## EFFECT OF HONEY ADDITION ON RHEOLOGICAL PROPERTIES OF PROBIOTIC SOY YOGHURT

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Yoghurt is a complex rheological system with 3-dimensional network of chains and clusters of casein micelles in which water is entrapped. The gel structure of set-style yoghurt is influenced by many factors that include milk composition, primary dry matter content, protein content and composition, heat treatment of milk. Protein content of milk has the greatest influence on the gel strength, viscosity and syneresis. The aim of this study was to investigate the effect of honey addition at different concentrations (0, 2, 4 and 6%) on rheological properties of fermented soymilk products manufactured by probiotics inoculum during 21 day of storage. Rheological analyses were studied by dynamic viscosity, syneresis and water holding capacity. The increase in the honey addition in milk increased viscosity and water holding capacity, while it decreased the syneresis of soy probiotic yoghurt over 21 day of storage.

Keywords: soy yoghurt, honey, rheology, water holding capacity

## INTRODUCTION

The science of rheology has many applications in the field of food acceptability, food processing, and handling (1). Viscosity, especially non-Newtonian viscosity, is an important component of the quality of most fluid and semifluid foods (1). Furthermore, rheological measurements have been widely used to characterize the structure of fermented milk as well as soymilk gels.

Yoghurt is a complex rheological system with 3-dimensional network of chains and clusters of casein micelles in which water is entrapped. The gel structure of set-style yoghurt is influenced by many factors that include milk composition, primary dry matter content, protein content and composition, heat treatment of milk. Protein content of milk has the greatest influence on the gel strength, viscosity and syneresis.

Soymilk today gets more and more attention because of its extraordinary nutritive and health characteristics. Soymilk is intended for population that cannot consume cow's milk, due to lactose intolerance or allergies to cow's milk proteins. Soybean milk contains raffinose, stachyose, pentanal, n-hexanal and phytoestrogens. Consequently, soymilk-based yoghurt offers a considerable appeal to a growing segment of consumers with certain dietary and health concerns.

Soy yoghurt presents fermented soymilk made with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Due to general dislike of the flavor of soy yoghurt, often described as "beany", it is not widely accepted by consumers.

*Lactobacillus acidophilus, Bifidobacterium* sp. and *Lactobacillus casei* are classified as probiotics since they are thought to exert beneficial health effects in the host by modulating the intestinal microflora (2). Recently, it was shown that some strains of *Bifidobacterium* are able to decrease the levels of pentanal and n-hexanal responsible for the beany flavour (3, 4). Therefore, to improve the growth of probiotic bacteria and production of organic acid, soymilk needs to be supplemented with various prebiotics such as raffinose or inulin or a combination of glucose and raffinose (4, 5, 6).

Recently, there has been an increasing interest in the use of natural food products into diet. Of these substances, bee products such as honey are among some of the most remarkable and versatile nutrients. Honey is a functional food that has a unique composition, antimicrobial properties and bifidogenic effect (7). It is composed primarily of fructose and glucose, fructooligosaccharides (4 to 5 %) that serve as prebiotic agents (8). Beside the sugars, small amounts of amino acids, lipids, along with some vitamins and minerals imparts improves its high nutritional value (9). Due to its healthy and natural image, honey has been widely used as a substitute sweetener in various foods. Several studies reported that honey had beneficial effects on the fermented milks through the improvement of bifidobacteria viability

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in probiotic fermented milks and longer shelf life (10, 11, 12). Honey may be an ideal sweetener for yoghurt due to its sugar concentration, low pH and a variety of beneficial nutritional properties.

Achieving the optimal rheological properties of yoghurt could be made by using different functional food ingredients such as whey protein concentrate (13) whey protein isolate (14), guar gum (15), a range of oligosaccharides, especially fructo-oligosaccharides. Previously rheological tests have shown that all soy yoghurts, irrespective of supplementation, had solid-like gel characteristics, but raffinose/glucose addition resulted in firmer products (16). Several researches have focused on the effect of fortifying yoghurt with honey also (12, 17, 18).

This work is aimed at producing a probiotic soy yoghurt supplemented with honey at different concentration (2 %, 4 % and 6 %) and at studying the effect of added honey on rheological properties of probiotic soy yoghurt during storage at  $4 \pm 1$  °C for 21 days.

## MATERIALS AND METHODS

#### Materials

Soymilk (2.2 % fat, 3.6 % proteins, 2.0 % carbohydrate) obtained from "ALNATURA" (EU, Germany) was used for the production of yoghurt samples. The initial pH of the soya milk was 6.85 (±0.05). Physical, chemical and microbiological characteristics of milk samples were entirely in accordance with the pertinent standards (Regulation of milk, dairy product, composites milk product and starter culture, Serbia, Belgrade, 2002). Linden honey "Krnjevac" (Krnjevo, Serbia), controlled quality (Regulation of honey, honey bee products and preparations, Serbia, Belgrade, 2003) was used in three different concentrations. Mixed probiotic starter culture DriSet BIOFLORA ABY 424: 70 % w/w Streptococcus thermophilus, 10 % w/w Lactobacillus bulgaricus, 10 % w/w Lactobacillus acidophilus, 10 % w/w Bifidobacterium ssp. (Vivolac Culture Corporation, Indiana, USA) was applied to achieve a concentration of 0.0025% in manufacturing yoghurt samples.

#### Yoghurt manufacturing

Soymilk was heated to 37°C and honey was added in three concentrations: 2 % w/v 4 % w/v and 6 % w/v. The milk was inoculated (37°C) with the chosen probiotic starter and incubated at the same temperature until pH 4.5 to 4.6 was reached. Fermentations were stopped by rapid cooling to 20°C and the samples of fermented milk were placed in a cold storage at 4°C±1. All experiments were repeated in triplicates, each analysis was done in duplicate and the average values were calculated.

#### Methods of analyses

After manufacturing, yoghurt samples were analyzed by measuring viscosity, syneresis and water-holding capacity (WHC). Viscosity was measured using a Brookfield DV-E viscosimeter (Brookfield Engineering Laboratories, Stoughton, MA, USA). The viscometer was operated at 20 rpm (spindle #4). Each result was recorded in mPa's after a 30 s rotation, during 3 min. Syneresis was determined by whey separation, and it was expressed in mL of whey separated during filtration of 50 g sample for 3 hours, at room temperature (19). Water-holding capacity was determined according to the procedure introduced by Guzman-Gonzalez et al. (20). A sample of about 20 g of yoghurt (Y) was centrifuged for 10 min at 1250x g at 4°C. The whey expelled (W) was removed and weighed. The water-holding capacity was calculated as:

WHC (%) = 
$$(Y - W) / Y \times 100$$

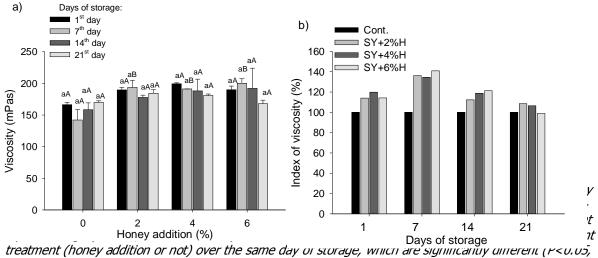
[1]

Analyses of the produced samples were carried out on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of storage at 4°C±1. The average value of 3 measurements was taken for further analysis. Two-way analysis of variance (ANOVA) was performed along with the Holm-Sidak test for comparison of means, with a probability of the results at a level of P<0.05. Values of different tests were expressed as the mean ± standard deviation (x ± SD). The data were analyzed by using SigmaPlot 11.0 (Sysstat Software, Inc. USA).

### **RESULTS AND DISCUSSION**

The rheological property of apparent viscosity of a product can significantly influences the acceptance and consumer purchase intention.

Viscosities of yoghurt samples over storage time are presented in Figures 1 and 2. Probiotic soy yoghurt viscosities of all samples were in the range: 152.20-200.21 mPas (Figures 1, 2a).



Holm-Sidak test).

Improving of viscosity by adding honey during the production of soy yoghurt was evident even after the first day of storage (Figures 1a, 2a), especially at the level of 4 % honey. Honey addition improved viscosity of probiotic soy yoghurt, after 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days of storage, at all added level of honey, respectively.

Table 1. Results of two way analy	sis of var	iance for yoghurt	t viscosity and stol	rage days	
Source of Variation	DF	SS	MS	F	Р
Yoghurt sample viscosity	3	5016.702	1672.234	12.910	< 0.001
Storage days	3	479.768	159.923	1.235	0.330
Yoghurt samples viscosity x storage day	9	2212.887	245.876	1.898	0.126
Residual	16	2072.455	129.528		
Total	31	9781.812	315.542		

Table 2. Multiple comparison procedures (Holm-Sidak method) after performing two-way ANOVA for viscosity of
different yogurt samples (within a different treatment) and particularly storage days. The results showed in table are
chosen for days where statistically significant difference is observed (P>0.05)

	Compariso	ns for yoghu	rt samples within 7 <sup>#</sup>	h day of storage	
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant
SY+4%H vs. SY+2%H	2.375	0.209	0.837	0.050	No
SY+6%H vs. SY+2%H	6.625	0.582	0.569	0.025	No
SY+4%H vs. Cont.	49.083	4.313	< 0.001	0.013	Yes
Cont. vs. SY+2%H	51.458	4.521	< 0.001	0.010	Yes
SY+6%H vs. Cont.	58.083	5.104	< 0.001	0.009	Yes
SY+4%H vs. SY+6%H	9.000	0.791	0.441	0.017	No

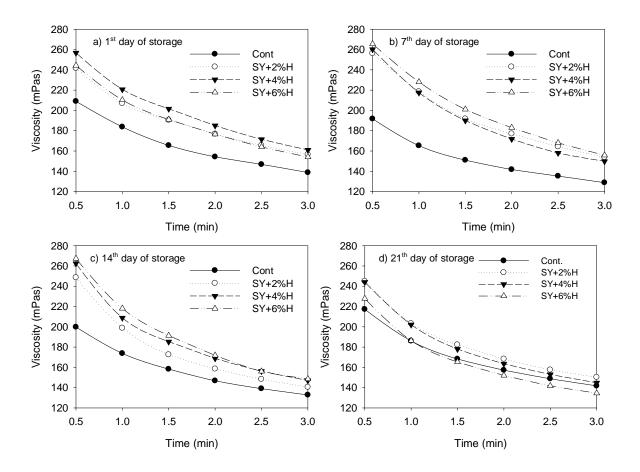
During the storage time, viscosity of soy yoghurts with honey addition slightly increased compared to control soy yoghurt. Honey supplemented soy yoghurts had the highest viscosity on the 7<sup>th</sup> day of storage, compared to control, and after that time viscosity slightly decreased (Figures 1a,b).

Produced soy milk yoghurt samples supplemented with 4% honey had the highest viscosity (180.88-199.42 mPas), while the control soy yoghurt had the lowest viscosity (152.13-169.88 mPas), but without statistically significant difference (P>0.05) (Figure 1, Tables 1, 2). These results showed that honey could improve yophurt viscosity, which improved the overall quality of products. An increase in viscosity by adding 4% honey to yoqhurt was reported previously by Wedad and Owayss (17).

The obtained results for index of viscosity showed that the viscosity of probiotic soy yoghurt samples containing honey were slightly increased (Figure 1b), which might be due to the possible high content of solids in honey, which led to an increase in both the viscosity and curd tension in analysed yoghurt samples. However, this viscosity changes induced by addition of different quantity of honey appeared to have no significant differences (P>0.05) compared to the control yoghurt, regardless of storage period (Figure 1b, Tables 1, 2).

As results show, control probiotic soy yogurt had the smallest viscosity on 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days of storage. However, control soy yoghurt had the smallest viscosity decrease during shearing (Figure 2) over 21 days of storage: for the 1<sup>st</sup> day viscosity decreased by 72.25 mPas; for the 7<sup>th</sup> day: 63 mPas; for the 14<sup>th</sup> day: 67 mPas and for the 21<sup>st</sup> day viscosity decreased by 75.5 mPas. The most pronounced viscosity decrease during shearing time appeared in the soy yoghurt with 4% honey addition on 1<sup>st</sup>, 7<sup>th</sup> and 21<sup>st</sup> day (viscosity decreased by 95.0 mPas, 110.5 mPas and 99.25 mPas, respectively), while soy yoghurt with 6 % honey addition had the most pronounced viscosity decrease on the 14<sup>th</sup> day of storage (viscosity decreased by 118.75 mPas) (Figure 2).

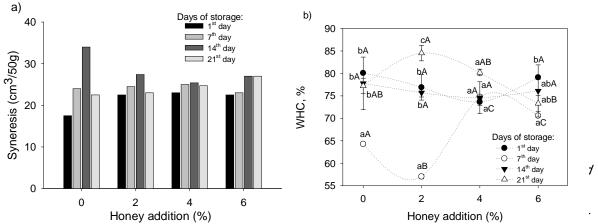
Honey added soy yoghurt had higher viscosity than control soy yoghurt after 0.5 min over shearing from 1<sup>st</sup> until 14<sup>th</sup> day of storage. However, the difference between viscosities of all yoghurt samples during the rest of shearing time became smaller, particularly after 2.5 min of shearing (Figure 2).



*Figure 2. Viscosity change of probiotic soy yoghurt with (and without) honey addition during shearing time on the 1<sup>st</sup> day of storage (a), 7<sup>th</sup> day (b), 14<sup>th</sup> day (c) and 21<sup>st</sup> day of storage (d) (SY-soy yoghurt; H-honey).* 

The less pronounced viscosity change of probiotic soy yoghurt was on the 21<sup>st</sup> storage day, regardless of honey addition. Donkor et al. (16) reported that supplementation with raffinose/ glucose produced firmer soy yoghurts. Due to the presence of nondigestible dietary fibre (oligosaccharides), honey probably could have similar effect on physicochemical properties of yoghurt as fructooligosaccharides, whose characteristics are well known (21, 22). Furthermore, it was previously shown that supplementation of milk with combination of honey/WPC and honey/inulin (23, 24) in yoghurt manufacturing, induced an increase in viscosity of yoghurt compared to control sample, over 21 day of storage.

Change of syneresis and WHC of probiotic soy yoghurt samples is presented in Figure 3. Probiotic soy yoghurt had syneresis in the range 17.5-34.0 ml/50g, while water holding capacity was in the range from 57.30 - 84.52 %. Despite the fact that honey could have high water holding capacity (25) no change in WHC were observed after 1<sup>st</sup> day of storage (Figure 3b). Surprisingly, the lowest syneresis also had control sample after 1<sup>st</sup> day of storage (Figure 3a). The most pronounced changes in WHC were observed on the 7<sup>th</sup> day and at the end of storage.



capital letters denote the results, within a different treatment (honey addition or not) over the same day of storage, which are significantly different (P<0.05, Holm-Sidak test).

Source of Variation	DF	SS	MS	F	Р
Yoghurt sample WHC	3	20.621	6.874	1.093	0.381
Storage days	3	726.517	242.172	38.505	< 0.001
Yoghurt samples WHC x storage day	9	532.222	59.136	9.402	< 0.001
Residual	16	100.630	6.289		
Total	31	1379.98	944.516		

Table 3. Results of two way analysis of variance for yoghurt WHC and storage days

With the increasing concentration of honey addition WHC increased, especially at level 6 % honey. Generally, over storage time WHC increased slightly (Figure 3b). Furthermore, lower content of honey (2 %) yielded to lower porosity of protein matrix, weaker protein interactions and strength of protein network, which resulted in lower WHC. Our results are particularly opposite to previous results of Sert et al. (18) which showed that increasing in sunflower honey concentration (2, 4 and 6 %) decreased syneresis and increased WHC, significantly.

However, soy yoghurt with 4% honey had the most stable values of syneresis and WHC over storage time (Figure 3), while the less stable WHC had soy yoghurt with 2% honey and less stable syneresis had control yoghurt. Addition of 4 and 6% honey significantly increase (P < 0.05) WHC compared to control soy yoghurt after 7<sup>th</sup> and 21<sup>st</sup> day of storage (Figure 3b, Tables 3, 4, 5). Stable values of WHC of yoghurt samples enriched with honey during storage time could be explained by the fact that WHC can be increased by adding stabilisers that interact with the protein network, which in this case is honey. In most honeys, fructose predominates (25), and fructose has a high water-binding capacity so it may react with many starches.

Table 4. Multiple comparison procedures (Holm-Sidak method), after performing two-way ANOVA for WHC: different storage days versus particularly soy yogurt sample (within a same treatment). The results showed in table are chosen for yoghurt samples where statistically significant difference is observed (P>0.05)

Comparisons for storage days within control soy yogurt	

	Comparis	sons for stora	nge days within cont	rol soy yogurt	
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1 vs. 7	15.805	6.302	< 0.001	0.009	Yes
14 vs. 7	13.505	5.385	< 0.001	0.010	Yes
21 vs. 7	12.950	5.164	< 0.001	0.013	Yes
1 vs. 21	2.855	1.138	0.272	0.017	No
1 vs. 14	2.300	0.917	0.373	0.025	No
14 vs. 21	0.555	0.221	0.828	0.050	No
	Com	parisons for .	storage days within	SY+2%H	
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
21 vs. 7	27.490	10.962	< 0.001	0.009	Yes
1 vs. 7	19.855	7.917	< 0.001	0.010	Yes
14 vs. 7	18.680	7.449	< 0.001	0.013	Yes
21 vs. 14	8.810	3.513	0.003	0.017	Yes
21 vs. 1	7.635	3.044	0.008	0.025	Yes
1 vs. 14	1.175	0.469	0.646	0.050	No

	Com	barisons for .	storage days within	SY+6%H	
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1 vs. 7	8.525	3.399	0.004	0.009	Yes
1 vs. 21	5.785	2.307	0.035	0.010	No
14 vs. 7	5.520	2.201	0.043	0.013	No
1 vs. 14	3.005	1.198	0.248	0.017	No
14 vs. 21	2.780	1.109	0.284	0.025	No
21 vs. 7	2.740	1.093	0.291	0.050	No

Comparisons for storage days within SY+6%H

Table 5. Multiple comparison procedures (Holm-Sidak method) after performing two-way ANOVA for WHC of different yogurt samples (within a different treatment) and particularly storage days. The results showed in the table are chosen for yoghurt samples where statistically significant difference is observed (P>0.05)

	Compariso	ons for yogur	t samples within 7 <sup>th</sup>	day of storage	
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant
SY+4%H vs. SY+2%H	17.730	7.070	< 0.001	0.009	Yes
SY+6%H vs. SY+2%H	13.550	5.403	< 0.001	0.010	Yes
SY+4%H vs. Cont.	10.490	4.183	< 0.001	0.013	Yes
Cont. vs. SY+2%H	7.240	2.887	0.011	0.017	Yes
SY+6%H vs. Cont.	6.310	2.516	0.023	0.025	Yes
SY+4%H vs. SY+6%H	4.180	1.667	0.115	0.050	No
	Compariso	ns for yogurt	t samples within 21st	<sup>t</sup> day of storage	
Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant
SY+2%H vs. SY+6%H	11.200	4.466	< 0.001	0.009	Yes
SY+2%H vs. Cont.	7.300	2.911	0.010	0.010	No
SY+4%H vs. SY+6%H	6.890	2.747	0.014	0.013	No
SY+2%H vs. SY+4%H	4.310	1.719	0.105	0.017	No
Cont. vs. SY+6%H	3.900	1.555	0.139	0.025	No
SY+4%H vs. Cont.	2.990	1.192	0.251	0.050	No

However, our finding points the stability of produced soy yoghurt enriched with honey as a very important characteristic during the refrigerated storage.

## CONCLUSION

From the foregoing results, it could be concluded that, probiotic soy yoghurt can be successfully produced with 4 % honeys as ingredient. The 4 % honey addition to soy milk increased viscosity and WHC, while it decreased the syneresis of soy probiotic yoghurt during 21 day of storage. The knowledge obtained from this study could be applied in the development of honey enriched probiotic soy yoghurts as a novel formulation for functional fermented food. Our results are also the contribution to a better understanding of the variation in physicochemical properties of soy yoghurt regarding the honey addition.

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# UTICAJ DODATKA MEDA NA REOLOŠKE OSOBINE PROBIOTIČKOG SOJINOG JOGURTA

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Jogurt je kompleksni reološki sistem predstavljen kao trodimenzionalni matriks isprepletenih kazeinskih micela i lanaca unutar kojih se nalazi voda. Struktura gela čvrstog jogurta zavisi od mnogih faktora, uključujući sastav mlijeka, sadržaj suve materije i proteina, te termičkog tretmana mlijeka. Poznato je da sadržaj proteina u mlijeku ima najsnažniji uticaj na jačinu gela, viskozitet i sinerezu.

Cilj ovoga rada je da se ispita uticaj dodatka meda (0, 2, 4 i 6 %) na reološke osobine probiotičkog sojinog jogurta tokom 21 dana skladištenja. Reološki parametri su praćeni mjerenjem viskoziteta, sinereze i sposobnosti zadržavanja vode kod proizvedenih uzoraka. Rezultati su pokazali da se, sa povećanjem koncentracije meda u sojinom jogurtu, povećavao viskozitet i sposobnosti zadržavanja vode, a smanjivao intenzitet sinereze tokom 21 dana skladištenja.

Ključne riječi: sojin jogurt, med, reologija, sposobnosti zadržavanja vode

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