly disrupt the network, which lowers the amount of microgels with enclosed pores to hold serum and results in favoring syneresis [51,59]. Low shearing intensity creates a more heterogeneous gel structure that has also been associated with higher induced syneresis [6,51].

4. Conclusions

This study has been conducted with a technical scale to better modelize an industrial line of production. The investigation of the FC and lactose formulation on stirred yogurt cooled down using either THX or PHX system showed that both the choice of the cooling system and FC had a major impact on yogurt rheological properties and induced syneresis. Globally, the use of THX, which produced a slower cooling rate and less intense shearing than PHX, increased both firmness and induced syneresis. While increasing TS using lactose did not have a major effect on non-fat stirred yogurts, increasing the FC content by more than 2.6% (w/w) led to reduced syneresis, higher firmness, viscosity and thixotropy. The standardization at 3.9% (w/w) FC changed radically stirred yogurt properties, especially concerning its firmness during storage when it was smoothed by the THX or the PHX system. It was the only yogurt that was firmer when smoothed with the PHX compared to the THX, indicating a different network reorganization after cooling. From the perspective of commercial yogurt production, our results imply that the cooling system plays a significant role on the yogurt texture and should be considered for process optimization. Moreover, when formulating the yogurt, adjusting the solid contents using lactose had a very limited impact on the final product properties.

In the present study, the stirred yogurt structure was not determined and should be considered for a future study to explore the relationship between the structure and yogurts' properties. Another research question would be to understand how the interaction between the formulation and post-fermentation shearing processes is impacted by other parameters such as fermentation kinetic or the effect of homogenization before or after the heat treatment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/dairy4010008/s1, Figure S1: Lactobacilli population evolution during storage of non-fat stirred yogurt standardized at 14% (YC) or 16.5% (Y0.0) total solids; and for yogurt standardized at 16.5% total solids and 1.3% (Y1.3), 2.6% (Y2.6), 3.9% (3.9) fat content; Figure S2: Stirred yogurt firmness evolution depending on storage time and standardization (non-fat stirred yogurt standardized at 14% [YC] or 16.5% [Y0.0] total solids; yogurt standardized at 16.5% total solids and 1.3% [Y1.3], 2.6% [Y2.6], 3.9% [3.9] fat content), and the cooling device used during stirring/smoothing process (tubular heat exchanger [THX], plate heat exchanger [PHX]); Table S1: Milk formulation targets.

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