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Food industry

MILK AND DAIRY PRODUCTS

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SCIENTIFIC PAPER

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PHYSICOCHEMICAL AND TEXTURAL CHARACTERISTICS OF FERMENTED DAIRY BEVERAGES DURING STORAGE

The aim of this study was to examine the influence of three types of non-conventional starters: kombucha inoculum, microfiltrated kombucha inoculum and concentrated kombucha inoculum on physicochemical and textural changes of fermented dairy beverages during storage. The research revealed that the chemical composition of these samples is different. During the 10 days of storage lactose content decreased, but galactose and (L)-lactic acid content increased in all fermented dairy beverage samples. pH value of samples ranged from 4.05 to 4.25 after ten days of storage. The differences between the values of textural characteristics of fermented dairy beverages are not significant during storage.

Key words: fermented dairy beverages • chemical characteristics • textural properties • storage

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INTRODUCTION

Fermented milk beverages represent a very diverse group of highly nutritional value products obtained by milk fermentation with the application of the appropriate starter cultures. In addition to traditional thermophilic starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*) great progress in modern technology of fermented milk beverages is made by the development of new types of probiotic bacteria, most commonly by *Lactobacillus* and *Bifidobacterium* strains. Fermented milk beverages containing probiotics and/ or prebiotics have a positive impact on the health status of the human body. They represent one of the most prominent examples of functionnal foods (Tamime, Robinson, 2004; Tamime, 2006; Tamime et al., 2006; Milanović et al., 2009; Iličić, 2010).

The application of kombucha (non-conventional starter) in the technology of fermented dairy products is a very interesting area for research, in terms of biochemical and structural transformations of milk. Kombucha is a symbiotic association of yeast and acetic acid bacteria, traditionally cultivated on a sweetened black tea, but also it may be cultivated on other substrates such as: dark beer, coca-cola, vine (red or white), topinambur extract, echinacea, mint and Rtanj tea, whey, lactose, and others (Petrović, Lončar., 1996; Dufresne, Farnoworth, 2000; Malbaša et al., 2009, Iličić et al., 2012). This symbiotic association of yeast and acetic acid bacteria causes, by complex biochemical transformations, sugar fermentation and production of primary metabolites: galactose, fructose, glucose, acetic acid, lactic acid, gluconic acid, glucuronic acid, glycerol, some vitamins of complex B and vitamin C, katehin, and small amount of ethanol (Jajabalan et al., 2007; Malbaša, 2009; Milanović et al., 2002). In recent researches kombucha has been applied in combination with probiotics for milk fermentation, with the aim to improve physicochemical and textural characteristics of new kombucha fermented milk products (Milanović et al., 2012). The quality of kombucha fermented milk beverages depends on different factor, where packaging conditions are the most important. Pejic et al. (2012) revealed that the fermentation during storage of kombucha fermented beverages packed under MAP conditions is slower, and its effect on the product characteristics was not pronounced.

The aim of this study was to investigate the influence of type and concentration of kombucha inoculum: a) cultivated on black tea, b) concentrated using microfiltration, and c) concentrated by evaporation, on the milk fermentation of 0.9% fat content. A special phase of the research included the monitoring of biochemical transformations of milk components under the influence of kombucha, and textural changes of the beverage during storage.

MATERIALS AND METHODS

Fermented milk beverages production

Homogenized and pasteurized cow's milk was provided by AD Imlek, Division Novi Sad Dairy of the following characteristics: 0.9 g/100g milk fat, 9.85 g/100g dry matter, 3.15 g/100g protein, 4.74 g/100g lactose and 0.78 g/100g ash.

Kombucha inoculum was prepared according to procedure published by Malbaša et al. (2009). Kombucha was cultivated on black tea (*Camellia sine-*

sis - 1.5 gL^{-1}) with saccharose concentration of $70gL^{-1}$. The tea was cooled to room temperature, after which inoculum from previous fermentation was added in concentration of 10% (v/v). Incubation was performed at 25°C for 7 days. pH value of kombucha inoculums were 3.07±0.02.

Three types of kombucha inoculums as a starter culture were used:

1. Kombucha inoculum
2. Kombucha inoculum concentrated with membrane filtration, using the apparatus containing the tubular ceramic membrane module (200 nm) at the process parameters: temperature 25ºC, excess pressure on the input and output modules 40 kPa and a flow of 5 L/min. The degree of concentration was 2.3.
3. Kombucha inoculum concentrated by evaporation - the concentration of kombucha (dry matter 6.5%) was carried out under vacuum at a temperature of 40°C, until dry matter reached 68%.

In 1000 mL of milk heated to 42ºC three types of prepared inoculums were added: a) 10% kombucha inoculum taken immediately after cultivation (KB), b) 10% kombucha inoculum concentrated by microfiltration (MFB) and c) 1.5% kombucha inoculum concentrated by evaporation (CKB). Fermentation lasted until pH reached 4.5, and after that gel was cooled to a temperature of 8ºC, and homogenized by an electric mixer. All products were stored for 10 days in a refrigerator at temperature of 4ºC. The production of the samples was repeated 3 to 5 times.

The analyses of milk and fermented dairy beverages

The following physicochemical analyses: dry matter (TS) by oven drying (ISO 6731, IDF 21, 2010), total protein (TP) by Kjeldahl method (ISO 8968-1: ISO 8968-1:2008), fat by Gerber method (ISO/IDF, 105, 1981) (AOAC, 2005) were determined in all samples (Carić et al., 2000). The content of lactose, galactose and L(+)–lactic acid were carried out using the enzyme tests (lactose/galactose-test K-LACGAR 12/05 and L-lactic acid-test K-DLATE 11/05) Megazyme, Ireland (Bergmeyer, 1988).

Total sugar content was calculated by the following formula:

$$TS=DM-(TP+MF+Ash).$$

The energy value of fermented dairy beverages was calculated as follows:

EV= (% proteins • 4.4 + % milk fat • 9.3 + % total sugar• 4.1) • 4.186 (kJ/100g)

Textural characteristics

Textural characteristics of fermented dairy beverages were examined by Texture Analyzer TA.XPplus (Micro Stable System, England) at temperature of +4ºC. The force of compression was measured by using a disc A/BE with a diameter of 35 mm, and weight extension bar whose load is 5kg. It was used the option "Return to Start". Speed disk displacement before and during the test was 1.0 mm/s. Disk has exceeded the distance of 30 mm.

Statistical analysis

Statistical analysis of results was carried out with the computer software program "Statistica” and were expressed as average, standard deviation of values obtained at three independent experiments STATISTICA version 6 (2001) (StatSoft Inc, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Chemical composition and energy value of functional fermented dairy beverages produced from milk with 0.9% fat and by using three types of kombucha inoculums are shown in Table 1.

The dry matter content in the functional fermented beverages is different among the samples produced with the same and different types of inoculum. The samples produced by microfilmtrated inoculum have the lowest average dry matter content and total sugars, while the samples produced with the use of kombucha have the slightly higher content. On the other hand, in sample produced by kombucha inoculum concentrated by evaporation, dry matter content and total sugars is 20% higher compared to samples produced with microfiltrated kombucha inoculum.

Lactose content in all samples decreased during storage, with different dynamics depending on the type of used inoculum. The greatest differences in lactose concentration are on ten day storage. The lowest changes were obtained in the samples produced with concentrated kombucha inoculum (Fig.1a). These changes are the result of the total number and the ratio of acetic acid bacteria and yeast in the used inoculums. Also, Belloso-Morales and Hernandez-Sanchez (2003) investigated metabolic activities of kombucha cultivated on black tea, reconstitution whey, sweet and acid whey and followed decreasing of lactose content during 100 hours of fermentation. Their results had similar trend as fermented milk samples produced with kombucha inoculums.

The content of galactose in all samples slightly increases during storage (Fig. 1b). All fermented milk with kombucha inoculums have the same trend of galactose content during ten days storage. On the other hand, the results of Belloso-Morales and Hernandez-Sanchez (2003) showed that total content of glucose and galactose decreased in all sample (black tea, sweet and acid whey) during 100 hours of fermentation.

The content of L-lactic acid (Fig. 1c) was significantly higher than the content of D-lactic acid (Fig. 1d), and it decreases in fermented beverages.

Table 1. CHEMICAL COMPOSITION AND ENERGY VALUE OF FERMENTED DAIRY BEVERAGES
Tabela 1. HEMIJSKI SASTAV I ENERGETSKA VREDNOST FERMENTISANIH MLEČNIH NAPITAKA

Sample Uzorak	COMPONENT CONTENT SADRŽAJ KOMPONENTE (g/100g)					Energy value Energetska vrednost (kJ/100g)
	Dry matter Suva materija	Milk fat Mlečna mast	Total proteins Ukupni proteini	Total sugars Ukupni šećeri	Ash Pepeo	
KB	10.92±0.33	0.8	2.56±0.22	6.78±0.19	0.68±0.02	198.55
MKB	9.63±0.18	0.8	2.78±0.10	5.27±0.10	0.68±0.03	176.69
CKB	11.38±0.45	0.8	3.19±0.06	6.57±0.18	0.72±0.04	206.55

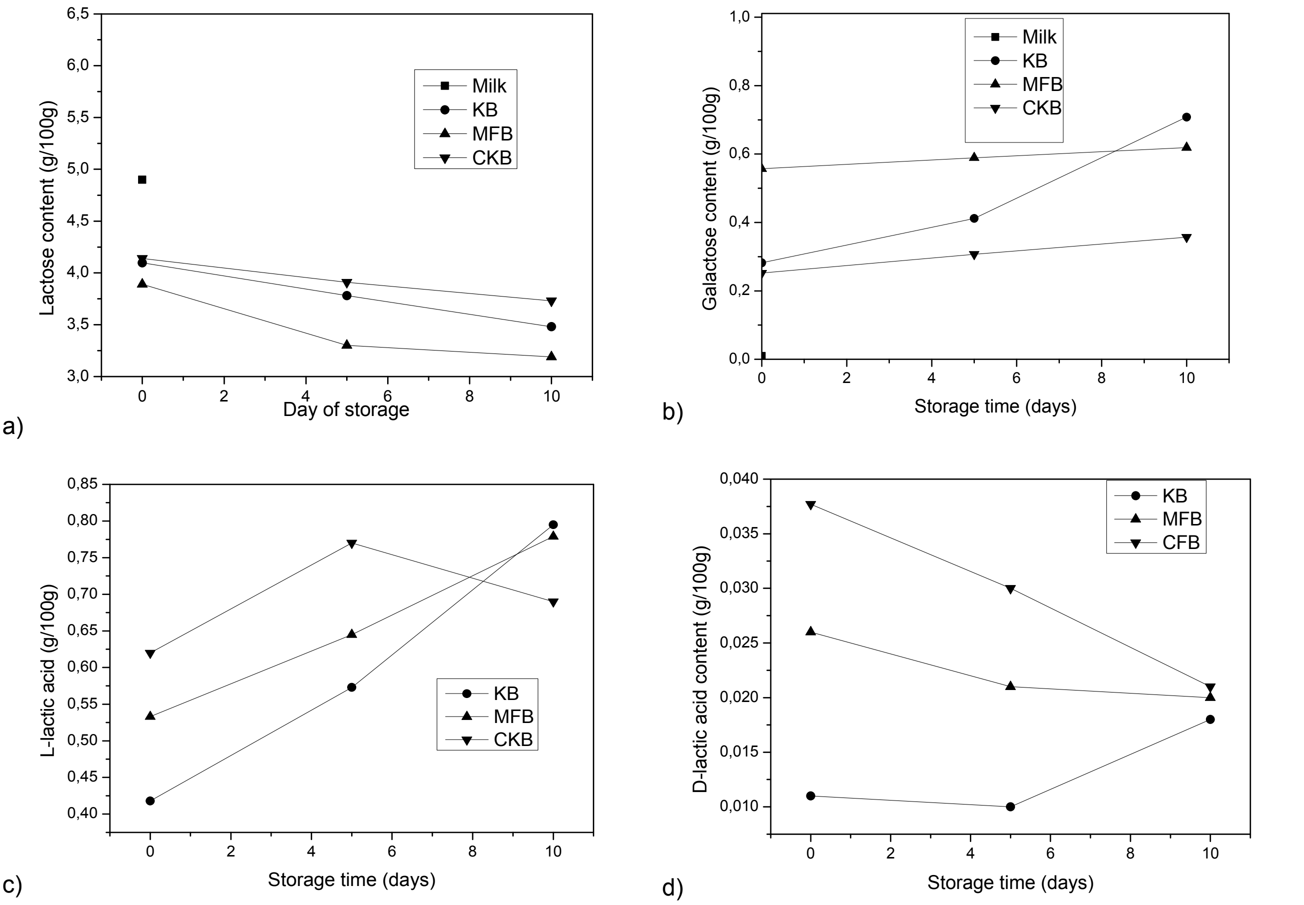

Figure 1. CHANGES OF COMPONENTS OF FERMENTED MILK BEVERAGES PRODUCED FROM MILK WITH 0.9% FAT USING NATIVE INOCULUM, INOCULUM CONCENTRATED BY MICROFILTRATION AND INOCULUM CONCENTRATED BY EVAPORATION DURING STORAGE: A) LACTOSE B) GALACTOSE C) L-LACTIC ACID D) D-LACTIC ACID.

Slika 1. PROMENE SADRŽAJA KOMPONENTI FERMENTISANIH MLEČNIH NAPITAKA PROIZVEDENIH IZ MLEKA SA 0,9% MASTI PRIMENOM NATIVNOG INOKULUMA, INOKULUMA KONCENTRISANOG MIKROFILTRACIJOM I INOKULUMA KONCENTRISANOG UPARAVANJEM TOKOM SKLADIŠTENJA: A) LAKTOZA B) GALAKTOZA C) L-MLEČNA KISELINA D) D-MEČNA KISELINA

It is known that the ratio of D-lactic acid and L-lactic acid varies depending on the composition of the starter culture; yoghurt usually contains 45-60% L-lactic acid and 40-55% D-lactic acid. Investigating 269 samples of commercial yoghurt, in which the ratio of D-lactic acid and L-lactic acid varied from 0.34 up to 8.28 (L-lactic acid is predominantly represented) (Tamime, 2006).

pH value of the samples during 10 days of storage decreases, and it is very uniformly in all fermented dairy beverages produced with the same type of inoculum. pH value is declining the fastest in samples produced with the kombucha concentrated by evaporation (Fig. 2).

Textural characteristics of kombucha fermented milk beverages during storage are shown in Fig.3. It is evident that firmness of samples ranges from 13.9 g to 14.2 g after production (Fig.3a). The firmness of all samples was about 2% higher after 10 day of storage. The highest consistency was noticed in kombucha fermented milk after production, while consistency of fermented milk with microfiltrated and concentrated kombucha inoculums have the same trend as firmness. Firmness after production of kombucha fermented samples is in accordance with the results of low fat kombucha beverages with 1% (w/w) fat content (Ilicic et al., 2012) and low fat probiotic yoghurt (Milanović et al., 2009), but it is 13% lower compared with the texture of kombucha beverage produced from milk of 2.2% fat content (Ilicic et al., 2012).

CONCLUSION

Chemical composition and energy value of fermented dairy beverages produced by using three types of kombucha inoculums is differrent among the samples.

Lactose content in all samples decreased during storage, with different dynamics depending on the type of used inoculum.

The content of L-lactic acid was significantly higher than the content of D-lactic acid and it decreases in fermented dairy beverages. Textural characteristic of kombucha fermented milk samples are not significantly different. Kombucha fermented milk beverages had the best physicochemical and textural characteristics during storage.

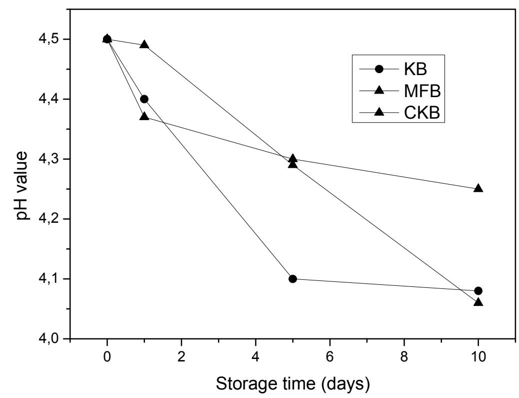

Figure 2. PH VALUE CHANGES DURING STORAGE OF FERMENTED DAIRY BEVERAGES PRODUCED BY USING DIFFERENT KOMBUCHA INOCULUMS

Slika 2. POMENA pH VREDNOSTI TOKOM SKLADIŠTENJA UZORAKA PROIZVEDENIH PRIMENOM RAZLIČITIH KOMBUHA INOKULUMA

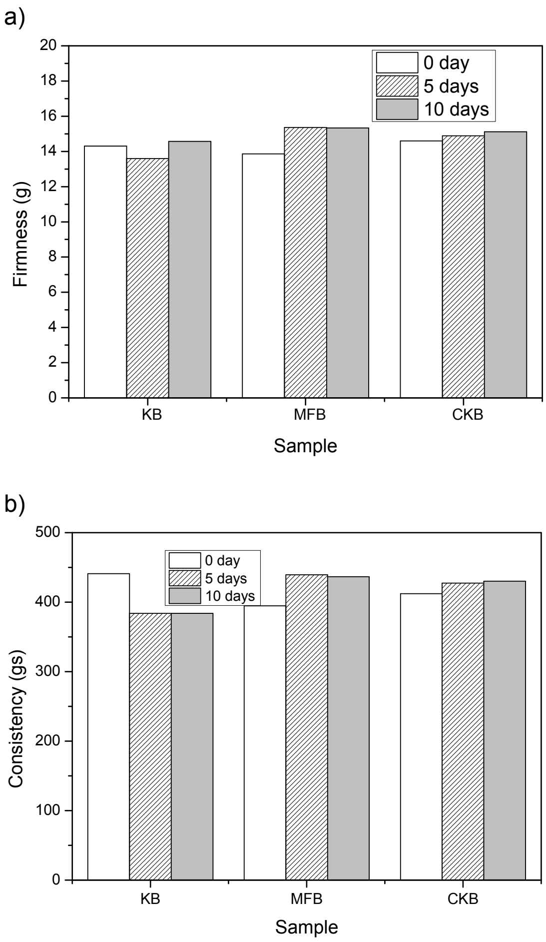

Figure 3. THE CHANGES OF TEXTURAL CHARACTERISTICS DURING STORAGE OF FERMENTED DAIRY BEVERAGES PRODUCED BY USING DIFFERENT KOMBUCHA INOCULUMS: A) FIRMNESS B) CONSISTENCY

Slika 3. POMENA TEKSTURALNIH KARAKTERISTIKA TOKOM SKLADIŠTENJA FERMENTISANIH MLEČNIH NAPITAKA PROIZVEDENIH PRIMENOM RAZLIČITIH KOMBUHA INOKULUMA: A) ČVRSTOĆA B) KONZISTENCIJA

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IZVOD

FIZIČKOHEMIJSKE I TEKSTURALNE KARAKTERISTIKE FERMENTISANIH MLEČNIH NAPITAKA TOKOM SKLADIŠTENJA

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Cilj ovog rada bio je da se ispita uticaj tri tipa nekonvencionalnih startera: kombuha inokulum, mikrofiltrirani kombuha inokulum i koncentrisani kombuha inokulum na fizičkohemijske i teksturalne promene dobijenih fermentisanih mlečnih napitaka tokom skladištenja. Rezultati ovog istraživanja ukazuju da postoje razlike u hemijskom sastavu između uzoraka. Tokom 10 dana skladištenja sadržaj laktoze opada, dok se sadržaj galaktoze i (L)-mlečne kiseline povećava u svim uzorcima. pH vrednost uzoraka nakom 10 dana skladištenja kretala se u intervalu od 4,05 do 4,25. Teksturalne karakteristike fermentisanih mlečnih proizvoda nisu značajno promenjene tokom 10 dana skladištenja.

Ključne reči: fermentisani mlečni napitak • hemijske karakteristike • tekstura • skladištenje

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ORIGINALNI NAUČNI RAD

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UTICAJ NEKIH PROCESNIH PARAMETARA NA ANTIOKSIDATIVNU AKTIVNOST FERMENTISANIH MLEČNIH PROIZVODA OD KOMBUHE

Cilj ovog rada je bio ispitivanje uticaja temperature fermentacije i koncentracije inokuluma kombuhe, kultivisane na čaju od majčine dušice, na antioksidativnu aktivnost i kvalitet dobijenih fermentisanih mlečnih proizvoda.

Antioksidativna aktivnost kombuha fermentisanih mlečnih proizvoda je ispitivana merenjem uticaja na DPPH i hidroksi radikale. Pored toga, kvalitet ovih proizvoda je praćen i standardnim metodama analize hemijskog sastava, fizičko-hemijskih karakteristika, kao i senzornom ocenom, predviđenim važećim Pravilnikom za takvu vrstu proizvoda.

Vreme fermentacije potrebno da se postigne željena vrednost pH od 4,5 opada sa porastom temperature fermentacije.

Antioksidativna aktivnost na DPPH radikale za proizvode dobijene na temperaturi 40ºC opada sa porastom koncentracije inokuluma, dok je na temperaturi od 43ºC suprotan trend. Antioksidativna aktivnost na hidroksi radikale opada sa porastom koncentracije inokuluma na obe primenjene temperature.

Na osnovu senzorne analize ustanovljeno je da napitak proizveden na 43ºC sa 10% inokuluma ima najbolje senzorne karakteristike, što proizvod čini najprihvatljivijim za konzumente, a najveća antioksidativna aktivnost na hidroksi radikale čini takođe proizvod od kombuhe najzdravijim za ishranu.

Key words: antioksidativna aktivnost • kombuha • majčina dušica • fermentisani mlečni proizvodi

UVOD

Fermentisani mlečni proizvodi zauzimaju značajno mesto u ishrani čoveka, jer ih odlikuje visoka nutritivna vrednost i terapeutska svojstva. Dobijaju se fermentacijom iz mleka pomoću odabranih mikroorganizama (starter kultura). Pored tradicionalnih startera (*Streptococcus thermophilus* i *Lactobacillus delbrueckii* spp. *bulgaricus*) danas se sve više upotrebljavaju probiotske bakterije najčešće iz roda *Lactobacillus* i *Bifidobacterium* (Tamime i Robinson, 2004). Savremena istraživanja ispituju mogućnost primene kombuhe – nekonvencionalnog startera za fermentaciju mleka u proizvodnji funkcionalne hrane (Belloso-Morales i Hernández-Sánchez, 2003; Malbaša *et al.* , 2009; Malbaša *et al.*, 2011).

Kombuha je simbioza kvasaca i bakterija sirćetne kiseline. Kultiviše se uobičajeno na zaslađenom crnom ili zelenom čaju koje prevodi u prijatan, nakiseo i slabo gaziran osvežavajući kombuha napitak. Napitak je bogat različitim biološki aktivnim supstancama kao što su: monosaharidi, isparljive kiseline, vitamini, enzimi, aminokiseline, alkohol, biogeni amini i neki antibiotici (Malbaša, 2009). Pored tradicionalnih podloga za rast kombuhe, ona može da fermentiše i na koka-koli, pivu, kafi, melasi, ekstraktima lekovitog bilja, a takođe i na mleku (Malbaša *et al.*, 2011). Fermentacijom kombuhe na mleku nastaje proizvod, koji je prema svojim fizičko-hemijskim i senzornim karakteristikama najsličniji kefiru i jogurtu (Malbaša *et al.* , 2009; Milanović *et al.*, 2012; Iličić *et al.*, 2012).

Majčina dušica je vrlo korisna lekovita biljka. Pomaže kod bolesti disajnih organa, jača želudac i nervni sistem. Čaj od majčine dušice deluje antibakterijski i spazmolitički, opušta grčeve. Koristi se kod crevnih i želučanih tegoba, te kod dijareje. Čaj od majčine dušice može se piti i kod stomačnih poremećaja kod žena, a pripremljen u obliku kupke ili obloga koristi se za lečenje kožnih bolesti, te kod opšte slabosti nervnog sistema (Fecka i Turek, 2008).

Antioksidanti su supstance koje mogu da zaštite ćelije od oštećenja koja nastaju pod dejstvom slobodnih radikala. Slobodni radikali su molekuli visoke reaktivnosti koji nastaju svakodnevno u organizmu kao proizvodi razlaganja kiseonika u procesu oksidacije u ćelijama. Reagujući sa mnogim drugim molekulima u organizmu, oni menjaju njihovu strukturu i time ih čine nesposobnim da vrše svoju funkciju. Antioksidanti reaguju sa slobodnim radikalima, stabilizuju ih, i na taj način štite ćelije od oštećenja (Halliwell, 2011). Mnoge studije su dokazale da antioksidanti pomažu u prevenciji kancera, srčanih bolesti, čuvanju memorije, smanjuju rizik od degenerativnih bolesti, umanjuju bol, zamor u mišićima, sprečavaju neka oboljenja oka, čak pomažu i protiv nastanka bora (Kaur i Kapoor, 2001).

Cilj ovog rada je ispitivanje uticaja temperature fermentacije i koncentracije inokuluma kombuhe, dobijenog kultivacijom na čaju od majčine dušice, na antioksidativnu aktivnost, hemijske, fizičko-hemijske i senzorne karakteristike fermentisanih mlečnih proizvoda. Kombuha je kultivisana na čaju majčine dušice zaslađenom sa 7% saharoze. Nakon toga je mleko sa 2,8% mlečne masti inkubirano sa 10, 12,5 i 15% (v/v) navedenog inokuluma na temperaturama 40 i 43^{o}C.

MATERIJAL I METODI

Inokulum

Inokulum za fermentaciju mleka je dobijen kultivacijom kombuhe na čaju

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od majčine dušice (MD), koji je pripremljen na sledeći način: u 1 L ključale česmenske vode dodato je 70 g saharoze i 1,5 g majčine dušice. Pripremljeni čaj je ohlađen na sobnu temperaturu, proceđen, a zatim je dodato 100 mL inokuluma iz prethodne fermentacije, odnosno 10% (v/v) fermentativne tečnosti kombuhe. Čaša je prekrivena tkaninom propusnom za vazduh. Inkubacija kombuhe je izvedena na sobnoj temperaturi, tokom 7 dana.

Proizvodnja fermentisanih mlečnih proizvoda

Za proizvodnju fermentisanih mlečnih proizvoda u laboratorijskim uslovima korišćeno je pasterizovano, homogenizovano mleko sa 2,8% mlečne masti, proizvođača „AD IMLEK" Beograd, ogranak „Novosadska mlekara", Novi Sad.

U mleko sa 2,8% mlečne masti dodato je 10, 12,5 i 15% (v/v) inokuluma kombuhe, redom. Fermentacija je izvedena na 40 i 43˚C do postizanja vrednosti pH od 4,5. Gel je zatim ohlađen na temperaturu od 8˚C i homogenizovan mešalicom. Dobijeni su uzorci označeni sa MD40-10, MD40-12,5, MD40-15, MD43-10, MD43-12,5 i MD43-15 zavisno od čaja na kome je izvedena fermentacija kombuhe, temperature na kojoj je izvedena fermentacija mleka i koncentracije u kojoj je inokulum dodat mleku.

Metode analize

Inokulumu korišćenom za fermentaciju mleka određeni su pH, suva materija i pepeo, prema standardnim metodama (Carić *et al.*, 2000).

Mleku, koje je korišćeno za proizvodnju fermentisanih mlečnih proizvoda i dobijenim fermentisanim mlečnim proizvodima određeni su pH vrednost, kiselost, suva materija, pepeo, mlečna mast, ukupni proteini i laktoza, prema standardnim metodama (Carić *et al.*, 2000).

Fermentisanim mlečnim proizvodima praćeni su i sinerezis surutke (Atamer *et al.*, 1996) i sposobnost vezivanja vode (Guzman-Gonzalez *et al.*, 1999), a data je i njihova senzorna ocena.

Antioksidativna aktivnost mleka i fermentisanih mlečnih proizvoda od kombuhe je ispitana merenjem uticaja na DPPH (Živković *et al.*, 2009) i hidroksi radikale (Deeseenthum i Pejović, 2010).

Proizvedeni uzorci su analizirani nakon proizvodnje.

REZULTATI I DISKUSIJA

Analiza inokuluma kombuhe i mleka

Inokulumu je određena vrednost pH=3,26, dok je sadržaj suve materije iznosio 6,85%, a pepela 0,03%.

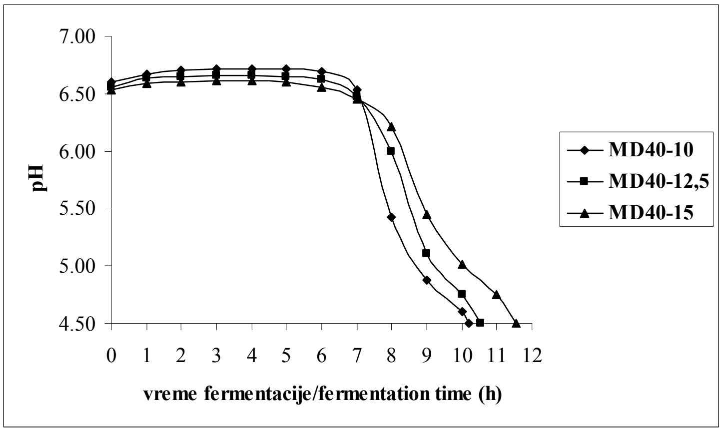
Rezultati analize kvaliteta mleka korišćenog u proizvodnji kombuha fermentisanih mlečnih proizvoda dati su u Tabeli 1.

Tabela 1. KARAKTERISTIKE MLEKA

Table 1. CHARACTERISTICS OF MILK

parametar kvaliteta	Mleko
pH	6,93
suva materija (%)	11,96
pepeo (%)	0,71
mlečna mast (%)	2,80
proteini (%)	3,45
laktoza (%)	4,18
kiselost (˚SH)	6,20

Na osnovu prikazanih rezultata utvrđeno je da kvalitet mleka odgovara važećem Pravilniku o kvalitetu proizvoda od mleka i starter kultura (2010).



Slika 1. TOK FERMENTACIJE MLEKA U UZORCIMA FERMENTISANIH MLEČNIH PROIZVODA DOBIJENIH NA 40°C

Figure 1. FERMENTATION PROCESS OF MILK-BASED KOMBUCHA PRODUCTS OBTAINED AT 40°C

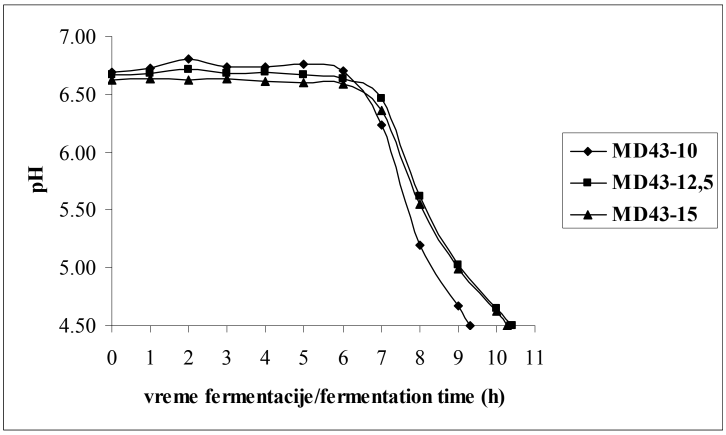
Promene vrednosti pH tokom fermentacije mleka

Tok fermentacije mleka sa 2,8% mlečne masti u procesu proizvodnje fermentisanih mlečnih proizvoda na temperaturama od 40ºC i 43ºC za sve tri koncentracije inokuluma prikazan je na slikama 1 i 2.

Fermentacija mleka je zaustavljena u svim uzorcima nakon postizanja pH=4,5. Vreme potrebno da se u procesu proizvodnje uzoraka postigne krajnja vrednost pH na temperaturi 40ºC kreće se od 10,21 h (10% inokuluma) do 11,55 h (15% inokuluma), a na temperaturi 43ºC se kreće od 9,33 h (10% inokuluma) do 10,40 h (12,5% inokuluma). Na temperaturi 40ºC prvih 6 sati nije zapažena nikakva značajna promena vrednosti pH. Narednih sat vremena zabeležen je blagi pad vrednosti pH, a tokom narednih sati vrednost pH je brže opadala. Na temperaturi od 43ºC prvih 5 sati takođe nema značajnih promena vrednosti pH. Narednih sat vremena, kao i na temperaturi od 40ºC, zabeležen je blagi pad vrednosti pH, a dalje je vrednost pH brže opadala. Prema ukupnom vremenu potrebnom da se postigne pH od 4,5 i toku fermentacije možemo zaključiti da se sa povišenjem temperature skraćuje vreme fermentacije. Sa slika 1 i 2 se uočava da su sve krive zavisnosti pH od vremena fermentacije imale sigmoidalni oblik, što je u saglasnosti sa literaturom (Malbaša i sar., 2009).

Analiza fermentisanih mlečnih proizvoda

U tabelama 2 i 3 prikazan je hemijski sastav kombuha fermentisanih mlečnih proizvoda nakon proizvodnje.



Slika 2. TOK FERMENTACIJE MLEKA U UZORCIMA FERMENTISANIH MLEČNIH PROIZVODA DOBIJENIH NA 43°C

Figure 2. FERMENTATION PROCESS OF MILK-BASED KOMBUCHA PRODUCTS OBTAINED AT 43°C

Tabela 2. HEMIJSKI SASTAV I FIZIČKO-HEMIJSKE KARAKTERISTIKE KOMBUHA FERMENTISANIH MLEČNIH PROIZVODA DOBIJENIH NA 40°C

Table 2. CHEMICAL COMPOSITION AND PHYSICOCHEMICAL CHARACTERISTICS OF MILK-BASED KOMBUCHA PRODUCTS OBTAINED AT 40°C

parametar kvaliteta	MD40-10	MD40-12,5	MD40-15
suva materija (%)	10,70	10,69	10,28
pepeo (%)	0,64	0,66	0,67
mlečna mast (%)	2,86	2,75	2,40
ukupni proteini (%)	3,37	2,90	3,09
laktoza (%)	3,13	3,64	3,77
kiselost (˚SH)	34,00	36,30	34,50
SVV (%)	58,21	55,00	53,20
sinerezis (mL)	29,00	28,00	29,00

Tabela 3. HEMIJSKI SASTAV I FIZIČKO-HEMIJSKE KARAKTERISTIKE KOMBUHA FERMENTISANIH MLEČNIH PROIZVODA DOBIJENIH NA 43°C

Table 3. CHEMICAL COMPOSITION AND PHYSICOCHEMICAL CHARACTERISTICS OF MILK-BASED KOMBUCHA PRODUCTS OBTAINED AT 43°C

parametar kvaliteta	MD43-10	MD43-12,5	MD43-15
suva materija (%)	11,18	10,65	10,44
pepeo (%)	0,68	0,65	0,67
mlečna mast (%)	2,86	2,75	2,40
ukupni proteini (%)	2,76	2,63	2,88
laktoza (%)	4,04	3,93	4,06
kiselost (˚SH)	32,00	32,40	33,40
SVV (%)	47,50	45,23	48,50
sinerezis (mL)	30,00	30,00	30,00

Poređenjem hemijskih i fizičko-hemijskih karakteristika dobijenih proizvoda ne uočava se značajna razlika u sadržaju suve materije i pepela, čije prosečne vrednosti iznose 10,66% i 0,66%. Prosečan sadržaj mlečne masti u proizvodima je 2,67%, a laktoze 3,76% što je niža vrednost u odnosu na mleko, dok je prosečan sadržaj ukupnih proteina 2,93% (tabele 2 i 3). Kiselost fermentisanih mlečnih proizvoda prosečno iznosi 33,77ºSH, a vrednost pH 4,22. Prosečna vrednost sinerezisa je 29,33 mL, dok vrednosti sposobnosti vezivanja vode ukazuju na dobar kvalitet proizvoda (tabele 2 i 3).

Senzorna analiza fermentisanih mlečnih proizvoda

Senzorna ocena dobijenih fermentisanih mlečnih proizvoda je dobijena od strane 7 kvalifikovanih ocenjivača.

Svi uzorci su bez izdvojene surutke na površini. Boja uzoraka je ujednačena i tipična za datu vrstu proizvoda. Konzistencija proizvoda je takođe karakteristična za datu vrstu proizvoda. Ukus fermentisanih mlečnih proizvoda koji su proizvedeni na 40ºC je kiseo, a kiselost raste sa povećanjem koncentracije inokuluma. Kod uzoraka koji su proizvedeni na 43ºC kiseo ukus je blaži, a takođe se kiselost povećava sa porastom koncentracije inokuluma.

Iz navedenih podataka može se zaključiti da proizvod dobijen na 43ºC sa 10% inokuluma ima najbolje senzorne karakteristike.

Antioksidativna aktivnost na DPPH radikale

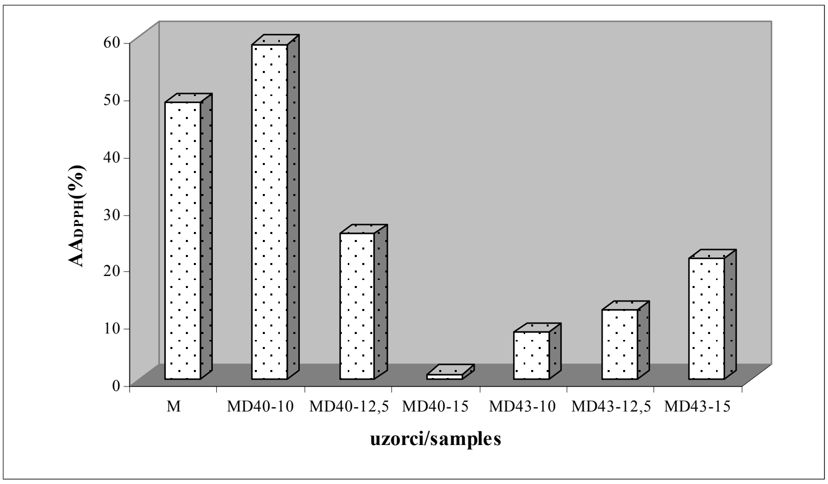
Rezultati analize antioksidativne aktivnosti mleka i fermentisanih mlečnih napitaka dobijeni spektrofotometrijski merenjem uticaja na DPPH radikale prikazani su na slici 3.

Poređenjem antioksidativnih aktivnosti mleka i proizvedenih uzoraka na DPPH radikale može se uočiti povećana antioksidativna aktivnost uzorka MD40-10, dok je kod ostalih uzoraka antioksidativna aktivnost smanjena u odnosu na mleko. Takođe se može zaključiti da antioksidativna aktivnost na temperaturi od 40ºC opada sa porastom koncentracije inokuluma, dok je na temperaturi od 43ºC situacija obrnuta, tj. antioksidativna aktivnost raste sa porastom koncentracije inokuluma (slika 3).

Prosečna vrednost antioksidativne aktivnosti na DPPH radikale je veća kod proizvoda dobijenih fermentacijom na 40°C.

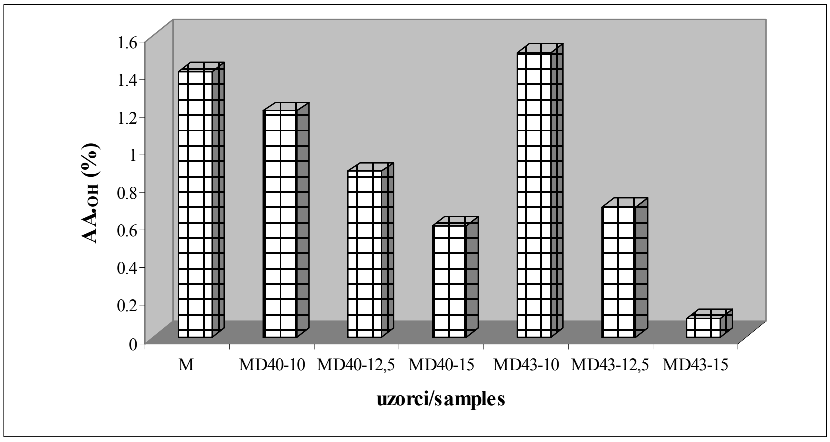
Antioksidativna aktivnost na hidroksi radikale

Rezultati analize antioksidativne aktivnosti mleka i fermentisanih mlečnih napitaka dobijeni spektrofotometrijski merenjem uticaja na hidroksi radikale prikazani su na slici 4.



Slika 3. ANTIOKSIDATIVNA AKTIVNOST MLEKA I FERMENTISANIH MLEČNIH PROIZVODA NA DPPH RADIKALE

Figure 3. ANTIOXIDANT ACTIVITY OF MILK AND FERMENTED MILK PRODUCTS TO DPPH RADICALS



Slika 4. ANTIOKSIDATIVNA AKTIVNOST MLEKA I FERMENTISANIH MLEČNIH PROIZVODA NA HIDROKSI RADIKALE

Figure 4. ANTIOXIDANT ACTIVITY OF MILK AND FERMENTED MILK PRODUCTS TO HYDROXYL RADICALS

Sa slike 4 se može uočiti povećana antioksidativna aktivnost na hidroksi radikale kod uzorka MD43-10, dok je kod ostalih uzoraka antioksidativna aktivnost smanjena u odnosu na mleko, kao i da antioksidativna aktivnost opada sa porastom koncentracije inokuluma na obe radne temperature.

Prosečna vrednost antioksidativne aktivnosti na hidroksi radikale je veća kod proizvoda dobijenih fermentacijom na 40^{o}C (osim uzorka sa dodatkom 15% inokuluma kombuhe), što je zabeleženo i kod antioksidativne aktivnosti na DPPH radikale.

ZAKLJUČAK

Fermentisani mlečni proizvodi su proizvedeni dodatkom starter kulture mleku sa 2,8% mlečne masti na temperaturama 40 i 43ºC u količini od 10% v/v, 12,5% v/v i 15% v/v. Kao starter kultura korišćena je tečnost kombuhe dobijena kultivacijom kombuhe na ekstraktu majčine dušice.

Vreme fermentacije za koje se postigne željena vrednost pH od 4,5 opada sa porastom temperature fermentacije.

Hemijske karakteristike fermentisanih mlečnih napitaka neznatno se menjaju u zavisnosti od temperature fermentacije i odgovaraju važećem Pravilniku za tradicionalni jogurt.

Od fizičko-hemijskih karakteristika sinerezis fermentisanih mlečnih napitaka je iznosio prosečno 29,33 mL, dok je sposobnost vezivanja vode visoka, što ukazuje na dobar kvalitet proizvoda.

Na osnovu senzorne analize ustanovljeno je da napitak proizveden na 43ºC sa 10% inokuluma ima najbolje senzorne karakteristike.

Antioksidativna aktivnost mleka i fermentisanih mlečnih proizvoda je merena spektrofotometrijski dejstvom na DPPH i hidroksi radikale. Antioksidativna aktivnost na DPPH radikale je najveća kod uzorka proizvedenog sa 10% inokuluma na temperaturi 40ºC. Antioksidativna aktivnost na DPPH radikale na temperaturi 40ºC opada sa porastom koncentracije inokuluma, dok je na temperaturi 43ºC suprotan trend.

Antioksidativna aktivnost na hidroksi radikale je najveća kod uzorka proizvedenog sa 10% inokuluma na temperaturi 43ºC. Pored toga, može se uočiti opadanje antioksidativne aktivnosti sa porastom koncentracije inokuluma na obe temperature.

Prosečne vrednosti antioksidativnih aktivnosti su veće kod proizvoda dobijenih fermentacijom na 40^{o}C.

ZAHVALNICA

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SUMMARY

INFLUENCE OF SOME PROCESS PARAMETERS ON THE ANTIOXIDANT ACTIVITY OF KOMBUCHA FERMENTED MILK PRODUCTS

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The aim of this manuscript is the investigation of influence of fermentation temperature and kombucha inoculum concentration to antioxidant activity of fermented milk products. Kombucha inoculum is produced on wild thyme extract sweetened with sucrose.

Antioxidant activity to hydroxyl and DPPH radicals is monitored using appropriate spectrophotometric methods. Quality of the obtained fermented milk products is followed using standard methods in accordance to actual Regulation.

Fermentation time necessary to achieve pH of 4.5 was decreasing with increased process temperature.

Antioxidant activity to DPPH radicals for the products obtained at 40ºC was decreasing with increased inoculum concentration, while the trend was opposite at 43ºC. Antioxidant activity to hydroxyl radicals was lower on both fermentation temperature when kombucha inoclum concentration was higher.

Sensory analysis found that the best product is obtained at 43ºC with the addition of 10% of inoculum.

Ključne reči: antioxidant activity • kombucha • wild thyme • fermented milk products

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ORIGINAL SCIENTIFIC PAPER

UDK: 637.146.3 : 547.455.65

RHELOGICAL AND SENSORY PROPERTIES OF PROBIOTIC YOGHURT MADE OF COW'S, GOAT'S AND SOYMILK ENRICHED WITH INULIN

The aim of this work was to examine yoghurt processing of cow, goat and soy milk enriched with inulin (2% i 4%). The quality of produced yoghurt was followed by comparing pH value during fermentation and storage time, viscosity, syneresis and sensory properties during 20 days of storage. Homogenised UHT milks: cow's milk, goat's milk and soymilk were used for the production of yoghurt samples. 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 samples were incubated at 37°C 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 5 °C, during 20 days of storage. The obtained results have shown that the shorter fermentation time had samples made of goats milk, than from the cow's milk, while the soymilk samples had the longest fermentation time. The lowest viscosity, followed by the highest syneresis had the goat's yoghurt samples, while the yoghurt samples made of cow's milk had the highest viscosity. The soy yoghurt samples enriched with inulin had the lowest syneresis and the best sensory properties. In general, inulin addition to milk for yoghurt production brought shorter fermentation time, and improved rheological and sensory properties of all produced yoghurt samples.

Key words: cow's milk, goat's milk • soymilk • probiotic yoghurt • inulin • viscosity • sensory properties

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INTRODUCTION

Gel strength and viscosity are important quality indicators related to consistency and mouth feel of fermented dairy products (Lewis, 1996). Like most food gels, yoghurt is a viscoelastic material. Stirred yoghurt behaves as a viscoelastic fluid while set yoghurt behaves as a viscoelastic solid. The gel structure of set yoghurt is fragile and can be broken easily when subjected to mechanical stress. Yoghurt is not a true thixotropic material as the breakdown is not completely reversible after release of applied stress (Harte et al., 2007). Achieving the optimal physical, rheological and sensory properties of low fat yoghurt could be made by using different functional food ingredient such as whey protein concentrate (Antunes et al., 2005; Aziznia et al., 2008; Milanović et al., 2009) whey protein isolate (Isleten et al, 2006), guar gum (Yamamoto et al., 1990; Yoon et al., 2007), a range of oligosaccharides, especially fructo-oligosaccharides. The frequently used functional food ingredient is inulin, a non-digestible fructan that offers a unique combination of interesting nutritional properties and important technological benefits (Suzuki and Chatterton, 1993) through improving rheological and sensory properties (Gueven et al, 2005.; Aryana et al., 2007.; Paseephol et al., 2008). Another added benefit of inulin that is often capitalized on yoghurt is the prebiotic effect, which may serve to reinforce or enhance the action of probiotic cultures typically added to yoghurt (Niness, 1999). Among the probiotic lactic acid bacteria, *Bifidobacterium* sp. have been the main focus of research (Shin et al., 2000; Akalin et al., 2004), although *Lactobacillus* has been also used widely in dairy products and should deserve attention due to their established health-promoting effects (Shah, 2001).

Goat's milk and soymilk today gets more and more attention because of its extraordinary nutritive and health characteristics. Goat's milk has an important dietetic and therapeutic advantages, while 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. Some strains of *Bifidobacterium* are able to reduce the concentration of raffinose and stachyose, eliminating the potential cause of flatulence, and also decrease the levels of pentanal and n-hexanal responsible for the beany flavour (Desai et al., 2002; Tsangalis and Shah, 2004). Soy yoghurt is a fermented soymilk made with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*. There are several reports of improving sensory quality of goat's and soy yoghurt by adding inulin, which can partly masked the goat's flavour and beany flavour of fermented milk (Božanić et al., 2001, 2002; Donkor et al., 2007a,b; Rinaldoni et al., 2012).

The aim of this study was to examine addition of 2% and 4% inulin on the rheological and sensory properties of probiotic yoghurt made of different type of milk: cow's, goat's and soymilk. Advantages of fermentation of goat's and soymilk are described, especially with probiotic lactic acid bacteria.

MATERIALS AND METHODS

Materials

Homogenised cow's milk (2.8% fat, 3.2% proteins, 4.6% lactose), obtained from „VITALIA, IMLEK“, d.d. Mlijekoprodukt (Kozarska Dubica, Bosnia and Herzegovina), goats milk ("Vindija" d.d. Varaždin, Hrvatska; 3% fat, 2.8% proteins, 4.5% lactose) and soymilk (ALPRO SOJA, Alpro Comm, Wevelgem, Belgium, 1.8% fat, 3% proteins, 2.5% carbohydrate) were used for the production of yoghurt samples. Sucrose was added to the soymilk to reach concentration of 4.6% sugar. The initial pH of the cow's milk was 6.26 (±0.01), pH of the goats milk was 6.33 (±0.02) and pH of the soya milk was 6.40 (±0.04). Physical, chemical and microbiological characteristics of milk samples were entirely in accordance with the pertinent standards.

Mixed probiotic starter culture Dri-Set 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.

Inulin (Fibruline® Instant Cosucra Groupe Warcoing S.A., Belgium) contained min 90 % inulin, max 10 % fructose, glucose and sucrose, max 0,3% ash.

Yoghurt manufacturing

Samples of cow's milk, goat's milk and soymilk were heated to 55ºC and inulin was added in two concentrations: 2% w/w and 4% w/w. As the control samples were considered those without any addition. The milk was cooled to the optimal temperature (37°C), inoculated with the chosen yoghurt 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 5 °C ±1. Each trial was repeated three times.

Methods of analyses

After manufacturing, yoghurt samples were analyzed by measuring pH value, viscosity, syneresis, lactic acid and sensory properties. pH was measured using pH 510/mV Meter (Eutech Instruments, England) during fermentation and during 20 days of storage. Lactic acid is calculated on the basic titratable acidity (Sabadoš, 1996) during 20 days of storage. 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 the centrifugation method (Keogh and O'Kennedy, 1998) with some modifications. For serum separation centrifuge was used SIGMA 2-6 Laboratory Centrifuges (Osterode am Harz, Germany). A sample of about 30 g of yogurt was centrifuged for 10min at 3000 rpm and the separated whey was removed and weighed. The analyses of the produced samples were carried out the 1st, 10th and 20th day of storage at 5ºC.

Sensory evaluation of yoghurt was profiled after 1st and 20th day in the cold store. The sensory properties of yoghurt were performed by 5 trained panelists using the International Dairy Federation method (IDF, 1984). The sensory attributes consisted of flavour, odour, general appearance, colour and consistency, and the coefficients of significance (Fv) were: 2.4 for taste; 0.4 for odour; 0.2 for appearance and for colour and 0.8 for consistency. Maximum score was 20, and the sensory scores were awarded for each attribute using a rating scale ranging between 1 and 5.

The average value of 3 measurements was taken for further analysis. Values of different tests were expressed as the mean ± standard deviation (x ± SD).

RESULTS AND DISCUSSION

Fermentation

During the first 120 min of fermentation, the pH value of cow's milks supplemented with inulin was the same as in control (Fig.1A), while after 120 min pH value decreased faster than in control samples and fermentation lasted shorter (about 235 min). As far as goat's milk is considered, generally it had the shortest time of fermentation (180-190 min) compared to cow's or soymilk samples, but there are no changes in fermentation time due to inulin addition to goat's milk (Fig.1B). As it is evident from the Fig.1C, the samples prepared from soymilk had the longest fermentation time (300-325 min), compared to cow's or goat's milk. Inulin addition induced shorter fermentation time (for

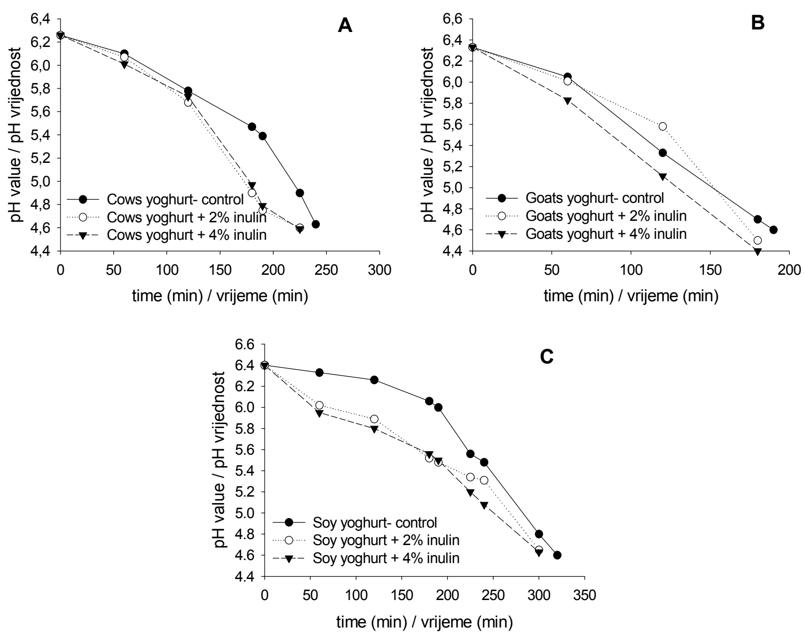

Figure 1. FERMENTATION TIME OF PROBIOTIC YOGHURT MADE OF COW (A), GOAT (B) AND SOYMILK (C) FORTIFIED WITH INULIN

Slika 1. VRIJEME FERMENTACIJE PROBIOTIČKOG JOGURTA PROIZVEDENOG OD KRAVLJEG (A), KOZJEG (B) I SOJINOG MLIJEKA (C) OBOGAĆENOG SA INULINOM about 25 min) in soymilk compared to control samples.

The shortest time of fermentation of goat's milk could be due to lower concentration of protein in milk compared to cow and soymilk, which was also reported by Božanić et al. (2001). Inulin addition induced shorter fermentation time in cow and soymilk samples. No significant change in fermentation time as a result of inulin addition in cow and goat milk (Božanić et al., 2002), which is in agreement with the obtained results for goat milk. Despite added inulin to soymilk, the lack of lactose, which are insufficient to sustain the LAB growth, brought longer fermentation time of soy yoghurt, which were previously reported (Donkor et al., 2007a,b; Iancu et al., 2010). Furthermore, according to the results of Iancu et al. (2010) the strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* is faster than the other lactobacilli in producing lactic acid and mentioned authors recommended it to be used for fermenting soy milk with added inulin.

This could explain relatively short fermentation time of soy yoghurt, which was also reported for probiotic soy yoghurt (Yang and Li, 2010), while other authors reported much longer fermentation time of soy yoghurt, about 8-20 hours long (Donkor et al., 2007a,b; Iancu et al., 2010).

pH and lactic acid

The pH and lactic acid (%) of all samples slightly changed during storage period (Tables 1 and 2). The highest pH value during 20 days of storage had control samples (in range from 4.54 to 4.25) of all used milks (Table 1). The pH values were lower in the yoghurts with inulin addition than in controls, regardless of milk type, which is in agreement with some results (Božanić et al., 2001; 2002; Stijepić et al., 2008; 2011; Iancu et al., 2010). However, Gueven et al. (2005) found that there is no effect of addition of inulin on the titratable acidity and pH of fat free yogurt. The more stable pH value during storage time had soy yoghurt, regardless of inulin addition. The prolonged stability of pH value, followed by low lactic acid content of these samples could be explained mainly because soymilk lacks free monosaccharides and disaccharide lactose (Liu, 1997).

The data obtained for lactic acid was found contained (Table 2) showed that lactic acid is produced during storage time. The highest content of lactic acid was contained in yoghurt made of cow milk (from 0.675 to 0.864%), while the soymilk yoghurt contained the lowest lactic acid content (from 0.450 to 0.648%). Goat yoghurt had lactic acid content in range from 0.617 to 0.745%. In general, it is observed an increase in lactic acid content with increasing concentration of added inulin, regardless of milk type. Lactic acid content in the inulin enriched soy yoghurt increased steadily during storage. Pinthong et al. (1980), Favaro Trindade et al. (2001) and Donkor et al. (2005) also observed an increase in lactic acid production after addition of glucose, sucrose and inulin, respectively.

Viscosity and syneresis

Viscosities of yoghurt samples are presented in Figures 2. Produced yoghurt samples from cow milk had the highest viscosity, in the range: 172.11 - 231.67 mPas (Fig.1.A), while the goat yoghurt (Fig.2B) had the lowest viscosity, in the range: 15.29 - 29.38 mPas.

Soy yoghurt viscosities of all samples were in range: 75.21 - 190.65 mPas (Fig.2C). Inulin addition slightly improved viscosity of cow and goat yoghurt, after 10 days of storage, at level of 4% and 2% inulin, respectively. Improving of viscosity by adding inulin during the production of soy yoghurt was evident even after the first day of storage (Fig.2C), especially in the sample with 4% inulin. During the storage time, viscosity of goat yoghurt slightly increased, regardless of inulin addition, while as for cow and soy yoghurt the highest viscosity of all samples were after 10 days of storage and after that time viscosity slightly decreased.

The observed results correspond to information given in literature: incre

Table 1. pH VALUE OF PROBIOTIC YOGHURT MADE OF COW, GOAT AND SOYMILK ENRICHED WITH INULIN DURING 20 DAY OF STORAGE

Tabela 1. pH VRIJEDNOST U PROBIOTIČKIM JOGURTIMA PROIZVEDENIM OD KRAVLJEG, KOZJEG I SOJINOG MLIJEKA, UZ DODATAK INULINA TOKOM 20 DANA SKLADIŠTENJA

	Days of storage/Dani skladištenja		
Samples/Uzorci	1	10	20
Cows yoghurt-control	4.40±0.11	4.14±0.10	4.00±0.12
Cows yoghurt+2%inulin	4.33±0.10	3.99±0.08	3.82±0.09
Cows yoghurt+4%inulin	4.33±0.07	4.00±0.09	3.86±0.06
Goats yoghurt-control	4.54±0.08	4.08±0.08	3.84±0.08
Goats yoghurt+2%inulin	4.38±0.05	3.97±0.08	3.78±0.07
Goats yoghurt+4%inulin	4.34±0.12	3.96±0.09	3.72±0.09
Soy yoghurt-control	4.25±0.11	4.13±0.08	4.03±0.09
Soy yoghurt+2%inulin	4.23±0.09	4.11±0.07	4.00±0.03
Soy yoghurt+4%inulin	4.26±0.11	4.17±0.06	4.05±0.05

Table 2. LACTIC ACID CONTENT (%) OF PROBIOTIC YOGHURT MADE OF COW, GOAT AND SOYMILK ENRICHED WITH INULIN DURING 20 DAY OF STORAGE

Tabela 2. SADRŽAJ MLIJEČNE KISELINE (%) U PROBIOTIČKIM JOGURTIMA PROIZVEDENIM OD KRAVLJEG, KOZJEG I SOJINOG MLIJEKA, UZ DODATAK INULINA TOKOM 20 DANA SKLADIŠTENJA

	Days of storage/Dani skladištenja		
Samples/Uzorci	1	10	20
Cows yoghurt-control	0.675±0.04	0.695±0.05	0.715±0.06
Cows yoghurt+2%inulin	0.765±0.04	0.787±0.08	0.844±0.03
Cows yoghurt+4%inulin	0.720±0.02	0.760±0.03	0.864±0.04
Goats yoghurt-control	0.617±0.04	0.647±0.01	0.691±0.09
Goats yoghurt+2%inulin	0.657±0.01	0.686±0.03	0.701±0.06
Goats yoghurt+4%inulin	0.675±0.01	0.711±0.03	0.745±0.02
Soy yoghurt-control	0.450±0.04	0.527±0.08	0.603±0.06
Soy yoghurt+2%inulin	0.491±0.04	0.549±0.07	0.614±0.03
Soy yoghurt+4%inulin	0.518±0.02	0.563±0.06	0.648±0.05

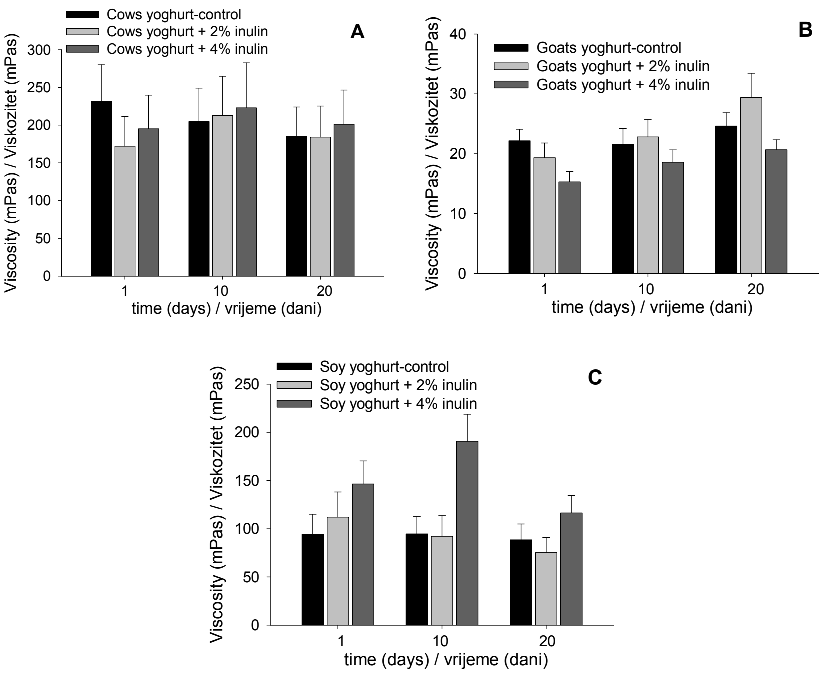

Figure 2. VISCOSITY OF PROBIOTIC YOGHURT MADE OF COW (A), GOAT (B) AND SOYMILK (C) FORTIFIED WITH INULIN DURING 20 DAY OF STORAGE

Slika 2. VISKOZITET PROBIOTIČKOG JOGURTA PROIZVEDENOG OD KRAVLJEG (A), KOZJEG (B) I SOJINOG MLIJEKA (C) OBOGAĆENOG SA INULINOM TOKOM 20 DANA SKLADIŠTENJA

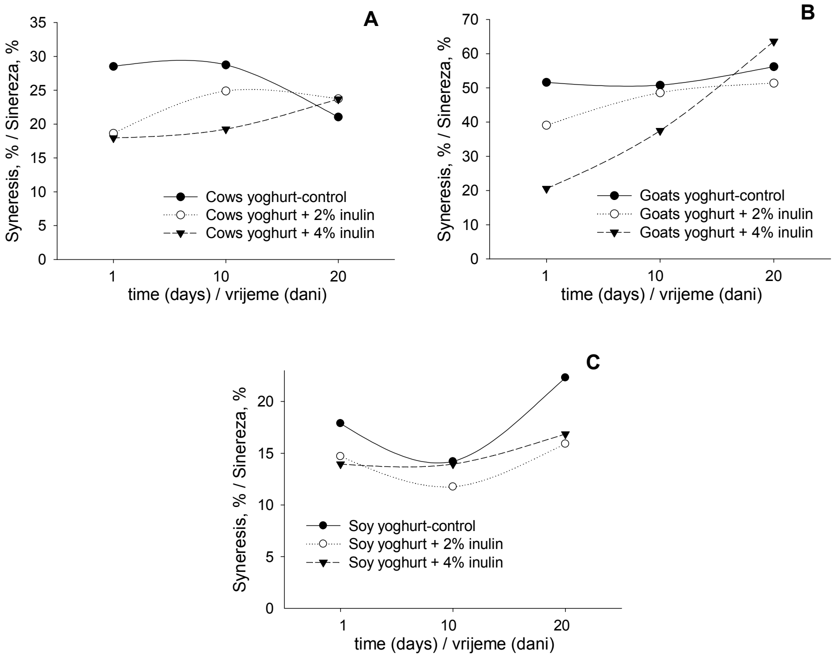

Figure 3. SYNERESIS (%) OF PROBIOTIC YOGHURT MADE OF COW (A), GOAT (B) AND SOYMILK (C) FORTIFIED WITH INULIN DURING 20 DAY OF STORAGE

Slika 3. SINEREZA (%) PROBIOTIČKOG JOGURTA PROIZVEDENOG OD KRAVLJEG (A), KOZJEG (B) I SOJINOG MLIJEKA (C) OBOGAĆENOG SA INULINOM TOKOM 20 DANA SKLADIŠTENJA

asing the inulin concentration in yoghurt up to 4% its viscosity also increases (Kip et al., 2006; Beitane and Ciprovica, 2012). Furthermore, several authors (Stijepić et al., 2008; Božanić et al., 2001, 2002) reported differrent results: decrease of viscosity of goat yoghurt with inulin concentration up to 2%, which is in agreement with our results.

The change in syneresis of yoghurt samples are presented in Figure 3. The highest syneresis had yoghurt samples made of goat milk (Fig.3.B) in range of 20.54 to 63.60%, while the cow yoghurt had syneresis in range from 17.95 to 28.53% (Fig.3.A). Surprisingly, the lowest syneresis had soy yoghurt (Fig.3.C) in range from 11.75 to 22.30%. As it is evident from the Fig.3., inulin addition lowering the syneresis of yoghurt samples, regardless of milk type, especially on 10th day of storage, which was previously reported (Gueven et al., 2005; Aryana et al., 2007; Stijepić et al., 2011). A possible explanation for these observations would be well-known fact that inulin has a high water holding capacity (Douglas, 2005). It was observed a slight increase in syneresis of cow and goat inulin enriched yoghurt, at the end of storage compared to control. According to several authors (Božanić et al., 2002), increasing in inulin concentration decrease syneresis, while Gueven et al., (2005) found that concentration of inulin higher than 1.5% in yoghurt could lead to higher syneresis, which could explain increase in syneresis of cow and goat yoghurt at the end of storage.

Sensory characteristics

Table 3 presents the sensory evaluation values of the produced yoghurts after 1st and 20th day of storage.

Soy yoghurt samples with inulin addition had the best average flavour scores at the end of storage. Inulin partly masked the goat's flavour of fermented milk, which was previously reported (Božanić et al., 2001, 2002; Stijepić et al., 2008). However, goat yoghurt samples had the lowest flavour and consistency scores. Yoghurt made of cow milk had a flavour typical for fermented products, but less desirable than the flavour of samples enriched with inulin. The most obvious characteristic according to the panelists was very desirable flavour of soy yoghurt

Table 3. SENSORY QUALITY OF PROBIOTIC YOGHURT MADE OF COW, GOAT AND SOYMILK ENRICHED WITH INULIN AFTER 1ST AND 20TH DAY OF STORAGE (n=5)

Tabela 3. SENZORSKI KVALITET PROBIOTIČKOG JOGURTA PROIZVEDENOG OD KRAVLJEG, KOZJEG I SOJINOG MLIJEKA, UZ DODATAK INULINA NAKON 1. I 20. DANA SKLADIŠTENJA (n=5)

	Samples/Uzorci	Appearance/ Izgled	Colour/ Boja	Odour/ Miris	Consistency/ Konzistencija	Flavour/ Ukus	Σ
1st day of storage/ 1. dan skladištenja	Cows yoghurt-control	1.0	1.0	2.0	4.0	11.4	19.4
	Cows yoghurt+2%inulin	1.0	1.0	2.0	4.0	11.6	19.6
	Cows yoghurt+4%inulin	1.0	1.0	2.0	4.0	11.6	19.6
	Goats yoghurt-control	1.0	1.0	2.0	2.2	7.8	14.0
	Goats yoghurt+2%inulin	1.0	1.0	2.0	2.4	7.9	14.3
	Goats yoghurt+4%inulin	1.0	1.0	2.0	2.6	7.9	14.5
	Soy yoghurt-control	1.0	1.0	2.0	4.0	11.2	19.2
	Soy yoghurt+2%inulin	1.0	1.0	2.0	4.0	12.0	20.0
	Soy yoghurt+4%inulin	1.0	1.0	2.0	4.0	12.0	20.0
20th day of storage/ 20. dan skladištenja	Cows yoghurt-control	1.0	1.0	2.0	4.0	11.4	19.4
	Cows yoghurt+2%inulin	1.0	1.0	2.0	4.0	11.6	19.6
	Cows yoghurt+4%inulin	1.0	1.0	2.0	4.0	11.6	19.6
	Goats yoghurt-control	1.0	1.0	2.0	2.0	7.6	13.6
	Goats yoghurt+2%inulin	1.0	1.0	2.0	2.2	7.8	14.0
	Goats yoghurt+4%inulin	1.0	1.0	2.0	2.4	7.8	14.2
	Soy yoghurt-control	1.0	1.0	2.0	4.0	11.0	19.0
	Soy yoghurt+2%inulin	1.0	1.0	2.0	4.0	11.8	19.8
	Soy yoghurt+4%inulin	1.0	1.0	2.0	4.0	11.8	19.8

sample enriched with inulin. Similar results, for soy yoghurt enriched with 50 g/l inulin, characterized as nice smell, flavor and color, and the sample with higher global acceptability, were reported by Rinaldoni et al. (2012). Furthermore, in terms of mouth feel, Donkor et al. (2007b) found that the supplemented products either with 2% inulin or raffinose/glucose in control and probiotic soy yoghurts were acceptable. Generally, the addition of inulin improved overall sensory quality of yoghurt samples.

CONCLUSION

The obtained results revealed that inulin addition had positive effects on the rheological, physico-chemical and sensory properties of produced yoghurt from different milk types. The knowledge obtained from this study could be applied to the development of novel formulation for functional food.

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IZVOD

REOLOŠKE I SENZORSKE KARAKTERISTIKE PROBIOTIČKOG JOGURTA PROIZVEDENOG OD KRAVLJEG, KOZJEG I SOJINOG MLIJEKA OBOGAĆENOG INULINOM

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U radu je ispitana mogućnost proizvodnje kravljeg, kozjeg i sojinog jogurta sa dodatkom inulina (2% i 4%). U proizvedenim jogurtima praćene su promjene pH vrijednosti tokom fermentacije i skladištenja, te promjene viskoziteta, sinereze i senzorskih osobina tokom 20 dana skladištenja. Za proizvodnju jogurta korištena su UHT sterilna mlijeka: kravlje mlijeko, kozje i sojino mlijeko. Fermentacija mlijeka vršena je dodatkom 0,0025% mješovite probiotičke kulture sastavljene od *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* i *Bifidobacterium* ssp., na temperaturi 37°C do postizanja pH vrijednosti 4,6. Nakon hlađenja, uzorci su čuvani na +5°C, a mjerenja su vršena 1., 10. i 20. dana u 3 ponavljanja. Dobijeni rezultati su pokazali da je najbrže fermentisalo kozje mlijeko, zatim kravlje mlijeko, dok je feremenatcija sojinog mlijeka trajala najduže. Najniži viskozitet i najvišu sinerezu imali su uzorci jogurta proizvedeni od kozjeg mlijeka, dok su uzorci jogurta od kravljeg mlijeka imali najviši viskozitet. Jogurt proizveden od sojinog mlijeka sa dodatkom inulina imao je najnižu sinerezu tokom skladištenja i najbolje senzorske ocijene. Generalno, dodatak inulina je ubrzao proces fermentacije, te uticao na poboljšanje reoloških i senzorskih karaktersitika svih vrsta proizvedenih jogurta.

Ključne reči: kravlje mlijeko • kozje mlijeko, sojino mlijeko • probiotički jogurt • inulin • viskozitet • senzorika

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ORIGINAL SCIENTIFIC PAPER

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CHANGES IN THE BASIC YOGHURT COMPONENTS DURING STORAGE

The aim of the research was to identify the chemical, biochemical, sensor and microbiological characteristics of the yogurt of three leading Macedonian brands purchased from the retail centers, as well as the changes that occur during the storage of 20 days. Ready to eat packed products were stored at 4 and 8°C during 20 days, and analyzes on the 1st, 5th, 10th, 15th, and 20th day were conducted. During four iterations, the following parameters were analysed: active acidity (pH), titration acidity, lactose, proteins, dry matter, ash, milk fats and sensory analysis.

According to the research it was determined that the storage temperature and storage duration, significantly affects the changes of the titration acidity, pH, and the content of lactose ($p>0.01$), while not influence the percentage of dry matter, fat free dry matter, milk fats, ash and proteins. The products show some deviations at different times of the year, i.e. the smallest variations were shown in the chemical composition, and larger in terms of acidity, which indicates inadequate standardization in technological process or variations in temperature of cooling and storage. All the variations influenced the products to have different taste in certain periods of the year.

Sensory analysis, performed on the second day, showed a good results in all three variants, and most pondered points got brand 1 (V1), while the 20-th day analysis could not be conducted because all three varieties stored at 4 and 8°C, showed unacceptable changes in the texture and taste.

Key words: yoghurt • quality • temperature • storage

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INTRODUCTION

The fermented dairy products are traditional products which are present in the capacities for milk production, the markets and also on the table. One of the most consumed fermented products in our country is the yoghurt. Under the term yoghurt we imply the product which is produced by fermentation of the pasteurized milk with adequate starter cultures. The quality and the safety of the yoghurt can be defined by a large number of criteria which include: microbiological, chemical, physical, and alimentary characterristics. As a result to this, the quality and the safety can be determined with a large number of tests from different degrees of objectivity, however everyone has a purpose to determine if the product is:

- Safe for humane diet (especially in sense of the microbiological and chemical contamination)
- In accordance with all legal demands
- Capable to meet the designated validity date without being broken
- With high organoleptic standards

The storage of the yoghurt, at temperatures lower than 10°C and the maintaining of these temperatures until the day of the wholesale/retail, insures lowering of the microbiological and biochemical reactions that are happening in the yoghurt. All the negative changes which can occur in yoghurt are result of the disregarding of the prescribed measures and regulations for the yoghurt's keeping and storage. The fermented products are products with high biological, nutritional and diet value which have the opportunity to be kept for a longer period of time. As a result of good nutritional and therapeutical attributes, which they possess, are belong to the functional food because (Tamime, 2006).

The nutritive value of the yoghurt is high because it contains all the necessary components which are necessary in physiological functions of the human organism. The yoghurt is rich with protein, fat, minerals, small amount of carbohydrates, vitamins and stimulating substances that are result of lactic acid fermentation.

The cooling of the yoghurt at temperatures lower than 10°C and maintaining these temperatures until the product sold, the microbiological and biochemical reactions makes it slower in the yoghurt.

The yoghurt is stored at a temperature of 0 to 10°C (±1°C) and the same temperature needs to be on while it is transported, but the tolerance during the transport is ± 2.5°C. Most of the major producers in the world are storing, transporting and allowing retail storing of the yoghurt at temperatures below 10°C.

During the long distance transport, it comes to yoghurt-shaking that can lead to a reduction of its viscosity that is difficult to exceed.

The yoghurt is consumed at a temperature about 10°C, while at higher temperatures it loses its freshness which comes also to reducing the viscosity.

Keeping the yoghurt in the supermarkets at room temperature about 20°C is not good, because it allows quickly to raise the temperature in the yoghurt itself and its rapid decay (Kakurinov et al. 2007).

MATERIALS AND METHODS

Studies in the thesis were conducted on samples of yoghurt which was produced in 3 different milk-processing capacities which are working with cutting edge technology and have implemented a food safety system.

The yoghurt testing, 1 liter packaging, were bought from markets immediately after production when they were placed in the retail and distribution network.

However, the survey included 3 yoghurts produced by different manufacturers (V1, V2 and V3) that were stored for a 20 days period of time, at 4°C temperature (V1-4, V2-4 and V3-4) and at temperature of 8°C (V1-8, V2-8 and V3-8). The chemical and biological studies were carried out in 4. The samples were examined and analyzed in the Institute of Public Health in Bitola and the in laboratory for milk at the Faculty of Agricultural Sciences and Food – Skopje. According to this survey, it was covered 6 variants:

- V1-4 yoghurt storage at 4°C
- V2-4 yoghurt storage at 4°C
- V3-4 yoghurt storage at 4°C
- V1-8 yoghurt storage at 8°C
- V2-8 yoghurt storage at 8°C
- V3-8 yoghurt storage at 8°C

Each variant is examined individually over a period of 20 days, more precisely the first, the tenth, and the twentieth day.

The chemical analyses included examination of the essential ingredients in the yoghurt according to the following methods:

- Determination of fats – Soxhlet-Henkel method
- Determination of proteins – Kiedahl method
- Determination of lactose – chloramines T IDF/ ISO /AOAC method
- Determination of ash – a method according to the IDF/ISO/AOAC combustion at 550°C
- Determination of dry matter according to the drying method at 105°C

The tests were conducted on the first day after the production of yoghurt, also on the tenth and the twentieth day.

For assessment of the biochemical changes in the yoghurt, the following tests were conducted:

- Titratable acidity – according to the Soxhlet-Henkel method modified by Moress,
- Active acidity – with pH meter,

Surveys were conducted on the dynamics of the titratable acidity on the first, fifth, tenth, fifteenth, and twentieth day.

The sensor analyses were conducted on the second and the twentieth day by the quantitive descriptive analysis, where a scale of 1 to 10 evaluated the taste, smell, colour, and consistency of the product.

While performing the organoleptic tests, it was taken a strict care to create and maintain optimal working conditions. The yoghurt samples were in original packaging from the manufacturer, in bottles of 1 litter and tetra pack bottles also of 1 litter.

Statistical analysis of the results

The statistical analysis of the results includes the following methods:

- Descriptive statistics (summarizing of the results, frequencies, descriptive researches and the descriptive statistical correlations).
- The influence of the temperature factors, variants and storage were examined using general linear model (General Linear Model – GLM), where all the factors were independently analyzed and in all the factors where statistical significance was set out, it was set out with so-called post hoc testing using the Bonfferoni test.
- Statistical significance, T – test.

RESULTS AND DISCUSSION

pH dynamics

The results of the dynamics of the active acidity (pH) in the yoghurt variants during storage at 4 and 8 °C are presented in table 1.

Based on the obtained results, it can be concluded that the initial acidity among the manufacturers is different, namely V1 shows the slightest acidity compared to other variants.

During storage, gradually the acidity was increasing in all 3 variants, but with different intensity. Namely, at the V1-4 the active acidity in the first day was 4.31 and the twentieth day 4.11 while at the V1-8 active acidity of the twentieth day was lower and amounted to 4.07. In the first day of the active acidity at the V2-4 was 4.25, and the twentieth day was 4.02 and at 8°C was 4.00 and there for in V3-4 the active acidity in the first day was 4.19 and the twentieth day 4.03 while at the V3-8 on the twentieth day was 3.99.

Comparing the results of the variants stored at 4 and 8°C, it can be concluded that the variants which were stored at 8°C showed lower pH compared to variants that were stored at 4°C. The differences in the pH, in the yoghurt samples, storage with various temperature, are highly statisticcally significant ($p < 0.01$).

Additionally, the pH differences among the 3 variants were tested with the post hoc analysis (Bonfferoni test), where the differences among all the variants are highly significant. With the post hoc analysis we showed that the differences in the average pH values, between the storage days (1-20)

Table 1. DYNAMICS OF ACTIVE ACIDITY (pH) IN THE YOGHURT VARIANTS DURING STORAGE

Tabela 1. DINAMIKA AKTIVNE KISELOSTI (pH) U VARIJANTAMA JOGURTA TOKOM SKLADIŠTENJA

Days	pH - dynamics			
	V1-4		V1-8	
	$\bar{x}\pm S_{\bar{x}}$	Cv	$\bar{x}\pm S_{\bar{x}}$	Cv
1	4,31±0,020	1,36	4,31±0,020	1,36
5	4,29±0,009	0,65	4,27±0,004	0,30
10	4,23±0,005	0,37	4,19±0,002	0,19
15	4,18±0,003	0,25	4,15±0,012	8,67
20	4,11±0,004	0,32	4,07±0,015	1,10
	V2-4		V2-8	
1	4,25±0,019	1,28	4,25±0,019	1,28
5	4,21±0,019	1,32	4,17±0,023	1,56
10	4,15±0,024	1,68	4,12±0,023	1,63
15	4,09±0,023	1,65	4,06±0,025	1,8
20	4,02±0,027	1,94	4.00±0,027	1,95
	V3-4		V3-8	
1	4,19±0,010	0,68	4,19±0,010	0,68
5	4,14±0,094	0,64	4,12±0,072	0,49
10	4,10±0,056	0,39	4,08±0,054	0,38
15	4,07±0,010	0,71	4,04±0,088	0,61
20	4,03±0,077	0,54	3,99±0,049	0,34

are statistically significant, with increasing the time of storage and increasing the difference with the level of the significance. The pH differences between the day 1-5 and 5-10 are statistically significant (<0.05) while the difference in the pH between 15-20 days (-0.0398) is statistically insignificant (p>0.05). The remaining differences in the pH values between the storage days are highly statistically significant (p<0.01). Results are consistent with the Husain et.al. (2009) where the pH was 4.22, Abdulrahman et. al. (1998) with pH 4.3 (4.1 – 4.5) and Rodrigues et al. (2010) with a pH of 4.1 to 4.4, while Shalid et al. (2002) obtained slightly higher pH of 4.55 and Zamberlin et al. (2007) where the pH of the yoghurt ranged from 4.2 to 4.5.

All tested varieties of yoghurt are in accordance with the Rulebook of the quality of the milk and milk products (Official gazette of the Republic of Macedonia no. 96, 2011) according to which the yoghurt that is put on the market must not have active acidity of less than 4.0.

Titratable acidity dynamics

The results from the monitoring of the titratable acidity of the yoghurt variants during storage at 4°C and 8°C are presented in Table 2.

Based on the results from the Table 2, the dynamics of the titratable acidity gradually increases over a period of 20 days and in all variants that are stored at 4 and 8°C.

At the V1-4 titratable acidity in the period of 20 days increases by 8.47°SH (21.8%), at the V1-8 the titratable acidity with in 20 days increases by 10.45°SH (26.39%), the titratable acidity at the V2-4 in period of 20 days increases to 11.85°SH (29.11%) while in the period from tenth to twentieth day to 5.95°SH (12.76%).

The titratable acidity at V3-4 in period of 20 days increases to 11.43°SH (27.83%), at the V3-8 the titratable acidity in period of 20 days increases to 12.85°SH (31.28%).

Compared with the results of the variants which were stored at temperature of 4 and 8°C, the increase of the tutricional acidities higher in variants which were stored at 8°C in terms of variants stored at 4°C which shows that the higher temperature of storage adversely affects the quality of the yoghurt with which the durability

Table 2. DYNAMICS OF THE TITRATABLE ACIDITY OF THE YOGHURT VARIANTS DURING STORAGE

Tabela 2. DINAMIKA TITRACIONE KISELOSTI U VARIJANTAMA JOGURTA TOKOM SKLADIŠTENJA

Days	Titratable acidity (°SH)			
	V1-4		V1-8	
	$\bar{x}\pm S_{\bar{x}}$	Cv	$\bar{x}\pm S_{\bar{x}}$	Cv
1	38,80±0,69	5,03	38,80±0,69	5,03
5	40,12±0,67	4,76	41,05±0,66	4,61
10	43,30±0,29	1,93	44,05±0,39	2,55
15	45,02±0,46	2,92	46,22±0,38	2,36
20	47,27±0,36	2,17	49,25±0,52	2,99
	V2-4		V2-8	
1	40,70±0,77	5,38	40,70±0,77	5,38
5	42,37±0,63	4,27	43,15±0,56	3,71
10	45,02±0,52	3,31	46,60±0,38	2,33
15	47,30±0,75	4,52	48,80±0,88	5,14
20	50,63±0,53	2,99	52,55±0,53	2,90
	V3-4		V3-8	
1	41,07±0,59	4,07	41,07±0,59	4,07
5	42,87±0,36	2,02	44,45±0,71	4,57
10	46,85±0,26	1,57	48,10±0,16	0,95
15	48,87±0,20	1,19	49,72±0,23	1,34
20	52,50±0,51	2,77	53,92±0,51	2,71

decreases. With post hoc showed that the differences in average values of the titrabile acidity between the days of storage (1-20) are highly statically significant, with increasing the time of storage increases the difference of the level of significance. The differences of values in the titratable acidity between the days are highly significant (p<0.01). The differences in the titrabile values in the samples of yoghurt that are stored in various temperature are highly significant (p<0.01).

In addition, the differences of the values of the titrabile acidity in the 3 variants were tested with post hoc analysis (Bonfferoni test), where the differences between all variants are highly significant. From the obtained results, the dynamics of the tutracional acidity can be concluded with a minor deviations, the results coincide with the research of the Petrovic and Manci (2006) where the tutricional acidity on the first day amounted 35°SH, while according to Bjelačic et al. (2004) averaged 38.26°SH (highest 42°SH, and lowest 34.40°SH) and according to Vilušic (2003) the titratable acidity of the yoghurt in the first day of storage at 4°C amounted 41.50°SH, while on the 21st day increased to 49.50°SH (due to the tutricional acidity in the raw materials).

While the results did not match the research of Shalid et al. (2002) where the tutricional acidity was significantly higher and amounted 51.5°SH, and then Abdulrahman et.al. (1998) where there is a large variation in tutricional acidity yoghurt samples (25.5 to 53.3°SH) and with the tests of the Ekram et al. (2011) where on the ninth day the tutricional acidity amounted 54.6°SH.

The occurrence of certain discrepancies regarding the tutricional acidity, due to differences in production technology, application of starter cultures, the quality of the raw materials, the season and the demand by the consumers for more acidic taste.

According to the Book of rules, the tutricional acidity of the yoghurt which circulates should not exceed more than 55°SH, so that the variants which were the subject of examination meet the criterion.

Lactose dynamics

Based on the results shown in Table 3, the lactose dynamics is determined that in all varieties that are stored at 4 and 8°C, the lactose has a downward trend.

The variants of the lactose dynamics stored at 4 and 8°C the higgest decrease in percentage of lactose in yoghurt variants stored at 4°C are the most incentive in V3-4 (62.26%), followed by V1-4 (55.4%) and after that V2-4 (48.5%). In the samples stored at 8°C the most intensive decrease is shown in V3-8 (66.03%), followed by V1-8 (60.39%) and V2-8 (52.69%).

Table 3. DYNAMICS OF LACTOSE CONTENT IN THE YOGHURT VARIANTS DURING STORAGE

Tabela 3. DINAMIKA SADRŽAJA LAKTOZE U VARIJANTAMA JOGURTA TOKOM SKLADIŠTENJA

Days	Lactose dynamics(%)			
	V1-4		V1-8	
	$\bar{x} \pm S_{\bar{x}}$	Cv	$\bar{x} \pm S_{\bar{x}}$	Cv
1	2,02±0,03	4,43	2,02±0,03	4,43
10	1,77±0,04	7,91	1,32±0,09	20,5
20	0,90±0,01	3,97	0,80±0,02	10,4
	V2-4		V2-8	
1	1,67±0,08	13,9	1,67±0,08	13,9
10	1,36±0,11	23,9	1,29±0,11	25,4
20	0,86±0,03	12,3	0,79±0,03	12,5
	V3-4		V3-8	
1	1,59±0,01	1,92	1,59±0,01	1,92
10	0,85±0,023	7,77	0,79±0,02	10,6
20	0,60±0,017	8,11	0,54±0,01	6,1

Statistically significant difference has not been established between all varieties stored at 4 and 8°C on the tenth and the twentieth day. The biggest initial and final value of the percentage of the lactose is found in V1-4 and V1-8, while the lowest initial and final value was determined in V3-4 and V3-8. The obtained results from the lactose monitoring did not match with the research of many authors, and that is mainly due to the different technological procedure for the yoghurt production in South Africa and Sudan. Thus, according to Abdulrahman et al. (1998), the lactose was 2.6%, while according to Ekram et al. (2011) the lactose was 2.34% which is slightly higher compared with our results.

Dynamics of ash content, dry matter and milk fat

Based on the results that are presented, it was determined that the percentage of **ash** in all variants stored at 4 and 8°C, ranges from 0.69% to 0.73%.

Compared with the results of other authors there are no major deviations and they are around 0.9%, according to Abdulrahman (1997), and according to Ekram (2011) the ash was 0.66%.

Based on the results obtained, it was concluded that the amount of **protein** in the yoghurt ranges from 3.29% to 3.48%, in contrast to our results; other authors when they examined the protein content found higher values, so with Abdulrahman et al (1997) the protein moved in the range of 4.3% (3 to 4,7%), where among Ekram et al. (2011) was 3.89% and in Simun et al (2007) the protein ranged from 3.9% to 4.3%.

The **dry matter** in the yoghurt variants stored at 4 and 8°C ranges from 11.6 to 12.00 %. In all variants there was no statistically significant deviation, and there is no statistically significant difference either between the variants , or between days. According to Simun et al. (2007) the percentage of dry matter ranged from 10.53% to 10.73%, and according to Ekram et al. (2011), this percentage ranged from 10.95% to 11.51% which compared to our research, there is not much differrence in the obtained results.

The percentage of **milk fat** in all variants stored at 4 and 8°C, over all iterations are identical and amounted to 3.3%. Other authors found a lower percentage of milk fat. Thus, in the tests of Ekram et al. (2011) this percentage was around 3.06% and according to other authors is significantly larger, for example with Abdulrahman al. (1997) the content of milk fat in the yoghurt averaged of 3.9%. The obtained deviations are mainly due to the different technological procedure for the production of yoghurt beyond our borders.

Sensory analysis of the yoghurt variants

Sensory analysis, performed on the second day, showed a good results in all three variants, and most pondered points got brand 1 (V1), while the 20-th day analysis could not be conducted because all three varieties stored at 4 and 8°C, showed unacceptable changes in the texture and taste (Table 4).

Table 4. TOTAL POINTS AWARDED FOR SENSORY ANALYSIS OF YOGHURT VARIANTS AT 4° C AFTER 1. DAY

Tabela 4. UKUPNE OCENE SENZORNE ANALIZE VARIJANTI JOGURTA NA 4°C NAKON 1. DANA

Variants	Look	Consistency	Color	Odor	Taste	Total points
V1	1.79	4.19	1	1.83	7.69	15.9
V2	1.84	3.69	1	1.34	6.26	13.9
V3	1.47	4.26	1	1.59	6.02	14.6

CONCLUSION

Based on the surveys, the quality of yoghurt presented in the domestic market, we can give the following conclusion:

1. The examined variants of yoghurt stored at 4 and 8°C are showing constant chemical composition and there are no significant differrences between the days of storage or between the variants of yoghurt, or the dry matter was about 12%, no fat dry matter 8,5%, proteins 3,4%, milk fat 3,3% and the amount of ash 0,7%. The only change is in the lactose content.
2. The lactose dynamics from the first to the twentieth day, in all 3 variants of yoghurt, stored at 4 and 8°C, significantly decreases. The largest lactose percentage has V1 (2.02%), while V3 has the lowest percentage (1.59%). The active acidity (pH), in the stored samples at 4 and 8°C from the first to the twentieth day, is declining in all three variants where the greatest initial value has V1 (4.31), and the lowest V3 (4.19). During 20 days of storage, the variants that are storage at 4°C showed less acidity, however the higher pH compared to the variants which were stored at 8°C, and between them there is a statistically significant differentce at the 5, 10, 15, and the 20th day of the storage at the level of $p<0.05$.
3. By tracking the dynamics of titracional acidity, it is noted that it grows significantly from the first to the 20th day in all varieties stored at 4 and 8°C, and mainly due to the lactose transformation into lactic acid. Variants which were stored at 8°C sho-

wed greater tutracional acidity compared to those who were stored at 4°C, and between them there is a statistically significant difference on the 5, 10, 15, and the 20th day at level of p<0.05.

4. With the sensor analysis variants, the highest number of points was given to V1 (15.9), followed by V3 (14.6) and V2 which had the lowest given points (13.9). On the 20th day it was unable to perform the sensor analysis due to large changes in the smell and the consistency.

5. The examined yoghurt products on the market in the Republic of Macedonia are with different quality, which sometimes doesn't meet the requirements according to the good manufacturing practice. This is mainly due to the poor quality of raw milk, inadequate sanitation program in dairy processing plants and improper temperature storage of the final product. This means that the producers should pay close attention to the standard operating procedures for the yoghurt production and thus need to ensure safety and the consistent quality of the final products, also the market to take care of the hygiene and the storage temperature.

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IZVOD

PROMENE OSNOVNIH KOMPONENATA JOGURTA TOKOM SKLADIŠTENJA

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Cilj istraživanja u radu je bio da se identifikuju hemijske, biohemijske, senzorne i mikrobiološke karakteristike jogurta proizvedenih u tri vodeće makedonske mlekare, (kupljenih u maloprodajnim centarima), kao i promene koje se dešavaju u toku 20 dana skladištenja na 4 i 8°C. Utvrđeno je da temperatura i period skladištenja značajno utiču na promene titracione kiselosti, pH i sadržaja laktoze (p> 0,01), dok ne utiče na sadržaj suve materije, mlečne masti, pepela i proteina. Proizvodi pokazuju odstupanja u različitim godišnjim dobima, a najmanje varijacije su u hemijskom sastavu, dok su veće u pogledu kiselosti, što ukazuje na nedovoljnu standardizaciju tehnološkog procesa, ili varijacije temperature hlađenja i skladištenja. Senzorna analiza drugog dana pokazala je dobre rezultate, dok 20-og dana analiza nije sprovedena, jer su svi uzorci bili senzorno neprihvatljivi.

Ključne reči: jogurt • kvalitet • temperature • skladištenje

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UTICAJ ULTRAZVUKA NA FERMENTACIJU KOZIJEG MLEKA SA DODATKOM KONCENTRATA PROTEINA SURUTKE

Specifičan proteinski sastav i puferni kapacitet kozijeg mleka utiču na produženo vreme fermentacije i lošiju strukturu čvrstog jogurta. Kako bi se dobio čvrsti jogurt od kozijeg mleka zadovoljavajućih reoloških karakteristika, neophodno je povećati sadržaj suve materije bez masti što se može postići dodatkom koncentrata proteina surutke. U cilju skraćenja trajanja fermentacije, u radu je ispitivana mogućnost primene ultrazvučnog tretmana kozijeg mleka sa dodatkom KPS u proizvodnji čvrstog jogurta. Za proizvodnju čvrstog jogurta korišćene su tri serije uzoraka kozijeg mleka sa dodatkom 1% KPS podvrgnute različitim tretmanima: A – standardni tretman; B – ultrazvuk 200W/10 min i C – 400W/10 min.

Vršeno je ispitivanje fizičko-hemijskih karakteristika sirovog mleka, mleka nakon dodatka 1% KPS, termički tretiranih mleka, i mleka nakon ultrazvučnih tretmana. Takođe, u toku fermentacije praćena je promena viskoziteta i pH vrednosti.

Na osnovu dobijenih rezultata utvrđeno je da je fermentacija najkraće trajala kod uzoraka C, koji su ujedno imali najviše vrednosti viskoziteta na kraju fermentacije.

Key words: kozije mleko • koncentrati proteina surutke • ultrazvuk • fermentacija

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UVOD

Jogurt od kozijeg mleka je tradicionalan proizvod u područjima iz kojih fermentisani mlečni proizvodi vode poreklo. Osnovni parametri kvaliteta jogurta su ukus i konzistencija. Konzistencija jogurta u velikoj meri zavisi od strukture proteinske mreže koja je relativno slaba. Takođe, specifičan proteinski sastav utiče na lošiju strukturu fermentisanih napitaka od kozijeg mleka čijom se fermentacijom stvara polutečni koagulum, što otežava proizvodnju čvrstog jogurta od kozjeg mleka (Herrero and Requena, 2006; Park and Guo, 2006).

Osnovni hemijski sastav kozijeg mlijeka je sličan kravljem i zavisi od genotipa koza, ishrane, redosleda i stadijuma laktacije (Božanić et al., 2002). Prosečno, kozje mleko sadrži 12.2% suve materije od čega 3.8% čini mlečna mast, 3.5% proteini, 4.1% laktoza i 0.8% mineralnih materija (Park, 2006). Karakterističan miris i ukus kozjeg mleka koji je mnogim potrošačima neprihvatljiv i rezultat je specifičnog sastava mlečne masti, može se umanjiti fermentacijom mleka pod dejstvom bakterija mlečne kiseline.

Dijametar masnih kapljica kozjeg mleka je manji u odnosu na masne globule kravljeg mleka. Prema Slačanac et al. (2010) oko 65% masnih kapljica kozijeg mleka je prečnika manjeg od 3.0 μm. Takođe, kozije mleko sadrži više masnih kiselina kratkih i srednjih lanaca i odlikuje se nedostatkom aglutinirajućih proteina koji izazivaju agregaciju masnih kapljica i izdvajanje pavlake (Jandal, 1996). Kapronska, kaprilna i kaprinska kiselina (C_6, C_8, C_{10}) čine oko 20% masnih kiselina kozjeg mleka za razliku od svega 6% u kravljem mleku (Božanić et al., 2002). Povišeni sadržaj ove tri masne kiseline uzrok je izraženijeg ukusa i mirisa kozjeg mleka u odnosu na kravlje mleko (Park, 2006; Raynal-Ljutovac et al., 2008; Slačanac et al., 2010).

Kazeinske micele kozijeg mleka se razlikuju od kazeinskih micela kravljeg mleka po veličini, hidratisanosti i mineralizaciji. Kazeinske micelle kozijeg mleka sadrže više kalcijuma, neorganskog fosfora, manje su termički stabilne, manje rastvorljive i brže gube β-casein u odnosu na kazeinske micele kravljeg mleka (Park et al. 2007).

Kozije mleko sadrži 3.0-4.5% proteina (Antunac et al., 2000; Jandal, 1996). Kao i u kravljem mleku, kazein kozijeg mleka se sastoji od četiri frakcije: α_{s1}-CN, α_{s2}-CN, β-CN i κ-CN. Najzastupljenija kazeinska frakcija je β-kazein sa oko 50%. U odnosu na kravlje, kozije mleko ima manje α_{s1}-kazeina što smanjuje njegova alergena svojstva (Park and Guo, 2006; Raynal-Ljutovac et al., 2008). U pogledu sadržaja i strukture κ-kazeina ne postoje značajnije razlike između kozijeg i kravljeg mleka. Kozije mleko, takođe, karakteriše viši sadržaj serum proteina i neproteinskog azota u odnosu na kravlje mleko (Antunac et al., 2000; Sarić et al., 2005). Manji udeo kazeinskog azota i veličina kazeinskih micela utiču na lošiju strukturu fermentisanih napitaka od kozijeg mleka (Park et al., 2007). U poređenju sa kravljim, kozije mleko ima veći puferni kapacitet usled višeg sadržaja serum proteina i neproteinskog azota (Antunac et al., 2000; Božanić et al., 2002; Park and Guo, 2006).

Sadržaj proteina, termički tretman, prisustvo mlečne masti, stabilizatori i egzopolisaharidi su faktori koji utiču na strukturu proteinskog matriksa jogurta. Da bi se dobila zadovoljavajuća konzistencija čvrstog jogurta od kozjeg mleka, neophodno je povećati sadržaj suve materije bez masti. U cilju poboljšanja reoloških karakteristika čvrstog jogurta od kozjeg mleka vrši se koncentrisanje mleka membranskim procesima, dodavanje želatina ili pektina, obranog mleka u prahu, koncentrata proteina surutke, korišćenje starter kultura koje proizvode egzopolisaharide i dr. (Martín-Diana et al., 2003; Tamime and Robinson, 2000).

Serum proteini i proizvodi tipa koncentrata i izolata proteina surutke odlikuju se visokom sposobnošću želiranja i vezivanja vode, emulgovanja, obrazovanja i stabilizacije pene, i na ovim osobinama se zasniva njihova primena u industriji mleka (Jovanović et al., 2007). Dodatakom koncentrata proteina surutke u proizvodnji jogurta dobija se proizvod koji ima veći viskozitet i pokazuje smanjeni sinerezis (Lucey et al., 1999; Maćej et al., 2007; Tratnik, 1998). Nativni proteini surutke imaju malo uticaja na konzistenciju, međutim, denaturacija proteina surutke (prisutnih u mleku i dodatih u obliku KPS) tokom termičkog tretmana mleka dovodi do porasta viskoziteta. S obzirom da se pH vrednost tokom fermentacije mleka približava izoelektričnoj tački proteina surutke, denaturisani proteini surutke u termički tretiranom mleku su podložni agregaciji tokom acidifikacije (Lucey et al. 1999).

Ultrazvuk je jedna od novih metoda koja se može koristiti u različitim procesima u industriji mleka. Ultrazvuk visoke snage se primenjuje kao pomoć kod membranskih procesa, za uništavanje mikroorganizama, homogenizaciju mleka, poboljšavanje teksture mlečnih proizvoda, poboljšavanje funkcionalnih i tehnoloških karakteristika proteina itd. (Režek Jambrak et al. 2009).

Prema Riener et al. (2009a, 2009b) jogurt proizveden od mleka koje je tretirano ultrazvukom ima bolju teksturu usled smanjenja veličine čestica proteina i homogenizacije mleka. U poređenju sa jogurtom proizvedenim od netretiranog mleka, jogurt proizveden od mleka koje je tretirano ultrazvukom ima veći viskozitet i kapacitet vezanja vode, i takođe pokazuje manji sinerezis (Vučić et al., 2010, 2011, Wu et al. 2001).

Cilj ovog istraživanja je da se ispita uticaj ultrazvučnog tretmana kozijeg mleka sa dodatkom koncentrata proteina surutke na fizičko-hemijske karakteristike mleka i tok fermentacije u proizvodnji čvrstog jogurta.

MATERIJAL I METODI

U istraživanju je korišćeno kozije mleko sa farme „Beocapra“, Kukujevci. Sirovo kozje mleko je termički tretirano na 92°C/10 min. Fermentacija je vršena na 43°C korišćenjem starter kulture FD-DVS YFL812 Yo-Flex Chr. Hansen, Danmark. Nakon postizanja pH vrednosti 4.6 dobijeni uzorci su podvrgnuti hlađenju, a zatim skladišteni na temperaturi 4°C.

Uzorci jogurta su proizvedeni od kozjeg mleka kome je 1h pre termičkog tretmana dodato 1% koncentrata proteina surutke Textrion Progel 800, DMV International, Holandija. uzorci jogurta A proizvedeni su po standardnom tehnološkom procesu proizvodnje, dok su uzorci jogurta B i C proizvedeni od kozjeg mleka koje je nakon termičkog tretmana tretirano ultrazvukom u trajanju od 10 minuta, snage 200W (uzorak B) i 400W (uzorak C), na temperaturi 43°C. Za tretman mleka ultrazvukom korišćeno je ultrazvučno vodeno kupatilo frekvencije 35kHz, Raypa, Španija.

Ispitivan je hemijski sastav sirovog mleka, mleka sa dodatkom 1% KPS, termički tretiranog mleka, kao i mleka nakon ultrazvučnog tretmana.

Kod svih uzoraka mleka vršene su sledeće analize: suva materija metodom sušenja na 102±2ºC (Carić i sar., 2000), mlečna mast metodom po Gerberu (IDF 105:1981; Carić i sar., 2000), proteini metodom po Kjeldahu pomoću Kjeltec sistema (IDF 20B: 1993), laktoza titracionom metodom (IDF 28:1974), mineralne materije (Carić i sar., 2000), titraciona kiselost mleka po Soxhlet-Henkel-u (°SH) (Carić i sar., 2000), i pH vrednost pH-metrom sa kombinovanom elektrodom model *Consort C 931*.

U toku fermentacije ispitivan je viskozitet mleka pomoću rotacionog viskozimetra: Visco Basic+R, Fungilab (Španija) konstantnom brzinom spindla (60 ob/min) na svakih 30 min, počevši od momenta inokulacije (nulto očitavanje) pa sve dok se pH vrednost ne spusti do 4,6. Ispitivanje je vršeno u dva ponavljanja, pri čemu je za svako ponavljanje korišćen novi uzorak. U istom vremenskom intervalu vršeno je i praćenje pH vrednosti pH-metrom sa kombinovanom elektrodom model *Consort C 931*.

Istraživanje je vršeno u laboratoriji za tehnologiju mleka na Poljoprivrednom fakultetu u Beogradu.

REZULTATI I DISKUSIJA

Hemijski sastav mleka

Kod svih uzoraka ispitivan je sastav sirovog mleka, mleka nakon dodatka 1% koncentrata proteina surutke, termički tretiranog mleka, i mleka nakon ultrazvučnog tretmana (uzorci B i C). Rezultati iz ovog dela istraživanja prikazani su u Tabeli 1.

Iz podataka prikazanih u Tabeli 1., kod svih ispitivanih uzoraka, može se uočiti porast sadržaja suve materije i sadržaja proteina nakon dodavanja koncentrata proteina surutke. Takođe, na porast sadržaja suve materije utiče i termički tretman mleka, usled koga dolazi do isparavanja dela vode iz mleka. Kod uzoraka mleka B i C nakon ultrazvučnog tretmana uočen je mali porast sadržaja mlečne masti, što je verovatno posledica delovanja ultrazvuka koji dovodi do razaranja masnih globula i homogenizacije mleka (Vercet et al. 2002; Herceg et al. 2009). Međutim, porast sadržaja mlečne masti zabeležen u ovom istraživanju nije veliki, što se može objasniti dužinom trajanja ultrazvučnog tretmana. Istraživanja Vučić et al. (2010) su pokazala da kraće trajanje ultrazvučnog tretmana (5 min) povoljnije utiče na homogenizaciju i dovodi do značajnijeg povećanja sadržaja mlečne masti.

Povećanje titracione kiselosti mleka zabeleženo je kod svih uzoraka nakon dodavanja 1% KPS i rezultat je povećanja sadržaja suve materije bez masti usled pufernog kapaciteta dodatih proteina. Međutim, nakon tretmana ultrazvukom zabeleženo je smanjenje titracione kiselosti mleka koje je izraženije kod uzoraka tretiranih ultrazvukom snage 400W.

Fermentacija mleka

Fermentacija mleka je najznačajnija operacija u proizvodnji jogurta tokom koje dolazi do nastajanja gela čiju osnovu čini proteinski matriks. Shodno tome osobine dobijenog gela, kako fizičko-hemijske tako i senzorne, u velikoj meri zavise od toka fermentacije. Potrebno je da pad pH vrednosti tokom fermentacije bude postepen kako bi se mreža gela pravilno formirala.

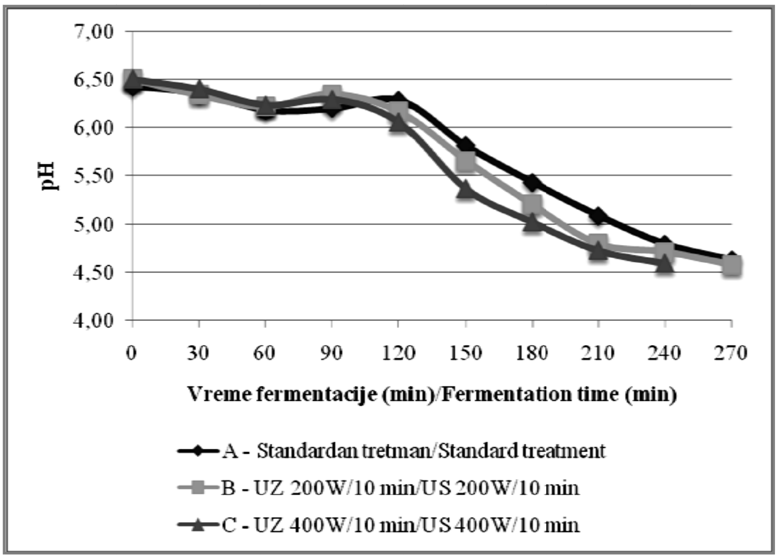
Fermentacija se odvijala na 43°C do postizanja pH vrednosti 4,6 kada je prekidana hlađenjem, jer bi dalji porast kiselosti doveo do kontrakcije gela i izdvajanje surutke na površini jogurta. Tok fermentacije praćen je me-

Tabela 1. HEMIJSKI SASTAV KOZIJEG MLEKA KORIŠĆENOG ZA PROIZVODNJU ČVRSTOG JOGURTA
Table 1. CHEMICAL COMPOSITION OF GOAT MILK USED FOR PRODUCTION OF SET-STYLE YOGHURT

Uzorak/Sample	Pokazatelj/ Parameter	Sirovo mleko/ Raw milk	Mleko sa dodatkom 1% KPS/ Milk supplemented with 1% WPC	Termički tretirano mleko/ Heat treated milk	Mleko tretirano ultrazvukom/ Ultrasound treated mik
A – Standardan tretman/ Standard treatment	SM/TS (%)	11.14±0.02	11.88±0.05	12.15±0.02	-
	Mast/Fat (%)	3.10±0.00	3.10±0.00	3.20±0.00	-
	SMbM/TSNF (%)	8.05±0.02	8.78±0.05	8.95±0.02	-
	Proteini/Proteins (%)	3.06±0.06	3.77±0.01	3.92±0.08	-
	Laktoza/Lactose (%)	4.49±0.00	4.63±0.00	4.63±0.00	-
	Pepeo/Ash (%)	0.90±0.00	0.93±0.01	0.96±0.01	-
	Kiselost/Acidity (°SH)	6.77±0.06	7.21±0.12	7.35±0.00	-
	pH	6.52±0.00	6.54±0.01	6.47±0.00	-
B – UZ 200W/10 min/ US 200W/10 min	SM/TS (%)	11.24±0.05	12.19±0.05	12.49±0.02	12.57±0.02
	Mast/Fat (%)	3.10±0.00	3.10±0.00	3.20±0.00	3.22±0.03
	SMbM/TSNF (%)	8.14±0.05	9.09±0.05	9.29±0.02	9.35±0.03
	Proteini/Proteins (%)	2.72±0.07	3.41±0.01	3.53±0.07	3.52±0.03
	Laktoza/Lactose (%)	4.70±0.00	4.75±0.06	5.02±0.05	4.91±0.00
	Pepeo/Ash (%)	0.78±0.00	0.83±0.01	0.87±0.01	0.84±0.01
	Kiselost/Acidity (°SH)	5.67±0.33	5.08±0.00	5.86±0.00	5.54±0.12
	pH	6.82±0.00	6.77±0.00	6.70±0.00	6.68±0.01
C – UZ 400W/10 min/ US 400W/10 min	SM/TS (%)	10.92±0.34	12.06±0.04	12.38±0.03	12.39±0.03
	Mast/Fat (%)	2.93±0.06	2.93±0.06	3.03±0.06	3.07±0.06
	SMbM/TSNF (%)	8.29±0.09	9.12±0.10	9.34±0.07	9.32±0.08
	Proteini/Proteins (%)	2.69±0.02	3.43±0.04	3.56±0.04	3.52±0.03
	Laktoza/Lactose (%)	4.77±0.00	4.81±0.05	4.77±0.00	4.84±0.10
	Pepeo/Ash (%)	0.78±0.00	0.81±0.01	0.83±0.01	0.83±0.01
	Kiselost/Acidity (°SH)	5.25±0.07	5.70±0.05	5.73±0.23	5.01±0.13
	pH	6.87±0.00	6.83±0.00	6.73±0.00	6.71±0.01

renjem pH vrednosti i viskoziteta, od momenta inokulacije a zatim na svakih 30 minuta do pH 4,6. Dobijeni rezultati su prikazani slikama 1, 2, i 3.

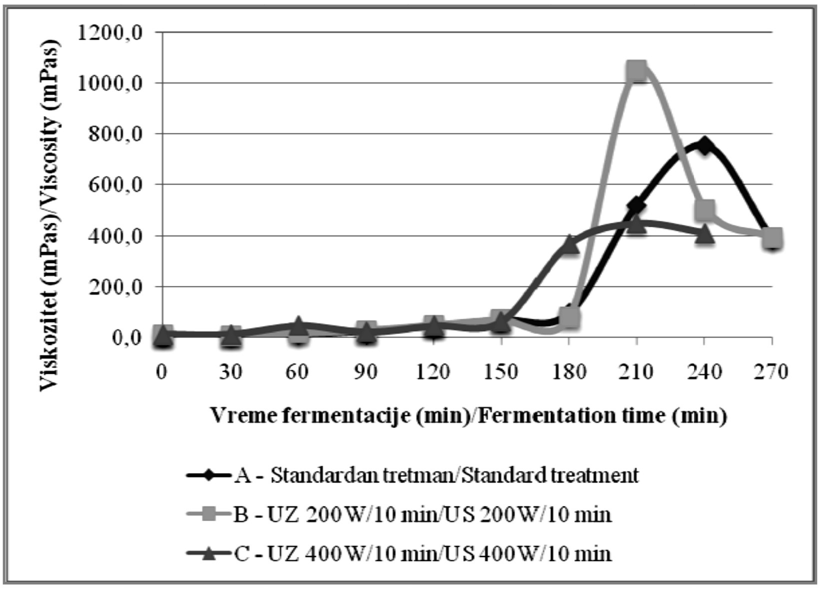
Najznačajniji faktori koji utiču na formiranje kiselog kazeinskog gela su sadržaj kazeina, pH i sadržaj kalcijuma u mleku. Pri niskim pH vrednostima kalcijum disocira iz kazeinskih micela i dolazi do anuliranja negativnog naelektrisanja micela što dovodi do agregacije micela i formiranja gela. Pri pH 4.6 formirana je trodimenzionalna proteinska mreža u koju su uklopljene ostale komponente mleka (Jumah et al., 2001, Maćej et al., 2007).



Slika 1. PROMENA pH VREDNOSTI U TOKU FERMENTACIJE

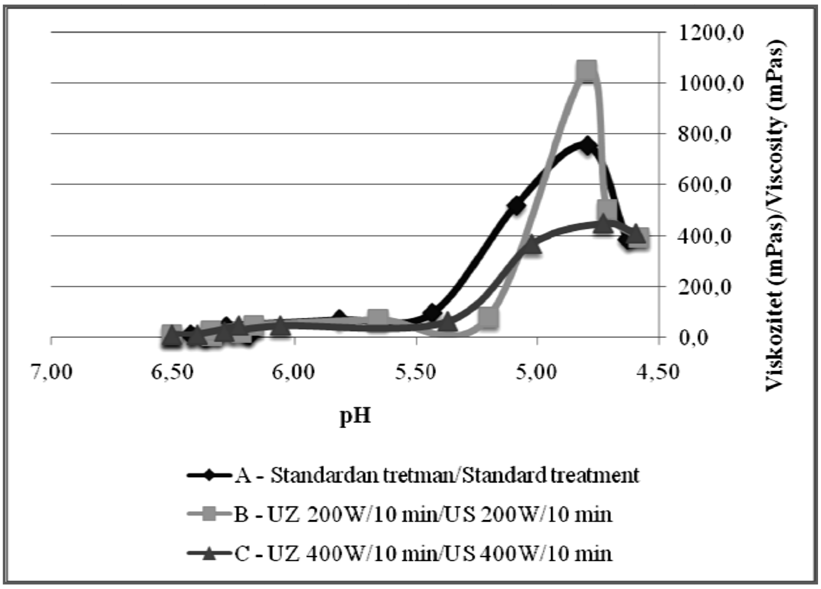
Figure 1. pH CHANGE DURING FERMENTATION

Fermentacija kozijeg mleka sa dodatkom 1% KPS trajala je 270 min za uzorke A i B, odnosno 240 min za uzorke C. Duža fermentacija u odnosu na kravlje mleko može se objasniti proteinskim sastavom kozijeg mleka i dodatkom KPS. Naime, zbog većeg udela serum proteina puferni kapacitet kozjeg mleka je viši u odnosu na kravlje mleko, što uslovljava sporiji pad pH vrednosti tokom fermentacije (Božanić et al., 2002). Promena pH vrednosti tokom fermentacije je ujednačena kod svih ispitivanih uzoraka. Takođe, kao posledica pufernog kapaciteta kozijeg mleka, kod svih uzoraka je uočen blag porast pH vrednosti. Kod uzoraka proizvedenih na standardan način porast pH vrednosti je zabeležen od 90-og do 120-og minuta fermentacije, dok se kod uzoraka tretiranih ultrazvukom porast pH vrednosti uočava u periodu od 60-og do 90-og minuta fermentacije. Opiranje promeni pH kod uzoraka koji su tretirani ultrazvukom takođe se može objasniti povoljnim dejstvom koje ultrazvuk ima na osobine proteina surutke (Režek Jambrak i sar., 2009).



Slika 2. PROMENA VISKOZITETA U TOKU FERMENTACIJE

Figure 2. VISCOSITY CHANGE DURING FERMENTATION



Slika 3. PROMENA VISKOZITETA U TOKU FERMENTACIJE U ZAVISNOSTI OD pH VREDNOSTI

Figure 3. VISCOSITY CHANGE DURING FERMENTATION DEPENDING ON pH VALUES

Iako sadržaj suve materije ima značajan uticaj na reološke karakteristike jogurta, viši viskozitet mleka u toku fermentacije pripisuje se povećanom kapacitetu vezivanja vode od strane proteina (Tammime and Robinson, 2000). Promena viskoziteta tokom fermentacije je rezultat agregacije kazeinskih micela i formiranja gela. Fermentaciju mleka u proizvodnji jogurta karakteriše faza indukcije (stacionarni viskozitet), logaritamska faza (maksimalno povećanje viskoziteta) praćena fazom metastabilne ravnoteže u kojoj je viskozitet konstantan i fazom sinerezisa u kojoj dolazi do smanjenja viskoziteta (Maćej et al., 2007; Tammime and Robinson, 2000). Kod uzoraka A i B u toku prvih 180 minuta fermentacije viskozitet pokazuje trend blagog rasta (faza indukcije). Viskozitet uzoraka A u periodu od 180-og do 240-og minuta raste do maksimalne vrednosti od 756.8 mPas usled agregacije kazeinskih micela, nakon čega je u narednih 30 min fermentacije zabeležena faza sinerezisa. Viskozitet uzoraka A je na kraju fermentacije iznosio 385.7 mPas. Maksimalna vrednost viskoziteta uzoraka B iznosila je 1050.7 mPas i zabeležena je nakon 210 min fermentacije. Kod uzoraka C stacionarna faza fermentacije trajala je 150 min, a maksimalan viskozitet je kao i kod uzoraka B utvrđen nakon 210 min fermentacije. Na kraju fermentacije najveći viskozitet zabeležen je kod uzoraka C – 411.9 mPas.

Promene viskoziteta uzoraka mleka u toku fermentacije u zavisnosti od pH vrednosti pokazuju sličan trend kao i promene viskoziteta prikazane na slici 2. Tokom pada pH vrednosti od 6.5 do 5.4 (kod uzoraka A i C), odnosno 5.2 kod uzoraka B, nije zabeležen značajniji porast viskoziteta, s obzirom da su u ovom pH opsegu kazeinske micele uniformne u pogledu veličine i distribucije (Hassan et al, 1995; Jumah et al., 2001; Maćej et al., 2007). Tokom fermentacije kravljeg mleka maksimalna vrednost viskoziteta uočava se pri pH 5.4 - 5.3. U ovoj fazi dolazi do povezivanja malih agregata kazeinskih micela u krupnije agregate (tzv. grozdove) i lance, te do približavanja novonastalih agregata i lanaca (Hassan et al, 1995). Međutim, usled specifičnog proteinskog sastava kozijeg mleka, dodatog koncentrata proteina surutke i primenjenog ultrazvučnog tretmana, maksimalne vrednosti viskoziteta u toku fermentacije uzoraka ispitivanih u ovom istraživanju zabeležene su pri nižim pH vrednostima: za uzorak A - 4,80, uzorak B - 4,80 i uzorak C - 4,73. Niže vrednosti viskoziteta kod uzorka mleka tretiranog ultrazvukom u toku fermentacije pri pH 5,4 - 5,3 mogu se objasniti smanjenjem veličina čestica proteina usled delovanja ultrazvuka (Riener et al., 2009). Smanjenje viskoziteta kod svih uzoraka zabeleženo je u fazi sinerezisa pri pH 4,8 - 4,6 kada dolazi do delimične dezintegracije kazeinskih micela i formiranja manjih agregata kazeina koje odlikuje poroznija struktura (Jumah i sar., 2001.)

ZAKLJUČAK

Na osnovu dobijenih rezultata utvrđeno je da je fermentacija najkraće trajala kod uzoraka koji su tretirani ultrazvukom snage 400W. Uzorci C su ujedno imali najviše vrednosti viskoziteta na kraju fermentacije.

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SUMMARY

THE INFLUENCE OF ULTRASOUND ON FERMENTATION OF GOAT MILK SUPPLEMENTED WITH WHEY PROTEIN CONCENTRATES

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Due to specific chemical composition of goat milk one of the main problems in production of goat milk yogurt is weakness and lack of consistency, as well as prolonged fermentation time. To obtain a satisfactory consistency of set-style yogurt made of goat milk, an increase in the content of non-fat solids is required. For this purpose supplementation of milk with whey protein concentrates can be used. Possibility of application of ultrasound treatment of goat milk supplemented with 1% WPC was used in order to shorten fermentation time.

Three series of samples were produced from goat milk supplemented with 1% WPC: A - standard treatment, B - ultrasound treatment 200W/10 min and C - ultrasound treatment 400W/10 min.

Physicochemical properties of raw milk, milk supplemented with 1% WPC, heat treated milk and milk after ultrasound treatment were investigated. Furthermore, during fermentation change of viscosity and pH were investigated.

Based on data analysis it was concluded that the samples C had the shortest time of fermentation. At the end of fermentation highest viscosity values also had samples C.

Ključne reči: goat milk • whey protein concentrates • ultrasound • fermentation

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UTICAJ GEOMETRIJE PROMOTORA TURBULENCIJE NA POVEĆANJE KAPACITETA TOKOM KONCENTRISANJA MLEKA

Primena membranskih procesa u prehrambenoj industriji je ograničena zbog smanjenja kapaciteta usled prljanja membrane. Adsorbovani i nataloženi proteini na površini i/ili u porama membrane, stvaraju dodatni otpor prenosu mase i smanjuju kapacitet kroz membranu. Promena hidrodinamičkog režima uvođenjem promotora turbulencije u membranski modul predstavlja jedan od načina da se smanji prljanje. U ovom radu je ispitan uticaj promotora turbulencije različitog geometrijskog oblika i približno iste karakteristične dimenzije na povećanje kapaciteta tokom koncentrisanja obranog mleka i smanjenje otpora usled prljanja. Kao promotori turbulencije su korišćeni uvrnuta traka, Keniks statički mikser i promotor čiji su elementi oblika sečiva, Koflo mikser. Pokazalo se da se otpor usled prljanja smanji za red veličine u odnosu na rad bez promotora, pri čemu dolazi i do smanjenja potrošnje energije za 28 do 50%. Promena oblika putanje strujnica u slučaju sva tri promotora ima ključni uticaj na smanjenje prljanja i poboljšanje selektivnih karakteristika membrane.

Ključne reči: promotori turbulencije • koncentrisanje mleka • otpor prljanja • selektivnost membrane

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UVOD

Koncentrisani mlečni proizvodi nastaju delimičnim uklanjanjem vode iz mleka. Mali sadržaj vode u mlečnim proizvodima onemogućuje razvoj mikroorganizama i omogućuje dugotrajnije skladištenje. Najčešće primenjivan postupak za koncentrisanje mleka je isparavanje vode i uklanjanje nastale pare (Carić, 1988). Sa zahtevom da se energetski invazivne tehnologije zamene tehnologijama sa malim utroškom energije, dolazi do zamene procesa isparavanja procesom membranske filtracije.

Membranski procesi (ultrafiltracija, elektrodijaliza i dr.) su već sedemdesetih godina imali industrijsku primenu za koncentrisanje mleka, a sa razvojem membranske tehnologije primenjivali su se i u proizvodnji drugih prehrambenih proizvoda. Ultrafiltracija, kao najzastupljeniji membranski proces, koristi se za ugušćivanje mleka, koncentrisanje surutke i u proizvodnji sireva. Reversna osmoza se, takođe, koristi za koncentrisanje mleka i surutke, dok se mikrofiltracija najčešće koristi za prečišćavanje surutke i uklanjanje bakterija ali i za odvajanje kazeina od proteina surutke (Saboya i Maubois, 2000).

Veća komercijalna primena mikrofiltracije je još uvek ograničena usled nedovoljno velikih flukseva kroz membranu. Smanjen kapacitet uslovljen prljanjem membrane tokom filtracije je jedna od negativnih karakteristika membranskih procesa (Popović i sar., 2011). Opadanje fluksa filtrata je naročito izraženo tokom koncentrisanja i frakcionisanja proteina mleka i surutke, gde dolazi do prljanja membrane usled adsorpcije i taloženja proteina na površini i u porama membrane (Knops i sar., 1992).

Glavnu oblast istraživanja u membranskim tehnologijama predstavlja povećanje kapaciteta procesa, odnosno povećanje fluksa filtrata kroz membranu. Pored mehaničkih i hemijskih metoda, za unapređenje procesa membranske filtracije se koriste i hidrodinamičke metode koje povećavaju turbulenciju i izazivaju nestacionarno strujanje kroz membranu (Brans i sar., 2004, Hilal i sar., 2008, Krstić i sar., 2002, 2003). Umetanje različitih geometrijskih elemenata, promotora turbulencije, u membranski modul je hidrodinamička metoda kojom se menja režim strujanja i oblik strujnica. Promotori intenziviraju turbulenciju u membrani što smanjuje koncentraciju proteina u blizini zida membrane i na samoj membrani, te na taj način preventivno smanjuju prljanje membrane. Geometrijski oblik promotora turbulencije utiče na hidrodinamički režim i sliku strujanja. Upotrebom glatke šipke u keramičkoj membrani pri filtraciji 3% rastvora dekstrana, Mavrov i sar. (1992) su postigli povećanje fluksa od dva puta dok su primenom promotora koničnog oblika ostvarili poboljšanje od šest puta. Promotori u obliku zavrtnja različitog hoda primenjeni su u ultrafiltraciji mleka, mikrofiltraciji pekarskog kvasca i nanofiltraciji boja (Bellhouse i sar., 2001) pri čemu su postignuta povećanja fluksa do deset puta u odnosu na rad bez promotora pri istim radnim uslovima. Primenom Keniks statičkog miksera u koncentrisanju obranog mleka, Krstić i sar.(2002, 2003) su ostvarili poboljšanje fluksa od 700%. Iako se pokazalo da Keniks mikser daje veće povećanje fluksa u odnosu na druge promotore turbulencije, povećanje pada pritiska i velika promena transmembranskog pritiska duž membrane čine ga manje atraktivnim u odnosu na druge promotore.

Oblik i dimenzije elemenata promotora turbulencije utiču na formiranje strujnog polja u membrani. Primenom glatke šipke dolazi samo do lokalnog ubrzavanja fluida usled smanjene površine poprečnog preseka za proticanje, čime se narušava formiranje graničnog sloja i smanjuje prljanje. Promotori u obliku sečiva, kao što je Koflo mikser, ne blokiraju centar membrane celom dužinom već samo na mestima gde se ukrštaju sečiva. Kod promotora helikoidnog oblika, kao što su uvrnute trake i Keniks statički mikser, tok strujnica se menja iz pravog u helikoidni usled čega dolazi do struganja i odnošenja čestica sa površine membrane.

Smanjenje prljanja membrane i povećanje fluksa filtrata koji se postižu upotrebom promotora turbulencije, praćeni su i povećanim padom pritiska što uzrokuje povećanje potrošnje energije. Pojava pada pritiska utiče i na ekonomsku isplativost primene promotora kao i na performanse procesa zbog neuniformnosti transmembranskog pritiska duž membrane. Analiza unapređenja fluksa nije dovoljan parametar za karakterizaciju ekonomske isplativosti zbog čega je neophodno odrediti i specifičnu potrošnju energije po jedinici zapremine dobijenog filtrata.

U ovom radu je ispitan uticaj geometrije promotora u uslovima koncentrisanja obranog mleka kao i njihov uticaj na potrošnju energije u datim uslovima. Primenom promotora turbulencije može doći do izostanka formiranja dinamičke membrane usled čega bi došlo do propuštanja frakcija micela kazeina manjih od prečnika pora membrane. Stoga je ispitan uticaj primene promotora turbulencije na selektivnost membrane i propuštanje komponenata.

MATERIJAL I METODE

Laboratorijska aparatura za mikrofiltraciju/ultrafiltraciju je korišćena za ispitivanja koncentrisanja delimično obranog, pasterizovanog mleka sa 3,2% proteina mleka (Dukat, Somborska mlekara). Eksperimenti su izvođeni na kompozitnoj keramičkoj membrani sa aktivnim slojem od ZrO_2 (Gea Exekia, Francuska), unutrašnjeg prečnika 6,8 mm, srednje veličine pora 0,1 μm i aktivne površine 46,2 cm^2.

Operativna temperatura za sve eksperimente je iznosila 50±0,5°C i kontrolisana je rashladnim fluidom u plaštu napojnog suda. Napojna struja je transportovana pomoću višestepene centrifugalne pumpe (CM-9, Grundfos, Nemačka). Operativni protok je praćen pomoću ultrazvučnog merača protoka (DFXL, Dynasonics,USA) dok je pomoću digitalnih manometara postavljenih na ulazu i izlazu iz cevnog membranskog modula praćen transmembranski pritisak (TMP). Digitalna vaga (EG 1500-2M, Kern, Nemačka) povezana na personalni računar (PC) je korišćena za kontinualno merenje mase filtrata.

U eksperimentalnom istraživanju primenjeni su promotori turbulencije različitog geometrijskog oblika (slika 1) čije su karakteristike prikazane u tabeli 1.

Analiza uklanjanja naslaga sa površine i iz pora membrane izvršena je na osnovu otpora prljanja membrane i to ukupnog otpora usled prljanja, R_f, kao i pojedinačnih otpora usled prljanja koji zaostaje nakon ispiranja membrane.

Ukupni hidraulički otpor prljanja (R_{tf}) može se podeliti na otpor čiste membrane (R_m) i otpor usled prljanja membrane (R_f):

$$R_{tf} = R_m + R_f \qquad (1)$$

Otpor čiste membrane (R_m) i ukupni otpor prljanja (R_{tf}) mogu se odrediti iz fluksa čiste vode J_w i fluksa filtrata J_f:

$$R_m = \frac{TMP}{\mu_w J_w} \qquad (2)$$

$$R_{tf} = \frac{TMP}{\mu_f J_f} \qquad (3)$$

gde μ_w i μ_f predstavljaju dinamičke viskozitete destilovane vode i filtrata, respektivno.

Kako bi se sagledao uticaj promotora turbulencije na potrošnju energije izračunata je relativna potrošnja energije (ER):

$$ER = \frac{E_{NTP} - E_{TP}}{E_{NTP}} \cdot 100(\%) \qquad (4)$$

gdje je specifična potrošnja energije sa i bez promotora definisana kao gubitak hidrauličke snage po jedinici zapremine filtrata:

$$E = \frac{P}{J_f A_{ac}} \qquad (5)$$

Gubitak hidrauličke snage se može izraziti kao proizvod primenjenog protoka (Q) i apsolutnog pada pritiska kroz membranu (△p):

$$P = Q \cdot \Delta p \quad (W) \qquad (6)$$

Selektivne karakteristike membrane su određene preko propuštanja ukupnih proteina (UP), proteina surutke (PS) i kazeina (K) kroz membranu tokom koncentrisanja mleka. Koncentracija ukupnih proteina se određuje na osnovu sadržaja ukupnog azota koga sadrže i druge materije koje nisu proteini (aminokiseline i peptidi) te je selektivnost izražena u odnosu na propuštanje pravih proteina (PP).

Koncentracije proteina određene su analizom filtrata nakon koncentrisanja i izračunavane su iz sadržaja azota određenog Kjeldal metodom na sledeći način:

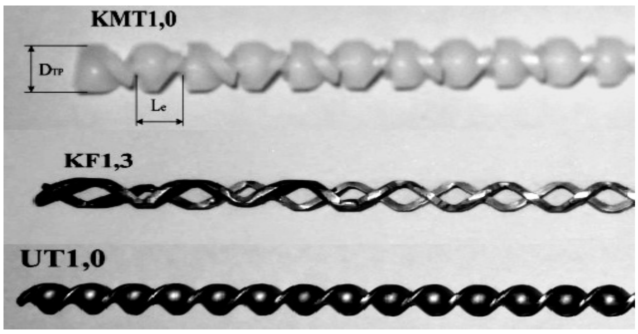
$$C_{UP} = UN \cdot 6{,}38\ (\%) \qquad (7)$$

$$C_{PP} = (UN - NPN) \cdot 6{,}38\ (\%) \qquad (8)$$

$$C_K = (UN - NKN) \cdot 6{,}38\ (\%) \qquad (9)$$

$$C_{PS} = (NKN - NPN) \cdot 6{,}38\ (\%) \qquad (10)$$

Stepen propuštanja određenih komponenata izračunavan je iz odno-



Slika 1. PROMOTORI TURBULENCIJE

Figure 1. TURBULENCE PROMOTERS

Tabela 1. KARAKTERISTIKE PROMOTORA TURBULENCIJE

Table 1. CHARACTERISTICS OF TURBULENCE PROMOTERS

Oznaka	KMT1,0	UT1,0	KF1,3
Dužina promotora L_{TP} (mm)	250	241	242
Prečnik promotora D_{TP} (mm)	6,32	6,5	6,2
Karakteristična dimenzija $O_{TP}=L_e/D_{TP}$	1,0	1,0	1,3

sa koncentracija odgovarajuće komponente u filtratu na kraju procesa i koncentracije u napojnoj smeši na početku procesa:

$$\text{Prop. komp.} = \frac{C_{komp,fil}}{C_{komp,ns}} \cdot 100\ (\%) \quad (11)$$

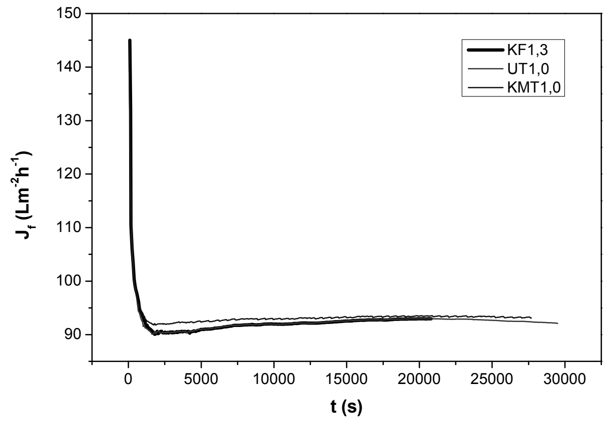
REZULTATI I DISKUSIJA

Koncentrisanje mleka

Rezultati dobijeni ispitivanjem u uslovima potpune recirkulacije napojne smeše su pokazali da se upotrebom promotora turbulencije povećava kapacitet procesa tako da je pretpostavljeno da će i u uslovima koncentrisanja promotori takođe povećati fluks filtrata. Kako fluks filtrata utiče na kapacitet i efikasnost celog procesa, poređenje uticaja tipa promotora u uslovima koncentrisanja napojne smeše izvršeno je pri istom fluksu filtrata. Odnosno, na osnovu rezultata tokom ispitivanja u uslovima potpune recirkulacije, radni uslovi za svaki od promotora tokom ispitivanja koncentrisanja su odabrani tako da se pri istom TMP od 50 kPa protoci odaberu tako da se dobiju isti fluksevi filtrata. Tako je za uvrnutu traku (UT1,0) i Koflo mikser (KF1,3) koncentrisanje izvođeno pri protoku od 3,0 $Lmin^{-1}$ dok je u slučaju Keniks miksera (KMT1,0) primenjen protok od 1,9 $Lmin^{-1}$. Eksperimenti su izvođeni do zapreminskog stepena koncentrisanja (VCF) 2.

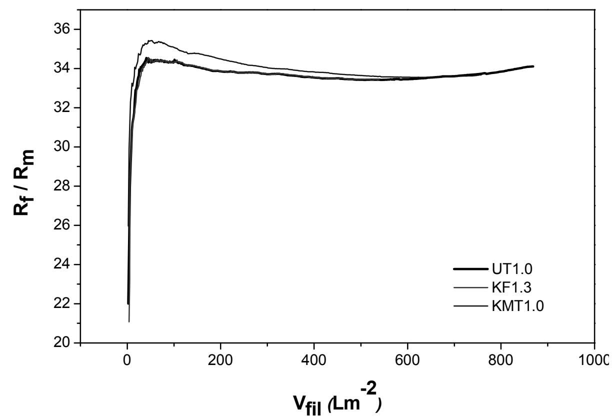
Na slici 2. prikazani su fluksevi filtrata u funkciji vremena. Tokom koncentrisanja se postižu stabilni pseudo-stacionarni fluksevi do VCF = 2, bez obzira na to koji od odabranih promotora je primenjen. Da bi se tokom koncentrisanja bez promotora postigao isti kapacitet, neophodno je primeniti protok od 10 $Lmin^{-1}$. Primenom KMT 1,0 isti fluks filtrata se postiže primenom 5 puta manjeg protoka dok je u slučaju UT1,0 i KF1,3 potrebno primeniti tri puta manji protok od 3 $Lmin^{-1}$. Pri različitim protocima ostvaruje se približno isti fluks filtrata od oko 90 $Lm^{-2}h^{-1}$.

Pozitivan uticaj promotora turbulencije na fluks filtrata može se objasniti povećanjem turbulencije, ali i karakterističnim načinom proticanja kroz membranu. U prisustvu uvrnute trake i Keniks miksera usled smanjenja poprečnog preseka membrane dolazi do promene toka iz aksijalnog u helikoidni usled čega dolazi do odnošenja čestica sa površine membrane. Periodična promena položaja elemenata



Slika 2. PROMENA FLUKSA FILTRATA SA VREMENOM

Figure 2. TIME DEPENDENCY OF FLUX



Slika 3. PROMENA OTPORA PRLJANJA SA FILTRIRANOM ZAPREMINOM

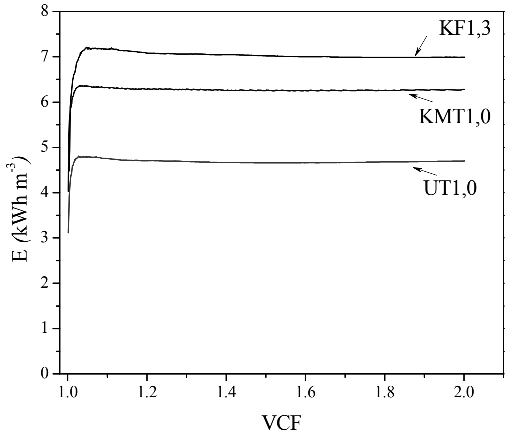
Figure 3. THE REDUCTION OF FOULING VERSUS FILTERED VOLUME

kod Keniks miksera dovodi i do stvaranja vrtloga koji dodatno povećavaju brzinu u blizini membrane što dodatno smanjuje prljanje zbog čega je sa ovim promotorom i moguće ostvariti željeni kapacitet pri manjem protoku u odnosu na druge promotore. U slučaju Koflo miksera centar membrane je blokiran samo na mestima gde se ukrštaju sečiva. Raspodela fluida nakon svakog elementa i sudaranje tokova u centralnom delu obezbeđuju karakterističan tok strujnica koje omogućavaju da se naruši i skine sloj nataložen na površini membrane.

Uticaj geometrije promotora na potrošnju energije tokom koncentrisanja

Da bi potvrdili uticaj promotora turbulencije na smanjenje prljanja izračunati su ukupni otpori usled prljanja na osnovu promene fluksa filtrata. Na slici 3. prikazana je promena normalizovanih vrednosti ukupnog otpora prljanja (R_f / R_m) sa filtriranom zapreminom (V_{fil}). Filtrirana zapremina predstavlja zapreminu filtrata po jedinici aktivne površine membrane.

Dobijene krive se praktično ne razlikuju po obliku. Otpor naglo raste u prvih par minuta koncentrisanja. Linearna zavisnost normalizovanog otpora R_f / R_m od filtrirane zapremine u prvim minutima ukazuje da je otpor usled formiranog sloja dominantni otpor prljanja, odnosno koncentraciona polarizacija i nataložene micele kazeina na površini membrane dominantno utiču na permeabilnost membrane. U narednih deset minuta otpor sporije raste i dolazi do odstupanja od linearne zavisnosti. Ovo odstupanje ukazuje na povećanje nepovratnog prljanja u odnosu na povratno. Već nakon deset do petnaest minuta se uspostavlja pseudo - stacionarno stanje. Otpor usled prljanja se može smanjiti za red veličine upotrebom promotora turbulencije. Pri protoku od 1,9 $Lmin^{-1}$ i TMP od 50 kPa otpor usled prljanja za KMT1,0 je oko $2x10^{12}$ m^{-1} dok se pri istim uslovima pri potpunoj recirkulaciji



Slika 4. PROMENA SPECIFIČNE POTROŠNJE ENERGIJE SA VCF

Figure 4. SPECIFIC ENERGY CONSUMPTION VERSUS VCF

bez upotrebe promotora turbulencije dobija otpor od 2,5x10^{13}m^{-1}. Dakle, bez obzira na geometrijsku strukturu, sva tri promotora obezbeđuju dovoljno smanjenje prljanja tokom koncentrisanja, odnosno, obezbeđuju dovoljan nivo turbulencije da se njihovom primenom efikasno izvodi proces koncentrisanja.

Delovanje promotora sastoji se u tome da se u određenim vremenskim intervalima narušava granični sloj i meša fluid u graničnom sloju koji se formira na površini membrane. Na ovaj način se smanjuje otpor usled prljanja usled povećanja mehaničkog napona smicanja i smanjenja veličine graničnog sloja.

Kako se promotori turbulencije razlikuju po padu pritiska, neophodno je uporediti i specifičnu potrošnju energije. Tokom izvođenja koncentrisanja radni uslovi poput TMP i pada pritiska bili su stabilni, te je za odabrane protoke bilo moguće postići stabilne stacionarne flukseve. Pad pritiska od oko 80 kPa postiže se u slučaju KMT1,0 iako je u tom slučaju primenjen najmanji radni protok. U slučaju UT1,0 i KF1,3 pri protoku od 3,0 Lmin^{-1}, pad pritiska je manji i iznosi 40 i 60 kPa, respektivno.

Promena specifične potrošnje energije sa promenom VCF je prikazana na slici 4. Potrošnja energije se po uspostavljanju stacionarnog fluksa ne menja značajnije tokom koncentrisanja za sva tri promotora. Najveća potrošnja energije se postiže u slučaju KF1,3, dok je potrošnja najmanja za UT1,0 promotor iako je u slučaju ova dva promotora primenjen isti protok za postizanje istog fluksa tokom koncentrisanja.

Iako KMT1,0 uzrokuje najveći pad pritiska, gubitak hidrauličke snage je manji u odnosu na KF1,3 zbog manjeg protoka, pa je time i specifična potrošnja energije manja za ovaj mikser. UT1,0 uzrokuje manji pad pritiska pri istom protoku u odnosu na KF1,3 što uzrokuje i manju potrošnju energije. Generalno, UT1,0 uzrokuje najmanji gubitak hidrauličke snage od svih primenjenih promotora zbog čega se pokazao kao najefikasniji u pogledu specifične potrošnje energije.

U tabeli 2. može se zapaziti da se primenom promotora postiže smanjenje potrošnje energije tokom koncentrisanja do VCF = 2. Najveće uštede od oko 50% se postižu primenom UT1,0. KMT1,0 je nešto manje efikasan sa uštedom od prosečno 36%, dok se u slučaju KF1,3 promotora postižu najmanje uštede energije od 28%.

Tokom koncentrisanja napojne smeše primena promotora turbulencije se pokazala efikasnom, naročito u slučaju primene uvrnute trake. U uslovima koncentrisanja uvrnuta traka obezbeđuje dovoljnu promenu načina strujanja kako bi se poboljšao prenos mase i smanjilo prljanje uz pad pritiska koji je prihvatljiv sa stanovišta potrošnje energije.

Uticaj geometrije promotora na selektivnost membrane

Kako bi se odredila selektivnost membrane određivana je koncentracija azota u uzorcima napojne smeše i u uzorcima filtrata. Koncentracije komponenata su izračunate pomoću jednačina 7 - 10, dok je propuštanje izračunato primenom jednačine 11. Bitnu karakteristiku membrane predstavlja i selektivnost (S) tj. odnos sadržaja proteina surutke i kazeina u permeatu. Sastav mleka, odnosno napojne smeše, je bio: UP 3,19%, PP 3,01, K 2,88%, PS 0,14%.

Određivanje koncentracije komponenata u filtratu je vršeno nakon koncentrisanja napojne smeše do VCF = 2 i to pri istom otporu usled prljanja. Stepen propuštanja membrane zavisi od karakteristika membrane i od karakteristika sistema koji se filtrira ali i od samih radnih uslova. Napon smicanja, čija veličina zavisi od protoka, utiče na prljanje membrane kao i primenjeni TMP, samim tim i selektivnost membrane zavisi od ovih uslova.

U tabeli 3. su prikazani rezultati za stepen propuštanja i selektivnost membrane. Propuštanje proteina je smanjeno primenom promotora turbulencije u odnosu na membranu bez promotora. Selektivnost membrane je relativno mala, iako se mora uzeti u obzir i da je koncentracija proteina surutke u napojnoj smeši veoma mala. Činjenica da je mleko homogenizovano delimično utiče na smanjenje propuštanja, s obzirom da je prisutan i izvestan udeo micela većeg prečnika od 300 do 800 nm kojim se zadržavaju manje čestice. Stepen propuštanja proteina surutke iznosi oko 60-80% i kazeina do 1% što je u okvirima vrednosti dobijenih od strane drugih autora (Piry i sar., (2008), Jimenez-Lopez i sar., (2008), (2011)).

Primenom promotora turbulencije, ostvareno je smanjenje propuštanja a selektivnost membrane je povećana, ali pravilnosti u odnosu na geometriju nisu uočene. Do smanjenja propuš-

Tabela 2. SMANJENJE POTROŠNJE ENERGIJE TOKOM KONCENTRISANJA, VCF = 2.

Table 2. REDUCTION OF ENERGY CONSUMPTION DURING MILK CONCENTRATION, VCF = 2

VCF	ER (%)		
	UT1,0	KMT1,0	KF1,3
1,1	53	37	28
1,2	53	37	29
1,5	52	36	28
2,0	51	35	27

Tabela 3. PROPUŠTANJE I SELEKTIVNOST MEMBRANE TOKOM KONCENTRISANJA

Table 3. PERMEABILITY AND SELECTIVITY OF MEMBRANE DURING MILK CONCENTRATION

Propuštanje (%)	NTP	UT1,0	KMT1,0	KF1,3
UP	9,6	7,6	7,8	8,1
PP	4,9	3,4	3,6	3,9
K	1,3	0,9	0,7	0,9
PS	81	57	67	69
S	2,8	3,0	4,7	3,6

tanja dolazi zahvaljujući odnošenju čestica sa površine membrane usled povećanja napona smicanja i specifičnog helikoidnog toka u graničnom sloju. Prenos mase sa zida membrane u masu fluida je na ovaj način povećan čime se smanjuje koncentracija komponenata u graničnom sloju, a time i mogućnost za njihov prolaz u filtrat.

Sachdeva i sar., (1997) kao i Jimenez-Lopez i sar., (2008) su ustanovili da postoji kritični napon smicanja koji obezbeđuje stanjivanje sloja istaloženih većih čestica poput kazeina usled čega može doći do formiranja sloja koji čine manje čestice, poput proteina surutke. Na ovaj način se smanjuje poroznost istaloženog sloja i smanjuje propuštanje čestica manjeg prečnika. Primenom promotora napon smicanja je dovoljno veliki da smanji prljanje i propuštanje. Dakle, promena obika strujnica u slučaju sva tri promotora ima ključni uticaj na prljanje i selektivne karakteristike membrane.

ZAKLJUČAK

Rezultati dobijeni ispitivanjem u uslovima koncentrisanja napojne smeše su pokazali da se upotrebom promotora turbulencije ostvaruje takav način proticanja da se isti kapacitet može postići pri znatno manjim brzinama proticanja u odnosu na rad bez promotora. Za dobijanje istog fluksa filtrata, upotrebom KMT1,0 potrebno je primeniti pet puta manji protok u odnosu na rad bez promotora dok je rad sa uvrnutom trakom i Koflo mikserom moguć pri tri puta manjem protoku.

Analiza prljanja membrane je pokazala da se primenom promotora smanjuje ukupni otpor prljanja membrane. Takođe je pokazano da koncentraciona polarizacija i formiranje sloja od micela kazeina predstavljaju dominantne otpore prljanja. Svi tipovi promotora uzrokuju promenu načina strujanja u membrani koja obezbeđuje poboljšanje prenosa mase u graničnom sloju i smanjuju otpor prljanja za red veličine u odnosu na konvencionalni način rada.

Ispitivanja potrošnje energije tokom upotrebe promotora turbulencije i poređenje sa slučajem bez njihove upotrebe u uslovima koncentrisanja napojne smeše su pokazala da se upotrebom promotora može ostvariti značajna ušteda energije. Primena uvrnute trake UT1,0 je najisplativija jer omogućava smanjenje potrošnje energije od oko 50% pri čemu je i pad pritiska najmanji. Selektivnost membrane je povećana dok je propuštanje proteina mleka smanjeno u prisustvu promotora turbulencije. Rezultati u ovom radu su pokazali da je upotrebom promotora turbulencije moguće izvođenje procesa sa ekonomski prihvatljivim vrednostima fluksa filtrata pri malim brzinama proticanja.

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SUMMARY

INFLUENCE OF TURBULENCE PROMOTERS GEOMETRY ON MILK CONCENTRATION

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The application of membrane processes is limited by capacity reduction due to membrane fouling. Adsorbed and deposited proteins at the membrane surface and/or in the membrane pore create additional mass transfer resistance and reduce the process capacity. One of the ways to reduce the fouling is to change the hydrodynamic regime by introduction of turbulence promoters in membrane module. The objective of this study was to investigate the influence of turbulence promoter with different geometric shape and approximately the same aspect ratio on the process capacity during concentration of milk and reduction of fouling. Twisted tape, Kenics static mixer and blade type Koflo mixer were used as turbulence promoters. In the presence of turbulence promoters, fouling resistance was an order of magnitude lower and energy consumption was reduced for 28 to 50%. The alternation of streamline path induced by the presence of all three promoters has major impact on fouling reduction and improving the selective characteristics of membrane.

Key words: turbulence promoters • milk concentration • fouling resistance • membrane selectivity

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ORIGINAL RESEARCH PAPER

UDK: 637.12:632.95

THE INFLUENCE OF MILK FAT, TEMPERATURE AND TIME OF STORAGE ON ORGANOCHLORINE PESTICIDES IN COW'S MILK

Persistent organic pollutants (POPs) are organic compounds that resist photolytic, biological and chemical degradation. Organochlorine pesticides (OCPs) are being extensively used in tropical countries in malaria control programs and against livestock ectoparasites and agricultural pests. They contain carbon, hydrogen and chlorine. Cow milk accumulates residues of these pesticides when they eat contaminated food, by inhaling contaminated air and by absorbing of pesticides sprayed on the skin of lactating cows.

The research of seven OCPs is performed in raw cow's milk taken from Pelagonia from 10 individual farms. Tasted dairy fat is important for the quality of milk and the presence of OCPs that are soluble in fat milk. Different temperatures were applied in order to investigate influence of temperature on the presence of OCPs. According to investigations fat ranges from 3.75 - 3.9%, whereas the solubility and the presence of OCPs is differrent and ranges from 0-2%, with the exception of DDT which may be encountered and to 6% at a temperature of 71-74°C. Examined OCPs in milk kept for 6 days in the refrigerator until it's spoiling, DDT and then the most abundant and least aldrin. The determination was done by Gas chromatograph from "Agilent Technologies'', and the degree of correlation has been demonstrated high accuracy of the method and absorption of pesticides sprayed on the skin of lactating cows.

Most chlorinated insecticides are relatively resistant to processing techniques used for milk and dairy products. Once residues get into milk they are stable and difficult to remove; therefore, the best policy is to prevent their entrance into milk by proper and careful management of the dairy cow.

In this paper the research is carried out by qualitative and quantitative determination of milk serum which commonly used non ionized organochlorine insecticides in milk.

Key words: organochlorine • pesticides • OCPs • temperature • cow milk • milk fat

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INTRODUCTION

Persistent organic pollutants (POPs) are organic compounds that resist photolytic, biological and chemical degradation. OCPs are being extensively used in tropical countries in malaria control programs and against livestock ectoparasites and agricultural pests (G.G. Pandit, 2002). They contain carbon, hydrogen and chlorine. The extensive use of organochlorine pesticides (OCPs) in agriculture and livestock result in environmental contamination. The organochlorine pesticides enter the food chain as a result of their lipophilic properties, where the concentration can be increased up to 70,000 times, thus causing a potential health risk for consumers (Ministry of Environment and Physical Planning, 2006)

Cow milk accumulates residues of these pesticides when they eat contaminated food, by inhaling contaminated air and by absorbing of pesticides sprayed on the skin of lactating cows (Saxena, 2012).

An analysis was conducted for Lindan, Heptahlor, Aldrin, Dieldrin, Endosulfan, Endrin, and DDT for the determination in cow's milk samples.

The group OCPs include pesticides containing chlorine, for example:

Lindan (γ-1,2,3,4,5,6 – hexachlorociclohexane or γ-HCH) is synthetically acquired chlorinated pesticide, and the other two isomers are byproducts of lindane and are characterized by high levels of presence in the environment. p,p'-DDT[1,1,1-trichloro-2,2,-bis-(p-chlorophenyl)ethylene], p,p'-DDE[1,1-dichloro-2,2,-bis-(p-chlorophenyl)ethylene], p,p'-DDD [1,1-dichloro-2,2,-bis-(p-chlorophenyl)ethane]. p,p'-DDE и p,p'-DDD occur during chemical transformations of p,p'-DDT, which is the first synthetically acquired pesticide.

Aldrine, with empirical formula is classified as moderately persistent and the degradation time in water and soil ranges from 20 days to 1,6 years depending on environmental conditions. Dieldrine ($C_{12}H_8Cl_6O$) is characterized by high persistency in soil, with half-life of around 3-4 years. After that it is deposited in the organisms.

Endrine ($C_{12}H_8Cl_6O$) is characterized with high persistency in the soil. Sometimes the half-life of Endrine is up to 12 years.

In the soil, plants and animal organizms, heptachlor ($C_{10}H_5Cl_7$) is metabolized into heptachlorepoxide, which is proven to be more persistent and cancerogenic than heptachlor. The half-life is around 9 months to 2 years.

DDT-dichlorodiphenyltrichloroethane or p,p'-DDT, ($C_{14}H_9Cl_5$), is characterized by high stability and persistency, and with half-life in soil from 10-15 years after application, which is the main reason for it to be found anywhere in the world. In the environment DDT is degraded into DDD and DDE.

When lindane ($C_6H_6Cl_6$) is used in agriculture, it is estimated that 12-13% of it evaporates in the atmosphere, and can be washed away by rainfall, thus it is deposited in surface water and in subterranean water reservoirs.

Endosulfane ($C_9H_6Cl_6O_3S$) has a moderate potential for bioaccumulation. It is degraded more rapidly compared to other OCPs the estimated time of full degradation of endosulfan is from 9 months to 6 years.

MATERIALS AND METHODS

The research was conducted on raw cow milk and soured-milk products, in a period over three months (December, January and February), from farms located in the Pelagonia region.

Table 1. QUANTITY (w/w%) OF OCPs DEPENDING ON THE APPLIED TEMPERATURE AND MILK FAT IN RAW COW MILK FROM THE PELAGONIA REGION

Tabela 1. KOLIČINA OCPs (w/w%) U SIROVOM MLEKU U ZAVISNOSTI OD PRIMENJENE TEMPERATURE I SADRŽAJA MASTI U REGIONU PELAGONIJE

Name of pesticide	Month	Milk fat (%)	w/w% of t = 4°C 24 hours	w/w% of t = 63-65ºC 30 min.	w/w% of t = 71-74ºC 15 sec.	w/w% of t =89-100ºC 1sec.	w/w% of t = - 18ºC 24 hours
Lindan	December 2009	3.75	0.09	0.07	0.07	0.07	0.08
	January 2010	3.9	0.13	0.11	0.08	0.07	0.07
	February 2010	3.9	0.02	0.08	0.08	0.08	0.07
Heptahlor	December 2009	3.75	0.10	0.09	0.14	0.08	0.10
	January 2010	3.9	0.12	0.15	0.20	0.20	0.06
	February 2010	3.9	0.05	0.30	0.20	0.07	0.08
Aldrin	December 2009	3.75	0.02	0.02	0.02	0.01	0.02
	January 2010	3.9	0.03	0.03	0.02	0.02	0.03
	February 2010	3.9	0.01	0.03	0.02	0.02	0.02
Dieldrin	December 2009	3.75	0.02	0.02	0.02	0.02	0.03
	January 2010	3.9	0.03	0.04	0.03	0.03	0.03
	February 2010	3.9	0	0.03	0.02	0.03	0.02
Endosulfan	December 2009	3.75	0.02	0,04	0.04	0.04	0.03
	January 2010	3.9	0.05	0.06	0.041	0.04	0.04
	February 2010	3.9	0.01	0.04	0.04	0.03	0.03
Endrin	December 2009	3.75	0.03	0.07	0.06	0.05	0.06
	January2010	3.9	0.04	0.06	0.04	0.03	0.05
	February 2010	3.9	0.01	0.05	0.05	0.04	0.05
DDT	December 2009	3.75	0.09	0.17	0.18	0.16	0.20
	January 2010	3.9	0.25	0.24	0.28	0.21	0.30
	February 2010	3.9	0.05	0.20	0.13	0.12	0.14

In this paper research is carried out of qualitative and quantitative determination of milk serum which commonly used non ionized organochlorine insecticides in milk. Chemical analysis was examined for the presence of milk fat.

Except for raw milk, the research found the presence of OCPs in milk warm-up on the temperatures below:

- Temperature of 63-65^{0}C for 30 min
- Temperature of 71-74^{0}C for 15 sec
- Temperature of 89-100^{0}C for 1 sec

It was also monitored the presence of organochloride pesticides in milk kept frozen at a temperature of -18^{0}C for 24 hours, milk kept at 4^{0}C for 24 hours, and milk kept for one week at 4^{0}C.

Milk fat was examined by the Acidobutirometric method by Gerber, which is based on the dissolution of all ingredients from milk with sulfuric acid, except milk fat which separates on the surface. Fat separation is facilitated by adding amilalchocol that reduces surface tension, and is acelerated by centrifugation, for the determination of milk fat a graduated butirometri was used to show the percentage of fat and appropriate centrifuge.

In order to check the accuracy of the method used above, in the milk from each region was exactly added of the known quantities of p,p'-DDT, which previously made the determination of OCPs. Validation of the accuracy of the method was done by the value of the analytical yield (R).The apparatus that was used to determinate the concentration of organochloric pesticides in milk is a gas chromatograph from "Agilent Technologies", the model GC 7890N equipped with appropriate software system.

RESULTS AND DISCUSSION

Determination of OCPs in raw cow milk

Organochlorine pesticides are made of chromatographic analysis present in raw cow milk from the Pelagonia region, and were monitored for their amounts and calculated in %, during a period of the three months (December 2009, January and February 2010). Their quantities are measured (w/w%) OCPs in milk depending on content fat of milk content. They are also measured on w/w% for all OCPs in raw cow milk, depending on the applied temperature, temperatures adequately applicable for standardization and pasteurization of milk and their storage time. The following temperatures were applied:

- 4^{o}C in 24 hours
- 63-65^{o}C for 30 minutes
- 71-74^{o}C for 15 seconds
- 89-100^{o}C for 1 second
- Milk kept at a temperature of -18^{o}C in a time of 24 hours.

The content of the pesticides in milk all decreased as the treatment time progressed, indicating degradation of the pesticides (Li-Ying Bo, 2011). Sterilization process had an efficient role on the degradation or elimination of pesticide residues (Abou Donio, 2010).

In order to increase the accuracy of the values obtained for the tested parameters, they are presented in tables and graphs below. The values in table 1 are average values of three repetitions depending on the applied temperature and milk fat in raw cow milk from the Pelagonia region for the months December 2009, January and February 2010.

Most chlorinated insecticides are relatively resistant to processing techniques used for milk and dairy products. Once residues get into milk they are stable and difficult to remove; therefore, the best policy is to prevent their entrance into milk by proper and careful management of the dairy cow (Liaska, 1968)

In Figure 1 we can see that pesticides from the group Lindan milk in terms of its fat at all applied temperatures are present in 2%, except when heated milk

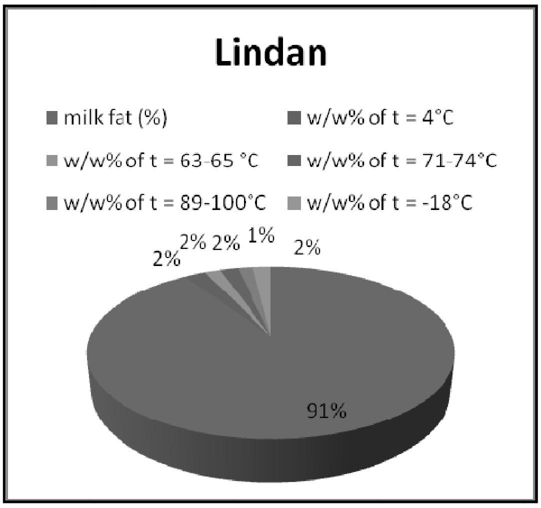

Figure 1. THE PRESENCE OF LINDAN IN MILK FAT (w/w%)

Slika 1. UDEO LINDANA U MLEČNOJ MASTI (w/w%)

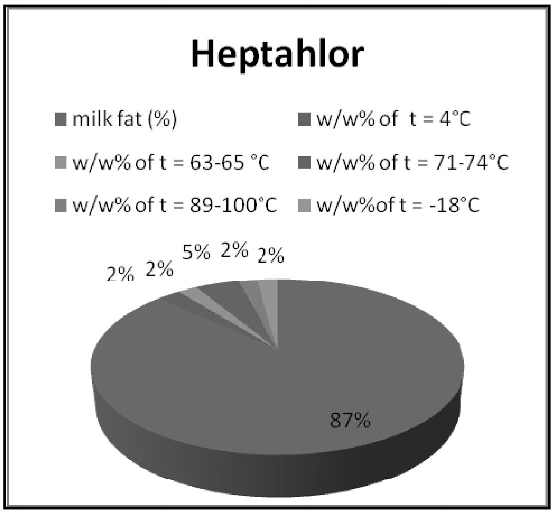

Figure 2. SOLUBLE OF HEPTAHLOR IN MILK FAT (w/w%)

Slika 2. UDEO HEPTAHLORA U MLEČNOJ MASTI (w/w%)

from 89-100°C where its amount has reduced to 1%.

In Figure 2 we can see that the pesticide in the group of Heptahlor applied temperatures have an impact on their presence, there was a small difference in the sample of milk which was kept for 24 hours at 4ºC and the sample was heated to a temperature of 71-74ºC where they were present in an amount of 5%.

The amount of residue destroyed varied with processing treatment and nature of the insecticide residue. Spray drying destroyed in excess of 80% of the Lindane residues in raw milk while sterilization caused essentially no change in lindane residues. Heptachlor was destroyed more easily than heptachlor epoxide (Liaska, 1968). According to (Ashnagar, 2009) the amount of Lindane (0,042 mg/kg) and DDT (0, 28 mgkg) exceeded the standard limits recommended by FAO/WHO.

Pesticides from the group Aldrin (figure 3) in raw cow's milk kept for 24 hours at a temperature of -18ºC and at a temperature of 4ºC which were present in amounts of 1%, while in milk heated to other temperatures had no presence of pesticides in this group.

In Figure 4 it can be seen that the pesticide Dieldrin in raw cow's milk was not present, only in milk samples heated to certain temperatures this pesticide has been shown in a minimal amount of 1%.

In Figure 5 it can be seen that the pesticide Endosulfan in raw cow's milk was not present, only in milk samples heated to certain temperatures this pesticide has been shown in a minimal amount of 1%.

Pesticides from the group of Endrin (figure 6) in raw cow's milk and milk heated to high temperature of 89-100ºC were present in amounts of 1%, while in the other samples of raw milk heated to certain temperatures were present in 2%.

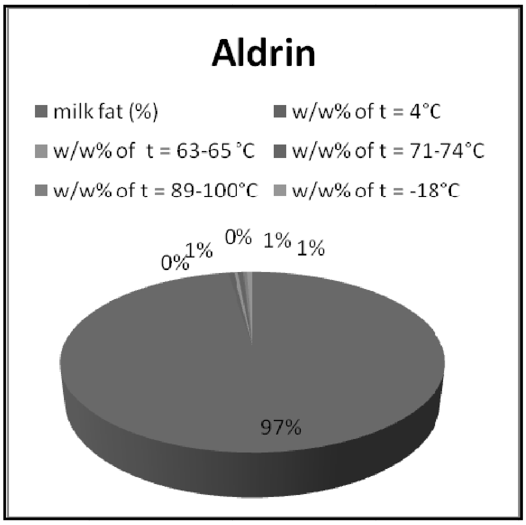

Figure 3. THE INFLUENCE OF ALDRIN IN FAT (w/w%)

Slika 3. UDEO ALDRINA U MASTI (w/w%)

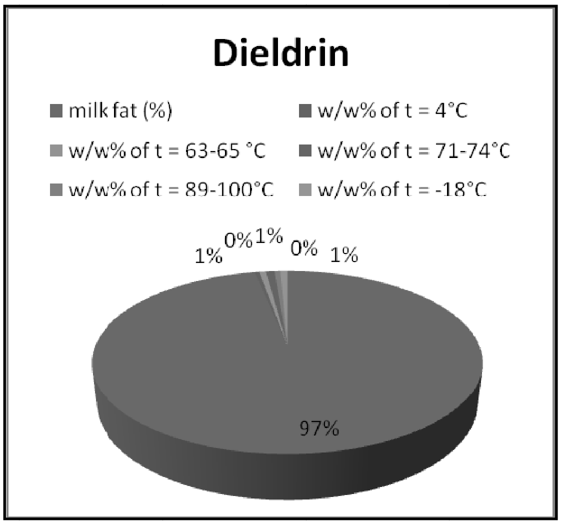

Figure 4. SOLUBLE OF DIELDRIN IN FAT (w/w%)

Slika 4. UDEO DIELDRINA U MASTI (w/w%)

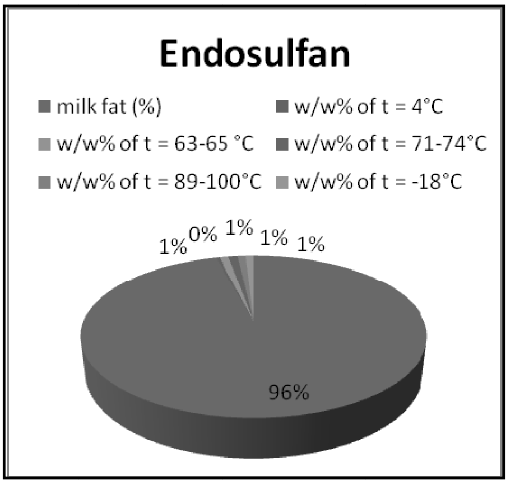

Figure 5. THE INFLUENCE OF ENDOSULFAN IN FAT (w/w%)

Slika 5. UDEO ENDOSULFANA U MASTI (w/w%)

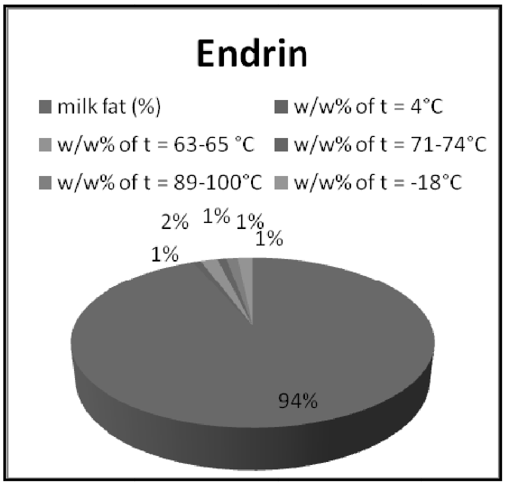

Figure 6. THE INFLUENCE OF ENDRIN IN FAT (w/w%)

Slika 6. UDEO ENDRINA U MASTI (w/w%)

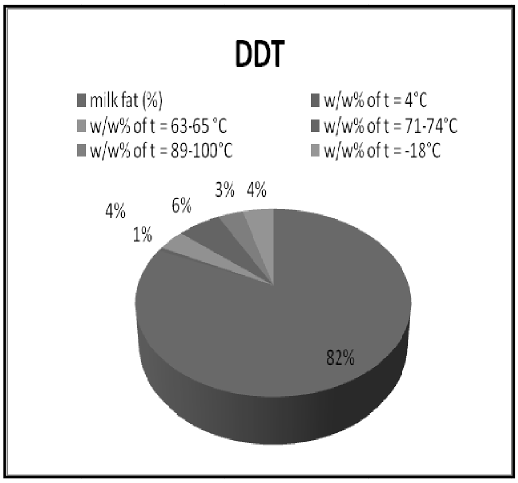

Figure 7. THE INFLUENCE OF DDT IN MILK FAT (w/w%)

Slika 7. UDEO DDT U MASTI (w/w%)

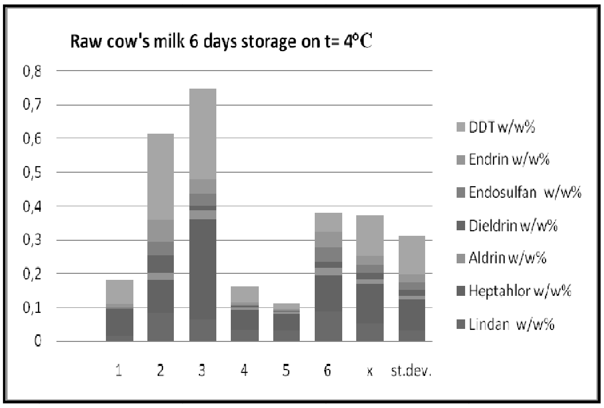

Figure 8. OCPS IN RAW COW'S MILK KEPT IN THE REFRIGERATOR AT A TEMPERATURE OF 4ºC

Slika 8. OCPS U SIROVOM MLEKU ČUVANOM U FRIŽIDERU NA 4ºC

Table 2. CORRELATION COEFFICIENT (R) DEPENDENCE OCPS IN RAW COW MILK IN DIFFERENT TEMPERATURES (°C) AND FAT CONTENT (%)

Tabela 2. KOEFICIJENT KORELACIJE (R) PRISUSTVA OCPS U KRAVLJEM MLEKU U ZAVISNOSTI OD TEMPERATURE I SADRŽAJA MLEČNE MASTI

Name of pesticide	w/w% t = 4°C 24 hours	w/w% t =63-65°C 24 hours	w/w% t = 71-74°C 15 sec.	w/w% t =89-100°C 1 sec.	w/w% t = -18°C 24 hours
Lindan	-0,53	0,77	0,99	0,93	-1,00
Heptahlor	-0,25	-0,25	1,00	0,43	-0,80
Aldrin	-0,31	0,94	0,93	0,82	0,69
Dieldrin	1	0,24	0,45	0,97	-0,48
Endosulfan	0,36	0,38	-0,65	0,97	-0,20
Endrin	-0,43	-0,78	-0,92	-0,75	-1,00
DDT	0,56	0,79	0,14	0,036	0,15

Pesticide DDT (figure 7) are pesticides that are represented by the largest amount in raw cow's milk from Pelagonia, so that the milk heated to a certain temperature their attendance is up to 4%, while in raw cow's milk kept 24 hours in the refrigerator were present with1%.

The dependence of OCPs on the present milk fat is processed by the correlation coefficient (r). Calculated (r) shows that there is a proportional relationship between the two sizes and the correlation coefficient has a positive value.

According to the results in milk kept at a temperature of 4°C for 24 hours in pesticides Dieldrin achieved perfect cor-

Table 3. STATISTICAL PROCESSING OF THE RESULTS OCPS IN RAW COW'S MILK KEPT IN THE REFRIGERATOR AT A TEMPERATURE OF 4ºC

Tabela 3. STATISTIČKA OBRADA REZULTATA ODREĐIVANJA OCPS U SIROVOM MLEKU ČUVANOM U FRIŽIDERU NA 4ºC

Number of days	Lindan (w/w%)	Heptahlor (w/w%)	Aldrin (w/w%)	Dieldrin (w/w%)	Endosulfan (w/w%)	Endrin (w/w%)	DDT (w/w%)
1	0.02	0.08	0	0	0.00	0.01	0.07
2	0.08	0.10	0.02	0.05	0.04	0.06	0.25
3	0.06	0.30	0.02	0.01	0.04	0.04	0.27
4	0.03	0.06	0.01	0.00	0.00	0.01	0.05
5	0.03	0.05	0.01	0.00	0.00	0.00	0.02
6	0.09	0.10	0.02	0.02	0.04	0.04	0.06
$\bar{x}$	0.05	0.11	0.01	0.02	0.02	0.03	0.12
SD	0.03	0.09	0.01	0.02	0.02	0.02	0.11

relation and the correlation coefficient is 1.

The determination of OCPs in raw cow's milk kept in the refrigerator until it's used by date. The measurement was done w/w% OCPs in raw cow's milk kept in the refrigerator at a temperature of 4°C during one week until it's used by date. In terms of days of storage a statistical processing of the results by applying the mean value and standard deviation was performed.

As seen from the data presented in Table 3, the highest mean OCP in raw cow's milk kept for a week at a temperature of 4°C in refrigerator reach group pesticide DDT 0.12, and the minimum value was pesticide group Aldrin so 0.02. Minimum value of the standard deviation (SD) group has pesticides Aldrin 0.01, while the maximum value has DDT with 0.11.

From the figure 8 for pesticides of group Lindan minimum quantity is 0.02% which is determined on the first day of its storage in the refrigerator, while the maximum amount of 0.09% is determined on the sixth day during storage of raw cow milk. Pesticides from the group Heptahlor minimum quantity is 0.05% proven on the fifth day, while the maximum amount is proven on the third day 0.30% of it's scaling in the refrigerator at a temperature of 4ºC. Pesticide groups Aldrin and Dieldrin on the first day in storage milk in the refrigerator is not proven, while in Aldrin maximum quantity is shown on the third day 0.02% and in Dieldrin on the second day 0.05%.

Pesticides from the group Endosulfan minimum quantity is proven on the fifth day 0.00% while maximum quantity is 0.04% on the second day of milk storage. Maximum quantity (0.06%) of the pesticides from the group Endrin was detected on the second day of storage of the milk in the refrigerator. Pesticides from the group DDT with minimum quantity was proven on the fifth day of 0.02%, while the maximum amount of the third day was 0.27% of the storage of milk.

CONCLUSION

1. A Gas chromatography method for determining OCPs in milk and dairy products has been developed, which may be used for routine analysis in everyday laboratory practice. This method is characterized by its accuracy, speed, simplicity and reproducibility.
2. The acquired values of the analytical yield R, with which the accuracy of the method is tested, are within the limits from 98.113599% to 101.83674%. This appoints to the fact that the method is quantitative and accurate.
3. From the tested OCPs in raw cow's milk from the Pelagonia region, most present is DDT and least present is Aldrin.
4. In raw cow's milk, kept at 4ºC for one week until spoiled, also most present is DDT with average value of 0.12. The least present is Aldrin with average value of 0.02.

It can be concluded that in Pelagonia region from Macedonia insectcides for the protection of plants, mostly present DDT are still applied. It is necessary to perform these tests in order to inform population that healthy food is necessary for human diet health.

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IZVOD

UTICAJ MLEČNE MASTI, TEMPERATURE I PERIODA SKLADIŠTENJA NA ORGANOHLORNE PESTICIDE U KRAVLJEM MLEKU

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Dugotrajne organske zagađujuće supstance (POPs) su organska jedinjenja koja su otporna na fotolitičke, biološke i hemijske degradacije. Organohlorni pesticidi (OCPs) se intenzivno koriste u tropskim zemljama u programima kontrole malarije, protiv malarije stoke i poljoprivrednih ektoparazita, štetočina. Sadrže ugljenik, vodonik i hlor. Kravlje mleko akumulira ostatke ovih pesticida kroz kontaminiranu hranu, udisanjem kontaminiranog vazduha i apsorbcije pesticida sa prskane kože tokom laktaciji krava.

Istraživanje sedam OCPs je vršeno u sirovom kravljem mleku uzetom iz Pelagonije od 10 individualnih gazdinstava. Analize mlečne masti su važna za kvalitet mleka i prisustvo OCPs koji su rastvorljivi u mlečnoj masti.

Različite temperature su primenjene u cilju ispitivanja uticaja temperature na prisustvo OCPs. Istraživanja su vršena na uzorcima sa opsegom mlečne masti od 3,75 do 3,9%, dok je rastvorljivost i prisustvo OCPs različita i kreće se od 0-2%, sa izuzetkom DDT koji se našao i na 6% na temperaturi od 71^{o}-74^{o}C. Ispitivani OCPs u mleku se čuvao 6 dana u frižideru do kvarenja. Određivanje je vršeno gasnim hromatografom "Agilent Technologies", a stepen korelacije je pokazao visoku preciznost metoda i apsorpciju pesticide sa prskane kože krava tokom laktacije.

Većina hlornih insekticida je relativno otporna na tehnološke procese i tehnike koje se koriste u preradi mleka i mlečnih proizvoda. Kada se ostaci već nađu u mleku stabilni su i teško ih je ukloniti, dakle, najbolja politika je da se spreči njihov ulazak u mleko pravilnom i pažljivom mužom i uzgojom mlečnih krava.

U ovom radu je sprovedeno kvalitativno i kvantitativno određivanje seruma mleka koji se uobičajno koristi nejonizovane organohlorne insekticide u mleku.

Ključne reči: goat milk • whey protein concentrates • ultrasound • fermentation

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ORIGINAL RESEARCH PAPER

UDK: 637.133:637.3

THE INFLUENCE OF THE THERMAL TREATMENT IN PLATE HEAT EXCHANGERS ON CHEMICAL COMPOSITION OF MILK DESIGNED FOR CHEESE PRODUCTION

Milk is a liquid with a complex structure, consisting of proteins, fats, carbohydrates, minerals and vitamins that are necessary for human organism,which have a high biological value. Cheese is a dairy product in solid or semisolid consistency, with characteristics such as appearance, texture and taste depending on the origin of milk, heat treatment, the content of milk fat, starter cultures and duration of ripening. One of the most important stages in the processing of milk intended for cheese production is heat treatment, which aims are to destroy microorganisms, to inactivate enzymes and to improve the technological properties of milk. However, thermal processing of milk affects on the physicochemical characteristics of milk. Heat treatment of milk is performed in machines called heat exchangers, which by construction can be of plate, tubular or scraped- type. Changes that occur in milk are different, depending on applied temperature and duration of heating, and these parameters differ in the type of cheese that is produced.

Key words: heat treatment • heat exchangers • milk • cheese • chemical changes

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INTRODUCTION

Milk has always been one of the most important components in the diet of humans and animals, ranging from the fact that milk is a biological fluid that is produced from the milk gland in female mammals.

Usually, major components in fresh milk are found in the following ratio:

- Water 87.5%
- Dry matter 12.5%
- Fat 3.2% to 5.5%
- Protein 2.6% to 4.2%
- Lactose 4.6% to 4.9%
- Minerals (ash) 0.6% to 0.8% (Presilski, 2006).

Milk is an ideal base for multiplication of bacteria, because it milk is perishable food. The milk is exposed to high temperature in order to destroy microorganisms, to inactivate enzymes, to improve technological properties and concentration of solids in milk.

Heat exchangers are devices that are used to transfer heat between two or more fluids, between a solid surface and the fluid, between the solid particles and the fluid, at different temperatures and in thermal contact (Ramesh et al. 2003).

According to Jean-Paul Julien (1985), three different are used mostly in the dairy industry:

- Plate heat exchangers
- Tubular heat exchangers
- Scraped - surface heat exchangers.

During the thermal process of milk, which is mostly performed in plate heat exchangers, often proteins degrade and precipitate so forms fouling, which has a major impact on the efficiency of heat treatment and desired characteristics of the product (De Bonis et al., 2006).

Depending on the production of dairy products, there are several different methods of milk heating. A process of milk heating to 71.7°C for a period of 15s to 20s, is called pasteurization, sterilization at 138°C for a second. There is also the third method of heating, known as termalization, which is performed at temperature of 60 to 65°C for a period of 15 to 30s.

The following temperature procedures are used during cheese production on the territory of Macedonia:

- Hard cheese - the manufacture of this type of cheese is made by heating the milk at temperature of 37.8°C (first scalding), and further process is adding hot water at the temperature of 95°C to 98°C in the curd (second scalding).
- Kashkaval - heat treatment is performed at a temperature of 63°C to 65°C for a period of 15 to 20 seconds, in the further stages of processing follows another heat treatment with the adding salting solution heated at temperature of 71°C to 72°C in the curd.
- White soft cheese - the production of this type of cheese, milk is heated to the temperature of 70°C for a period of 30 minutes (Presilski, 2004).

Cheese that is made from unpasteurized milk is considered to have better flavor and aroma, but most producers must use pasteurized milk. The bacteria that affect the quality of cheese, such as coliform bacteria that can cause so-called "blowing" of cheese and unpleasant taste, are destroyed by pasteurization. Commonly applied procedure of pasteurization is HTST (High Temperature Short Time) which is performed at a temperature of 72-73°C for a period of 15-20 seconds (Bylung, 1995).

According Presilski (2006), the taste, smell, consistency and appearance of the cheese produced from milk pasteurized at a temperature of 70 to 72°C with retention of 15 to 20 minutes are the best and typical, so this pasteurization is recommended in cheese practice.

The heating of the milk affects the structure of the membrane of milk fat, with changes in its density and composition of the lipoprotein complex.

Most changes that occur during heating of milk are associated with the protein fraction, which affected the structure of the products. The changes are the following:

- Denaturation of whey proteins and connecting with casein micelles (interaction of β-lactoglobulin with κ-casein),
- Transfer of calcium and phosphate in casein micelles,
- Aggregation of casein micelles at temperatures above 110°C,
- Changes in proteins in the membrane of fat globules.

MATERIALS AND METHODS

As a test material was used bulk cow milk intended for cheese production in the milk factory "Sirma and Pelister" in Prilep. Thirty samples of milk at random choice were taken, where the total quantity of milk was 45 tons. All experimental samples were properly and timely taken for analysis. Prior to heat treatment of milk, measuring of chemical parameters of each sample separately was done. After heat treatment of the milk, which was performed in plate heat exchangers SORDI, the samples were again taken for analysis of the chemical characteristics. Examination of parameters for water content, dry matter, fats, dry matter without fats, protein, lactose, was perfomed with high precision by using ultrasonic analyzers Lactoscan, Milkana and Microlab 6P. Heat treatment used in the manufacture of cheese is different depending on the technology. Milk was heated in three different temperature procedures and a temperature of 37.8°C in the production of hard cheese, then at the temperature 65°Cin production of kashkaval and at the temperature of 72.2°C in the production of white soft cheese.

Data obtained from the analysis were grouped into three different groups depending on the type of cheese that is produced.

RESULTS AND DISCUSSION

The values of the studied parameters in milk are presented in Tables 1-3.

The average chemical composition (water, dry matter, dry matter without fats, protein, fat, lactose) for tested milk before and after thermal treatment, which was intended for hard cheese production is shown in Table 1.

Table 1. AVERAGE CHEMICAL COMPOSITION OF TESTED MILK INTENDED FOR PROCESSING OF HARD CHEESE BEFORE AND AFTER THERMAL TREATMENT

Tabela 1. PROSEČAN HEMIJSKI SASTAV MLEKA KORIŠĆENOG ZA PROIZVODNJU TVRDIH SIREVA PRE I POSLE TERMIČKOG TRETMANA

Chemical parameters	Milk intended for the production of hard cheese	
	Before heating	After heating 37.8°C
Water (%)	88.08	88.15
Dry matter (%)	11.92	1.85
Dry matter without fat (%)	8.36	8.29
Protein (%)	3.20	3.16
Fats (%)	3.56	3.56
Lactose (%)	4.75	4.66

N =10 Total number of samples

Table 2. AVERAGE CHEMICAL COMPOSITION OF TESTED MILK INTENDED FOR PROCESSING OF KASHKAVAL BEFORE AND AFTER THERMAL TREATMENT

Tabela 2. PROSEČAN HEMIJSKI SASTAV MLEKA KORIŠĆENOG ZA PROIZVODNJU KAČKAVALJA PRE I POSLE TERMIČKOG TRETMANA

Chemical parameters	Milk intended for the production of kashkaval	
	Before heating	After heating 65°C
Water (%)	87.74	87.81
Dry matter (%)	12.26	12.19
Dry matter without fat (%)	8.46	8.40
Protein (%)	3.22	3.20
Fats (%)	3.80	3.79
Lactose (%)	4.70	4.62

N =10 Total number of samples

Table 3. AVERAGE CHEMICAL COMPOSITION OF TESTED MILK INTENDED FOR PROCESSING OF SOFT WHITE CHEESE BEFORE AND AFTER THERMAL TREATMENT

Tabela 3. PROSEČAN HEMIJSKI SASTAV MLEKA KORIŠĆENOG ZA PROIZVODNJU BELIH MEKIH SIREVA PRE I POSLE TERMIČKOG TRETMANA

Chemical parameters	Milk intended for the production of white soft cheese	
	Before heating	After heating 72.2°C
Water (%)	87.75	87.87
Dry matter (%)	12.25	12.13
Dry matter without fat (%)	8.42	8.35
Protein (%)	3.25	3.21
Fats (%)	3.81	3.78
Lactose (%)	4.71	4.68

N =10 Total number of samples

For production of hard cheese, milk was subjected to the first scalding at 37.8°C. Before the scalding the percenttage of water was 88.08%. After the thermal treatment the percentage of water was 88.15%. The percentage of dry matter in milk before scalding was 11.92%, while after the end of I scalding the decline was observed i.e.11.85%. The amount of dry matter without fats before was 8.36%, and after I scalding amounted to 8.29%. The percentage of protein before the I scalding was 3.20%, and after I scalding the obtained value was 3.16%. Fat percentage before the thermal treatment of milk was 3.56% and amounted to 3.56 % at the end of heat treatment. Changes are noted in the amount of lactose in milk.Before heat treatment lactose content was 4.75%, and after thermal treatment its value was 4.66%.

The average chemical composition of the milk that was intended for the production of cheese is shown in Table 2.

For production of kashkaval, milk was subjected to thermal treatment at 65°C. Before thermal treatment the percentage of water was 87.72%, while after heat treatment the percentage of water was 87.81%. The percentage of dry matter in milk before heat treatment was 12.26%, while after the end of thermal treatment the decline was observed and that number is 12.19%. The amount of dry matter without fats before heat treatment was 8.46%, and after thermal treatment reached 8.40%. The protein content before the thermal treatment was 3.22%, and after heat treatment the estimated value was 3.20%. Fat content did not show any significant changes before (3.80%) and after thermal treatment (3.79%). After thermal treatment the lactose content in milk was 4.62% (before heat treatment lactose content was amounted to 4.70%).

The average chemical composition of the milk that was intended to produce a soft white cheese is shown in Table 3. During the production of white soft cheese milk was subjected to thermal treatment of 72.2°C. Before thermal treatment the percentage of water was 87.75%. while after heat treatment the percentage of water was 87.87%. The percentage of dry matter in milk before heat treatment was 12.25%, while after the end of thermal treatment the decline was observed and that number is 12.13%. The amount of dry matter without fats before heat treatment was 8.42%. and after thermal treatment it was 8.35%. Decline is observed in the percentage of protein. before thermal treatment it was 3.29%, and after heat treatment it was 3.21%. Fat content before the thermal treatment of milk was 3.81% and after treatment it was 3.79 %. Changes are noted in the amount of lactose in milk which was 4.71%, and after thermal treatment its value was 4.68%.

According to Wang et al. (2007) plate heat exchangers as a result of their structure has a number of advantages compared to other heat exchangers:

- Plate exchangers can be used for very viscous fluids, which tend to move in laminar regime as a result of their rotating features. In that way the impact on alleviating deposition was registered.
- The high heat transfer coefficient and the opposite way allow these heat exchangers to work with approximately the same temperature (~ 1°C).
- The rate of deposition of plate heat exchangers varies from 0.1 to 0.25 as compared to other heat exchangers where it is 1.

If any other type of heat exchanger is used, it is expected that the values obtained from research would be about 25% to 90% higher. That would mean a significant reduction in quality of thermal treated milk.

CONCLUSION

The following conclusions could be drawn:

- During the thermal treatment of milk in the plate exchanger designed for the production of hard cheese, kashkaval and white soft cheese the increasing of water content, but reducing the percentage of dry matter, dry matter without fats, protein, fats and lactose were noticed.
- After thermal treatment of milk intended for production of hard cheese there is an increase in the percentage of water 0.068%. There is an increase of water content - 0.066% in milk intended for production of kaskaval, and 0.116% in the production of white soft cheese. The increase of the percenttage of water in the heat treated milk has a negative impact on the durability of products.
- After thermal treatment of milk there is a reduction in the percentage of dry matter and of 0.068% in milk for production of hard cheese, 0.066% of milk for kashkaval production and 0.116% of milk for production of white soft cheese. The reduction of dry matter in milk directly affects on the reduction of quantity in cheeses which is dependent on the amount of milk protein and fat.
- After thermal treatment of milk there is a reduction in the percentage of dry matter without fats and 0.068% of milk for production of hard cheese, 0.058% of milk for kashkaval production and 0.068% in the milk for production of white soft cheese. Reducing of dry matter without fats is due to incipient denaturation of proteins, degradation of lactose; change the balance of salts in milk which is expressed through the deposition of calcium three-phosphate and three-calcium citrate.
- After the thermal treatment of milk there is a reduction in the percentage of milk fat: 0.004% of milk for production of hard cheese, 0.008% of milk in kashkaval production and 0.028% of milk in production of white soft cheese. Major changes in milk fat were observed in milk which is treated to produce a soft white cheese due to higher temperature. An impact on reduction of the agglutinins activity in which fat droplets are glued and by thermal processing separated, so influence the greater participation in forming sediment.
- There is a reduction in the percentage of protein - 0.046% of milk for production of hard cheese, 0.024% of milk to produce kashkaval and 0.042% of milk in the production of white soft cheese. The loss of protein in milk is the fullest expression that is intended for the production of white soft cheese due to the application of higher temperatures and longer heating period of retention of milk. When milk is heated above 70°C the SH-groups of β-lactoglobuline are activited and thus creates disulphide bridges (SS). This explains aggregation and deposition of proteins on the surface of heat exchanger.

After thermal treatment of milk the reduction in the percentage of lactose - 0.03% for thermal treatment of milk for production of hard cheese, 0.076% for thermal treatment of milk to produce kashkaval cheese and 0.07% in white soft cheese production, was observed. The reduction of lactose due to the crystallization process and its participation in forming sediment.

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IZVOD

UTICAJ TERMIČKOG TRETMANA U PLOČASTIM IZMENJIVAČIMA TOPLOTE NA HEMIJSKI SASTAV MLEKA ZA PROIZVODNJU SIREVA

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Mleko je tečnost složenog sastava, sadrži proteine, masti, ugljene hidrate, minerale i vitamine koji su neophodni za ljudski organizam, a imaju visoku biološku vrednost. Sir predstavlja mlečni proizvod u čvrstom ili polučvrstom stanju, čije karakteristike kao što su izgled, tekstura i ukus zavise od vrste mleka, termičke obrade, sadržaja mlečne masti, starter kulture i perioda zrenja. Jedna od najvažnijih faza u obradi mleka namenjenog za proizvodnju sira je toplotni tretman, koji ima za cilj da uništi mikroorganizme, inaktiviše enzime, poboljša tehnološke osobine mleka. Međutim, termička obrada mleka utiče na fizičko-hemijske karakteristike mleka.

Termička obrada mleka vrši se u izmenjivačima toplote, različitih konstrukcija. Promene mleka koje se javljaju tokom termičkog tretmana su različite u zavisnosti od primenjene temperature i trajanja tretmana.

Ključne reči: termička obrada • izmenjivači toplote • mleko • sir • hemijski sastav

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MOGUĆNOST PROIZVODNJE TRADICIONALNOG SIRA ČABRENJAKA U POLUINDUSTRIJSKIM UVJETIMA

U Bosni i Hercegovini, do danas, u domaćinstvima su sačuvane tradicionalne recepture autohtonih sireva. Tehnologija većine ovih sireva je jednostavna i prilagođena skromnim uvjetima domaćinstva. Presudnu ulogu u zaštiti sireva, koji su se prenosili sa generacije na generaciju, predstavlja tradicija i iskustvo.

U ovom radu objašnjen je postupak proizvodnje sira čabrenjaka i ispitana mogućnost njegove proizvodnje u poluindustrijskim uvjetima. Čabrenjak je u osnovi svježi sir, u koji se nakon dobijanja umiješuju razni dodaci.

Sol je neizostavan dodatak, a može se dodati paprika (slatka i ljuta), bijeli luk i dr. Nakon tog postupka, sir se stavlja da odstoji nekoliko dana u drvenoj kačici, nakon čega se može konzumirati.

Sir čabrenjak proizveden u mljekari “MFS Trade” d.o.o, Doboj Istok, pakiran i čuvan u čabru imao je vrlo visoke prosječne ocjene senzorskih svojstava. Opći izgled i boja su bodovani sa maksimalnim ocjenama. Konzistencija je ravnomjerno je sitno-zrnata i nije otpuštala sirutku. Miris sira blago kiseo i prihvatljiv. Uzorci sira pakirani u čašicu i vrećicu su nešto slabije senzorski ocijenjeni, sa postojanim karakterističnim svojstvima za proizvod.

Shodno rezultatima, i u poluindustrijskoj proizvodnji preporučuje se upotreba drvenog čabra, radi boljeg kvaliteta i senzorskih svojstava, većeg prinosa i duže trajnosti proizvoda.

Key words: sir čabrenjak • tradicija • tehnologija • kvalitet

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UVOD

U Bosni i Hercegovini su tradicionalni mliječni proizvodi, među njima i sirevi, sačuvani do danas, a sami proizvodni postupci nisu se značajnije promijenili. Kod nas je sirarstvo, još uvijek, umijeće, jer se tradicionalna proizvodnja zadržala u domaćinstvima u mnogim krajevima (Prpić i sur., 2003), a vrlo malo sira se proizvodi industrijski.

Među najstarije autohtone sireve Bosne i Hercegovine spada sir čabrenjak (tarenik, tucenik, čabrenik), koji se čuva u kačicama. Na području sjeveroistočne Bosne za ovu vrstu sira koristi se naziv sir iz čabra ili čabrenjak. Uglavnom se konzumira u domaćinstvima, kao namirnica na koje je seosko stanovništvo naviklo, sa specifičnim i prepoznatljivim organoleptičkim svojstvima.

Tradicionalni način proizvodnje podrazumijeva proizvodnju sira uglavnom od svježeg kravljeg mlijeka, korištenje prirodne ili autohtone mikrobne kulture, prirodne uvjete zrenja, jedinstven oblik i posebne dodatke siru, i uglavnom ručnu prozvodnju sira.

Tehnologija tradicionalnog načina proizvodnje sira čabrenjaka u domaćinstvima je jednostavna. Uglavnom se proizvodi za potrebe domaćinstva u zimskom periodu, zbog temperaturnih pogodnosti, jer se sir u tom periodu mogao duže čuvati. Svježe pomuženo i procijeđeno mlijeko se presipa u posude, i ostavi da prirodno fermentira. Nakon nekoliko dana (2-3 dana) djelovanja autohtone mikroflore mlijeka, odvaja se kajmak (pavlaka), a kiselo mlijeko se zagrijava na laganoj vatri do koagulacije istog. Potom se vrši ocjeđivanje gruša, pri čemu se odvaja sirutka pomoću cjediljke. Dobro iscijeđeni svježi sir se soli sa 3-4% soli, te umiješuju dodaci po želji, paprika i/ili bijeli luk. Sir se onda prebacuje u čabar (drvena kačica), dobro se nabije i posoli dodatno po površini. Zatim se stavi vlažna krpa, dospe sirutka i poklopi drvenim poklopcem.

Nakon par dana, može se koristiti, a dužim stajanjem dobija se izraženija aroma i bolja struktura sira.

Zadatak ovog rada je bilo ispitivanje mogućnosti proizvodnje sira čabrenjaka u poluindustrijskim uvjetima, njegova valorizacija i postavljanje tehnoloških normativa u proizvodnji ovog sira.

MATERIJAL I METODI

Za proizvodnju sira čabrenjaka korišteno je mlijeko mljekare “MFS Trade” d.o.o., Doboj Istok, dopremljeno sa otkupnog područja mljekare. Mlijeko je po prijemu mehanički i toplinski obrađeno i čuvano u tanku na temperaturi od 4-5°C do momenta proizvodnje.

Za direktnu inokulaciju u mlijeko za sir korištena je visoko koncentrirana, standardizirana, duboko smrznuta, liofilizirana kultura Lyofast M 036 N (Sacco, Italija) koja sadrži *Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* i *Leuconostoc.* Za koagulaciju mlijeka korišteno je i sirilo u prahu Microbial coagulant, Microclerici (Italija).

Kao dodatak siru korištena je samo sol (4 g soli/kg sira).

Sir čabrenjak je proizveden i čuvan u mljekari “MFS Trade” d.o.o. u Doboj Istoku.

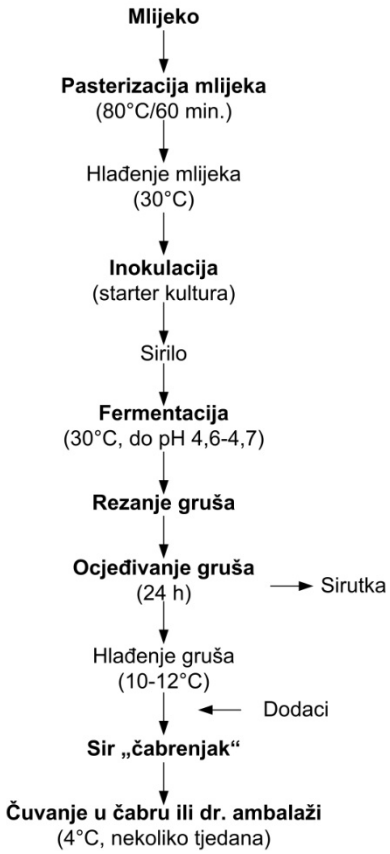
Oznake uzoraka i vrsta ambalaže u kojoj je pakiran i čuvan sir čabrenjak su: A – čabar, B – čašica, C – vrećica.

Svježe mlijeko, dopremljeno sa otkupnih područja je prethodno termički obrađeno na 80°C u trajanju od 60 minuta.

Mlijeko za proizvodnju sira je nakon pasterizacije i hlađenja čuvano u spremniku i prije daljnjeg korištenja dobro promiješano radi osiguranja jednolične kvalitete mlijeka tokom pokusa. Tehnološki proces proizvodnje ovog sira prikazan je shematski na slici 1.

Svi pokusi su rađeni od obranog mlijeka sa 0,1% mliječne masti. U prethodno pasterizirano, obrano mlijeko sa 0,1% mliječne masti, temperature 25°C, dodato je 1,2% mikrobne kulture Lyofast M 036 N i 0,1% sirila.

Fermentacija mlijeka, trajala je oko 19 sati na 30°C, do postizanja pH ~ 4,6 - 4,7.



Slika 1. SHEMATSKI PRIKAZ PROIZVODNJE SIRA ČABRENJAK

Figure 1. SHEMATIC DIAGRAM OF ČABRENJAK CHEESE PRODUCTION

Tabela 1. FIZIČKOKEMIJSKI SASTAV MLIJEKA KORIŠTENOG ZA PROIZVODNJU SIRA ČABRENJAK

Table 1. PHISICOCHEMICAL COMPOSITION OF MILK USED FOR ČABRENJAK CHEESE PRODUCTION

Parametri/Parameters	Sadržaj/Content
Mliječna mast/Milk fat (%)	0,1
Suha tvar bez masti/Fat free dry matter (%)	8,61
Laktoza/Lactose (%)	4,70
Proteini/Proteins (%)	3,23
Pepeo/Ash (%)	0,72
Gustoća/Density (g/cm^3)	1028,01
Točka ledišta/Freezing point (°C)	-0,517
pH vrijednost/pH value	6,67

Tabela 2. KEMIJSKI SASTAV SIRA ČABRENJAKA

Table 2. CHEMICAL COMPOSITION OF ČABRENJAK CHEESE

Parametar/parameters (%)	Uzorak/Sample		
	A	B	C
Mliječna mast/Milk fat	0,50	0,35	0,32
Proteini/Proteins	15,91	15,52	14,16
Pepeo/Ash	4,62	4,00	5,21
Voda/Water	75,56	76,66	77,77
Suha tvar/Dry matter	24,44	23,34	22,23
Suha tvar bez masti/Fat free dry matter	24,10	23,02	21,91
Voda u suhoj tvari bez masti/Water in fat free dry matter	75,90	76,98	78,09
Prinos/Yield	24,70	24,10	23,90

Potom je izvršeno rezanje gruša, brzo hlađenje i cijeđenje sirutke preko plastičnih posuda u vidu cjediljki (24 sata). Nakon završenog ocjeđivanja sirnog gruša, odnosno postizanja željene konzistencije, u svježi sir je dodatato 4% soli.

Uzorak sira A je punjen u drvenu posudu „čabar”, po čemu je ovaj sir i dobio naziv, a zatim je stavljen poklopac, a na njega posuda napunjena vodom, da bi se pritisnuo sir.

Tako pripremljeni sir je ručno punjen i pakiran u čašice od 100 ml, koje su stavljene u plastične vrećice, a zatim vakumirane (uzorak B), dok je uzorak C direktno pakiran u plastične vrećice od 500-600 gr, koje su potom vakumirane. Svi uzorci su skladišteni na sobnoj temperaturi od oko 25°C par dana, a nakon toga preneseni u hladnjaču na temperaturu od oko 4-6°C. Izuzetak je sir u čabru, koji je ostao još nekoliko dana na temperaturi oko 25°C.

Fizičko-kemijski sastav mlijeka za proizvodnju sira čabrenjaka ispitan je pomoću Lactoscan MCC50.

Analiza sira čabrenjaka provedena je sljedećim metodama: mliječna mast po Gerberu, suha tvar sušenjem na 105°C (IDF ISO 43/2:1982), proteini po Kjeldahlu, laktoza po formuli: ST – (UP + MM +Pe). Aktivna kiselost (pH) potenciometrijski primjenom pH metra Testo 206-pH2 Set; Kiselost sira čabrenjaka mjerena je nakon 7, 14 i 21 čuvanja u hladnjaku.

Senzorska svojstva uzoraka sira čabrenjaka ocijenila je grupa od 5 ocjenjivača, nakon 7, 14 i 21 dan čuvanja. Senzorska ocjena je prosječna ocjena ocjenjivača.

REZULTATI I DISKUSIJA

U tabeli 1. prikazan je fizičko-kemijski sastav obranog mlijeka sa 0,1% mliječne masti, korištenog za proizvodnju sira čabrenjaka.

Kao i u ranijim istraživanjima (Bašić i sur., 2003), korišteno mlijeko imalo je zadovoljavajući fizičko-kemijski sastav.

Tabela 3. pH VRIJEDNOST SIRA ČABRENJAKA TIJEKOM ČUVANJA

Table 3. pH VALUE OF ČABRENJAK CHEESE DURING STORAGE

Uzorak/Sample	Vrijeme (dani)/Time (days)		
	7	14	21
A	4,78	4,64	4,57
B	4,80	4,54	4,50
C	4,88	4,63	4,55

Zagrijavanjem kiselog mlijeka gruš se stvarao brzo, uz jednolično izdvajanje sirutke iz sirne mase (Bašić i sur., 2005; Vilušić i sur., 2006; Vilušić i sur., 2008), a kvaliteta sira ovisila o svojstvima korištenog mlijeka i uvjetima procesa proizvodnje (Tratnik, 1998).

Prema Pravilniku o proizvodima od mlijeka i starter kulturama (Službeni glasnik BiH 21/11), svježi sir od mlijeka treba imati 69% do 85% vode u bezmasnoj suhoj tvari sira. Uzorci sira čabrenjaka u potpunosti zadovoljavaju ove zahtjeve, sa sadržajem vode u suhoj tvari bez masti u granicama od 75,90-78,09% (tabela 2). Prema tome, vidi se da sir čabrenjak sadrži jako veliku količinu vode i spada u meke sireve.

S obzirom da je korišteno obrano mlijeko sa 0,1% m. m., udio mliječne masti u uzorcima sira se kretao od 0,32 do 0,5%. Sir čabrenjak je bogat izvor proteina, sa sadržajem od 14,16 do 15,91%, i nalazi se u granicama literaturnih podataka (Vilušić, 2007).

Sadržaj pepela je u varirao od 4,00-5,21%, a čine ga mineralne tvari i dodaci (so u koncentraciji do 4%).

Veliki problem u proizvodnji svježih sireva je pitanje održivosti kvaliteta svježeg sira, ovisno o uvjetima čuvanja. Podaci o promjeni kiselosti sira čabrenjaka nakon 7., 14., 21. dana čuvanja u hladnjaku dati su u tabeli 3.

Iz tabele 3 je vidljiv blagi pad kiselosti tijekom čuvanja u hladnjaku, što je tipično za svježe sireve i druge fermentirane mliječne proizvode. Uzorak A čuvan u tradicionalnom čabru imao je najmanji pad pH vrijednosti, što vjerojatno ukazuje na pozitivan utjecaj posude na prirodnu mikrofloru i biokemijske procese koji se odvijaju tijekom čuvanja.

Struktura i teksturalna svojstva mliječnih proizvoda su primarni kriteriji njihove ocjene kvalitete.

Uzorci proizvedenog sira čabrenjaka su senzorski ocijenjeni nakon 7., 14. i 21. dana čuvanja, a prosječne vrijednosti uzoraka sira prikazane su u tabeli 4.

Uzorak sira A, čuvan u drvenom čabru, najbolje je senzorski ocijenjen, sa maksimalnim ocjenama za opći izgled i boju. Površina sira bila je jednolično bijele do svijetložućkaste boje i nije ispuštala sirutku. Miris je slabo kiseo i bez stranih primjesa. Dužim čuvanjem odnosno zrenjem proizvod dobiva bolja senzorska svojstva.

Na površini uzoraka sira, pakiranih u čašicu i vrećicu (B i C), uočeno je malo izdvojene sirutke, što nije svojstveno za proizvod pakiran u tradicionalnom drvenom čabru. Miris i okus uzoraka B i C je bez stranih primjesa i srednje kiseo.

Trajnost svježih sireva je inače ograničena na oko 4 tjedna pri temperaturama 4-8°C, dok sir čuvan u čabru može imati znatno duži rok trajanja.

Prema svemu navedenom, i u poluindustrijskoj proizvodnji preporučuje se korištenje drvenog čabra, radi postizanja bolje konzistencije i sensorskih svojstava, većeg prinosa i duže trajnosti proizvoda.

ZAKLJUČAK

Prema tradicionalnoj recepturi proizveden je sir čabrenjak od mlijeka sa 0,1% mliječne masti i dodatak soli, u poluindusrijskim uvjetima.

Sir čabrenjak čuvan u čabru imao je vrlo visoke prosječne ocjene senzorskih svojstava. Opći izgled i boja su bodovani sa maksimalnim ocjenama. Konzistencija je ravnomjerno sitno-zrnasta i nije otpuštala sirutku. Miris sira blago je kiseo i prihvatljiv.

Uzorci sira pakirani u čašicu i vreću su nešto slabije senzorski ocijenjeni, ali ipak sa jasno izraženim i karakterističnim svojstvima za proizvod.

Shodno rezultatima, u poluindustrijskoj proizvodnji sira čabrenjaka, preporučuje se upotreba drvenog čabra, radi postizanja boljeg kvaliteta i senzorskih svojstva, većeg prinosa i duže trajnosti proizvoda.

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Tabela 4. SENZORSKA SVOJSTVA SIRA ČABRENJAKA

Table 4. SENSORY PROPERTIES OF ČABRENJAK CHEESE

Uzorak Sample	Vrijeme (dani) Time (days)	Opći izgled General appearance (1,0)	Boja Colour (2,0)	Konzistencija Consistency (4,0)	Miris Odour (3,0)	Okus Flavour (10,0)	Σ (20,0)
A	7	1	2	3,67	2,80	8,97	18,44
	14	1	2	4,00	2,43	9,67	19,10
	21	1	2	4,00	2,27	9,17	18,44
B	7	0,93	1,93	3,17	2,67	8,30	17,00
	14	0,83	1,93	3,60	2,27	8,67	17,30
	21	1,00	2	4,00	2,37	8,30	17,67
C	7	0,93	1,93	3,43	2,60	7,97	16,86
	14	0,83	1,83	3,93	2,20	8,67	17,46
	21	0,93	1,90	4,00	2,37	8,30	17,60

SUMMARY

POSSIBILITY OF ČABRENJAK CHEESE PRODUCTION IN SEMI-INDUSTRIAL CONDITIONS

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In Bosnia and Herzegovina, to date, households have saved the traditional recipe of authentic cheeses. The technology of most of these cheeses is simple and adapted to conditions of modest households. A crucial role in protecting the cheeses, which were passed down from generation to generation, is a tradition and experience.

This paper describes the process of čabrenjak cheese production and possibility of its production in semiindustrial conditions. Čabrenjak is basically fresh (cottage) cheese, in which after receiving various additives can be added. Salt is an essential supplement, and can be added peppers (sweet and hot), garlic, etc. After this procedure, the cheese is put to rest for several days in wooden čabar, after which it can be consumed.

Čabrenjak cheese produced in the dairy “MFS Trade” d.o.o Doboj Istok has a very high average of sensory properties. The general appearance and color were scored with a maximum. The consistency is uniformly fine-grained, whey is not reversed. Cheese samples packaged in glass and bag are lower sensorial evaluation, but with a persistent characteristic properties of the product.

Based on the results, in semi-industrial production of čabrenjak cheese recommended to use wooden container čabar, in order to achieve better quality and sensory properties, higher yield and longer shelf life of products.

Key words: cheese čabrenjak • tradition • technology • quality

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MASNOKISELINSKI SASTAV KOZJIH SIREVA

U cilju definisanja masnokiselinskog sastava sireva od kozjeg mleka, analizirane su četiri vrste kozjeg sira i jedna vrsta kravljeg sira, primenom gasne hromatografije – masene spektrometrije. Najzastupljenije masne kiseline u analiziranim uzorcima kozjih sireva bile su palmitinska, kaprinska, miristinska i oleinska, koje su zajedno činile oko 65% ukupnih masnih kiselina. U poređenju sa sirom od kravljeg mleka, kozji sirevi su sadržali značajno više masnih kiselina kratkog niza, kao i ukupnih zasićenih masnih kiselina. Kravlji sir se pokazao bogatijim u sadržaju stearinske kiseline i mononezasićenih masnih kiselina. U pogledu sadržaja ukupnih aterogenih masnih kiselina (laurinske, miristinske i palmitinske), nema značajne razlike između kozjih sireva i kravljeg sira.

Key words: kozji sirevi • masnokiselinski sastav • gasna hromatografija-masena spektrometrija

UVOD

Tokom poslednjih dvadeset godina svetska proizvodnja kozjeg mleka porasla je za 69%, odnosno na 12,2 miliona tona (FAO, 1998). Povećana potražnja za kozjim mlekom, a samim tim i mlečnim proizvodima napravljenim od kozjeg mleka, povezuje se sa različitim faktorima (Haenlein, 2004). Jedan od njih vezuje se za pojavu sve većeg broja ljudi alergičnih na kravlje mleko, tj. na protein iz kravljeg mleka (Walker, 1964). Smatra se da je alergija na kravlje mleko često oboljenje koje preovlađuje kod 2,5% dece tokom prve tri godine života (Businco and Bellanti, 1993), a javlja se u 12-30% slučajeva kod novorođenčadi mlađih od tri meseca (Lothe et al., 1982). Usled kompleksnosti sastava proteinskog dela mleka, tj. postojanja genetskih polimorfizama kazeina i proteina surutke, teško je utvrditi koji je protein glavni uzrok alergije. Prema nekim istraživanjima (Park, 1994), smatra se da je β-laktoglobulin, protein surutke, koji se ne nalazi u ljudskom mleku, glavni uzročnik alergije na kravlje mleko. Konzumiranje kozjeg umesto kravljeg mleka dovelo je do poboljšanja u 30-40% slučajeva, a u određenim studijama sprovedenim kod dece tokom 5 meseci, deca koja su konzumirala kozje mleko nadmašila su decu koja su konzumirala krvalje mleko u telesnoj masi, visini, skeletnoj mineralizaciji, nivou vitamina A, kalcijuma, tiamina, riboflavina, niacina i hemoglobina u krvi (Haenlein, 2004). Studije sprovedene na pacovima koji su umesto kravljim hranjeni kozjim mlekom, pokazale su da je došlo do povećanog iskorišćenja masti telesne mase, a smanjenja nivoa holesterola, dok su nivoi triglicerida i HDL (high density lipoprotein – dobar holesterol), ostali uobičajeni (Alferez et al., 2001). Zaključeno je da se konzumiranjem kozjeg mleka smanjuje ukupan nivo holesterola i nivo LDL (low density lipoprotein – lošeg holesterola), usled većeg prisustva masnih kiselina srednjeg lanca (36% u kozijem mleku prema 21% u kravljem), čime se smanjuje sinteza endogenog holesterola.

Sir predstavlja jednu od prvih namirnica koju su proizveli ljudi, a prema istorijskim podacima njegova upotreba stara je oko 4000 godina (Loewenstein et al., 1980; Fox i McSweeney, 2004). Kozji sir potiče iz Mesopotamije. Smatra se da su prvo nastali meki sirevi, a kasnije su u mediteranskim zemljama nastale različite vrste tvrdih sireva (Park, 2001; Fox, 2011).

Mlečna mast predstavlja veoma važnu komponentu sira, a njen sastav i zastupljenost masnih kiselina dosta je obrađivan u literaturi. Ustanovljeno je da mlečna mast ima ključnu ulogu u razvoju odgovarajućeg ukusa sira tokom zrenja, što je demonstrirano u studijama sa sirevima napravljenim od obranog mleka, i od mleka gde je mlečna mast zamenjena drugim lipidima. Kod takvih sireva nije došlo do razvitka odgovarajućeg ukusa (Foda et al., 1974). Mlečna mast takođe deluje i kao rastvarač za komponente ukusa koje su rastvorljive u mastima, omogućavajući njihovo zadržavanje u siru i otpuštanje tokom njegovog konzumiranja (Manning, 1974; Olson i Johnson, 1990; Wijesundera i Drury 1999; Fox, 2011).

Sirevi od kozjeg mleka su visoko cenjene namirnice, između ostalog, zbog senzornih karakteristika, koje obično zavise od kompozicije velikog broja jedinjenja. Ova jedinjenja su proizvodi kompleksnih biohemijskih procesa, od kojih je jedan od najvažnijih lipoliza. Lipolizom se oslobađaju masne kiseline odgovorne za karakterističnu aromu kozjih sireva: heksanska, oktanska i dekanska (zbog čega su poznate i kao kapronska, kaprilna i kaprinska, redom), kao i neke razgranate poput 3-metil-butanske, 4-

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metil-oktanske i 4-etil-oktanske kiseline (Ramírez et al., 2011; Trujillo et al., 2001).

Pored uticaja mlečne masti na karakteristike sira, treba istaći i njen zdravstveni aspekt, koji određuje sastav masnih kiselina. Poslednjih decenija u fokusu nacionalnih dijetetskih preporuka preporučuje se smanjenje unosa masti, kako bi se smanjio rizik od koronarnih bolesti. Međutim, jasno je da tip masti ima važniju ulogu u određivanju rizika od koronarnih bolesti nego ukupna količina masti u hrani. Različite masne kiseline imaju različit uticaj na ljudski organizam. Dok masne kiseline sa kratkim i srednjim lancem ne utiču na lipoproteine plazme, konzumiranjem zasićenih masnih kiselina, posebno onih sa 12-16 ugljenikovih atoma, povećava se ukupan nivo holesterola kao i LDL frakcija. Stoga se u cilju prevencije koronarnih oboljenja preporučuje smanjenje unosa zasićenih masnih kiselina dugog lanca (Bahrami i Vanak, 2008). Mononezasićene masne kiseline i određene polinezasićene masne kiseline imaju važnu ulogu u prevenciji i lečenju kardiovaskularnih bolesti, hipertenzije, dijabetesa, artritisa i drugih inflamatornih i autoimunih bolesti i tumora (Shenghua et al., 2011). Prema podacima nekih istraživanja, u mlečnoj masti identifikovano je preko 437 različitih masnih kiselina (Collins et al., 2003). Odnos pojedinih masnih kiselina u mlečnoj masti zavisi od više faktora (stadijum laktacije, način ishrane i dr.), a u proseku sadržaj zasićenih masnih kiselina iznosi 70% od ukupne količine masti, mononezasićenih oko 27%, a polinezasićenih oko 3% (Tratnik i Božanić, 2012).

S obzirom na porast potrošnje proizvoda od kozjeg mleka, kao i pomenute uticaje masnih kiselina na ljudsko zdravlje, cilj ovog rada bio je određivanje masnokiselinskog sastava različitih vrsta kozjih sireva dostupnih na našem tržištu.

MATERIJAL I METODI

Uzorci

Analizirano je pet vrsta sira dostupnog u prodaji, i to četiri kozja sira i jedna vrsta kravljeg sira. Sastav masnih kiselina u uzorcima određen je primenom gasne hromatografije-masene spektrometrije (GC-MS). Analizi uzoraka prethodila je priprema uzoraka, koja je obuhvatala usitnjavanje, ekstrakciju masti po Soxhlet-u i pripremu metilestara.

Priprema metilestara

Priprema metilestara izvedena je brzom modifikovanom metodom (Kravić et al., 2006). U epruvetu sa čepom pomoću staklenog štapića stavljeno je 5 kapi ekstrahovane masti iz uzorka, rastvoreno u 2,4 cm^3 n-heksana, a zatim je epruveta mućkana oko 10 sekundi. Nakon toga, dodato je 0,6 cm^3 2 mol/dm^3 rastvora KOH u metanolu i epruveta je mućkana 20 sekundi. Začepljena epruveta uronjena je u vodeno kupatilo zagrejano na 70ºC i držana jedan minut, mereno od momenta kada rastvor u epruveti počne da ključa. Epruveta je zatim izvađena i mućkana začepljena još 20 sekundi. U epruvetu je dodato 1,2 cm^3 1 mol/dm^3 rastvora HCl u metanolu, epruveta je blago promućkana, nakon čega se sačeka da se sadržaj u epruveti rasloji. Dodato je 3 cm^3 n-heksana, a zatim su metilestri iz gornjeg sloja dekantovani u čistu epruvetu i razblaženi sa n-heksanom do ukupne zapremine pripremljenog uzorka od ≈ 5 cm^3. Za analizu je korišćena zapremina uzorka od 1 µl.

GC – MS analiza

Za analizu metil-estara masnih kiselina korišćen je gasni hromatograf (Hewlett-Packard HP 5890 Series II GC) u kombinaciji sa masenim spektrometrom (Hewlett-Packard HP 5971 A MSD). Razdvajanje metil-estara masnih kiselina izvedeno je primenom kapilarne kolone SP-2560 (Supelco), dužine 100 m, unutrašnjeg prečnika 0,25 mm, sa slojem stacionarne likvidne faze od 0,20 µm. Kao gas nosač korišćen je helijum protoka 0,56 cm^3/min. Maseni spektri snimani su SCAN tehnikom, u intervalu *m/z* 40-400 a.m.u. Kvalitativno određivanje izvedeno je na osnovu masenih spektara i retencionih vremena. Kvantitativno određivanje izvedeno je u skladu sa AOAC metodom (Official Methods of Analysis 28.067,1984), pri čemu je za definisanje korekcionih faktora korišćen standardni rastvor smeše 37 metilestara proizvođača Supelco (37 component FAME Mix, 47885-U).

REZULTATI I DISKUSIJA

Sastav masnih kiselina u analiziranim uzorcima dat je u Tabeli 1. Sadržaji masnih kiselina su izraženi kao relativni sadržaj pojedinih masnih kiselina u odnosu na ukupne masne kiseline. Prikazani rezultati predstavljaju srednju vrednost ± standardna devijacija tri ponovljene analize za svaki uzorak. Kao što se iz Tabele 1. može videti, najzastupljenije masne kiseline u uzorcima kozjih sireva bile su palmitinska sa sadržajem od 19,98% do 20,90%, kaprinska (13,12 - 16,68%), miristinska (12,33 - 15,11%) i oleinska (13,67 - 16,24%). Ove masne kiseline su činile oko 65% ukupnih masnih kiselina. Do sličnih rezultata došlo se i u ranijim studijama (Trujillo et al., 2001; Poveda and Cabezas, 2006), a prema istraživanjima Stibilj et al. (2006), navedene masne kiseline bile su najzastupljenije i u kozjem mleku.

Relativni sadržaj ukupnih zasićenih (saturated fatty acids - SFA), mononezasićenih (monounsaturated fatty acids - MUFA) i polinezasićenih masnih kiselina (polyunsaturated fatty acids - PUFA) u ispitanim uzorcima dat je na Slici 1.

Relativni sadržaj zasićenih masnih kiselina u uzorku kravljeg sira (68,16%) bio je niži u odnosu na sadržaj SFA u uzorcima sira od kozjeg mleka, gde se kretao u opsegu od 77,88% do 79,91%.

Na Slici 2. dat je prikaz sadržaja aterogenih masnih kiselina, masnih kiselina kratkog lanca i sadržaj stearinske kiseline u analiziranim uzorcima. Zbirni sadržaj laurinske, miristinske i palmitinske, koje se smatraju aterogenim masnim kiselinama, u ispitivanim uzorcima se kretao u intervalu od 39,01% do 43,23%. Nije bilo značajne razlike u sadržaju aterogenih masnih kiselina u kozjim sirevima i kravljem siru. Sadržaj stearinske kiseline, koja se smatra neutralnom u smislu aterogenih svojstava, bio je za oko 2 puta viši u uzorku kravljeg sira (12,99%), u odnosu na kozje sireve (4,68-7,13%). Međutim, sadržaj zasićenih masnih kiselina kratkog lanca (C4 - C10), koje su lako svarljive kiseline, a ne poseduju aterogena svojstva, bio je značajno viši u uzorcima kozjih sireva, što je i očekivano s obzirom da su kapronska, kaprilna i kaprinska kiselina glavni nosioci karakteristične arome kozjeg mleka. Kozji sirevi su sadržali od 22,23% do 27,32% masnih kiselina kraćeg lanca, dok je sadržaj ovih kiselina u kravljem siru iznosio svega 6,82% (Slika 2.).

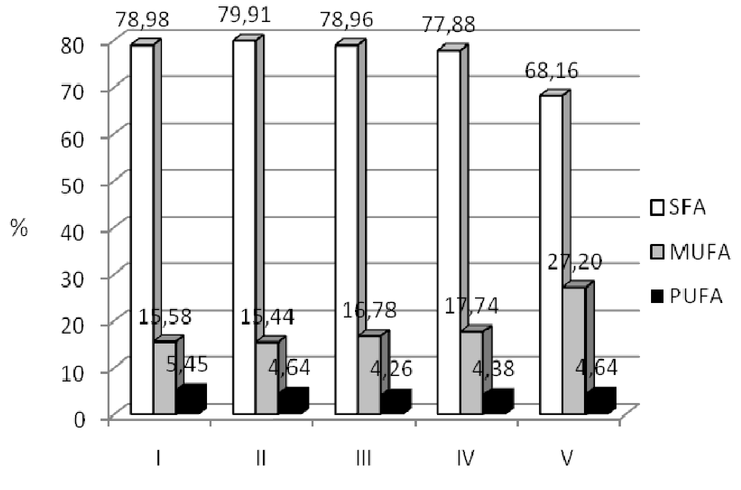
Masne kiseline razgranatog lanca, karakteristične za preživare – *iso*- i *anteiso*-C15:0, *iso*- i *anteiso*-C17:0 i *iso*-C16:0, detektovane su u svim analiziranim uzorcima. Sadržaj ovih kiselina kretao se u opsegu od 2,31% do 3,07%.

Sadržaj mononezasićenih masnih kiselina u kozjim sirevima se kretao od 15,44% do 17,74%, dok je kravlji sir sadržao znatno više MUFA (27,20%). U svim uzorcima najzastupljenija masna kiselina iz ove grupe bila je oleinska ki-

Tabela 1. MASNOKISELINSKI SASTAV KOZJIH SIREVA I KRAVLJEG SIRA

Table 1. FATTY ACID COMPOSITION OF GOAT'S CHEESES AND COW'S CHEESE

Broj uzorka Sample number	**I**	**II**	**III**	**IV**	**V**
Vrsta uzorka Sample	**Koziji sir 1 Goat's cheese 1**	**Koziji sir 2 Goat's cheese 2**	**Koziji sir 3 Goat's cheese 3**	**Koziji sir 4 Goat's cheese 4**	**Kravlji sir Cow's cheese**
Relativni sadržaj masnih kiselina (%) Fatty acid content (% of total fatty acid)					
C 4:0	1,11 ± 0,04	1,28 ± 0,05	1,04 ± 0,03	1,22 ± 0,00	1,93 ± 0,03
C 6:0	4,27 ± 0,08	4,52 ± 0,34	4,62 ± 0,07	4,14 ± 0,06	1,43 ± 0,01
C 8:0	3,73 ± 0,21	4,84 ± 0,78	4,16 ± 0,13	4,14 ± 0,05	0,93 ± 0,05
C 10:0	13,12 ± 0,43	16,68 ± 0,36	14,78 ± 0,74	15,24 ± 0,27	2,53 ± 0,08
C 11:0	0,37 ± 0,01	0,49 ± 0,04	0,42 ± 0,02	0,25 ± 0,02	-
C 12:0	7,08 ± 0,28	6,70 ± 0,19	7,57 ± 0,40	7,59 ± 0,63	3,65 ± 0,14
C 13:0	0,19 ± 0,02	0,17 ± 0,02	0,26 ± 0,02	0,55 ± 0,03	-
C 14:0	15,11 ± 0,23	12,33 ± 0,75	14,81 ± 0,92	14,22 ± 0,79	12,54 ± 0,17
C 15:0 i	0,55 ± 0,01	0,50 ± 0,04	0,55 ± 0,03	0,49 ± 0,02	0,62 ± 0,02
C 15:0 a	0,43 ± 0,02	0,42 ± 0,02	0,51 ± 0,02	0,46 ± 0,02	0,51 ± 0,02
C 14:1	0,28 ± 0,01	0,27 ± 0,02	0,42 ± 0,04	0,62 ± 0,02	1,23 ± 0,01
C 15:0	1,42 ± 0,05	1,47 ± 0,05	1,75 ±0,12	1,72 ± 0,11	2,00 ± 0,05
C 16:0 i	0,19 ± 0,02	0,35 ± 0,01	0,04 ± 0,01	0,23 ± 0,01	0,35 ± 0,01
C 16:0	20,88 ± 0,51	19,98 ± 0,87	20,85 ± 0,96	20,90 ± 0,97	25,98 ± 0,24
C 17:0 i	1,06 ± 0,09	0,81 ± 0,03	1,10 ± 0,16	0,99 ± 0,03	0,51 ± 0,01
C 17:0 a	0,61 ± 0,02	0,74 ± 0,05	0,87 ±0,06	0,17 ± 0,01	0,32 ± 0,01
C 16:1	-	-	0,12 ± 0,02	2,31 ± 0,03	3,91 ± 0,02
C 17:0	1,72 ± 0,08	1,25 ± 0,09	0,74 ± 0,04	0,88 ± 0,02	1,49 ± 0,07
C 17:1	0,67 ± 0,02	0,65 ± 0,02	-	1,15 ± 0,06	0,54 ± 0,01
C 18:0	7,13 ± 0,10	6,65 ± 0,23	4,90 ± 0,13	4,68 ± 0,16	12,99 ± 0,12
C 18:1	14,62 ± 0,91	14,52 ± 0,82	16,24 ± 0,50	13,67 ± 0,75	21,52 ± 0,07
C 18:2	4,34 ± 0,17	3,71 ± 0,35	4,26 ± 0,03	4,38 ± 0,10	4,64 ± 0,08
C 20:0	-	0,55 ± 0,02	-	-	0,37 ± 0,03
C 18:3 n3	1,10 ± 0,05	0,93 ± 0,03	-	-	-
C 22:0	-	0,16 ± 0,01	-	-	-



SFA – saturated fatty acids/zasićene masne kiseline, MUFA – monounsaturated fatty acids/mononezasićene masne kiseline; PUFA – polyunsaturated fatty acids/polizasićene masne kiseline

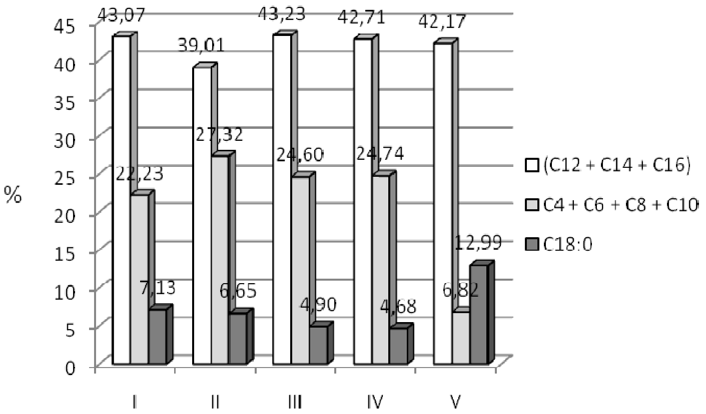
Slika 1. RELATIVNI SADRŽAJ ZASIĆENIH, MONO- I POLINEZASIĆENIH MASNIH KISELINA U KOZJIM SIREVIMA I KRAVLJEM SIRU

Figure 1. RELATIVE CONTENT OF SATURATED, MONO- AND POLYUNSATURATED FATTY ACIDS OF GOAT'S CHEESES AND COW'S CHEESE

selina. U pogledu sadržaja polinezasićenih masnih kiselina nije bilo značajnih razlika između kozjih sireva i kravljeg sira. Sadržaj PUFA kretao se u intervalu od 4,26% do 5,45%. Najzastupljenija masna kiselina iz ove grupe bila je linolna, sa najvećim sadržajem u uzorku kravljeg sira (4,64%). Esencijalna linolenska kiselina je detektovana u dva uzorka kozijeg sira u količinama od oko 1%.

ZAKLJUČAK

Najzastupljenije masne kiseline u analiziranim uzorcima kozjih sireva bile su palmitinska, kaprinska, miristinska i oleinska, koje su zajedno činile oko 65% ukupnih masnih kiselina. Kozji sirevi sadrže oko četiri puta više lakosvarljivih neaterogenih masnih kiselina kratkog lanca (buterna, kapronska, kaprilna i kaprinska kiselina) dok je ukupan sadržaj zasićenih masnih kiselina u kozjim sirevima nešto viši u odnosu na kravlji sir. Kravlji sir se pokazao bogatijim stearinskom kiselinom i mononeza-



Slika 2. SADRŽAJ ATEROGENIH MASNIH KISELINA (C12 + C14 + C16), MASNIH KISELINA KRATKOG LANCA (C4 + C6 + C8 + C10) I STEARINSKE KISELINE U KOZJIM SIREVIMA I KRAVLJEM SIRU

Figure 2. THE CONTENT OF ATHEROGENIC FATTY ACIDS (C12 + C14 + C16), SHORT CHAIN FATTY ACIDS (C4 + C6 + C8 + C10) AND STEARIC ACID IN GOAT'S CHEESES AND COW'S CHEESE

sićenim masnim kiselinama. U pogledu sadržaja ukupnih aterogenih masnih kiselina nema značajne razlike između kozjih i kravljeg sira.

ZAHVALNICA

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SUMMARY

FATTY ACID COMPOSITION OF GOAT'S CHEESES

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In order to define the fatty acid composition of goat's cheeses available on the Serbian market, four types of goat's cheese and one type of cow's cheese were analysed using gas chromatography – mass spectrometry. The most abundant fatty acids in all samples of goat's cheese were palmitic, capric, myristic and oleic, representing about 65% of total fatty acids. Compared to cow's cheese, all goat's cheese samples contained significantly higher values of short chain fatty acids and total saturated fatty acids. Cow's cheese was richer in stearic acid and monounsaturated fatty acids. In terms of the total content of atherogenic fatty acids (lauric, myristic and palmitic acid), there was no significant difference between goat's and cow's cheese.

Ključne reči: goat's cheese • fatty-acid composition • gas chromatography-mass spectrometry

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RIZIK PRISUSTVA STAFILOKOKA U SIREVIMA

Koagulaza pozitivni sojevi izolovani iz autohtonih sireva su okarakterisani u odnosu na njihov enterotoksogeni potencijal i profil fenotipske antimikrobne rezistencije. Primenom VIDAS tehnologije, od 73 koagulaza pozitivna soja stafilokoka analiziranih u našem radu, potencijal produkcije enterotoksina je pokazalo 22 (30,14%) sojeva. Rezistencija na jedan ili više odabranih antibiotika je utvrđena kod 11 (50%) sojeva, od kojih se 6 (54,54%) karakterisalo svojstvom multiple rezistencije. Svojstvo enterotoksogenosti i antimikrobne rezistencije ispitivanih koagulaza pozitivnih stafilokoka predstavlja snažan argument u karakterizaciji rizika njihovog prisustva u sirevima.

Key words: stafilokoke • enterotoksogeni potencijal • antimikrobna rezistencija • rizik

UVOD

Prema podacima Evropske agencije za bezbednost hrane, enterotoksini stafilokoka se navode kao uzrok trovanja u 293 (55,4%) od 525 slučajeva alimentarnih trovanja u 2009. godini. Ispitivanja sprovedena u periodu od 1993. do 1998. godine pokazuju da je 4,8% registrovanih slučajeva stafilokoknih trovanja u Evropi povezano sa konzumacijom mleka i proizvoda od mleka (Lopez-Pedemonte i sar., 2007).

Sa aspekta bezbednosti hrane, najvažnija karakteristika koagulaza pozitivnih stafilokoka je produkcija enterotoksina. Koagulaza pozitivni sojevi, i to pre svega *Staphylococcus aureus*, su jasno implicirani u slučajevima alimentarnih intoksikacija (Hennekinne i sar., 2011). U naučnoj zajednici ne postoji usaglašen stav o enterotoksogenom potencijalu koagulaza negativnih sojeva stafilokoka.

Do danas je otkriven 21 tip stafilokoknih enterotoksina (SE) i enterotoksinima sličnih tipova (**S**taphylococcal **E**nterotoxin-**l**ike (**SEl**) (Hennekine i sar., 2011). U 95% slučajeva stafilokoknih trovanja implicirani su klasični enterotoksini (A, B, C, D, i E) (Schelin i sar., 2011), s time da se u najvećem broju slučajeva dokazuje enterotoksin A (Bergdoll, 1989; Mossel i sar., 1995). Prema današnjem naučnom konsenzusu, količina enterotoksina potrebna za ispoljavanje tipičnih simptoma trovanja u korelaciji je sa približnim brojem stafilokoka od 10^5 CFU/g namirnice, što je pri mikrobiološkoj karakterizaciji sireva ujedno i vrednost postavljena kao hazard od strane evropske (European Comission Regulation (EC) No. 1441/2007), ali i naše legislative (Sl. glasnik RS, br. 72/10).

Antimikrobna rezistencija, zahvaljujući perzistentnoj cirkulaciji rezistentnih sojeva bakterija u okruženju, ali i kroz lanac hrane, predstavlja ozbiljan problem globalnih razmera. Prema postojećoj, tzv. "rezervoar" teoriji, lanac hrane se može smatrati jednim od glavnih puteva transmisije rezistentnih mikroorganuzama između populacije ljudi i životinja (Witte, 1997). Sojevi *S. aureus* i koagulaza negativnih stafilokoka poreklom iz sireva u najvećem broju pokazuju rezistenciju na tetraciklin, eritromicin, hloramfenikol i linkomicin (Perreten i sar., 1998). Kako u određenim slučajevima, molekularnu bazu rezistencije na antibiotike predstavlja ekstrahromozomalna DNK, odnosno mobilni genetski elementi, takvi rezistentni sojevi stafilokoka mogu poslužiti kao rezervoari gena rezistencije, gde se rizik posledičnog prenosa takvih gena na druge mikroorganizme u okruženju (komenzale, oportuniste ili patogene) ne može isključiti (Teale, 2002).

Cilj ovog rada jeste da se sojevi koagulaza pozitivnih sojeva stafilokoka, izolovani iz autohtonih sireva poreklom od sirovog mleka, okarakterišu u odnosu na njihov enterotoksogeni potencijal i profil fenotipske antimikrobne rezistencije. Time bi na osnovu podataka o enterotoksogenom potencijalu i fenotipu antimikrobne rezistencije bila moguća preliminarna karakterizacija rizika.

MATERIJAL I METODI

Materijal ispitivanja predstavljali su koagulaza pozitivni sojevi stafilokoka (ukupno 73 soja), izolovani iz sireva predstavljenih tradicionalnom tehnologijom, poreklom od sirovog mleka.

Određivanje enterotoksogenosti koagulaza pozitivnih sojeva *Staphylococcus* spp.

Koagulaza pozitivne stafilokoke su testirane na potencijal produkcije enterotoksina upotrebom VIDAS tehnologije (Vitek Immuno Diagnostic Assay System, bioMérieux, France), koja koristi princip **E**nzyme **L**inked **F**luorescent **A**ssay testa (ELFA), a prema in-

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strukcijama proizvođača. Sojevi koagulaza pozitivnih stafilokoka su kultivisani u **B**rain **H**eart **I**nfusion (BHI) bujonu (37ºC/24h). Po završenoj inkubaciji obavi se centrifugovanje na 7000 g pri 4ºC/10 min. 500 µl supernatanta dodaje se u odgovarajuća udubljenje VIDAS strip – a (Staph enterotoxin II, SET 2, bio-Merieux), a potom se strip ubaci u aparat Mini VIDAS (Vitek Immuno Diagnostic Assay System, bioMérieux, France) i po obavljenoj kalibraciji, aparat izvrši očitavanje i izbaci listu dobijenih vrednosti.

Ispitivanje fenotipske rezistencije na odabrane antibiotike kod izolovanih sojeva stafilokoka primenom disk difuzione metode i E-testa

Ispitivanje osetljivosti/rezistencije sojeva stafilokoka je obavljeno primenom disk difuzione metode, sledeći preporuku Instituta za kliničke i laboratorijske standarde (Clinical and Laboratory Standard Institution - CLSI, bivši National Committee for Clinical Laboratory Standards - NCCLS) (NCCLS, 2002), time da su korišćeni BBL diskovi impregnirani odgovarajućim antibiotikom (BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs) (Becton, Dickinson and Company, Le Pont de Claix, France). Primenjeni su sledeći antibiotici: eritromicin (15 µg), tetraciklin (30 µg), gentamicin (10 µg), penicilin (10 IU), hloramfenikol (30 µg), ampicilin (10 µg), oksacilin (1 µg), vankomicin (30 µg), neomicin (30 µg), ciprofloksacin (5 µg).

Za određivanje minimalne inhibitorne koncentracije za tetraciklin, penicilin i oksacilin kod enterotoksogenih sojeva gde je disk difuzionom metodom potvrđena fenotipska rezistencija, korišćeni su E test stripovi ((AB Biomerieux, Solna, Sweden) na osnovu instrukcija proizvođača.

REZULTATI I DISKUSIJA

Primenom VIDAS tehnologije, od 73 koagulaza pozitivna soja stafilokoka analiziranih u našem radu, potencijal produkcije enterotoksina je pokazalo 22 (30,14%) sojeva. Morandi i sar. (2007) ispitujući enterotoksogeni potencijal 107 koagulaza pozitivna soja poreklom iz proizvoda od mleka, sposobnost ekspresije enterotoksina utvrđuju kod 52% izolata. Normanno i sar. (2007) ispitujući enterotoksogeni potencijal sojeva stafilokoka izolovanih iz proizvoda od mleka i mesa, sposobnost produkcije enterotoksina utvrđuju kod 125 (59,8%) sojeva.VIDAS metoda (Vitek Immuno Diagnostic Assay System, bioMérieux, France) je brza i jednostavna za izvođenje, ali njenom primenom se detektuju samo SAE-SEE, ali ne i drugi, u skorije vreme, okarakterisani enterotoksini (Chiang i sar., 2008). Pored toga, rezultati se izražavaju kao pozitivni i negativni, i ne postoji mogućnost diferencijacije različitih enterotoksina. Princip metode je imunološka reakcija (EFLA), čija osetljivost zavisi od količine produkovanog toksina, tako da postoji mogućnost da se slabo produkujući enterotoksogeni sojevi, ne detektuju (Cunha i sar., 2006). Prema Schmitz i sar. (1998), u kliničkoj praksi, svaki soj stafilokoka koji nosi gen za produkciju enterotoksina trebao bi se smatrati potencijalnim producentom enterotoksina kako se toksogeneza *in vivo* ne može isključiti.

Enterotoksogeni sojevi su dalje analizirani u odnosu na profil antimikrobne rezistencije, gde je 11 (50%) sojeva pokazalo rezistenciju na jedan ili više odabranih antibiotika, od kojih se 6 (54,54%) karakterisalo svojstvom multiple rezistencije (rezistencija na dva i više antibiotika).

Zastupljenost sojeva stafilokoka u odnosu na fenotipsku rezistenciju ispoljenu prema jednom, dva ili više odabranih antibiotika prikazana je u tabeli 1.

Najčešći fenotipovi antimikrobne rezistencije kod sojeva stafilokoka podrazumevaju rezistenciju na vankomicin, penicilin i ampicilin (54,54%), dok se rezistencija na tetraciklin utvrđuje kod 3 (27,27%), odnosno 2 (18,18%) soja u slučaju rezistencije na oksacilin. Fenotip rezistencije na hloramfenikol i ciprofloksacin nosi po jedan izolat stafilokoka. Perreten i sar. (1998) ispitujući osetljivost koagulaza negativnih stafilokoka izolovanih iz namirnica kao najčešće fenotipove utvrđuju rezistenciju na hloramfenikol, tetraciklin, eritromicin i linkomicin. Naši rezultati su saglasni rezultatima ispitivanja Resch i sar. (2008), gde se kao dominatni profil rezistencije utvrđuje rezistencija na penicilin, oksacilin, ampicilin, ali i na linkomicin, tetraciklin, i fusidiksičnu kiselinu. U radu Spanu i sar. (2010) sojevi *Staphylococcus aureus* izolovani iz sireva proizvedenih od sirovog ovčijeg mleka pokazuju rezistenciju na ampicilin (36,1%), penicilin (33,3%), tetraciklin (11,1%) i kloksacilin (2,8%).

Rezistentna populacija stafilokoka je izložena proceduri određivanja minimalne inhibitorne koncentracije (MIC) za penicilin, tetraciklin i oksacilin primenom E testa, gde se 3 soja karakterisalo rezistencijom na penicilin i 1 soj je pokazao rezistenciju na tetraciklin. Rezistencija na oksacilin nije potvrđena (tabela 2).

Tabela 1. FENOTIPSKA REZISTENCIJA IZOLOVANIH SOJEVA STAFILOKOKA U ODNOSU NA ODABRANE ANTIBIOTIKE

Table 1. ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATED STAPHYLOCOCCAL STRAINS

Rezistencija na Resistance to	**Rezistentni sojevi stafilokoka Resistant strains of staphylococci**		**Zastupljenost sojeva u odnosu na rezistenciju prema antibioticku/antibioticima *-profili rezistencije Antimicrobial resistance profiles**
	broj number	%	
1 antibiotik 1 antibiotic	5	45,45	Van
2 antibiotika 2 antibiotics	1	9,09	P/Am
3 antibiotika 3 antibiotics	2	18,18	Te/P/Am (1) P/Am/Cip (1)
4 antibiotika 4 antibiotics	3	27,27	P/Tet/Am/Van/(1) P/C/Am/Ox (1) Te/P/Am/Ox (1)

* E – eritromicin; Te – tetraciklin; Gm – gentamicin; P – penicilin; C - hloramfenikol; Am – ampicilin; Ox – oksacilin; Va – vankomicin; N – neomicin; Cip – ciprofloksacin

Tabela 2. REZISTENTNA POPULACIJA STAFILOKOKA PREMA REZULTATIMA ODREĐIVANJA MIC ZA PENICILIN I TETRACIKLIN

Table 2. RESISTANT POPULATION OF STAPHYLOCOCCI ACCORDING TO RESULTS OF MIC EVALUATION FOR TETRACYCLINE AND PENICILLIN

Antibiotik Antibiotic	MIC (µg/mL)	Broj rezistentnih sojeva Number of resistant strains
Staphylococci spp.		
tetraciklin tetracycline	R ≥ 1	1
penicilin penicillin	R ≥ 0.125	3

Rezultati određivanja antimikrobne rezistencije ispitivanih izolata stafiloka opravdavaju primenu disk difuzionog testa kao orijentacione, odnosno „screening" metode, ali u objektivnoj proceni rezistencije sasvim sigurno je opravdano uvođenje jedne od kvantitativnih metoda, odnosno određivanje MIC-a.

ZAKLJUČAK

Na osnovu utvrđenog enterotoksogenog potencijala, i svojstva multiple rezistencije na odabrane antibiotike, prisustvo koagulaza pozitivnih stafilokoka u sirevima predstavlja biološki hazard. Potrebna su daljna ispitivanja kako bi se u potpunosti spoznao složeni mehanizam ekspresije enterotoksina stafilokoka u samom matriksu namirnica, ali i utvrdila genetska baza antimikrobne rezistencije.

ZAHVALNICA

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SUMMARY

THE RISK OF STAPHYLOCOCCI PRESENCE IN CHEESES

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Coagulase positive staphylococci isolated from autochthonous cheeses were screened regarding their enterotoxigenic potential and antimicrobial resistance profile. By applying the VIDAS techology, out of 73 coagulase positive staphylococcal isolates, 22 (30,14%) strains showed enterotoxigenic potential. The 50% analysed strains showed antimicrobial resistance properties at least at one of antibiotics tested, and among those resistant population 54,54% strains were characterized by multiple resistance. The obtained results presented the strong argument in risk characterization.

Key words: staphylococci • enterotoxigenic potential • antimicrobial resistance • risk

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RAZGRADIVOST POLIETILENSKIH FOLIJA U VEŠTAČKIM I PRIRODNIM USLOVIMA

Različiti proizvodi industrije prerade mleka se pakuju u brojne, različite ambalažne materijale formirane u atraktivnu ambalažu, koja zadovoljava potrebe potrošača. Osim primarne ambalaže, značajna je i upotreba sekundarnih ambalažnih materijala u obliku transportnih kutija, podloški, kao i skupljajućih folija.

Svi ambalažni materijali svojim ekološkim svojstvima utiču na životnu sredinu. Smanjenje uticaja iskorištene i odbačene ambalaže na životnu sredinu moguće je većom primenom razgradivih ambalažnih materijala. Novu generaciju ambalažnih materijala predstavljaju biopolimeri, jestiva ambalaža i razgradivi ambalažni materijali za pakovanje različitih proizvoda. Sintetski polimeri postaju razgradivi uz dodatak aditiva za razgradnju. Dodati aditiv čini plastiku biorazgradivom tako što joj menja neke hemijske i fizičke karakteristike.

Cilj ovog rada je ispitivanje razgradivosti polietilenskih folija, kojima je dodat aditiv za razgradnju, a koje se koriste u prehrambenoj industriji. Za uzorak je odabrana polietilenska folija, kojoj je dodat aditiv, čija je razgradivost ispitivana u prirodnim i veštačkim uslovima. Pre i nakon izlaganja prirodnim i veštačkim uslovima ispitana su zatezna svojstava, maseni protok rastopa, kao i strukturne karakteristike polimerne folije. Rezultati su pokazali smanjenje zateznih svojstava ispod 5% od početnih vrednosti, povećanje karbonilnog indeksa, kao i povećanje MFR-a nakon tretmana za uzorke tretirane na oba načina, na osnovu čega je zaključeno da je ispitivana folija razgradiva. Postizanje razgradivosti u prirodnim uslovima trajalo je 4 nedelje (28 dana), a u veštačkim, laboratorijskim uslovima 120 sati (5 dana).

Key words: ambalaža • polimeri • razgradivi materijali • ekologija

UVOD

Dosta je literaturnih podataka o tradicionalnim materijalima i postupcima pakovanja proizvoda industrije prerade mleka. Polimerni ambalažni materijali su vodeći u prehrambenoj industriji (Lox, 1992), pre svega zahvaljujući svojim dobrim fizičko mehaničkim i barijernim svojstvima prema nepovoljnim faktorima spoljašnje sredine, kao što su vlaga, kiseonik, mikroorganizmi, lakoći prerade, kao i relativno povoljnoj ceni. Ovi materijali su veoma zastupljeni za pakovanje hrane, lekova, kozmetike, deterdženata i hemikalija. Oko 30% plastike širom sveta se koristi kao ambalaža. Ovakav vid upotrebe ima visoku stopu rasta od 12 odsto godišnje (Sabir, 2004). Velika proizvodnja i primena polimernih ambalažnih materijalan ima za posledicu iscrpljivanje neobnovljivih sirovina (nafte), kao i zagađenje životne sredine (Lazić, 2011).

Pored uticaja iskorišćene i odbačene ambalaže, na narušavanje životne sredine utiče i ceo životni ciklus ambalaže, koji se ogleda kako u iskorištenju neobnovljivih izvora sirovina i energije, tako i u štetnim emisijama u vazduh i vodu u procesima proizvodnje, primene i uklanjanju iskorištene ambalaže (Lazić i Novaković, 2010).

Izražena je potreba za novim materijalima, zbog nagomilavanja ambalažnog otpada i negativnog uticaja na životnu sredinu. Relativno nova generacija ambalažnih materijala, koji se proizvode iz obnovljivih izvora i podložni su samorazgradnji su biopolimeri i jestiva ambalaža. Takođe se od sintetskih polimera, uz dodatak aditiva za biorazgradnju, proizvode i razgradivi ambalažni materijali, namenjeni za primenu u prehrambenoj industriji (Lazić i Gvozdenović, 2007).

Biopolimeri su polimeri dobijeni iz biomase (iz obnovljivih poljoprivrednih sirovina, ili su životinjskog porekla, ili se dobijaju iz otpada prerade morske hrane, iz mikrobnih izvora). U biorazgradive polimere spadaju i jestivi filmovi (Krkić et al., 2012). Pored toga što se dobijaju iz obnovljivih izvora, materijali koji su biorazgradivi, razlažu se do sastojaka neškodljivih za okolinu: CO_2, vodu i kvalitetan kompost, pod uticajem prirodne mikroflore, kiseonika i vlage (Ojeda et al., 2009). Termin biorazgradivost znači da u biosferi postoji barem jedan enzim koji ubrzava razgradnju hemijskog lanca datog polimera (Lox, 1992; Lazić et al., 2008). Za sada je proizvodnja biorazgradivih polimera skuplja od polimera dobijenih iz naftnih derivata.

Jedan od pravaca poboljšanja ekološkog statusa sintetskih polimera je proizvodnja razgradivih materijala uz upotrebu aditiva koji uz delovanje spoljnih faktora, vlaga, kiseonik i UV zračenje, ubrzavaju razgradnju makromolekula do oligomera i monomera. Aditivi koji ubrzavaju ovaj proces i ubrzavaju biodegradaciju se nazivaju prodegradanti. Upotreba prodegradanata je stara tehnologija koja se komercijalno koristi poslednjih godina (Ammala et al., 2011).

Dodati aditiv čini plastiku biorazgradivom tako što joj menja neke hemijske osobine. On reaguje sa kiseonikom iz vazduha i time razlaže plastiku na male molekule. Prodegradant može da se meša sa različitim standardnim plastičnim masama, kao što su polietilen i polipropilen, tokom faze modeliranja.

Sklonost plastičnih proizvoda da podlegnu razgradnji indukovanoj UV radijacijom, toplotom ili ozonom je povećana dodavanjem prodegradanata u te polimere. Pored ovih degradacija, biorazgradnja nudi najefikasniji i najatraktivniji put za upravljanje otpadom u životnoj sredini (Singh i Sharma, 2008).

Degradacija polimera se dešava zbog nekoliko mehanizama i njihovih kombinacija: fotolitički, termalni, mehanički, hidrolitički, oksidativni, biološ-

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ki, sa poslednjim mehanizmom koji podrazumeva biološke procese, poznatim kao "mineralizacija". Mineralizacija je neophodna kako bi proces degradacije bilo kog polimera bio kompletan, a bez akumulacije konstituenata sa nepoznatim i rizičnim uticajem na životnu sredinu (Krzan et al., 2006).

Tokom degradacije polimer se prvo konvertuje u svoje monomere, a onda se ti monomeri mineralizuju. Hidro-biorazgradivi i foto-biorazgradivi polimeri se razgrađuju kroz dvostepen proces - prvo nastupa hidroliza ili fotorazgradnja, posle koje sledi biorazgradnja ostatka. Takođe postoje i polimeri koji se razgrađuju u samo jednoj fazi (Krzan et al., 2006).

Prvi korak razgradnje je depolimerizacija, ili cepanje lanca, korak u kome je polimer pretvoren u manje fragmente - oligomere. Depolimerizacija je značajno povezana sa pogoršanjem fizičkih karakteristika, kao što su boja, krtost i fragmentacija. Hidroliza i/ili oksidacija su primeri ovog koraka razgradnje polimera. Ovaj prvi korak je bitan zato što materijali sastavljeni od makromolekula,velike molekulske mase ne mogu da prođu kroz spoljašnje membrane živih ćelija (Sivan, 2011). Najštetniji efekat je vizuelan, gubitak mehaničkih karakteristika polimera, gubitak rastegljivosti, mehaničkog integriteta i snage uz smanjenje njihove prosečne molekulske težine (Abadal et al., 2006).

Drugi korak, poznat kao mineralizacija, se odvija unutar ćelija mikroorganizama gde se mali fragmenti polimera - oligomeri razlažu do CO_2, vode i biomase, ukoliko se mineralizacija odvija u aerobnim uslovim, ili CH_4, CO_2 i biomase, u slučaju anaerobnih uslova (Krzan i sar., 2006). Biorazgradnja se zasniva na upotrebi plastičnog supstrata kao izvora ugljenika za metabolizam mikroorganizama.

Karakteristike polimera, kao što su njegova pokretljivost, kristalinitet, molekularna masa, vrsta funkcionalnih grupa i supstituenata u njegovoj strukturi, kao i plastifikatori ili aditivi koji se dodaju polimerima, igraju važnu ulogu u njegovoj degradaciji. Pored karakteristika polimera i prirode predtretmana, faktor koji utiče na tok biodegradacije je i vrsta mikroorgnizama u postupku mineralizacije (Shah i sar., 2008).

MATERIJAL I METODI

Kao uzorak korišćene su polietilenske biorazgradive folije namenjene za primenu u prehrambenoj industriji, označene kao HD-PE (polietilen visoke gustine), debljine 0.020 mm, kojima je dodat aditiv za biorazgradnju.

Polovina uzoraka izložena je delovanju atmosferskih prilika na otvorenom prostoru (imitacija deponije smeća), dok je druga polovina postavljena u komoru za ubrzano starenje.

Izlaganje uzoraka UV zračenju u uređaju za ubrzano starenje, „Weathering tester QUV/spray" izvršeno je prema standardima SRPS G.S2.519: 2011, SRPS G.S2.520:2011 i SRPS G.S2.664:2011. Pripremljene epruvete uzoraka se postavljaju na odgovarajuće metalne nosače uređaja. Uzorci su u uređaju za ubrzano starenje izloženi delovanju ciklusa: 20h UV zračenja pri temperaturi 50 ºC i 4h kondenzacije pri temperaturi 40 ºC (ciklus A, SRPS G.S2.520.).

Ispitivanje zateznih svojstava i **utvrđivanje završne tačke razgradnje ispitivanjem zatezanja,** pre i nakon tretmana. Ispitivanja se vrše prema standardima SRPS G.S2.737: 2011 i SRPS G.Z2.010:2011. Zatezna svojstva su ispitana na aparatu „Instron 4301" sa podešenim početnim razmakom klema 50 mm i brzinom razmicanja klema 100 mm/min. Rezultati su dati kao % izduženja pri kidanju. Epruvete za ispitivanje su pripremljene isecanjem minimum 5 traka uzorka dimenzija (15x100) mm, pomoću uređaja za pripremu uzoraka „Film-/Paper Model Strip Cutter". U završnoj tački razgradnje materijal dostiže tačku krtosti. U ovoj tački minimum 75% epruveta uzorka ima vrednost izduženja koja je ≤5% početne vrednosti.

Određivanje strukturnih karakteristika uzorka infracrvenom spektrofotometrijom (FTIR), prema standardu SRPS G.S1.511:2011. Spektri su određeni FTIR spektrofotometrijom, na aparatu FTIR Spektrofotometar, model „Nicolet iS10". Karbonilni indeks (CI) pika se računa kao:

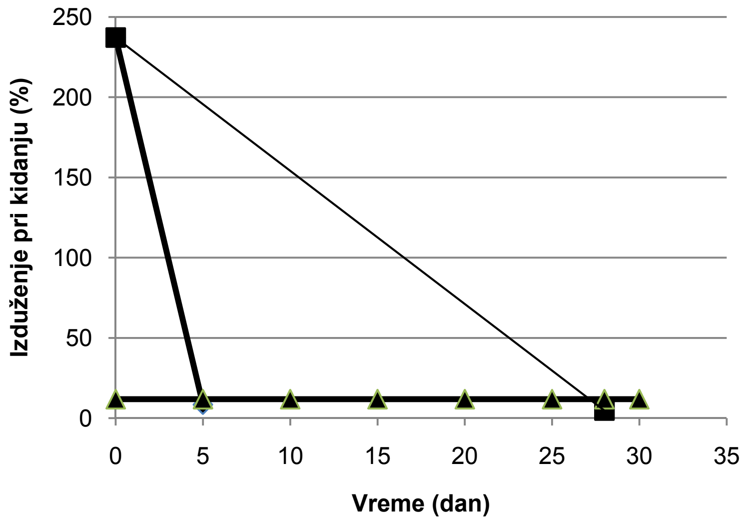
$$CI = A_{1713} / A_{1463} \qquad (1)$$

gde je A_{1713} apsorbanca očitana na $1713 cm^{-1}$, a A_{1463} apsorbancija očitana na $1463 cm^{-1}$. Kao dokaz razgradnje neophodno je da vrednost karbonilnog indeksa nakon tretmana bude veća od početne vrednosti.

Određivanje masenog protoka rastopa uzorka, pre i posle tretmana prema standardu SRPS G.S2.521: 2011. Maseni protok rastopa (MFR) je određen na aparatu Ekstruzioni plastomer „Tinius Olsen MP600", prema proceduri A-190/2.16. Rezultat se izražava u jedinici g/10min. Dokaz razgradnje je povećana vrednost MFR posle tretmana, koja ukazuje na smanjenje molekulske mase i/ili stepena umreženosti polimera.

REZULTATI I DISKUSIJA

Izlaganje uzoraka delovanju UV zračenja i kondenzaciji, bilo u prirodnim ili veštačkim uslovima, dovodi do narušavanja strukture polietilenskih lanaca tako što se oni skraćuju na kraće



Slika 1. IZDUŽENJE PRI KIDANJU (%)

Figure 1. ELONGATION AT BREAK (%)

Tabela 1. VREDNOSTI APSORBANCE OČITANE NA 1463 I 1713 cm^{-1}

Table 1. ABSORBANCE VALUES AT 1463 AND 1713 cm^{-1}

	Pre tretmana	Vreme tretmana u prirodnim uslovima	Vreme tretmana u veštačkim uslovima
	0 h	28 dana	5 dana
A_{1713}	0.0514	0.0060	0.0808
A_{1463}	0.5360	0.0383	0.4940
CI	**0.0958**	**0.1567**	**0.1636**

oligomere. Neophodno je postići skraćivanje lanaca kako bi fragmenti lanaca postali dostupni mikroorganizmima u drugoj, biotičkoj fazi razgradnje. Narušavanje strukture praćeno je promenom fizičkih i hemijskih karakteristika, kao što su zatezna svojstva, strukturne karakteristike i promene masenog protoka rastopa polimera.

Rezultati ispitivanja zateznih svojstava uzorka i utvrđivanje završne tačke razgradnje su prikazani na slici 1. Narušavanjem strukture, materijal postaje krt što uslovljava niže vrednosti izduženja pri kidanju tokom degradacionog perioda.

Na grafiku su prikazane srednje vrednosti izduženja pri kidanju, pre i tokom tretmana u uređaju za ubrzano starenje i u prirodnim uslovima. Linija paralelna sa x-osom predstavlja tačku krtosti uzorka, tj, momenat kada je došlo do razgradnje materijala. Njena vrednost iznosi 11.86%. U završnoj tački razgradnje materijal dostiže tačku krtosti. U ovoj tački minimum 75% epruveta uzorka ima vrednost izduženja koja je ≤5% početne vrednosti.

Ispitivani uzorak tretiran u prirodnim uslovima dostigao je tačku krtosti (razgradnje) nakon 4 nedelje (28 dana), dok je uzorak tretiran u laboratorijskim uslovima dostigao tačku krtosti nakon 120 sati (5 dana).

Promene strukturnih karakteristika su definisane promenom karbonilnog indeksa. Rezultati određivanja strukturnih karakteristika prikazani su u tabeli 1.

Dobijeni rezultati pokazuju porast karbonilnog indeksa sa 0.0958 na 0.1567 nakon tretmana u prirodnim uslovima i porast karbonilnog indeksa sa 0.0958 na 0.1636 nakon tretmana u veštačkim uslovima, što je posledica i dokaz razgradnje ispitivanih uzoraka folija.

Narušavanja strukture polietilenskih lanaca, odnosno skraćivanje lanaca na kraće oligomere uslovljava povećanje indeksa tečljivosti, odnosno masenog protoka rastopa. U tabeli 2 prikazani su rezultati određivanja masenog protoka rastopa (MFR).

Prikazani rezultati pokazuju porast vrednosti masenog protoka rastopa sa 0.212 na 3.912 nakon tretmana, razgradnje, u prirodnim uslovima i porast vrednosti masenog protoka rastopa sa 0.212 na 3.946 nakon tretmana, razgradnje, u veštačkim uslovima. Porast masenog protoka rastopa polimera (MFR-a) je posledica smanjenja molekulske mase polimera i dokaz razgradnje ispitivanih uzoraka.

ZAKLJUČAK

Jedan od pravaca poboljšanja ekološkog statusa ambalaže je veća primena prirodnih biopolimera ili sinteza razgradivih sintetskih polimera. Postojeći materijali (PE, PP, PS...) se mogu modifikovati raznim dodacima čiji je zadatak da obezbede brzu fotodegradaciju velikih makromolekula u kraće ugljovodonične lance, koji mogu biti podložni biodegradaciji.

U laboratorijskim uslovima moguće je prepoznati i pratiti proces degradacije koji bi se dešavao u prirodi, ali ubrzan nekoliko puta. Rezultati su pokazali smanjenje zateznih svojstava ispod 5% od početnih vrednosti, povećanje karbonilnog indeksa, kao i povećanje MFR-a nakon tretmana u komori za uzorke i nakon odlaganja u prirodi. Na osnovu ovih rezultata može se zaključiti da je ispitivana polietilenska folija, koja nalazi primenu u prehrambenoj industriji, razgradiva. Nakon 120h (pet dana) izlaganja veštačkom starenju u komori za ubrzano starenje potvrđena je biodegradabilnost, dok je izlaganjem uzorka folije prirodnim, atmosferskim uslovima spoljašnje sredine, biorazgradivost postignuta nakon 4 nedelje (28 dana).

Sa ekološkog aspekta, veliki je značaj pojave razgradivih ambalažnih materijala. Istraživanja idu u pravcu poboljšanja karakteristika, kako bi se proširila mogućnost primene ovih materijala za pakovanje različitih proizvoda, kao sekundarna, ali i kao primarna ambalaža.

Tabela 2. MASENI PROTOK RASTOPA (g/10 min)

Table 2. MELT MASS FLOW (g/10 min)

	Pre tretmana	Posle tretmana u prirodnim uslovima	Posle tretmana u veštačkim uslovima
MFR	**0.212**	**3.912**	**3.946**

ZAHVALNICA

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SUMMARY

POLYETHYLENE FOIL DEGRADABILITY IN ARTIFICIAL AND NATURAL CONDITIONS

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Different products of milk processing industry are packed in numerous various packaging materials formed into an attractive package that meets consumer needs. Besides primary packaging, the use of secondary packaging materials in the form of transport boxes, washers, and shrinking foils is also significant.

All packaging materials with their environmental attributes affect the environment. Reducing the impact of used and discarded packaging on the environment may be possible by using biodegradable packaging materials. New generation of packaging materials are biopolymers, edible packaging and biodegradable packaging materials for different products packaging. Synthetic polymers become biodegradable with the addition of additives for degradation. Added additive makes plastic biodegradable because it changes some chemical and physical properties of plastics.

The aim of this paper is to examine the degradation of polyethylene foils, to which an additive was added, and which are used in the food industry. Polyethylene foil, to which an additive was added, was selected as a sample. The degradability of this sample was examined in natural and artificial conditions. Before and after exposure to natural and artificial conditions, tensile properties, melt mass flow and structural properties of the polymer film were tested. The results showed decrease in tensile properties below 5% of the initial value, increase in carbonyl index and increase in MFR after samples were treated in both ways. So it could be concluded that the examined foil is degradable. Achieving degradation in natural condition lasted 4 weeks (28 days) and in artificial laboratory conditions 120 hours (5 days)

Key words: packaging • polymers • degradable materials • ecology

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KNJIGE: Tamime, A.Y., Robinson, R.K. (2004): Yoghurt Science and Technology, Woodhead publishing limited, Cambridge, England, p. 619.

POGLAVLJE U KNJIZI: Kindstedt, P., Carić, M., Milanović, S. (2004): Pasta Filata Cheeses. Chapter 11, in: Cheese: Chemistry, Physics and Microbiology, 3rd ed.: Major Cheese Groups-vol. 2, eds. P.F. Fox, et al., Elsevier Science Ltd., London, 251-277.

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