# Microbiological quality of Portuguese yogurts

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**The microbiological quality of four brands of natural yogurts and two probiotic yogurts available in the Portuguese market, was evaluated during the shelf-life period. Although the specific flora decreased during storage it was always within the range of recommended values. No coliforms and an insignificant number of fungi were detected.**

**Keywords:** yogurt; *Lactobacillus bulgaricus*; *Streptococcus thermophilus*; *Lactobacillus acidophilus*; *Bifidobacterium* spp

## Introduction

Yogurt may be defined as the end product of a controlled fermentation of high solids whole milk with a symbiotic mixture of *Streptococcus salivarius* subsp *thermophilus* (termed *S. thermophilus* hereafter) and *Lactobacillus delbrueckii* subsp *bulgaricus* (termed *L. bulgaricus* hereafter). *L. bulgaricus* degrades casein supplying peptides and aminoacids to the weakly proteolytic streptococci. Growing more rapidly at the beginning, *S. thermophilus* lowers the redox potential and slightly acidifies the milk. These conditions are stimulatory for *L. bulgaricus* that acidifies the milk even more [14]. Together, the two species ferment almost all the lactose to lactic acid and flavour the yogurt with diacetyl (*S. thermophilus*) and acetaldehyde (*L. bulgaricus*).

Unlike *Lactobacillus acidophilus* and bifidobacteria, most strains of *L. bulgaricus* and *S. thermophilus* are highly sensitive to gastric acid and bile salts, and show poor survival during transit through the gastro-intestinal tract to the colon [7]. A number of health benefits have been claimed for *L. acidophilus* and bifidobacteria due to the ability of these organisms to establish themselves amongst the colonic microflora and they are increasingly being incorporated into dairy products. It seems reasonable to assume that the beneficial effects of these probiotic bacteria can be expected only when viable cells are ingested.

In the past some studies indicated that the hydrogen peroxide produced by yogurt cultures might be detrimental to the viability of added cells of *L. acidophilus* [2]. In addition, the viability of *Lactobacillus* and *Bifidobacterium* species diminishes markedly during refrigerated storage [18].

The main objective of this study was to evaluate and compare the microbiological quality of different yogurts available in the Portuguese market in terms of the viability of the natural flora and the presence of contaminants during the shelf-life period; pH values were also determined as another quality indicator.

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## Materials and methods

Four different brands of natural solid (set) yogurts (A, B, C, D) and two different brands of natural probiotic yogurts containing *L. acidophilus* (E) and *Bifidobacterium* spp (F) were analyzed. Two different lots of each brand were analyzed. Yogurts were obtained in the market 1 week after production and transported to the laboratory at refrigeration temperature. Products were maintained at 4°C and individual pots of the same batch code analyzed until the sell-by-date (approximately 4 weeks after production).

## pH analyses

The pH values of the yogurts were measured at 20°C using a Crison 2002 pH meter after calibrating with fresh pH 4.0 and 7.0 standard buffers.

## Microbiological analyses

Two 10-g samples of yogurt were diluted with 90 ml of sterile 0.1% w/v peptone water (Lab M, Bury, UK). After uniform mixing, subsequent serial decimal dilutions were prepared in 9 ml of sterile 0.1% w/v peptone water. Duplicate plates of each set of dilutions were prepared. These procedures were performed in duplicate for each of two batches of yogurt.

### *Streptococcus salivarius* subsp *thermophilus*

Enumeration was performed according to NP1864 [9] on M17 agar (Lab M) using the pour plate technique. Plates were incubated aerobically at 37°C for 48 h.

### *Lactobacillus delbrueckii* subsp *bulgaricus*

Enumeration was performed according to NP1864 [9] on acidified (pH 5.4 with 100% glacial acetic acid) MRS agar (Lab M) using the pour plate technique. Plates were incubated under microaerophilic conditions (produced by burning a candle to extinction in a closed container) at 37°C for 72 h.

### *Bifidobacterium* spp

Enumeration was performed on de Man, Rogosa, Sharp (MRS) agar with the addition of 4.476% v/v NNPL solution using the pour plate technique [6]. Plates were incubated under anaerobic conditions at 37°C for 72 h. NNPL solution contains per 100 ml: 0.030 g nalidixic acid (Sigma, St Louis, MO, USA); 0.20 g neomycin (Sigma); 0.25 g paromomycin (Sigma); 6.00 g LiCl (Merck, Frankfurt, Germany).

### *Lactobacillus acidophilus*

Enumeration was performed on MRS agar in which glucose was substituted by an equal amount of maltose [1], using the pour plate technique. Plates were incubated under microaerophilic conditions at 37°C for 72 h.

### *Yeasts and moulds*

Enumeration was performed according to NP 1934 [10] on rose bengal agar with chloramphenicol (Lab M) using the spread plate technique. Plates were incubated under aerobic conditions at 23°C for 5 days.

### *Coliforms*

The presence of coliforms was examined according to NP 1935 [11] using lactose broth and brilliant green broth as growth media. Turbidity, colour changes and production of gas were all presumptive evidence of the presence of coliform organisms.

## Results and discussion

Higher pH values were observed in probiotic yogurts than in the traditional yogurts (Tables 1–3). Brand C yogurt was an exception since it was the only product which had pH values lower than 4.0 (Table 1). No significant variations in pH values were observed during storage at 4°C.

According to Radke-Mitchell and Sandine [14], the balance between lactobacilli and streptococci is critical for yogurt flavour development. When streptococci predominate, a mild acid flavour with a fuller aroma from diacetyl and acetaldehyde results, whereas a predominance of lactobacilli gives a sharply acidic flavour and a good yogurt aroma. Pette and Lolkema [12] reported that for proper flavour development the initial ratio of *S. thermophilus* to *L. bulgaricus* should be in the range of 1:1 to 3:1. It is generally agreed that this ratio should be approximately 1:1 [15].

As may be seen in Table 1, with the exception of product C, *S. thermophilus* was always present in higher numbers than *L. bulgaricus*. In product C, there was a predominance of *L. bulgaricus*, confirmed by the low pH of the product.

Puhan *et al* [13] indicated that viability of *S. thermophilus* and *L. bulgaricus* in yogurt was dependent upon pH. Numbers of *S. thermophilus* increased in yogurts with an initial pH greater than 4 until the pH decreased below 4, and the numbers then diminished rapidly. Numbers of *L. bulgaricus* either remained constant or increased for the first 10–20 days with an initial pH greater than 4 and then decreased [13]. These results were not confirmed by our results in which an increase, maintenance or decrease in the numbers of *S. thermophilus* and *L. bulgaricus* does not seem to be related to pH (Table 1). Numbers of *L. bulgaricus* decreased faster than did those of *S. thermophilus* (Table 1) but both were consistently greater than $10^7$ CFU $g^{-1}$ which is the recommended value by the Portuguese norms [9] and the International Dairy Federation [3]. The yogurt organism survival curves were quite different for the different brands tested. According to Medina and Jordano [8], this is possibly due to manufacturing practices and the strains of starter cultures utilised by the different manufacturers.

*L. acidophilus*, *L. bulgaricus* and *S. thermophilus* in product F (Table 2) and *Bifidobacteria* spp and *S. thermophilus* in product G (Table 3), all showed a decline in the number of survivors during storage. *L. bulgaricus*, the main factor

**Table 1** Survival of *L. bulgaricus* and *S. thermophilus* and pH evolution in natural solid yogurts during storage at 4°C

| Brand | Time (weeks) | *L. bulgaricus* (CFU $g^{-1}$ ($\times 10^7$)) | *S. thermophilus* (CFU $g^{-1}$ ($\times 10^7$)) | pH |
|---|---|---|---|---|
| A | 1 | 6.3 | 100 | 4.2 |
| A | 2 | 1.0 | 20 | 4.2 |
| A | 3 | 4.0 | 251 | 4.2 |
| A | 4 | 4.0 | 100 | 4.2 |
| B | 1 | 0.8 | 400 | 4.1 |
| B | 2 | 1.0 | 1259 | 4.1 |
| B | 3 | 2.0 | 1259 | 4.1 |
| B | 4 | 1.0 | 1000 | 4.0 |
| C | 1 | 1000 | 316 | 3.8 |
| C | 2 | 398 | 316 | 3.8 |
| C | 3 | 1000 | 794 | 3.8 |
| C | 4 | 40 | 100 | 3.7 |
| D | 1 | 16 | 794 | 4.2 |
| D | 2 | 10 | 200 | 4.2 |
| D | 3 | 4.0 | 126 | 4.2 |
| D | 4 | 0.8 | 158 | 4.1 |

**Table 2** Survival of *L. bulgaricus*, *S. thermophilus* and *L. acidophilus* and pH evolution in natural probiotic yogurts (brand E) during storage at 4°C

| Time (weeks) | *L. bulgaricus* (CFU $g^{-1}$ ($\times 10^7$)) | *S. thermophilus* (CFU $g^{-1}$ ($\times 10^7$)) | *L. acidophilus* (CFU $g^{-1}$ ($\times 10^7$)) | pH |
|---|---|---|---|---|
| 1 | 158 | 6309 | 40 | 4.4 |
| 2 | 63 | 3981 | 16 | 4.4 |
| 3 | 20 | 1995 | 32 | 4.4 |
| 4 | 4.0 | 631 | 25 | 4.3 |

**Table 3** Survival of *L. bulgaricus*, *S. thermophilus* and *Bifidobacterium* spp and pH evolution in natural probiotic yogurts (brand F) during storage at 4°C

| Time (weeks) | *L. bulgaricus* (CFU $g^{-1}$ ($\times 10^7$)) | *S. thermophilus* (CFU $g^{-1}$ ($\times 10^7$)) | *Bifidobacterium* spp (CFU $g^{-1}$ ($\times 10^7$)) | pH |
|---|---|---|---|---|
| 1 | ND | 1585 | 63 | 4.3 |
| 2 | ND | 1585 | 16 | 4.3 |
| 3 | ND | 1259 | 0.16 | 4.3 |
| 4 | ND | 943 | 0.25 | 4.3 |

ND, Not detected.

responsible for *L. acidophilus* and *Bifidobacterium* spp, mortality [17], was not present in product G. The decline was, however, much more rapid for bifidobacteria compared to *L. acidophilus*. Suggested minimum levels of probiotic bacteria in yogurt are $10^6$ CFU $g^{-1}$ [5]. It was observed that both products contained probiotic organisms in greater numbers than those suggested. The correlation between high numbers of *S. thermophilus* and a sufficient level of *Bifidobacterium* spp (Table 3), confirmed that *S. thermophilus* could be beneficial for *Bifidobacterium* spp as an oxygen scavenger [4]. As expected, due to the low pH and presence of living lactic acid bacteria in the yogurts, no coliforms were detected.

Certain yeasts play an important role in the spoilage of fermented products. Since milk is pasteurised before yogurt production, the presence of yeasts in yogurt is caused by recontamination processes during manufacture [16], and can be a problem in fruit-containing yogurts. The maximum number of moulds found was $6.8 \times 10^2$ CFU $g^{-1}$ (Brand A) and the number of yeasts was always $<1.0 \times 10^2$ CFU $g^{-1}$, which according to the Portuguese norms, is an acceptable value.

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