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Dedicated to Professor Luminița Silaghi-Dumitrescu on the occasion of her 65th anniversary

ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF SOME COMMERCIAL FRUIT- FLAVOURED YOGURTS

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ABSTRACT. There is currently an increasing interest in developing functional foods and in the use of natural food antioxidants as health promoting additives. The purpose of our study was to compare the antioxidant potential and the total phenolic content of 12 commercial available fruit flavoured yogurts from three different producers present on the Romanian diary market. The free radical scavenging capacity was evaluated using the 2, 2-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) assay and the total phenolic content (TPC) was determined by Folin-Ciocalteu method. The TPC of the investigated samples varied between 362.3 and 926.7 µg gallic acid equivalents/mL yogurt and the antioxidant activity (AA) was in the range of 197.1÷ 653.8 µM Trolox. A positive linear correlation between the antioxidant activity and the total phenolic content (R^2 = 0.915; 0.912; 0.687) was established for the yogurt samples.

Keywords: *fruit-flavoured yogurt, polyphenols, antioxidant activity*

INTRODUCTION

Fermented foods have been consumed since ancient times. Fermentation was used to improve the shelf-life of some foods. The most famous examples of consumed fermented products are bread, cheese and wine [1]. Beside these, yogurt has also an important place, being one of the most popular traditionally consumed fermented foods, firstly consumed in the Middle Asia and nowadays widely spread in the whole world. Yogurt is a basic diary product, produced by some thermophilic lactic acid generating bacteria such

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as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus* during the slow fermentation of milk lactose [2]. Compared to milk, yogurt has increased nutritional properties being a good source of proteins, vitamins (thiamine, riboflavin, niacin, nicotinic acid, folic acid, ascorbic acid), minerals and microelements (calcium, phosphorus, zinc, magnesium) [3]. Yogurt is considered a healthy food being an excellent source of probiotics, energy and calcium. In addition, consumption of yogurt has proved to be beneficial to the immune system, inflammatory diseases, allergies, cancer prevention, reduction of constipation, regulation of blood pressure, weight control, obesity, decrease of cholesterol absorption and many others [4-6]. Moreover, lactose intolerant people can consume yogurt since during fermentation milk lactose is converted to lactic acid [7].

Yogurt is often flavoured or fortified to create value-added products, as yogurt's flavour gained lately an important role on consumers' demand. Flavouring of yogurt can be achieved by addition of synthetic compounds or natural ingredients such as fruit juices or fruit pulp. The enrichment with various plant derived health beneficial ingredients can improve the nutritional properties of fermented diary food products.

Strawberry is the leading fruit used in Europe for flavouring yogurt [2]. Other fruits, such as peach, apricot, apple, banana, orange, plum, cherries are also widely used to enhance the flavour of yogurt. Apart of giving different tastes to yogurt, these fruit based additives are an important source of active compounds which provide specific health benefits including antioxidant, anti-inflammatory and antimicrobial activities [8, 9]. Despite its nutritional characteristics, yogurt as well as other dairy products are extremely poor in phenolic compounds. Hence, adding fruits reach in phenolics improves the antioxidant activity of yogurt [10].

Antioxidant compounds protect the human organisms from oxidative damages of the free radicals and play an important role in prevention of some degenerative diseases. The most active antioxidants of fruits are polyphenols such as anthocyanins, flavonols, catechins and carotenoids and vitamins. Among these, phenolics have received lately an increased attention, fruits reach in these compounds being intensively used as functional ingredients in the food industry.

Despite the widely consumption of fruit flavoured yogurt and its improved nutritional properties, there are not many studies reporting the antioxidant capacity and phenolic content of these commercial available dairy products [11].

The aim of our study was to assess the antioxidant activity and the total phenolic content of 12 commercial available fruit flavoured yogurt samples. The samples were chosen in order to include fruits with powerful antioxidant capacity such as strawberry, sour cherries, wild berries and apricots and were provided by different producers.

RESULTS AND DISCUSSION

The total phenolic content (TPC) of 12 fruit flavoured yogurts was estimated using the Folin-Ciocalteu reagent. The TPC of the investigated samples varied from one producer to another and among the added fruits (Figure 1). Elevated TPC values were recorded for the wild berry and the sour cherry flavoured yogurts, the highest phenolic content being determined for the sour cherry flavoured yogurt from producer 3 (926.7 µg gallic acid equivalents/mL yogurt) while the lowest value was 362.3 µg gallic acid equivalents/mL for the apricot yogurt from producer 2. The differences in the total phenolic content might be due to the different phenolic profile of the fruits used for flavouring the yogurt as well as to the different fruit content of the investigated yogurts which varied from one producer to another (Table 1).

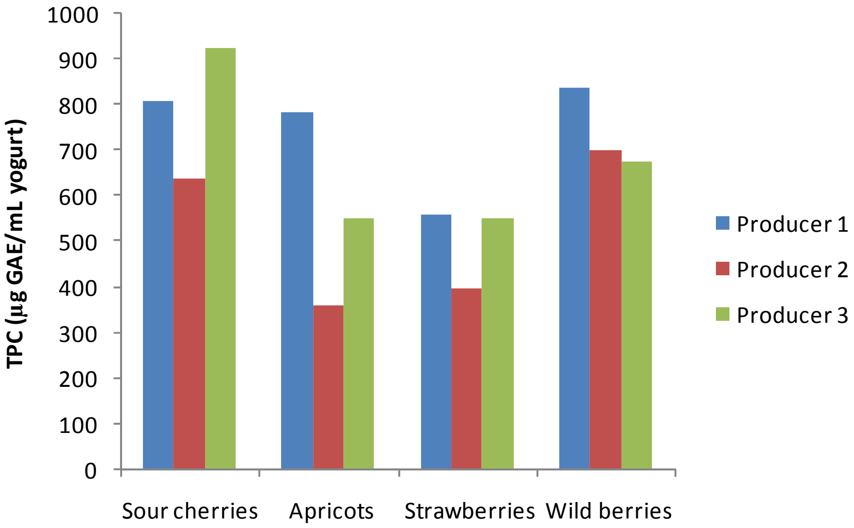

Figure 1. Total phenolic content (µg GAE/mL yogurt) of investigated yogurts

The antioxidant capacity (AA) of the 12 investigated fruit flavoured yogurts was determined using the ABTS radical cation method, based on the free radical scavenging capacity of antioxidant molecules. The total antioxidant capacity of the studied yogurts was assessed using a dose response curve for $ABTS^{+\cdot}$ as function of stock solutions of Trolox standard (concentration from 50 to 800 µM) and expressed as µM Trolox equivalents. The obtained values of AA for the investigated samples presented a large variation, ranging from 197.1 to 653.8 µM Trolox. The highest levels of the antioxidant capacity were obtained for yogurts from producer 1 (Figure 2).

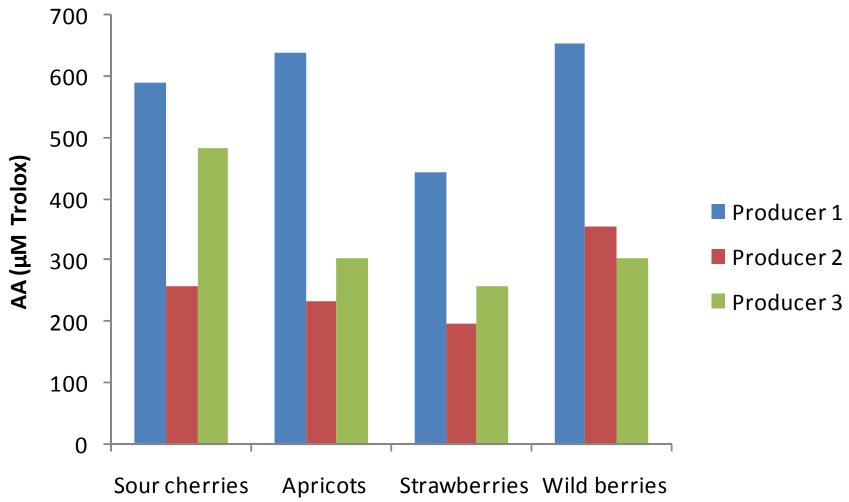

Figure 2. Antioxidant activity (µM Trolox) of investigated yogurts

Wild berries flavoured yogurt from producer 1 exhibited the highest radical scavenging capacity comparable to that of apricots flavoured yogurt from the same producer (653.8 and 639.4 µM Trolox, respectively). Comparing yogurts with added wild berries from all three producers is notable that yogurts from producers 2 and 3 presented similar antioxidant activities (355.7 and 302.8 µM Trolox, respectively) values that are almost half than that of yogurt from producer 1. Generally, the lowest levels of the antioxidant capacity of investigated samples were obtained for the yogurts from producer 2, with wild berries added yogurt as the only exception. Strawberry flavoured yogurts presented the lowest values of antioxidant activity in all the investigated cases.

By comparing the values of antioxidant activities it's important to take into account the free radical scavenging capacity of the contained fruits in the analyzed yogurts. It's well known that wild berries posses a stronger antioxidant activity than cultivated strawberries or apricots [12, 13]. The literature studies on the antioxidant activity of commercial fruit flavoured yogurts are poor and the reported data were obtained by applying different assays such as DPPH scavenging method, β-carotene bleaching inhibition and ferric reducing antioxidant power assay [11]. There are no reported data regarding evaluation of the antioxidant activity of commercial fruit yogurts using the ABTS assay.

A positive linear correlation between the antioxidant capacity and total phenolic content of the investigated yogurts was established (Figure 3).

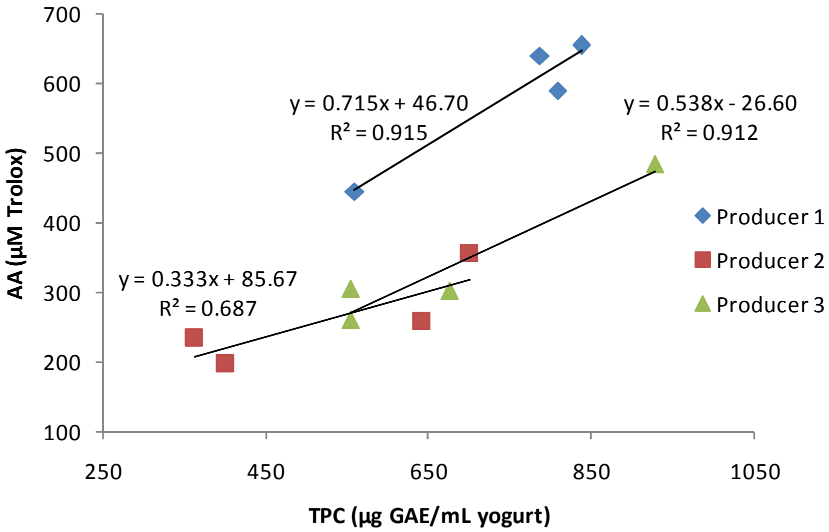

Figure 3. Correlation between total phenolic content and antioxidant activity of investigated yogurts

The high linear correlation coefficients R^2 (0.915 for yogurts of producer 1 and 0.912 for yogurts of producer 3) clearly indicated that the phenolic compounds from the added fruits significantly contributed to the antioxidant capacity of the derived dairy products. The results are consistent with those reported by other research groups [14, 15]. The poor correlation between the two investigated parameters of yogurts from producer 2 (R^2 = 0.687) may be explained by the fact that the total phenolic content does not necessarily incorporate all the antioxidants present in the samples. Some antioxidants (such as citric acid) are added in the fermented dairy products of producer 2 as labelled by producer.

CONCLUSIONS

The present study delivers key information on the total phenolic content and antioxidant capacity of some commercial available fruit flavoured yogurts, less investigated until now. Wild berry yogurts showed the highest AA value, while the highest TPC was recorded for sour cherry flavored yogurt. The radical scavenging capacity decreased in the order: wild berry > apricots > sour cherry > strawberry. Addition of fruits to yogurt enhances its nutritional properties due to their high antioxidant content.

EXPERIMENTAL SECTION

Yogurt samples

Twelve fruit flavoured yogurts were purchased from a local market from Cluj-Napoca, Romania. The samples were selected taking into account the addition of fruits with high antioxidant capacity. The composition of the chosen yogurt samples is given in Table 1. All samples were kept refrigerated at 2°C and analyzed at 24 h after purchase.

Table 1. Composition of the investigated yogurts as labeled by producer

Producer	Type of yogurt	Composition
1	**Sour cherries**	Pasteurized milk, sugar, sour cherry 2%, glucose-fructose syrup, colors (aronia and carrot juice), flavor, milk proteins, corn modified starch, thickener (pectin), lactic ferments
	Apricots	Pasteurized milk, sugar, apricot 2%, colors (carrot and red pepper extracts, β-carotene), flavor, milk proteins, corn modified starch, thickener (pectin), lactic ferments
	Strawberries	Pasteurized milk, sugar, strawberry 2%, glucose-fructose syrup, colors (red beet and carrot juice, β-carotene), flavor, milk proteins, corn modified starch, thickener (pectin), lactic ferments
	Wild berries	Pasteurized milk, sugar, wild berries 2.3% (blackberry, strawberry, raspberry, blueberry, black elderberry), colors (black carrot and red beet concentrated juice, hibiscus), flavor, milk proteins, corn modified starch, thickener (pectin), lactic ferments
2	**Sour cherries**	Pasteurized milk, sugar, sour cherry 2% (sour cherry pulp, sour cherry concentrated juice, water, modified starch, stabilizer: pectin, acidifying agent: citric acid), color (carrot concentrated juice), milk proteins, lactic ferments
	Apricots	Pasteurized milk, sugar, apricot 2% (apricot pulp, apricot concentrated juice, water, modified starch, flavor, stabilizer: pectin, acidifying agent: citric acid), colors (carrot concentrated juice, β-carotene), milk proteins, lactic ferments
	Strawberries	Pasteurized milk, sugar, strawberry 2% (strawberry pulp, strawberry concentrated puree, water, modified starch, stabilizer: pectin, flavor, acidifying agent: citric acid), colors (carrot concentrated juice, β-carotene), milk proteins, lactic ferments
	Wild berries	Pasteurized milk, sugar, wild berries 2% (black currant, blackberry, raspberry and strawberry concentrated juice, water, modified starch, stabilizer: pectin, flavor, acidifying agent: citric acid), colors (aronia, grapes and carrot concentrated juice), milk proteins, lactic ferments

Producer	Type of yogurt	Composition
3	**Sour cherries**	Semi-skimmed yogurt, sugar, sour cherry 3.7%, glucose-fructose syrup, color (carrot concentrated juice), flavor
	Apricots	Semi-skimmed yogurt, sugar, apricot 3.7%, glucose-fructose syrup, color (pumpkin and apple concentrate), flavor
	Strawberries	Semi-skimmed yogurt, sugar, strawberry 3.7%, glucose-fructose syrup, color (carrot concentrated juice), flavor
	Wild berries	Semi-skimmed yogurt, sugar, wild berries concentrated juice (raspberry 2.3%, strawberry 1.8%, blackcurrant 0.7%, red currant 0.5%), glucose-fructose syrup, color (carmin), flavor

Chemicals and reagents

All chemicals and reagents were purchased from Merck (Darmstadt, Germany), were of analytical grade and were used without further purification. A TYPDP1500 Water distiller (Techosklo LTD, Držkov, Czech Republic) was used to obtain the distilled water.

Extraction procedure

A volume of 10 mL yogurt was stirred with 10 mL ethanol:water mixture (60:40) at room temperature for 30 minutes. The resulting mixture was centrifuged at 5000 rpm for 15 minutes in a Hettig 1004 EBA 21 centrifuge (Germany). The collected supernatant was stored at 2°C and further used to evaluate the total phenolic content and antioxidant activity of the samples.

Determination of total phenolic content

The TPC was determined by the method of Singleton [16] using the Folin-Ciocalteu reagent.

Yogurt extract (0.3 mL) was mixed with 0.2 N Folin-Ciocalteu reagent (1.5 mL). After 5 min, 1.2 mL of 0.7N Na_2CO_3 solution were added. The mixture was incubated at room temperature for 2 hours and then the absorbance was measured at 765 nm, using an UV-VIS Perkin Elmer Lambda 25 double beam spectrophotometer against a blank sample as reference. Quantitative determinations were performed based on a five points standard calibration curve (10; 25; 50; 75; 100 µg/mL) of gallic acid in 80% methanol. The results were expressed as µg galic acid equivalents (GAE)/mL yogurt.

Determination of antioxidant capacity

The $ABTS^{\cdot+}$ method of Re et al. [17], slightly modified was used to determine the antioxidant activity of the examined yogurts. 360 mg of ABTS were dissolved in 100 mL distilled water. The ABTS radical cation was generated by mixing ABTS solution (7mM) and of potassium persulfate solution (2.45 mM) in equal volumes. The resulting solution was kept in the dark for 24 h. To 6 mL of diluted $ABTS^{\cdot+}$ solution (absorbance around 0.8), 0.1 mL yogurt extract were added. After 15 minutes, the decrease of the absorbance of $ABTS^{\cdot+}$ was monitored at 734 nm. A calibration curve of the standard -Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (50-800 µM Trolox), was used to determine the antioxidant capacity of the samples. The AA was expressed in µM Trolox equivalents.

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