Abstract: One of the most popular dairy products worldwide is yogurt, which flavor is highly influenced by lactic acid content. Yogurts with fruit preparations contain polyphenols, secondary plant metabolites with proven antioxidant properties. As there is no data regarding neither lactate nor polyphenol content in commercial yogurts in Serbia, this study aimed to determine lactate and polyphenol levels, as well as antioxidant activity in selected yogurts. A total of 15 plain and 5 fruit yogurts with strawberry preparations were analyzed. Lactate contents were from 0.83 to 1.33%. Three plain yogurts in plastic containers differed in lactate content. There was no difference in lactate content among the same plain yogurts packed in plastic and Tetra Pack containers. Fruit yogurts with strawberry preparations differed in total polyphenol content in the range from 6.84 to 29.11mg GAE/100g and antioxidant properties were determined by reducing power test (from 0.22 to 0.79) and DPPH assay (from 28.13 to 87.23%), while there was no difference regarding lactate. Our results provided new information about the levels of lactate, total polyphenols and antioxidant activity of selected commercial yogurts available on the Serbian market.

Key words: plain yogurt, fruit yogurt, strawberry, packaging

INTRODUCTION

Yogurt has been used traditionally in the Balkan region (Teneva Angelova, Balabanova, Boyanova & Beshkova, 2018) and it is popular worldwide, due to its health benefits and sensory properties (Chen et al., 2017; Scibisz, Ziarno & Mitel, 2019; Deshwal, Tiwari, Kumar, Raman & Kadyan, 2021; Tolu & Altin, 2021). It is produced from milk, which undergoes initial treatment, standardization, homogenization, heat treatment, fermentation, and cooling. Fermentation is the most important step of the process for the formation of the texture and flavor of yogurt. It is based on the activity of starter cultures Streptococcus thermophilus subsp. Thermophiles (STT) and Lactobacillus delbrueckii subsp. Bulgaricus (LDB), gram positive bacteria. STT are aerotolerant and thermod tolerant, which enables them to grow faster at the beginning. They hydrolyze lactose from milk into glucose and galactose, of which the first is converted into pyruvate. It is then converted into lactic acid, by the activity of lactic acid dehydrogenase. According to TS 1330 Yogurt Standard, lactic
Based on taste, yogurts can be plain and flavored (Weerathilake, Rasika, Ruwanmali & Munasinghe, 2014). Fruit yogurts are made by the addition of fruit preparations (Cheng, 2010; Jayasinghe, Fernando, Jayamanne & Hettiarachchi, 2015), usually in the amount from 5 to 25% (Ścibisz et al., 2019). Different commercial yogurts are available on market with preparations from strawberries, forest fruits, raspberries, apples, peaches, and plums. The addition of fruit improves the taste of yogurt (Amal, Eman & Nahla, 2016) and literature data point out that strawberry is one of the most popular flavors of yogurt (Vahedi, Tehrani & Shahidi, 2008; Routray & Mishra, 2011; Jaster et al., 2018; Salehi, 2021). Strawberry (Fragaria x ananassa Duch.), a plant from Rosaceae family, represents one of the most important agro-industrial crops globally (Aaby, Mazur, Nes, & Skrede, 2012) and it contains high amounts of polyphenols, plant metabolites with well-documented antioxidant activity (Salazar-Orbea, García-Villalba, Tomás-Barberán & Sánchez-Siles, 2021). The level of polyphenols in fresh strawberry fruit depends on growth conditions, cultivar, and stage of maturity (Aaby et al., 2012; Mahmood, Anwar, Abbas & Saari, 2012; Nöwicka, Kucharska, Sokól-Lętowska & Fecka, 2019; Salazar-Orbea et al., 2021). Strawberries are also used for the production of jams, jellies, juices, or other products (Salazar-Orbea et al., 2021) in which the content of polyphenols can vary depending on processing and storage (Mahmood et al., 2012; Salazar-Orbea et al., 2021). At this moment, there is still not enough scientific data to set recommendations for optimal dietary intake (Olas, 2018; Fraga, Croft, Kennedy & Tomás-Barberán, 2019). However, dose-dependent effects of polyphenols have been reported in numerous studies (Frejnagel & Juskiewicz, 2011; Murakami, 2014; Ounnas et al., 2017; Zec et al., 2017).

The aims of this study were manifolds: 1) to determine lactic acid content in selected commercial plain and fruit preparations-added yogurt available on the Serbian market; 2) to evaluate whether the package influenced lactate content in plain yogurt; 3) to analyze total polyphenol levels in fruit yogurts with strawberry preparations and their contribution to antioxidant activities.

**MATERIALS AND METHODS**

**Material sampling**

A total of 20 samples (15 plain and 5 fruit yogurts with strawberry preparations) were purchased in a supermarket (in Belgrade, Serbia) in September 2021. The sample planning and their collection and preparation were according to instructions given in the EuroFIR guide (Oseredzuk, Salvini, Roe & Moller, 2009). Characteristics of the yogurts that were available on labels are provided in Table 1.

Three commercially available yogurts, in both plastic (polypropylene 200 ml cup with aluminium foil closure) and Tetra Pak (250 ml) containers, were chosen to analyze whether the types of packaging material could have influenced lactate content.

Samples were stored at +4 °C for 7 days (total of six days after opening).

**Preparation of yogurts extracts**

Methanolic and 80% ethanolic extracts of fruit-added yogurts were prepared. Briefly, 2 g of fruit-added yogurts were measured and an appropriate volume of organic solvents was added (8 ml), vigorously mixed for 1 min and left for 12 hours at 4 °C. The next day, extracts were vortexed three times for 1 min and then centrifuged at 5000 rpm for 10 min. Extracts were separated from precipitates and their volumes were measured and further used for the determination of polyphenols and antioxidant activities during the same day.
**Determination of lactic acid**

Lactic acid content was determined according to Borschchevskaya, Gordeeva Kalinina and Sineokii (2016). Briefly, tenfold dilution of yogurts samples in distilled water was made. After centrifugation at 3000 rpm for 10 min, 50 µL of supernatant was added to 950 µL of 0.2% iron (III)-chloride solution and absorbance was measured at 390 nm after 5 min of incubation using a spectrophotometer UV-1800 (Shimadzu Corporation, Kyoto, Japan).

The standards were prepared by diluting lactate (Sigma-Aldrich, Germany) in range from 0.25-10.0 g/L and linear curve equation obtained $y = 0.12x + 0.014$, with $r^2 = 0.99$.

**Total polyphenol content**

Total polyphenol content was determined using a slightly modified Folin-Ciocalteu (FC) method (Blainski, Lopes & de Mello, 2013). In brief, 125 µL of the sample was mixed with 125 µL of FC reagent and incubated for 6 minutes, at room temperature.

Then 1000 µL of 7% Na2CO3 was added and the mixture was incubated for 90 minutes, in the dark, at room temperature. Subsequently, 500 µL of distilled water was added and the absorbance was measured at 760 nm, with gallic acid used as standard.

**Antioxidant activity**

**DPPH test**

Measurement of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl) was performed according to the slightly modified method of Blois (1958). Briefly, 640 µL of 0.15 mM DPPH solution was added to the 160 µL mL of the sample, the mixture was in-cubated for 30 min at room temperature and the absorbance was measured at 517 nm. DPPH scavenging activity (%) was determined according to the following formula: 

$$PPH(\%) = 100 \times \left( \frac{ABS_{blank} - ABS_{sample}}{ABS_{sample}} \right)$$

where $ABS_{blank}$ is absorption of blank sample and $ABS_{sample}$ is absorption reading of sample.

**Reducing power test**

The assessment of reducing power was performed according to the method by Oyaizu (1986), reported by Kim, Cho, Yeon, Choi and Lee (2019), with slight modifications. In brief, we mixed 250 µL of yogurt samples, 50 µL of 0.2 M phosphate buffer (pH 6.6) and 50 µL of 1% K$_3$Fe(CN)$_6$ and incubated it for 20 min at 50 °C. Then we added 50 µL of 10% trichloroacetic acid and centrifuged the mixture at 865xg for 10 min. The amount of 300 µL of supernatant was mixed with 400 µL of distilled water and 100 µL 0.1% FeCl$_3$ and incubated.
for 10 min at room temperature. Subsequently, the absorbance was measured at 700 nm.

**Statistical analysis**

Six replicates were made for each of the samples and the results were presented as mean values and standard deviation. Differences between means were analyzed using ANOVA and Tukey's post-hoc test. Pearson test was used for the assessment of the correlation between total polyphenols and antioxidant activity. Statistically significant results were those with the p value less than 0.05. Data analysis was performed using the software GraphPad Prism (version 9, San Diego, California).

**RESULTS AND DISCUSSION**

**Characteristics of the collected samples**

The samples of plain yogurts showed high variability for fat content being in a range from 0.50-3.20g/100 g and consequently in saturated fat from 0.35-2.58 g/100 g (Table 1). At the same time, in yogurts P1, P6 and P12 probiotic bacteria were added and other had only starter cultures. The total energy value of fruit-added yogurts was higher compared to the plain ones (mean value 77.8±9.3 kcal/100 g compared to 48.9±4 kcal/100 g, respectively) due to higher added sugar content as there was no direct association between sugar amounts and percentage of fruit preparation. Furthermore, the content of fruit varied from 7-18%, therefore the consumption of 200 g of fruit yogurt would lead to a dietary intake of 14-36 g of strawberry preparation.

**Lactate content in plain and fruit yogurts**

The most abundant organic acid in yogurts is lactic acid (Vénica et al., 2014), which highly contributes to their refreshing and tart flavor (Cheng, 2010). Recent researches indicate possible beneficial effects of lactic acid, on the reinforcement of the function of the intestinal barrier (Flint, Duncan, Scott & Louis, 2015), modulation of innate immunity (Iraporda, Romanin, Rumbo, Garrote & Abraham, 2014) and inflammation (Hoque, Farooq, Ghani, Gorelick & Mehal, 2014). According to literature data, the lactic acid content in yogurt could be very variable, as the authors reported values from 0.36 to 2.0% (Fernandez-Garcia & McGregor, 1994; Ekinci & Gurel, 2008; Güler & Park, 2011; Cruz et al., 2012; Vénica et al., 2014; Tolu & Altun, 2021). The obtained mean values for lactate contents were from 0.83 to 1.17%, but only sample P3 was statistically significantly different from P1 (p = 0.02) and P2 (p = 0.03) (Fig. 1). The results for lactate content in plain yogurts are similar with those reported for Turkish standard yogurts (0.6 - 1.6%) (Güler & Park, 2011), and with 1% of those obtained by Fernandez-Garcia and McGregor (1994).

Additionally, there were no statistically significant differences when comparing plain yogurts’ samples P8, P10 and P12 in plastic (polypropylene cup with aluminium foil closure) and Tetra Pak containers. Although Saint-Eve, Lévy, Le Moing, Ducruet and Souchon (2008) observed differences in the post-acidification of yogurts stored in glass and polypropylene containers, indicating the influence of different package materials, our result suggests that both plastic (polypropylene) and Tetra Pak containers could have a similar influence on quality maintenance considering lactate content (Fig. 1).

Furthermore, there was no change in lactate levels 6 days after the opening of samples in Tetra Pak containers after storage at +4 °C in the refrigerator (Fig. 2). The period of 7 days was chosen as it is stated on the label of Tetra Pak samples that they can be used within this period. As samples in polypropylene 200 ml containers are usually opened and consumed immediately, our results indicate that yogurts in Tetra Pack containers will not increase lactate concentration if they are stored for 7 days, which could be important information for consumers. However, our observations are limited due to the very small number of samples. The samples of commercial fruit yogurts with strawberry preparations investigated in this study contained from 7% to 18% of strawberry preparations. There was no statistically significant difference in the lactate content among the fruit-added yogurts samples, with mean values in a range from 0.98 to 1.33% (Fig. 1) Additionally, the fruit yogurts with strawberry preparations investigated in our study did not contain lower levels of lactate compared to the plain yogurt counterparts (Fig. 1). Moreover, sample S1 had higher lactate concentration compared to those of plain yogurts’ samples P3 (p = 0.01) and P7 (p = 0.03) and post-fermentation production of lactate occurring in fruit yogurts (Ścibisz et al., 2019) could, at least partly, contribute to observed, levels of lactate in our fruit yogurts’ samples.
To our best knowledge, there is only one recent study investigating lactate content in fruit yogurts with strawberry preparations (Ścibisz et al., 2019) reporting lower lactate levels (about 0.6%) than those observed in our study, but it is worth noting the yoghurts in the mentioned study by Ścibisz and coworkers contained more fruits (about 20% of strawberry preparation). The number of investigated samples in our study is small (n = 5) and lactate content could significantly vary, so it may be important to routinely monitor its content.

**Total polyphenols and antioxidant activity in fruit yogurts with strawberry preparations**

The concentrations of polyphenols were analyzed in both methanolic and ethanolic extract to enable a more efficient comparison of our results with available data from other studies since some authors have been using methanolic extract, and other ethanolic extracts. The total polyphenol contents did not differ comparing methanolic and 80% ethanolic extracts and their levels were in a range from 6.84 to 29.11 mg GAE/100g (Table 2). Significant differences among the samples of fruit yogurts with strawberry preparations regarding their total polyphenol content were found (Table 2).

Many factors could influence total polyphenol content in strawberry preparations such as the type of fruit cultivar, agricultural conditions, as well as processing and storage conditions of the product (Aaby et al., 2012; Mahmood et
Table 2.
Total polyphenol content and antioxidant activity in fruit yogurts with strawberry preparations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total polyphenol content (mg GAE/100g)</th>
<th>DPPH (%)</th>
<th>Reducing power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methanolic extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>12.60±0.32c</td>
<td>53.20±1.63c</td>
<td>0.36±0.02d</td>
</tr>
<tr>
<td>S2</td>
<td>12.12±0.17c</td>
<td>46.35±0.91d</td>
<td>0.37±0.01c</td>
</tr>
<tr>
<td>S3</td>
<td>17.00±0.34b</td>
<td>81.71±1.82b</td>
<td>0.79±0.04b</td>
</tr>
<tr>
<td>S4</td>
<td>27.30±1.75a</td>
<td>87.23±0.15a</td>
<td>4.40 (0.22±0.01b)a</td>
</tr>
<tr>
<td>S5</td>
<td>6.84±0.21d</td>
<td>29.10±1.86b</td>
<td>0.34±0.005d</td>
</tr>
<tr>
<td></td>
<td>Ethanolic extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>13.29±0.56c</td>
<td>50.59±0.72c</td>
<td>0.33±0.003d</td>
</tr>
<tr>
<td>S2</td>
<td>13.80±0.64c</td>
<td>47.34±0.49d</td>
<td>0.41±0.009c</td>
</tr>
<tr>
<td>S3</td>
<td>18.60±0.97b</td>
<td>72.79±0.90b</td>
<td>0.69±0.03b</td>
</tr>
<tr>
<td>S4</td>
<td>29.11±2.47a</td>
<td>87.09±0.79a</td>
<td>4.40 (0.22±0.02b)a</td>
</tr>
<tr>
<td>S5</td>
<td>8.89±0.59d</td>
<td>28.13±1.93e</td>
<td>0.30±0.02d</td>
</tr>
</tbody>
</table>

Results are given as mean ± standard deviation (n = 3). # sample was 20 fold diluted and extrapolated; P- plain yogurt; S- fruit yogurt with strawberry preparation;

Statistical significance (p <0.05) in each column is presented with different letters.

All aforementioned factors could have been affected the total content of polyphenols in yogurts from this study. At this moment, to our best knowledge, there is no data regarding the total polyphenol content of fruit yogurts with strawberry preparations neither in the most comprehensive polyphenol data base Phenol explorer nor in the Serbian food composition database. So, the obtained results could be useful to extend existing databases, which might be important for future public health investigations (Ocke et al., 2021). However, in several studies, the total polyphenol concentrations and antioxidant activity of strawberries and their extracts have been analyzed (Aaby et al., 2012; Mahmood et al., 2012; Nowicka et al., 2019; Salazar-Orbea et al., 2021). The reported levels for total polyphenols in these studies have shown high variability ranging from 8.45 to 208.58 mg/100g of total polyphenols. As the average daily intake of polyphenols is about 1000 mg/daily (Olas, 2018; Fraga et al., 2019), dietary intake of commercial fruit yogurts with strawberry preparations could provide substantial amounts of these antioxidants substances, especially, for the consumer who prefer commercial products over fresh fruits. However, the total polyphenol content showed no correlation with fruit preparations percentage. Polyphenols are considered to be the main contributors to antioxidant activity in fruit yogurts with strawberry preparation (Citta et al., 2017), but since they are present only in plants, in the current study, their concentrations were not determined in the samples of plain yogurt.

Numerous diseases, such as cardiovascular, neurodegenerative and cancer can result from oxidative stress (Basu et al., 2016; Olas, 2018). Antioxidative activity can be measured using various tests that are cost-effective and easily reproducible. They are based on one of the reactions: hydrogen atom transfer or electron transfer (Huang, Ou & Prior, 2005). The DPPH test is based on the transfer of hydrogen, which is donated by antioxidants to DPPH free radicals. On the other hand, the reducing power assay is based on an electron transfer reaction from potassium ferricyanide (Fe$^{3+}$) to a substance with antioxidant potential (Bhalodia, Nariya, Acharya & Shukla, 2013).

Literature data shows the common use of these tests alone or in combination for the assessment of the antioxidant activity of various plants (Nowicka et al., 2019; Dinkçi, Aktaş, Akdeniz & Sirbu, 2021; Pavlović et al., 2021; Batinić et al., 2022). Results for antioxidative activity determined by the DPPH test and
reducing power for methanolic and ethanolic extracts are presented in Table 2. In our study, the sample with the highest polyphenol content (S4) showed the highest antioxidant activity measured by the DPPH test and reducing power test (Table 2). However, this sample did not contain the highest percentage of fruit preparation (Table 1). On the other hand, the fruit yogurt with the lowest amount of fruit preparation contained fewer polyphenols than other samples and had the lowest DPPH value (Tables 1 and 2). The control sample (plain yogurts) had lower values for DPPH (2.84±0.11%) and reducing power (0.07±0.02). There were statistically significant correlations between total polyphenols and DPPH in both methanolic ($r^2 = 0.9128$; $p = 0.03$) and 80% ethanolic ($r^2 = 0.9537$; $p = 0.01$) extracts. On the other hand, they were not demonstrated for reducing power assay regarding both methanolic ($r^2 = -0.1161$; $p = 0.8$) and ethanolic extracts ($r^2 = -0.1234$; $p = 0.8$). However, the main limitation of our study is the small number of analyzed samples. In contrast, Nowicka et al. (2019) reported a low correlation between total polyphenols and DPPH ($r^2=0.038$), but higher for the ABTS test ($r^2=0.444$) in 90 different strawberry cultivars.

Numerous literature data point out that the effects of strawberry polyphenols’ dietary intake were dose-dependent (Alvarez-Suarez et al., 2011; Basu et al., 2016; Huang et al., 2022). Huang et al. (2022) reported that strawberry polyphenols (flavonols, flavanols and phenolic acid), in a dose from 16 to 64 µg GAE/mL, decreased NO production and the expression of proinflammatory proteins iNOS and c-FOS in RAW 264.7 macrophage treated with lipopolysaccharides. Therefore, fruit yogurts containing strawberry polyphenols could be potentially used as part of an anti-inflammatory diet, depending on the level of certain polyphenols, but further human intervention studies are necessary. Additionally, strawberry polyphenols and anthocyanins dose-dependently weakened gastric lesions in rats (Alvarez-Suarez et al., 2011). A human intervention study reported that a high daily dose of strawberries (50 g) elevated whole blood glutathione in obese subjects compared to a daily intake of 25 g of strawberries (Basu et al., 2016). Thus, the content of fruit preparation and polyphenols in commercial yogurts on the Serbian market could be of critical importance for their health effects.

There are not much literature data about commercial fruit yogurts considering their total polyphenols content and antioxidant activity. Pereira, Barros and Ferreira (2013) investigated 14 samples of yogurts available in Portugal, but there were not any only strawberry-added. One of the analyzed samples in their study contained 6% of strawberry preparation, which is lower compared to our samples but it was labeled as “strawberry-kiwi” yogurt (Pereira et al., 2013). However, the DPPH and reduction power values from ethanolic extract were presented in different units than ours disabling direct comparison of the results. Also, Moldovan, Iasko and David (2016) investigated total polyphenols and the antioxidant activity in methanolic extracts of 12 commercial yogurts available in Romania, among which three were “strawberry yogurts”, which contained about 400-500 µg GAE/ mL (approximately 40 mg GAE/100 g), which are far higher values compared to our results. So, our data may represent the first and most valuable information for consumers about the polyphenolic content and antioxidant activity of fruit yogurts with strawberry preparations available on the Serbian market.

CONCLUSIONS

Plain yogurts available in Serbian markets, either in plastic or Tetra Pak containers, did not differ in lactate content. Additionally, there was no difference in lactate content comparing strawberry-added and plain yogurts. Finally, statistically significant differences regarding the content of total polyphenols and antioxidant activities of fruit yogurts with strawberry preparations were observed. It can be suggested that labeling fruit-added yogurts for the content of polyphenols may be a useful strategy to better describe and provide far better information to consumers regarding antioxidant activity and consequently potential health benefits.

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SADRŽAJ LAKTATA, UKUPNIH POLIFENOLA I ANTIOKSIDATIVNA AKTIVNOST ODABRANIH KOMERCIJALNIH JOGURTA SA DOMAĆEG TRŽIŠTA


Univerzitet u Beogradu, Institut za medicinska istraživanja, Centar izuzetnih vrednosti u oblasti istraživanja ishrane i metabolizma, 11129 Beograd, Dr Subotića 4, Srbija

Sažetak: Jogurt je jedan od najpopularnijih mlečnih proizvoda širom sveta, čiji je ukus pod velikim uticajem sadržaja mlečne kiseline. Jogurti sa voćnim preradevinama sadrže polifenole, sekundarne biljne metabolite sa dokazanim antioksidativnim svojstvima. Kako ne postoje podaci o količini laktata i sadržaju polifenola u komercijalnim jogurtima u Srbiji, cilj ovog istraživanja bio je da se utvrdi nivo laktata i polifenola, kao i antioksidativna aktivnost u odabranim jogurtima. Analizirano je 15 običnih i 5 voćnih jogurtova sa preradevinama od jagoda. Sadržaj laktata bio je od 0,83 do 1,33%. Tri obična jogurtova u plastičnoj ambalaži su se razlikovala po sadržaju laktata. Nije bilo razlike u sadržaju laktata među istim običnim jogurtima upakovanim u plastične i Tetra Pack kontejnere. Voćni jogurt sa preradevinama od jagoda razlikovali su se u ukupnom sadržaju polifenola koji se kretao u rasponu od 6,84 do 29,11 mg GAE/100 g i po antioksidativnim svojstvima utvrđenim testom redukcije moći sa Fe³⁺ jonima (od 0,22 do 0,79) i DPPH testom (od 28,13±1,93 do 87,23±0,15%), dok nije bilo razlike u pogledu laktata. Naši rezultati dali su nove informacije o nivoima laktata, ukupnih polifenola i antioksidativne aktivnosti odabranih komercijalnih jogurta dostupnih na domaćem tržištu.

Ključne reči: obični jogurt, voćni jogurt, jagoda, pakovanje

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