Extended storage of cold raw milk on yogurt manufacturing

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Abstract – The objective of this work was to evaluate the effect of the extended cold storage of raw milk on the kinetics of fermentation on yogurt production, as well as on the product's microbiological and physicochemical properties during shelf life. Three treatments were evaluated: yogurts made with raw milk stored for 4, 72, and 168 hours. Kinetics of fermentation was assessed through the lactic acid production rate, growth rate of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and time to reach pH 5.0 and 4.5. The physicochemical and microbiological characteristics of raw milk and yogurts were also analyzed during cold storage at 4°C. The microbial quality of raw milk was affected by the storage time of 168 hours, with a decreasing tendency in the mesophiles:psychrotrophs ratio. Extended storage of raw milk beyond 72 hours negatively affects yogurt production, despite the low initial bacterial count, decreasing lactic acid production, *S. thermophilus* growth rate, pH, and protein content. Therefore, to optimize fermentation and yogurt shelf life, the maximum storage time for raw milk at 4°C should not exceed 72 hours.

Index terms: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, fermentation, kinetics, psychrotrophs.

Armazenamento prolongado de leite cru refrigerado sobre a produção de iogurte

Resumo – O objetivo deste trabalho foi avaliar o efeito do armazenamento prolongado de leite cru refrigerado na cinética de fermentação na produção de iogurte, bem como nas características físico-químicas e microbiológicas do produto durante sua vida útil. Três tratamentos foram avaliados: iogurte feito com leite cru armazenado por 4, 72 e 168 horas. A cinética de fermentação foi analisada por meio da taxa de produção de ácido lático, da velocidade de crescimento de *Streptococcus thermophilus* e *Lactobacillus bulgaricus*, e do tempo até atingir pH 5,0 e 4,5. As características físico-químicas e microbiológicas do leite cru e do iogurte também foram avaliadas durante o armazenamento refrigerado a 4°C. A qualidade microbiológica do leite cru foi influenciada pelo tempo de armazenamento de 168 horas, com tendência de decréscimo na proporção mesófilos:psicrotróficos. O armazenamento prolongado do leite cru acima de 72 horas influencia negativamente a produção de iogurte, mesmo com contagem bacteriana inicial baixa, com diminuição na taxa de produção de ácido lático, na velocidade de crescimento de *S. thermophilus*, no pH e na percentagem de proteína. Portanto, para otimização da fermentação e da vida útil do iogurte, o tempo de armazenamento do leite cru a 4°C não deve exceder 72 horas.

Termos para indexação: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, fermentação, cinética, psicrotróficos.

Introduction

Milk cooling in dairy farms and the bulk hauling system are common practices within the dairy production chain of many countries to maintain the microbiological quality of raw milk until arrival at the industry. However, the heat-resistant enzymes involved in the growth of psychrotrophic bacteria severely affect the quality of dairy products such as yogurts and other fermented milks (Decimo et al., 2014).

Even though some topics on spoilage microorganisms have been extensively explored, including *Pseudomonas* spp. and their proteolytic potential (Scatamburlo et al., 2015), only a few studies have investigated the maximum cold storage time of raw material to prevent industrial losses. It has been reported that raw milk with high bacterial counts, especially of psychrotrophic microorganisms, may impair the production and yield of yogurts, reducing the products' shelf life and acceptance (Al-Kadamany et al., 2003; Shin et al., 2014). However, there is no knowledge of studies, at a dairy plant level, that clearly indicate how cold storage of raw milk could diminish losses due to psychrotrophic bacteria growth.

During the past 30 years, many researchers have studied the effects of cold storage conditions on the microbiological and physicochemical characteristics of milk, but only some have considered the effect of cold storage on the processing properties of dairy products. It was observed that the cold storage of raw milk results in casein dissociation, in the release of inorganic calcium from the micelles, and in the increased activities of plasmins and associated enzymes, i.e., proteases and lipases, affecting shelf life and ultimately the quality of heat-processed dairy products (Malacarne et al., 2013). Furthermore, the cleavage of the fat globule membrane by phospholipases to glycerol and fatty acids leads to milk fat degradation (Lopez et al., 2010). It should be noted, however, that these studies are mainly focused on cheese yield, not on how raw milk storage can affect the quality of processed yogurts related to fermentation and shelf life (Leitner et al., 2008).

Besides low yield, microbiological contamination of raw milk can also result in sensory defects and reduced shelf life. Çakmakçi et al. (2012) observed satisfactory sensory scores for freshly made yogurts, which, however, decreased after seven days of storage, resulting in higher acidity, which is less preferred by the consumer.

Therefore, since psychrotrophic microbiota of milk may negatively affect yogurt production and decrease shelf life, further studies on their effect on dairy products can help industries reduce losses and optimize the fermentation process.

The objective of this work was to evaluate the effect of the extended cold storage of raw milk on the kinetics of fermentation on yogurt production, as well as on the product's microbiological and physicochemical characteristics during shelf life.

Materials and Methods

The raw milk used throughout the experiment was obtained from one farm with enclosed milking facilities and a plate precooler, selected as it complied with well-established criterion for quality raw milk production: good milking and herd management practices; and low somatic cell and total bacterial counts at a bulk tank level.

After milking and cooling at 4°C, 5 L of milk from the farm tank were aseptically collected and immediately hauled, at 4°C, to the dairy pilot plant of Universidade do Norte do Paraná. Raw milk was kept refrigerated at 4°C throughout the entire evaluation period for 4, 72, and 168 hours before being pasteurized at 62°C, for 30 min, and processed to yogurt. Therefore, the yogurt was manufactured with the same milk batch, which was subjected to different storage times, resulting in three treatments: yogurts produced from raw milk kept for 4 (D0), 72 (D3), and 168 hours (D7) at 4°C.

Raw milk was analyzed at D0, D3, and D7 for: physicochemical characteristics, which included pH, titratable acidity, total protein, fat, lactose, total solids, and ash content; and microbiological characteristics, in this case, the enumeration of aerobic mesophilic microorganisms, psychrotrophs, coliforms, and *Escherichia coli*.

At D0, milk somatic cell count, using the Somacount 300 instrument (Bentley Instruments, Inc., Chaska, MN, USA), and antibiotic residue analysis, with the Snap Beta-Lactam ST and Snap Duo Beta-Tetra tests (Idexx Laboratories, Inc., Westbrook, ME, USA), were also assessed.

pH was measured by a potentiometer and acidity by titration with Dornic solution (0.1111 N NaOH), using phenolphthalein as the indicator. Nitrogen content was estimated by the micro-Kjeldahl method followed by conversion (×6.38) to crude protein content (Wehr & Frank, 2004), whereas Gerber's method was used to estimate fat content (Wehr & Frank, 2004). Total solids were measured by oven drying at 105°C for 16 hours, and ash was determined according to Wehr & Frank (2004).

A Petrifilm AC plate (3M Company, St. Paul, MN, USA) was used to enumerate aerobic mesophilic flora at 37°C for 48 hours. Psychrotrophic microorganisms were enumerated by surface plating of the milk sample onto plate count agar (PCA) medium at 21°C for 25 hours. A Petrifilm EC plate (3M Company, St. Paul, MN, USA) was used to enumerate total coliforms and *E. coli*, respectively, at 37°C for 24 hours and at 37°C for 48 hours. The Petrifilm system was used according to the manufacturer's instructions.

For yogurt manufacturing, pasteurized milk at 62°C was cooled to 42°C and promptly inoculated with a *Streptococcus thermophilus* and *Lactobacillus bulgaricus* lyophilized yogurt culture (FD-DVS YF L812 Yo-Flex, Chr. Hansen Holding A/S, Hoersholm, Denmark) at 1% (wt/wt). Subsequently, inoculated milk was poured into 1,000-mL sterile glass bottles and into 18-cm sterile screw-cap test tubes, then incubated at 45°C.

pH was monitored during milk renneting until pH 4.5 (pH end point); the milk was then cooled at 4°C and kept stored until further analysis.

Kinetics of fermentation from each batch was determined by placing 10 to 20 mL of each freshly inoculated yogurt (D0, D3, and D7) in 20 sterile test tubes, kept incubated at 43°C. Two tubes (duplicate) at a time were taken every 30 min for pH and Dornic acidity determination. Another aliquot was used for the microbiological analysis, which was assessed once every subsequent hour.

For further physicochemical analysis and storage effect evaluation, 300 mL of each freshly inoculated yogurt (D0, D3, and D7) were packed into three sterile screw-cap glass containers.

To assess fermentation kinetics (20 tubes) throughout the fermentation process, an aliquot was analyzed as to: lactic acid production rate, in g L^{-1} h^{-1}; growth rate of *S. thermophilus* and *L. bulgaricus*, in colony-forming unit (CFU) L^{-1} h^{-1}; and time to pH 5.0 and to 4.5 in minutes, which was evaluated every 30 min.

To calculate the lactic acid production rate, at 30-min intervals, two tubes were retrieved from incubation for pH and titratable acidity (in duplicate) determination, in order to plot the fermentation curve, as follows: $dP/dt = [(\ln P_3 - \ln P_1) / (t_3 - t_1)] \times P_2$, in which ln is the Naperian logarithm; P is the lactic acid concentration (g L^{-1}); P_1 is the lactic acid concentration at time t_1; P_2 is the lactic acid concentration at time t_2; P_3 is the lactic acid concentration at time t_3; and t is the fermentation time (Sinclair & Cantero, 1990).

To determine the growth rate of *S. thermophilus* and *L. bulgaricus*, at every hour of fermentation, one test tube was removed from the incubation chamber to enumerate *S. thermophilus* and *L. bulgaricus* populations in duplicate analysis. For *L. bulgaricus*, this was done by plate count with de Man-Rogosa-Sharpe (MRS) agar, with the addition of acetic acid, at pH 5.4, incubated anaerobically at 37°C for 72 hours; and, for *S. thermophilus*, with lactose-M17 agar aerobically incubated at 37°C for 48 hours (Wehr & Frank, 2004). The following formula was used: $dP/dt = [(\ln X_3 - \ln X_1) / (t_3 - t_1)] \times X_2$, in which ln is the Naperian logarithm; X_1 is the population of microorganisms at time t_1; X_2 is the population of microorganisms at time t_2; X_3 is the population of microorganisms at time t_3; and t is the fermentation time (Sinclair & Cantero, 1990).

For determination of the physicochemical and microbiological properties of the yogurts during shelf life, three yogurt samples of each treatment (D0, D3, and D7) were stored at 4°C and evaluated at 24 hours, 7 days, and 15 days after manufactured. Titratable acidity, pH, total protein, fat, lactose, total solids, ash, as well as *L. bulgaricus*, *S. thermophilus*, yeast, mold, total coliforms and *E. coli* counts, were determined as previously described. A Petrifilm YM plate (3M Company, St. Paul, MN, USA) was used to enumerate yeasts and molds at 24°C for 72 hours.

Data were subjected to the Kolmogorov-Smirnov test, and visual inspection of the distribution was used to check normality and homoscedasticity (Drezner et al., 2010). Normal distribution was not observed and, therefore, the numerical variables (enumeration of aerobic mesophiles, psychrotrophs, and coliforms) were categorized as ordinal scale by logarithmic transformation.

Observed differences in the microbiological enumeration and physicochemical characteristics of raw milk and yogurts during storage were assessed by the Wilcoxon paired test, at 5% probability. The variables of each treatment were also compared in pairs using the same statistical approach, and the experiment was performed fivefold to account for randomized effects.

Results and Discussion

A decrease in aerobic mesophilic bacteria was observed when compared with the initial counts in raw milk after 168 hours of storage at 4°C, from 4.38 to 4.05 log CFU mL^{-1}, respectively (Table 1). However, the number of psychrotrophic bacteria remained the same throughout the seven-day storage period.

The mesophiles:psychrotrophs ratio also decreased during raw milk storage. Although from D0 to D3, the average ratio remained unchanged, there was a decrease to 0.88 at D7. In the present study, a trend for the reduction of the aerobic mesophilic population was observed during the cold storage of raw milk (Table 1). This pattern is related to the adaptation of microorganisms to refrigeration (Souza et al., 2009) and has also been reported by Malacarne et al. (2013) and O'Connell et al. (2016). For most psychrotrophs, optimum growth temperatures range from 20 to 30°C, since these are primarily mesophilic microorganisms capable of adapting to milk cooling temperatures. In addition, psychrotrophic microbiota selection occurs at around 4°C, producing heat-resistant enzymes and, consequently, increasing rancidity and bitter taste, besides decreasing dairy product yield (Stoeckel et al., 2016).

In the European Union and the United States, milk quality regulations standardized total bacterial count for dairy manufacturing at ≤100,000 CFU mL^{-1} (European Comission, 2004; Grade…, 2011). However, current Brazilian ordinance limits total bacterial count in raw milk to 300,000 CFU mL^{-1} (Brasil, 2011). Therefore, all batches of raw milk used in the present study complied with the Brazilian statutory regulation.

Total coliforms and *E. coli* in raw milk remained unchanged during the entire storage period (Table 1). Coliforms ranged from 1 to 3.56 log CFU mL^{-1}, whereas *E. coli* ranged from <1 to 10 CFU mL^{-1}. Since trading of raw milk for direct consumption in Brazil is forbidden, neither coliforms nor *E. coli* CFU limits are established in the country's milk ordinance.

No differences were found for raw milk physicochemical parameters at 4°C during the storage period (Table 1). Titratable acidity, pH, fat, and protein content remained constant during the seven days of storage. Owing to the low CFU initially observed in the raw milk and to its storage temperature, only minor changes on those parameters were observed due to bacterial spoilage (psychrotrophs). However, expected changes in further physicochemical properties, such as water-soluble nitrogen, nonprotein nitrogen, peptide nitrogen, free amino acids with the production of heat-stable proteases and lipases, would be likely to occur with time (Mankai et al., 2012). These latter parameters, however, were beyond the scope of the present study and, therefore, were not assessed.

The lactic acid production rate during fermentation was affected by the increase of raw milk storage time (Table 2). Even though no differences were observed between yogurts produced from raw milk stored for 4 and 72 hours, there was a decrease in the rate of lactic acid production with storage for 168 hours.

The growth rate of *S. thermophilus* was also affected by raw milk storage (Table 2). The bacteria showed limited activity due to the storage time lapse,

Table 1. Median, mean ($\bar{X}$), standard deviation (σ), and range (⊢) of pH, titratable acidity, fat, total protein, aerobic mesophilic bacteria (Am) and psychrotrophic bacteria (Psy) counts, aerobic mesophiles:psychrotrophs ratio (Am:Psy), and total coliform and *Escherichia coli* (Ec) counts in raw milk stored for 4 (D0), 72 (D3), and 168 (D7) hours at 4ºC[(1)].

Physicochemical and microbiological characteristics	D0		D3		D7	
	Median	$\bar{X}$±σ	Median	$\bar{X}$±σ	Median	$\bar{X}$±σ
pH	6.70	6.69±0.03 6.64⊢6.72	6.79	6.75±0.10 6.62⊢6.84	6.68	6.71±0.08 6.63⊢6.83
Titratable acidity (ºD)	16.30	16.68±2.01 14.0⊢20.0	16.30	16.36±0.74 15.0⊢18.0	16.00	16.36±0.96 15.0⊢18.0
Fat (%)	3.40	3.52±0.39 3.20⊢4.10	3.45	3.40±0.29 3.00⊢3.70	3.40	3.38±0.15 3.20⊢3.60
Total protein (%)	3.15	3.13±0.20 2.90⊢3.32	3.49	3.45±0.38 3.03⊢3.79	3.38	3.26±0.30 2.86⊢3.54
Aerobic mesophilic bacteria (log CFU mL^{-1})	4.38a	4.32±0.22 4.10⊢4.63	4.34a	4.34±0.24 4.11⊢4.65	4.05b	4.17±0.26 3.89⊢4.57
Psychrotrophic bacteria counts (log CFU mL^{-1})	4.67	4.57±0.25 4.18⊢4.86	4.74	4.65±0.34 4.19⊢4.92	4.69	4.73±0.19 4.51⊢5.01
Am:Psy ratio	0.94b	0.95±0.06 0.88⊢1.03	0.92b	0,93±0.05 0.88⊢0.98	0.89a	0.88±0.03 0.84⊢0.91
Total coliform (log CFU mL^{-1})	1.78	2.09±0.94 1.30⊢3.50	1.90	2.15±0.79 1.70⊢3.56	1.15	1.74±1.05 1.00⊢3.43
Escherichia coli (CFU mL^{-1})	3.00	4.20±3.35 2.00⊢10.00	1.00	2.20±2.17 <1.0⊢5.00	1.00	3.00±4.06 <1.0⊢10.0

[(1)]Medians followed by different letters, lowercase in the lines, differ by Wilcoxon's test, at 5% probability.

causing the decrease in lactic acid yield, which was probably related to the storage of milk prior to its pasteurization, when free fatty acids might have been released by psychrotrophic enzymes, inhibiting the growth of lactic acid bacteria (Beal et al., 2001; Huang et al., 2010). Polyunsaturated fatty acids, such as free linoleic acid and oleic acid, are reported to lead *S. thermophilus*, *Lactobacillus* GG (probiotic), and *Lactobacillus casei Shirota* to cell death (Kankaanpää et al., 2001). This is important since the acidifying ability of *S. thermophilus* decreases yogurt pH (Lecomte et al., 2016).

The fermentation time span among D0, D3, and D7 yogurts did not differ. The time lapse to pH 5.0 was of 3 hours and to the final pH 4.5 of 4.2 hours, with an average time span of 2.2 hours to reach the highest lactic acid production (Figure 1). Oliveira et al. (2009) investigated milk fermentation using *S. thermophilus* and *L. bulgaricus* and found an average time lapse of 3.68 hours to pH 5.0, of 5.47 hours to the final pH 4.5, and of 3 hours to the highest acidification rate.

Viable cell counts of *S. thermophilus* and *L. bulgaricus* increased during the fermentation process in all treatments. However, *S. thermophilus* counts (7 to 8 log CFU mL^{-1}) remained higher than those of *L. bulgaricus* (2 to 3 log CFU mL^{-1}) throughout the entire fermentation process (Figure 2). A ratio of 5:1 (chain:chain) of *S. thermophilus* to *L. bulgaricus* is desired in yogurt and can be achieved at an optimum pH of 6.4 and temperature of 46°C (Aghababaie et al., 2015).

It was also observed that the storage time span of raw milk negatively affected the pH of the produced yogurts during shelf life (Table 3), decreasing the pH median from 4.41, on the first day of storage, to 3.93 after 15 days of storage. However, this difference was solely observed for the D7 yogurt, which leads to the assumption that, regardless of its initial bacterial count, the manufacturing viability of aged raw milk to yogurt can be reduced, as found in the present study.

The decrease in pH leads to a substantial sour taste in yogurt. Although sourness is an expected characteristic of yogurt, excessive acidification (post-acidification) may result in lower acceptance, syneresis, and variations in aroma volatile metabolites, especially in acetic acid and 2-butanone, as well as in nonvolatile ones, decreasing the organoleptic quality of yogurt (Çakmakçi et al., 2012; Settachaimongkon et al., 2016).

Table 2. Median, mean ($\bar{X}$), standard deviation (σ), and range (⊢) of lactic acid production rate (L.acid), growth rate of *Streptococcus thermophilus* (St) and *Lactobacillus bulgaricus* (Lb), time lapse to pH 5.0 ($t_{pH\ 5.0}$) and to pH 4.5 ($t_{pH\ 4.5}$), time lapse to the highest lactic acid production rate (Tmax L.acid), and time lapse to the highest growth rate of *S. thermophilus* (Tmax St) and *L. bulgaricus* (Tmax Lb) in yogurts produced from raw milk stored for 4 (D0), 72 (D3), and 168 (D7) hours at 4ºC[1].

Physicochemical and microbiological characteristics	D0		D3		D7	
	Median	$\bar{X}$±σ	Median	$\bar{X}$±σ	Median	$\bar{X}$±σ
L.acid ($g\ L^{-1}\ h^{-1}$)	1.89a	1.70±0.69 0.13⊢2.83	2.02a	1.84±0.73 0.14⊢3.26	1.73b	1.62±0.68 0.13⊢3.60
St (log CFU $L^{-1}\ h^{-1}$)	8.92ab	8.88±0.27 8.28⊢9.10	9.00a	9.07±0.21 8.86⊢9.44	8.84b	8.83±0.14 8.54⊢8.97
Lb (log CFU $L^{-1}\ h^{-1}$)	1.95	1.93±0.37 1.37⊢2.41	2.18	2.22±0.39 1.78⊢2.79	2.25	2.18±0.53 1.28⊢2.79
$t_{pH\ 5.0}$ (min)	186	180±23.66 150⊢210	186	180±23.66 150⊢210	174	180±12.65 150⊢180
$t_{pH\ 4.5}$ (min)	258	240±25.30 240⊢300	270	252±25.30 210⊢270	240	240±28.28 210⊢270
Tmax L.acid (min)	150	144±12.65 120⊢150	120	138±25.30 120⊢180	120	126±12.65 120⊢150
Tmax St (min)	180	165±27.78 120⊢180	150	150±32.01 120⊢180	120	135±27.78 120⊢180
Tmax Lb (min)	180	160±30.98 120⊢180	180	180±0.00 180⊢180	180	180±0.00 180⊢180

(1)Medians followed by different letters, lowercase in the lines, differ by Wilcoxon's test, at 5% probability.

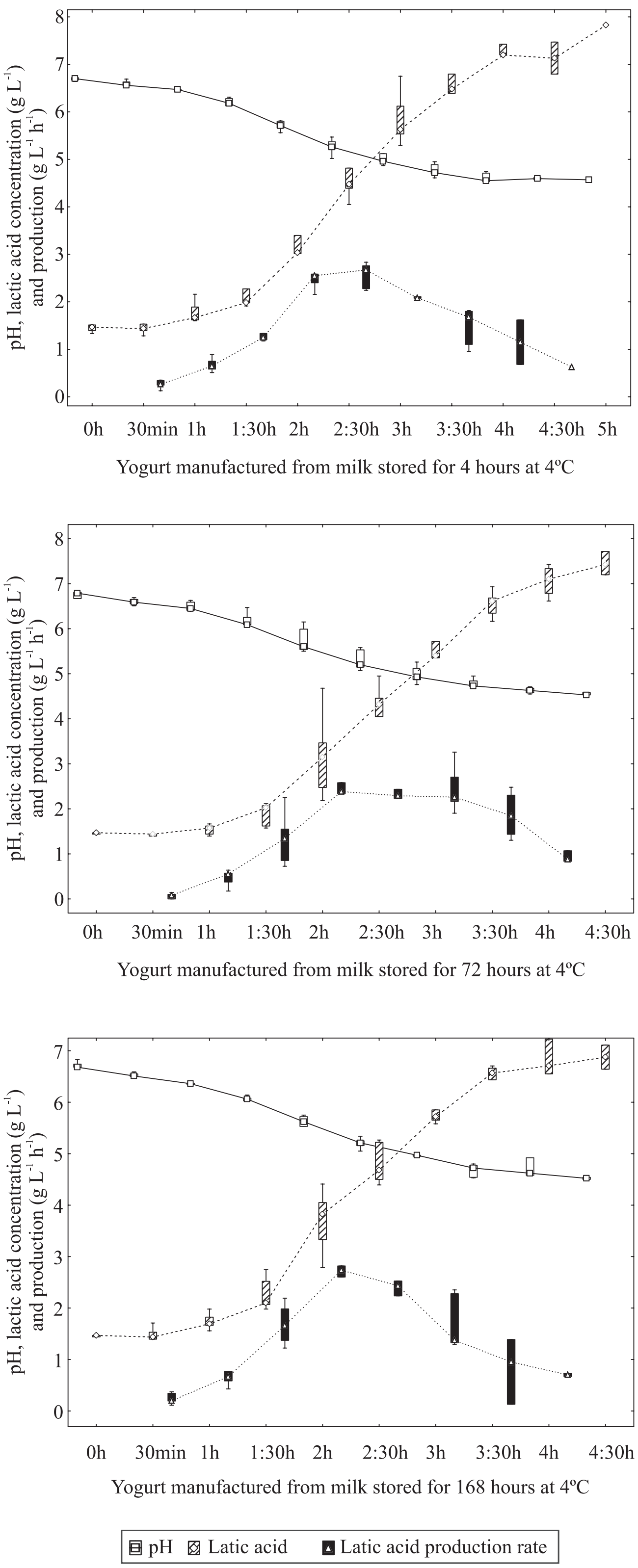

Figure 1. pH, lactic acid concentration, and lactic acid production rate during fermentation of yogurts manufactured with raw milk stored for 4, 72, and 168 hours at 4ºC.

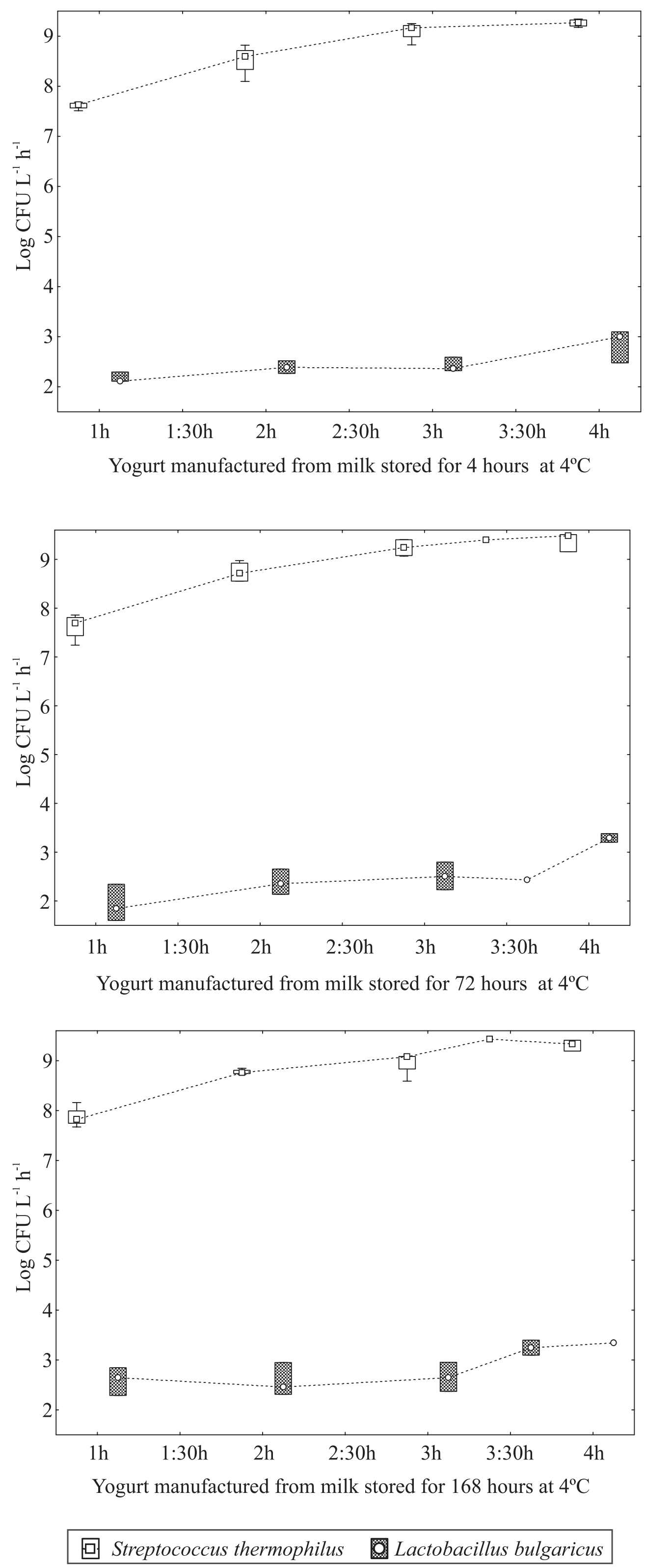

Figure 2. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* populations in yogurts manufactured with raw milk stored for 4, 72, and 168 hours at 4ºC.

The main reasons for post-acidification are the higher proportion of lactobacilli to streptococci and proteolysis (Rajapaksha et al., 2013). Since no changes were observed for the enumeration of *S. thermophilus* and *L. bulgaricus* in the present study, it is presumed that the protein degradation of the yogurt produced from raw milk stored for an extended period of time contributed to the acidification of the product.

The protein content of the yogurts stored for 15 days decreased when raw milk was stored for 72 and 168 hours (Table 3). Therefore, the extended storage of raw milk can affect the nutritional aspects of yogurt during shelf life, particularly when yogurt storage time must also be extended under refrigeration.

D7 raw milk produced the yogurt with the lowest protein content (Table 3). Yamazi et al. (2013) also reported low protein contents and decreased yield in cheese from raw goat milk kept refrigerated for more than 48 hours. According to Perin et al. (2012), when raw milk is stored at 4°C, for an extended time period, the dairy product yield is reduced due to the growth of proteolytic psychrotrophic bacteria within 48 hours. As over 80% of the psychrotrophic microorganisms in raw milk may determine proteolysis, the remaining proteolytic activity must be considered even when milk is pasteurized or subjected to ultra-high temperature treatment prior to yogurt production (Nörnberg et al., 2010).

It should be highlighted that protein is not only regarded as an important nutrient, it also standardizes the identity of dairy products. For instance, protein percentages in fermented milks are required to be at least 2.9% in Brazil (Brasil, 2007). Consequently, raw milk storage can also play a key role in ensuring the availability of solids in raw milk, in order to comply with yogurt standards. Viable *S. thermophilus* and *L. bulgaricus* CFUs are also important for yogurt shelf life and identity. In the present study, the yogurt

Table 3. Median, mean ($\bar{X}$), standard deviation (σ), and range (⊢) of pH, titratable acidity, fat and total protein, as well as of *Streptococcus thermophilus* (St), *Lactobacillus bulgaricus* (Lb), total coliforms (Ct), *Escherichia coli* (Ec), and yeast and mold (Ym) counts, during shelf life of 1, 8, and 15 days of yogurts produced from raw milk stored for 4 (D0), 7 (D3), and 168 (D7) hours at 4ºC[1].

Physicochemical and microbiological characteristics	1 day		8 days		15 days	
	Median	$\bar{X}$±σ	Median	$\bar{X}$±σ	Median	$\bar{X}$±σ
			D0, raw milk stored for 4 hours at 4ºC			
pH	4.35	4.38±0.08	4.37	4.34±0.10	4.33	4.25±0.18
Titratable acidity (ºD)	74.5	75.5±3.26	76.3	75.5±1.53	77.0	76.6±2.72
Fat (%)	3.10	3.16±0.13	3.20	3.08±0.36	3.30	3.10±0.57
Total protein (%)	3.25	3.31±0.31	3.64	3.56±0.19	3.47A	3.42±0.25
St (log CFU mL^{-1})	9.15	9.16±0.08	9.10	9.12±0.07	9.09	9.02±0.20
Lb (log CFU mL^{-1})	3.35	3.27±0.46	3.53	3.58±0.13	3.50	3.39±0.29
Ct/Ec/Ym (CFU mL^{-1})	<1	<1±0.00	<1	<1±0.00	<1	<1±0.00
			D3, raw milk stored for 7 hours at 4ºC			
pH	4.40	4.39±0.06	4.38	4.36±0.10	4.30	4.30±0.10
Titratable acidity (ºD)	75.5	76.1±2.75	75.5	76.4±2.22	76.8	77.3±1.53
Fat (%)	3.10	3.08±0.26	2.90	2.86±0.40	3.20	3.14±0.68
Total protein (%)	3.41ab	3.37±0.37	3.53a	3.72±0.55	3.34bA	3.14±0.47
St (log CFU mL^{-1})	9.36	9.38±0.19	9.18	9.17±0.16	9.11	8.88±0.93
Lb (log CFU mL^{-1})	3.46	3.45±0.18	3.64	3.52±0.32	3.41	3.40±0.40
Ct/Ec/Ym (CFU mL^{-1})	<1	<1±0.00	<1	<1±0.00	<1	<1±0.00
			D7, raw milk stored for 168 hours at 4ºC			
pH	4.41a	4.38±0.16	4.31a	4.26±0.22	3.93b	4.11±0.29
Titratable acidity (ºD)	77.0	77.0±4.51	74.0	77.5±5.85	77.3	77.3±3.62
Fat (%)	3.30	3.14±0.36	3.30	3.28±0.16	3.40	3.50±0.41
Total protein (%)	3.16	2.99±0.42	3.20	3.24±0.34	3.11B	3.09±0.29
St (log CFU mL^{-1})	9.17	8.93±0.69	9.24	8.82±0.87	9.14	9.09±0.09
Lb (log CFU mL^{-1})	3.65	3.61±0.61	3.37	3.40±0.15	3.50	3.53±0.25
Ct/Ec/Ym (CFU mL^{-1})	<1	<1±0.00	<1	<1±0.00	<1	<1±0.00

[1]Medians followed by different letters, lowercase in the lines and uppercase in the columns, differ by Wilcoxon's test, at 5% probability.

bacterial counts within 15 days of storage were not different, regardless of which raw milk (D0, D3, or D7) was used (Table 3). It was observed that *S. thermophilus* and *L. bulgaricus* counts ranged from 9.09 to 9.36 log CFU mL^{-1} and from 3.35 to 3.65 log CFU mL^{-1}, respectively, which complied with the minimum lactic acid bacteria counts established for this product (Brasil, 2007).

Conclusions

1. The extended storage of raw milk at 4°C beyond 72 hours negatively affects yogurt manufacturing, regardless of the initial bacterial count, and decreases the lactic acid production rate and *Streptococcus thermophilus* growth rate during the fermentation process.

2. The extended storage of raw milk for more than 72 hours also affects yogurt shelf life by decreasing its pH and protein content.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for financial support.

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Received on May 24, 2016 and accepted on August 18, 2016