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Food Research International 33 (2000) 799–805

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Viability of lactic acid microflora in different types of yoghurt

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Received 14 January 2000; accepted 4 April 2000

Abstract

The suitability of three culture media (Skim milk agar, M17 agar and MRS agar, incubated under aerobic and anaerobic conditions) to obtain total and differential counts of yoghurt bacteria, was assayed. A total of 25 samples of different yoghurt types (set, skimmed set, drinking, and set with "dulce de leche") obtained with the culture IA, were used. The lactic microflora viability was studied at 6°C and 12°C. Skim milk agar (aerobiosis) was the most useful medium for total and differential counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Cell viability depended on the yoghurt type and the storage temperature. On the basis of a minimum value of 10^7 CFU g^{-1}, the shelf life of yoghurts at 6°C was longer than 60 days, with the exception of the drinking type (45 days). At 12°C, shelf life was identical for the skimmed drinking yoghurt but lower for the other yoghurt types. Both the storage temperature and the yoghurt type should be taken into account when shelf life is specified on the basis of the lactic microflora content.

Keywords: Yoghurt; Lactic acid bacteria; Shelf life; Storage

1. Introduction

Since the 1960s, the industrial production of fermented milks — especially yoghurt — has increasingly developed worldwide. Several factors account for the success of yoghurt: its natural image, its organoleptic characteristics (fresh and acidulated taste and characteristic flavour), nutritional, prophylactic and therapeutic properties, and its moderate cost (due to the high productivity of the production lines) (Roissart & Luquet, 1994).

Yoghurt is the dairy product for which demand has had the highest increase in Argentina. In 1980, yoghurt consumption per person per year was 1.8 l, reaching the quantity of 6.1 l (SAGPA, 1996) in 1992. In 1995, the volume of milk used in Argentina for yoghurt production (18.05 millions of l) accounted for 3% of the total industrialized milk (Edipres, 1996).

The existence of viable lactic acid bacteria in a high concentration in fermented milks like yoghurt has been correlated with several benefits for the consumer's health: a higher lactose tolerance (IDF, 1988a; Martini et al.,1987; SYNDFRAIS, 1984), a benefic balance of the intestinal microflora (IDF, 1988a; Nakasawa & Hosono, 1992; Wood, 1992), antimicrobial activity (IDF, 1988a; Rasic & Kurmann, 1978; SYNDFRAIS, 1984; Wood, 1992), stimulation of the immune system (IDF, 1988a; Roissart & Luquet, 1994), anti-tumoral effect (Bottazzi, Friend & Shahani, 1985; IDF, 1988a; Wood, 1992) and anti-cholesterolemic effect (IDF, 1988a; SYNDFRAIS, 1984; Wood, 1992).

France and Spain established the requirement of a minimum viable lactic acid bacteria number during yoghurt's shelf life of 5×10^8 CFU/ml. Other countries have established values of 10^6 CFU/ml (Switzerland and Italy), 10^7 CFU g^{-1} (Japan) and 10^8 CFU g^{-1} (Portugal) (IDF, 1988a). In Argentina, the new legislation included in the "MERCOSUR technical rules of identity and quality of fermented milks" (Pagano, 1998) has been mandatory since April 1998 and establishes a minimum content of viable lactic acid bacteria of 10^7 CFU g^{-1} during yoghurt's shelf life. Although there are mandatory levels for viable microflora, there are some important topics that have not been taken into account in the legislation, e.g. different types of commercial yoghurts.

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0963-9969/00/$ - see front matter
PII: S0963-9969(00)00101-0

The local importance of yoghurt with additives such as stabilizers, flavouring ingredients, sweeteners, preservers, etc., against the plain yoghurt, raises some doubts about the actual viable bacteria concentration when the product reaches the consumers.

This lack of information triggers the need for studies leading to the establishment of criteria about the shelf life of the different types of yoghurt that are formulated nowadays. Besides, in Argentina, some still existing deficiencies in the cold chain distribution allow the appearance of post-acidification problems in the product, which causes an excessive decrease of pH and the modification of the viability of the lactic acid bacteria.

The aim of the present work was to find a methodology for the count of viable lactic microflora in yoghurt and to study its variation during refrigerated storage of different yoghurt types.

2. Materials and methods

2.1. Lactic acid starter and culture conditions

A commercial lyophilized mixed culture of *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (30/70), identified as IA, was used for the industrial manufacture of yoghurt.

The mixed culture was used in two different ways: as direct inoculation or semi-direct inoculation culture, depending on the type of yoghurt produced. When it was used in the direct form, and according to the manufacturer's instructions, 500 ml of heat-treated milk (15 min — 90°C) were inoculated and kept 1 h at 30°C; then it was inoculated to the production batch. For the indirect inoculation (bulk culture), 50 l of heat-treated milk (15 min — 90°C) were inoculated and kept at 40°C until it reached 55–60° Dornic; then it was cooled to 8°C and stored refrigerated until it was added to the production batch (2% v/v).

2.2. Yoghurt types

All the yoghurt samples were taken from an ordinary yoghurt production from a factory in the surroundings of Santa Fe City (Argentina). The different types of yoghurt studied were: (1) Whole Set Yoghurt, produced with whole milk (3.2%v/v fat content), sugar, skimmed powder milk, stabilizer, natural colour and flavour; (2) Skimmed Set Yoghurt, elaborated with skimmed milk (0.2%v/v fat content max.), skimmed powder milk, textured proteins of serum, stabilizer, aspartame and flavour; (3) Whole Drinking Yoghurt, manufactured with whole milk (3.2%v/v fat content), sugar, stabilizer, natural colour and flavour; (4) Skimmed Drinking Yoghurt: produced with skimmed milk (0.2%v/v fat content max.), skimmed powder milk, textured proteins of serum, stabilizer, aspartame and flavour and (5) Whole Set Yoghurt with "dulce de leche" (concentrated product of heated milk and sugar), produced with whole milk (3.2%v/v fat content), sugar, skimmed powder milk, "dulce de leche", stabilizer and flavour.

Direct inoculation was used to produce Set Yoghurt and Drinking Skimmed Yoghurt with IA starter. Indirect inoculation (bulk culture) was used to manufacture Drinking Whole Yoghurt with the same starter. All yoghurts were aseptically packaged.

2.3. Culture media and media performance

The performance of three culture media to count and differentiate yoghurt bacteria was evaluated. These media were: Skim milk agar (Marshall, 1992), acidified MRS agar (Biokar, Beauvais, France) (IDF, 1988b) and M17 Agar (Biokar, Beauvais, France) (IDF, 1988b).

The suitability of the media was tested by plating decimal dilutions of yoghurt samples. A total of 25 samples was studied (five of each type of manufactured yoghurt). Thus, a sample of 1 g was decimally diluted in sterile peptone water (0.1%) and 0.1 ml aliquot dilution plated over the different media, in triplicate. Plates were incubated 72 h at 37°C under two different conditions: aerobic and anaerobic (GasPak System-Oxoid, Basingstoke, Hampshire, UK).

Enumeration of *S. thermophilus* and *L. bulgaricus* was carried out regarding the morphological differences of the colonies. The identity of the colonies was confirmed as established by the FIL-IDF (IDF, 1988b; 1991).

2.4. Statistical analysis

The results were statistically compared using the Test for Homogeneity of Variances (Miller, Freund & Johnson, 1992) and Duncan and Student's Tests (Miller & Miller, 1993).

2.5. Cell viability during cold storage

Lactic acid microflora viability was studied at storage temperatures of 6 and 12°C for each type of yoghurt. The samples for viability assays were taken at the end of the production line, in ordinary production runs. In the case of set yoghurts, 20 cups of 200 g were taken and stored at 6°C (10 cups) and 12°C (10 cups). For the drinking yoghurts, plastic pouches of 1 l, packaged consecutively, were stored (10 of them at each temperature).

During the cold storage of the samples (60 days), cell counts of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* in Skim milk agar and pH measurements (pH meter Orion SA720) were carried out every 7 days. The identity of the colonies was checked (IDF, 1988b). Counts of coliform bacteria (IDF, 1985a) and yeasts and moulds (IDF, 1985b) were performed every 15 days. All these trials were performed in duplicate.

3. Results

3.1. Media performance

Table 1 shows the count values obtained on the different media for total lactic acid bacteria, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*.

On Skim milk agar (aerobic incubation) an excellent colony differentiation between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was achieved. The former gave circular opalescent white colonies with well-defined borders, while, *L. delbrueckii subsp. bulgaricus* gave irregular flat translucent colonies, bigger in size than the colonies of streptococci, with non-defined borders. After an anaerobic incubation, it was not possible to differentiate between both types of colonies, because of the opalescent aspect of the Petri dish due to the precipitation of casein.

On M17 agar, under both incubation conditions assayed, it was possible to recognize only one type of colony that was bigger under anaerobic (mean diameter 1 mm) than in aerobic conditions (mean diameter < 1 mm). They looked circular in shape, opalescent white and with well-defined borders. All these colonies belonged exclusively to *S. thermophilus*, as it was seen at microscopic observation.

On MRS agar, it was possible to distinguish — though with some difficulty — two kinds of colonies under anaerobic conditions. The first ones were circular in shape, opalescent and with well-defined borders and belonged to *S. thermophilus*. The others were rounded, duller, flat and with non-defined borders, which corresponded to *L. delbrueckii* subsp. *bulgaricus*.

The statistical analysis of total lactic acid microflora counts obtained on the different culture media assayed (Table 1) allowed to establish that the mean values obtained on Skim milk agar, under both incubation conditions, were not significantly different ($P > 0.01$). The same result was obtained for MRS agar under both conditions. Comparing these four values among them, it was seen that counts obtained on MRS agar (aerobiosis) were significantly lower ($P < 0.01$).

The counts of *S. thermophilus* obtained on M17 agar, incubated under aerobic and anaerobic conditions did not differ significantly ($P > 0.01$). When these values were compared with those obtained on Skim milk agar (aerobiosis) and MRS agar (anaerobiosis), the last one resulted significantly lower than the other ones.

Finally, the counts of *L. delbrueckii* subsp. *bulgaricus* obtained on Skim milk agar (aerobiosis) and MRS agar (anaerobiosis) resulted statistically similar ($P > 0.01$).

3.2. Microflora viability during cold storage

Coliforms, moulds and yeasts were not detected in any of the samples studied. Figs 1–5 show the viability of the lactic acid microflora and the changes in pH in the different types of yoghurts at the two storage temperatures, during approximately two months. In all the cases, at the beginning of the experiment, yoghurts showed counts of total lactic acid microflora higher than 10^8 CFU g^{-1}. Numbers of *S. thermophilus* were higher than the *L. delbrueckii* subsp. *bulgaricus* ones.

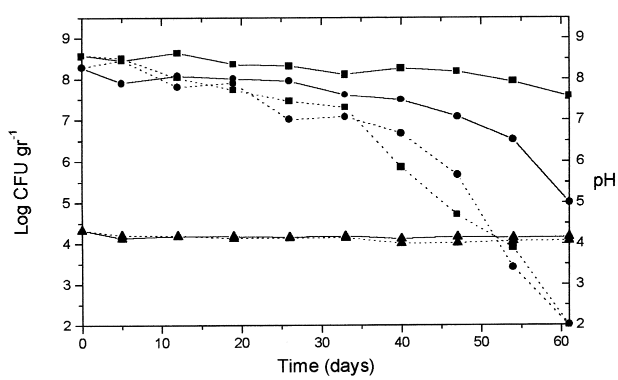

Fig. 1. Changes in pH values (▲) and viable cell counts of *S. thermophilus* (■) and *L. delbrueckii* subsp. *bulgaricus* (●) in Whole Set Yoghurt at 6°C (solid lines) and 12°C (dotted lines).

Table 1
Yoghurt cell counts (log CFU g^{-1})[a] of total lactic acid microflora, *S. thermophilus* and *L. delbreuckii* subsp. *bulgaricus* in different culture media and incubation conditions

Cell count	Culture media/incubation condition (3 days at 37°C)					
	Skim milk agar		MRS agar		M17 agar	
	Aerobiosis	Anaerobiosis	Aerobiosis	Anaerobiosis	Aerobiosis	Anaerobiosis
Total lactic acid microflora	$8.62\pm0.36^{a}_{1}$	$8.55\pm0.44^{a}_{1}$	7.54 ± 0.56^{b}	$8.11\pm0.65^{b}_{1}$	–	–
S. thermophilus	$8.58\pm0.37_{2}$	u[b]	u	7.72±0.81	$8.47\pm0.63^{c}_{2}$	$8.54\pm0.55^{c}_{2}$
L. delbrueckii subsp. *bulgaricus*	$7.29\pm0.56_{3}$	u	u	$7.38\pm0.53_{3}$	ng[c]	ng

[a] Mean value ± standard deviation of 25 samples. Values with the same letter or number did not differ significantly ($P > 0.01$).
[b] u, Unable to differentiate.
[c] ng, No growth.

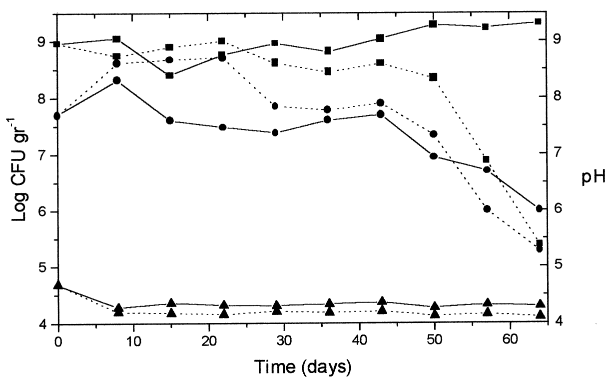

Fig. 2. Changes in pH values (▲) and viable cell counts of *S. thermophilus* (■) and *L. delbrueckii* subsp. *bulgaricus* (●) in Skimmed Set Yoghurt at 6°C (solid lines) and 12°C (dot lines).

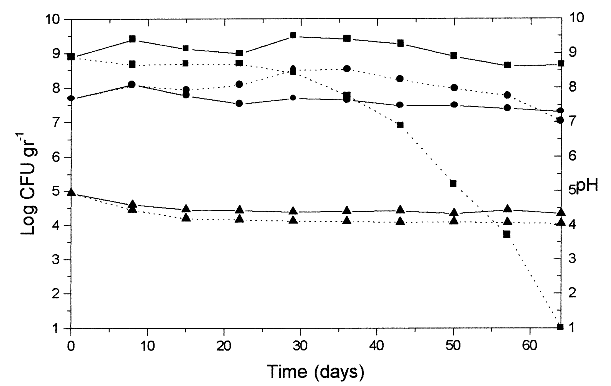

Fig. 4. Changes in pH values (▲) and viable cell counts of *S. thermophilus* (■) and *L. delbrueckii* subsp. *bulgaricus* (●) in Skimmed Drinking Yoghurt at 6°C (solid lines) and 12°C (dotted lines).

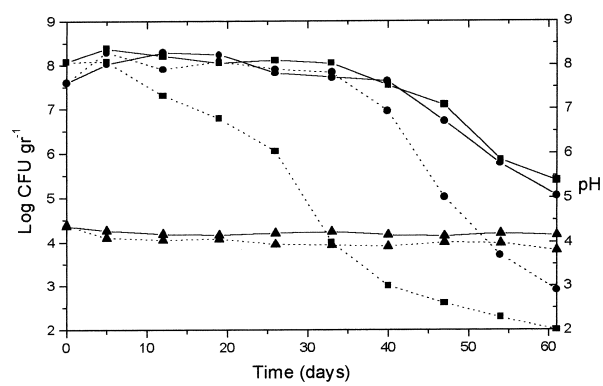

Fig. 3. Changes in pH values (▲) and viable cell counts of *S. thermophilus* (■) and *L. delbrueckii* subsp. *bulgaricus* (●) in Whole Drinking Yoghurt at 6°C (solid lines) and 12°C (dotted lines).

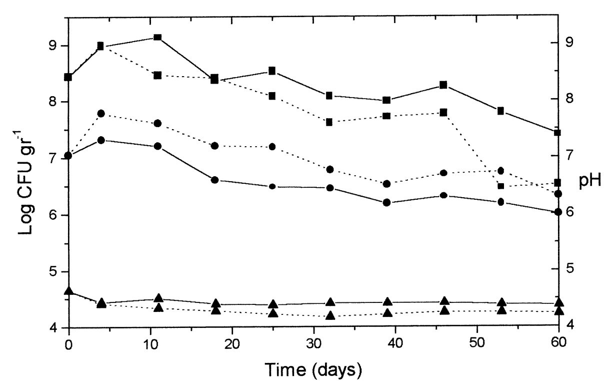

Fig. 5. Changes in pH values (▲) and viable cell counts of *S. thermophilus* (■) and *L. delbrueckii* subsp. *bulgaricus* (●) in Set Yoghurt with "dulce de leche" at 6°C (solid lines) and 12°C (dotted lines).

In Whole Set Yoghurt (Fig. 1), the level of streptococci decreased approximately 1 log cycle in 60 days at 6°C, meanwhile, at 12°C the cellular viability was dramatically affected and it fell almost to zero. On the other hand, the viability of the lactobacilli was very different, showing a steady decrease at both temperatures (3 and 6 log cycles after 60 days, at 6 and 12°C, respectively). The initial pH value (4.3) stabilized rapidly in 4.15 at 6°C (throughout the experiment) but it decreased to 4.0 at 12°C.

The evolution of the lactic acid microflora in Skimmed Set Yoghurt (Fig. 2) showed that at 6°C the streptococci were capable to remain viable and even to reproduce, and their count increased slightly from their initial level (10^9 CFU g^{-1}). However, at 12°C the initial cell counts remained the same for approximately 40 days and then they decreased sharply, reaching 5×10^5 CFU g^{-1} at the end of the refrigerated storage. In this kind of product, lactobacilli increased its population in 1 log order during the first 10 days of cold storage, maintaining values of 5×10^8 CFU g^{-1} at 12°C during the following 15 days. Later on there was a steady decrease to 10^5 CFU g^{-1} at the end of the storage period. On the other hand, at 6°C, *L. delbrueckii* subsp. *bulgaricus* counts were stable at 5×10^7 CFU g^{-1} between day 15 and 45, but counts diminished later. The initial pH (4.7) decreased to 4.25 after 10 days and then it remained constant.

For the Whole Drinking Yoghurt (Fig. 3), the changes in cell counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were similar at 6°C, starting from values that ranged from 5×10^7 to 10^8 CFU g^{-1}. After 40 days of cold storage, these numbers decreased to approximately 10^5 CFU g^{-1}. At 12°C, *L. delbrueckii* subsp. *bulgaricus* showed the same behavior described above, although the cell counts on day 60 were significantly lower (10^3 CFU g^{-1}) than those revealed in the yoghurt type previously described. On the other hand, *S. thermophilus* was very influenced at this temperature since the initial cell count (10^8 CFU g^{-1}) decreased steadily from the first day to 10^2 CFU g^{-1} on day 60. The initial pH value (4.35) decreased slowly to 6°C in 10 days and it remained at 4.2 until the end of the experience. At 12°C, the pH showed values of approximately 4.0 during the whole cold storage.

Fig. 4 shows the results obtained for Skimmed Drinking Yoghurt. The viability of *L. delbrueckii* subsp.

bulgaricus was not modified. In practice, at both temperatures, the initial value of cell count (5×10^8 CFU g^{-1}) remained constant during the cold storage. It was even possible to observe a slight growth, more noticeable at 12 than at 6°C. *S. thermophilus* showed a good cell viability at 6°C, maintaining its viable cell counts between 5×10^8 and 5×10^9 CFU g^{-1} for two months, but at 12°C it was adversely influenced by the product characteristics since starting from day 30 its cell counts diminished to zero (60 days). The pH values decreased from approximately 5 to 4.3 and 4.0, at 6 and 12°C, respectively.

In Set Yoghurt with "dulce de leche" (Fig. 5), *S. thermophilus* showed a smooth growth (approximately 0.5 log cycles) at the beginning of the storage to decrease finally 1.5 and 2.5 log cycles at 6 and 12°C, respectively. In this type of product, the initial level of *L. delbrueckii subsp. bulgaricus* (10^7 CFU g^{-1}) was significant lower than the level of streptococci. These values showed a little growth during the first days of cold storage, but then they decreased approximately 2.5 log cycles, at both temperatures assayed. The pH showed an initial value of 4.6 and then it decreased up to 4.4 and 4.2 at 6 and 12°C, respectively.

Regarding these results, Table 2 shows the periods, in days, through which the different types of manufactured yoghurts had total lactic acid cell contents higher than the minimum suggested of 10^7 CFU g^{-1} (Pagano, 1998). For the yoghurts stored at 6°C, it was possible to see that the lactic acid microflora had an excellent viability during at least two months of cold storage, except for the Whole Drinking Yoghurt, which has a level of lactic acid bacteria lower than 10^7 CFU g^{-1} after 45 days. At 12°C, the different types of yoghurts kept a total lactic acid microflora content higher than 10^7 CFU g^{-1} during shorter periods than at 6°C, except for the Skimmed Drinking Yoghurt, that showed similar values (>60 days) at both conservation temperatures. At 12°C the cell viability was more affected in whole yoghurts (Drinking and Set). In Whole Set Yoghurt, the period of microbiological aptitude decreased by half (>60 days at 6°C and 35 days at 12°C).

Table 2
Periods (days) throughout the different type of yoghurts kept a lactic acid microflora content higher than 10^7 CFU g^{-1} (minimum required by the MERCOSUR legislation for fermented milks)

Type of yoghurt	Storage temperature (°C)	
	6	12
Whole Set	>60	35
Skimmed Set	>60	55
Whole Drinking	45	40
Skimmed Drinking	>60	>60
Whole Set with "dulce de leche"	>60	50

4. Discussion

Different culture media have been used for total and differential counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in yoghurt, such as Elliker agar, with the addition of Tween 80 and 2, 3, 5-triphenil tetrazolium chloride (Matalon & Sandine, 1986; Rasic & Kurmann, 1978) and $LAPT_{g10}$ agar (Peral de Portillo, Amoroso & Oliver, 1988). Several other media has been also previously reviewed (Radke-Mitchell & Sandine, 1984). In 1988, inter-laboratory results of a study performed by the IDF were published. The use of acidified-MRS and M17 agar for selective count of cocci and lactobacilli in yoghurt, respectively (IDF, 1988b) was suggested.

Previous studies carried out in our laboratory (Reinheimer, Binetti, Quiberoni, Bailo, Rubiolo & Giraffa, 1996; Reinheimer, Suarez, Bailo & Zalazar, 1995) demonstrated that Skim milk agar was an excellent medium for the count of lactic acid bacteria. For this reason, its performance was assayed for differential and total counts in yoghurt, and results were compared with the medium recommended by the IDF (1988b). This work demonstrated that M17 agar is a selective culture medium for *S. thermophilus*, in accordance with the IDF document. However, MRS agar allowed the growth of both types of lactic acid species, even though counts of *S. thermophilus* were significantly lower ($P<0.01$) than those obtained on M17 agar. On the other hand, the differentiation of cocci and bacilli in MRS agar was only possible-though with difficulty- in anaerobiosis. On Skim milk agar it was possible to clearly differentiate and enumerate colonies of both starter bacteria. As regards cellular recovery, the counts of both organisms in this medium did not differ significantly ($P>0{,}01$) from those obtained on M17 agar (*S. thermophilus*) and MRS agar-anaerobiosis (*L. delbrueckii* subsp. *bulgaricus*). These results indicate that it is possible to replace the use of M17 and MRS agars by Skim milk agar, which is cheaper and easier to prepare.

The beneficial effects of the regular ingestion of yoghurt on the consumers's health have always been related to the presence of a high concentration of viable lactic acid bacteria in the product (Bottazzi et al., 1985; IDF, 1988a). Because of this, several countries have established minimum values of lactic acid bacteria for yoghurts and/or fermented milks during shelf life. These values range from 1×10^6 to 5×10^8 CFU g^{-1} (IDF, 1988a). The recommended value for MERCOSUR was recently placed (Pagano, 1998) within this range.

The viability of the lactic acid bacteria in yoghurt is related to the characteristics of the product (as acidity and chemical composition) as well as the storage temperature. Canganella, Ziletta, Sarra, Massa and Trovatelli (1992) studied this subject in Italian yoghurts maintained at 4°C during 60 days. In all the cases, the counts of lactobacilli were kept above 10^7 CFU g^{-1} up to 30–32 days, while streptococci counts were always lower than this value, except in yoghurt with fruits, that had a higher pH value. In this case, the high osmotic pressure produced by the addition of sweeteners and jams seemed to be the cause of the low viability of *L. delbrueckii* subsp. *bulgaricus*.

Studies made in France (Roissart & Luquet, 1994) showed that the shelf life of yoghurt must be 20 days at 5°C, taking into consideration a minimum level of viable lactic acid microflora of 10^8 CFU g^{-1}. At 22 and 30°C, this period decreased to 2 days, and it was observed that as temperature rose, the proportion of lactobacilli over streptococci diminished. Another research work in the United Kingdom (Davis, Ashton & McCaskill, 1971) compared the viability of the lactic acid bacteria in yoghurt with fruits with respect to plain yoghurt stored at 5 and 15°C. Even after 28 days at 5°C, the counts of the viable microflora remained above 10^8 CFU g^{-1}, while at 15°C, the counts decreased after 15 days (until 1×10^2 CFU g^{-1}). The pH values, in all the cases, were between 3.8 and 4.0.

In contrast to European studies, this work revealed that *S. thermophilus* counts in all the types of studied yoghurts were higher than the *L. delbrueckii* subsp. *bulgaricus* ones, in almost 1 log order. This might be due to, in first place, the use of a starter with dominant *S. thermophilus*, that is common in the local market. The use of industrial starters with low proportion of *L. delbrueckii* subsp. *bulgaricus* allows the production of yoghurt with a reduced acidity and with lesser risks of post acidification. Also, the high concentration of sugars in several types of yoghurt (typical of Argentinian yoghurts) might have affected the bacilli viability. This fact was mainly observed in yoghurt with "dulce de leche".

The initial pH value of the yoghurts resulted to be dependent on the type of yoghurt manufactured. In general, the skimmed yoghurts (Drinking and Set) showed higher values than whole yoghurts. The lowest initial pH value (4.3) belonged to Whole Set Yoghurt, while the highest value was observed for Skimmed Drinking Yoghurt (5.0). Set Yoghurt with "dulce de leche" showed an intermediate value (4.6). In all the assays, the final pH value at 12°C resulted slightly lower than at 6°C. In whole yoghurts (Set and Drinking), as well as Set with "dulce de leche", the initial pH value was slightly modified during the 60 days of cold storage, but in skimmed yoghurts (Set and Drinking) this variation was significant (almost 0.5 units of pH).

The highest content of *S. thermophilus* remained at 6°C during refrigerated storage in all types of yoghurt, except for the Whole Drinking yoghurt where the concentration of both microorganisms showed similar values and evolution. The concentration of lactic acid microflora decreased at both temperatures of storage, but the rate of decrease was more important in yoghurt kept at 12°C. However, in the case of Skimmed Set yoghurt, it was possible to observe a moderate increase in *S. thermophilus* counts at 6°C.

Although the international standards (IDF, 1988a) recommends for yoghurts a conservation temperature not higher than 8°C, the MERCOSUR legislation (Pagano, 1998) established a temperature not higher than 10°C. The long distances between production and consumption of these products in our country, added to the tropical and subtropical temperatures and to not-always acceptable refrigeration conditions, were the factors considered. In accordance with our studies, from the microbiological point of view, the aptitude periods for the types of yoghurts studied could be longer than 2 months at 6°C, except for the Whole Drinking yoghurt (45 days). At 12°C, a temperature which is closer to the actual temperature of storage in markets, this period decreased significantly to 35 days for Whole Set Yoghurt; whereas the other periods were not modified (Skimmed Drinking Yoghurt) or reduced to values from 40 to 55 days. However, it is important to highlight that in these kind of yoghurts, complying with the minimal count of lactic acid microflora of 10^7 CFU g^{-1} during product shelf life implies to have only one lactic acid species as viable microflora. This situation was verified for the Whole Drinking Yoghurt, where the level of streptococci dropped dramatically at 12°C; and for the Set Yoghurt with "dulce de leche", where the same happened to lactobacilli at 6°C.

5. Conclusions

This work demonstrated that Skim milk agar was an adequate culture medium for the total and differential counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in yoghurt. This culture medium, which is cheap and easy to prepare, gave an excellent cellular recovery and differentiation.

The content of viable lactic acid microflora and its evolution during cold storage resulted dependent on the type of yoghurt and the temperature of storage. Whole yoghurts (Set and Drinking) showed to be, mainly at 12°C, the types of yoghurt that strongly affected the viability of the lactic acid microflora. In Whole Set Yoghurt, as the storage temperature duplicated the microbiological aptitude period was practically reduced by half ($>$ 60 days at 6°C and 35 days at 12°C).

In Skimmed Set Yoghurts, lactobacilli viability was significantly affected, while in Drinking Yoghurt the conservation at 12°C influenced the level of streptococci. In yoghurt with "dulce de leche", the higher concentration of sugars resulted to be an inhibitory factor for lactobacilli.

From these results, it could be concluded that the type of yoghurt as well as the storage temperature must be considered to set the shelf life of the product.

Acknowledgements

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica from Argentina (Project PICT No. 09-00000-00747).

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