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Original scientific paper

CHANGES IN THE VISCOSITY OF COW AND SOY YOGURT DURING STORAGE WITH THE APPLICATION OF INULIN

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Abstract: *The aim of this work was to examine changes in the viscosity of cow's and soy probiotic yogurt with different inulin content (IN) during 10 days of storage at 5°C. Cow and soy milk without additives and with the addition of 1.0% IN and 2.0% IN were fermented with 0,005g/l inoculum of mixed probiotic starter culture DriSet BIOFLORA, ABY 438: Lactobacillus acidophilus, Streptococcus thermophilus and Lactobacillus bulgaricus (Vivolac Culture Corporation, Indiana, USA). Different varieties of cow (cy) and soy (sy) yogurt (Cont; 1%IN-cy; 2%IN-sy; sy; 1%IN-sy; 2%IN-sy) were produced. Viscosity changes were monitored on the 1st, 5th and 10th storage days. The viscosity of the samples was measured during 3 min (the value was read every 30 s) at a rotation speed of the spindle (Ø4) of 20 rpm, using a BROOKFIELD Digital Viscometer, DV-E (Brookfield Engineering Laboratories, USA). Changes in pH during fermentation and storage were also observed using a laboratory pH meter (pH HI2002 Meter, HANNA Instruments, USA). All yogurt samples produced from cow's milk fermented in 5 hours, and samples produced from soy milk in 8 hours, which means that inulin supplementation did not affect the rate of fermentation. During storage at 5°C, slightly higher pH values were recorded in samples with the addition of 1%IN,sy and 2%IN,sy, compared to all other samples, but stability was observed inside each sample individually, during 10 storage days. The viscosity produced from cow's milk, regardless of additives, had statistically significantly higher values ($p<0.05$) compared to the viscosity values of soy yogurt. Inside the samples, no significant changes in viscosity were observed from the 1st to the 10th day of storage.*

Keywords: *viscosity, cow and soy yogurt, inulin, storage time*

Introduction

The consumption of fermented milk in the world and in our country has increased multiple in recent years. The reason for the rise in yogurt consumption, as the most popular product in the group of fermented milks, is the increase in its nutritional and health value while retaining desirable physical and sensorial properties during storage [1]. Yogurt production is based on controlled lactic acid fermentation of lactose into lactic acid, with partial protein coagulation. It comes to the change in the acidity of the yogurt, by the action of yogurt bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are added during the production process, and further leads to a change in the viscosity and product structure, and thus the tendency to syneresis.

The quality of yogurt depends on physical, microbiological and technological factors, but primarily on the texture and rheological properties of the formed gel. Viscosity is one of the rheological parameters whose properties are determined by the type of

microbiological culture and previous thermal treatment of milk [2], chemical composition of milk [3], fermentation kinetics, inoculum amount, temperature and duration of fermentation [4], initial viscosity of milk, performed homogenization, method standardization of milk [5] procedures with yogurt after fermentation, etc.

On the other hand, in the production of yogurt, primarily due to its sensorial properties, different types of supplements are used. Among the significant supplements certainly is included the indigestible polyfructan inulin, which achieves its prebiotic effect by selectively stimulating the growth and activity of non-pathogenic bacteria, mainly bifidobacteria and lactobacilli [6], suppresses harmful bacteria (clostridia) [7]. Inulin has multiple purposes, so it can be used as a low-energy sweetener, and due to its properties to stabilize the structure of the water phase, it can be used as a substitute for milk fat, giving yogurt firmness and a full fat-like taste in the mouth [8; 9; 10; 11]. In addition, inulin can influence the modification of the textural properties of the final product [12]. The amount in which inulin is added is also important. The optimal amount is up to 1%, since its larger addition negatively affects the sensory properties of yogurt and causes increased syneresis [8].

In the production of fermented milk, as a suitable substitute for cow's milk, soy milk is becoming increasingly important, especially for people who are allergic to cow's milk and people with a vegetarian and vegan diet. Although soy milk is considered unfavorable to consumers due to its bean flavor, its palatability is improved by fermentation. In addition to improving organoleptic properties, fermentation contributes to better nutritional value [13] and antioxidant capacity of the beverage [14, 15]. Some results suggest that fermentation by lactic acid bacteria could increase the bioactivity of soy milk [16]. Soy and cow's milk have very little in common, but both contain high-value proteins. Certainly, the amino acid profile of soy milk differs from the amino acid profile of cow's milk protein. Soy proteins contain all the amino acids needed for human nutrition, except amino acids with sulfur (methionine and cystine). Soy yogurt is produced by fermenting soy milk with the help of a starter culture mixture consisting of *Streptococcus thermophilus*, *Lactobacillus delbureckii* ssp. and *Bifidobacterium* spp. [17]. These bacteria have α -galactosidase enzyme activity [18] that allows them to utilize sugars such as the oligosaccharides raffinose and stachyose, and sufficient proteolytic activity to support growth in soya milk [19]. Fermentation itself reduces the proportion of stachyose and raffinose, which can cause digestive disorders in the body [20], and the health value of the product increases significantly. In addition, isoflavone aglycols and peptides, which are produced in soy milk during fermentation, have anticancer effects [21], they help to prevent osteoporosis and cardiovascular diseases [22], they reduce the risk of diabetic diseases and prevent chronic inflammation [23; 24] and affect the lowering of cholesterol in the blood and the prevention of fat accumulation in the liver [25].

The aim of the work was to examine changes in the viscosity of cow and soy probiotic yogurt with different inulin content (IN) during 10 days of storage at a temperature of 5°C.

Material and methods

Milk and inulin

- It was used homogenized cow's milk "Moja Kravica" (2.8% fat, 3.0% protein, 4.5% lactose) produced by Mlijekoprodukt d.o.o. Kozarska Dubica (Bosnia and Herzegovina) and soy milk, "dmBioPflanzendrink, Soja Drink natur" (1.9% fat, 3.2% protein, 1.8% carbohydrates, 0.03% salt) produced by Karlsruhe (EU, Germany). The initial pH of cow's milk was 6.64 (± 0.01), density 1.030, and the pH of soy milk was 6.99 (± 0.01).
- Inulin (Fibruline® Instant Cosucra Groupe Warcoing S.A., Belgium) contains min 90% inulin, max 10% fructose, glucose and sucrose, max 0.3% ash.

Cultures

A mixed probiotic culture DriSet BIOFLORA, ABY 438: *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Vivolac Culture Corporation, Indiana, USA) was used in a concentration of 0.005 g/l.

Yoghurt Manufacturing

After heating cow's and soy milk to 55°C, inulin (IN) was added in two concentrations: 1.0% v/v, and 2.0% v/v. Then the milk was cooled to 37°C and inoculated with 0.005g/l inoculum of mixed probiotic culture. The prepared samples were incubated at the same temperature until reaching pH of 4.6. After fermentation, the samples are quickly cooled to 20°C and placed in a refrigerator at 5°C ± 1 . Each examination was repeated three times. The design of the experiment and the product code are presented in Table 1. The samples of cow's yogurt without any addition were considered as control samples.

Table 1. Codes of different yogurt mixes present in this study

Code*	Treatment
Contr	Control cow's yogurt
1%IN-cy	Cow's yogurt with 1.0 % inulin (10 g kg ⁻¹ IN)
2%IN-cy	Cow's yogurt with 2.0 % inulin (20 g kg ⁻¹ IN)
sy	Soy yogurt without any addition
1%IN-sy	Soy yogurt with 1.0% inulin (10 g kg ⁻¹ IN)
2%IN-sy	Soy yogurt with 2.0% inulin (20 g kg ⁻¹ IN)

*cy- cow's yogurt, IN-inulin, sy-soy yogurt

Methods of Analysis

After manufacturing, yogurt samples were analyzed by measuring pH value during fermentation and over 10 storage days. The pH value was measured with a laboratory pH meter (pH HI2002 Meter, HANNA Instruments, USA).

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.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed with the Tukey test for comparison of mean values, with the probability of results at the level of $P < 0.05$. Data were analyzed using Excel 2016.

Results and Discussion

Fermentation of milk samples

The efficiency of the starter culture in different milk and with different concentrations of inulin (1 and 2%) was followed by changes in the pH value during fermentation (Figure 1). In all samples, the fermentation was stopped at close to pH 4.6, but the listed pH samples reached in different times. During the first two hours, the pH gradually decreased in all samples, and in the third hour, a much faster decrease in the pH value was recorded. After the third hour, a mild decrease in pH is established again.

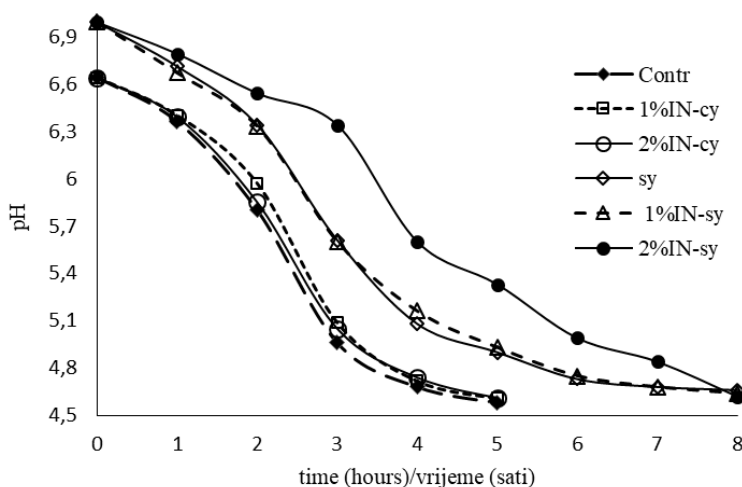


Figure 1. Fermentation time of probiotic yogurt made of cow's milk and soymilk fortified with inulin (1.0 and 2.0 %)

In relation to the type of milk, the decrease in pH was faster in cow's milk than in soy milk, which is in agreement with some previous results [26]. However, different additions of inulin did not significantly affect the fermentation time. Thus, all 3

samples of cow's milk (control and samples with 1% and 2% IN) fermented in 5 hours, while all samples of soy milk fermented in 8 hours. These results are not in agreement with the results of Iancu et Nicolau [27], in which the fermentation time decreases with an increase in the percentage of inulin during yogurt production. Also, the results obtained by Stijepić et al [26] do not agree with these results, where in soy milk, a higher percentage of inulin addition of 1.5% and 3.0% influenced faster fermentation compared to samples with 0.5% inulin and the control sample.

Change in pH during storage

Changes in pH during storage of traditional and soy yogurt with the addition of 1% or 2% inulin are shown in Figure 2.

Although fermentation in all samples was stopped at pH close to 4.6, after the 1st storage day, different pH values were observed for cow's and soy yogurt. While soy samples with 1% and 2% inulin maintained the same pH value (4.64 and 4.62 respectively), the other samples recorded a certain decrease in pH (for Contr sample 4.26, for samples: with 1%IN-cy 4.29, with 2% IN-yc 4.37 and with sy 4.37). It can also be noted that on the 1st storage day there is a statistically significant difference ($p < 0.05$) between all samples, except between soy samples with 1%IN-sy and 2%IN-sy, and between soy yogurt samples without additives (sy) and cow's yogurt with 2%IN-cy.

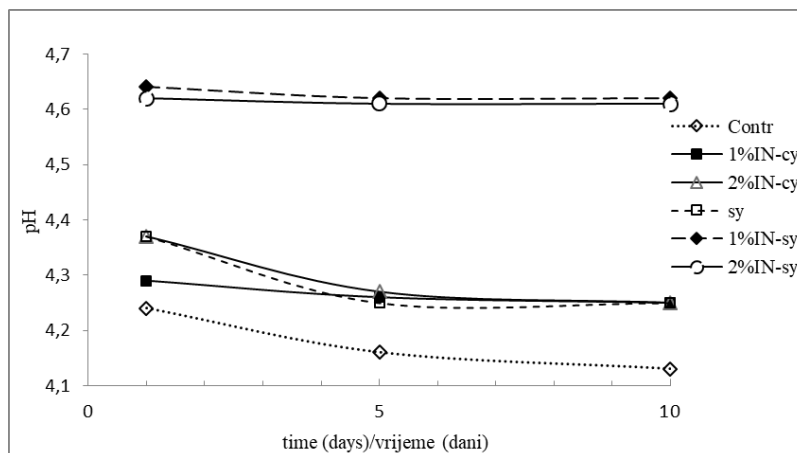


Figure 2. Change in pH of probiotic cow's and soy yogurt with different additions of inulin (IN) during storage

There were similar ratios between the samples at the end of the 10th day of storage, with the fact that the samples produced from soy milk with inulin additives showed better stability, where there was no change in pH. In other samples, a certain decrease in pH value was observed, and a statistically significant difference ($p < 0.05$) was noticed in soy (sy) and control yogurt (Contr) between the 1st and 5th day of storage (Table 2).

Table 2. Calculated statistics of Tukey's test for pH during storage of cow's and soy yogurt with different additions of inulin (IN)

Storage days	pH value yogurt samples					
	Contr	1%IN-cy	2%IN-yc	sy	1%IN-sy	2%INsy
1	aA	abA	bA	bA	cA	cA
5	aB	abA	abB	abB	cA	cA
10	aB	bA	bB	bB	cA	cA

^{ab} – different lowercase letters indicate a statistically significant difference between different samples on the same storage day (Tukey, $P < 0.05$)

^{AB} – different capital letters indicate a statistically significant difference between the same samples on different storage day (Tukey, $P < 0.05$)

Change in viscosity during storage

Viscosity is one of the most important rheological parameters that can significantly affect the sensory properties and acceptability of the product by consumers. Values of apparent viscosity of cow's and soy yogurt with different additions of inulin are shown in Figure 3. The best results, with the highest viscosity values, were shown by samples of cow's yogurt with 2% IN-cy, whose values from the 1st to the 10th day of storage ranged from 155.7 mPas to 154.3 mPas. On the other hand, the lowest viscosity values were in samples with 2% IN-sy from 111.5 mPas on the 1st day of storage to 115.5 mPas on the 10th day of storage.

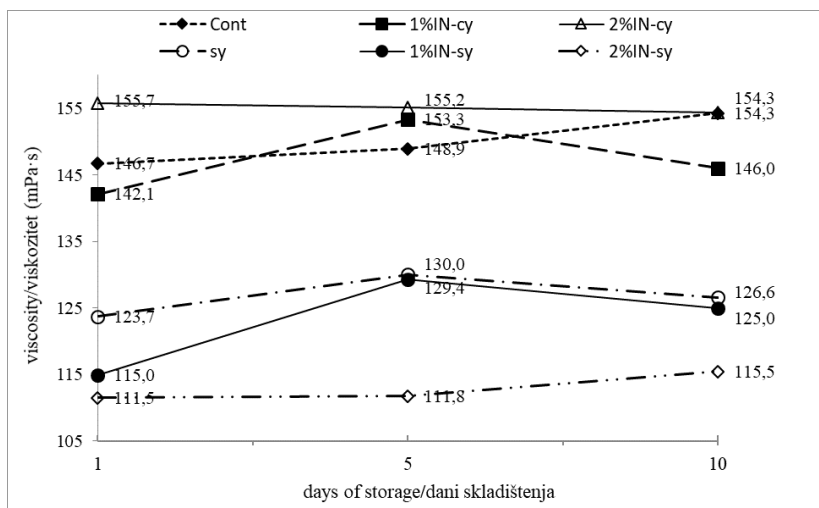


Figure 3. Viscosity changes of cow's and soy yogurt with different additions of inulin during 10 days of storage

Observing the yogurt samples, produced from cow's milk, it can be concluded that different additions of inulin did not have a statistically significant effect ($p > 0.05$) on the increase in viscosity during the entire storage time. This is consistent with the results of Akalin et al. [28] who did not find any effect of addition of inulin-type

fructooligosaccharides on the viscosity values of probiotic yogurt. Also, some research showed that the addition of 2% of inulin [29], or 0.7% or 2.7% inulin [30] generally did not significantly affect the apparent viscosity of yogurt during 21 storage days. In contrast, Helal et al. [31] found that the apparent viscosity of yogurt increased by adding inulin up to 2% to yogurt, while the results of Rezaei et al. [32] showed a significant effect of the addition of 1% and 2% inulin on the viscosity improvement of frozen yogurt.

On the other hand, in soy yogurt samples with an increase in inulin concentration, there was a decrease in viscosity. Thus, it was observed that there are statistically significantly lower viscosity values of samples with 2%IN-sy ($p<0.05$) compared to samples without additives (sy) and with 1%IN-sy. Some earlier studies have shown similar results [12, 26].

If we compare the viscosity of yogurt samples produced from cow's milk compared to those produced from soy milk, it is observed that the viscosity of all soy yogurt samples is statistically significantly lower ($p<0.05$) compared to the viscosity of cow yogurt samples (Table 3).

In general, all yogurt samples, regardless of the amount of inulin and the type of milk from which they were produced, showed very good viscosity stability from the 1st to the 10th day of storage ($p>0.05$).

Table 3. Calculated statistics of the Tukey test for viscosity during storage of cow's and soy yogurt with different additions of inulin (IN).

Storage days	viscosity yogurt samples					
	Contr	1%IN-cy	2%IN-yc	sy	1%IN-sy	2%INsy
1	abA	bA	aA	cA	cdA	cdA
5	aA	aB	aA	bA	bB	cA
10	aB	abA	aA	cA	cB	dA

ab – different lowercase letters indicate a statistically significant difference between different samples on the same storage day (Tukey, $P<0.05$)

AB – different capital letters indicate a statistically significant difference between the same samples on different storage day (Tukey, $P<0.05$)

Conclusion

Different additions of inulin did not significantly affect the fermentation time, so all samples of cow's yogurt fermented in 5 hours, and soy yogurt in 8 hours. Better stability during storage was shown by the samples produced from soy milk with added inulin, in which there was no change in pH, while in all other samples a slight decrease was recorded from the 1st to the 5th day of storage. Analysis of variance showed that there are significant differences in viscosity between the studied samples of cow's and soy yogurt. Tukey's test determined that different additions of inulin did not statistically significantly affect the increase in viscosity of cow yogurt during storage,

while in soy yogurt samples with an increase in inulin concentration, there was a decrease in viscosity.

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PROMJENE VISKOZITETA KRAVLJEG I SOJINOG JOGURTA TOKOM SKLADIŠTENJA UZ PRIMJENU INULINA

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Sažetak: Cilj ovog rada bio je ispitati promjene viskoziteta kravljeg i sojinog probiotskog jogurta sa različitim sadržajem inulina (IN) tokom 10 dana čuvanja na temperaturi 5°C. Kravlje i sojino mlijeko bez dodataka i sa dodatkom 1,0% IN i 2,0% IN je fermentisano sa 0,005g/l inokuluma probiotske starter kulture DriSet BIOFLORA, ABY 438: *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Vivolac Culture Corporation, Indiana, USA). Proizvedene su različite varijante kravljeg (cy) i sojinog (sy) jogurta (Cont; 1%IN-cy; 2%IN-sy; sy; 1%IN-sy; 2%IN-sy). Promjene viskoziteta praćene su 1., 5. i 10. dana skladištenja. Viskozitet uzoraka mjereno je tokom 3 min (očitanje je vrijednost svakih 30 s) pri brzini rotacije vretena (Ø4) od 20 o/m, korištenjem BROOKFIELD Digital Viscometer, DV-E (Brookfield Engineering Laboratories, USA). Praćene su i promjene pH vrijednosti tokom fermentacije i skladištenja pomoću laboratorijskog pH-metra (pH HI2002 Meter, HANNA Instruments, USA). Svi uzorci jogurta proizvedeni od kravljeg mlijeka su fermentisali za 5 sati, a uzorci proizvedeni od sojinog mlijeka za 8 sati, što znači da suplementacija inulinom nije uticala na brzinu fermentacije. Tokom skladištenja na 5°C nešto više pH vrijednosti zabilježene su kod uzoraka sa dodatkom 1%IN,sy i 2%IN,sy, u odnosu na sve ostale uzorke, ali je unutar svakog uzorka pojedinačno uočena stabilnost tokom 10 dana skladištenja. Viskozitet proizveden od kravljeg mlijeka, bez obzira na dodatke, imao je statistički značajno veće vrijednosti ($p < 0,05$) u odnosu na vrijednosti viskoziteta sojinog jogurta. Unutar samih uzoraka, ne primjećuju se značajne promjene u viskozitetu od 1. do 10. dana skladištenja.

Ključne riječi: viskozitet, kravlji i sojin jogurt, inulin, vrijeme skladištenja