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Full Length Research Paper

Microbiological quality of yoghurt commercialized in Viçosa, Minas Gerais, Brazil

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Yoghurt consumption has been increasing since 1980 decade due to the search for healthy foods by consumers, including Brazil. In order to evaluate the quality of the yoghurt commercialized in Viçosa, Minas Gerais, Brazil, 36 samples were collected from markets and analyzed for coliforms (total and thermotolerant) and lactic acid bacteria (LAB) enumeration and pH, considering different times for expiration. Coliforms were not detected at levels higher than 0.3 MPN/g, and only 7 (19.4%) samples showed LAB counts lower than 10^7 CFU/g. However, the mean pH observed in these samples (4.4) was not significantly different when compared to samples with higher LAB counts, suggesting addition of weak starter cultures during the processing. In the samples with less than 15 days for expiration the LAB counts and pH values were 7.4 log CFU/g and 4.1, respectively, and they were lower when compared to samples with more than 16 days of commercial viability, but without significant differences. The obtained results indicate adequate quality of yoghurt commercialized in Viçosa.

Key words: Yoghurt, quality, coliforms, lactic acid bacteria, pH.

INTRODUCTION

The consumption of fermented milks by man dates from the beginning of civilization, once residues of these products were found in pottery fragments from Neolithic, Bronze and Iron Ages settlements in Britain (McKinley, 2005). It is accepted that the initial consumption of fermented or cultured milk products, such as yoghurt, butter and cheese, occurred around the time as they were recognized as effective means of prolonging the shelf-life of milk (Ross et al., 2002; Tamine and Robinson, 2007).

One of the most traditional cultured milk is the yoghurt, which is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Steinkraus, 1997; Tamine and Robinson, 2007). This product is widely consumed worldwide, mainly in northern European countries, Balkans, Middle-East and Indian sub-continents (Tamine, 2002). The main reasons pointed out for yoghurt consumption is the cultural and the increasing search for healthy foods (Kalantzopoulos, 1997; McKinley, 2005; Álvarez-León et al., 2006; Cueva and Aryana, 2008), and in Brazil, such consumption has been increasing since 1980's.

The yoghurt production is quite simple, and the rising consumption of this product has led to the development of automated and sophisticated equipment for the Industrial processing. Despite the practicability, the current processing equipments must provide proper quality and safety (Salinas, 1986). The maintenance of these secure characteristics is essential even in the post-production steps, like storage, transport and commercialization. Microbiological parameters are generally used to verify these conditions, especially by coliforms and lactic acid bacteria (LAB) enumeration (Tamine and Robinson, 2007). At commercial points, yoghurts produced in Brazil must contain at least 10^7 colony formers unit per gram (CFU/g) of LAB and less than 10 most probable number per gram (MPN/g) of thermotolerant coliforms for indicative samples (Brasil, 2000, 2001). Coliforms quantification allows the verification of raw milk quality and the efficiency of the processing, and LAB enumerating indicates the levels of added starter culture and its

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development during the storage and shelf-life. In addition, the measurement of acidity is also observed in order to evaluate the preservation of yoghurt status, varying from 0.6 to 1.5 g of lactic acid per 100 g of product, and the temperature of preservation in dairy industries and markets must not be higher than 10°C (Brasil, 2000).

The objective of this study was to evaluate the microbiological quality of yoghurts from different brands available in Viçosa market, considering the levels of contamination by total and thermotolerant coliforms and counts of viable LAB in different shelf-life periods; pH values were also determined as another quality indicator.

MATERIAL AND METHODS

Sample collection and dilution

Thirty-six yoghurt samples from different brands and shelf-lives, with either the addition of fruit pulp or not, were obtained in markets from Viçosa, Minas Gerais, Brazil. All samples were kept under refrigeration condition during the transport to laboratory, when they were diluted serially in decimal scale using sterile NaCl 0.85% until 1:10,000,000.

Microbiological analysis

All microbiological analysis was conducted according to Wehr and Frank (2002) using culture media from Oxoid Ltda. (Basingstoke, England). Coliforms were quantified using the Multiple Tubes Technique (3 tubes per series) using undiluted and 1:10 and 1:100 diluted samples. The presumptive step was conducted with lauryl sulfate tryptose broth (35°C for 48 h) followed by the confirmation of total coliforms using brilliant green bile broth (35°C for 48 h). Thermotolerant coliforms were confirmed using *Escherichia coli* broth (44.5°C for 48 h) and tryptone broth (35°C for 48 h) followed by indol production detection. The levels of contamination by total and thermotolerant coliforms were obtained according to the combination of positive results in each dilution using a Table of Most Probable Number and the results were expressed as MNP/g.

For LAB enumerating, the dilutions 1:1,000,000 and 10,000,000 were plated on de Man, Rogosa and Sharpe agar by pour plate technique with incubation in anaerobic conditions (Anaerobac, Probac do Brasil , São Paulo, S.P., Brazil) at 35°C for 48h. The obtained results were expressed in CFU/g.

pH analyses

The pH values of the yoghurts were measured at 20°C using a Bel W3B pH meter (Bel Engineering, Monza, Italy) after calibrating with fresh pH 4.0 and 6.86 standard buffers.

Statistical analysis

The obtained results were analyzed considering the official parameters established by Brazilian Agriculture and Health Departments (Brasil, 2000, 2001). In addition, the mean counts of microbiological parameters (converted in $\log_{10}$) and pH were calculated and compared by analysis of variance (ANOVA and Tukey, $P < 0.05$) considering the time for expiration (shelf-life) and levels of LAB. Associations between levels of contamination and shelf-life periods were also made. All statistical analysis was processed in statistica 7.0 software (Stat Soft, Inc., Tulsa, OK, U.S.A.).

RESULTS AND DISCUSSION

Only 7 samples showed microbiological parameters in disagreement with official standards (Brasil, 2000, 2001), and all of these presenting LAB counts lower than 10^7 CFU/g (Table 1). All samples showed levels of coliforms (total and thermotolerants) lower than 0.3 MPN/g, indicating adequate heating treatment of milk and high hygienic standards of processing and packaging that precluded recontamination of the product.

Coliforms detection or enumerating are often used as parameters for evaluating the yoghurt quality in different countries (Brasil, 2000, 2001; Con et al., 1996; Nogueira et al., 1998; Tamine and Robinson, 2007). The obtained results are similar when compared to other studies in Lebanon (Al-Kadamany et al., 2003), Turkey (Con et al., 1996) and Portugal (Nogueira et al., 1998). However, there have been instances where much higher counts of coliform group were found in yoghurt samples, with frequency varying from 35 to 80% (Con et al., 1996).

Until this moment, there are no published studies focusing on coliforms enumerating or detection in Yoghurts produced and commercialized in Brazil, being impossible a broad comparison of the results obtained.

In almost all dairy industries, heating the milk is the most widely used unit operation in the manufacture of yoghurt. The heating treatment of milk at 85 - 90°C for 4 - 20 s to 30 min (high pasteurization) is usually applied in yoghurt production and it is sufficient to kill the majority, if not all, of the autochthonous microorganisms of raw milk (Con et al., 1996; Tamine, 2002; Olson and Aryana, 2007), including the coliforms. In Brazil this heating treatment is the preferred in yoghurt production once raw milk has notorious poor microbiological quality (Nero et al., 2004; Arcuri et al., 2006), being necessary suitable procedures to avoid any interference in the development of the added starter cultures.

Low levels of LAB in fermented products can occur due to inappropriate conditions of storage leading to uncontrolled development of the starter cultures (Tamine, 2002; Tamine and Robinson, 2007), with consequent acid production. However, the mean pH values considering the levels of LAB counts were not significantly different when compared by Tukey ($F = 0.16$; $df = 2$; $p = 0.85$) (Figure 1), suggesting that small amounts of the starter cultures were inoculated in the samples with LAB counts lower than 10^7 CFU/g. These findings were similar to the ones observed by Pereira et al. (2007) and Lin et al. (2006) studying viable LAB in yoghurts.

The yoghurt shelf-life can also interfere in the quality of this product. Associated to this, when yoghurt is kept in inappropriate conditions the LAB from starter cultures tends to increase their development provoking high acidity

Table 1. Frequencies of yoghurt samples in agreement and disagreement with Brazilian standard microbiological parameters.

	Thermotolerant coliforms n (%)	Lactic Acid Bacteria n (%)	Total n (%)
Agreement	36 (100.0)	29 (80.6)	29 (80.6)
Disagreement	0 (0.0)	7 (19.4)	7 (19.4)
Official standard	10 MPN/g *	Higher than 10^7 CFU/g **	

Brasil, 2001** Brasil, 2000.

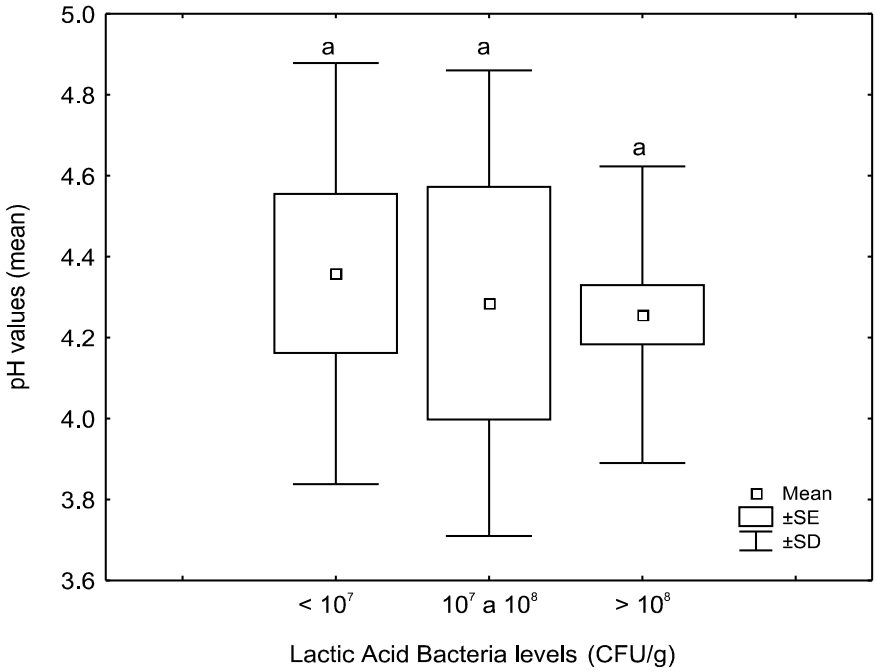

Figure 1. Mean values of pH considering different levels of lactic acid bacteria counts in yoghurt samples. Values with same letters did not differ significantly (Tukey, $P < 0.05$).

and consequently the killing of themselves (Tamine and Robinson, 2007). The mean values of pH and LAB counts considering different times for expiration of the samples are presented in Table 2, and no differences between these parameters were observed. Considering all samples, the mean of LAB counts was 7.9 log CFU/g and it was possible to observe a decrease in these counts in samples with less than 15 days to finish their viability (Table 2). The same behavior of LAB in yoghurt was observed by Çon et al. (1996) and Aryana and McGrew (2007), who described that the decrease in numbers of LAB may have occurred due to the accumulation of ambient lactic acid. During the storage, the added LAB starter culture is still able to develop and produce acids, causing, as a result, a decrease in pH.

Although the pH is not an official parameter to verify the quality of yoghurt (Brasil, 2000, 2001), it can be measured in order to allow additional information. The pH of the samples varied from 3.59 to 5.24 (mean of 4.28), slightly similar to the results obtained from Moreira et al. (1999). As for LAB, it can be observed that the pH tended to decrease in the samples with less than 15 days for expiration, confirming the continuous development of the LAB cultures with consequent acid production (Table 2).

However, no significant differences between the pH of LAB means were observed, suggesting that this development occurred at expected speed. The same pH decrease was observed by Al-Kadamany et al. (2003) and Nogueira et al. (1998) during the storage of yoghurt at 4°C.

Conclusions

According to the results of this study, it can be considered that the yoghurt commercialized in Vicosa presented adequate microbiological quality considering the low levels of contamination by coliforms microorganisms.

Table 2. Statistical parameters of pH and Lactic Acid Bacteria (LAB) in yoghurt samples, considering different times for expiration.

Days for expiration	pH			LAB (log CFU/g)		
	Mean ± SD*	Min.**	Max.***	Mean ± SD	Min.	Max.
More than 31	4.3 ± 0.41 [a]	3.71	5.08	7.9 ± 0.96 [a]	6.51	9.21
16 to 30	4.3 ± 0.47 [a]	3.59	5.24	8.0 ± 1.06 [a]	6.44	9.67
Less than 15	4.1 ± 0.12 [a]	3.96	4.30	7.4 ± 1.42 [a]	5.81	8.99

Values in each column with same letters did not differ significantly (Tukey, $P < 0.05$)
* Standard deviation, ** Minimum, *** Maximum

However, some samples with low counts of LAB were observed, suggesting inoculation of starter cultures in small amounts once no significant differences were observed in the pH and LAB mean values in different times for expiration.

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