PROPORTION OF THE MICROFLORA OF LACTOBACILLUS AND STREPTOCOCCUS GENERA IN YOGHURTS OF DIFFERENT DEGREES OF CONDENSATION

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Abstract

Investigations were carried out on yoghurts of different content of dry matter, which were manufactured applying Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus at 1:1 ratio and then subjected to storage at refrigerated conditions for 21 d. Lactobacillus and Streptococcus bacterial counts as well as their mutual proportions were determined in these yoghurts. It was found, that the higher degree of condensation of the processed milk, the more thermophilic yoghurt bacteria were determined in the final product. Significant changes in the number and mutual ratio of the microflora of both species took place during yoghurt storage. The above-mentioned proportions were most uniform in the yoghurt obtained from milk condensed with 3% addition of powdered skim milk, whereas the highest discrepancies – in the yoghurt manufactured from non-condensed milk.

Key words: yoghurt, lactic acid bacteria, storage.

The crucial point in the process of yoghurt manufacture is to try and maintain the appropriate number and mutual proportions of characteristic microflora, most frequently represented by bacteria from the Lactobacillus and Streptococcus genera (11, 12). The extent and possibilities of their growth in milk under processing, in the course of fermentation and during storage of the produced yoghurt curd, depend on many factors. The most important of them include: the size and type of the applied inoculum, incubation and storage time and temperature, the presence of undesirable microflora and its enzymes and nutrient availability in the medium. The introduction into the processed milk of appropriate quantities of technological additives or their mixtures, at carefully balanced proportions, provides its dry matter with essential quantities of lactose and amino acids necessary for the growth of microflora (2, 14). Optimal numbers and proportions of yoghurt bacteria create therapeutic characteristics of the final product and influence acidity, texture and sensory properties. These features, in turn, have impact on the evaluation results of the overall yoghurt desirability (3, 4, 9).

The objective of the performed investigations was to determine mutual proportions of bacterial counts of Lactobacillus and Streptococcus genera in yoghurts, which were manufactured from milk of different degrees of condensation and stored for three weeks at refrigerated conditions.

Material and Methods

The experimental material was a natural yoghurt manufactured at industrial conditions from the highest quality condensed and non-condensed milk. When condensing the processed milk, powdered skim milk was introduced in the amount of 1, 3 or 5% in relation to milk volume, which allowed increasing its content of dry matter from 12.57% to 13.57, 15.61 and 16.98%. The introduced powdered skim milk contained 96.2% dry matter, including 33.40% protein and 51.64% lactose. Raw non-condensed or condensed milk was pasteurised at 86°C for 3 s and then cooled down to the temperature of 43°C and inoculated with the thermophilic bacterial culture of the Direct Vat Set type (DVS) of traditional strain composition of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus at 1:1 ratio in the amount of 50 activity units to 250 dm³ of processed milk, which corresponded to a 2% addition of activated working starter (Chr.Hansen’s Laboratory Denmark A/S). The inoculated milk was incubated at 43°C. The incubation was terminated after 6 h by an instantaneous reduction of the temperature to 6°C and, for the next 18 h, the manufactured yoghurt was allowed to mature at this temperature. The obtained natural yoghurt was stored 21 d at 8±1°C. The experiment was based on six production batches. Four yoghurt samples were collected from each batch (n=24). Yoghurt microflora counts were determined directly after the termination of production and after 7, 14 and 21 d of storage.

The Lactobacillus bacteria in the examined yoghurts were determined using De Man, Rogosa and Sharpe (MRS) selective and acidified media.
supplemented with 1 cm$^3$ of Tween 80 (BTL) to stimulate the growth of the determined bacteria. Ice-cold acetic acid was added to the media to ensure that its active acidity after sterilisation, expressed in pH units was 5.4, as measured at 25±1°C. Inoculated upturned plates were incubated at 37±1°C for 72 h in anaerobic conditions (7).

*Streptococcus* bacteria counts in the examined yoghurts were determined using the agar medium M17 (Merck) according to Terzaghi (15). In order to secure medium acidity after sterilisation ranging from 7.1 to 7.2 at 25±1°C, 0.1 M solution of sodium hydroxide or 0.1 M solution of hydrochloric acid was added to the dissolved components of the medium. Inoculated plates for the determination of the *Streptococcus* bacteria in the examined yoghurts were incubated at 37±1°C for 48 h (7).

Results of measurements and assays, obtained in the course of the performed experiments, were then subjected to statistical analysis using the Excel calculation sheet of the EAV program, ELSQ. The obtained results were employed as the basis for the determination of linear and multiple regressions using least squares method, coefficient of determination and verification of hypotheses at the set level of significance $P=0.05$ (5).

**Results**

On the basis of the performed investigations, it was found that, with the increase in the degree of condensation of the processed milk, the number of bacteria of the *Lactobacillus* and *Streptococcus* genera in the yoghurt increased immediately after its manufacture (Table 1). However, no significant differences were observed in the number of bacteria of the *Lactobacillus* genus in the yoghurt manufactured from the condensed milk with 3 and 5% addition of powdered skim milk. After the end of production, significantly more bacteria of the *Lactobacillus* genus than those of *Streptococcus* were determined in the yoghurt manufactured from the non-condensed milk (Table 1). Reversed proportions were found in the yoghurt with the highest dry matter content.

The total bacterial count underwent significant decline during the storage of the examined yoghurts (Table 2). Regardless of the extent of their condensation, after 21 d of storage, the total bacterial count of both types of bacteria in the stored yoghurts was significantly lower than their numbers determined directly after their manufacture. After storage, in yoghurts manufactured from non-condensed milk or from the milk supplemented with 1% powdered skim milk, significantly more *Lactobacillus* bacteria than those of *Streptococcus* ones were determined (Table 2). Later on, no statistically significant influence of the extent of condensation with 3 or 5% addition of powdered skim milk on the number of bacteria from the *Lactobacillus* genus and those of the *Streptococcus* genus was found in the examined yoghurts.

### Table 1

<table>
<thead>
<tr>
<th>Addition SMP (%)</th>
<th><em>Lactobacillus</em></th>
<th><em>Streptococcus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.75 $^{Aa}$</td>
<td>7.63 $^{Aa}$</td>
</tr>
<tr>
<td>1</td>
<td>8.27 $^{Ab}$</td>
<td>8.30 $^{Ab}$</td>
</tr>
<tr>
<td>3</td>
<td>8.51 $^{Ac}$</td>
<td>8.57 $^{Ac}$</td>
</tr>
<tr>
<td>5</td>
<td>8.59 $^{Ac}$</td>
<td>8.73 $^{Bd}$</td>
</tr>
</tbody>
</table>

A-B, a-d - various small letters in columns entered for the same determination and various capital letters in rows represent statistically significant differences at the level of $P=0.05$.

### Table 2

<table>
<thead>
<tr>
<th>Addition SMP (%)</th>
<th><em>Lactobacillus</em></th>
<th><em>Streptococcus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.50 $^{Aa}$</td>
<td>5.15 $^{Aa}$</td>
</tr>
<tr>
<td>1</td>
<td>6.66 $^{Bb}$</td>
<td>6.39 $^{Ab}$</td>
</tr>
<tr>
<td>3</td>
<td>7.56 $^{Ac}$</td>
<td>7.47 $^{Ac}$</td>
</tr>
<tr>
<td>5</td>
<td>7.61 $^{Ac}$</td>
<td>7.60 $^{Ac}$</td>
</tr>
</tbody>
</table>

A-B, a-c - various small letters in columns entered for the same determination and various capital letters in rows represent statistically significant differences at the level of $P=0.05$. 
The analysis of the course of the regression lines describing the percentage share of both bacterial genera in the yoghurts revealed their dependence on the storage time (Figs 1 – 4). The most approximate value of percentage share of both bacteria after production and 21 days of storage was observed in the case of the yoghurt manufactured from the condensed milk with 3% powdered skim milk (Fig. 3). This trend occurred not only immediately after the manufacture of yoghurt but was maintained throughout the storage period. However, half through this period, a change in the proportion of the two types of bacteria occurred. In the case of the yoghurt with the lower dry matter content, the change in the proportions of the two types of bacteria took place before day 7 of storage, whereas in the case of the yoghurt with the highest dry matter content – after day 14 of refrigerated storage.

**Discussion**

Following the fermentation of the processed milk inoculated with traditional cultures at 42°C, by the time the curd reached the pH value of 4.6 to 4.8, the number of bacteria, including those of *Lactobacillus*, increased 1.5 to 2 times reaching, in the final product, the level of $10^8 - 10^9$ CFU·g$^{-1}$. In addition, the authors also found 2- to 3-fold lower number of rods in yoghurts stored for 4 weeks at 6°C than in yoghurts directly after their production (9).

![Fig. 1](image1.png) Percentage changes in the bacterial proportion of *Lactobacillus* and *Streptococcus* genera during the storage of yoghurts manufactured from non-condensed milk.

![Fig. 2](image2.png) Percentage changes in the bacterial proportion of *Lactobacillus* and *Streptococcus* genera during the storage of yoghurts manufactured from milk condensed with the addition of 1% powdered skim milk.
It is evident from literature data, concerning the proportion of individual bacterial strains in the final product which are not identical with their proportions in the applied bacteria culture, that the proportion of Str. thermophilus bacteria in the total acidifying microflora is higher than that of L. delbrueckii subsp. bulgaricus bacteria. Bielecka and Majkowska (3), determined in a natural yoghurt 1.4×10⁸ CFU·g⁻¹ of Str. thermophilus bacteria and 9.9×10⁸ CFU·g⁻¹ of L. delbrueckii subsp. bulgaricus bacteria, despite the fact that the milk was inoculated with a culture of identical proportion of these bacteria. Similar results were reported by other authors investigating yoghurts obtained from processed milk which was inoculated with a traditional culture consisting of Str. thermophilus and L. delbrueckii subsp. bulgaricus at 1:1 ratio, after 5 h of incubation at 42°C (6). The number of each of the above-mentioned types of bacteria determined by those authors was: 3.6×10⁸ and 2.2×10⁸ CFU·g⁻¹, respectively. Also Beal et al. (2), who inoculated processed milk with a traditional bacterial culture of identical numbers of bacilli and streptococci, in the finished product obtained fewer bacteria from the Lactobacillus genus (1.2×10⁸) than from the Streptococcus genus (5.3×10⁸).

In the abundant literature on the subject, there is a lot of information concerning the symbiosis of these two bacterial genera and their proportion in the natural yoghurt (11, 12, 13). The observed complex phenomenon of symbiosis consists in the production of the formic acid and pyruvates as well as in the reduction of the redox potential by the Streptococcus bacteria, which favour the growth of bacilli. On the other hand, the more proteolytic bacilli, with their slower growth but more acidifying properties, release amino acids from.
proteins (valine, in particular) and, in so doing, they stimulate the growth of streptococci. Amoroso et al. (1) reported that the optimal ratio between bacilli and streptococci in yoghurts should range from 1:1 to 1:2. A significant reduction in the number of lactic fermentation bacteria in yoghurts during their storage from $10^8$ to $10^7$ or even to $10^5$ CFU·g$^{-1}$ during their shelf life was also reported by other researchers (8, 10). According to Żbikowski (16), after 21 d of storage, of all the yoghurt microflora, the total of 45% of bacteria undergo quantitative changes and in the case of yoghurts manufactured using probiotic cultures – 41.1%. The author showed that during the same period of storage of yoghurt manufactured from milk inoculated with a monoculture, the survival of *L. delbrueckii* subsp. *bulgaricus* bacteria was lower than that of *Str. thermophilus*. Investigations carried out by Beal et al. (2) revealed that the survival of milk fermentation bacteria could fluctuate in the range of 40 to 75% and, as a rule, more bacteria from the *Lactobacillus* genus survive than those from the *Streptococcus* genus.

**References**

1. Amoroso M.J., Manca De Nadra M.C., Oliver G.: Glucose, galactose, fructose, lactose and sucrose utilisation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* isolated from commercial yoghurt. Milchwissenschaft 1988, 43, 626-629.