Growth and activity of probiotic bacteria in fermented milks fortified with polyphenol extract from strawberry by-product

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Abstract. The effect of polyphenol-enriched extract from a strawberry by-product (SPE) on lactic acid production and the viability of Lactobacillus delbrueckii subsp. bulgaricus S19 (Lb. bulgaricus), Lactobacillus rhamnosus YW (Lb. rhamnosus) and Streptococcus thermophilus S13 (Str. thermophilus) in fermented milks during refrigerated storage was investigated. The fermented milks, with SPE (samples S1 and S2) and without the addition of the SPE (samples K1 and K2), were stored at 4±2°C for 15 days. A constant tendency to decrease in the pH values from 4.40±0.06 to 4.05±0.11 and an increase in the lactic acid concentration from 8.33±0.10 g/L to 11.12±0.41 g/L, respectively, were observed. The residual lactose content of the control and test samples at the end of the refrigerated storage was in the range of 31.44±0.39 g/L. The addition of polyphenol-enriched extracts did not significantly affect (P>0.05) the viability of Lactobacillus spp. and Str. thermophilus during the refrigerated storage.

Keywords: fermented milks, functional foods, post-acidification, probiotic bacteria, strawberry polyphenols.

1. Introduction

Fermented milks have high consumers’ acceptance and positive health promoting ingredients such as ingested live microorganisms (probiotics) included in starter cultures [1]. The efficiency of probiotic bacteria depends on their viability and activity [2]. According to National Yoghurt Association (NYA) regulations the viability of yoghurt starter cultures must be maintained thought storage period [3]. It is well known that the probiotic microorganisms are widely used in the dairy industry [4] and it has been proven that the ingredients that are rich in phenolic compounds can increase the survival of probiotic cultures in milk products [5]. On the other hand, experimental data indicate that polyphenols may influence the risk of developing the chronic disease and cardiovascular disease [6] due to their antioxidant and anti-inflammatory properties [7]. Also, phenolic compounds are known as antimicrobial agents inhibiting the growth of pathogenic bacteria [8] as well as lactic acid bacteria [9].

Strawberries are a widely consumed fruits in fresh forms and industrially used as raw material with proven high content of phenolic compounds [10, 11]. Strawberry by-products can be successfully used as a source of bioactive compounds. Incorporating into food products, especially in probiotics fermented milks, make them one of the most important categories of functional foods [12]. On the other hand, it has been found that in the production of probiotic functional products polyphenols may influence the...
metabolic activity of microorganisms as well as the in vitro and in vivo viability of probiotic lactic acid bacteria [13]. In order to increase the health benefits of functional fermented milk, the incorporation of polyphenol-enriched extracts can be used [14]. Some studies have evaluated the influence of enrichment of fermented milks with by-products or extracts from different plants [15, 16]. However, there are no studies concerning evaluation of growth and activity of probiotic bacteria in fermented milks fortified with polyphenol-rich extracts from strawberry by-products. In this context, the objective of this study was to evaluate the influence of a polyphenol extract from strawberry by-products on the growth and activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13 in fortified fermented milks during cold storage period.

2. Materials and methods

2.1. Materials

2.1.1. Polyphenol extract

The strawberry pomaces were obtained from Santulita Ltd. (Sofia, Bulgaria). The press residues were transferred to the laboratory and stored frozen at -18°C until used to produce the polyphenol extracts. Polyphenol-enriched extract was obtained following the method described by [17].

2.1.2. Bacterial strains

The starter cultures of lactic acid bacteria used in the present study consisted of *Lactobacillus delbrueckii* subsp. *bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13. They belong to the laboratory collection of the Department of Microbiology at the UFT, Plovdiv.

2.1.3. Fermented milk samples

The control and experimental batches of fermented milks were prepared following the method described by [18]. The cow’s milk (M = 3.7%) was heated to 95°C for 15 min, cooled to t=45±1°C and divided into two lots: one experimental lot (samples S1 and S2) fortified with polyphenols to 0.39 mg GAE/mL using SPE, and an unfortified control lot (samples K1 and K2). The experimental and control batches of milk were inoculated with 2% Bulgarian yoghurt starter culture consisted of *Lb. bulgaricus* : *Str. thermophilus* in ratio of 1:5 (samples K1 and S1) and starter culture consisted of *Lb. bulgaricus* : *Lb. rhamnosus* : *Str. thermophilus* in ratio of 0.5:0.5:5 (samples K2 and S2). All samples were packaged in containers and incubated at t=44±1°C until the pH reached 4.6. At the end of the incubation, the fermented milks were cooled down to approximately 4°C and then stored at the same temperature for 15 days.

2.2. Methods

2.2.1. Microbiological analysis

The viable cell counts of *Str. thermophilus* S13, *Lb. bulgaricus* S19 and *Lb. rhamnosus* YW were conducted following the method described by [18]. M17 and MRS (Merck, Darmstadt, Germany) media were used for cultivation of lactic acid bacteria. The methodology described in Standard EN ISO 7889 [19] was followed. The samples were prepared according to ISO 8261:2001 [20].

2.2.2. Physicochemical analysis

The pH values of the samples were determined using a digital MS 2011 pH meter (Microsyst, Plovdiv, Bulgaria) equipped with a Sensoret pH electrode (Garden Grove, CA, USA). The titratable acidity was measured by the titration method [21]. The residual lactose content was calculated on the basis of the results from initial lactose content in milk and lactic acid formation during the storage period.
2.2.3. Statistical analysis
At least three replicates analytical determinations were obtained for each data point. Data were analyzed using one-way ANOVA performed with Microsoft Excel, as described by [18].

3. Results and discussion
The results of the microbiological evaluation and physicochemical analysis of test fermented milks during refrigerated storage are presented on Figure 1 and Figure 2.

![Figure 1](attachment:image1.png)

**Figure 1.** Growth and acidification activity of the starter culture during the storage period of the control sample – K1 (A) and polyphenol-enriched sample – S1 (B).

From the 1st to the 15th day, a constant tendency to decrease in the pH values from 4.40±0.06 to 4.05±0.11 and an increase in the lactic acid concentration from 8.33±0.10 g/L to 11.12±0.41 g/L, respectively, were observed. All fermented milk samples showed a more intense decrease in pH and lactic acid concentration from the day 1 to day 9 and a slower change between the 9th and 15th day (Figure 1 and Figure 2). The residual lactose content of the control and test samples at the end of the refrigerated storage was in the range of 31.44±0.39 g/L. It could be seen that (Figure 1 and Figure 2) the pH values and lactic acid concentration in the controls and polyphenol-enriched fermented milks did not differ significantly (P>0.05) during the cold storage. The results indicate that the addition of polyphenol
extracts from the strawberry presses residues in fermented milk did not affect the post-acidification process. Our results were in agreement with other studies, which indicated that during the storage period of polyphenol-enriched fermented milks the pH values were similar to those of the respective control samples [22].

Figure 2. Growth and acidification activity of the starter culture during the storage period of the control sample – K2 (A) and polyphenol-enriched sample – S2 (B).

Caleja et al. [23] report that the analysis of this parameter in fermented milk is very important in terms of product safety. In our study, the presence of *Lactobacillus rhamnosus* YW in the starter culture did not influence significantly (P>0.05) the lactic acid production during the refrigerated storage, as evidenced by the similar pH values and lactic acid concentration of batches K1 and K2 as well as S1 and S2 (Figure 2).

The enrichment of fermented milks with plant by-products is a good approach to improve the viability of lactic acid bacteria [24]. Muniandy et al. [25] investigated the effect of adding green, white and black tea extracts on the viability of *Lb. bulgaricus* and *Str. thermophilus* in fermented milk during 15 days cold storage. The authors found that the addition of tea extracts did not significantly affect the viability of lactic acid bacteria. In the present study, good survival of probiotic strains of lactic acid bacteria during the storage period was observed. No significant differences (P>0.05) in the
Streptococcus thermophilus S13 counts in the control and polyphenol-enriched fermented milks was observed. Similar results were reported by other authors [14]. A slight decrease in the lactobacilli count was found, which for the entire storage period was within about Log 0.5. It was found that the presence of Lactobacillus rhamnosus YW in the starter culture was associated with a higher count of lactobacilli at the end of the refrigerated storage (samples K2 and S2). This could be due to its better survival during refrigerated storage compared to Lactobacillus delbrueckii subsp. bulgaricus S19.

The mean viable count of lactic acid bacteria in control fermented milks and the fermented milks with SPE are shown in Table 1.

Table 1. Total count of lactic acid bacteria in polyphenol-enriched fermented milks during the storage period.

| Parameters                      | Total count of lactic acid bacteria, CFU/mL<sup>a</sup> |
|---------------------------------|--------------------------------------------------------|
|                                 | 1 day        | 3 day        | 6 day        | 9 day        | 12 day       | 15 day       |
| **Samples**                     |             |             |             |             |             |             |
| K1                              | 3.6±0.18.10<sup>8</sup> | 3.7±0.19.10<sup>8</sup> | 3.7±0.20.10<sup>8</sup> | 3.8±0.18.10<sup>8</sup> | 3.8±0.20.10<sup>8</sup> | 3.2±0.16.10<sup>8</sup> |
| S1                              | 2.7±0.14.10<sup>8</sup> | 2.0±0.10.10<sup>8</sup> | 1.3±0.10.10<sup>8</sup> | 1.5±0.12.10<sup>8</sup> | 1.4±0.11.10<sup>8</sup> | 2.5±0.14.10<sup>8</sup> |
| K2                              | 3.8±0.19.10<sup>8</sup> | 3.6±0.14.10<sup>8</sup> | 4.2±0.21.10<sup>8</sup> | 3.8±0.19.10<sup>8</sup> | 3.4±0.17.10<sup>8</sup> | 3.6±0.18.10<sup>8</sup> |
| S2                              | 3.9±0.20.10<sup>8</sup> | 3.5±0.18.10<sup>8</sup> | 3.0±0.15.10<sup>8</sup> | 2.5±0.15.10<sup>8</sup> | 1.9±0.12.10<sup>8</sup> | 2.7±0.14.10<sup>8</sup> |

<sup>a</sup> mean ± SD (n = 3).

On the 15th day of refrigerated storage a high level of Str. thermophilus and Lactobacillus spp. counts in the range of 3.0±0.4.10<sup>8</sup> CFU/mL was observed (Table 1), which is important for the functional characteristics of the fermented milk. During the refrigerated storage, no significant difference (P>0.05) in the total count of lactic acid bacteria between the control and experimental samples was observed (Table 1). These results indicated that, the addition of polyphenol-enriched extracts from strawberry press residues (SPE) did not affect the survival of the probiotic lactic acid bacteria during 15 days of cold storage. Our results were consistent with previous studies [16, 26] which found that the population of the addition of Streptococcus thermophilus and Lactobacillus spp. remained stable during 14 days of cold storage of the fermented milks containing grape seed extract.

4. Conclusion
The results reported in the present study showed that the fortification of milk with polyphenols extracted from strawberry press residues had no significant effect on the post-acidification during 15 days of refrigerated storage. The two types of polyphenol-fortified yogurt samples (S1 and S2) preserved a high Str. thermophilus and Lactobacillus spp. counts in the range of 3.0±0.4.10<sup>8</sup> CFU/mL throughout the refrigerated storage. Such a high count of viable probiotic bacteria ensures the potential health benefits of regular consumption for consumers. In addition, the utilization of waste from fruit industries can help to reduce the environmental impacts caused by its disposal. The findings of the present investigation would be applied for the utilization of strawberry press residues which are readily available as wastes from Agro-food industry, into source of phenolic bioactive compounds. On the basis of the results from the research work an appropriate combination of polyphenols from strawberry by-products and lactic acid bacteria strains for production of new functional fermented milks are suggested.

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