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Effect of Goji Berries and Honey on Lactic Acid Bacteria Viability and Shelf Life Stability of Yoghurt

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Abstract

The probiotic properties and the viability of lactic acid bacteria of fermented dairy products can be improved by addition of bioactive compounds originating from natural sources (e.g. goji berries). This study aimed to evaluate how goji berries and honey affect the sensorial quality of yoghurt, the chemical properties, the viability of lactic acid bacteria (LAB) and the concurrent microflora development. Two types of yoghurts (yoghurt with goji berries and yoghurt with honey and goji berries) were developed. The addition of honey affected the entire yoghurt microflora including LAB, manifesting bactericidal effect. The addition of goji berries maintained the viability of LAB at probiotic levels (10^6-10^7 log CFU/ml) during 21 days of storage; compared to classic yoghurt, LAB viability decreased during storage at 10^3 log CFU/ml. Goji berries also improved sensory acceptance of consumers. The results obtained in this study collect information that enables the use of goji berries as enhancer of probiotic levels in yoghurt, while honey can provide bacteriostatic/bactericidal effect for contaminants.

Keywords: consumer acceptance, chemical parameters, plant extracts, probiotic

Introduction

Fermented dairy products are popular because of the health benefits provided by the ingestion of probiotics generated by the consumption of these products (Butel, 2014; Goktepe *et al*., 2006; Guarner and Schaafsma, 1998; Khalid, 2011; Sanders, 2003; Wang, 2009). Among all of the fermented dairy products yoghourt is the most consumed (Cruz *et al*., 2010; Cruz *et al*., 2013; de Oliveira, 2014; Saint-Eve *et al*., 2006), probably due to the positive perception on the market as being seen by the consumers as a functional dairy product containing living microorganisms like lactic acid bacteria (LAB), streptococci, bifidobacteria or their combinations, coming from the starter cultures, recognised as ingredients that promote human health (Davis, 2014; Goktepe *et al*., 2006; Kent *et al*., 2014; Khalid, 2011; Ouwehand *et al*., 2015; Rastall *et al*., 2002; Sanders *et al*., 2010). The market generated a need for fermented milk products that are fermented and processed in new conditions or enriched with bioactive compounds (Sun-Waterhouse *et al*., 2013; Zamfir *et al*., 2006).

Studies regarding the addition of different categories of bioactive molecules in yoghurt, including free-cell of probiotics, entrapped in different matrices and symbiotic forms (Brinques *et al*., 2011; Chavarri *et al*., 2010; Krasaekoopt *et al*., 2003; Lourens-Hattingh *et al*., 2001; Pinto *et al*., 2012; Stanton *et al*., 2001) and a wide range of plant extracts with various active properties as red berries (Breme *et al*., 2014; Cruz *et al*., 2010; Ścibisz *et al*., 2012; Sun-Waterhouse *et al*., 2013), grape and grape seed extracts (Chouchouli *et al*., 2013; Coda *et al*., 2012; Karaaslan *et al*., 2011; Tseng *et al*., 2013), pomegranate peel extract (El-Said *et al*., 2014), tea extracts (Jaziri *et al*., 2009; Ye *et al*., 2012) could be easily found. Researchers attempted to make the yoghurt a better environment for LAB and a source of bioactive compounds by addition of valuable molecules (Breme *et al*., 2014; do Espírito Santo *et al*., 2011).

The nutritional impacts of LAB and health benefits still continue to arise the interest of scientists who discover new potentials as food and valuable ingredients. Systems that can emphasize the great potential of probiotics are of interest. Saccharides are a good source food for these valuable bacteria, being utilized mostly as probiotics (Rastall *et al*., 2002; Teitelbaum *et al*., 2002; Wang, 2009). The benefits brought by carotenoid consumption include reduction of cancer risk or cardiovascular diseases (Pintea *et al*., 2005; Pintea *et al*., 2011; Socaciu *et al*., 2000), improving vision (Pintea *et al*., 2011) and a healthy tan looking effect.

Polyphenols possess strong antioxidant activities being free radical scavengers, electron donors and strong metal chelators (Andjelković *et al*., 2006), helping in the prevention of lipid peroxidation (Vodnar *et al*., 2014). Several reports have shown

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Table 1. Experimental design-treatments and responses

Treatments	Responses	Treatments	Responses
Sensory evaluation		Physicochemical and microbiological analysis	
YC (classic yoghurt)	X	YC_i (classic yoghurt-initial)	xx
YG_3 (yoghurt with 3% goji berries)	X	YG_{7i} (yoghurt with 7% goji berries-initial)	xx
YG_5 (yoghurt with 5% goji berries)	X	YHG_{7i} (yoghurt with honey and 7% goji berries-initial)	xx
YG_7 (yoghurt with 7% goji berries)	X	YC_m (classic yoghurt-middle of storage-14th day of storage)	xx
YHG_3 (yoghurt with honey and 3% goji berries)	X	YG_{7m} (yoghurt with 7% goji berries-middle of storage-14th day of storage)	xx
YHG_5 (yoghurt with honey and 5% goji berries)	X	YHG_{7m} (yoghurt with honey and 7% goji berries-middle of storage-14th day of storage)	xx
YHG_7 (yoghurt with honey and 7% goji berries)	X	YC_f (classic yoghurt-final of storage-21st day of storage)	xx
		YG_{7f} (yoghurt with 7% goji berries-final of storage-21st day of storage)	xx
		YG_{7f} (yoghurt with 7% goji berries-final of storage-21st day of storage)	xx

that polyphenols prevent the proliferation of degenerative diseases, clearly improving the condition of oxidative stress biomarkers (Bunea *et al.*, 2013; Chedea *et al.*, 2010).

Lycium barbarum (goji berries or wolfberries, *Solanaceae* family) represent a rich source of chemical, having health promoting properties: ocular neuroprotecti (Srinivasan, 2014), hepato-protective (Liu *et al.*, 2015), antitumoral (How *et al.*, 2014; Martínez *et al.*, 2014), antioxidative and immunomodulatory effects (Xiao *et al.*, 2012). These properties are related to the saccharides, caroteinoids and some phenolics in the soluble fraction (Bondia-Pons *et al.*, 2014; Inbaraj *et al.*, 2010; Wang *et al.*, 2010; Xiao *et al.*, 2012; Yang *et al.*, 2013). Honey contains phenolic acids and their derivates, flavonoids and hydrogen peroxide (Brudzynski, 2006; Brudzynski *et al.*, 2011); it has high osmolarity, low pH and water activity (Voidarou *et al.*, 2011). Thus, honey could provide good bacteriostatic or bactericide effect. Research showed that the redox potential can be reduced by supplementing yoghurt with bioactive compounds from natural sources (Perna *et al.*, 2014; Zalibera *et al.*, 2008). Moreover, the viability of LAB (*L. bulgaricus* and *S. thermophilus*) could be increased by reducing the redox potential with addition of bioactive compounds from natural sources (Zalibera *et al.*, 2008).

In this study it was investigated how goji berries and honey affected the sensorial quality, the chemical properties, the viability of lactic acid bacteria and concurrent microflora in yoghurt. Two types of yoghurt were obtained with different concentrations of goji berries and honey.

Materials and methods

Yoghurt preparation

Whole milk was provided by UASVM farm together with an analysis bulletin (fat content - 3.87%; protein content - 3.40%; crioscopic point: - 0.60 °C; non-fat dry matter - 9.10%; density - 1.0295 g/cm^3). Classic yoghurt was prepared starting from whole milk (3.5% fat), pre-heated (homogenized) at 50-65 °C (150-200 atm), pasteurized at 85-90 °C (maintained for 20-30 min) and cooled at 45-48 °C. Starter mezophylic culture Lyofast Y450B (Sacco, Cadorago, Italy) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (ratio 1:1) was added (5 units at 250 L milk which correspond to 0.5 x 10^{12} CFU/ml) to start fermentation. The yoghurt was stored at 43-45 °C for 3 hours, pre-cooled at 18-20 °C, cooled at 2-8 °C and stored at this temperature for further analysis (Jimborean and Țibulcă, 2013).

The classic yoghurt was supplemented with 3%, 5% and 7% (w/w) goji berries, after the inoculation with starter culture. The same technology was used for yoghurt with honey and goji berries; polyfloral honey 3% (w/w) was added before fermentation and goji berries in amount of 3%, 5%, and 7% (w/w) after fermentation. Goji berries and honey were purchased from a local market.

Total phenolic content of goji berries

Goji berries (2.5 g) were cut in small pieces, homogenized using a rotary magnetic stirrer with 10 mL distilled water, centrifuged at 3,000×*g* for 10 min. The supernatant was analyzed spectrophotometrically using Folin-Ciocâlteu method. Aliquots of 2.375 mL distilled water were mixed with 0.025 mL extract, 0.150 mL Folin-Ciocâlteu reagent and 0.450 mL Na_2CO_3 (7.5%). Absorbance was read at 750 nm (Biotek multiplate reader) after keeping the samples for 2 hours in the dark. Results were expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} goji berries.

Experimental design

Two types of yoghurt were obtained in this study: yoghurt with goji berries (YG) and yoghurt with honey and goji berries (YHG). Classic yoghurt (YC) was the control sample. Goji berries were added in proportions of 3%, 5% and 7% (w/v) in classic yoghurt (YG_3, YG_5 and YG_7) and in yoghurt with honey (YHG_3, YHG_5 and YG_7). Samples codification and experimental design are shown in Table 1.

Yoghurt with 3%, 5% and 7% goji berries (YG_3, YG_5, YG_7) and yoghurt with honey (3%) and goji berries (YHG_3, YHG_5, YHG_7), were sensory evaluated. Further studies (chemical and microbiological analysis) were conducted on classic yoghurt, yoghurt with 7% goji berries, and yoghurt with honey and 7% goji berries during a shelf life of 21 days at 4 °C. The samples were analyzed initial (i), in the 14th day of storage (m) and in the 21st day of storage (f). Fat, proteins, lactic acid, lactose, glucose, fructose, sucrose, total sugars, total solids and solids non-fat were tested. *Salmonella* spp., *Enterobacter* spp. and *Escherichia coli* were determined as microbial contaminants and *Streptococcus thermophilus* and *Lactobacillus bulgaricus* as lactic acid bacteria.

Sensory evaluation of yoghurt

A 9-point hedonic test was used to determine consumer's preference of yoghurt. Yoghurt with 3%, 5% and 7% goji berries (YG_3, YG_5, YG_7) and yoghurt with honey (3%) and goji berries (YHG_3, YHG_5, YHG_7) were sensory evaluated. A panel of 30 trained assessors (male and female) participated to this study. The response categories ranged from 1-extreme dislike, to 9-extreme like.

Chemical analysis of yoghurt

10 mL of yoghurt were homogenized using a stomacher (Bagmixer®-100MiniMix®, Interscience, Arpents, France) before the chemical analysis, as sample preparation.

The chemical content (fat, proteins, lactic acid, lactose, glucose, fructose, sucrose, total sugars, total solids and solids non-fat) of the yoghurt was determined using the MilkoScan FT2 analyser (Foss, Hillerød, Denmark).

The method was based on a mathematic procedure that allowed splitting the interferogram in sinus functions, each one representing a wavelength. The interferogram was then introduced in a spectrophotometer and converted in a larger spectral image of the sample. The results were expressed as percentage.

Determination of lactic acid bacteria in yoghurt

Lactic acid bacteria in yoghurt with 7% goji berries and in yoghurt with honey and goji berries was initially determined, after the 14th day of storage and after the 21st day of storage. Yoghurt samples were ten-fold diluted, placed on MRS broth/M17 broth, (Oxoid, Basingstoke, UK) and incubated in anaerobic conditions for 72 h at 37 °C (*L. bulgaricus)* and for 48 h at 37 °C (*S. thermophilus)*. Plates containing 30 to 300 CFU were counted. The confirmation was made by specific test (Gram affinity, colony aspect and catalase +).

Determination of microbial pathogens in yoghurt

Three strains of Gram negative bacteria (*Salmonella* spp., *Enterobacter* spp. and *Escherichia coli)* and one Gram positive strain (*Staphylococcus* spp.) were tested for yoghurts and aqueous extract of goji (5 g goji berries in 45 mL physiological serum). Honey was microbiological evaluated according to the same methodology as goji berries.

Identification of Salmonella spp.

The presence of *Salmonella* was determined according to SR ISO 6579/1997 method. For the pre-enrichment stage, the sample was suspended in Buffered Peptone Water (Laboratorios Conda, Madrid, Spain). For the enrichment stage, 1.0 mL of sample was inoculated on RVS broth (Merck, Darmstadt, Germany) and incubated at 42 °C for 24 h. The isolation was made by inoculating the bacterial suspension obtained in the enrichment phase on XLD Agar (Oxoid, Basingstoke, UK) and Brilliant Green Agar (modified CM0329, Oxoid, Basingstoke, UK). The incubation was made at 35-37 °C for 20-24 h (another 24 h if necessary). The confirmation was made on characteristic colonies using selective growth mediums. The results were expressed as colony forming units per gram (CFU 25 ml^{-1}).

Identification of Enterobacter spp.

The presence of *Enterobacter* was made according to SR-ISO 21528-2/2007 method. Briefly, 1.0 mL of the diluted sample was transferred to a sterile Petri dish. Aliquots of 15 mL of Violet Red Bile Glucose Agar (Lab M Ltd., Lancashire, UK) were poured over the sample and maintained at 45±1 °C. In order to ensure semi-anaerobic conditions, another 15 mL of VRBGA agar were poured into the Petri dish. Incubation was made at 35 °C for 24 h. The results were expressed as Log CFU/ml.

Identification of Escherichia coli

The presence of *E. coli* was determined according to SR ISO 7251/1996. 1.0 mL of the diluted sample was uniformly distributed into a sterile Petri dish and then TBX Agar (Oxoid, Basingstoke, UK) was poured and mixed. The incubation was made at 35 °C for 24 h. The results were expressed as Log CFU/ml.

Identification of Staphylococcus aureus

SR EN ISO 6888-2/A-1/2005 standard method was used. Briefly, 1.0 mL of the diluted sample was transferred to a sterile Petri dish covered with Baird-Parker agar (Oxoid, Basingstoke, UK) supplemented with Egg Yolk Tellurite Supplement (SR 00540, Oxoid, Basingstoke, UK) and spread using a Drigalsky spatula. The results were expressed as Log CFU/ml.

Statistical analysis

Statistical analysis of data was performed by Minitab Statistical software version 16.1.0 (LEAD Technologies, Inc.). The analysis of variance was assessed by two-way ANOVA and significant differences among the means of samples were analyzed by Tukey's test with a 95% confidence level.

Results and discussions

Total phenolic content of goji berries

The total phenolic content (TPC) of goji berries water extract obtained in this study was 132.26 mg GAE 100 g^{-1} goji berries. The study of Hunaefi *et al.* (2012) reports that phenolic compounds are secondary metabolites that can interfere with the LAB fermentation process through their antioxidant properties.

Donno *et al.* (2014) evaluated TPC of various cultivars of fresh goji berries and determined values ranging from 255.87 to 281.91 mg GAE g^{-1} fresh weight (FW), while Medina *et al.* (2011) obtained higher values in dry goji berries extracted in ethanol (895 mg GAE g^{-1} DW). Differences can be attributed to genotype, cultivars (Donno *et al.*, 2014), extraction type, mainly solvent polarity (Medina *et al.*, 2011) processing and method sensibility.

Sensory evaluation by Hedonic test

The sensory evaluation showed that the consumers preferred yoghurt with 7% goji berries (8.21 points on hedonic scale). Yoghurt with goji berries and honey was less accepted by consumers. Yoghurt with honey and 7% goji berries scored 7.4 points, while yoghurt with honey and 3% goji berries scored the lowest 6.9 points on hedonic scale (Fig. 1).

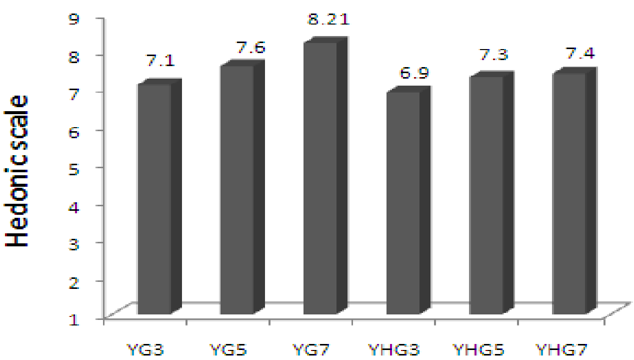

Fig. 1. Graphical representation of the sensory evaluation of yoghurt, according to the hedonic scale

Table 2. Results of the physicochemical evaluation of tested yoghurts

Sample	Fat (%)	Proteins (%)	Lactic acid (%)	Lactose (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugars (%)	Total solids (%)	Solids non-fat (%)
YC_i	4.51±0^d	3.66±0^f	0.61±0.01^{cd}	4.77±0^a	0.04±0.01^e	0.00±0^f	0.20±0^f	4.14±0^g	13.84±0^i	9.24±0^h
YG_{7i}	4.50±0^d	3.73±0^e	0.61±0^{bcd}	4.72±0.01^b	0.02±0^e	0.03±0^e	0.24±0^e	4.17±0^f	13.96±0^g	9.31±0^g
YHG_{7i}	4.52±0.01^d	3.67±0.01^f	0.60±0.01^d	4.74±0^b	0.02±0^e	0.00±0^f	0.21±0^f	4.15±0.01^{fg}	13.90±0^h	9.25±0^h
YC_m	4.32±0^e	4.18±0.01^b	0.63±0^{bc}	3.82±0^d	0.35±0.01^{ab}	1.96±0^c	3.45±0.01^a	8.76±0.01^a	20.91±0.01^a	16.40±0.01^c
YG_{7m}	4.20±0^f	4.22±0^a	0.63±0.01^{bc}	3.65±0.01^f	0.27±0^d	2.06±0^a	3.45±0.01^a	8.72±0.01^b	20.85±0.01^c	16.56±0.01^a
YHG_{7m}	4.22±0.01^f	4.23±0.01^a	0.63±0^b	3.76±0.01^e	0.35±0^a	2.01±0^b	3.40±0.01^b	8.74±0.01^{ab}	20.88±0.01^b	16.49±0.01^b
YC_f	5.51±0.01^a	4.14±0.01^c	1.17±0.0^a	3.87±0.01^c	0.30±0^c	1.32±0^d	2.35±0^c	7.13±0.01^c	20.16±0.01^d	14.46±0.01^f
YG_{7f}	5.42±0.01^c	4.08±0.01^d	1.17±0.0^a	3.82±0.01^d	0.27±0^d	1.32±0^d	2.31±0^d	6.99±0^e	20.06±0.01^f	14.56±0.01^d
YHG_{7f}	5.46±0.01^b	4.13±0^c	1.17±0.0^a	3.87±0^c	0.33±0^b	1.31±0^d	2.31±0^d	7.04±0.01^d	20.13±0.01^e	14.52±0.01^e

*Values are presented as mean ± standard deviation of three replicates; Different letters within columns indicates statistically significant differences at p<0.05 (Tukey's test)

1) YC_i (classic yoghurt-initial); YG_{7i} (yoghurt with 7% goji berries-initial); YHG_{7i} (yoghurt with honey and 7% goji berries-initial); YC_m (classic yoghurt-14th day of storage);

2) YG_{7m} (yoghurt with 7% goji berries-14th day of storage); YHG_{7m} (yoghurt with honey and 7% goji berries-14th day of storage);

3) YC_f (classic yoghurt-21st day of storage); YG_{7f} (yoghurt with 7% goji berries-21st day of storage); YHG_{7f} (yoghurt with honey and 7% goji berries-21st day of storage)

Table 3. Effects of yoghurt type, storage time and their first-degree interaction on fat (%), proteins (%), lactic acid (%), lactose (%), sucrose (%), glucose (%), fructose (%), total sugars (%), total solids (%), solids non-fat (%) and their percentage contribution

Factor	Fat (%)	Proteins (%)	Lactic acid (%)	Lactose (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugars (%)	Total solids (%)	Solids non-fat (%)
Yoghurt type (YT)										
YC	4.8^a	4.0^b	0.8^a	4.2^a	0.2^a	1.1^c	2.0^a	6.7^a	18.3^a	13.4^c
YG	4.7^c	4.0^a	0.8^a	4.1^c	0.2^b	1.1^a	2.0^a	6.6^c	18.3^b	13.5^a
YHG	4.7^b	4.0^a	0.8^a	4.1^b	0.2^a	1.1^b	2.0^b	6.6^b	18.3^a	13.4^b
SD/Contribution (%)	***/0.34	**/0.12	n.s./0	***/0.72	***/2.32	***/0.05	***/0.01	***/0.01	**/0.001	***/0.02
Storage time (ST)										
Initial	4.5^b	3.7^c	0.6^c	4.7^a	0.0^c	0.0^c	0.2^c	4.2^c	13.9^c	9.3^c
Middle stage of storage	4.2^c	4.2^a	0.6^b	3.7^c	0.3^a	2.0^a	3.4^a	8.7^a	20.1^b	16.5^a
Final stage of storage	5.5^a	4.1^b	1.2^a	3.8^b	0.3^b	1.3^b	2.3^b	7.0^b	20.9^a	14.5^b
SD/Contribution (%)	***/99.48	***/98.59	***/99.96	***/98.98	***/96.33	***/99.91	***/99.98	***/99.97	***/99.98	***/99.98
YT x ST										
SD/Contribution (%)	*/0.17	***/1.26	n.s./0.01	***/0.29	***/1.30	***/0.04	***/0.01	***/0.02	***/0.02	***/0.003

1) Different letters indicates statistically significant differences at p<0.05 (Tukey's test)

2) Significant differences (SD) are denoted by asterisks: *p<0.05; **p<0.01; ***p<0.001; n.s. $p \geq 0.05$, non-significant

3) YC (classic yoghurt); YG (yoghurt with 7% goji berries); YHG (yoghurt with honey and 7% goji berries)

In general, fruity yoghurts are popular among consumers (Kailasapathy *et al.*, 2008). Senaka Ranadheera *et al.* (2012) also reported a higher preference of yoghurts supplemented with fruits. No previous report evaluated the sensory attributes of fruity yoghurt supplemented with honey. In this study the addition of honey influenced negatively the consumer's perception.

Chemical evaluation of yoghurts

Chemical analysis evaluated the effects of fortification and shelf life stability on yoghurt (Table 2). The effects of yoghurt type, storage time and crossed treatment interaction on chemical properties were analyzed by two-way ANOVA and Tukey's test. Significant differences were observed in chemical attributes dependent on yoghurt type, storage time and cross treatment interaction (Table 3).

Sucrose had the highest percentage of contribution of yoghurt type (2.32%, p<0.001); sucrose accumulated in yoghurts towards the end of shelf life. The quantity of sucrose increased during storage for all types of yoghurt. Sucrose from honey was solubilized in yoghurt and lead to high values of sugars (Tewari *et al.*, 2004) (Table 2). This increase might occur due to efflux of intracellular carbohydrates associated with the disaccharide metabolism and can be observed in case of LAB. The lowest percentage contribution on yoghurt type was determined for total solids followed by fructose, total sugars and solid non-fat.

An increasing tendency for glucose and fructose was observed until the 14th day of storage because saccharides from honey and goji berries were solubilized. This can be also explained by the fact that lactose was decomposed by lactic acid bacteria. Moreover, LAB have the ability to decrease the carbohydrates content by fermentation process.

Storage influenced the content of lactic acid with a contribution of 99.96%. The content of lactose decreased and lactic acid increased during storage (lactose is decomposed into lactic acid). Lactic acid showed insignificant differences in relation to yoghurt type, but a direct contribution to storage time and an increasing tendency was found towards the end of shelf life (Table 3).

Storage time influenced significantly the content of total solids, solids non-fat, fructose (99.98%), total sugars (99.97%) and glucose (99.91%). Lactic acid bacteria consumed glucose and fructose resulting in low quantities at the end of the storage. In case of yoghurt with 7% goji berries, glucose decreased from 2.06% to 1.32%, while fructose decreased from 3.45% to 2.35%. In yoghurt honey and with 7% goji berries, glucose decreased from 2.01% to 1.31% and fructose from 3.40% to 2.31%. Classic yoghurt showed a similar decreasing tendency for glucose and fructose, from 1.96% to 1.32% and from 3.45% to 2.35%, respectively.

Solid non-fat, total solids and total sugars increased until the 14th day of storage and decreased in the 21st day of storage; the type of yoghurt had little influence on these parameters. The total sugar content for yoghurt with 7% goji berries ranged from 4.17% (initial) to 8.72% in the 14th day of storage and decreased at 6.99% in the 21st day of storage. The same tendency was observed in case of total solids and solid non-fat (Table 2). The higher amount of total solids (including fat and protein content) was found in yoghurts in the 21st day of storage.

Table 4. Microbiological evaluation of yoghurt, goji berries and honey

Contaminant	Samples										
	YC_i	YC_m	YC_f	YG_{7i}	YG_{7m}	YG_{7f}	YHG_{7i}	YHG_{7m}	YHG_{7f}	Goji berries	Honey
Salmonella log CFU/ 25ml	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli log CFU/ml	ND	ND	ND	ND	$0.35x10^2±1.77$	$0.55x10^2±1.77$	ND	ND	$0.72x10^2±2.0$	$0.35x10^3±1.77$	ND
Enterobacter log CFU/ ml	ND	ND	ND	ND	$0.6x10^2±0.44$	$0.35x10^2±1.77$	ND	ND	$0.4x10^2±1.11$	$1.6x10^3±0.44$	ND
S. aureus log CFU/ ml	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lactic acid Bacteria											
Str. thermophilus log CFU/ ml	$1.38x10^7$	$1.46x10^6$	$1.50x10^3$	$2.14x10^8$	$1.99x10^7$	$1.76x10^7$	$1.7x10^7$	$1.63x10^6$	$2.06x10^5$	NA	NA
L. bulgaricus log CFU/ ml	$1.59x10^7$	$1.51x10^6$	$1.33x10^3$	$2.17x10^8$	$2.71x10^7$	$1.67x10^7$	$1.30x10^8$	$1.33x10^6$	$1.71x10^5$	NA	NA

*Values are presented as mean ±standard deviation of three replicates
[1)] YC_i (classic yoghurt-initial); YG_{7i} (yoghurt with 7% goji berries-initial); YHG_{7i} (yoghurt with honey and 7% goji berries-initial)
[2)] YC_m (classic yoghurt-14th day of storage); YG_{7m} (yoghurt with 7% goji berries-14th day of storage); YHG_{7m} (yoghurt with honey and 7% goji berries-14th day of storage)
[3)] YC_f (classic yoghurt-21st day of storage); YG_{7f} (yoghurt with 7% goji berries-21st day of storage); YHG_{7f} (yoghurt with honey and 7% goji berries-21st day of storage

Crossed treatment interaction revealed significant differences in the content of sucrose (1.30% contribution, $p<0.001$) and proteins (1.26% contribution, $p<0.001$), while the smallest percentage of contribution was determined in case of solids non-fat (0.003%, $p<0.001$) and fructose (0.02%, $p<0.001$).

Lactic acid bacteria

Lactic acid bacteria concentration remained at probiotic value level (10^6-10^7 Log CFU/ml) (Shiby *et al.,* 2013) in yoghurt with 7% goji berries addition during storage and decreased in yoghurt with honey and 7% goji berries (Table 4).

S. termophillus concentration decreased during storage in all yoghurt types because of its sensitivity to lactic acid. A slight maintenance of *S. termophillus* concentration was observed during the first week of storage. One of the most important properties of lactic acid bacteria is their ability to decrease the carbohydrates content by fermentation. Until the 14th day of storage, lactic acid bacteria had sufficient carbohydrates to synthesize lactic acid (Tables 2 and 4); in the 21st day of storage, lactic acid bacteria viability decreased and once with it the ability to metabolize carbohydrates.

The growth of probiotics/prebiotics and yoghurt starter culture in the presence of fruit juices is strain specific (Vinderola *et al.,* 2002). The data presenting the growth and viability of lactobacilli in this particular medium is scarce (Kailasapathy *et al.,* 2008; Vinderola *et al.,* 2002).

The production of lactic acid by *Lactobacillus* is influenced by the medium pH. Chookietwattana (2014) reported that at an initial pH of 6.5 the lactic acid production was high, whereas at a pH of 5.0/5.5 the production of lactic acid was prohibited.

The same evolution in lactic acid bacteria concentration was observed by Michael *et al.* (2010), while Rotar *et al.* (2007) reported a significant decrease of the viable germs to 10^3 log CFU/ml at the end of the storage period in classic yoghurt.

Microbiological evaluation of yoghurt

Salmonella spp. and *Staphylococcus* spp. were absent in all types of yoghurt (Table 3). Contamination with *E. coli* was determined in goji berries yogurts. In the 14th day of storage the yoghurt with goji berries resulted positive for contamination. The presence of *E. coli* was noted in case of yoghurt with honey and goji berries in the 21st day of storage.

The presence of *Enterobacter* was detected in the 21st day of storage for yoghurt with honey and goji berries. The initial levels of *Enterobacter* spp. in goji berries was $1.6x10^3$ log CFU/ml; these values were reduced in the 21st day of storage at $0.4x10^2$ log CFU/ml, proving bacteriostatic effect by adding honey. *Salmonella* spp. and *Staphylococcus* spp. were absent in all types of yoghurt (Table 3). Contamination with *E. coli* was determined in goji berries. In the 14th day of storage the yoghurt with goji berries resulted positive for contamination. The presence of *E. coli* was noted in case of yoghurt with honey and goji berries in the 21st day of storage. Literature reports the bactericide/bacteriostatic effect of honey (Brudzynski *et al.*, 2012; Brudzynski *et al.,* 2011; Voidarou *et al.*, 2011).

Conclusions

The addition of goji berries (7%) improved the sensory quality of classic yoghurt and increased the consumer's acceptance. Quality parameters (chemical parameters) were maintained during storage. Goji berries improved the lactic acid bacteria evolution and maintained the prebiotic value of yoghurt during storage. Concurrent microflora (contaminants) appeared when goji berries were added. The results obtained in this study collect information that enables the use of goji berries as enhancer of probiotic levels in yoghurt.

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