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Address of Editorial office:

Agricultural Science and Technology
Faculty of Agriculture, Trakia University
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Telephone.: +359 42 699330
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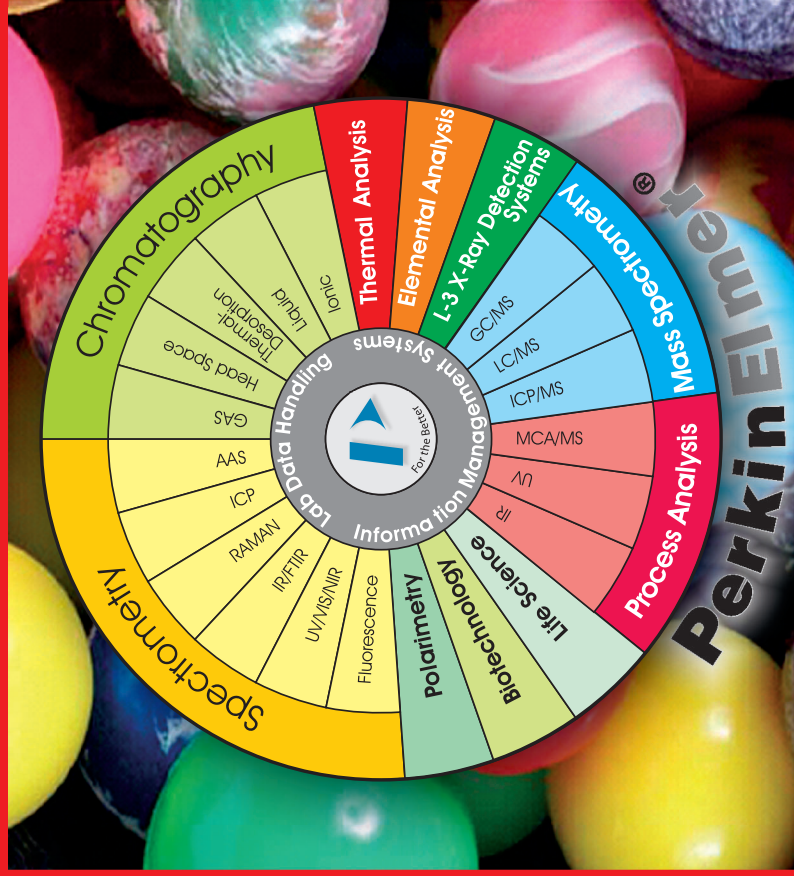
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Quality and Safety

Quality characteristics of yogurt supplemented with nuts

S. Boycheva¹, N. Naydenova^{2*}, G. Mihaylova², T. Dimitrov², D. Pavlov³

¹Department of Biochemistry and Microbiology, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

²Department of Dairy Science, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

³Department of Crop Science, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract. The aim of the present study was to investigate the effects of supplements of pieces of walnuts without and with honey and hazelnuts on the count of lactic acid bacteria, dynamic of acidity, and the syneresis of yogurt. It was prepared yogurt from cow's milk, supplemented by pieces of walnuts, without and with honey and hazelnuts. Our experimental results show that supplements reduced the time for coagulation with 2.3 to 15.7%, and slightly increased the titratable acidity of yogurt during the storage. All supplements lead to increasing of extracted milk serum and syneresis index during the storage. The highest effect was obtained from walnut + honey supplementation. Enrichment of milk with plant biologically active substances has positive effect on the microbial activity. Total count of lactic acid bacteria increased significantly at coagulum, almost twice - with the supplementation of walnut and hazelnut, and almost three times - with walnut plus honey.

Keywords: yogurt, lactic acid bacteria, titratable acidity, nuts

Abbreviations: LAB – lactic acid bacteria, SCC – somatic cell count, CFU – colony forming units

Introduction

Functional foods are new direction in the science with a big importance recently for development of human healthy nutrition. There are many aspects of functional foods and as a consequence term it has no exact definition. It is usually described as "food which promotes health beyond providing basic nutrition" (Rogelj, 2000).

Yogurt is a traditional food in many countries and especially in Bulgaria. The healing properties of fermented milk products, including yogurt are determined by live lactic acid bacteria (LAB). They introduced in human intestines through fermented milk products as yogurt which has many nutritional and therapeutic benefits to the consumers. LAB stimulates immune system (Meydani and Ha, 2000; Oyetayo and Oyetayo, 2005; Denev, 1996, 2000, 2006) by enhancing antibody content (Zeuthen et al., 2006). Vitamins and enzymes produced by LAB contribute to host metabolism. Their antimicrobial substances control the proliferation of undesired pathogens in the intestinal ecosystem (Chomakov and Boycheva, 1993; Boycheva, 1997; Denev, 2007). LAB possess antimutagenic, antitoxic, antitumor, hypocholesterolemic effects, and other health and nutritional benefits (Fernandes et al., 1987; Rafter, 2002; Denev, 1996, 2000, 2006; Parvez et al., 2006; Andrade and Borges, 2009).

Milk products are basic product that can be enriched with different bioactive ingredients. The presence of water and lipids in them allows the involvement of water- and fat-soluble nutrients that increase optimal physiological effect in the body.

Nuts are natural food that can supply the body with some important components. They are an excellent source of protein and can be used as an alternative to meat, especially as vegetarian

meals. They are particularly rich in natural antioxidant nutrients, including ascorbate (vitamin C) and the tocopherols (vitamin E). A sufficient supply with antioxidants from diet might help prevent or delay the occurrence of pathological changes associated with oxidative stress (Giugliano, 2000). Another valuable component of nuts is folic acid, which is necessary for cell division and formation of erythrocytes. Folic acid has been shown to prevent birth defects (Wardlaw, 1999). Nuts are a great source of some microelements such as copper, magnesium, manganese, selenium and zinc that are rarely found in other food products.

The aim of the present work was to study the effects of supplements of pieces of walnuts without and with honey and hazelnuts on the count of lactic acid bacteria, dynamic of acidity, and the syneresis of yogurt.

Materials and methods

Fresh milk and starter culture

Fresh cow's milk obtained from the experimental base at Trakia University and starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* ready for direct inoculation were used for yogurt preparation.

Yogurt preparation

The cow's yogurt was prepared in laboratory conditions. The milk was pasteurized (95 °C/30 min), cooled to 45°C and inoculated with 1.5% yogurt starter, consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. The raw milk was divided into four lots described as variants of the experiment as

* e-mail: lambev_iremka@abv.bg

follows: 1. Control (without supplementation); 2. Walnut pieces (10%); 3. Hazelnut pieces (10%); 4. Walnut pieces (10%) + honey (5%). Supplements were incorporated prior to adding the yogurt starter culture. The samples were then cultivated at 42°C until coagulation, then cooled and stored in a refrigerator at 4-6°C.

Physicochemical analyses

The titratable acidity of milk was determined at the 1st and the 2nd h after milk culturing, at the time of coagulation, and after storage in a refrigerator for 24 h, and 10 days respectively, by titration with 0.1N sodium hydroxide (AOAC, 1990). The syneresis of yogurt was studied immediately after the coagulation and after storage of milk at 4-6°C for 24 h and 10 days respectively (Shidlovskaya, 1979).

Optical microscopic analyses

The counts of LAB (*L. delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) in coagulated milk were enumerated by direct counting on microscopic preparations using the method of Breed (A.P.H.A., 1916). The preparations were made from 1:10 dilutions immediately after the coagulation of milk and after storage at 4-6°C for 24 h, and 10 days respectively. The counts of lactic acid rods and cocci were determined individually in 50 observation fields.

Table 1. Composition and properties of raw milk (n=5)

Indexes	$\bar{x}$	S $\bar{x}$
Dry matter, %	13.48	0.124
Fat, %	4.66	0.059
Solids non fat, %	8.82	0.038
Protein, %	3.52	0.046
Lactose, %	4.7	0.032
Density	1.0288	0.0004
Titrable acidity, °T	16.70	0.239

Statistical analysis

For statistical analysis, ANOVA was performed on all the batches and the corresponding replications, using statistical software (Statistica 6.0).

Results and discussion

Fresh milk quality

The quality of yogurt depends on the quality of the fresh cow's milk. In relation to the basic components – dry matter, protein, fat, lactose, and solids non fat the cow's milk used for producing of yogurt in this experiment has a high quality concerning to the requirements of the Bulgarian regulations (Table 1). The total bacterial count and the somatic cell count (SCC) are important quality indices by which can be estimated milk hygiene, the health of lactating animals and the suitability of cow's milk for production of milk products, including yogurt.

The used raw milk is a good quality regarding to the requirements of the Bulgarian state standard (Table 2). The obtained results for total bacterial count, LAB, coliforms and psychrotrophs correspond to these of other authors (Dimitrov et al., 2002; Chomakov and Boycheva, 1986). The count of bacteria after pasteurization was highly reduced and the milk was used as high quality base for yogurt concerning to the requirements of Bulgarian state standard for pasteurized cow's milk.

Table 2. Microflora of cow's milk (cfu*/cm³)

Indexes	Raw milk (n=5)	Pasteurized milk (n=5)
Total bacterial count	3 x 10 ⁴	7.25 x 10 ²
Lactic acid bacteria	3 x 10 ⁵	1.7 x 10 ³
Coliforms	6 x 10 ²	0
Psychrotrophs	-	1.1 x 10 ³

* CFU - colony forming units

Titritable acidity

During the first hour of culturing, the titratable acidity of milks supplemented with pieces of walnuts-10%, hazelnuts-10%, and walnuts- 10% + honey 5%, was higher than that of the control (Table 3). The raise in acidity in the sample with walnut pieces and hazelnut pieces was 20.7% and 22% respectively. By the 2nd hour this trend

Table 3. Acidity dynamics of yogurt supplemented with nuts and honey (n=5)

Indexes	Supplements			
	Control	10% walnut pieces	10% walnut pieces and 5% honey	10% hazelnut pieces
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Initial acidity, °T	16.7 ± 0.239	16.7 ± 16.7	0.239 ± 0.239	16.7 ± 0.239
Acidity at the 1 st h of culturing, °T	23.7 ± 2.766	28.6 ± 28.9	7.972 ± 2.071	26.8 ± 2.363
Acidity at the 2 nd h of culturing, °T	35.0 ± 3.221	43.9 ± 45.0	6.598 ± 5.102	43.9 ± 4.029
Acidity at coagulation, °T	59.4 ± 4.989	61.9 ± 56.5	3.303 ± 11.304	47.4 ± 2.596
Acidity after 24 h	81.5 ± 3.340	88.5 ± 77.6	5.246 ± 5.986	73.3 ± 4.800
Acidity after 10 days	105.1 ± 8.498	106.7 ± 109.7	10.459 ± 6.001	97.9 ± 5.765
Time for coagulation, min	153.0 ± 11.937	138.4 ± 148.0	12.942 ± 9.018	129.0 ± 5.676

was the same. At the time of milk coagulation the acidity of the sample with walnut pieces and control was similar (61.9°T and 59.4°T respectively). Titratable acidity of the samples with hazelnut pieces and that with honey and walnut pieces were lower than other (56.5T and 47.4T). Cow's yogurt with walnut pieces and honey coagulated at lower titratable acidity compared to control yogurt. The supplementation with honey significantly influenced milk acid level in the final product. These data are in contrast to those of Varga (2006), who found insignificant effect in yoghurt with acacia honey.

Milk coagulation first occurred in the sample walnut pieces and honey (129 min) followed by yogurt with walnut pieces (138.4 min). The fermentation process was 16% shorter than control when 5% honey was added to the milk. In our view, this was due to the presence of biologically active substances (vitamins, amino acids and other growth factors) in walnut pieces and honey. Yogurt with hazelnut pieces coagulated 5 min faster than control. After storage of yogurt in refrigerator for 24 h and 10 days, the samples with walnut pieces exhibited slower increase in acidity that was beneficial with regard to yogurt stability.

Lactic acid bacteria count

The experimental data about LAB count showed that by the time of coagulation, these bacteria were the most numerous in the sample with walnut pieces and honey – 1.1×10^7 (Table 4). This was valid for both rods and cocci. These results are in contrast to those of Roymyan et al. (1996), who found a considerable inhibition in the growth of *L. delbrueckii* ssp. *bulgaricus* when testing the influence of honey addition on the yogurt's starter cultures. This trend was also preserved after 24h and 10 days of milk storage at 4-6°C. Such

observations are in agreement with those of Chick et al. (2001), who reported that pasteurized clover honey at the level of 5% generates similar improved growth of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* and honey did not exert an inhibitory effect at this level. The initial viable counts of *L. delbrueckii* ssp. *bulgaricus* were found to be approximately twice lower than those of *S. thermophilus* in all yogurt samples. According to Desmazeaud (1983), the better growth of *S. thermophilus* in milk could be explained by its lower nutritional requirements compared to lactobacilli in milk. In fact, *S. thermophilus* requires few amino acids and is able to synthesize branched-chain amino acids (Garault et al., 2000), and its growth can probably be supported by free amino acids and peptides present in milk (Letort et al., 2002). In contrast, *L. delbrueckii* ssp. *bulgaricus* is much more in need from a nutritional point of view than *S. thermophilus* (Letort and Juillard, 2001), and its optimal growth depends on the supply of essential factors (CO₂, pyruvate, formate) produced by *S. thermophilus* (Tamine and Robinson, 1999).

The ratio between rods and cocci in cow's fermented milk by the time of coagulation was smallest in yogurt with hazelnut pieces (1:3), followed by the control yogurt (1:2.4). During the storage, it increased up to 1:1.5 by the 24th h and 10 days in all samples. The rods to cocci ratio in high-quality yogurt should be from 1:1 to 1:2.7 (Persic, 1991), and our data were within this range.

Enrichment of yogurt with honey is recommended because honey is a natural sweetener with a wide range of beneficial nutritional properties. In addition, at a concentration of approximately 3%, it highly improves the sensory characteristics of yogurt without having an inhibitory effect on starter bacteria (Varga,

Table 4. Lactic acid bacterial counts in cow's yogurt supplemented either with walnuts or hazelnuts* (n=5)

Indexes	Control	Supplements		
		10% walnut pieces	10% walnut pieces and 5% honey	10% hazelnut pieces
At coagulation				
<i>L. bulgaricus</i> (1)	110 400	245 600	366 400	163 200
<i>S. thermophilus</i> (2)	262 400	451 200	755 200	492 000
Ratio (1:2)	1:2.4	1:1.8	1:2	1:3
Total count	372 800	669 800	1 121 600	655 200
After 24 h				
<i>L. bulgaricus</i> (1)	283 200	279 200	399 200	276 000
<i>S. thermophilus</i> (2)	612 000	599 200	736 000	608 800
Ratio (1:2)	1:2.2	1:2.1	1:1.8	1:2.2
Total count	895 200	878 400	1 135 200	884 800
After 10 days				
<i>L. bulgaricus</i> (1)	268 000	220 800	515 200	235 200
<i>S. thermophilus</i> (2)	534 400	484 000	756 800	523 200
Ratio (1:2)	1:2	1:2.2	1:1.5	1:2.2
Total count	802 400	705 600	1 272 000	758 400

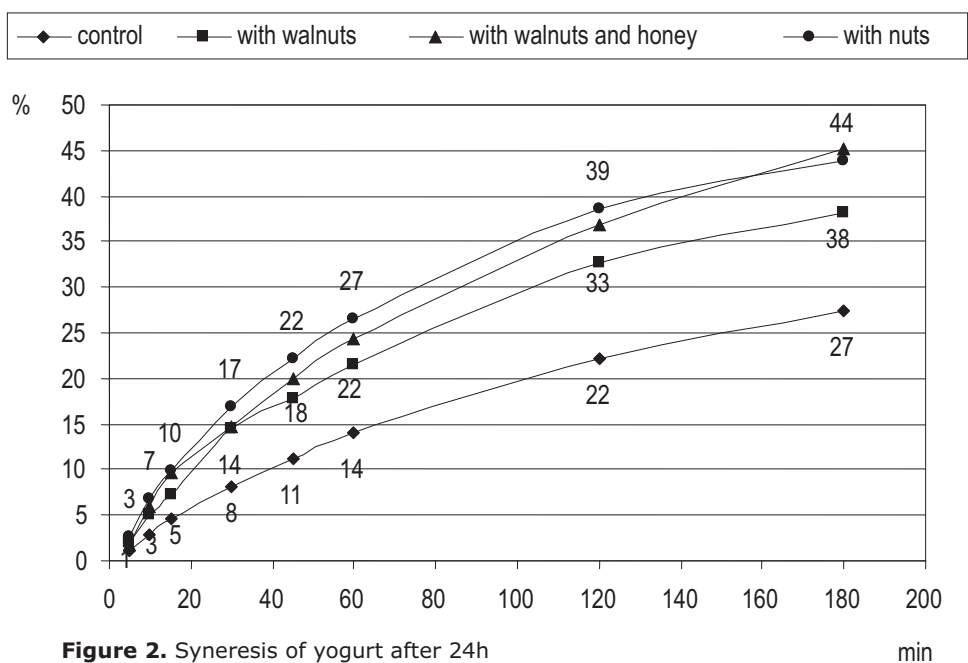
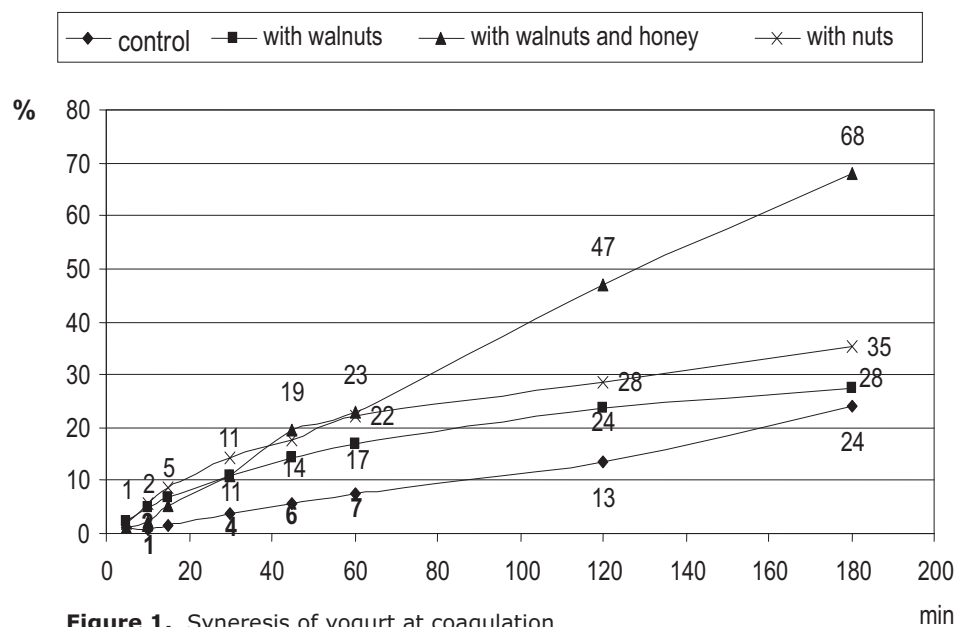
* (x 10³/ml)

2006). Honey contains fructooligosaccharides which act as prebiotics in the gut, therefore honey could be of great value in the concept of functional foods (Slavin, 1997).

Syneresis of yogurt

Significant differences were observed among the control and experimental variants regarding to the amount of the milk serum after coagulation (Figure1). The differences in the amounts of separated liquid were clear after 40th min and they increased up to the end of the third hour. The amount of the separated serum during

coagulation was the lowest for the control group and the highest for yogurt supplemented with walnuts (10%) + honey (5%). Incorporation of honey (5%) increased the amount of serum with comparison to variant of supplementation of walnut (10 %) without honey. The serum accumulation was influenced in different manner from the supplemented substances during the storage. After 24 h of storage at 4-6°C the amount of serum separated in the control group and the variants with addition of walnut and hazelnut has a trend for increasing (Figure 2). Supplementation of yogurt with walnut + honey has not significant impact on serum and the amount of



separated serum in significantly lower compared to the other supplements. After 10 days storage (Figure 3) milk serum for the control was the same compared to measurement at 24h, while at the same time for the variant of supplementation with walnut + honey

was increased with 3 ml. The period for complete coagulation is the longest for the control may be due to the positive effect of biologically active compounds into the supplemented substances.

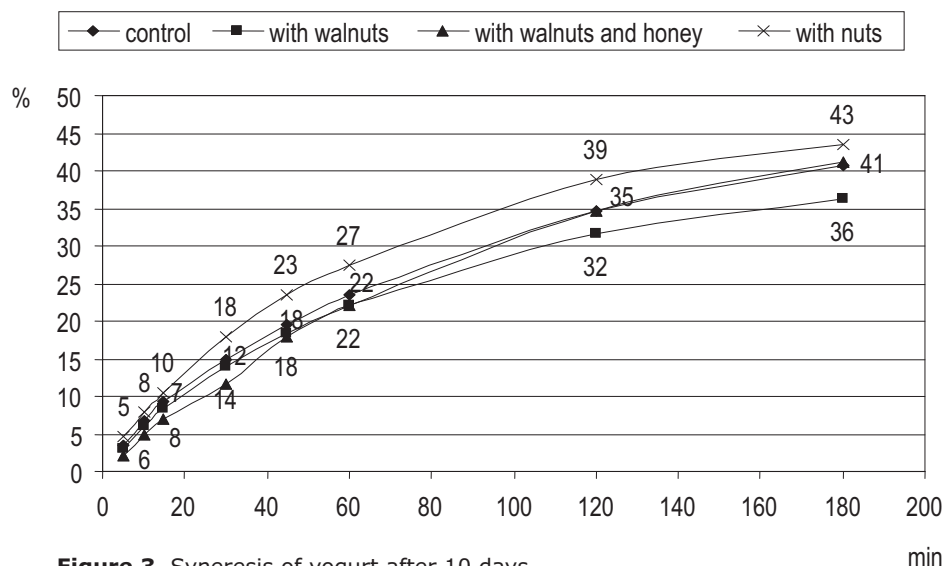


Figure 3. Syneresis of yogurt after 10 days

Conclusion

Supplementation of nuts has a positive effect on the quality characteristics of yogurt. Supplements lead to reducing the time for coagulation with 2.3 to 15.7 %. Supplements slightly increased the titratable acidity of yogurt during the storage. All supplements lead to increasing of extracted milk serum and syneresis index during the storage. The highest effect was obtained from walnut + honey supplementation. Enrichment of milk with plant biologically active substances has positive effect on the microbial activity. Total number of LAB increased significantly at coagulum, almost twice- with the supplementation of walnut and hazelnut, and almost three times - with walnut plus honey.

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