

Lactic acid beverage fortified with goji berry

Tatyana Balabanova^{1*}, Mihaela Ivanova¹, Ivan Ivanov², Milena Dimitrova¹, Maria Dushkova³, Radka Vlaseva¹

¹*Department of Milk and Dairy Technology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria*

²*Department of Organic and Inorganic Chemistry, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria*

³*Department of Department of Process Engineering, Technical Faculty, University of Food Technologies, Plovdiv, Bulgaria*

*Corresponding author: tbg_georgieva@yahoo.com

Abstract

Balabanova, T., Ivanova, M., Ivanov, I., Dimitrova, M., Dushkova, M. & Vlaseva, R. (2018). Lactic acid beverage fortified with goji berry. *Bulgarian Journal of Agricultural Science*, 24(5), 885–890

A lactic acid beverage fortified with ground goji berries has been developed. The sequence of technological operations, the chemical, microbiological, organoleptic profile and antioxidant activity of the product during cold storage have been established. No statistically significant differences ($p \leq 0.5$) characterizing the rate of the lactic acid process, acidification, total active acidity and residual lactose have been found in the test samples. The results of the microbiological analysis show that the low storage temperatures have a decisive impact on the intensity of microbial growth and correlate with acid formation. The data obtained for antioxidant activity show that the metal-reducing activity of the lactic acid beverage containing goji berries is more pronounced than the radical scavenging activity indicator.

Keywords: milk beverage; goji berry; fermentation; antioxidant activity

Introduction

In recent years, scientific interest in nutrition has been focused on developing and eating healthy foods enriched with products that have beneficial effects on the human body and might lower the risk of various diseases. Milk and dairy products are characterized by a high nutritional and biological value and are a staple food in human nutrition. The increasing interest of modern consumers in maintaining a good health status has stimulated the manufacture of a diverse range of milk-based products.

Goji berry, also known as wolfberry, is the fruit of the *Lycium chinense* and *Lycium barbarum* plants, closely related to the *Solanaceae* family. The healing properties of the goji

berry fruits come from the biologically active components they contain: polyphenols, carotenoids and polysaccharides. Goji berry fruits have been found to contain a large number of vitamins, amino acids and trace elements (Dong et al., 2012), and they are an excellent source of antioxidants (Leong and Shui, 2002). Various studies (Wong et al., 1994; Chang, 2002) have shown that the polysaccharides contained in goji berries possess immunomodulatory properties that determine their medicinal properties. The second large group of metabolites are carotenoids, the content of which increases during fruit ripening (Piao et al., 2005). Goji berries are rich in β -carotene – 19.6 mg/100 g (Chang et al., 2010) and also contain small amounts of glutamine, asparagine, stigmasterol, cholestanol, lupeol, taurine, the minerals

K, Ca, Zn, Fe, Co, Mn, Se, Mg and B₂, B₁, C vitamins (Potterat, 2010).

The aim of present study was to observe the dynamics of changes during storage in the basic physico-chemical, microbiological, organoleptic and antioxidant activity of a lactic acid beverage supplemented with ground goji berry fruits.

Material and Methods

Sample preparation

For the purposes of the experiment, the lactic acid drink was obtained from raw cow's milk. The milk was standardized to a fat content of $3.0 \pm 0.1\%$, followed by homogenization at $55 \div 60^\circ\text{C}$ and pressure 14-17 MPa, pasteurization at $93 \div 95^\circ\text{C}$ with a residence time of $15 \div 20$ min. The heat-treated milk was cooled to a temperature of $44 \div 45^\circ\text{C}$ and fermented with 2% traditional Bulgarian starter containing selected strains of lactic acid bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) obtained from Lactina Ltd. The milk was thermostated for 4-4.5 h and afterwards cooled down to 20°C . Two parallel beverages were prepared from the lactic acid product thus obtained:

– **Sample A** (control) with additions of 30.0% of water, 0.1% of stabilizer (guar gum), and 8.0% of sugar;

– **Sample B** contained the same components like sample A and 1.0% of ground berries.

After the addition of ingredients, both samples were divided into portions and stored at $1 \div 4^\circ\text{C}$ for 20 days. During the period of cold storage, the dynamics of the following indicators were tracked:

Chemical analysis

The pH of the samples was measured using a pH meter (model MS 2000, Mycosist, Plovdiv, Bulgaria) with a glass electrode (Sensorex, Garden Grove, USA) standardized at 20°C in the range 7.01–4.01.

Titrateable acidity (TA) of samples was determined by the Thorner's method (BNS 1111-80) and expressed as percent of lactic acid.

Residual lactose – Determination of residual lactose:

$$\begin{aligned} \text{Gres. lact.} &= \text{Glac.} - \text{Gdegr.lac.} \\ \text{Gdegr.lac.} &= [(\Delta\text{Tbeg.} - \Delta\text{Tcur.}) * 0,009 * 342] / 360 \quad (1) \end{aligned}$$

where:

Gres. lact – residual quantity of lactose;

Glac. – initial quantity of lactose;

Gdegr.lac. – degraded quantity of lactose;

$\Delta\text{Tbeg.}$ – beginning initial titrateable acidity;

$\Delta\text{Tcur.}$ – current titrateable acidity.

Microbiological analysis

Total lactic acid bacteria count (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*): sample preparation was conducted according to IDF Standard 122C:1996. The suitable dilutions were inoculated into selective agars M17 and MRS, as described in IDF Standard 117B:1997.

Statistical analysis

Computer processing of the results was performed using the program Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by the LSD method. The results are presented as mean values \pm SD ($n = 3$).

Antioxidant activity

Sample preparation: 4 ml of ethanol were added to 2 g of product (6 ml final volume). The sample was stirred and placed in an ultrasonic bath (45 kHz) for 10 min, then centrifuged to precipitate the proteins at 4500 rpm for 10 min. The supernatant was used to determine the antioxidant activity.

Radical scavenging activity ABTS method: the ABTS radical was prepared by mixing ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (7 mM in DH_2O) and potassium persulfate (2.45 mM in water) in equimolar amounts and kept in darkness for 16 h. Prior to analysis, 2 ml of the ABTS radical was dissolved in ethanol in a ratio of 1:30 to obtain a final adsorption of $1.0 \div 1.1$ at 734 nm. For analysis: 0.15 ml of the test extract was mixed with 2.85 ml of freshly prepared ABTS solution. The reaction mixture was incubated in the dark for 15 min at 37°C . The adsorption reduction was spectrophotometrically read at 734 nm (Petkova et al., 2012).

Metal-reducing activity CuPRAC method: The reaction was initiated by mixing 1 ml of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1 ml of Neocuproin (7.5 mM in methanol), 1 ml of 0.1M ammonium acetate buffer at pH 7.0; 0.1 ml of the test extract and 1 ml of DH_2O . The reaction mixture was incubated for 20 min at 50°C in the dark. After cooling the mixture, the absorbance of the resulting compound was read at 450 nm (Petkova et al., 2012).

Spectrophotometer: a CamSpec M107 spectrophotometer was used to measure the decrease in absorbance to determine the antioxidant activity of the lactic acid beverage at a wavelength of 734 nm for the ABTS methods and 450 nm for the CuPRAC method.

Organoleptic evaluation of fermented milks

The evaluation criteria measured the following indicators: taste and flavour – 40 points, texture – 20 points, appearance – 20 points, colour – 20 points (maximum total score – 100 points), according to BNS 15612-83.

Results and Discussion

Fig. 1 shows the change in the values of the active (pH) and total (percent of produced lactic acid) acidity during storage of the lactic acid beverages.

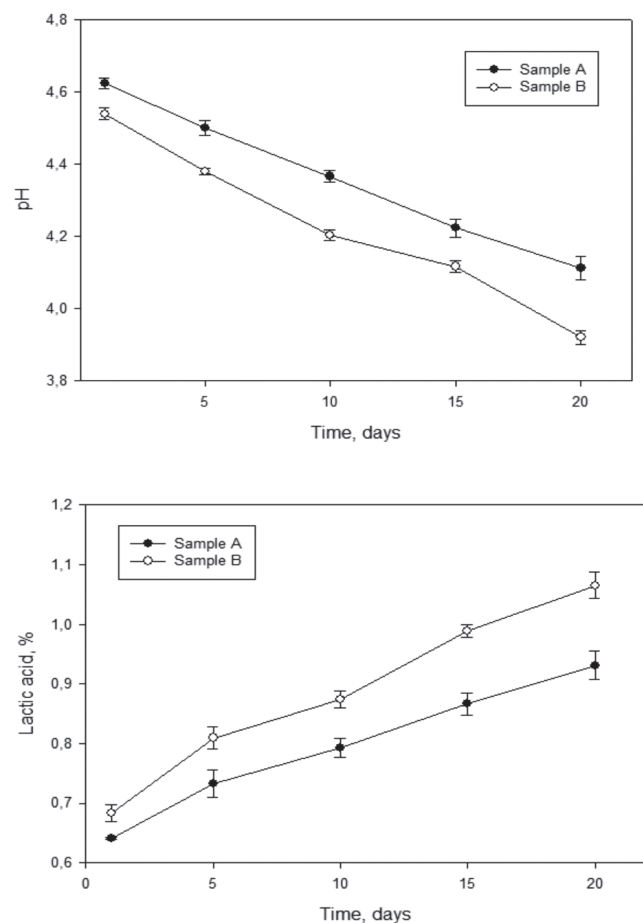


Fig. 1. Change in acidity during storage

It is obvious from the figure that the decrease in pH values in the different periods of study is more distinct in the lactic acid beverage containing goji berry, which is probably due to the added fruits in sample B. Nevertheless, the observed active acidity values were approximately 0.5 units lower for both lactic acid beverages. As a result of lactic acid fermentation of lactose, which is mainly produced by starter lactic acid bacteria, lactic acid and other organic acids accumulated. This process begins during fermentation and continues during sample storage. The results indicate that the amount of lactic acid accumulated on the first day of storage was 0.639% for sample A and 0.675% for sample B. These

results correlate with the results of Bueno et al. (2014). During storage, a steady tendency towards a gradual increase in the amount of lactic acid was observed. At the end of the study period, the total acidity indicator had increased by approximately 0.3% and 0.4% for samples A and B, respectively. The obtained data reflect the lactic acid accumulation and the related rate of post-acidification during storage in both samples tested (A and B).

During storage, the decrease in the amount of lactose directly correlated with the change in acidity in both samples. The data presented in Fig. 2 reveal a gradual change of that indicator between the 1st and 20th day of storage. The low storage temperature significantly inhibited the fermentation processes and by the end of storage this indicator had changed by about 0.3% and 0.5% on average for samples A and B, respectively.

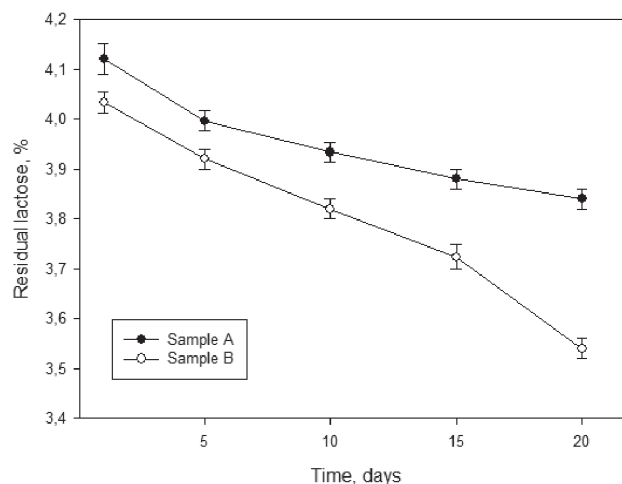


Fig. 2. Change in the amount of lactose in samples during storage

Fig. 3 shows the dynamics in the development of the lactic acid microflora during sample storage. The results obtained indicate that the total number of lactic acid bacteria at the beginning of the process was 8.5 log and 8.4 log cfu/g for samples A and B, respectively. These values suggest good development of the starter lactic acid bacteria in the previous stages of the technological process.

A small decrease in the amount of lactic acid microflora was observed in the samples during the first five days of storage. That tendency was more noticeable in the lactic acid beverage containing goji berries. A smoother change in the amount of lactic acid microflora was observed between 5 and 15 days of sample A storage, whereas for sample B a sharper decrease of about 2.0 log cfu/g was detected. This

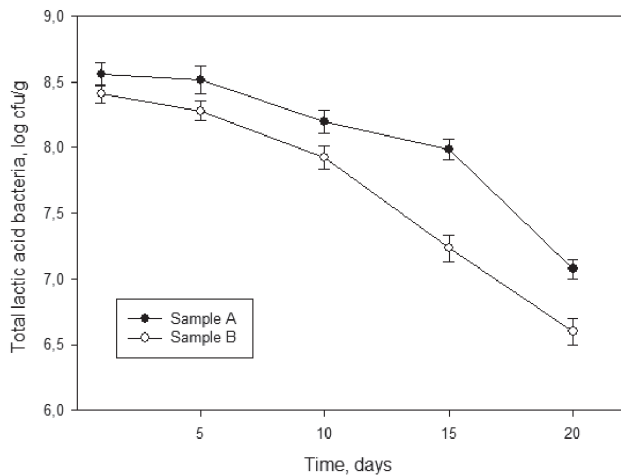


Fig. 3. Total lactic acid bacteria during storage

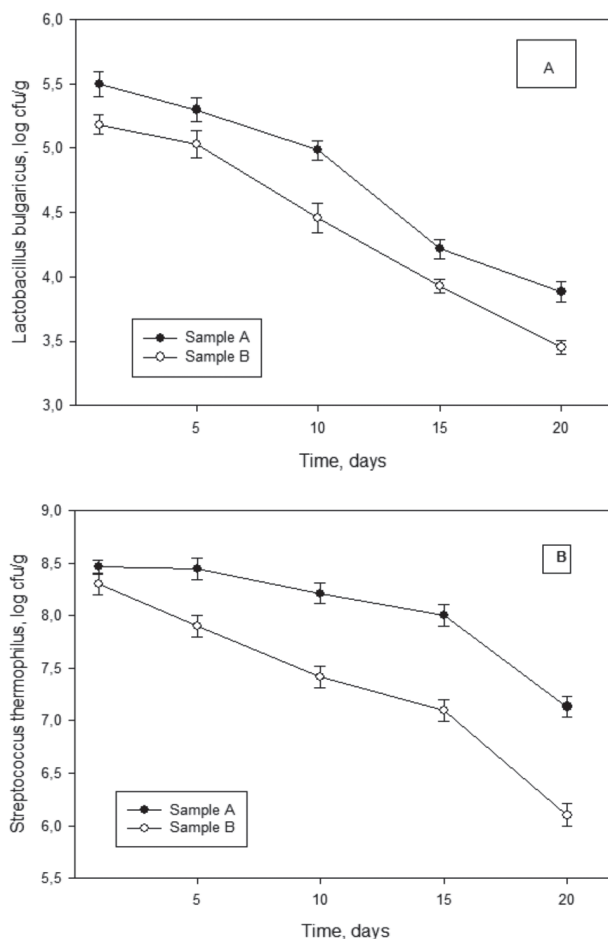


Fig. 4. Change in the total number of lactobacilli (A) and streptococci (B) during storage

tendency remained steady until the end of storage, with a higher concentration of lactic acid microflora recorded in sample A. The lower concentrations of bacterial biomass detected can be explained by the relatively more intense acid formation in sample B. Larger amounts of lactic acid inhibit the growth of lactic acid bacteria in the early stages and thus cause earlier reduction in their concentration. According to Rotar et al. (2014, 2015), after the 21st day of storage, the amount of lactic acid bacteria is in the range of 10^6 - 10^7 cfu/g.

The results about the change in the total number of lactobacilli and streptococci in the samples are presented in Fig. 4. In both experimental samples, lactic acid microflora is represented mainly by the streptococci that are present in the starter.

The change in the number of lactobacilli and streptococci in the experimental samples followed similar trends to those found for the total number of lactic acid bacteria (Fig. 3). The results (Fig. 4A) show that lactobacilli are highly sensitive to low temperatures and their number in the samples significantly declined during storage. Thus, at the end of storage, the number of lactobacilli decreased by 1.6 log for sample A, and by 1.7 log units for sample B. At the beginning of the storage process, the number of streptococci was 8.4 log and 8.2 log units for samples A and B, respectively. For the entire study period the decrease in their number was approximately 1.4 log for sample A and by 2.2 log units for sample B. Due to their higher resistance to low temperatures, lactobacilli remained the predominant lactic acid microflora until the end of storage in both experimental samples.

Antioxidant activity of a dairy food is important both for the shelf life of the product as well as for protection from oxidative damage in the human body. Grazyna et al. (2017) also reported that the high antioxidant potential of milk is determined in both lipophilic (conjugated linoleic acid, α -tocopherol, β -carotene, vitamins A and D3, coenzyme Q10, phospholipids) and hydrophilic antioxidants (proteins, peptides, vitamins, minerals and trace elements). Consumers have more concerns and recommendations to use natural antioxidants from food sources rather than synthetic antioxidants which have been restricted because of their toxic and carcinogenic effects (Alenisan et al., 2017).

Two methods of analysis to determinate the antioxidant potential were used: the radical scavenging activity ABTS method and the metal-reducing activity CuPRAC method, both methods being independent of each other. The results are given as mmol Trolox equivalents (mM TE) per 100 g of product (Table 1).

Niero et al. (2017) reported that a high total antioxidant

Table 1
Determination of antioxidant activity of sample A and B

Sample Method	Sample A		Sample B	
	ABTS mM TE/100 g	CuPRAC mM TE/100 g	ABTS mM TE/100 g	CuPRAC mM TE/100 g
Day 1	48.7 ± 0.8	60.0 ± 1.1	108.9 ± 1.0	364.3 ± 8.8
Day 5	32.7 ± 0.9	51.0 ± 5.0	93.0 ± 0.9	336.2 ± 2.2
Day 10	26.5 ± 0.3	44.6 ± 4.1	87.1 ± 0.9	313 ± 3.0
Day 15	24.1 ± 0.5	40.3 ± 4.1	82.3 ± 1.0	302.1 ± 4.1
Day 20	18.5 ± 0.3	36.1 ± 4.5	75.2 ± 0.9	298 ± 3.5

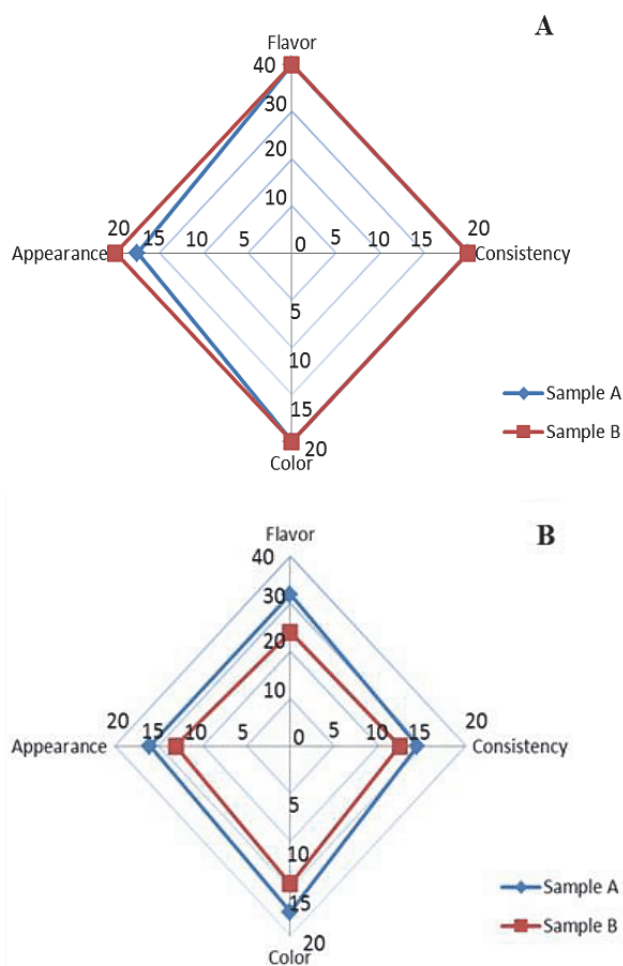


Fig. 5. Organoleptic evaluation of samples on days 1 (A) and 20 (B) of storage

activity was found for raw milk (29.31- 44.72 mM TE/L). Our result from control sample A is in accordance with Niero et al. (2017). The addition of plant-based antioxidants in dairy food has met acceptance for the retardation of oxida-

tion in dairy products. The data presented in the Table 1 show that the addition of goji berries in the lactic acid product are characterized by a more pronounced metal-reducing activity (364.3 mM TE/100g) than the radical scavenging activity (108 mM TE/100g). The results about the antioxidant activity of the lactic acid beverage during storage show significantly higher values in the samples fortified with goji berry than in the control sample. These results are also confirmed by studies by other researchers, who found that the polyphenols contained in the fruits have a high antioxidant activity (Dong et al., 2012; Alenisan, et al., 2017). For a longer storage period the antioxidant activity of the product decreased. The results obtained are in correlation with the data reported by Gutierrez et al. (2008).

The results of the organoleptic evaluation of samples were followed on days 1 and 20 of storage at 1±4°C (Fig. 5). The results obtained on the first day of storage indicate a high organoleptic score for the samples. On day 20 of storage, the control received a higher overall score. A change in taste and aroma was detected in sample B. The sensory panel perceived the acidity and specific flavour to be stronger for similar types of lactic acid beverages. Deviations were found in two other organoleptic indicators – texture and appearance. Separation of serum was observed on the surface of the beverage, which requires that it should be shaken well prior to consumption. There was also a slight change in the colour of the lactic acid beverage containing goji berry fruit.

Conclusion

The results of the study on the preparation of a lactic acid beverage fortified with ground goji berry fruit allow for the following conclusion:

There is a possibility of expanding the range of health lactic acid products with prophylactic and healing properties with an additional beneficial functional effect of goji berries on the human body.

References

- Alenisan, M. A., Alqattan, H. H., Tolbah, L. S., & Shori, A. B.** (2017). Antioxidant properties of dairy products fortified with natural additives: A review. *Journal of the Association of Arab Universities for Basic and Applied Sciences*, 24(1), 101-106.
- Bueno, L., Silva, T. M., Perina, N. P., Bogsan, C., & Oliveira, M. N.** (2014). Addition of Strawberry, Raspberry and "Pitanga" Pulps Improves the Physical Properties of Symbiotic Yoghurts. *Chemical Engineering Transactions*, 38, 499-504.
- Chang, R.** (2002). Bioactive polysaccharides from traditional Chinese medicine herbs as anticancer adjuvants. *The Journal of Alternative & Complementary Medicine*, 8(5), 559-565.
- Chang, R. C. C., Ho, Y. S., Yu, M. S., & So, K. F.** (2010). Medicinal and nutraceutical uses of wolfberry in preventing neurodegeneration in Alzheimer's disease. In: *Recent Advances on Nutrition and the Prevention of Alzheimer's Disease*, 169-185.
- Dong, J. Z., Wang, S. H., Zhu, L., & Wang, Y.** (2012). Analysis on the main active components of *Lycium barbarum* fruits and related environmental factors. *Journal of Medicinal Plants Research*, 6(12), 2276-2283.
- Grażyna, C., Hanna, C., Adam, A., & Magdalena, B. M.** (2017). Natural antioxidants in milk and dairy products. *International Journal of Dairy Technology*, 70(2):1-14.
- Gutiérrez, J., Barry-Ryan, C., & Bourke, P.** (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International journal of food microbiology*, 124(1), 91-97.
- Leong, L. P., & Shui, G.** (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, 76(1), 69-75.
- Niero, G., Penasa, M., Currò, S., Masi, A., Trentin, A. R., Cas-sandro, M., & De Marchi, M.** (2017). Development and validation of a near infrared spectrophotometric method to determine total antioxidant activity of milk. *Food Chemistry*, 220, 371-376.
- Petkova N., Vrancheva, N, Ivanov, R., Denev, I., Pavlov, P. & Alexieva, A.** (2012). Determination of biologically active substances in topinambur tubers (*Helianthus tuberosus* L.). In: 50 years FoodRDI. International Scientific-Practical Conference "Food, Technologies & Health" Proceedings Book, Plovdiv, Bulgaria, pp. 49-54 (Bg).
- Piao, M., Murata, Y., Zhu, B., Shimoishi, Y., & Tada, M.** (2005). Changes in carotenoid content and its composition during maturation of *Fructus lycii* fruits. *Japanese Journal of Food Chemistry and Safety*, 12(1), 35-39.
- Potterat, O.** (2010). Goji (*Lycium barbarum* and *L. chinense*): phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta Medica*, 76(01), 7-19.
- Rotar, A. M., Vodnar, D. C., Bunghez, F., Catunescu, G. M., Jimborean, M., & Semeniuc, C. A.** (2015). Effect of goji berries and honey on lactic acid bacteria viability and shelf life stability of yoghurt. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43(1), 196-203.
- Rotar, A. M., Semeniuc, C., Bunghez, F., Jimborean, M., & Pop, C.** (2014). Effect of different storage period on lactic acid bacterias from goji yogurt and goji yogurt with honey. *Bulletin UASVM Food Science and Technology*, 71(1), 75-76.
- Wong, C. K., Leung, K. N., Fung, K. P., & Choy, Y. M.** (1994). Immunomodulatory and anti-tumour polysaccharides from medicinal plants. *Journal of International Medical Research*, 22(6), 299-312.
- BNS 1111-80, 1980. Milk and milk products. Determination of acidity (Bg).
- BNS 15612-83, 1983. Milk and milk products. Dairy products sensory evaluation (Bg).
- IDF Standard 122C:1996. Milk and Milk Products – Preparations of Samples and dilutions for microbiological examination, IDF 122C:1996. International Dairy Federation, Brussels, Belgium.
- IDF Standard 117B:1997. Milk and Milk Products. Yogurt – Enumeration of characteristic microorganisms – Colony-count technique at 37 degrees C (*Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*) are present and viable. International Dairy Federation, Brussels, Belgium.