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SHORT COMMUNICATION

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF YOGHURT WITH GOJI BERRIES (*LYCIUM BARBARUM*)

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Abstract: The inclusion of fresh or processed fruit in yoghurt is a popular method of increasing the phenolic content of the product and improving its antioxidant capacity. This study shows that goji berries can be an alternative method of enriching dairy products with biologically active substances. Enriched dairy products with goji berries have a higher amount of polyphenol compounds and a higher antioxidant capacity compared to the control sample. Enriching dairy products (yoghurt) with natural antioxidants does not cause significant changes in the active acidity (*p*H) of yoghurt and its nutritional value. Enriched yoghurts with dried berries have a higher phenolic content and a higher antioxidant activity (63.30 ± 1.42 %) than the control (10.57 ± 0.13 %). The high antioxidant activity is due to the radical-absorbing effect of the bioactive compounds found in the enriched yoghurt. Hence, berry berries can be used to enrich dairy products (yoghurt) with biologically active substances and to improve their antioxidant properties. For the food industry, these conclusions may have an important role for yoghurt producers to develop an effective strategy for production of milk with high antioxidant capacity.

Keywords: *antioxidant activity, goji berry, total phenolic content, yoghurt*

INTRODUCTION

Yoghurt is a lactic acid product in which lactic acid fermentation takes place under the action of *Lactobacterium bulgaricum* and *Streptococcus thermophilus*. Lactic acid produced as a result of lactose degradation through the activity of lactic acid bacteria, destabilizes the casein-calcium phosphate complex. Under the action of the lactic acid the casein of milk together with the denatured whey proteins coagulates and forms a coagulum [1].

Yoghurt is rich in proteins, carbohydrates, amino acids and more. A number of dieticians and doctors recommend it for bone fractures and luxuries because of the pure calcium ions contained in it. The literature, however, states that dairy products have a limited content of bioactive compounds which may reduce its biological value. Recently, there has been increasing interest in fresh or dried fruits, herbs, vegetables, and other plant materials rich in phenols that can be added to dairy products in order to delay the oxidative degradation of lipids. This improves the quality and nutritional value of enriched dairy products [2, 3].

The inclusion of fresh or processed fruit in yoghurt is a popular method of increasing the phenolic content of the product and improving its antioxidant capacity [4, 5].

Goji berries fruits contain large amounts of antioxidants, carotenoids, vitamin A and zeaxanthin, rich in vitamins B, C and polysaccharides. Fruits have high protein content (10 %) and provide 18 different amino acids, of which 8 are indispensable. Also, goji berry contains components rich in glutathione, which are among the most effective antioxidants, aiding the proper functioning of cells in the human body [6, 7].

Some researchers [8] have shown that the consumption of edible flowers forms a new trend in human nutrition in Bulgaria. They can be used as potentially rich resources of natural antioxidants and colorants in functional foods that help prevent and treat diseases caused by oxidative stress.

The purpose of the present study is to obtain yoghurt with a high antioxidant capacity by adding dried fruit of *Lycium barbarum*.

MATERIALS AND METHODS

Materials

For the preparation of yoghurt with and without the addition of dried goji berries, cow's milk obtained from the region of Yambol, Bulgaria has been used with constant composition and properties (fat content - 3.6 %, active acidity *p*H - 6.6, dry non-fat bacteria (*Lactobacillus delbreukii ssp. bulgaricus, Streptococcus thermophilus*) for direct inoculation of Lactina Ltd, Bulgaria with lactic acid bacteria of more than 9.5×10^9 CFU·g^{-1}.

Dry fruit of *Lycium barbarum* purchased commercially was used. Before the application, the fruits were washed, dried and ground to 30-50 mm and added to milk at 2, 4 and 6 %.

Methods

Obtaining yoghurt without and with goji berries

After grading, taking and filtering, the milk is pasteurized at 70 - 72 °C for 25 minutes. The pasteurized milk is cooled to a temperature of 44 - 45 °C and is mixed with a lyophilized starter culture (*Lactobacillus delbreukii* ssp. *bulgaricus, Streptococcus thermophilus*). The amount of starter is 3 % of the weight of the milk. The inoculated milk is thermostated at 42 - 45 °C for 3.5 - 4.0 hours. When *p*H 4.6 - 4.7 is reached, the coagulated milk is homogenized (stirring), adding the fruits of goji berries in quantities of 2, 4 and 6 % (samples S1, S2, S3) and cooled to 18 - 20 °C per hour and a half, and then stored at 0 - 4 °C.

Chemical methods of analysis

Analysis of dry goji berries with regard to: Total phenolic content - the total phenol content is measured with a Folin-Ciocalteu reagent according to the procedure described by [9] in Gallic acid (GAE) per 100 g. Antioxidant activity - The assayed sample (150 µL) is mixed with 2850 µL of freshly prepared DPPH solution (0.1 mM in ethanol). The reaction was run for 15 min at 37 °C in the dark and the absorption measured at 517 nm. The antioxidant activity was calculated against a standard strain constructed with Trolox concentrations of 0.05 to 1.0 mM. The results are presented as mM Trolox equivalents (TE) per g dry extract [10].

Yoghurt studies in relation to - yoghurt without goji berries (control K); yoghurt with 2 % goji berries (S1); yoghurt with 4 % of goji berries (S2): yoghurt with 6 % of goji berries (S3). Active acidity, *p*H - potentiometrically, by *p*H meter (Model MS 2011, Microsyst, Plovdiv, Bulgaria) equipped with an electrode (*p*H electrode Sensorex, Garden Grove, CA, USA). Total phenolic content - The total phenol content of the yoghurt compound enriched with goji berries is determined by the Folin-Ciocalteu method by measuring the absorption at 715 nm in a UV-VIS 16 [11]. DPPH Radial Activity (RSA), the % - radiate activity of yoghurt enriched with goji berries was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method by measuring the absorption at 517 nm in a spectrophotometer [12].

All of the results are considered significant at a level of $P \leq 0.05$.

All used reagents and solvents were of analytical grade scale. All solvents as well as DPPH (1,1-diphenyl-2-picrylhydrazyl) reagent, a Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Steinheim, Germany).

RESULTS AND DISCUSSION

Table 1 shows the total phenolic content and antioxidant activity of the dry goji berries used in the experiment. The total phenol content is estimated in aqueous and methanol extracts of goji berry. From the results obtained, it is evident that the higher the content of common phenols in the methanol extracts than in the aqueous ones. The content of common phenols in methanol extracts (535.4 mg GAE / 100 g) is higher than found in literature data - 259.5 ± 23.25 mg GAE / 100 g [2]. Other authors, such as Mocan A. et

al. 2014 [10], indicate that the total phenol content of goji berries can also reach 6159.0 ± 54.12 mg GAE / 100 g.
The antioxidant activity of berry berries is also observed, which is also higher in methanol extracts 46.4 ± 1.2 mM (TE) / g. These results correlate with the results obtained for the total phenol content. The results for the antioxidant activity of berry berries do not differ from those found in the literature [13].

Table 1. *Chemical indicators of goji berries (Lycium barbarum)*

Fruits	Total phenolic [mg GAE/100g]		DPPH [mM(TE)/g]	
	water	methanol	water	methanol
Dried goji fruits	458.1±32.87	534.4±12.71	28.5±1.3	46.4±3.42

Various fruits or fruit extracts can be added to dairy products to improve their antioxidant capacity [14]. The results obtained show that the berries are a suitable additive for increasing the antioxidant activity of the yoghurt obtained.

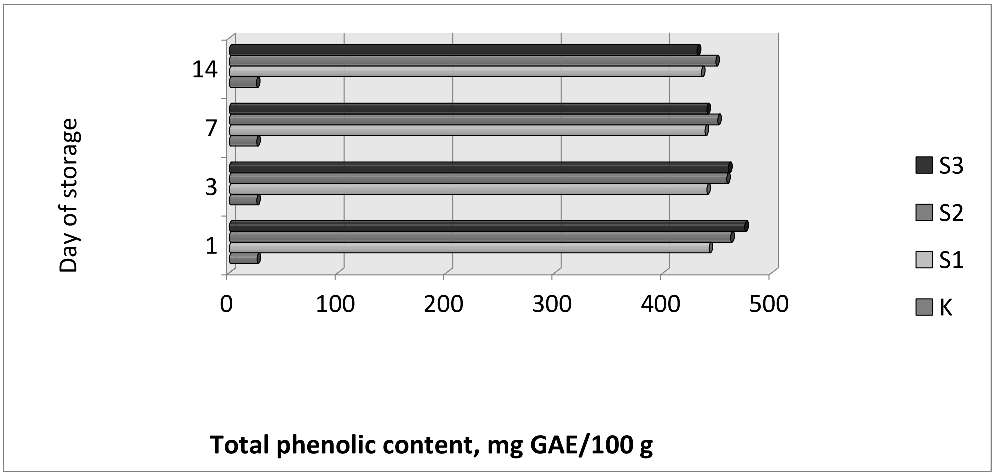

Figure 1. *Total phenol content of yoghurt, enriched with berry berries*
K-control sample, S1-2 % goji berries, S2-4 % goji berries, S3-6 % goji berries

The content of phenols in the yoghurt samples was monitored, with and without goji berries, during the storage period until the 14th day (Figure 1). The results obtained show that the total content of phenols in yoghurt samples with goji berries is higher than that of the control. The highest phenolic content was observed in sample S3 - 475.2 ± 22.5 mg GAE / 100g while at least in control - 25.5 ± 0.87 mg GAE / 100 g. During storage, the total phenolic content in each sample decreases, with the reduction being the most sensitive in sample S3 - from 475.2 ± 45.32 to 431.2 ± 23.5 mg GAE / 100 g. The lowest reduction in total phenolic content was observed in sample S2 – from 442.3 ± 12.62 to 435.2 ± 18.3 mg GAE / 100 g.
Using the DPPH method to determine the antioxidant activity of the yoghurt samples obtained, it was found that the addition of goji berries to the milk resulted in an increase in its antioxidant activity (Figure 2).
The lowest antioxidant activity was observed in a control sample of 10.57 ± 0.13 %, while in the other samples it increased, respectively, by increasing the amount of goji

berries. The yoghurt with the addition of 6 ± 0.01 % of goji berries (sample S3) showed the highest antioxidant capacity (63.30 ± 1.42 %) on the first day of storage, followed by S1 and S2 (32.25 ± 2.46 % and 42.52 ± 1.14 %). The high antioxidant activity is due to the radical absorbing effect of the bioactive compounds in the enriched yoghurt, having antioxidant properties.

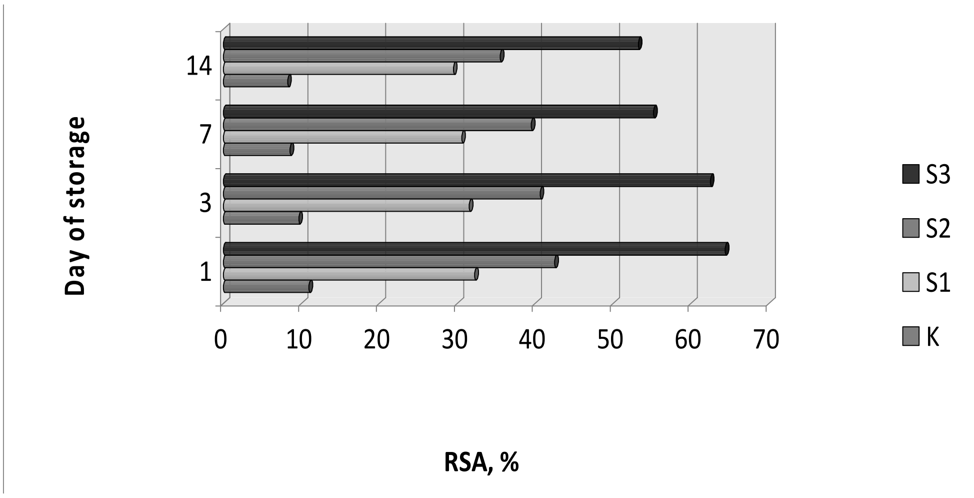

Figure 2. *The antioxidant activity of yoghurt enriched with goji berries*
K-control sample, S1-2 % goji berries, S2-4 % goji berries, S3-6 % goji berries

During sample storage, a decrease in antioxidant activity was observed, this decrease being highest for sample S3 on day 14, from 63.30 ± 3.81 % to 43.20 ± 1.24 % and lowest for sample S1, from 32.25 ± 2.26 % to 29.52 ± 1.42 %. Similar results for the reduction of antioxidant activity have been demonstrated by [15], in traditional dahi milk enriched with strawberry extract. Dahi milk is traditionally yogurt or fermented milk originating in India from buffalo or goat milk.
Figure 3 shows the active acidity (*p*H) of the yoghurt obtained without and with goji berries on the 1st, 7th and 14th day of storage. The *p*H values of the yogurt samples ranged from 4.6 ± 0.1 to 3.9 ± 0.16. Adding goji berries at the beginning of storage did not result in significant changes in *p*H values of the analyzed samples. Although some samples show fluctuations in *p*H values, an increase in acidity is observed at the end of storage. At the end of the storage period, the lowest *p*H was observed in sample S3-3.9.

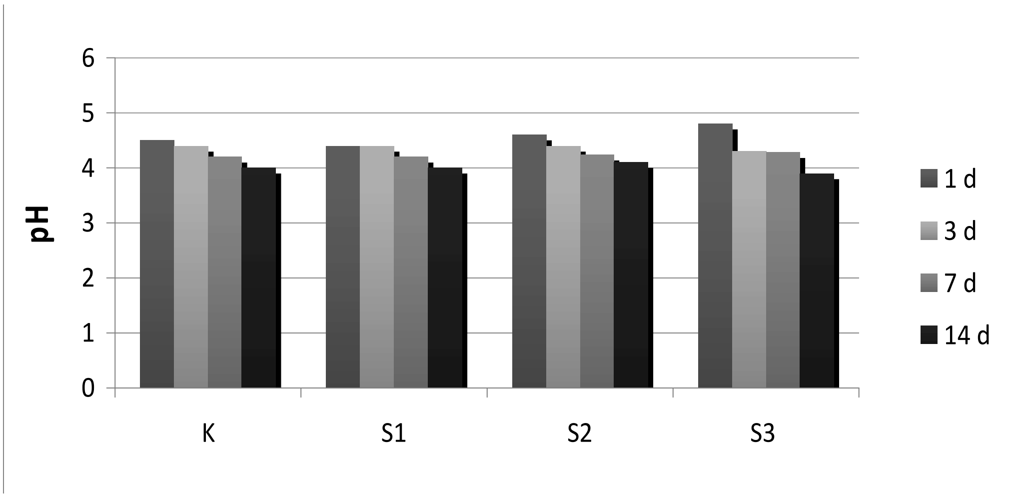

Figure 3. *Active acidity of yoghurt without and with goji berries*
K-control sample, S1-2 % goji berries, S2-4 % goji berries, S3-6 % goji berries

Tseng and Zhao [16] received similar results in the production of yogurt with added grape must.

CONCLUSIONS

This study shows that goji berries can be an alternative method of enriching dairy products with biologically active substances. Enriched dairy products with goji berries have a higher amount of polyphenol compounds and a higher antioxidant capacity compared to the control sample. During storage of the enriched milk, the total phenolic content and the antioxidant activity decrease in each sample, the reduction being the most significant in the sample with the addition of 6 % berries (sample S3). Despite the reduction in antioxidant activity in enriched milk, goji berries are a suitable additive for obtaining dairy products with acceptable flavors.
For the food industry, these findings may be important for yoghurt producers to develop an effective strategy for producing milk with high antioxidant capacity.

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