COMPOSITIONAL CHARACTERISTICS OF COMMERCIAL YOGHURT BASED ON QUANTITATIVE DETERMINATION OF VIABLE LACTIC ACID BACTERIA

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Yoghurt quality is particularly difficult to standardize because of the many forms, varieties, manufacturing methods, ingredients and consumer preferences that exist. Since these factors will always play an important role, it is unlikely that a uniform yoghurt quality concept will ever emerge, such as has been developed for other dairy products. There are a number of common denominators, however that have bearing on yoghurt quality. Since a number of producers are recognized within the broad category entitled yoghurt. This situation makes yoghurt an interesting, challenging, but also a confusing area to work in. The present investigation was undertaken to isolate from commercial yoghurt the strains involved in its manufacture and determine the characteristics of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. This study is concerned with the lactic acid bacteria (L. delbrueckii subsp. bulgaricus and S. thermophilus) growth in yoghurt from involving different procedures and with the determination of the number of lactic acid bacteria in dependence of the temperature and acidity in the period of storage. Predominant samples of yoghurt were with 11-10⁷/ml lactic acid lactococci (44.28%).

KEY WORDS: LAB, yoghurt, viable lactic acid bacteria, probiotics

INTRODUCTION

One of the first records of yoghurt consumption comes from the Middle East during the times of the Conqueror Ğenghis Khan in the 13th century, whose armies were sustained by this healthful food. Yoghurt and other fermented dairy products have long been a staple in the diets of cultures of the Middle East, Asia, Russia and Eastern European countries, such as Bulgaria. Yet, the recognition of yoghurts special health benefits did not become apparent in Western Europe and North America until the 20th century, as a result of research done by Dr. Elie Metchnikoff. Dr. Metchnikoff (12, 13) is the first researcher who proposed fermented dairy products with beneficial properties. Conducted research on the health benefits of lactic acid-producing bacteria and postu-
lated that the longevity of peoples of certain cultures, such as the Bulgarians, was related to their high consumption of yoghurt and fermented dairy products.

The benefits of yoghurt depends for „live active cultures“ or „living yoghurt cultures“. Yoghurt is made by fermenting milk with friendly bacteria, mainly Lactobacillus delbrueckii subsp.bulgarius and Streptococcus thermophilus. Yoghurt is a traditional food and beverage in many countries and especially in Serbia. Yoghurt consumption in Serbia has increased during the last decade. Product quality and satisfaction of consumer expectation are discussed since they are essential for the continued successful growth of the yoghurt market. Much emphasis is placed on yoghurt flavor, body, and texture. The specific objectives of the study were: a) to determine the effect of cell lactic acid bacteria L.delbrueckii subsp. bulgaricus and S. thermophilus on growth in yoghurt from a different producers and b) to determine the number of lactic acid bacteria of the temperature and acidity in the period of storage (1,2). Initial counts of Lactobacillus and Streptococcus in the samples of yoghurts were in the range from 8 to 1x10⁶ /g resp. Ratio of Lactobacillus : Streptococcus at the start of the test varied from 1:1 to 1: 2.7.

**EXPERIMENTAL**

**Yoghurt production**

Yoghurt is made by fermenting milk with friendly bacteria, in Serbia mainly with Lactobacillus delbrueckii subsp.bulgarius and Streptococcus thermophilus. The milk sugar or lactose is fermented by these bacteria to lactic acid which causes the characteristic curd to form. This process gives yoghurt its refreshingly tart flavor and unique pudding like texture. The yoghurt qualities were judged to be satisfactory without defective taste. The baccilli/cocci ratio in the pre-fermented milk, unstable with free cells, was stabilized when the strains were enterapped. The yoghurt starter cultures play an important role during the production of yoghurt.

High cell numbers to about 5-10⁷ C.F.U. ml⁻¹ with a steady bacilli/cocci ratio were present in the effluent milk. The starter culture for most yoghurt production is a symbiotic blend of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus in relation 50:50. Although they can grow independently, the rate of acid production is much higher when used together than either of the two organisms grown individually. Streptococcus thermophilus grows faster and produces both acid and carbon dioxide. The format and carbon dioxide produced stimulates growth of lactobacilli. On the other hand, the proteolytic activity of lactobacilli produces stimulatory peptides and amino acids for use by streptococci. These microorganisms are ultimately responsible for the formation of typical yoghurt flavor and texture. Yoghurt that contains live bacterial cultures may help to live longer and may fortify immune system. The yoghurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yoghurt mix to approximately 5.0. The lactobacilli are responsible for a further decrease to pH 4.0. The following fermentation products contribute to flavor:

- lactic acid
- acetaldehyde
acetic acid
- diacetyl

The acid also restricts the growth of food poisoning bacteria. During the yoghurt fermentation some flavors are produced, which give yoghurt its characteristic flavor.

**Cultured media for the enumeration of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus mixtures**

Yoghurt was sampled 1 day after manufacture and the samples were taken in the market. Samples from 2 replicate experiments were processed. There are at present several culture media for the differential enumeration of mixtures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus. The use of these media is important for the control of the relation between these two microorganisms in the yoghurt production starter medium, as well as in the follow-up of both populations during the production and later ripening of yoghurt. The number of lactobacilli was determined on the MRS agar (6) and the number of streptococci was determined on M17 agar medium after 48 h of incubation was investigated, making use of the different morphologies of the colonies as a means of different enumeration. Total bacterial count was determined by direct counting on a microscope glass. Material for the research was 70 samples of yoghurt from 9 different dairy producers. The samples were from Belgrade trade market. As a laboratory control we used the mixed cultures of *L. delbrueckii* subsp. bulgaricus and *S. thermophilus*. Cultures enumerated in this study were lactic acid starter cultured used for manufactured of yoghurt. Lactic acid bacteria were enumerated using Elliker (7, 8) and MRS (5, 6) solid agar plates, used for the isolation lactobacilli (3, 4). Reconstituted milk powder was used for the storage lactic acid bacteria in the refrigerator. Strains of lactic acid lactococci were aerobically transferred three times at 37°C for 48 h (BBL Gas Pak System). The species designation of isolated was confirmed by Gram stain colonial appearance. MRS agar plate was incubated at 37°C without further adjustment. Plates or tube prepared with MRS agar were placed in plastic bags in anaerobe conditions and then incubated. M17 agar plates were used for the detection of streptococci. Each plate was overlaid with the same solid medium and then incubated at 30-37°C. Duplicate plates were prepared for each medium for the required dilutions (7, 9). Plates were examined after 48 and 72 h. To evaluate the factors that might be responsible for excessive acid development during yoghurt storage, 9 brands of plain commercial yoghurt were purchased from local retail markets and stored at 8, 12, 20°C, and analyzed weekly for 288 hours to monitor changes in acidity (°SH), total viable *Lactobacillus* and *Streptococcus*, coliform, yeasts and moulds.

**Microorganisms**

Strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus isolated from commercial products were used. Commercial yoghurt samples were used with no modifications, mixing 1 g of yoghurt in 10 ml saline solution and effective successive dilutions from $10^{-2}$ to $10^{-6}$. 
Microorganisms: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from commercial yoghurt were grown at 43°C in M.R.S. and M17 agar.

Isolation and identification: The lactis acid bacteria were isolated by API system for lactobacilli and streptococci identification. The API system was incubated at 37°C for 72 h. The various colonies formed were identified. Criteria examined included: Gram reaction, cell morphology, catalasa reaction, growth at 50°C, acid production from lactose, sucrose, trehalose, maltose and manitol, thermoresistence to 63°C, growth in NaCl 2%, hydrolysis of arginine, and growth at pH 9.6.

**Culture medium and growth conditions**

Enumeration of microorganisms: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp.*bulgaricus* were enumerated by surface spreading 0.1 ml samples on Elliker agar medium and by direct microscopic count on the microscope slade.

**RESULT AND DISCUSSION**

The optimum growth temperature for *Lactobacillus delbrueckii* subsp. *bulgaricus* occurred at 45°C and *Streptococcus thermophilus* has its optimum between 40°C and 45°C. The optimum temperature of the mixed culture was 45°C. The growth of these cultures at different initial pH in Elliker medium shows that the optimum pH for pure and mixed cultures is from 6.5 to 8.0. In Table 1 the results of the chemical analyses and total bacterial count (on the microscope slide) in yoghurt from different producers are shown.

**Table 1. Relationship between *Streptococcus* and *Lactobacillus* levels and average acidity**

<table>
<thead>
<tr>
<th>Producers</th>
<th>Acidity (°SH)</th>
<th>Lactobacillus (L)</th>
<th>Streptococcus (S)</th>
<th>Relation S/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>40.00</td>
<td>65</td>
<td>45</td>
<td>1 : 1.4</td>
</tr>
<tr>
<td>(II)</td>
<td>43.00</td>
<td>70</td>
<td>30</td>
<td>1 : 2.33</td>
</tr>
<tr>
<td>(III)</td>
<td>41.00</td>
<td>73</td>
<td>27</td>
<td>1 : 2.7</td>
</tr>
<tr>
<td>(IV)</td>
<td>39.00</td>
<td>55</td>
<td>54</td>
<td>1 : 1.2</td>
</tr>
<tr>
<td>(V)</td>
<td>37.00</td>
<td>50</td>
<td>50</td>
<td>1 : 1</td>
</tr>
<tr>
<td>(VI)</td>
<td>42.00</td>
<td>65</td>
<td>45</td>
<td>1 : 1.44</td>
</tr>
<tr>
<td>(VII)</td>
<td>38.00</td>
<td>50</td>
<td>50</td>
<td>1 : 1</td>
</tr>
<tr>
<td>(VIII)</td>
<td>42.00</td>
<td>67</td>
<td>43</td>
<td>1 : 1.55</td>
</tr>
<tr>
<td>(IX)</td>
<td>40.00</td>
<td>71</td>
<td>29</td>
<td>1 : 2.44</td>
</tr>
<tr>
<td>Control</td>
<td>32.00</td>
<td>50</td>
<td>50</td>
<td>1 : 1</td>
</tr>
</tbody>
</table>
Fig. 1. Relationship between viable count of Streptococci/ Lactobacilli in the different commercial yoghurt samples

The results of a brief microbiological evaluation of yoghurt and of a consumer preference survey are presented also in Fig.1. Lactic acid bacteria are broadly used as a starter cultures for industrial production of fermented food. The requirement of Serbian Regulation is a minimum number of 10^6 viable yoghurt organisms (L. delbrueckii subsp. bulgaricus and S. thermophilus) must be present. The ratio of the yoghurt organisms is also important in determining the quality of yoghurt. In this study we determined the effect of cell for lactic acid bacteria (L. delbrueckii subsp. bulgaricus and S. thermophilus) on growth in yoghurt from a different procedures and made a quantitative estimation of the number of lactic acid bacteria the temperature and acidity in the period of storage. Material for the research were 70 samples of yoghurt from 9 different dairy producers.

The commercial samples were from the Belgrade trade market. Lactic acid bacteria were enumerate using Elliker solid medium agar plates, MRS solid agar plates for isolation of lactobacilli and M17 agar plates for the isolation of streptococci. There was a wide range in the total number of LAB. Predominant samples of yoghurt were with 11-10^7/ml LAB (44, 28%) of examined samples.

Yoghurt taste is most satisfactory after 72 hours of storage at 8°C when acidity was 44.14° SH. Coliforms, yeasts or moulds were not detected at any time during storage. There were consistent differences in °SH among brands with means ranging from 54.1 to 40.28 at 8°C, 63.1 to 47.74 at 12°C, 64.46 to 45.96 at 20°C indicating that it was possible for the pH of yoghurt to remain ≥ 8.0. All brands showed initial counts of Lactobacillus and Streptococcus in the range 8 to 1x10^6/g resp. Ratio of Lactobacillus: Streptococcus at the start of the test varied from 1 : 1 to 1 : 2.7. The decrease in counts of Streptococcus and Lactobacillus in most of brand stored at 20°C. It is apparent that strain of bother Streptococcus and Lactobacillus vary in their survival and in their ability to maintain a higher °SH in yoghurt during storage.
Table 2. Temperature storage of yoghurt and the number of viable lactobacilli and streptococci at the corresponding pH

<table>
<thead>
<tr>
<th>Temperature storage (°C)</th>
<th>Time of storage (hours)</th>
<th>Number of viable lactobacilli and streptococci in the samples of yoghurt (in 000000)</th>
<th>Acidity (°SH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>11-100</td>
<td>101-200</td>
</tr>
<tr>
<td>8</td>
<td>72</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>120</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>192</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>288</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>72</td>
<td>2</td>
<td>2</td>
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<td>12</td>
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<td>12</td>
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<td>12</td>
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<td>7</td>
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<td>20</td>
<td>288</td>
<td>8</td>
<td>2</td>
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</tbody>
</table>

The product is resulting from milk by fermentation with a mixed starter culture consisting only *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and these two organisms have a symbiotic relationship during the manufacture of yoghurt with the ratio of *Streptococcus thermophilus* to *Lactobacillus delbrueckii* subsp. *bulgaricus* constantly changing. Both organisms produce lactic acid as the main fermentation products. For a proper flavor development, the ratio of *Streptococcus thermophilus* to *Lactobacillus delbrueckii* subsp. *bulgaricus* should be in the range of 1:1 to 3:1. Consistency is an important attribute in evaluating the quality of yoghurt. There are numerous factors which affect consistency including milk composition, heat treatment, homogenization, use of stabilizer, yoghurt culture, and mechanical handling of the yoghurt.

**CONCLUSION**

During the past two decades, there has been renewed interest in the study of the nutritional and therapeutic aspects of dairy products (12, 13, 14). A majority of reviewed papers suggested potential therapeutic benefit following the consumption of fermented dairy products containing viable lactic acid bacteria (LAB) count and decreased coliform count in the intestine as observed in the analysis. The beneficial effect of normal gut flora temporarily brings yoghurt live microorganisms which have probiotics effects (3, 7). While numerous researchers have suggested that lactic cultures and cultured dairy products provide several nutritional and therapeutic benefits to the consumer there exist a few reports in which some of the benefits have been questioned. Lactic acid bacteria are broadly used as starter cultures for industrial production of fermented food. It has been known for many years that lactic acid bacteria may positively influence the gastrointestinal tract of human and other mammals. The beneficial effects include the inhibition of undesirable microorganisms, reduction in cholesterol level and reduction of the risk of colon cancer (10, 11, 14, 7).
REFERENCES


2. Amoroso M.J., Manca M.C. de Nadra, G. Oliver: Growth and sugars utilization by mixed cultures of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus Salivarus subsp, thermophilus isolated from Argentina, World Journal of Microbiology and Biotechnology 8 (1997) 50-54.


КВАНТИТАТИВНО ОДРЕЂИВАЊЕ БАКТЕРИЈА МЛЕЧНЕ КИСЕЛИНЕ КОМЕРЦИЈАЛНИХ УЗОРАКА ЈОГУРТА

Драгана Пешић-Микулец и Гордана Б. Никетић

Квалитет јогурта је тешко стандардизовати због великог броја и врста бактерија млечне киселине у процесу производње. Због свих ових особина уједначеност квалитета јогурта тешко се постиже. Испитивања у овом раду обухватали су изолацију, однос и број бактерија млечне киселине (L. delbrueckii subsp. bulgaricus и S. thermophilus) које се користе за производњу одређених варијетета јогурта карактеристичних за поднебље Србије. Одређиван је број бактерија млечне киселине у различитим комерцијалним узорцима јогурта. Квалитет узорака јогурта одређиван је у зависности од температуре и времена чувања. Код већег броја узорака јогурта нађено је 11-10⁷/мл живих бактерија млечне киселине. Укус и арома јогурта били су задовољавајући након 72 часа при температури складиштења од 8°C. Однос бактерија млечне киселине Streptococcus и Lactobacillus износио је од 1:1 до 1:2,7 код свежих узорака јогурта, да би даљим складиштењем на неповољним температурама дошло до поремећаја однosa до 3:1 стартер култура јогурта.

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