Prehrambena industrija

MLEKO I MLEČNI PROIZVODI

ČASOPIS SAVEZA HEMIJSKIH INŽENJERA SRBIJE I TEHNOLOŠKOG FAKULTETA U NOVOM SADU

VOL. 22 **BEOGRAD, 2011.** **BROJ 1**

Izdavači:
SAVEZ HEMIJSKIH INŽENJERA SRBIJE (SHI)
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Publikovanje časopisa finansijski su pomogli:
Ministarstvo prosvete i nauke Republike Srbije
Pokrajinski sekretarijat za nauku i tehnološki razvoj, AP Vojvodina, Republika Srbija

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Tehnološki fakultet
21 000 Novi Sad, Bulevar cara Lazara 1
Tel.: +381 21 485 3712
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Pretplata:
Za čitaoce u SRB 300 RSD
Za inostranstvo 30 Eura
Žiro račun: 840-1647666-56

Lektor:
Mr Jelena Jerković

Priprema i štampa:
VERZAL, Novi Sad
Tel./faks: 021/505-103

Tiraž: 250 primeraka

SADRŽAJ

Food industry

MILK AND DAIRY PRODUCTS

JOURNAL OF ASSOCIATION OF CHEMICAL ENGINEERS OF SERBIA AND FACULTY OF TECHNOLOGY NOVI SAD

VOL. 22 **BEOGRAD, 2011.** **No 1**

Publishers:
ASSOCIATION OF CHEMICAL ENGINEERS OF SERBIA (AChE)
FACULTY OF TECHNOLOGY UNIVERSITY OF NOVI SAD (FoT NS)

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The journal is financially supported by:

Ministry of Education and Science, Republic of Serbia

Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina, Serbia

Editorial:
Faculty of Technology, 21000 Novi Sad,
Bulevar Cara Lazara 1, Serbia
Tel: +381 21 485 3712
Fax: +381 21 450 413

Subscription:
300 RSD per issue or 30 Euro
Bank account: 840-1647666-56

Text proof reader:
M.Sc. Jelena Jerković

Prepress & printed by
VERZAL, Novi Sad
Tel./faks: +381 21 505 103

Copies: 250

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SCIENTIFIC PAPER

UDK: 637.344:628.165

EXPERIMENTAL AND NUMERICAL INVESTIGATIONS ON WHEY DESALINATION WITH NANOFILTRATION*

Whey is the by-product obtained when milk is transformed into cheese or casein. It is generated in large quantities world-wide: the annual volume of dairy whey produced globally exceeds 160 millions. Nanofiltration provides a demineralization performance that makes whey a suitable additive in human food formulas. This paper present robust and reliable simulation methods to predict the dynamics of batch membrane filtration processes dealing with multi-component systems such as whey. Experimental data was collected from lab nanofiltration investigations using sweet and acid whey. Our special interest is to employ data-driven models that minimize the necessary a-priori experiments and allow the conversion of raw data into useful information. We have integrated statistical tools and machine learning techniques in the proposed mathematical framework. We show that such techniques are capable to model multi-component systems where limited information on their true chemical composition is available.

Key words: desalination • diafiltration • nanofiltration • simulation • whey

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INTRODUCTION

Whey is the by-product obtained when milk is transformed into cheese or casein. This dairy stream represents an excellent source of functional proteins and peptides, lipids, vitamins, minerals, and lactose. It is produced in large quantities world-wide: the production of one ton of cheese generates approx. eight tons of liquid whey (Dec, 2007). The annual volume of dairy whey produced globally exceeds 160 millions of tons and it increases with an annual growth rate of 1-2% (Smithers, 2008). Depending on the type of cheese made and the corresponding casein precipitation procedure used, there are two main whey varieties: acid whey (pH <5) and sweet whey (pH 6−7). The acid type whey contains a higher amount of lactic acid and ash, calcium in particular. Table 1 shows the general composition of acid and sweet whey.

Nanofiltration membranes are a relatively recent development in the field of pressure-driven membrane separations, and their properties lie between ultrafiltration (UF) and reverse osmosis (RO). Due to its potential advantages, NF has gained a strong market position, brought about a number of patents, industrial research projects and commercial installations (Bessarabov and Twardowski, 2002). Starting in the late sixties, NF membrane processes have gradually found their way into industrial applications in various fields such as water softening, dye recovery, treatment of metal contained waste waters, oil-water separation, demineralization of whey, recycle of nutrients in fermentation processes, purification of landfill leachate, removal of sulfates from sea-water, bioproduct separation (Timmer, 2001).

NF systems are usually operated at medium pressures in the range of 10-50 bar, and have much higher water fluxes compared to RO membranes.

Nanofiltration can be applied for separation between ions with different valences and for separation of low- and high-molecular weight components. NF rejects uncharged, dissolved material and positively charged ions according to the size and shape of the molecule in question (Wagner, 2001). The degree of transportation of non-charged solutes through NF membranes is shown in Fig. 1.

NF membranes show diversity in separation behavior but they are common in rejecting multivalent ions (such as SO_2^{-4}, CO_2^{-3}, PO_3^{-4}, Mg^{2+}) in a higher degree, while in compari-

* This paper was presented as Session Lecture at International Conference JOINT EVENTS: 2nd CEFSER (Center of Excellence for Food Safety and Emerging Risks) Workshop *"Persistent organic pollutants in food and environment"*, 26th Symposium on Recent Developments in Dairy Technology and BIOXEN seminar *"Novel approaches for environmental protection"* at University of Novi Sad, Faculty of Technology, 8-10 September 2011.

Table 1. COMPOSITION OF SWEET-TYPE (CHEDAR CHEESE) AND ACID-TYPE WHEY (Sienkiewicz and Riedl, 1990)

Composition	Sweet whey	Acid whey
Water, %	93.3	95.6
Dry matter, %	6.70	6.42
Total protein, mg g^{-1}	0.60	0.53
Non-protein nitrogen, mg g^{-1}	0.34	0.34
Lactose, %	5.00	4.40
Ash, %	0.52	0.60
pH	6.10	4.70

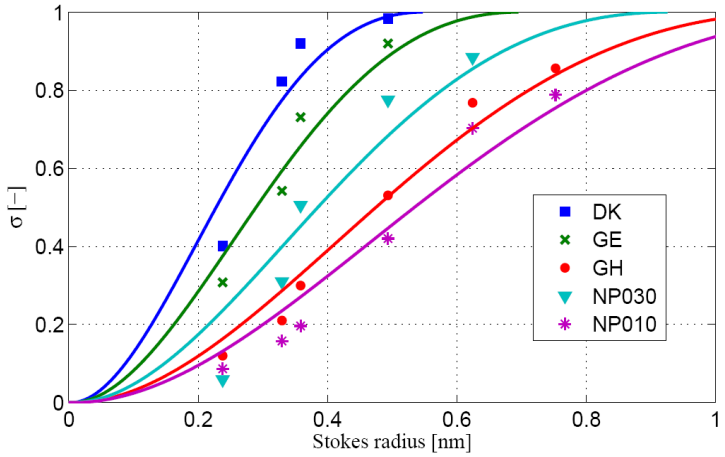

Figure 1. RELATIONSHIP BETWEEN REFLECTION COEFFICIENT σ AND STOKES RADII OF DIFFERENT SOLUTES (BUTANOL, RIBOSE, GLUCOSE, LACTOSE, PEG, AND DEXTRAN) FOR SEVERAL NANOFILTRATION MEMBRANES (DK, GE, AND GH FROM GE W&P TECHNOLOGIES, US, AND NP030 AND NP010 FROM MICRODYN-NADIR GMBH, GERMANY) (Kovács and Samhaber, 2008)

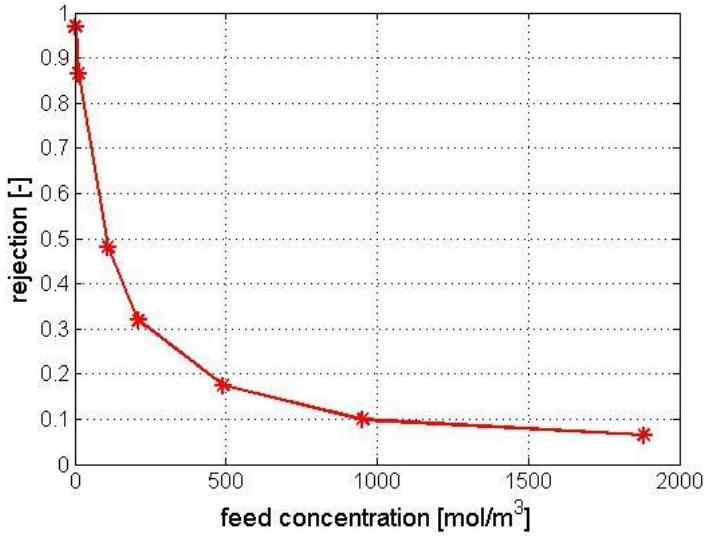

Figure 2. REJECTION OF THE MEMBRANE DESAL-DK5 (GE W&P TECHNOLOGIES, US) FOR NaCl AS A FUNCTION OF FEED CONCENTRATION (30 BAR, 25ºC, 0.55 M^2 SPIRAL-WOUND ELEMENT, 1 M^3H^{-1} RECIRCULATION FLOW-RATE). SOLID LINE IS FOR EYE-GUIDANCE (Kovács et al., 2008)

son, rejection of monovalent ions (Cl^-, Na^+ K^+) is much less. As represented in Fig.2, rejection of ionic species is highly concentration-dependent.

Whey represents a complex multi-component system. In processes of practical interest, the concentrations of both salts and organics present in whey are subject to change during operation, and a considerable interdependence in their permeation occurs. The modeling of nanofiltration of such a complex system is a difficult task.

Several physical models, such as the extended Nernst-Planck model (van der Horst et al., 1995), the Spiegler-Kedem model (Cuartas-Uribe et al., 2007), the solution-diffusion model (Minhalma et al., 2007), and the Donnan-steric-pore model (Cuartas-Uribe et al., 2007) have been employed to describe whey NF. However, these quantitative modeling techniques in their presented form can find only limited applications, because they are either restricted to model single components or simplified operation modes are considered (Román et al., 2011).

The most relevant factor that limits the applicability of physical models to filtration modeling is the available information on the solution itself. Due to costly chemical analysis of complex process streams, process engineers have often only limited information on several components. Moreover, many of the measured quantities are not solute-specific quantities; in fact, they represent certain collective features of a group of solutes of common types. Under such circumstances, the employment of empirical methods may be a reasonable alternative over physical models. This research work investigates robust data-driven modeling techniques to predict the dynamics of whey nanofiltration.

MATERIALS AND METHODS

Nanofiltration of both sweet whey and acid whey were experimentally investigated. In both cases, one-one single experiment was used to determine the parameters of the mathematical models. Then, two-two other experiments with different operational settings were conducted in order to validate the models.

A detailed description of the membrane filtration apparatus can be found in our previous work (Román et al., 2011). In brief, all experiments

were carried out at constant transmembrane pressure (20 bar), temperature (40°C), and cross-flow velocity (3.0 m s^{-1}) using a lab-scale apparatus equipped with a flat-sheet membrane module. The commercial polymeric membrane XN45 (TriSep Co., Goleta, CA, USA) was used for both sweet whey and acid whey NF. The schematic representation of batch membrane diafiltration setting is shown in Fig. 3.

Total soluble solid, lactose, protein, and fat content of both permeate and feed samples were analyzed. Determination of five major elements (Na, K, Ca, Mg, P) in milk was carried out by inductively coupled plasma mass spectrometry (ICP-MS). Conductivity of feed and permeate streams were also monitored.

Simulation procedure

A modular approach, shown in Fig. 4, is proposed to simulate desalination of whey.

In our previous work (Kovács et al., 2009) we have presented a general mathematical framework in a compact form to predict the dynamics of different configurations of batch membrane filtration processes. We have elaborated the common basis of the different operational modes, such as concentration, constant-volume dilution, or variable-volume dilution mode, and delivered a comprehensive model. The following initial-value problem can be formulated to represent the membrane system configuration:

$$\begin{cases} \frac{dV_f}{dt}(t) = u(t) - q(t) \\ V_f(0) = V_f^0 \end{cases} \quad (1)$$

and, for component *i*,

$$\begin{cases} V_f(t)\frac{dC_{f,i}(t)}{dt} = C_{f,i}(t)[q(t)R_i(t) - u(t)] \\ C_{f,i}(0) = C_{f,i}^0 \end{cases} \quad (2)$$

which describe the evolution in time of the volume in the feed tank V_f and of the feed concentration $C_{f,i}$ for *i=1,2...n.* V_f^0 and $C_{f,i}^0$ denote respectively the initial feed volume and the initial feed concentration of the solute *i.* The proportionality factor *α* (i.e. the ratio of diluant flow *u(t)* to permeate flow *q(t))* can be then adjusted to zero, unity, or a constant value between 0 and 1 in order to run the process in concentration mode, constant-volume dilution mode, or variable-volume dilution mode, respectively.

The estimation of flux *q* and rejections R_i presented in Eqs. (1) and (2) can be carried out separately using the most convenient method for the problem at hand. Thus, either mechanism-driven, or data-driven methods can be used without having to modify the proposed framework. We have investigated statistical tools, such as response surface methodology (RSM) and partial least-squares regression (PLSR), and machine learning techniques in order to estimate the dependence of flux and rejections on the feed composition such as

$$q = q(c_1, c_2 \ldots c_n) \quad (3)$$

$$R_i = R_i(c_1, c_2 \ldots c_n) \text{ for } i = 1, 2, \ldots n \quad (4)$$

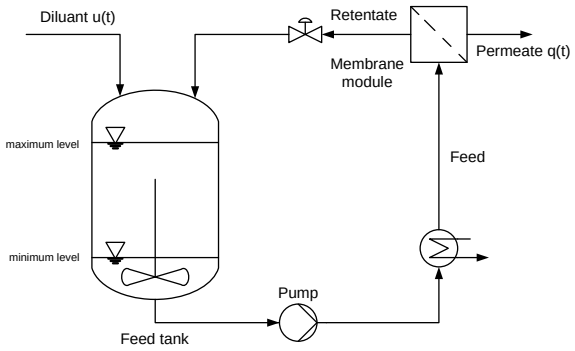

Figure 3. SCHEMATIC REPRESENTATION OF BATCH DIAFILTRATION SETTINGS

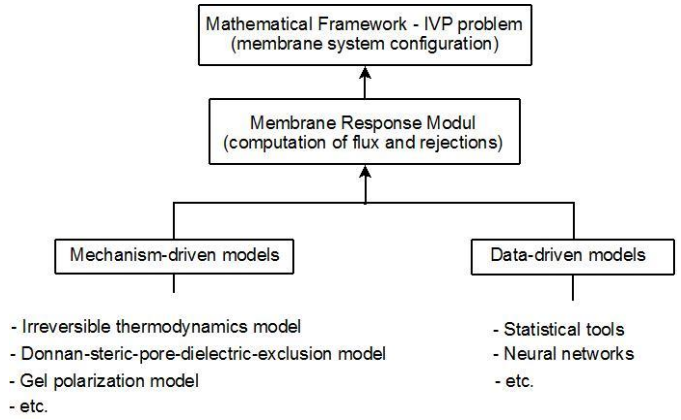

Figure 4. FLOW-CHART OF THE SIMULATION APPROACH

RESULTS AND DISCUSSION

The experimental data obtained from the experimental run no. 1 was processed with data-driven modelling techniques (RSM, PLSR and artificial neural networks). Then, the resulting quantitative expressions for flux *q* and rejections R_i were integrated in the proposed mathematical framework. Runge-Kutta integration scheme is implemented for integrating the feed volume along with the concentrations for the studied interval of the operational time. Figs. 5 and 6 are representative figures of the simulation outputs for whey diafiltration using response surface methodology and neural network approach, respectively. A good agreement between predicted and experimental results can be achieved.

CONCLUSION

A mathematical framework is provided for simulation of whey diafiltration processes. Either transport models or real-life experimental data can be employed, without having to modify the governing equations of the framework. The proposed procedure is applicable for different batch diafiltration concepts and for multi-component systems. We have proposed an experimental design that minimizes the a-priori experimentation and permits the extraction of high-quality information from the experimental data. To convert raw data into useful information, we have successfully employed statistical tools (RSM, PLSR) and artificial neural networks. The modeling technique has been validated with a number of process runs by adjusting diffe-

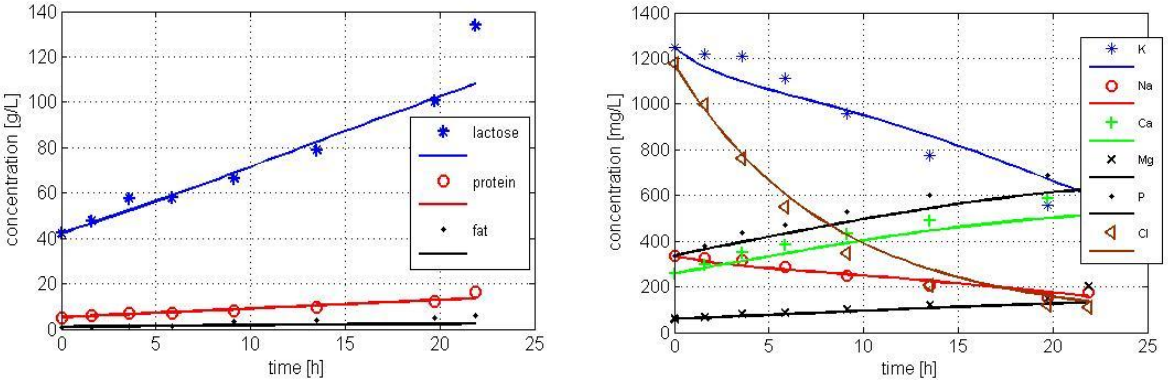

Figure 5. ORGANIC COMPOUNDS (LEFT SIDE) AND IONIC SPECIES (RIGHT SIDE) AS FUNCTION OF OPERATIONAL TIME FOR VARIABLE-VOLUME DIAFILTRATION WITH A=0.75. EXPERIMENTAL DATA ARE ILLUSTRATED WITH SYMBOLS AND ESTIMATED VALUES WITH CONTINUOUS LINES

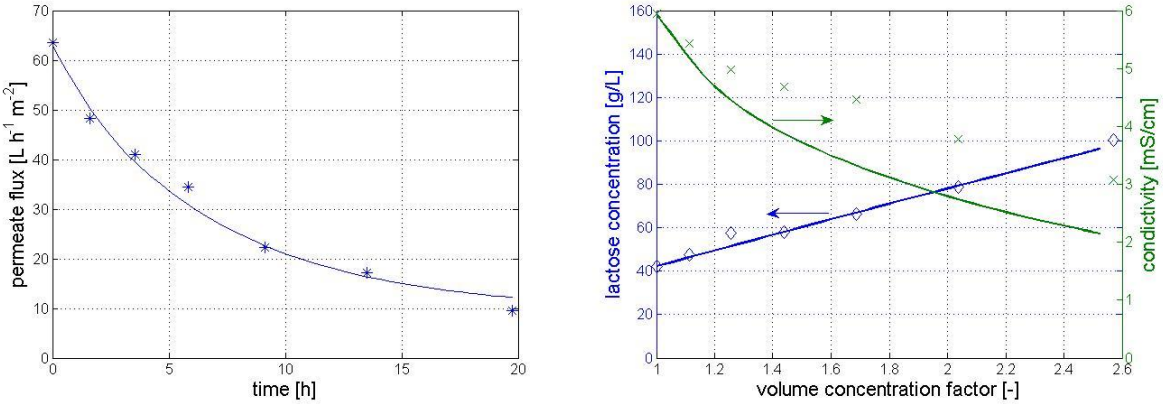

Figure 6. SIMULATION OF THE DYNAMICS OF SWEET WHEY DIAFILTRATION WITH ARTIFICIAL NEURAL NETWORK APPROACH. PERMEATE FLUX VS OPERATIONAL TIME (LEFT SIDE); CONDUCTIVITY AND LACTOSE CONCENTRATION AS FUNCTION OF VOLUME CONCENTRATION FACTOR (RIGHT SIDE). EXPERIMENTAL DATA ARE ILLUSTRATED WITH SYMBOLS AND ESTIMATED VALUES WITH CONTINUOUS LINES

rent diluant utilization schemes. A good agreement between simulated and measured filtration data was found.

ACKNOWLEDGEMENT

This research is a cooperative effort. The first author is grateful for the sponsorship of the Corvinus University of Budapest for providing a visiting scholarship (Corvinus Visiting Scholar Program, TÁMOP 4.2.1/B-09/1/KMR-2010-0005). We would also like to thank the Hessen State Ministry of Higher Education, Research and the Arts for the financial support within the Hessen initiative for scientific and economic excellence (LOEWE-Program).

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IZVOD

EKSPERIMENTALNA I NUMERIČKA ISPITIVANJA DESALINACIJE SURUTKE PRIMENOM NANOFILTRACIJE

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Surutka je sporedni proizvod dobijen tokom prerade mleka u sir ili kazein. Surutka se proizvodi u velikim količinama u celom svetu: godišnji obim surutke proizvedene u svetu prelazi 160 miliona tona. Primena nanofiltracije čini surutku pogodnim dodatkom u ljudskoj ishrani. U radu je data simulacija metode za predviđanje dinamike procesa membranske filtracije u višekomponentnim sistemima kao što je surutka. Eksperimentalni podaci su dobijeni tokom laboratorijske nanonfiltracije slatke i kisele surutke. Upotrebljeni su modeli koji minimiziraju neophodne apriori eksperimente i omogućavaju pretvaranje sirovih podataka u korisne informacije. Integrisani su statistički alati i tehnike učenja pomoću mašina u predloženom matematičkom okviru. Rezultati prikazuju da ovakve tehnike mogu da modeluju višekomponentne sisteme gde je dostupna limitirana informacija o njihovom stvarnom hemijskom sastavu.

Ključne reči: desalinacija • dijafiltracija • nanofiltracija • simulacija • surutka

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TARIK A. DIZDAREVIĆ

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SCIENTIFIC PAPER

UDK: 637.353:663.12] : 66.012

OCCURRENCE OF YEASTS AND MOULDS IN CHEESE AND MEASURES FOR GROWTH CONTROL*

Due to their role in cheese manufacture and ripening yeasts and moulds are attracting increasingly attention. While they are supposed to be useful in some cheese types production their presence in most of cheese varieties can be harmful for cheese quality and human health. Cheese defects can be more or less moderate such as changes of flavour, disintegration of the structure, open texture or slimy surface as result of yeasts activity or more serious such as production of toxic secondary metabolites (mycotoxins) that can do harm to consumers as result of moulds activity. Occurrence of yeasts and moulds during cheese production is almost unavoidable since they have ability to grow well at low temperatures, pH and water activity as well as at high salt concentration which all of them are limiting factors for most of bacteria. There are many sources of contamination both in manufacture and in ripening of cheese. They are: equipment surface, environment, floors, walls, workers aprons and hands but especially brine (yeasts) and air (moulds). *Debaromyces hansenii* is dominant species in most cases. Moulds can cause visible damage to the cheese with intensive lipolysis and proteolysis and *Penicillium* spp. is the main cause while *Aspergillus* spp. very often occurs. The best measure for prevention and control of contaminating yeasts and moulds growth is proper cleaning and sanitation of processing plant, ripening and storage rooms. Many treatments for growth of yeasts and moulds prevention are studied such as cooling, freezing, coating, modified atmosphere packaging, biopreservation or their combination. Some of them are quite useful but many factors influence efficiency.

Key words: cheese • moulds • yeasts • ripening • measures • prevention

INTRODUCTION

Yeasts and moulds have a positive role in many branches of food technology and are one of the important foundations that underpin modern biotechnology. In the production of cheese they have a positive effect on the characteristics of certain types of cheese. Therefore they are used as secondary starters for many types of cheese, such as blue-veined and white-surface mould cheese varieties or surface smear ripened cheese varieties. However, their presence in cheese is still generally considered as harmful as it can cause adverse effects on the sensory properties of cheese and cause harmful effects on human health.

Occurrence of yeasts and moulds in cheese

The presence of yeasts in cheese is not surprising considering that they are developing well at low temperatures, low pH, low water activity and high concentration of salt, which are precisely the conditions prevailing in most cheeses (Jakobsen and Narvhus, 1995; Seiler, 2003). Most yeasts develop well in hot, watery, sweet, sour and aerobic environments (Viljoen, 1996). The most dominant in cheeses is *Debaromyces hansenii* (Viljoen, 1996; Seiler, 2003). Similar to yeasts, moulds too tolerate low pH and low water activity better than bacteria and therefore they are more often the cause of spoilage of food products with increased acidity, including cheeses (Frisvad et al., 2007). In addition, many types of mould are microaerophilic and can grow in low oxygen environments such as the interior of cheese or in hermetic packages (Montagna, 2004). Yeast use carbohydrates as a source of energy and we can argue that they are the most capable in terms of utilization of glucose, while only certain types are capable of assimilation and fermentation of lactose and galactose. They have lipolytic and proteolytic enzymes and thus participate in the breakdown of fats and proteins. The feature that makes them significant in cheese ripening is the capacity to utilize lactates and thus contribute to increasing the pH of cheese (Jakobsen and Narvhus, 1995). Moulds secrete numerous proteolytic, amilolytic and lipolytic enzymes, and a number of products that affect the flavour of cheese are thus produced. Some species, such as *Penicillium roqueforti* and *P. camemberti*, can use lactates as a carbon source which causes a rise in pH, often to a neutral environment (Hutkins, 2006). Taste and odour defects that can occur as a result of the presence of contaminating yeasts can be characterized as fruity, bitter or yeasty "notes", ester-like smell, and moldy, putrid, overripe, alcoholic, musty, fermented, earthy, spicy, ammonia and sweet. The effects on the structure are reflected as gassy and open texture, while the enhanced lipolysis and proteolysis may cause the cheese surface to be softened, and the bark may become slimy (Jakobsen, Narvhus, 1996; Pereira-Dias et al. 2000; Seiler, 2003). Moulds cause changes in the structure, odour and flavour of cheeses, very often with side effects such as unpleasant odour and flavour, discolouration, decay and softened texture. However, the most important effect is the production of toxic secondary metabolites, mycotoxins (Filtenborg et al., 1996). Milk for cheese production is not a significant source of yeasts and moulds as they are rather poor com-

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* This paper was presented as Session Lecture at International Conference JOINT EVENTS: 2nd CEFSER (Center of Excellence for Food Safety and Emerging Risks) Workshop *"Persistent organic pollutants in food and environment"*, 26th Symposium on Recent Developments in Dairy Technology and BIOXEN seminar *"Novel approaches for environmental protection"* at University of Novi Sad, Faculty of Technology, 8-10 September 2011.

petitors against bacteria in cheeses. Sources of contamination include: equipment surfaces and the immediate environment, supplements and aids in the production line, brine, air in the production and ripening rooms, floors and walls, and workers` hands and working clothes. In most cases, brine is found to be the main source of yeast contamination, and in the case of mould contamination the most common source is the air in ripening and production rooms (Hocking and Faedo, 1992; Welthagen and Viljoen, 1998; Welthagen and Viljoen, 1999; Lund et al., 2003; Viljoen et al., 2003a; Viljoen et al., 2003b; Kure et al., 2004; Temelli et al., 2006). In most cases, the dominant yeast species in cheeses is *D. hansenii*. The count of yeasts in cheese can reach 5-6 log cfu/g while in some species it can be as high as 7-8 log cfu/g (Welthagen and Viljoen, 1999). This count varies and is certainly dependent on a number of factors: the type of cheese, ripening stage, the surface or inside of the cheese, season, etc. (Figure 1). Dominant moulds are usually *Penicillium* spp., and *Aspergillus* spp. occurs very often as well (Hocking and Faedo, 1992, Varnam and Sutherland, 1994).

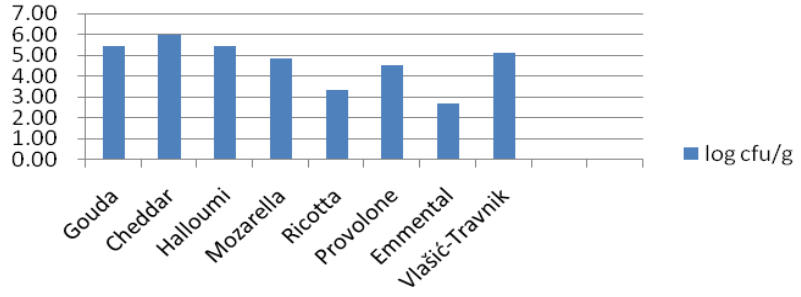

Figure 1. THE COUNT OF YEASTS AND MOULDS IN DIFFERENT RIPE CHEESE TYPES (Welthagen and Viljoen, 1998; Welthagen and Viljoen, 1999; Minervini et al., 2001; Papademas and Robinson, 2001; Alkić et al., 2008; Rantsiou et al., 2008)

Measures for growth control

The first and the best way to control the growth of contaminating yeasts and moulds is to apply the correct procedure for cleaning and sanitation of processing plants, storage and ripening rooms. The correct choice of sanitizer is important. Thus, chlorine has a more effective impact on the moulds in comparison to the peracetic acid or peroxides (Cousin, 2003). Freezing reduced the count of yeasts and moulds in a soft French-style cheese from pasteurized goat milk while cooling was less efficient. The study has shown that freezing can reduce the count of microorganisms and that the procedure is suitable for goat cheese without causing microbiological failure (Park et al., 2004).

Moulds that are formed in cheese are generally viewed as having a toxic nature. Depending on the type and characteristics, the toxins can pass into the depth of 1-2 cm from the surface but can also penetrate the inside of the cheese. For this reason, removal of bark is not effective, so the mould occurrence is prevented by regular washing of cheese surface, packing cheese in a film or coating it with paraffin wax. Primarily, the use of polyethylene natamycin antibiotic (pimaricin) is common. It is classified as a preservative, mould preventive, and antibiotic in food additive list (Var et al., 2006). In many countries natamycin and sorbate are the only permitted antifungal agents for surface treatment of cheese. Although sorbates effectively inhibit the growth of many spoilage organisms that occur on the surface of the product they have a number of disadvantages compared to natamycin. What is required is a relatively high concentration of sorbates to prevent mould growth, and additional problem is that common moulds develop a significant resistance to sorbates. While natamycin is retained on the cheese surface the sorbates migrate into the interior of the product, resulting in reduced effectiveness. In addition, the sorbates may have a negative impact on flavour, odour and colour of the product and may inhibit starter cultures. On the other hand, natamycin has a broad spectrum of activity against yeasts and moulds, prevents the formation of mycotoxins, does not create resistance, does not affect the starter bacteria of the surface ripening flora, it is effecttive at low concentrations, has a longer activity period with a slow release, it stays on the surface, and the application is simple - coating, casing, dipping or spraying, there is no negative effect on cheese quality, has no odour and taste, its use is allowed in most countries and it is chemically stable (Stark, 2007). Bacteriocin nisin is also widely used as a protective agent. Antimicrobial agents natamycin and nisin can be released from the synthetic lacquer coatings on polymer packaging film in quantities that could inhibit the sensitive forms of microorganisms on the surface of packaged food. Coextruded polyamide/polyethylene film coated with PVdC lacquer containing nisin and natamycin had inhibitory effect on the selected test moulds (*P. expansum*, and *Fusarium culmorum*). The film was not suitable for packing of surface-ripened cheeses, but was able to prevent the growth of spoilage microorganisms on the surface of packaged soft cheese (Hanušová et al., 2010). Some *Penicillium* species isolated from cheese are capable of sorbic acid degradation and produce 1,2-pentadiene, a volatile component with an unpleasant odour of hydrocarbon. The study was conducted to examine the effects of several antimycotics (K-sorbate, Na-benzoate, Ca-propionate, di-Na-ethylenediaminetetraacetic acid (EDTA) and natamycin) alone or in combination, in preventing the growth of five moulds isolated from Parmesan cheese and lemon-flavoured drink containning sorbate. The results showed that the protective system, which contains a reduced concentration of K-sorbate, in combination with other antimycotics, especially natamycin, has the potential to control the growth of moulds capable of producing 1,3-pentadiene (Mann & Beuchat, 2008). The use of antimicrobial agents (natamycin) and packaging material during ripening stage of Kashar cheese was also examined. After two months only the cheeses with a combination of natamycin and packaging showed no mould growth (Var et al., 2006). Modified atmosphere packaging (MAP) is a modern process developed in food industry to extend shelf life of products with higher moisture content. The gases used are carbon dioxide, nitrogen, carbon monoxide and sulfur dioxide. The most commonly used and perhaps the most effective gas is CO_2 in

combination with or without other gases. The use of modified atmosphere to prevent fungal growth and mycotoxin production in cheese was examined. Eight species of moulds were inoculated into cheese and then incubated under conditions of decreasing concentration of O_2 (from 5% to 0.5%) and increased concentration of CO_2 (20-40%). All of the tested moulds were grown in an atmosphere with 20 and 40% CO_2 with 1% or 5% O_2, but this growth was reduced by 20-80% depending on the species, compared with growth in air (Taniwaki et al., 2001). In examining the shelf-life of Cameros cheese, fresh cheese produced from pasteurized goat milk, packed in modified atmosphere, five different combinations of conditions of modified atmosphere (CO_2/N_2 mixtures and vacuum) were used, while the control cheese was packaged under air. Cheese packaged in an atmosphere of 50% CO_2 / 50% N_2 and 40% CO_2 / 60% N_2 showed the best result for the viability of cheese that kept good sensory properties (Gonzales-Fandos et al., 2000). The effect of MAP was studied on the shelf life of whey cheese Myzithra Kalathaki using the following variants: the vacuum pack, 20% CO_2 / 80% N_2, 40% CO_2 / 60% N_2, and 60% CO_2 / 40% N_2; pack under air used as control. With combinations of 40% CO_2 / 60% N_2, and 60% CO_2 / 40% N_2 yeasts and moulds remained below the detection limit (1.0 log cfu/g) even after 35 days. Thus, high concentrations of CO_2 had an effect on inhibiting the growth of yeasts and moulds (Dermiki et al., 2008). A particular problem occurs with preservation of fresh cheeses with high moisture content that are therefore highly susceptible to microbial failure. The combination of the active coating and modified atmosphere packaging is applied to extend the shelf life of fresh cheese Fior di Latte. Active coating was based on Na-alginate (8% wt/vol.), with the addition of lysozyme (0.25 mg/ml) and EDTA, disodium salt (EDTA-NA_2, 50 mM) while MAP was composed of 30% CO_2, 5% O_2 and 65% N_2. Moulds were not registered in any sample, and the like was observed for the yeasts so we can argue that the used combination improved the sustainability of Fiore di Latte cheese (Conte et al., 2008). Biopreservation as control of growth of one type of organisms by others has attracted much attention in recent years. Among the natural biological antagonists, the lactic acid bacteria (LAB) attract special attention. They produce antagonistic components capable of controlling pathogennic bacteria and undesirable spoilage microflora. The use of LAB to control mould growth can be an alternative to physical and chemical methods. Certain studies have shown that a good selection of LAB can help control the development of mould and extend the viability of many fermented products, and therefore reduce the risk to human health resulting from mycotoxins entering the body (Dalié et al., 2010). Active proteinaceous substances produced by *Lactobacillus paracasei ssp. paracasei* M3 strain used as a starter for Bulgarian yellow cheese showed bactericidal and fungistatic properties during testing in laboratory conditions against some of contaminating yeast-strains of *Candida albicans*, *C. pseudointermedia*, *C. blankii* and *S. cerevisiae* (Atanassova et al., 2003). The protective culture consisting *of Propionibacterium jensenii* SM1 and *L. paracasei ssp. paracasei* strains SM20, SM29 or SM63 showed inhibitory activity against yeasts on the surface of Gruyère cheese at refrigeration temperature (6^{o}C) without affecting the quality of the product (Schwenninger and Meile, 2004). Also, the isolates of heterofermentative lactobacilli isolated from Feta cheese showed antagonistic activity against moulds that grow on the surface of the cheese and yeast in cheese. Different degree of inhibition was detected against isolates of *P. candidum* and *D. hansenii*, but none of them showed activity against *S. cerevisiae* (Voulgari et al., 2010). Biological decontamination, which is considered as a good potential for control of mycotoxins in addition to LAB also involves the antagonistic abilities of some yeasts against moulds. It has been shown that *S. cerevisiae* and some lactic acid bacteria have the capacity to strongly bind different mycotoxins to cell wall components which can be explained as a feature of the strain (Shetty and Jespersen, 2006). Some strains of *D. hansenii* have an antagonistic effect on moulds. Inhibitory effect was shown on the contaminating moulds *Aspergillus sp*., *Byssochlamys fulva*, *B. nivea*, *Cladosporium sp*., *Eurotium chevallieri*, *P. candidum* and *P. roqueforti*, but it depended on the concentration of mould. The lower the concentration, the more effective was the yeast. However, when applied on mould-ripened cheeses, precautions should be taken because if an antagonistic yeast strain is present, it may have a negative effect on ripening and quality of cheese (Liu and Tsao, 2009). The inhibitory effect of fungal starter on the growth of contaminating moulds and production of their secondary metabolites were examined in laboratory media and Camembert cheese. Species *P. nalgiovense*, *P. camemberti*, *P. roqueforti* and *G. candidum* were selected as fungal starters. Fungal starters showed different level of competitiveness against the contaminating moulds. *P. nalgiovense* manifested inhibitory effect on the growth of contaminating moulds on the laboratory medium. *G. candidum* caused a significant inhibition of fungal contaminants on Camembert cheese and it is evident that it plays a significant role in competition with undesirable microorganisms (Nielsen et al., 1998).

CONCLUSION

The emergence of yeasts and moulds is a sporadic problem in cheese production. Their count varies depending on the type of cheese and production conditions. Prevention measures are different, and those are in the first place good hygiene practices and use of various measures, some of which yield successful results in preventing the occurrence of yeasts and moulds.

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IZVOD

POJAVA KVASACA I PLESNI U SIRU I MERE ZA KONTROLU RASTA

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Kvasci i plesni privlače sve više pažnje zbog svoje uloge u proizvodnji i zrenju sira. Dok bi oni trebalo da budu korisni u proizvodnji nekih vrsta sireva, njihovo prisustvo u mnogim vrstama sireva može biti štetno po kvalitet sira i ljudsko zdravlje. Nedostaci sira kao rezultat delovanja kvasaca mogu biti manje ili više umereni kao što su promena ukusa, strukture, otvorena tekstura ili sluzava površina sira ili ozbiljnije promene delovanja plesni kao što su proizvodnja sekundarnih toksičnih metabolita (mikotoksina). Pojava kvasaca i plesni za vreme proizvodnje sira je gotovo neizbežna jer oni imaju sposobnost rasta na niskim temperaturama, niskoj pH i niskoj aktivnost vode kao i pri visokoj koncentraciji soli, što su ograničavajući faktori za većinu bakterija. Postoje mnogi izvori zagađenja, kako u proizvodnji tako i tokom zrenja sira. To su: površina opreme, okolina, podovi, zidovi, kecelje i ruke radnika a posebno salamura (kvasci) i vazduh (plesni). *Debaromyces hansenii* je dominantna vrsta u većini slučajeva. Plesni mogu izazvati vidljiva oštećenja na siru sa intenzivnom lipolizom i proteolizom, pri čemu je *Penicillum spp.* glavni uzrok dok se *Aspergillus spp.* veoma često pojavljuje. Najbolja mera za prevenciju i kontrolu rasta zagađujućih kvasaca i plesni je odgovarajuće čišćenje i sanitacija tehnoloških linija za preradu mleka u sir i prostorija za zrenje i skladištenje. Mnogi tretmani za sprečavanje rasta kvasaca i plesni se izučavaju kao što su hlađenje, smrzavanje, upotreba premaza za zaštitu sira, pakovanje sira u modifikovanoj atmosferi, biokonzervisanje ili njihova kombinacija. Neki od njih su izuzetno korisni ali od brojnih faktora zavisi efikasnost.

Key words: sir • plesni • kvasci • zrenje • postupci • prevencija

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SCIENTIFIC PAPER

UDK: 637.1: 663.18

DAIRY PRODUCTS AND PROBIOTICS – PRESENT STATE*

Fermented dairy foods containing beneficial bacteria have been around for thousands of years. These bacteria are called probiotics which when administered in adequate amounts, confer a health benefit on the host. Scientists are investigating whether dairy products are the preferred delivery vehicle for these probiotic cultures. The evidence for the impact of probiotics on diverse end points of human health is mounting, driving the commercial development of products containing them. As a consequence of this explosion of scientific publications the list of patents and products on the market has been continuosly expanding. Main role of starter cultures in dairy industry consists in the production of organic acids, aromatic compounds and obtaining appropriate sensory characteristics. When it comes to probiotics all the above effects are negligible but the emphasis is placed on health effects. Therefore probiotic cultures have been exploited extensively by the dairy industry as a tool for the development of novel functional products. However, it is important that probiotic products meet appropriate international standards, and contain appropriately characterized organisms, in shelf-stable formulations that have been shown in well-designed clinical studies to confer defined health benefits on the consumer. This medical data will further dispel the doubts in certain scientific circles and at the same time provide consumers with the assurance that consuming foods with probiotics is an effective way to improve general health. This indicates that the industrial application of probiotics has a secure future.

Key Words: probiotics • dairy industry • application

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INTRODUCTION

In recent times, there has been a growing appreciation for important role of commensal microbes in human and animal health, through mediation of intestinal development and innate immunity, or digestion of food and protection of the host against disease (Gorbach, 2000). This had led to attempts to manipulate or augment the microorganisms through the use of probiotics "live microorganisms that when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Our knowledge of probiotics and their interactions with their hosts is constantly increased, so that a number of potential and in some cases absolutely proven beneficial effects exist, as a result of the consumption of live microbial cells, including immune modulation, prevention of the growth of pathogenic microorganisms, such as rotaviruses, *Clostridia difficile, Helicobacter pylori* and also prevention of vaginal infections, dental caries, respiratory infections, reduction of serum cholesterol and dermatitis. Since the health effects are specific to each strain, it cannot be expected that all strains possess these positive clinical effects. The most commonly used organisms in probiotic preparation are the lactic acid bacteria, which are found in large numbers in the gut of healthy people and animals and are in the words of the FDA, Generally Regarded as Safe. Organisms other than lactic acid bacteria, which are currently being used in probiotic preparations, include *Bacillus* sp. and yeast *Saccharomyces boulardi*i. Probiotic products are now available in different formulations with various strains of lactobacilli, *Bifidobacterium longum, Bifidobacterium infantis, Enterococcus faecium* and others with or without prebiotics like inulin and fructooligosaccharides (FOS). There are some ideal properties of the probiotic strains that would benefit human health and could be used in the probiotics industry. These include resistance to acid and bile; attachment to the human gut epithelial cells; colonization in the human intestine; production of antimicrobial substances, including bacteriocins; good growth characteristics and beneficial effects on the human health. One of the most important characteristics of a probiotic strain is that it must be nonpathogenic. Probiotics must also present some desirable characteristics, such as maintenance of viability during processing and storage, ease of application in products, and resistance to the physicochemical processing of the food (Ouwehand et al., 2002; Sanders, 2009). These bacteria should not be pathogenic, toxic, mutagenic, or carcinogenic in the host organism, must be antagonistic to pathogens and be genetically stable without a plasmid transfer mechanism, especially concerning antibiotic resistance; they must survive during digestion and possess the ability to adhere and colonize the gut mucosa, promoting immuno-stimulation without inflammatory effects (Schrezenmeir and de Vrese, 2001).

The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunemodulatory cells, and producing lactase. The leader in the application of probiotics is undoubtedly dairy industry and especially sector of fermented milks. Probiotic

* This paper was presented as Session Lecture at International Conference JOINT EVENTS: 2nd CEFSER (Center of Excellence for Food Safety and Emerging Risks) Workshop *"Persistent organic pollutants in food and environment"*, 26th Symposium on Recent Developments in Dairy Technology and BIOXEN seminar *"Novel approaches for environmental protection"* at University of Novi Sad, Faculty of Technology, 8-10 September 2011.

dairy products are expected to command the highest market share among all the probiotic foodstuffs accounting for almost 70% in the year 2009 and will reach a market size of almost $24 billion by the end of 2014.

The biggest markets for these products are Europe and Asia; the U.S. market has slowly but surely opened up to these products in the recent past and is expected to grow at a CAGR of 17% from 2009 to 2014, the biggest contributor being probiotic cultured drinks followed by probiotic yogurts (Markets and Market, 2009). Though the market base of probiotic products is comparatively lesser in the U.S., the market is expected to grow at an astounding rate of almost 14% in the same period driven by the large scale acceptance of - the probiotic yogurts in spoonable single serve packs, probiotic cultured drinks in single shot packaging form and probiotic dietary supplements.

Factors affecting the activity of probiotics

Production of high-quality functional food with various types of lactobacilli and bifidobacteria is a very complex task for food, especially when milk is used as a substrate for fermentation. These microorganisms are characterized by slow growth in milk and low acidogenic potential resulting in an extension of time of fermentation, whereas bifidobacteria are extremely sensitive to the presence of oxygen and low pH. In addition *L. delbrueckii* subsp*. bulgaricus* with acidogenic activity and the production of peroxide inhibits bifidobacteria while with proteolytic activity stimulate action of these bacteria. Bearing all this in mind probiotic bacteria are usually applied in the community with a yogurt culture, or one of these bacterial strains, usually *S. thermophilus*. Combination with mesophilic bacteria is also possible. This is achieved by intensifying the rate of growth of probiotics, saves the time of fermentation, improves sensory characteristics such as viscosity, taste and smell, and at the same time promotes the nutritional properties (Hatingh and Viljoen, 2001).

Milk as substrate for fermentation is subject to various treatments for various reasons and the most important are: getting the desired composition (standardization and homogenization, adding ingredients of milk, evaporation, ultrafiltration and reverse osmosis), the selection of appropriate heat treatments to obtain optimal conditions for starter culture growth, denaturation of whey protein to comply with the casein network and thus improve the viscosity of the product and deaeration of the substrate that provides better development of lactic acid bacteria and therefore the probiotics. On the other hand the possibility of dry matter adjustments associated with increased buffering capacity is to create optimal conditions for the microorganisms, since on one hand there is an increased amount of nutrients and on the other hand log phase is extended, allowing prolonged activity of starter cells. This also contributes to a stronger coagulum and weaken the separation of serum. One important feature of the cooled milk is a high oxygen content, which does not decrease during the thermal treatment, and if the content exceeds the concentration of 4 mg/kg it can promote slow growth of microaerophilic starter lactic acid bacteria. Because of this, the problem of milk deaeration and also reduction of mechanical treatments, which increase the oxygen content, should be given greater attention as these simultaneously improves homogenizers, and reduces the appearance of foam, improves f viscosity of fermented milks and removes odours (Obradovic, 2000).

The size of inoculum of probiotic bacteria is one of the key factors contributing to the desired number of living cells in the final product. One of the simplest and rational solutions to this problem is the application of concentrated cultures, which represent major advances in the application of starter cultures in industrial fermentation and especially in the dairy industry. It is safe to say that the modern milk processing is unthinkable without the use of these cultures, which are delivered in freeze-dried or frozen state. Therefore, it is important to report that these bacteria should be present in dairy foods to a minimum level of 10^6 CFU/g or the daily intake should be about 10^8 CFU/g, with the aim to compensate for the possible reduction in the number of the probiotic microorganisms during the passage through the gut (Shah, 2007). It is also important that the probiotic strains used are compatible with the lactic acid starter cultures conventionally used in the processing of dairy products (Sanders and Levy, 2011). Firstly, with regard to the chosen strains for both the yogurt and the process, these should be compatible with each other and between themselves, avoiding problems such as inhibition by acid, peroxide, bacteriocins and other metabolites that may affect logistics, process yield, and final product quality by slowing the acidification kinetics (Vinderola et al., 2002).

Probiotic dairy products

The pallet of fermented milks with probiotics is a very diverse and includes products that have a solid and liquid consistency, with added fruit, grains, vitamins, micro and macro elements, prebiotics which all contribute to improving the nutritive value of these products. It is also well known that yoghurt and other fermented milks are the most used media to incorporate probiotic bacteria in foods and in this regard, research studies have optimized sensory qualities to render a palatable product to consumers. What particularly attracted attention in recent years is the appearance of fermented drinks in plastic bottles or cardboard packaging volume 65-100 ml, which in fact contain the recommended daily dose of probiotics. Examples of this are Actimel and Activia (Danone), Gefilus (Valio), Actifit (Emmi), Cultura (Arla) and it is obvious that the concept of daily doses of probiotics is encountering more than a positive consumer response. These convenient shots, low in fat and high in fibres which help increase digestion, contain 30-40 billion probiotic cells. In most countries, these products are not considering as food and therefore are no competition to dairy products (Tamime, 2002).

There are many technological hurdles involved in the development and stability of probiotic cheeses. The major challenge associated with the application of probiotic cultures in the development of functional foods is their viability maintenance during processing (Heller et al., 2003). There are five identified hurdles which directly influence the maintenance of the functional activities of probiotic bacteria in cheese: addition of the probiotic inoculum, salting, packaging, ripening and storage conditions. But if this production is compared with fermented milks, it is evident that the cheeses have some advantages. It is known that the cheeses have a higher pH and increased buffering capacity

that prevents the increase in acidity which absolutely corresponds to probiotics, especially bifidobacteria. Also the structure of cheese which has embedded proteins and fat protects probiotic bacteria during passage through the upper parts of the gastrointestinal tract. But compared to yoghurt the problem for cheese, especially semi-hard and hard cheese, acting as carrier for probiotics result from the high fat and salt content and the relatively low recommended daily intake (Heller et al., 2003). It follows that the concentration of probiotics in cheese should be about four to five times higher than in yoghurt. However, this does not apply to fresh cheese, such as Cottage cheese, which can easily be adjusted to low fat and salt contents, and for which recommended daily intake is rather high. Low-fat fresh cheese may thus serve as a food with a high potential to be applied as a carrier for probiotics. The use of cheese as probiotic food carrier presents potential advantages and it is a valuable alternative for the dairy industry. Inspite the fact that the survival of probiotics in cheese is more than good a key question is what is the minimal dose of cheese to lead to the colonization of probiotics in gastrointestinal tract and thus their positive impact on health.

Ice creams are food products that show great potential for use as vehicles for probiotic cultures, with the advantage of being foods consumed by all age groups (Cruz et al., 2009). Although several factors in their processing stages should be optimized, to maintain the microorganisms in viable doses capable of providing therapeutic activity to consumers, these probiotic cultures usually do not modify significantly the sensory features of ice creams and frozen desserts. It depends on the microorganism and the technological conditions employed to develop the product. In summary, the decline in bacterial populations during freezing is caused by injury as a result of the process to which they were submitted, causing their death. In addition, mechanical stress caused by agitation and the incorporation of air might result in a smaller population of viable cells (Akin, 2005). Even though several studies have shown adequate viability of the probiotic cultures during storage of icecreams, more clinical studies on the cosumption of probiotic ice-creams are recommended. Also, it is important to confirm if, after long storage periods, the probiotic cultures are still able to confer the same health benefits already observed in other foods with shorter shelf-lives and higher storage temperatures, such as yoghurt and other fermented milks.

CONCLUSION

In the past decade dairy products with probiotics had a large expansion, some fermented milks have become market leaders, the use of prebiotics has become domesticated, but there is evident that there is still a certain kind of confusion when it comes to functional properties of these foods. Little is known about how the food matrix and product formulation impacts probiotic functionality, even though such information is essential to scientific understanding and regulatory substantiation of health benefits. The food format has the potential to affect probiotic survival, physiology, and potentially efficacy, but few comparative studies in humans have been conducted. Also the new data was providing insights into how different bacteria function at the genetic and molecular level in the body along with the non-nutritive potentialities. There are usually just couple of genes that are unique to each strain and isolating these is providing information about functionality. It is important information for probiotics, dairy products in the nutrition area alone. It is difficult to see how this kind of research can be done without genomics now. But the lack of validated biomarkers for probiotic end points is a big scientific challenge to establishing causal link between a probiotic and health benefit. This high quality clinical evidence is prerequisite for further development of probiotic product including those from dairy industry.

ACKNOWLEDGEMENT

This investigation is a part of the Project No. 46009 financially supported by Ministry of Education and Science, Republic of Serbia.

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IZVOD

MLEČNI PROIZVODI I PROBIOTICI- SADAŠNJE STANJE

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Fermentisani mlečni proizvodi koji sadrže korisne mlečne bakterije postoje već hiljadama godina. Ove bakterije se zovu probiotici koje kada se koriste u odgovarajućim dozama, pokazuju koristne efekte po zdravlje ljudi. Naučnici ispituju da li su mlečni proizvodi odgovarajuće sredstvo za prenos ovih probiotskih kultura. Dokaz za uticaj probiotika na ljudsko zdravlje je oslonac za komercijalni razvoj proizvoda koji ih sadrže. Kao posledica ove ekspolatacije naučnih publikacija, lista patenata i proizvoda na tržištu se konstantno povećava. Glavna uloga starter kulture u industriji mleka sastoji se od proizvodnje organskih kiselina, aromatičnih jedinjenja i postizanja odgovarajućih senzornih osobina. Kada je reč o probioticima svi spomenuti uticaji su zanemarljivi, ali se naglasak stavlja na zdravlje. Stoga probiotske kulture intenzivno iskorišćava Industrija mleka kao sredstvo za razvoj novih funkcionalnih proizvoda. Međutim, važno je da probiotski proizvodi zadovoljavaju odgovarajuće međunarodne standarde i sadrže karakterizovane organizme u formulacijama koje su produžene trajnosti, prikazane u dobro dizajniranim kliničkim studijama kako bi pružile definesane benefite po zdravlje potrošača. Ovakvi medicinski podaci će dodatno razbiti sumnje u određenim naučnim krugovima, a u isto vreme obezbediti potrošačima sigurnost da konzumiranje hrane sa probioticima jeste efikasan način za poboljšanje opšteg zdravstevnog stanja. Ovo ukazuje da industrijska primena probiotika ima sigurnu budućnost.

Ključne reči: probiotici • industrija mleka • primena

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SCIENTIFIC PAPER

UDK: 637.146.3:637.047:66.021.4

THE OPTIMISATION OF TEMPERATURE FOR TRANSGLUTAMINASE ACTIVATION IN PROBIOTIC YOGHURT PRODUCTION

Transglutaminase (TG) is an enzyme that increases formation of inter- and intramolecular bonds inside milk protein chains, which contributes to formation of firmer gel, decrease of syneresis, improvement of consistency and achievement of smoother yoghurt surface. The effect of different temperatures for TG activation on fermentation time, syneresis, water holding capacity, acidity, as well as viscosity of probiotic yoghurt has been examined. Yoghurt samples were produced from milk with 0.1% milk fat and addition of thermophylic starter culture DELVO-Yog MY-721 DSL (*Lactobacillus acidophilus, Bifidobacterium, Streptococcus thermophilus*). TG was added in milk in concentrations of 0.02%, 0.04% and 0.08% with or without activation. Temperatures used for TG activation were: 25ºC and 40ºC. It was found that the parameters of transglutaminase activation have a significant effect on fermentation time, syneresis, water holding capacity, as well as on viscosity of probiotic yoghurt.

Key words: yoghurt • probiotic starter cultures • transglutaminase • optimisation

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INTRODUCTION

Milk and various dairy products have important place in human food consumption. Fermented dairy products, especially new probiotic yoghurts have a large share on the market. Their nutritional and biological values contribute to the increasing popularity of this products. This positive image could be expanded further by adding nutraceutical ingredients such as native whey proteins to the yoghurt (Guggisberg et al., 2007).

Important role in milk fermentation process have starter cultures with their activity in technological process and production of fermented dairy products. They also give them certain sensory characteristics. For production of regular yoghurt mixed and simbiotic starter cultures are used, such as: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Probiotics (*Lactobacillus* and *Bifidobacterium*) are considered to be the third generation starter cultures. They are microorganizms isolated from a human intestinal tract. Latest forms of fermented milk beverages contain prebiotics (inulin, oligofructose). They are digestible food ingredients, which stimulate the growth and the viability of present probiotic starter cultures. Products that contain both probiotics and prebiotics can be called a functional food (Tamime, 2006)

Generally, rheological properties of yoghurt affect significantly the quality of the product. Various factors have a major contribution to yoghurt structure, but the most important are: the heat treatment/homogenization of the milk base (casein/whey protein ratio), the starter culture and technological parameters such as temperature, pressure, etc.(Sodini et al., 2004).

The presence of a significant amount of milk fat has a positive impact on gel strenght of yoghurt produced from whole milk. Present trend in dairy technology is production of "low-fat" (0.5-2% fat) and "non-fat" (less then 0.5% fat) yoghurt. Serious problem is achieving the specific properties that are important indicator of the quality of yoghurt: viscosity, texture, homogeneous consistency without release of the liquid phase – syneresis.

In order to produce yoghurt with low energy value, improved gel characteristics, good physical and chemical properties enzyme transglutaminase (TG) can be used. Enzymes are potential tools to increase the formation of covalent cross-links in proteinaceous food (Sodini et al., 2004, Bönisch et al., 2007; Milanović et al., 2007; Schorsch et al., 2000).

The enzyme transglutaminase is a transferase which form both inter and intra-molecular isopeptide bounds between proteins by cross-linking of the amino-acid residues of glutamine and lysine (Ikura et al., 1992; Nielsen 1995; Motoki and Seguro 1998). This contributes to formation of firmer gel, decrease of syneresis, improvement of consistency and achievement of smoother yoghurt surface. Although other enzymes have been used in different food structure engineering applications TG is the only commercial cross-linking enzyme available for dairy products. The enzyme transglutaminase naturally present in most animal tissues and body fluids, plays an important role in blood-clot formation (Yeh et al., 2006).With the availability of the enzyme from a microbial

source, its applications in the food industry are being widely researched.

Treatment of milk with TG improves its heat stability, probably by preventing dissociation of k-casein from the micelles through cross-linking of caseins (Hinz et al., 2007). The addition of TG can reduce proteins and milk fat to produce non-fat yoghurt with the same rheological characteristics as yoghurt from whole fat milk. There are two different ways of TG application simultaneously with or prior to the fermentation. In the first case, TG was simultaneously added with the starter culture. Pretreatment of milk with TG addition prior to fermentation demand additional process time as well as thermal inactivation step and has the advantage of a constant pH during the cross-linking reaction (Schorsch et al., 2000).

Recent researches have indicated that UHT treatment of micellar casein suspended in milk serum improves the cross-linking reaction by increasing isopeptide ε–(γ-glutamyl) lysine concentration (Rodriguey-Nogales, 2006). Generally, heat treatment of milk prior to cross-linking is necessary due to the reactivity of TG is very low in unheated or pasteurized milk, despite the high reactivity of the casein.

The aim of this study was to optimise the amounts of TG added in yoghurt for its production from milk with 0.1% fat content and to investigate influence of different TG activation temperatures on samples' physical and rheological characteristics.

MATERIALS AND METHODS

Milk

Skim cow's milk with 0.1% fat from AD Imlek, Division Novi Sad Dairy, Serbia, was used for the production of probiotic yoghurt. Milk characteristics are shown in Table 1.

Starter culture

Milk was inoculated with 0.00942% DELVO-Yog MY-721 DSL (Direct Set Lypophilised) starter culture and it was manufactured by DSM Food Specialties, Dairy Ingredients, Netherlands. Starter culture contained: *Lactobacillus acidophilus, Bifidobacterium* and *Streptococcus thermophilus*.

Transglutaminase

Enzyme transglutaminase (Ajinomoto Foods, Japan) was used for the improvement of the characteristics of probiotic yoghurt. TG was added to milk in concentrations of 0.02%, 0.04% and 0.08% with or without activation at two different temperatures (25ºC and 40ºC).

Table 1. MILK PHYSICOCHEMICAL CHARACTERISTICS

Tabela 1. FIZIČKO-HEMIJSKE KARAKTERISTIKE MLEKA

Total solids (%)	Milk fat (%)	Total proteins (%)	Ash (%)	Acidity (ºSH)	pH
8.67	0.10	3.27	0.76	6.23	6.59

(w/w). The enzyme was activated at two different temperatures (25ºC during 3h and 40ºC during 2h). Inactivation was done with heat treatment at 80ºC, which lasted for 1 minute. Milk was inoculated with starter culture at 42ºC. Fermentation lasted until pH reached 4.5 and then was cooled at 6ºC. The samples were homogenized and packed into appropriate packing.

Table 2. SAMPLES PRODUCED WITH AND WITHOUT TG ACTIVATION

Tabela 2. PROIZVEDENI UZORCI SA I BEZ AKTIVACIJE TRANSGLUTAMINAZE

TG addition, %	Without TG activation	With TG activation	
		25°C	40°C
0.02	2WA	2A25	2A40
0.04	4WA	4A25	4A40
0.08	8WA	8A25	8A40

* A- with TG activation, WA- without TG activation

Yoghurt production

Yoghurt samples were produced on a laboratory scale from skimmed milk of 0.1% milk fat. TG application was tested in two ways: with activation, when milk with TG was incubated prior to fermentation and without previous activation.

The enzyme transglutaminase was added into milk in three concentrations: 0.02%, 0.04% and 0.08%

The samples without TG activation were inoculated at 42ºC and transglutaminase was added at the same temperature. Fermentation lasted until pH reached 4.5 and then was cooled at 6ºC. This samples were also homogenized and after that packed.

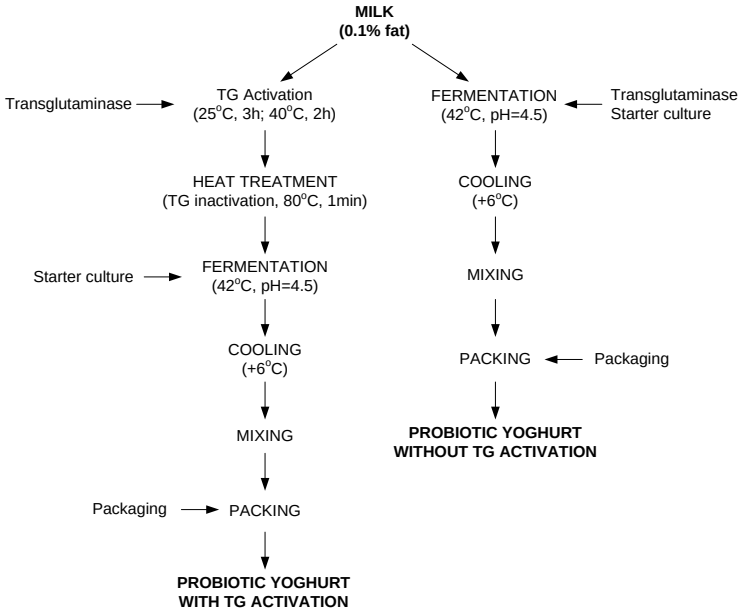

Figure 1. TECHNOLOGICAL PROCESS OF PROBIOTIC YOGHURT PRODUCTION WITH TRANSGLUTAMINASE

Slika 1. TEHNOLOŠKI PROCES PROIZVODNJE JOGURTA SA DODATKOM TRANSGLUTAMINAZE

Samples produced are shown in Table 2. Control sample was produced without TG addition (K).

Probiotic yoghurt flow chart with and without TG activation is represented on Figure 1.

Physical characteristics of a low fat yoghurt samples were analyzed after production.

Whey syneresis was measured by whey separation and it was expressed in mL of whey separated during filtration of 50 mL sample for 3 hours, at room temperature (Atamer et al., 1996).

Water-holding capacity of yoghurt (WHC) was determined according to a procedure introduced by Guzman-Gonzalez *et al.* (1999).

Total acidity was determinated by titration with a standard solution of sodium hydroxide and phenolphthalein as indicator (Carić et al., 2000).

Viscosity of the samples was determinated by Brookfield viscometer (Digital DV-E, Brookfield, England) under following conditions: temperature 20°C, sample 150g, spindle shape S03, spindle speed 6.0 rpm, mixing time 180s. Viscosity was determened after production.

RESULTS AND DISCUSSION

Fermentation time

Fermentation times (Figure 2) were the shortest in samples produced with TG activation at 40^{o}C and it lasted from 6h to 6.5h. These results are in accordance with the previous results (Milanovic et al., 2007). Control sample (produced without TG) and sample without TG activation had the longest fermentation time lasted 7.5h and 7.2h, respectively.

It is noticeable that fermentation time of samples with TG activation increased with increasing of TG concentration. Fermentation time of samples without TG activation decreasing with increasing of TG concentration. This could be due to negative influence of inactivated TG residues on starter culture in samples with activated TG. In samples without TG activation enzyme is not inactivated and positively influences fermentation time. TG activation temperature had signifycant influence on fermentation time. These results indicate that TG activa ted on higher temperature change protein micelles more intensively, which causes the shorter fermentation time.

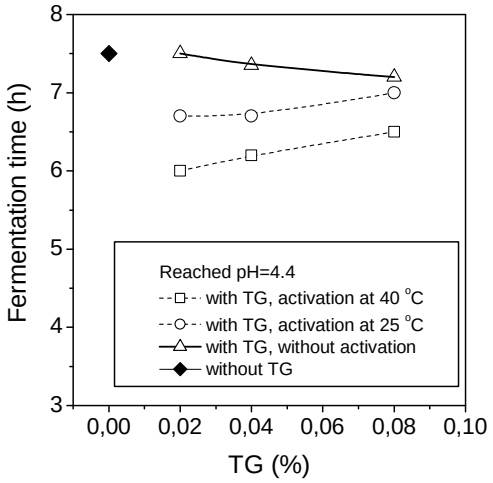

Figure 2. FERMENTATION TIME OF PROBIOTIC YOGHURT

Slika 2. VREME FERMENTACIJE PROBIOTSKOG JOGURTA

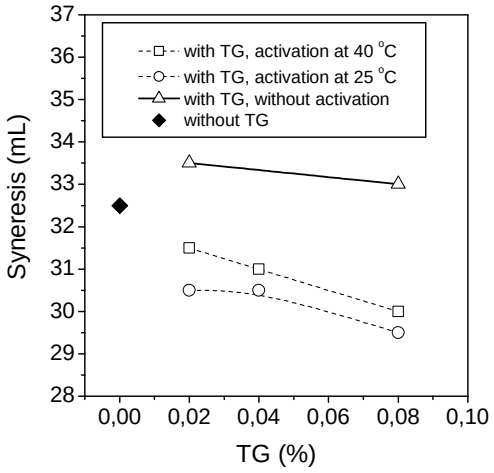

Figure 3. WHEY SYNEREZIS OF PROBIOTIC YOGHURT

Slika 3. SINEREZIS PROBIOTSKOG JOGURTA

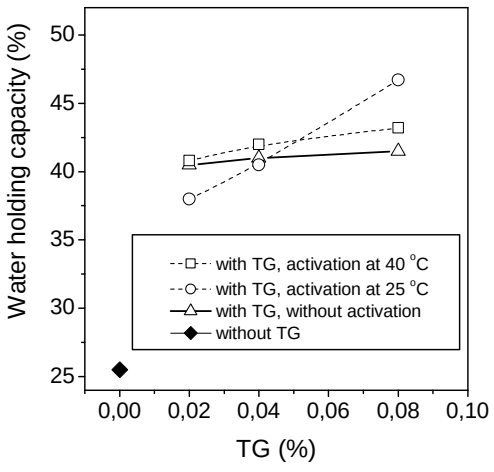

Figure 4. WATER HOLDING CAPACITY OF PROBIOTIC YOGHURT

Slika 4. SPOSOBNOST VEZIVANJA VODE PROBIOTSKOG JOGURTA

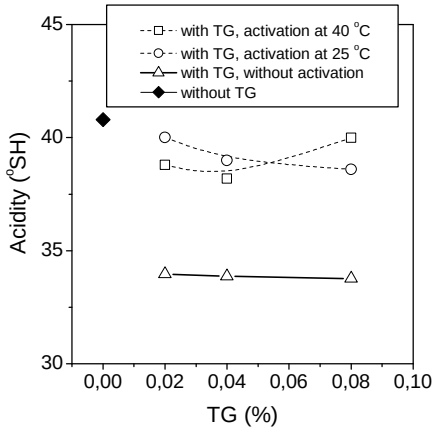

Figure 5. TOTAL ACIDITY OF PROBIOTIC YOGHURT

Slika 5. UKUPNA KISELOST PROBIOTSKOG JOGURTA

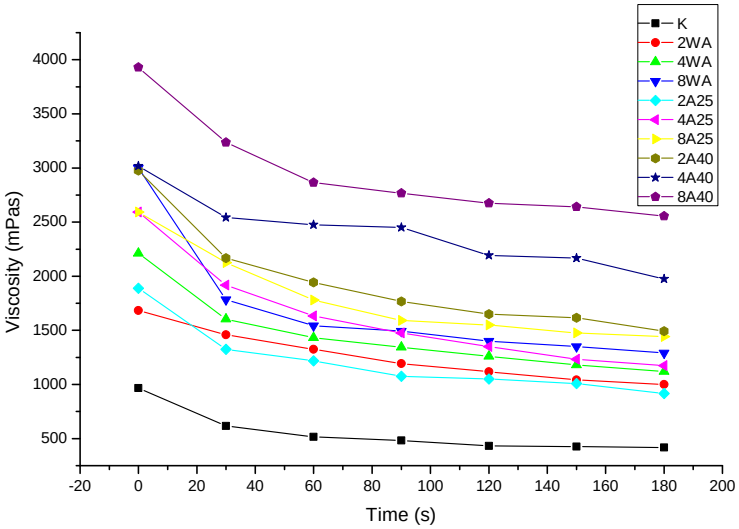

Figure 6. VISCOSITY OF PROBIOTIC YOGHURT

Slika 6. VISKOZITET PROBIOTSKOG JOGURTA

Whey syneresis

The obtained values of whey syneresis are shown in Figure 3. The lowest syneresis values were achieved in samples with TG activated at 25ºC. Activation temperature of 40ºC gave higher whey syneresis in analyzed samples. Samples without TG activation had the highest syneresis. These results showed that addition of TG without inactivation negatively influence samples syneresis.

Water holding capacity

Water holding capacity of samples is shown in Figure 4. It is evident that activation temperature had significant influence on water holding capacity. TG concentration applied had higher influence on water holding capacity of samples with TG activated at lower temperature (25°C) than of samples with TG activated at higher temperature (40°C). The obtained results implicate stronger influence of activation temperature than TG concentration. Sample without TG addition had the lowest water holding capacity which is in accordance with the previous results Milanović et al., 2009.

Total acidity

Analysis of total acidity showed that samples produced without TG activation had lower total acidity than samples produced with TG activation. Temperature of TG activation did not have significant influence on total acidity. Samples produced without TG had the highest total acidity.

Viscosity

TG concentration and activation temperature showed significant influence on samples viscosity (Figure 6). Samples produced with higher TG concentration had higher viscosity and their values linearly increased. Also samples produced with TG activated at higher temperature had higher viscosity values than samples produced with TG activated at lower temperature. Samples with TG activated at 40°C (2A40, 4A40 and 8A40) are more viscous than samples produced with TG activated at 25°C (2A25, 4A25 and 8A25). Samples produced without TG activation had similar viscosity with samples produced with TG activated at 25°C. The smallest viscosity had sample produced without TG (control sample).

CONCLUSION

The application of transglutaminase in the production of yoghurt with 0.1% fat, ("non fat yoghurt") allows to obtain samples with significantly improved physical characteristics and viscosity compared to samples produced without the use of transglutaminase. Specifically, there were established: significantly shorter process time, low syneresis, high water-holding capacity. Samples produced with activated TG have better physical characteristics and viscosity compared to samples produced without TG activation. Interaction of factors activation temperature and TG concentration indicate that concentration of 0.04% of TG gave the most stable results depending on activation temperature.

ACKNOWLEDGEMENT

Authors want to thank Ministry of Education and Science of Republic of Serbia for the financial support of researches presented in this article, Eureka! 3488 and Project No. 46009.

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IZVOD

OPTIMIZACIJA TEMPERATURE AKTIVACIJE TRANSGLUTAMINAZE U PROIZVODNJI PROBIOTSKOG JOGURTA

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Transglutaminaza (TG) je enzim koji povećava formiranje inter i intramolekularnih veza u lancima proteina mleka, što doprinosi formiranju čvršćeg gela, smanjenju sinerezisa, poboljšanju konzistencije. U radu je ispitan uticaj različitih temperatura aktivacije transglutamonaze na sinerezis, sposobnost vezivanja vode, kiselost i viskozitet probiotskog jogurta. Uzorci jogurta su proizvedeni od mleka sa 0,1% mlečne masti dodavanjem termofilne starter kulture DELVO-Yog MY-721 DSL (*Lactobacillus acidophilus, Bifidobacterium, Streptococcus thermophilus*). TG je dodata u mleko u koncentracijama od 0,02%, 0,04% i 0,08%, sa ili bez aktivacije. Temperature aktivacije TG su: 25°C u trajanju od 3h i 40ºC u trajanju od 2,5h. Utvrđeno je da su parametri aktivacije TG (temperatura i koncentracija) imale značajan uticaj na sinerezis, kapacitet vezivanja vode, kao i na viskozitet probiotskih jogurta.

Ključne reči: jogurt • probiotska starter kultura • transglutaminaza • optimizacija

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SCIENTIFIC PAPER

UDK: 663.88:665.12:637.146

INFLUENCE OF KOMBUCHA INOCULUM ON THE FATTY ACID COMPOSITION OF FERMENTED MILK PRODUCTS

Dairy products obtained by the fermentation of cow's milk with kombucha cultivated on stinging nettle and peppermint tea at two different fermentation temperatures were studied in order to investigate influence of inoculum on fatty acid composition. Analysis of fatty acid methyl esters was performed by gas chromatography. The saturated, mono and polyunsaturated fatty acid contents were within the ranges of 62.86-70.42, 27.85-32.62 and 1.56-4.25% of total fatty acids, respecttively. Among all investigated samples, milk fermented at temperature of 43ºC with kombucha cultivated on stinging nettle tea showed the most desirable fatty acid composition with the lowest saturated fatty acid and the highest mono and polyunsaturated fatty acid contents.

Key words: fatty acids • kombucha • fermented milk products

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INTRODUCTION

The nutritional importance of milk constituents places milk above other nutritive substances, because it contains all of the main nutrient groups (Lorand, 1913).

Milk fat is the most variable component of the milk constituents (Kadegowda, 2008). It is also an important dietary source of nutrients and energy, but during the past several decades milk fat has been considered as a risk factor for coronary heart diseases (CHD) and reduction in fat intake has been recommended. However, it has been cleared that types of fat have a more important role in determining risk of CHD than total amount of fat in the diet (Hu *et al.*, 2001). Various fatty acids have different effects on plasma lipids: short and medium chain fatty acids do not affect plasma lipoproteins, while consumption of saturated fatty acids (SFA), specifically saturated fats with 12-16 carbon atoms tend to increase plasma total and low density lipoprotein (LDL) cholesterol levels (He *et al.*, 2007). The fatty acid composition of milk fat typically comprises 70% saturated fatty acids, 25% monounsaturated fatty acids, and 5% polyunsaturated fatty acids. The carbon number of the fatty acids, their degree of unsaturation, and their positional distribution within the triacylglycerol molecules influence the nutriational and physical properties and consumer acceptance of foods contaiinning milk fat (Bobe *et al.*, 2007). Milk is ideal for human nutrition because of high content of short chain fatty acids which can be more easily attacked by the digestive enzymes. Although milk fat contains a relatively small amount of unsaturated fatty acids, it is an important source of essential fatty acids, especially arachidonic acid (Salamon *et al.*, 2009). Additionally, milk fat is a significant source of conjugated linoleic acids (CLA), which have recently been recognised as a nutrient that exerts important physiological effects (Gulati *et al.*, 2000).

Kombucha or the tea fungus is a symbiotic culture of acetic acid bacteria and fungi capable of producing a refreshing beverage with many beneficial effects on human health, by means of fermentation of sugared tea (Dufresne and Farnworth, 2000). Black and green tea are typical substrates for kombucha cultivation, however, it can be cultivated on different atypical nutrients such as coca-cola, wine, vinegar, extract of *Echinacea*, *Mentha* or molasses from sugar beat processing (Malbaša *et al.*, 2009).

Fermentation milk products contain all important food ingredients in such relation that a human body can optimally use them and because of that they belong to a group of very important food in human nutrition. They, as well as milk, contain all the basic ingredients needed for growth of a human body, for development, reproduction, maintenance and satisfying energy needs. During fermentation there is a change of some constituent of milk and with creating of new constituents, fermented products get new features compared to milk (Vitas *et al.,* 2010).

Composition of dairy products manufactured by adding pure cultures is determined to the greatest extent by the composition of the raw milk, since the cultures produce rather aroma materials and they affect fatty acid composition to a smaller extent. Kravić *et al.* (2011) noted changes in composition and contents of some

fatty acid during fermentation of cow's milk with kombucha starters cultivated on differrent inoculums, but still small number of researches exist about inoculum effect on the fatty acid composition of kombucha fermented milk products. Therefore, the aim of this study was to examine fatty acid composition of dairy product obtained by addition of kombucha cultivated on peppermint and stinging nettle tea to cow's milk at two different fermentation temperatures: 40ºC and 43ºC.

MATERIALS AND METHODS

Inoculum

Two different kombucha inoculums were prepared. The first inoculum was prepared with peppermint extract as follows: in 1 dm^3 of boiling tap water 70 g sucrose and 2.25 g peppermint tea (tea in bulk purchased at a local health food store) was added. After boiling for 5 minutes, the tea was cooled to room temperature, strained, and then 100 cm^3 inoculum from a previous fermentation was added. The glass jar was covered with fabric bandwidth for air. Kombucha incubation was performed at room temperature for 7 days. The second inoculum was prepared with stinging nettle extract (tea in bulk purchased at a local health food store) under the same conditions as the inoculum with peppermint extract.

Production of fermented milk products

Pasteurized and homogenized milk with 1.6% milk fat, from the producer "AD IMLEK" Belgrade, Department "Novosadska mlekara", Novi Sad, was used for the laboratory manufacture of fermented milk products by adding 10% (v/v) kombucha inoculum. Kombucha was added in milk at two different temperatures: 40ºC and 43ºC, until a pH value of 4.5 was reached. Obtained gels were cooled to the temperature of 8ºC and homogenized by mixing.

Lipid extraction

The extraction of fat was carried out as described by Havemose *et al.* (2004) with minor modifications. Fat was extracted from kombucha milk products (4 cm^3) by adding methanol (4 cm^3) and chloroform (4 cm^3). The mixture was shaken vigorously for 1 min and then centrifuged for 10 min. The lower phase containing the lipid fraction was isolated and evaporated to dryness under nitrogen.

Preparation of fatty acid methyl esters

The methylation of fatty acid extracted from milk-based kombucha products was carried out as described by Kravić *et al.* (2010) with minor modifications. Previously extracted fats were dissolved in 2.4 cm^3 of hexane. An aliquot (0.6 cm^3) of 2 mol dm^{-3} methanolic KOH solution was added. The tube was capped and vigorously shaken for 20 s and allowed to boil one min in water bath at 70°C. After 20s of shaking 1.2 cm^3 of 1 mol dm^{-3} HCl was added and gently stirred. After phase separation the upper phase containing the fatty acid methyl esters was decanted and 2 µl was used for further analysis.

Gas chromatography analysis

The analysis of fatty acid methyl esters was performed on a Becker 409 gas chromatograph equipped with a flame ionization detector (FID). Chromatographic resolution was achieved using a stainless steel column (3 m × 3 mm i.d.) packed with 10% SP-2330 on 100/120 mesh Chromosorb W AW (Supelco). The inlet temperature was 240ºC, and the carrier gas was nitrogen (99.9%) with constant flow rate of 15 cm^3 min^{-1}. Injected sample volumes were 2 µl. The analyses were performed at isothermal run with oven temperature of 180ºC. Identification of individual fatty acid methyl esters was based on relative retention times of commercial standard FAME Mix RM-6 (Supelco) and on dependence of Kovats index of relative retention times. Quantitative determination of separated fatty acid methyl esters was done using method 100%.

RESULTS AND DISCUSSION

Changes of pH value during fermentation of milk

Kombucha fermentation was monitored by measuring pH. Course of fermentation of milk with 1.6% milk fat in the production process of kombucha fermented milk products is shown in Figure 1. Samples were labelled as following, milk fermented with kombucha cultivated on peppermint tea: at a temperature of 40ºC - P40 and at a temperature of 43ºC - P43; milk fermented with kombucha inoculum cultivated on stinging nettle tea: at temperature of 40ºC – SN40 and at temperature of 43ºC – SN43. Fermentation was stopped after reaching the pH value of 4.5. Sample P43 achieved the desired pH value for 10.10 hours, sample P40 for 11.00 hours and samples SN40 and SN43 for 11.50 hours.

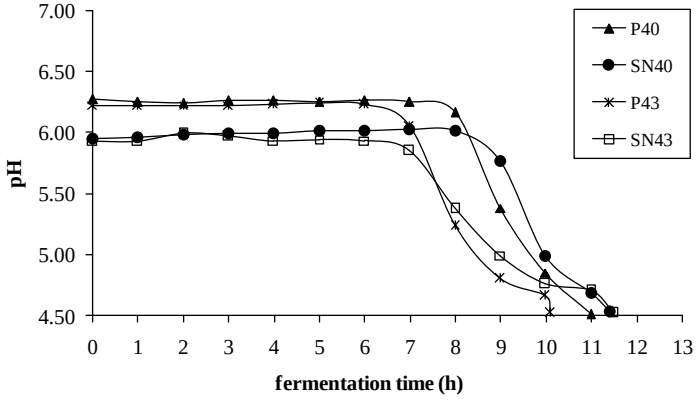

Figure 1. FERMENTATION PATTERN OF KOMBUCHA FERMENTED MILK PRODUCTS

Slika 1. FERMENTACIONI DIJAGRAM PROIZVODNJE KOMBUHA FERMENTISANIH PROIZVODA

Fatty acid composition of kombucha fermented milk products

The fatty acid composition of the milk and resulted four kombucha milk products are given in Table 1, as relative ratio of total fatty acid content. The presented results represent the

Table 1. FATTY ACID COMPOSITION OF MILK AND KOMBUCHA FERMENTED MILK PRODUCTS

Tabela 1. SASTAV MASNIH KISELINA MLEKA I KOMBUHA FERMENTISANIH MLEČNIH PROIZVODA

Sample	M1.6	SN40	SN43	P40	P43
Fatty acid content (% of total fatty acid)					
C4:0	1.23±0.07	1.31±0.08	1.27±0.05	1.24±0.04	1.17±0.07
C6:0	0.82±0.04	0.84±0.05	0.77±0.06	0.69±0.04	0.73±0.06
C8:0	0.37±0.03	0.23±0.02	0.18±0.01	0.27±0.02	0.32±0.02
C10:0	2.82±0.07	2.34±0.06	2.24±0.07	6.28±0.08	2.54±0.05
C11:0	0.24±0.02	0.10±0.01	0.17±0.01	0.23±0.02	0.24±0.02
C12:0	3.50±0.10	3.40±0.11	3.51±0.12	3.25±0.11	3.64±0.09
C13:0	0.19±0.02	0.09±0.01	0.58±0.03	0.14±0.01	0.08±0.01
C14:0	12.04±0.41	11.93±0.31	11.41±0.43	12.66±0.37	14.54±0.43
C14:1	2.81±0.11	2.73±0.09	2.23±0.08	2.26±0.11	2.13±0.12
C15:0	0.25±0.02	0.26±0.02	0.24±0.01	0.16±0.02	2.07±0.12
C16:0	32.36±0.43	32.87±0.62	31.23±0.82	32.21±0.71	34.50±0.52
C16:1	2.26±0.12	2.50±0.16	2.08±0.11	2.14±0.11	2.23±0.12
C17:0	0.27±0.02	0.33±0.03	0.34±0.03	0.22±0.01	0.66±0.05
C17:1	0.34±0.03	0.34±0.03	0.80±0.06	0.29±0.02	0.37±0.03
C18:0	11.59±0.18	11.45±0.33	10.94±0.27	10.21±0.17	9.95±0.26
C18:1	25.95±0.42	26.34±0.51	27.52±0.71	24.57±0.48	23.12±0.41
C18:2	2.79±0.07	2.93±0.11	4.25±0.14	3.19±0.09	1.56±0.07
SFA	65.68	65.13	62.86	67.55	70.42
MUFA	31.35	31.91	32.62	29.26	27.85
PUFA	2.79	2.93	4.25	3.19	1.56
UFA	34.14	34.83	36.87	32.45	29.40
AI	2.46	2.41	2.18	2.65	3.28

SFA – saturated fatty acids / zasićnene masne kiseline
MUFA – monounsaturated fatty acids / mononezasićene masne kiseline
PUFA – polyunsaturated fatty acids / polinezasićene masne kiseline
UFA – unsaturated fatty acid / nezasićene masne kiseline
AI – atherogenic index / aterogeni indeks

mean ± standard deviation of three replications for each sample.

As can be seen from Table 1 predominant fatty acids in milk and kombucha fermented milk products were palmitic (C16:0), followed by oleic acid (C18:1), myristic acid (C14:0) and stearic acid (C18:0), which together accounted for around 83% of total fatty acids. Samples P40 and P43 showed a higher relative content of SFA and a lower relative content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) compared to control milk. The relative content of SFA in samples SN40 and SN43 was lower than in control milk, while the relative content of MUFA in the same samples slightly increased. The relative content of PUFA was near two-fold higher in sample SN43 compared to control milk and slightly higher in sample SN40. Among investigated kombucha fermented milk products, those fermented at temperature of 43ºC with kombucha cultivated on stinging nettle tea showed the most desirable fatty acid composition with the lowest SFA content and the highest MUFA and PUFA contents compared to control milk sample and the other kombucha fermented milk products.

Saturated fatty acids with a chain length of C12:0-C16:0 are atherogenic, stearic acid is neutral, and oleic and polyunsaturated fatty acids have a lipid lowering effect (Kravić *et al.*, 2011). The sum of lauric (C12:0), myristic and palmitic acids was the highest in milk fermented with kombucha cultivated on peppermint tea at temperature of 43ºC (52.68%), which was higher than in control milk, while the lowest atherogenic fatty acids content was observed in sample SN43 (46.14%). In addition, sample SN43 showed the highest contents of oleic and linoleic (C18:2c) acids. Amount of stearic acid was ranged from 9.95% to 11.59%. Medium chain capric acid (C10:0) was about two-fold higher in sample P40 compared to milk and other kombucha fermented milk products.

The atherogenic index is the sum of concentrations of C12:0, C16:0, and 4 x C14:0 divided by the concentration of total unsaturated fatty acids (Kravić *et al.*, 2011) and it was proposed as a dietary risk indicator of lipids for cardiovascular diseases. According to this equation, all unsaturated fatty acids, regardless of their double-bond number, position, or configuretion, are considered to be equally effective in decreasing the risk for atherogenicity, primarily for lack of reliable information to assign more suitable coefficients to the individual acids. The results of this investigation indicate that milk fermented at temperature of 43ºC with kombucha cultivated

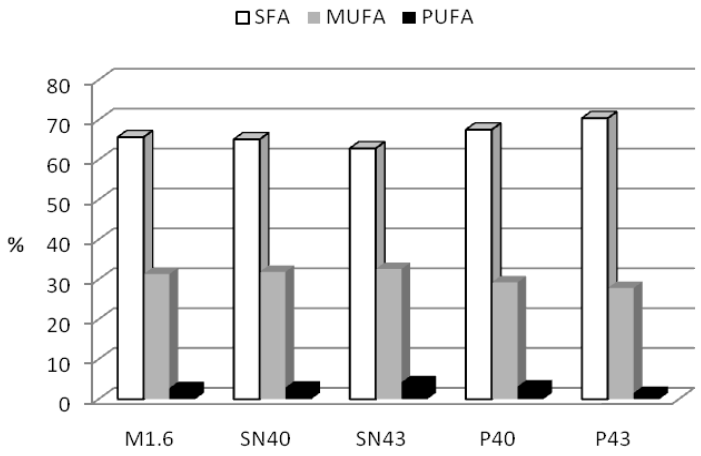

Figure 2. TOTAL CONTENT OF SATURATED, MONO AND POLYUNSATURATED FATTY ACIDS OF MILK AND KOMBUCHA FERMENTED MILK PRODUCTS

Slika 2. UKUPAN SADRŽAJ ZASIĆENIH, MONO I POLINEZASIĆENIH MASNIH KISELINA U MLEKU I KOMBUHA FERMENTISANIM MLEČNIM PROIZVODIMA

on stinging nettle tea has the lowest atherogenic index compared to all examined samples.

In Figure 2 total contents of SFA, MUFA and PUFA of control milk and kombucha fermented milk products is showed. It is evident that saturated fatty acid content slightly increases while polyunsaturated fatty acid content decreases with rising of fermentation temperature in samples obtainned by fermentation of milk with kombucha inoculum prepared with peppermint extract. Otherwise, in samples obtained by fermentation of milk with kombucha inoculum prepared with stinging nettle extract saturated fatty acid content decreases while polyunsaturated fatty acid content increases with rising of fermentation temperature.

CONCLUSION

This study demonstrates that milk fermented at a temperature of 43ºC with kombucha cultivated on stinging nettle tea probably has a more health-promoting fatty acid composition compared to milk and milk fermented with kombucha cultivated on peppermint tea. This product showed lower content of saturated fatty acids and higher content of unsaturated fatty acids compared to the same samples. It also had lower atherogenic index.

ACKNOWLEDGEMENT

This research is financially supported by the Ministry of Education and Science of the Republic of Serbia (Grant III 46009).

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IZVOD

UTICAJ INOKULUMA KOMBUHE NA SASTAV MASNIH KISELINA U FERMENTISANIM MLEČNIM PROIZVODIMA

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Mlečni napici proizvedeni fermentacijom kravljeg mleka pomoću kombuhe kultivisane na čaju koprive i mente na dve različite temperature fermentacije su analizirani u cilju ispitivanja uticaja inokuluma na sastav masnih kiselina. Analiza metil estara masnih kiselina izvedena je primenom gasne hromatografije. Sadržaj zasićenih, mono i polinezasićenih masnih kiselina kretao se u opsegu 62,86-70,42; 27,85-32,62 i 1,56-4,25%, redom. Među svim ispitivanim uzorcima, mleko fermentisano na 43ºC inokulumom kombuhe kultivisane na čaju koprive, pokazalo je najpovoljniji masno-kiselinski sastav sa najnižim sadržajem zasićenih masnih kiselina i najvišim sadržajem mono i polinezasićenih masnih kiselina.

Ključne reči: masne kiseline • kombuha • fermentisani mlečni proizvodi

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NAUČNI RAD

UDK: 637.146:663.88:665.12

MASNE KISELINE U MLEČNIM PROIZVODIMA DOBIJENIM POMOĆU KOMBUHE KULTIVISANE NA RTANJSKOM ČAJU

Cilj ovog rada je identifikacija i određivanje sadržaja masnih kiselina u mlečnim proizvodima dobijenim inkubacijom na različitim temperaturama, pomoću kombuhe kultivisane na rtanjskom čaju.

Masne kiseline su analizirane gasnom hromatografijom sa plameno-jonizacionim detektorom (GC-FID). Osim određivanja masnih kiselina, kvalitet dobijenih proizvoda je utvrđen i standardnim metodama analize hemijskog sastava u skladu sa važećim Pravilnikom.

Uzorak proizveden na najvišoj primenjenoj temperaturi (43°C) dostigao je vrednost pH od 4,5 za 10h, dok je fermentacija na temperaturama 37 i 40°C izvedena do 17h trajanja.

Najviši sadržaj nezasićenih masnih kiselina, kao i najniži aterogeni indeks proizvoda dobijenog fermentacijom na 43°C preporučuju napitak kao najzdraviji za ishranu.

Ključne reči: masne kiseline • mlečni proizvodi • kombuha • rtanjski čaj • GC-FID

UVOD

Kombuha je simbioza više vrsta kvasaca i sirćetnih bakterija, sposobna da metaboliše na različitim supstratima. Kvasci iz šećera stvaraju alkohol, a bakterije taj alkohol koriste kao izvor energije i pretvaraju ga u sirćetnu kiselinu. Pritom kvasci saharozu pretvaraju u glukozu i fruktozu i time omogućuju bakterijama da stvaraju glukonsku kiselinu, koja štiti kvasce od konkurentnih mikroorganizama. Vrsta kvasaca koja učestvuje u ovoj simbiozi zavisi od geografskog područja u kome se kombuha gaji. Kombuha je sposobna da iz malog broja komponenata stvori veliki broj različitih nutritivnih i farmakološki korisnih supstanci. Kombuha stvara i celuloznu biomasu kao proizvod sekundarnog metabolizma. Osim tradicionalnih podloga za rast kombuhe, kao što su zaslađeni crni i zeleni čaj, ona fermentiše i na koka-koli, pivu, kafi, ekstraktu topinambura, melasi, ekstraktima biljnih čajeva, a takođe i na mleku. Fermentacijom na mleku nastaje proizvod, koji je prema svojim fizičko-hemijskim i senzornim karakteristikama najsličniji kefiru i jogurtu (Malbaša, 2009).

Rtanjski čaj (*Satureja montana*, familija *Lamiaceae*) je endemična biljna vrsta i pokriva samo centralni deo Balkanskog poluostrva (Diklić, 1974). Rtanjski čaj je poznata lekovita biljka čiji ekstrakt pokazuje mnoga pozitivna fiziološka dejstva na ljudski organizam. Rtanjski čaj se koristi u narodnoj medicini za lečenje bolesti organa za disanje, varenje i mokraćnog sistema. Za spoljašnju upotrebu se preporučuje kod upala kože i sluzokože. Po nekim narodnim travarima ova biljka ima svojstva opšteg tonika koji jača organizam, ali to nije dokazano kliničkim ispitivanjima. Uspešno se koristi u lečenju bronhitisa, astme, kašlja i upale disajnih organa kod dece, kao i u lečenju starih osoba. Veoma je efikasan u lečenju dijabetesa, zato što gasi žeđ i ublažava bolove u gastrointestinalnom sistemu (Ćavar *et al*., 2008; Grosso *et al*., 2009).

Mleko je nutritivno visoko vredna namirnica, jer sadrži gotovo sve energetske, gradivne i zaštitne materije, u takvom kvantitativnom odnosu da ljudski organizam može optimalno da ih iskoristi. Fermentisani mlečni proizvodi predstavljaju prehrambenu namirnicu bogatu hranjivim materijama u odgovarajućem odnosu i lako dostupnu za ljudski organizam. U svom sastavu imaju sve komponente mleka, koje mogu biti uvećane koncentrisanjem ili obogaćene raznim dodacima. Primenjeni fermentativni procesi menjaju sastav mleka. Mlečno-kiselinska fermentacija utiče na stvaranje mlečne kiseline iz lakotoze, masnih kiselina iz mlečne masti i aminokiselina iz proteina (Milanović, 1997).

Masna kiselina je karboksilna kiselina, često s dugim nerazgranatim lancem. Masne kiseline mogu biti zasićene ili nezasićene. Velika većina prirodnih masnih kiselina ima paran broj ugljenikovih atoma, zato što u njihovoj biosintezi učestvuje acetil koenzim A, koji nosi sa sobom dva atoma ugljenika. Zasićene masne kiseline ne sadrže dvostruke kovalentne veze ili druge funkcionalne grupe u molekularnom lancu. Zasićene masne kiseline formiraju ravne lance atoma, i kao rezultat toga mogu se zgusnuto skladištiti u organizmu, što povećava količinu energije po jedinici zapremine. Masno tkivo čoveka i životinja sadrži velike količine dugolančanih zasićenih masnih kiselina. Nezasićene masne kiseline su kiseline sličnog oblika, osim što postoji jedna ili više alkenskih funkcionalnih grupa unutar

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lanca gde svaki alken zamenjuje jednostruku ugljenikovu vezu, $-CH_2-CH_2-$, u delu lanca sa dvostrukom vezom, -CH=CH-. Takve dvostruke veze mogu biti formirane u *cis* ili *trans* konfiguraciji. Ove razlike u geometriji između *cis* i *trans* oblika nezasićenih masnih kiselina, te između zasićenih i nezasićenih masnih kiselina igraju vrlo značajnu ulogu u biološkim procesima (u ljudskom organizmu) i u izgradnji bioloških struktura (u izgradnji ćelijske membrane) (Marenjak *et al.*, 2006). Kao dijetetski pokazatelj rizika lipida za kardiovaskularne bolesti predložen je aterogeni indeks (AI). Njegova niža vrednost ukazuje na pozitivan efekat na zdravlje ljudi (Kravić *et al*., 2011).

U ovom radu kombuha je kultivisana prvo na ekstraktu rtanjskog čaja zaslađenog sa 7% saharoze. Nakon toga je mleko sa 1,6% mlečne masti inkubirano sa 10% (v/v) navedenog inokuluma na temperaturama od 37, 40 i 43^{o}C. Cilj ovog rada je identifikacija i određivanje sadržaja masnih kiselina u mlečnim proizvodima dobijenim inkubacijom na različitim temperaturama, pomoću kombuhe kultivisane na rtanjskom čaju.

MATERIJAL I METODI

Inokulum

Inokulum za fermentaciju mleka je dobijen kultivacijom kombuhe na rtanjskom čaju, koji je pripremljen na sledeći način: u 1 L ključale česmenske vode dodato je 70 g saharoze i 1,5 g rtanjskog čaja. Pripremljeni čaj je ohlađen na sobnu temperaturu, procеđen, 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 mlečnih napitaka

Za proizvodnju mlečnih napitaka u laboratorijskim uslovima korišćeno je pasterizovano, homogenizovano mleko sa 1,6% mlečne masti, proizvođača „AD IMLEK“ Beograd, ogranak „Novosadska mlekara“, Novi Sad.

U mleko sa 1,6% mlečne masti je dodato 10% (v/v) inokuluma kombuhe. Fermentacija je izvedena na 37, 40 i 43^{o}C do postizanja vrednosti pH od 4,5 ili najduže do 17h trajanja. Gel je zatim ohlađen na temperaturu od 8^{o}C i homogenizovan mešalicom. Dobijeni su uzorci označeni sa R37, R40 i R43, zavisno od čaja na kome je izvedena fermentacija kombuhe i temperature na kojoj je izvedena fermentacija mleka.

Metode analize

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

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

Mlečnim napicima praćeni su i sinerezis surutke (Atamer *et al*., 1996) i sposobnost vezivanja vode (Guzman-Gonzalez *et al*., 1999).

Sastav i sadržaj masnih kiselina mleka i proizvedenih mlečnih napitaka je određen gasnom hromatografijom sa plameno-jonizacionim detektorom (eng. GC-FID). Uzorci za GC-FID su pripremljeni u skladu sa prethodno objavljenim eksperimentalnim rezultatima (Kravić i sar., 2011). Slepa proba je dobijena na sledeći način: u 20 mL mleka sa 1,6% mlečne masti je dodato 2 mL inokuluma kombuhe kultivisane na rtanjskom čaju i odmah je usledila priprema uzorka za GC-FID, tako da nije bilo fermentacije. Inokulum je dodat mleku u količini od 10% (v/v), kao i pri proizvodnji mlečnih napitaka. Dobijena slepa proba je označena sa R*. Slepa proba je pripremljena radi poređenja sadržaja masnih kiselina pre i posle fermentacije i procene uticaja procesa fermentacije na sastav i sadržaj masnih kiselina dobijenih proizvoda.

Proizvedeni uzorci su analizirani nakon proizvodnje.

Tabela 1. HEMIJSKI SASTAV MLEKA

Table 1. CHEMICAL COMPOSITION OF MILK

parametar kvaliteta / quality parameter	Mleko / Milk
pH / pH value	6,64
suva materija (%) / dry matter (%)	10,58
pepeo (%) / ash (%)	0,73
mlečna mast (%) / milk fat (%)	1,60
proteini (%) / proteins (%)	3,37
laktoza (%) / lactose (%)	4,03
kiselost (°SH) / acidity (°SH)	6,60

REZULTATI I DISKUSIJA

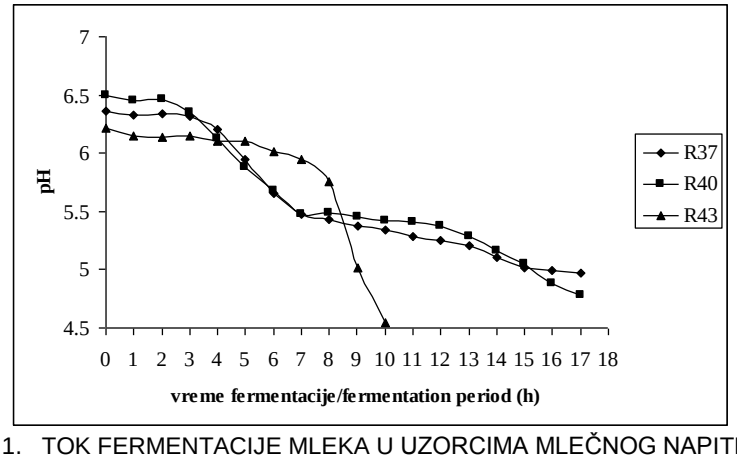
Analiza inokuluma

Inokulumu je određena vrednost pH od 4,37, dok je sadržaj suve materije bio 6,94%, a pepela 0,01%.

Analiza mleka

Rezultati analize kvaliteta mleka korišćenog u proizvodnji kombuha mlečnih napitaka dati su u tabeli 1.

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 MLEČNOG NAPITKA

Figure 1. FERMENTATION PROCESS OF KOMBUCHA MILK BEVERAGES

Tabela 2. HEMIJSKI SASTAV I FIZIČKO-HEMIJSKE KARAKTERISTIKE KOMBUHA MLEČNIH NAPITAKA

Table 2. CHEMICAL COMPOSITION AND PHYSICOCHEMICAL CHARACTERISTICS OF KOMBUCHA MILK BEVERAGES

parametar kvaliteta / quality parameter	R37	R40	R43
pH / pH value	5,04	4,79	4,50
suva materija (%) / dry matter (%)	10,04	9,92	10,23
pepeo (%) / ash (%)	0,67	0,67	0,66
mlečna mast (%) / milk fat (%)	1,54	1,54	1,54
proteini (%) / proteins (%)	2,73	2,33	3,31
laktoza (%) / lactose (%)	3,63	3,77	3,73
kiselost (°SH) / acidity (°SH)	23,20	22,80	29,80
SVV (%) / WHC (%)	15,50	20,40	29,35
sinerezis (mL) / syneresis (mL)	<10	24,00	34,00

Promene vrednosti pH tokom fermentacije mleka

Tok fermentacije mleka sa 1,6% mlečne masti u procesu proizvodnje kombuha mlečnih napitaka prikazan je na slici 1.

Fermentacija je zaustavljena nakon postizanja vrednosti pH od 4,5 (R43) i nakon 17h trajanja (R37 i R40). Fermentacija je prekidana posle 17h trajanja, zbog tehno-ekonomske neisplativosti. Vreme potrebno da se u procesu proizvodnje uzorka R43 postigne željena vrednost pH je 10 h. Uzorci R37 i R40 su nakon 17h dostigli vrednost pH od 4,97, odnosno 4,78, redom.

Značajnija promena vrednosti pH za uzorak R43 je zabeležena nakon 9h fermentacije.

Krive toka fermentacije ovog uzorka imaju karakterističan sigmoidalan oblik u skladu sa literaturnim podacima (Malbaša *et al.,* 2009).

Tok fermentacije uzoraka R37 i R40 nije bio karakterističan za ovaj tip proizvoda.

Analiza mlečnih napitaka

U tabeli 2 prikazan je hemijski sastav kombuha mlečnih napitaka nakon proizvodnje.

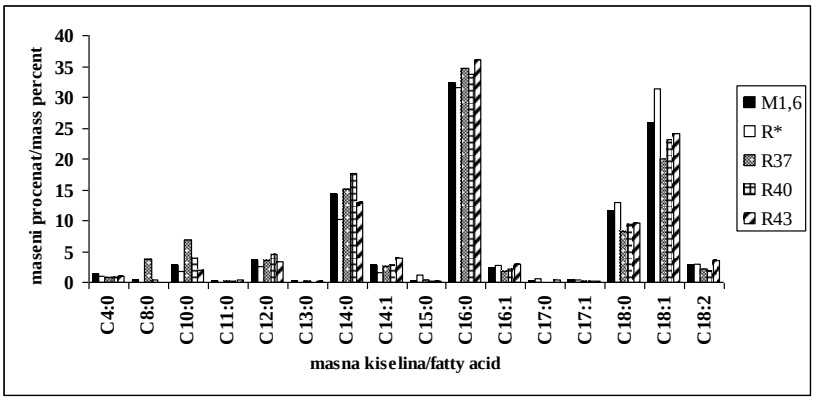
Uzorci R37 i R40 nisu dostigli vrednost pH od 4,5 karakterističnu za proizvodnju fermentisanih mlečnih proizvoda tipa jogurta i kefira. Vrednost pH, kiselost, sinerezis i sposobnost vezivanja vode uzoraka R37 i R40 nisu u skladu sa vrednostima karakterističnim za jogurt i kefir. Hemijski sastav i fizičko-hemijske karakteristike uzorka R43 su prema važećem Pravilniku bile u skladu sa vrednostima karakterističnim za jogurt i kefir.

Sastav i sadržaj pojedinih i ukupnih masnih kiselina mleka, slepe probe i tri mlečna napitka dat je na slikama 2 i 3.

U najvećem procentu, u svim ispitanim uzorcima, zastupljene su miristinska i palmitinska masna kiselina, od zasićenih kiselina, a od nezasićenih kiselina oleinska kiselina.

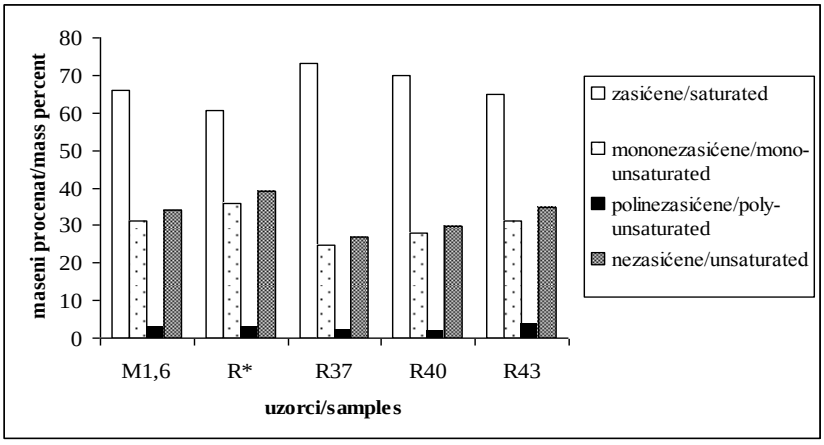
Tokom fermentacije se značajnije povećao sadržaj kapronske, laurinske, miristinske i miristoleinske kiseline.

U poređenju sa slepom probom, sadržaj zasićenih masnih kiselina je porastao u mlečnim proizvodima. Sadržaj mononezasićenih, polinezasićenih i nezasićenih masnih kiselina je veći u slepoj probi nego u mlečnim proizvodima. Osim najboljih hemijskih i fizičko-hemijskih karakteristika proizvoda R43, naročito je važan sadržaj nezasićenih masnih kiselina, koji je u tom proizvodu najveći. To je naročito bitno zbog fiziološkog delovanja ovih jedinjenja za humani organizam.



Slika 2. SASTAV I SADRŽAJ MASNIH KISELINA MLEKA, SLEPE PROBE I MLEČNIH PROIZVODA

Figure 2. COMPOSITION AND CONTENT OF FATTY ACIDS IN MILK, BLANK SAMPLE AND KOMBUCHA MILK BEVERAGES



Slika 3. SADRŽAJ UKUPNIH MASNIH KISELINA MLEKA, SLEPE PROBE I MLEČNIH PROIZVODA

Figure 3. CONTENT OF TOTAL FATTY ACIDS IN MILK, BLANK SAMPLE AND KOMBUCHA MILK BEVERAGE

ZAKLJUČAK

Primenom odgovarajućeg tehnološkog procesa proizvedeni su mlečni napici iz mleka sa 1,6% mlečne masti sa dodatkom 10% inokuluma kombuhe kultivisane na rtanjskom čaju zaslađenim saharozom.

Uzorak R43 dostiže vrednost pH od 4,5 za 10h, zbog čega je njegova proizvodnja ekonomski najisplativija.

Hemijski sastav i fizičko-hemijske karakteristike uzorka R43 su bile u skladu sa važećim Pravilnikom i svrstale ga u fermentisane mlečne proizvode.

Uzorak R43 se pokazao najboljim zbog najvećeg sadržaja nezasićenih masnih kiselina.

ZAHVALNICA

Autori se zahvaljuju Ministarstvu prosvete i nauke Republike Srbije za finansiranje istraživanja predstavljenih u ovom radu, projekat III-46009.

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SUMMARY

FATTY ACIDS IN MILK PRODUCTS OBTAINED BY KOMBUCHA CULTIVATED ON WINTER SAVORY TEA

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The aim of this study is identification and determination of fatty acids content in milk products obtained at different temperatures by kombucha cultivated on winter savory.

Fatty acids were analyzed by gass chromatography with flame ionization detector (GC-FID). Besides fatty acids determination, the quality of the obtained products was determinated by standard analysis methods of chemical composition according to the current Regulations. All analyses were performed after production.

The sample obtained at the highest applied temperature (43°C) reached the pH value of 4.5 in 10h, while the fermentation process at 37 and 40°C stopped after 17h.

The best milk-based beverage is the one obtained at 43°C because of the highest content of unsaturated fatty acids.

Key words: fatty acids • milk beverages • kombucha • winter savory tea • GC-FID

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NAUČNI RAD

FIZIČKO-HEMIJSKA I SENZORSKA SVOJSTVA PROBIOTIČKOG JOGURTA SA DODATKOM INULINA

UDK: 637.146.3:547.455.65

U radu je ispitan uticaj dodatka inulina (0,5%, 1% i 1,5%) i termičkog tretmana mlijeka (85°C/20' ili 95°C/10') na kiselost, viskozitet, indukovanu sinerezu i senzorska svojstva probiotičkog jogurta tokom 21 dana skladištenja. Za proizvodnju jogurta korišteno je svježe homogenizovano i djelimično obrano kravlje mlijeko standardizovano na 1,5% mliječne masti. Fermentacija mlijeka vršena je dodatkom 0,0025% odabrane 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., 7., 14. i 21. dana u 3 ponavljanja. Dobijeni rezultati su pokazali da se sa povećanjem sadržaja inulina vrijeme fermentacije uzoraka skraćuje, gel je finiji i ujednačenije kremaste strukture, osjeća se puniji okus, viskozitet se povećava, a izdvajanje surutke smanjuje. Različiti termički tretmani mlijeka nisu bitnije uticali na fizičkohemijska i senzorska svojstva probiotičkog jogutra tokom skladištenja.

Ključne reči: probiotički jogurt • inulin • kiselost • sinereza • viskozitet • senzorska svojstva

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UVOD

Fermentisani mliječni proizvodi danas čine najpopularniju grupu funkcionalne hrane. Njihova proizvodnja zasniva se na kontrolisanom mliječnokiselinskom vrenju laktoze u mliječnu kiselinu, uz djelimičnu koagulaciju proteina. Djelovanjem mikroflore dodate tokom procesa proizvodnje, dolazi do promjene kiselosti jogurta, što dalje dovodi do promjene viskoziteta i strukture proizvoda, pa tako i sklonosti sinerezi. S druge strane, kao posljedica procesa mliječnokiselinskog vrenja povećava se sadržaj mliječne kiseline, galaktoze, kalcijuma, fosfora, slobodnih aminokiselina i manjih peptida, te masnih kiselina (Gurr, 2006).

Osim visoke nutritivne vrijednosti sve se više važnosti pridaje potencijalnoj zdravstvenoj vrijednosti ovih proizvoda. Njihov pozitivan učinak na pojedine tjelesne funkcije uključuje poboljšanje metabolizma laktoze (Miller i sar., 2007), prevenciju i terapiju dijareje (Gill, 2003) i urogenitalnih infekcija (Reid i sar., 2001), prevenciju bolesti krvnih sudova (Liong i Shah, 2006), te poboljšanje opšteg stanja organizma. Zdravstvena svojstva fermentisanih mliječnih proizvoda, u odnosu na ostale mliječne proizvode, poboljšavaju se korištenjem probiotičkih bakterija (Fondén i sar., 2003; Saxelin i sar., 2003). Poznato je da ove bakterije imaju antikancerogeno dejstvo na cijeli digestivni sistem, obnavljaju crijevnu mikrofloru, stimulišu imunološki sistem, te imaju ulogu u prevenciji gastritisa uzrokovanog bakterijom *Helicobacter pylori* (Frece i sar., 2005). Analize koje su vršili Sazawal i sar. (2006) pokazale su da probiotičke bakterije smanjuju incidenciju dijareje nakon upotrebe antibiotika za čak 52%. Od probiotičkih bakterija najviše se koriste *Lactobacillus acidophilus* i *Bifidobacterum* spp.

Novi funkcionalni mliječni proizvodi, čija se svojstva temelje na njihovom visokovrijednom nutritivnom sastavu, razvili su se modifikacijom tradicionalnih formulacija, eliminacijom ili zamjenom određenih ingredijenata (mast, šećer) ili dodavanjem različitih sastojaka kao što su vitamini, dijetalna vlakna, fitosteroli, itd. (Fogliano i Vitaglione, 2005). Među značajne dodatke svakako se ubraja i nesvarljivi ugljeni hidrat inulin, jak bifidogeni prebiotik, koji svoje dejstvo ostvaruje selektivnim stimulisanjem rasta i aktivnosti nepatogenih bakterija, uglavnom bifidobakterija i laktobacila (Roberfroid i Slavin, 2000), a potiskuje štetne bakterije (clostridia) (Shanahan, 2000). Prebiotički efekat inulina zavisi od velikog broja faktora i djelimično od kompozicije intestinalne flore u svakoj individui. Zbog individualnih razlika, opšta odgovarajuća doza se ne može utvrditi (Roberfroid, 2005). Minimum unosa od 5g u toku dana smatra se dovoljnim da poveća udio bifidobakterija (Roberfroid i Slavin, 2000). Inulin ima višestruku namjenu pa se može koristiti kao niskoenergetski zaslađivač, a zbog svojih osobina da stabilizuje strukturu vodene faze, može se koristiti kao zamjena za mliječnu mast dajući čvrstoću i u ustima puni okus sličan mastima (Gueven i sar., 2005; Stijepić, 2008, 2009).Pored toga, inulin može da utiče na modifikovanje teksturalna svojstva finalnog proizvoda (Tungland i Meyer, 2002).

Cilj rada je bio ispitati uticaj dodatka inulina i termičkog tretmana mlijeka na kinetiku grušanja, te kiselost,

viskozitet, indukovanu sinerezu i senzorska svojstva probiotičkog jogurta tokom 21 dana skladištenja.

MATERIJAL I METODI

U istraživanjima je korišteno homogenizovano i djelimično obrano kravlje mlijeko standardizovano na 1,5% mliječne masti proizvođača "MILKO" d.d. Prijedor (BiH). Proizvedeni su, u laboratorijskim uslovima, čvrsti probiotički jogurti sa različitim dodacima inulina (0,5%, 1%; 1,5%) i kontrolni uzorak (K) bez dodatka. Uzorci su tretirani na 85ºC/20' ili 95ºC/10', a korišteni inulin (Fibruline® Instant Cosucra Groupe Warcoing S.A., Belgium) dodavan je prije termičkog tretmana. Za inokulaciju mlijeka korištena je odabrana probiotička kultura VIVOLAC DriSet BIOFLORA ABY 424: 70% *Streptococcus thermophilus,* 10% *Lactobacillus bulgaricus,* 10% *Lactobacillus acidophilus,* 10% *Bifidobacterium ssp.* (Vivolac Culture Corporation, USA). Fermentacija mlijeka je vođena, uz dodatak 0,0025% inokuluma, na 37°C do postizanja pH 4,6. Svakih sat vremena utvrđene su promjene pH vrijednosti. Nakon završene fermentacije, uzorci su hlađeni i čuvani u frižideru 21 dan na 5ºC. Analize su vršene svakih sedam dana skladištenja.

Titraciona kiselost određena je metodom po Soxshlet – Henkel-u (Carić i sar., 2000), a pH vrijednost potenciometrijski pomoću pH-metra (pH 510/mV Meter, Eutech Instruments Oakton, England). Promjene viskoziteta tokom skladištenja su mjerene pomoću viskozimetra BROOKFIELD Digital Viscometer, DV-E (*Brookfield Engineering Laboratories, Inc*., USA) pri brzini rotacije spindla (Ø4) od 30 obrt/min. Vrijednosti su očitavane svakih 30s, a u radu su korištene srednje vrijednosti viskoziteta izmjerene u toku 3 minute. Za određivanje intenziteta sinereze korištena je centrifuga *SIGMA 2-6 Laboratory Centrifuges* (Osterode, Germany). Uzorci su centrifugirani pri brzinama 1000, 2000 i 3000 o/min u trajanju od 10 min (modifikovana metoda po Keogh i O'Kennedy, 1998). Nakon toga određena je masa taloga preostalog nakon centrifugiranja, a na osnovu te vrijednosti izračunata je masa izdvojenog seruma. Procenat izdvojenog seruma se izračunava po formuli:

$$\% \text{ (w/w)} = (m_K - m_T) / m_M \times 100$$

gdje je:

m_K - masa kivete sa sadržajem;
m_T - masa taloga preostalog nakon centrifugiranja
m_M - masa sadržaja (jogurta) u kiveti.

Senzorska svojstva probiotičkog jogurta (ukus, konzistenciju, boju, miris i izgled površine) ocijenila je panel grupa od pet članova koristeći sistem od 20 ponderisanih bodova (ISO, 1985).

Sve analize su ponovljene tri puta, a rezultati su prikazani kao srednje vrijednosti. Za statističku obradu i grafički prikaz rezultata korišten je Microsoft®Excel 2003.

REZULTATI I DISKUSIJA

Fermentacija

Fermentaciju mlijeka pomoću specifičnih mikroorganizama i različitih dodataka prati tehnološka modifikacija i čitav niz strukturnih promjena, što uzrokuje i promjenu sastava, ukusa, izgleda, boje, mirisa i nutritivnih karakteristika mlijeka.

Rezultati promjena pH-vrijednosti mlijeka termički tretiranog na 85ºC/20' ili 95ºC/10' prikazani su u Tabeli 1.

Tabela 1. UTICAJ DODATKA INULINA NA PROMJENU pH MLIJEKA TOKOM FERMENTACIJE U PROIZVODNJI PROBIOTIČKOG JOGURTA

Table 1. THE INFLUENCE OF INULIN ADDITION IN pH VALUE CHANGE DURING MILK FERMENTATION IN PROBIOTIC YOGHURT PRODUCTION

% IN	TTM	IP	Vrijeme fermentacije mlijeka (min) / Fermentation time (min)								
			0	60	120	180	240	300	330	360	400
(K)	85ºC/20'	☒	**6,58**	**6,40**	**6,39**	**6,38**	**6,02**	**5,33**	**4,83**	**4,74**	**4,60**
		Sd	0,0000	0,0200	0,0153	0,0100	0,0100	0,0100	0,0252	0,0300	0,0058
		Cv	0,0000	0,3125	0,2392	0,1567	0,1567	0,1873	0,5214	0,6329	0,1249
	95ºC/10'	☒	**6,58**	**6,43**	**6,33**	**6,16**	**5,96**	**5,23**	**4,87**	**4,77**	**4,62**
		Sd	0,0000	0,0153	0,0252	0,0153	0,0252	0,0252	0,0208	4,7600	0,0153
		Cv	0,0000	0,2377	0,3978	0,1567	0,1661	0,1873	0,5214	0,0265	0,3309
0,5% IN	85ºC/20'	☒	**6,58**	**6,39**	**6,31**	**6,03**	**5,06**	**4,72**	**4,64**	**4,60**	
		Sd	0,0000	0,0115	0,0252	0,1528	0,1528	0,0153	0,0306	0,0153	
		Cv	0,0000	0,1806	0,3986	0,9569	0,9569	0,3234	0,6575	0,3323	
	95ºC/10'	☒	**6,58**	**6,41**	**6,31**	**6,18**	**5,75**	**5,27**	**4,95**	**4,84**	**4,54**
		Sd	0,0000	0,0208	0,0153	0,0153	0,0306	0,0252	0,0306	4,8467	0,0252
		Cv	0,0000	0,3244	0,2426	0,9569	0,1972	0,3234	0,6575	0,0208	0,5539
1% IN	85ºC/20'	☒	**6,58**	**6,34**	**6,28**	**5,82**	**4,99**	**4,69**	**4,57**		
		Sd	0,0000	0,0306	0,0208	0,0200	0,0200	0,0252	0,0361		
		Cv	0,0000	0,4816	0,3311	0,3436	0,3436	0,5370	0,7907		
	95ºC/10'	☒	**6,58**	**6,40**	**6,21**	**5,28**	**5,10**	**4,79**	**4,69**	**4,59**	
		Sd	0,0000	0,0252	0,0208	0,0252	0,1050	0,0208	0,0265	4,5933	
		Cv	0,0000	0,3918	0,3311	0,3436	0,1156	0,5370	0,7907	0,0252	
1,5 % IN	85ºC/20'	☒	**6,58**	**6,34**	**6,25**	**5,75**	**4,97**	**4,73**	**4,61**		
		Sd	0,0000	0,0252	0,0265	0,0153	0,0153	0,0300	0,0265		
		Cv	0,0000	0,3965	0,4226	0,2655	0,2655	0,6342	0,5727		
	95ºC/10'	☒	**6,58**	**6,40**	**6,23**	**5,33**	**5,11**	**4,75**	**4,68**	**4,59**	
		Sd	0,0000	0,0100	0,0300	0,0252	0,0794	0,0252	0,0306	4,5933	
		Cv	0,0000	0,1563	0,4815	0,2655	0,4024	0,6342	0,5727	0,0252	

U zavisnosti od količine dodatog inulina (IN) i termičkog tretmana mlijeka (TTM), tok i vrijeme fermentacije je bilo različito. Period prilagođavanja probiotičke kulture kod svih ispitivanih uzoraka trajao je oko tri sata, nakon čega je kod uzoraka sa dodatkom inulina dosta naglo krenula fermentacija. Najbrže su fermentisali uzorci sa 1% i 1,5% IN (5,5 sati), proizvedeni od TTM na 85ºC/20', dok je uzorak sa 0,5% IN fermentisao za 6 sati. Evidentna je razlika u toku i trajanju fermentacije između ovih uzoraka i K uzorka, koji je fermentisao za 7 sati. Slične rezultate su imali uzorci čije je ishodno mlijeko termički tretirano na 95ºC/10': dodatak 1% i 1,5% IN znatno je ubrzao tok i vrijeme fermentacije (6 sati), u odnosu na K i uzorak sa 0,5% IN (7 sati).

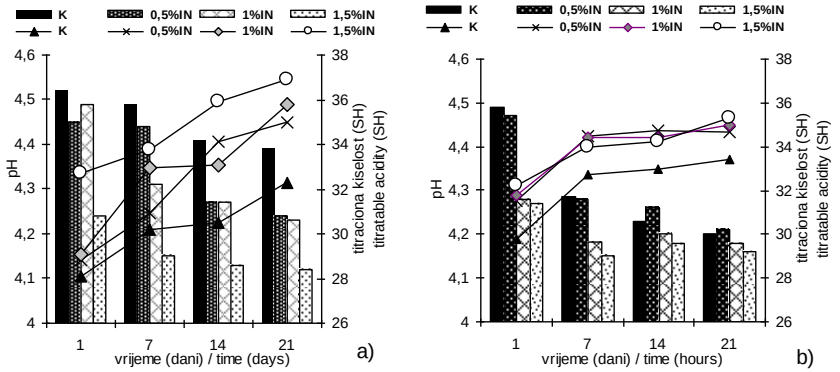
pH i titraciona kiselost

Rezultati promjena pH vrijednosti i titracione kiselosti probiotičkog jogurta tokom 21 dana skladištenja prikazani su na Slici 1. Tokom skladištenja ustanovljene su različite promjene pH vrijednosti probiotičkog jogurta.

Uzorci proizvedeni od mlijeka pasterizovanog na 85ºC/20' (ili 95ºC/10') imali su od 1. do 21. dana skladištenja srednje vrijednosti pada pH: kod K uzorka za 0,31 (ili 0,34) pH jedinica, kod uzorka sa 0,5% IN za 0,21 (ili 0,30) pH jedinica, kod uzorka sa 1% za 0,26 (ili 0,17) pH jedinica i za uzorak sa 1,5% IN za 0,12 (ili 0,19) pH jedinica. Najniža srednja vrijednost pH nakon 21 dana čuvanja, kod uzoraka proizvedenih od termički tretiranih mlijeka na 85ºC/20', zabilježena je kod uzorka sa 1,5% IN (4,12), kod uzorka sa 1% IN (4,23), zatim kod uzorka sa 0,5% IN (4,24), dok je pH vrijednost kod kontrolnog uzorka bila najviša (4,39). Vrijednosti nakon 21 dana skladištenja, za uzorke proizvedene od termički tretiranih mlijeka na 95ºC/10', kretale su se: za K uzorak (4,2), za uzorak sa 0,5% IN (4,21), za uzorak sa 1% IN (4,18) i za uzorak sa 1,5% IN (4,16).

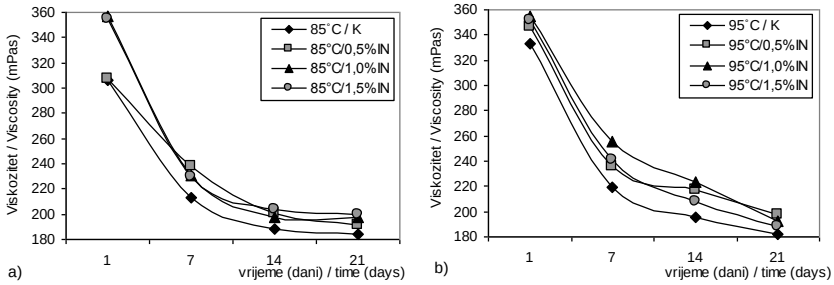
U procesu kiseljenja postoji odgovarajući odnos između titracione kiselosti i pH, jer produkti fermentacije, prije svega mliječna kiselina, uslovljavaju pad pH vrijednosti i porast stepena kiselosti (Kršev, 1989; Stijepić i sar. 2008).

Rezultati ovih ispitivanja, takođe, pokazuju odgovarajuću korelaciju između ova dva parametra. Najviše srednje vrijednosti titracione kiselosti (TTM 85ºC/20' ili 95ºC/10') nakon 21 dana čuvanja imali su uzorci kojima je dodato 1,5% IN (36,9ºSH ili 35,3 ºSH) i 1% IN (35,8ºSH ili 34,9 ºSH). Uzorak sa 0,5% IN imao je nešto nižu vrijednost (35,0ºSH ili 34,4 ºSH), a najnižu K uzorak (32,25ºSH ili 33,7 ºSH).



Slika 1. PROMJENA pH VRIJEDNOSTI I TITRACIONE KISELOSTI JOGURTA SA DODATKOM I BEZ DODATKA INULINA TOKOM 21 DANA SKLADIŠTENJA: A) TT 85ºC/20', B) TT 95ºC/10'

Figure 1. THE CHANGE OF pH AND TITRATABLE ACIDITY OF YOGHURT WITH INULIN ADDITION AND WITHOUT ANY ADDITION DURING 21 DAYS OF STORAGE



Slika 2. VRIJEDNOSTI VISKOZITETA ČVRSTOG PROBIOTIČKOG JOGURTA U ZAVISNOSTI OD PROCENTA DODATOG INULINA - IN (0,5%, 1% I 1,5%) I PRIMIJENJENOG TERMIČKOG TRETMANA MLIJEKA: 85ºC/20' (A) ILI 95ºC/10' (B) TOKOM 21 DANA SKLADIŠTENJA NA 5°C

Figure 2. VISCOSITY OF PROBIOTIC SET YOGHURT DEPENDING ON PERCENTAGE OF ADDED INULIN–IN (0,5%,1% AND 1,5%) AND APPLIED MILK HEAT TREATMENTS: 85°C/20' (A) OR 95°C/10' (B) DURING 21 DAYS OF STORAGE AT 5°C

Viskozitet

Promjene srednjih vrijednosti viskoziteta jogurta tokom 21 dana skladištenja, mjerene u periodu od 3 minuta, date su na Slici 2.

Pad viskoziteta uočava se kod svih uzoraka jogurta u toku skladištenja. To se objašnjava „starenjem" kiselog kazeinskog gela. Naime, u gelu dolazi do djelimičnog kidanja proteinske mreže i ispuštanja male količine surutke, što za posljedicu ima smanjenje viskoziteta tokom čuvanja proizvoda. Ukoliko rezultate viskoziteta, u periodu skladištenja posmatramo jedinstveno kao cjelinu, uočavamo da je veći pad viskoziteta tokom prvih 7 dana skladištenja, dok u kasnijem periodu dolazi do stabilizacije strukture gela i do blažeg opadanja viskoziteta jogurta do zadnjeg dana čuvanja. Generalno, najviše vrijednosti viskoziteta pokazao je uzorak sa dodatkom 1% inulina, bez obzira na termički tretman mlijeka.

Sinereza

Sinereza je pokazatelj smanjene stabilnosti teksture fermentisanih mliječnih proizvoda. Promjene intenziteta sinereze u ovim ispitivanjima vršene su pri 1000, 2000 i 3000 o/min u roku od 10 minuta (Tabela 2). Sinereza je

Tabela 2. PROCENAT IZDVOJENE SURUTKE U 100g UZORKA PROBIOTIČKOG JOGURTA PRI RAZLIČITIM BRZINAMA CENTRIFUGIRANJA (O/MIN) TOKOM SKLADIŠTENJA U ZAVISNOSTI OD DODATKA INULINA I TERMIČKOG TRETMANA MLIJEKA

Table 2. THE PROCENT OF SEPARATED SERUM (%) FROM 100g PROBIOTIC YOGHURT AT DIFFERENT CENTRIFUGATION RATE (O/MIN) DEPENDING ON PERCENTAGE OF ADDED INULIN AND APPLIED MILK HEAT TREATMENTS DURING STORAGE

Dani skladi-štenja / Storage days	% IN	Brzina centrifugiranja / Centrifugation rate (o/min)					
		1000		2000		3000	
		85ºC/20'	95ºC/10'	85ºC/20'	95ºC/10'	85ºC/20'	95ºC/10'
1	0,0	1,90±0,06	1,20±0,09	13,57±0,96	11,70±0,72	23,70±4,31	23,10±4,90
	0,5	0,00±0,00	0,00±0,00	11,60±0,67	10,47±0,57	19,13±3,29	20,00±3,01
	1,0	0,00±0,00	0,00±0,00	10,63±0,77	8,90±0,34	14,20±3,98	18,77±3,50
	1,5	0,00±0,00	0,00±0,00	10,23±0,39	8,40±0,44	15,07±2,99	16,53±3,08
7	0,0	2,14±0,07	1,80±0,01	15,30±0,98	11,80±0,87	25,63±4,61	23,50±3,98
	0,5	0,00±0,00	0,00±0,00	14,70±0,56	9,73±0,32	23,20±4,02	21,20±3,13
	1,0	0,00±0,00	0,00±0,00	11,47±0,34	8,93±0,65	21,43±4,00	18,20±3,69
	1,5	0,00±0,00	0,00±0,00	10,83±0,33	8,53±0,55	20,09±2,98	17,30±3,33
14	0,0	2,31±0,05	3,00±0,06	16,27±1,01	10,57±0,89	27,57±3,91	24,03±4,21
	0,5	2,00±0,01	2,20±0,05	13,83±0,86	10,17±0,49	24,40±2,95	22,50±4,01
	1,0	1,86±0,08	1,80±0,01	11,20±0,61	9,27±0,55	23,60±3,62	18,73±3,21
	1,5	1,33±0,10	1,50±0,01	10,03±0,68	8,30±0,50	23,13±3,48	18,53±3,87
21	0,0	3,46±0,12	3,20±0,09	17,00±1,03	11,22±0,91	29,00±4,33	25,32±4,06
	0,5	2,26±0,07	2,50±0,02	14,00±0,64	10,67±0,81	26,20±3,86	23,60±3,87
	1,0	2,00±0,02	2,20±0,01	12,00±0,51	9,50±0,61	24,00±3,61	20,20±3,91
	1,5	1,70±0,03	1,90±0,01	12,20±0,68	8,78±0,72	24,10±3,21	20,02±3,85

izražena procentom izdvojene surutke (w/w) nakon centrifugiranja uzoraka.

Na osnovu dobijenih rezultata može se uočiti da sa povećanjem intenziteta centrifugiranja raste izdvajanje surutke, bez obzira na dodatak inulina. Tako, pri brzini centrifugiranja od 1000 o/min nije došlo do bitnijih promjena u strukturi gela i procenat izdvojene surutke je vrlo mali. Sa povećanjem brzine centrifugiranja na 2000 o/min, uočava se da dolazi do određenog otpuštanja surutke tokom skladištenja kod svih uzoraka. Od 1. do 21. dana skladištenja (TTM 85ºC/20') sinereza je bila najizraženija kod K uzoraka od 13,57% ± 0,96 do 17,00% ± 1,03, dok kod uzorka sa 0,5% IN se kretala od 11,60% ± 0,67 do 14,00% ± 0,64, kod uzorka sa 1% IN od 10,63% ± 0,77 do12,00% ± 0,51 i kod uzorka sa 1,5% IN od 10,23% ± 0,39 do 12,20% ± 0,68. Uzorci proizvedeni od TTM 95ºC/10' pokazali su se stabilnijim, sa manje izraženom sinerezom: za K uzorak od 11,70% ± 0,72 do 11,22% ± 0,91, za uzorak sa 0,5% IN od 10,47% ± 0,57 do 10,67% ± 0,81, za uzorak sa 1% IN od 8,90% ± 0,34 do 9,50 ± 0,61 i za uzorak sa 1,5% IN od 8,40% ± 0,44 do 8,78% ± 0,72.

Pri brzini centrifugiranja na 3000 o/min pružaju se najbolji uslovi za procjenu karaktera gela. Pri ovoj brzini postiže se bolje pakovanje gela, formira se kompaktniji talog i veće istiskivanje surutke. Od 1. do 21. dana skladištenja nije došlo do bitnijeg povećanja sinereze (TTM 85ºC/20'). Rezultati za kontrolni uzorak (K) su se kretali od 23,70% ± 4,31 do 29,00% ± 4,33 izdvojene surutke, za uzorak sa 0,5% IN je od 19,13% ± 3,29 do 26,20% ± 3,86, za uzorak sa 1,0% IN od 14,20% ± 3,98 do 24,00% ± 3,61 i za uzorak sa 1,5% IN od 15,07% ± 2,99 do 24,10% ± 3,21 istisnute surutke. Pri oštrijem termičkom tretmanu mlijeka (95ºC/10') sinereza je, tokom skladištenja, bila slabijeg intenziteta u odnosu na blaži TTM. Rezultati za kontrolni uzorak (K) su se kretali od 23,10% ± 4,90 do 25,32% ± 4,06 izdvojene surutke, za uzorak sa 0,5% IN je od 20,00% ± 3,01 do 23,60% ± 3,87, za uzorak sa 1,0% IN od 18,77% ± 3,50 do 20,20% ± 3,91 i za uzorak sa 1,5% IN od 16,53% ± 3,08 do 20,02% ± 3,85 istisnute surutke.

Dobijeni rezultati pokazuju da se sa povećanim sadržajem inulina intenzitet sinereze smanjuje, što je u skladu sa istraživanjima Božanić i sar. (2002).

Senzorska analiza

Probiotički jogurti podvrgnuti su senzorskoj ocjeni petočlane grupe analitičara. Utvrđene su neznatne razlike u parametrima senzorskog ocjenjivanja uzoraka proizvedenih od mlijeka tretiranih na 85°C/20' ili 95°C/10', dok je različit dodatak IN imao izvjesnog uticaja tokom skladištenja (Tabela 3). Uzorci su prvo ocijenjeni vizuelno (izgled, boja), zatim su ocijenjeni miris i konzistencija i na kraju ukus.

S obzirom da je za analizirane uzorke korišteno djelimično obrano mlijeko, dodatak inulina je dijelom poslužio kao supstitucija za mliječnu mast, stvarajući kremastu teksturu (konzistenciju) proizvoda izvrsnih ukusnih svojstava, što je i ocijenjeno visokim ocjenama, a što je u skladu i sa nekim ranijim istraživanjima (El-Nagar i sar., 2002; Gueven i sar., 2005; Stijepić i sar.,2008). U toku cjelokupnog vremena skladištenja najprijatniju aromu, umjereno kiselkastu i osvježavajuću sa kremastom konzistencijom i sa homogenim, sjajnim i glatkim gelom imali su uzorci sa dodatkom 1% IN (pri oba TTM) i sa maksimalnih 20 ponderisanih bodova (PB). Dvadeset prvog dana skladištenja neznatno niže su ocijenjeni uzorci sa 1,5% IN (19,7 i 19,6 PB) zbog nešto lošijeg ukusa proizvoda (brašnast ukus) i uzorci sa 0,5% IN (19,4 PB) na račun slabije konzistencije. Kontrolni uzorci su najniže ocijenjeni sa 18,3 i 18,2 PB (TTM 85 C i 95C), što je posljedica lošije ocjene za konzistenciju i ukus.

Tabela 3. SENZORSKA PROCJENA JOGURTA BEZ DODATKA I SA DODATKOM INULINA – IN (0,5%,1% I 1,5%) TOKOM 21 DANA SKLADIŠTENJA NA 5°C

Table 3. SENSORY EVALUATION OF YOGHURT WITH AND WITHOUT INULIN ADDITION – IN (0.5%,1% AND 1.5%) DURING 21 DAYS OF STORAGE AT 5°C

Dani skladišt. *Storage days*	Osobine/ *Characteristics*	*TTM: 85°C/20'				*TTM: 95°C/10'			
		K	0,5%	1,0%	1,5%	K	0,5%	1,0%	1,5%
1.dan /*1 st day*	izgled/*appearance*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	boja/*colour*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	miris/*odour*	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
	konzistencija/ *consistency*	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0
	ukus/*flavour*	12,0	12,0	12,0	12,0	12,0	12,0	12,0	12,0
	Σ	20,0	20,0	20,0	20,0	20,0	20,0	20,0	20,0
7.dan /*7 th day*	izgled/*appearance*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	boja/*colour*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	miris/*odour*	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
	konzistencija/ *consistency*	3,4	3,5	4,0	4,0	3,5	3,4	4,0	4,0
	ukus/*flavour*	11,2	12,0	12,0	12,0	11,2	12,0	12,0	12,0
	Σ	18,6	19,5	20,0	20,0	18,7	19,4	20,0	20,0
14.dan /*14 th day*	izgled/*appearance*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	boja/*colour*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	miris/*odour*	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
	konzistencija/ *consistency*	3,4	3,4	4,0	4,0	3,5	3,5	4,0	4,0
	ukus/*flavour*	11,0	12,0	12,0	11,8	11,0	12,0	12,0	11,8
	Σ	18,4	19,4	20,0	19,8	18,5	19,5	20,0	19,8
21.dan /*21 st day*	izgled/*appearance*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	boja/*colour*	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	miris/*odour*	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
	konzistencija/ *consistency*	3,3	3,4	4,0	4,0	3,3	3,4	4,0	4,0
	ukus/*flavour*	11,0	12,0	12,0	11,7	10,9	12,0	12,0	11,6
	Σ	18,3	19,4	20,0	19,7	18,2	19,4	20,0	19,6

*TTM- termički tretman mlijeka / *TTM- milk heat treatments

ZAKLJUČAK

Kod oba termička tretmana mlijeka (85°C/20' ili 95°C/10') ustanovljeno je povoljno djelovanje dodatka inulina na ubrzanje fermentacije ispitivanih uzoraka, što je izraženo preko pH-vrijednosti. Tokom skladištenja nije bilo značajne razlike u promjeni kiselosti između svih ispitivanih uzoraka. Dodatak inulina uticao je na poboljšanje fizičkih osobina probiotičkog jogurta, smanjenje sinereze i rast viskoziteta. Povećan sadržaj inulina imao je povoljan uticaj i na parametre senzorskog ocjenjivanja što je rezultovalo blažim mirisom i izraženo punijim ukusom. Najbolje ocijenjeni uzorci su sa 1% inulina, bez obzira na termički tretman mlijeka.

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SUMMARY

PHYSICOCHEMICAL AND SENSORY PROPERTIES OF PROBIOTIC YOGHURT WITH INULIN ADDITION

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The aim of this study was to examine the influence of inulin addition (0.5%, 1% i 1.5%) and milk heat treatment on acidity, viscosity, induced syneresis and sensory properties of probiotic yoghurt during 21 days of storage. Yoghurt was manufactured from raw homogenised low fat milk (1.5%). The fermentation started after addition 0.0025% of probiotic starter: *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* i *Bifidobacterium* ssp. to milk at 37°C. After milk fermentation at pH 4.6, probiotic yoghurt samples were cooled and stored at 5°C. During 21 day of storage the change of pH, titratable acidity, viscosity, syneresis and sensory properties were observed. On the basis of the obtained results it can be concluded that yoghurt samples with inulin addition had shorter fermentation time, creamy texture, higher viscosity and lower syneresis. Different milk heat treatments had no significant influence on physicochemical properties of probiotic yoghurt during storage.

Key words: probiotic yoghurt • inulin • acidity • syneresis • viscosity • sensory properties

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NAUČNI RAD

UDK: 637.146.3+637.12'639]:637.142.2

UTICAJ KONCENTRATA PROTEINA SURUTKE NA KARAKTERISTIKE ČVRSTOG JOGURTA OD KOZJEG MLEKA TOKOM SKLADIŠTENJA

U radu je ispitivana mogućnost primene koncentrata proteina surutke u proizvodnji čvrstog jogurta od kozjeg mleka. Proizvedene su tri serije uzoraka: A – čvrsti jogut od kozjeg mleka; B – čvrsti jogurt od kozjeg mleka sa dodatkom 0,5% KPS i C – čvrsti jogurt od kozjeg mleka sa dodatkom 1% KPS.

Ispitivanje fizičko-hemijskih karakteristika i viskoziteta proizvedenih uzoraka jogurta vršeno je 1, 7, 14 i 21-og dana skladištenja.

Na osnovu dobijenih rezultata utvrđeno je da su najveće vrednosti viskoziteta 1, 7 i 14-og dana skladištenja imali uzorci čvrstog jogurta (A) dok je 21. dana skladištenja najviši viskozitet zabeležen kod uzoraka čvrstog jogurta proizvedenog od kozjeg mleka sa dodatkom 1% KPS. Najveću sposobnost vezivanja vode tokom celokupnog perioda skladištenja imali su uzorci C, dok je najmanja sklonost ka sinerezisu zabeležena kod uzoraka B.

Ključne reči: kozje mleko • čvrsti jogurt • koncentrati proteina surutke • viskozitet

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UVOD

Zbog veće nutritivne vrednosti i terapeutskih svojstava potrošnja kozjeg mleka i proizvoda od kozjeg mleka poslednjih godina raste. U poređenju sa kravljim, kozje mleko ima povećanu svarljivost i smanjena alergenska svojstva (Park i Guo, 2006). Specifičan miris i ukus kozjeg mleka koji je mnogim potrošačima neprihvatljiv, može se umanjiti fermentacijom mleka pod dejstvom bakterija mlečne kiseline. Jogurt je jedan od najpopularnijih fermentisanih mlečnih proizvoda čiji su osnovni parametri kvaliteta ukus i konzistencija. Konzistencija jogurta u velikoj meri zavisi od strukture proteinske mreže koja nastaje fermentacijom mleka dodatkom *Lactobacillus delbrueckii* ssp. *bulgaricus* i *Streptococcus thermophilus.*

Osnovni hemijski sastav kozjeg mleka je sličan kravljem i zavisi od genotipa koza, ishrane, redosleda i stadijuma laktacije (Božanić i sar., 2002). Prosečno, kozje mleko sadrži 3.8% mlečne masti, 3.5% proteina, 4.1% laktoze i 0.8% mineralnih materija (Park, 2006).

Jedna od značajnijih razlika između kozjeg i kravljeg mleka odnosi se na strukturu i sastav mlečne masti. Dijametar masnih čestica kozjeg mleka je manji u odnosu na masne globule kravljeg mleka i kreće se u intervalu 0,73-8,58 μm, a prema Slačanac i sar. (2010) oko 65% masnih čestica kozjeg mleka je prečnika manjeg od 3,0 μm. Takve manje masne kapljice su bolje raspoređene što omogućava bolju homogenost i svarljivost kozjeg mleka u odnosu na kravlje. Takođe, lipidna komponenta kozjeg mleka se razlikuje u količini masnih kiselina kratkih i srednjih lanaca. 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 i sar., 2002). Povišeni sadržaj ove tri masne kiseline uzrok je izraženijeg ukusa i mirisa kozjeg mleka u odnosu na kravlje mleko (Park, 2006; Raynal-Ljutovac i sar., 2008; Slačanac i sar., 2010).

Kao i kod kravljeg mleka, kazein kozjeg mleka sadrži iste proteinske frakcije: α_{s1}-CN, α_{s2}-CN, β-CN i κ-CN. Međutim, kozje mleko pokazuje značajne varijacije u sadržaju α_{s1}-CN od 2,7 g/L do svega 0,12 g/L (Park, 2006), dok je najzastupljenija kazeinska frakcija β-CN (Jandal, 1996; Park, 2006; Raynal-Ljutovac i sar., 2008). Takođe, kozje mleko karakteriše veći sadržaj proteina surutke i neproteinskog azota u odnosu na kravlje mleko (Antunac i sar., 2000; Pešić, 2011; Sarić i sar., 2005). Zbog većeg udela proteina surutke puferni kapacitet kozjeg mleka je viši u odnosu na kravlje mleko, što uslovljava sporiji pad pH vrednosti tokom fermentacije (Božanić i sar., 2002). Takođe, manji udeo kazeinskog azota utiče na lošiju strukturu fermentisanih napitaka od kozjeg mleka pa se fermentacijom kozjeg mleka stvara polutečni koagulum, što otežava proizvodnju čvrstog jogurta od kozjeg mleka (Herrero i Requena, 2006; Park i Guo, 2006). Da bi se dobila zadovoljavajuća konzistencija čvrstog jogurta od kozjeg mleka, neophodno je povećati sadržaj suve materije bez masti. Sadržaj proteina, termički tretman, prisustvo mlečne masti, stabilizatori i egzopolisaharidi su faktori koji utiču na strukturu proteinskog matriksa jogurta. 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 i dr. (Lucey, 2004; Martín-Diana i sar., 2003; Tamime i Robinson, 2000).

Proteini surutke 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 i sar., 2007). Koncentrati proteina surutke (KPS) dodaju se mleku pri proizvodnji jogurta kako bi se smanjio sinerezis i povećao viskozitet jogurta (Lucey i sar., 1999; Maćej i sar., 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. Prema Lucey i sar. (1997) denaturisani proteini surutke u termički tretiranom mleku su podložni agregaciji tokom acidifikacije, s obzirom da se pH vrednost tokom fermentacije mleka približava izoelektričnoj tački proteina surutke.

Cilj ovog istraživanja je da se ispita uticaj dodatka koncentrata proteina surutke na fizičko-hemijske karakteristike čvrstog jogurta od kozjeg mleka u toku skladištenja.

MATERIJAL I METODI

U istraživanju je korišćeno kozje mleko sa farme „Beocapra“, Kukujevci. Sirovo kozje mleko je termički tretirano na 92^{o}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 21 dan na temperaturi 4°C.

Uzorci jogurta A su proizvedeni od kozjeg mleka, dok su uzorci jogurta B i C proizvedeni od kozjeg mleka kome je 1h pre termičkog tretmana dodato 0,5%, odnosno 1% koncentrata proteina surutke Textrion Progel 800, DMV International, Netherlands.

Ispitivan je hemijski sastav sirovog mleka, mleka sa dodatkom 0.5% i 1% KPS, termički tretiranog mleka i jogurta tokom 21 dana skladištenja. Takođe, u toku skladištenja ispitivan je viskozitet jogurta. Istraživanje je vršeno u laboratoriji za tehnologiju mleka na Poljoprivrednom fakultetu u Beogradu.

Kod svih uzoraka mleka i jogurta vršene su sledeće analize: suva materija metodom sušenja na 102±2ºC (Carić i sar., 2000), metodom po Kjeldahu pomoću Kjeltec sistema (IDF 20B:1993), mlečna mast metodom po Gerberu (IDF 105:1981; Carić i sar., 2000), laktoza titracionom metodom (IDF 28:1974), mineralne materije (Carić i sar., 2000), titraciona kiselost mleka po Soxhlet-Henkel-u (°SH) (Carić i sar., 2000), titraciona kiselost jogurta potenciometrijskom metodom (IDF 150:1991), i pH vrednost pH-metrom sa kombinovanom elektrodom model *Consort C 931*.

U toku skladištenja kod uzoraka jogurta izvršeno je ispitivanje sposobnosti vezivanja vode prema metodi Parnell-Clunies (Riener i sar., 2010). Takođe je vršeno ispitivanje sinerezisa (Riener i sar., 2010).

Viskozitet uzoraka jogurta tokom skladištenja određen je pomoću rotacionog viskozimetra: Visco Basic+R, Fungilab (Španija), prema metodi koju su opisali Vučić i sar. (2010).

Proizvodnja svih varijanti čvrstog jogurta od kozjeg mleka (A, B i C) ponovljena je tri puta.

REZULTATI I DISKUSIJA

Hemijski sastav mleka i čvrstog jogurta

Kod svih uzoraka ispitivan je sastav sirovog mleka, termički tretiranog mleka, kao i sastav jogurta tokom skladištenja (1, 7, 14, i 21-og dana). Kod uzoraka proizvedenih od kozjeg mleka uz dodatak 0.5% i 1% koncentrata proteina surutke takođe je izvršeno ispitivanje mleka nakon dodatka KPS.

Kod uzorka kozjeg jogurta uočen je porast sadržaja suve materije i suve materije bez masti nakon termičkog tretmana mleka što je posledica isparavanja dela vode u toku tretmana (Tabela 1).

Kod svih uzoraka čvrstog jogurta od kozjeg mleka uočava se smanjenje sadržaja suve materije prvog dana skladištenja što je u saglasnosti sa rezultatima drugih autora (Denin Đurđevic i sar., 2002a, 2002b; Vučić i sar., 2010, 2011). Takođe, tokom skladištenja jogurta uočava se blago smanjenje udela laktoze, što se može objasniti konverzijom laktoze od strane bakterija mlečne kiseline.

Promene pH vrednosti i titracione kiselosti jogurta tokom skladištenja

Promena pH vrednosti je praćena tokom 21 dana skladištenja a dobijeni rezultati prikazani su na slici 1.

pH vrednost kozjeg jogurta se kretala od 4,42 prvog dana skladištenja do 4,27 nakon 21 dana skladištenja.

Tokom ispitivanog perioda skladištenja najizraženiju promenu pH vrednosti imali su uzorci jogurta proizvedeni od kozjeg mleka sa dodatkom 1% KPS. Najveći pad pH vrednosti zabeležen je u periodu od 1. do 7. dana skladištenja i iznosio je 0,36 pH jedinica, respektivno.

Međutim kod uzoraka jogurta od kozjeg mleka proizvedenih sa dodatkom 0,5% i 1% KPS u poslednjih sedam dana skladištenja došlo je do povećanja pH vrednosti za 0,05 i 0,03 pH jedinica, respektivno, što je verovatno rezultat proteolitičkih promena.

Tokom ispitivanog perioda skladištenja ispitivana je kiselost kozjeg jogurta (Slika 2).

Kod uzoraka čvrstog jogurta od kozjeg mleka (A) uočen je postepen porast titracione kiselosti tokom skladištenja,od 0,71% m.k. prvog dana do 0,82% m.k. dvadeset prvog dana skladištenja. Najmanja promena titracione kiselosti tokom skladištenja zabeležena je kod uzoraka čvrstog jogurta proizvedenog od kozjeg mleka sa dodatkom 0,5% KPS (B). Kod ovih uzoraka takođe je zabeleženo smanjenje titracione kiselosti od 0,01% u periodu od 1. do 7. dana skladištenja. Najizraženije povećanje titracione kiselosti tokom skladištenja uočava se kod uzoraka čvrstog jogurta proizvedenog od kozjeg mleka sa dodatkom 1% KPS (C). U toku prvih sedam dana skladištenja titraciona kiselost se povećala za 0,11%. Najviša titraciona kiselost nakon ispitivanog perioda skladištenja zabeležena je kod uzoraka C – 0,86% m.k., što je verovatno posledica povećanog rasta BMK kod uzoraka proizvedenih sa dodatkom KPS (Herrero i Requena, 2006).

Sinerezis i sposobnost vezivanja vode čvrstog jogurta

Sinerezis predstavlja izdvajanje tečne faze iz gela i zavisi od termičkog tretmana mleka, sadržaja suve materije, upotrebljenih stabilizatora, brzine acidifikacije, temperature acidifikacije, vrste startera i kiselosti koja nastaje kao rezultat delovanja bakterija mlečne kiseline (Denin Đurđević, 2001).

Sinerezis uzorka čvrstog jogurta od kozjeg mleka je ispitivan 1., 7., 14. i 21. dana skladištenja, a rezultati dobijeni u ovom delu istraživanja prikazani su na Slici 3.

Tabela 1. HEMIJSKI SASTAV UZORAKA ČVRSTOG KOZJEG JOGURTA

Table 1. CHEMICAL COMPOSITION OF GOAT'S YOGHURT SAMPLES

		Pokazatelj/ Parameter					
		SM/TS (%)	Mast/Fat (%)	SMbM/ TSNF (%)	Proteini/ Proteins (%)	Laktoza/ Lactose (%)	Pepeo/Ash (%)
A	Sirovo mleko/Raw milk	11,13±0,03	2,80±0,00	8,33±0,03	2,82±0,13	4,16±0,19	0,80±0,01
	Term. tret. mleko/Heat treated milk	11,64±0,19	3,00±0,00	8,64±0,19	2,92±0,06	4,69±0,00	0,81±0,09
	Jogurt 1.dan/Yoghurt 1st day	10,87±0,07	2,64±0,24	8,23±0,28	2,85±0,09	4,05±0,08	0,82±0,04
	Jogurt 7. dan/Yoghurt 7th day	10,88±0,11	2,86±0,24	8,02±0,13	3,01±0,05	4,12±0,08	0,84±0,01
	Jogurt 14. dan/Yoghurt 14thday	10,93±0,04	2,97±0,12	7,96±0,15	2,97±0,02	3,98±0,21	0,84±0,01
	Jogurt 21.dan/Yoghurt 21st day	10,97±0,10	2,86±0,24	8,11±0,14	3,02±0,05	3,77±0,23	0,86±0,03
B	Sirovo mleko/Raw milk	11,16±0,03	3,00±0,00	8,16±0,03	2,56±0,03	4,47±0,00	0,77±0,00
	0.5% KPS/0.5% WPC	11,46±0,03	2,93±0,06	8,53±0,09	2,91±0,05	4,44±0,05	0,81±0,01
	Term. tret. mleko/Heat treated milk	11,73±0,08	3,00±0,00	8,73±0,08	3,00±0,04	4,62±0,00	0,83±0,01
	Jogurt 1.dan/Yoghurt 1st day	11,14±0,09	2,64±0,00	8,50±0,09	3,02±0,04	4,15±0,00	0,83±0,01
	Jogurt 7. dan/Yoghurt 7th day	10,92±0,03	2,57±0,13	8,35±0,11	2,96±0,10	4,09±0,05	0,82±0,00
	Jogurt 14. dan/Yoghurt 14thday	11,06±0,14	2,42±0,00	8,64±0,14	3,01±0,06	4,13±0,00	0,83±0,00
	Jogurt 21.dan/Yoghurt 21st day	11,04±0,04	2,49±0,13	8,55±0,15	3,07±0,01	4,28±0,00	0,83±0,00
C	Sirovo mleko/Raw milk	10,60±0,06	2,80±0,00	7,80±0,06	2,42±0,04	4,32±0,04	0,75±0,00
	1% KPS/1% WPC	11,24±0,06	2,80±0,00	8,44±0,06	3,24±0,12	4,34±0,00	0,78±0,01
	Term. tret. mleko/Heat treated milk	11,49±0,07	2,90±0,00	8,59±0,07	3,32±0,04	4,43±0,04	0,81±0,00
	Jogurt 1.dan/Yoghurt 1st day	11,04±0,10	2,64±0,00	8,50±0,09	3,02±0,04	4,22±0,05	0,83±0,01
	Jogurt 7. dan/Yoghurt 7th day	10,65±0,08	2,57±0,13	8,08±0,09	3,20±0,05	4,14±0,04	0,85±0,01
	Jogurt 14. dan/Yoghurt 14thday	10,99±0,02	2,75±0,00	8,24±0,02	3,21±0,04	4,06±0,18	0,92±0,02
	Jogurt 21.dan/Yoghurt 21st day	10,90±0,11	2,71±0,13	8,18±0,07	3,01±0,11	3,75±0,05	0,87±0,03

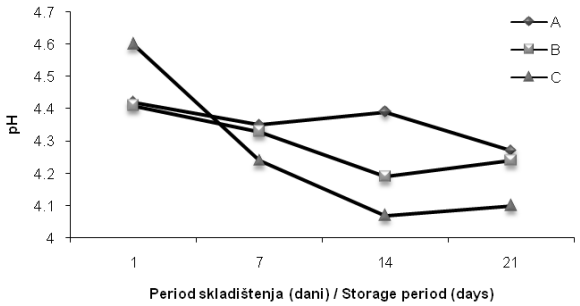
Legenda/Legend: A – čvrsti jogut od kozjeg mleka; B - čvrsti jogurt od kozjeg mleka sa dodatkom 0.5% KPS; C - čvrsti jogurt od kozjeg mleka sa dodatkom 1% KPS / A – set-style goat's yoghurt; B - set-style yogurt from goat's milk supplemented with 0.5% WPC; C - set-style yoghurt from goat's milk supplemented with 1% WPC

SM-suva materija / TS-total solids

SMbM-suva materija bez masti / TSNF(non fat)-total solids

Kod svih uzoraka čvrstog kozjeg jogurta u period od 1. do 14. dana skladištenja, zabeleženo je smanjenje sinerezisa, dok je u poslednjih sedam dana skladištenja uočeno izdvajanje veće količine surutke. Najveći sinerezis 21. dana skladištenja zabeležen je kod uzoraka C – 16,25mL. Izraženiji sinerezis 21. dana skladištenja je rezultat formiranja slabih veza u proteinskom matriksu tokom starenja gela.

Dodatakom koncentrata proteina surutke u mleko, u toku fermentacije dolazi do formiranja nežnijeg gela sa manje izraženim sinerezisom (Tamime i Robinson, 2000; Tratnik, 1998). Rezultati dobijeni u ovom delu istraživanja pokazuju da je sinerezis najslabije izražen kod uzoraka čvrstog jogurta proizvedenog od kozjeg mleka sa dodatkom 0,5% KPS. Najmanja količina izdvojene surutke zabeležena je 14. dana skladištenja i iznosila je 14,25 mL.



Slika 1. PROMENA PH VREDNOSTI UZORAKA JOGURTA TOKOM SKLADIŠTENJA, A - ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

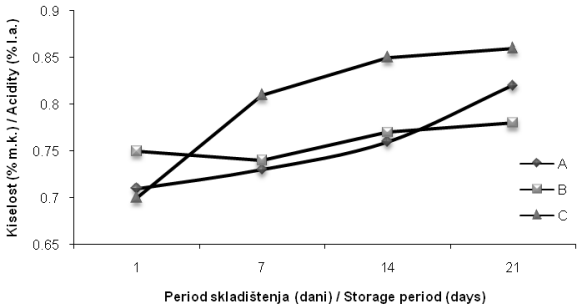
Figure 1. CHANGE OF PH VALUE IN YOGHURT SAMPLES DURING STORAGE, A – SET-STYLE GOAT'S YOGHURT; B - SET-STYLE YOGURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC

Najveću sposobnost vezivanja vode (Slika 4.) pokazuju uzorci čvrstog jogurta proizvedeni od kozjeg mleka sa dodatkom 1% KPS. Tokom ispitivanog perioda skladištenja kod uzoraka C je zabeležena minimalna promena sposobnosti vezivanja vode od 50,08% prvog dana do 49,76% dvadeset prvog dana skladištenja. Nasuprot tome, uzorci čvrstog jogurta proizvedeni od kozjeg mleka sa dodatkom 0,5% KPS imali su sličnu sposobnost vezivanja vode kao i uzorci čvrstog kozjeg jogurta tokom celokupnog perioda skladištenja.

Viskozitet čvrstog jogurta tokom skladištenja

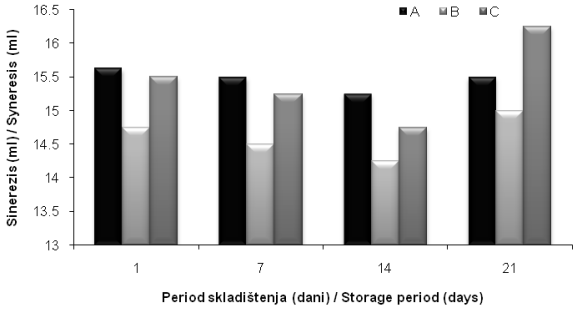
U ovoj fazi istraživanja ispitivan je uticaj vremena na promenu viskoziteta čvrstog jogurta proizvedenog od kozjeg mleka bez i sa dodatkom 0,5%, odnosno 1% KPS. Uzorci su ispitivani nakon 1, 7, 14 i 21 dana skladištenja.

Na slici 5. prikazana je promena viskoziteta uzoraka čvrstog jogurta u zavisnosti od vremena izlaganja konstantnoj sili smicanja (brzina obrtaja spindla 20 ob/min) prvog dana skladištenja.



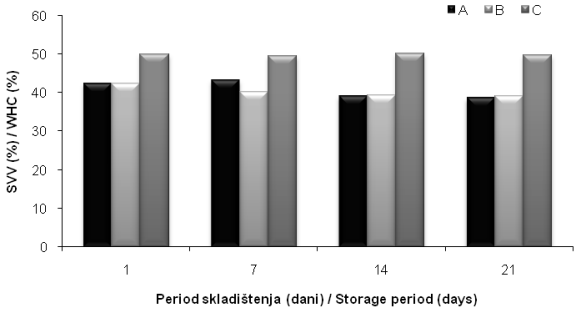
Slika 2. PROMENA TITRACIONE KISELOSTI UZORAKA JOGURTA TOKOM SKLADIŠTENJA, A – ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 2. CHANGE OF TITRABLE ACIDITY IN YOGHURT SAMPLES DURING STORAGE, A - SET-STYLE GOAT'S YOGHURT; B - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC



Slika 3. SINEREZIS UZORAKA JOGURTA, A - ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 3. SYNERESIS OF YOGHURT SAMPLES, A - SET-STYLE GOAT'S YOGURT; B - SET-STYLE YOGURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC



Slika 4. SPOSOBNOST VEZIVANJA VODE (SVV) UZORAKA JOGURTA, A - ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0,5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 4. WATER HOLDING CAPACITY (WHC) OF YOGHURT SAMPLES, A – SET-STYLE GOAT'S YOGHURT; B - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC

Nakon 1 dana skladištenja uzorci čvrstog kozjeg jogurta (A) pokazuju značajno smanjenje viskoziteta tokom merenja. Prva izmerena vrednost viskoziteta, nakon 30 s, bila je 2463,2 mPas, dok je nakon tri minuta vrednost viskoziteta opala na 810,2 mPas, što znači da je smanjenje viskoziteta iznosilo 1653,0 mPas. Ovako izraženo smanjenje viskoziteta uzoraka A rezultat je naglog narušavanja strukture, pa je nakon 180 s vrednost viskoziteta ovih uzoraka najmanja. Nasuprot tome, uzorci čvrstog jogurta proizvedeni od kozjeg mleka sa dodatkom 0,5% KPS (B) i 1% KPS (C) pokazuju linearno smanjenje viskoziteta tokom vremena. Na osnovu ovoga možemo zaključiti da manja početna vrednost viskoziteta uzoraka čvrstog jogurta proizvedenih od kozjeg mleka sa dodatkom KPS ima za posledicu i manje smanjenje viskoziteta tokom vremena. Takođe, povećan sadržaj proteina dovodi do intenzivnijih interakcija i povećanja protein-protein veza u gelovima proizvedenim sa dodatkom KPS, što povećava njihovu elastičnost (Damin i sar., 2009).

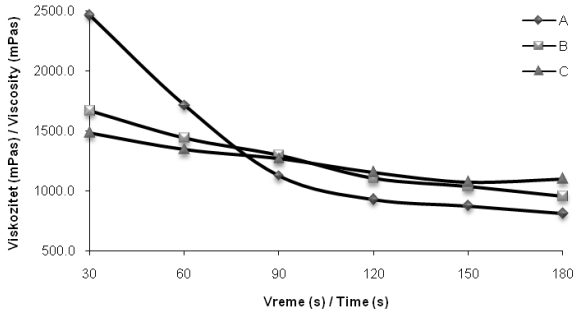
Promena viskoziteta čvrstog jogurta od kozjeg mleka sedmog dana skladištenja prikazana je na Grafikonu 4.

Sedmog dana skladištenja, najveću vrednost viskoziteta nakon tokom 180s delovanja sile imali su uzorci čvrstog kozjeg jogurta (A) - 1992,0 mPas. Uzorci čvrstog jogurta proizvedeni od kozjeg mleka sa dodatkom 1% KPS imali su najniže vrednosti viskoziteta. Nakon 30 s delovanja sile prosečan viskozitet uzoraka C je iznosio 1438,1 mPas, a vrednost viskoziteta nakon 180 s iznosila je 1239,6 mPas. Ukupno smanjenje viskoziteta tokom vremena, iznosilo je 198,5 mPas.

U odnosu na prvi dan skladištenja svi ispitivani uzorci su imali manje vrednosti viskoziteta. Usled promena proteinskog matriksa tokom skladištenja, nakon 7 dana kod svih uzoraka je zabeleženo manje narušavanje strukture tokom delovanja sile.

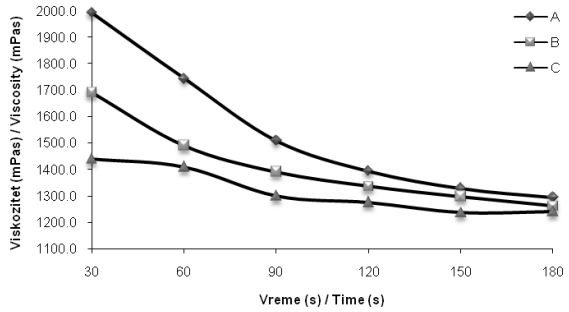
Na slici 7 prikazana je promena viskoziteta uzoraka čvrstog jogurta 14. dana skladištenja.

Iz podataka prikazanih na slici 7 može se uočiti da uzorci C pokazuju odstupanja u tiksotropnom ponašanju kiselog kazeinskog gela. Nakon 90 s zabeležena je najniža vrednost viskoziteta – 1027,2 mPas, što je za 157,1 mPas niža vrednost nego nakon 180 s delovanja sile. Najveće vrednosti vis-



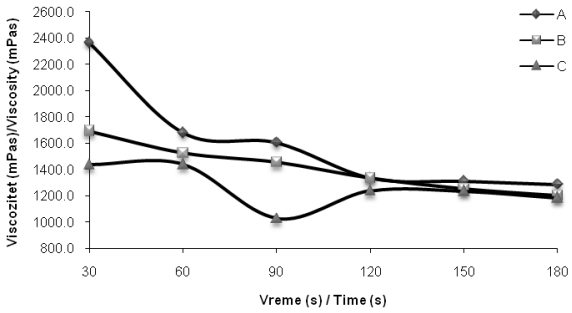
Slika 5. PROMENA VISKOZITETA UZORAKA JOGURTA 1. DANA SKLADIŠTENJA, A – ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 5. VISCOSITY CHANGE OF YOGHURT SAMPLES ON THE 1ST DAY OF STORAGE, A – SET-STYLE GOAT'S YOGURT; B - SET-STYLE YOGURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC



Slika 6. PROMENA VISKOZITETA UZORAKA JOGURTA 7. DANA SKLADIŠTENJA, A - ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 6. VISCOSITY CHANGE OF YOGHURT SAMPLES ON THE 7TH DAY OF STORAGE, A - SET-STYLE GOAT'S YOGURT; B - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC



Slika 7. PROMENA VISKOZITETA UZORAKA JOGURTA 14. DANA SKLADIŠTENJA, A - ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 7. VISCOSITY CHANGE OF YOGHURT SAMPLES ON THE 14TH DAY OF STORAGE, A - SET-STYLE GOAT'S YOGHURT; B - SET-STYLE YOGURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC

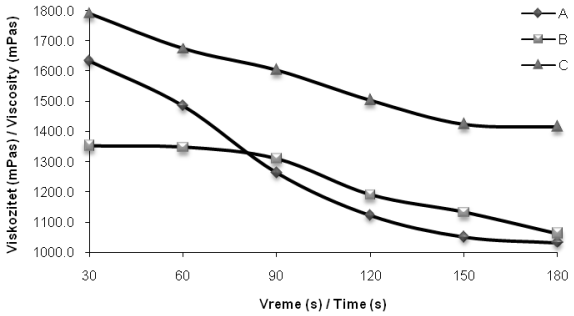
koziteta, kao i prvog i sedmog dana skladištenja zabeležene su kod uzoraka A, što je u saglasnosti sa istraživanjima Jelen i sar. (1987), koji su u svojim ispitivanjima proizvodili čvrsti jogurt od mleka sa izmenjenim odnosom kazein: serum proteini, i došli do zaključka da se sa povećanjem sadržaja serum proteina smanjuje viskozitet i čvrstina gotovog proizvoda.

Promena viskoziteta čvrstog jogurta od kozjeg mleka 21. dana skladištenja prikazana je na slici 8.

Nakon 21 dana skladištenja, svi ispitivani uzorci čvrstog kozjeg jogurta pokazuju tiksotropno ponašanje. Uzorci čvrstog jogurta proizvedeni od kozjeg mleka sa dodatkom 0.5% KPS (B) imali su najmanju izmerenu početnu vrednost viskoziteta (1352,4 mPas), koja je za 315,5 mPas, 337,9 mPas i 340,8 mPas manja nego kod gelova starih 1, 7 i 14 dana, respektivno. Iako su uzorci C tokom skladištenja 1., 7. i 14. dana imali najmanje zabeležene vrednosti viskoziteta, nakon 21 dana skladištenja viskozitet ovih uzoraka viši je u odnosu na uzorke A i B. Takođe, 21. dana skladištenja zabeležena je najviša početna vrednost viskoziteta uzoraka čvrstog jogurta proizvedenog od kozjeg mleka sa dodatkom 1% KPS – 1790,2 mPas, dok je nakon 180 s vrednost viskoziteta iznosila 1416,0 mPas.

ZAKLJUČAK

Na osnovu rezultata dobijenih u ovim istraživanjima može se zaključiti da je najslabije izražen sinerezis zabeležen 14. dana skladištenja kod svih proizvedenih uzoraka čvrstog jogurta od kozjeg mleka. Najmanju sklonost ka izdvajanju surutke tokom ispitivanog perioda skladištenja imali su uzorci čvrstog jogurta proizvedeni od kozjeg mleka sa dodatkom 0,5% KPS. Najveća sposobnost vezivanja vode zabeležena je kod uzoraka čvrstog jogurta proizvedenih od kozjeg mleka sa dodatkom 1% KPS. Najviše prosečne vrednosti viskoziteta u periodu od prvog do četrnaestog dana skladištenja imali su uzorci čvrstog jogurta od kozjeg mleka, dok je 21. dana skladištenja najviši viskozitet zabeležen kod uzoraka čvrstog jogurta proizvedenog od kozjeg mleka sa dodatkom 1% KPS.



Slika 8. PROMENA VISKOZITETA UZORAKA JOGURTA 21. DANA SKLADIŠTENJA, A - ČVRSTI JOGURT OD KOZJEG MLEKA; B - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 0.5% KPS; C - ČVRSTI JOGURT OD KOZJEG MLEKA SA DODATKOM 1% KPS

Figure 8. VISCOSITY CHANGE OF YOGHURT SAMPLES ON THE 21ST DAY OF STORAGE, A – SET-STYLE GOAT'S YOGHURT; B - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 0.5% WPC; C - SET-STYLE YOGHURT FROM GOAT'S MILK SUPPLEMENTED WITH 1% WPC

ZAHVALNICA

Rad je deo istraživanja na projektu III 46009 finansiranom od strane Ministarstva prosvete i nauke Republike Srbije.

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SUMMARY

THE INFLUENCE OF WHEY PROTEIN CONCENTRATES ON CHARACTERISTICS OF SET-STYLE YOGHURT MADE FROM GOAT MILK DURING STORAGE

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The possibility of whey protein concentrates application in the production of goat milk's set yoghurt was investigated. Three series of samples were produced: A – set-style goat's yoghurt; B - set-style yoghurt from goat's milk supplemented with 0.5% WPC; C - set-style yoghurt from goat's milk supplemented with 1% WPC.

Physicochemical properties and viscosity of produced samples were investigated on the 1st, 7th, 14th and 21st day of storage.

Due to data analysis it was concluded that the highest viscosity on 1st, 7th and 14th day of storage had set-style goat's yoghurt samples (A), while on 21st day of storage highest viscosity was recorded in samples of set-style yoghurt from goat's milk supplemented with 1% WPC (C). The highest water holding capacity during storage period had samples C, while the least syneresis had samples B.

Key words: goat milk • set-style yoghurt • whey protein concentrates • viscosity

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UDK: 640.23: [637.03+637.12

PRISTUPI MERENJU ODRŽIVOSTI PROIZVODNJE MLEKA

U radu se istražuju pristupi, načini i indikatori merenja održivosti poljoprivrednih gazdinstava sa dominantnom linijom proizvodnje kravljeg mleka. Koncept održivog razvoja poljoprivrede nije definitivan i očekuje se njegov dalji razvoj. Ključna posledica evolucije koncepta održivog razvoja je da su do sada primenjivane metode za merenje održivosti zasnovane na znatnom pojednostavljivanju teorije. Kvalitetan odabir indikatora u okviru ekonomske, ekološke i društvene dimenzije održivosti, kao i primena savremenijih metoda omogućuje adekvatnije merenje i praćenje održivosti proizvodnje mleka na nivou poljoprivrednog gazdinstva.

Ključne reči: poljoprivredno gazdinstvo • proizvodnja mleka • održivost proizvodnje • indikatori

UVOD

Kroz istoriju su ostale zabeležene mnoge civilizacije koje su nestale zbog neodrživosti korišćenih sistema proizvodnje hrane. Takvi sistemi poljoprivredne proizvodnje su tokom vremena najčešće dovodili do erozije zemljišta, iscrpljivanja hranljivih materija u zemljištu i visoke zaslanjenosti poljoprivrednog zemljišta. Kada bi korišćene površine postale neupotrebljive za poljoprivrednu proizvodnju, preostajalo bi je još uvek dovoljno rezervnog zemljišta gde se tadašnje stanovništvo moglo preseliti i nastaviti sa poljoprivrednom proizvodnjom. Međutim, danas su takve mogućnosti iscrpljene, a luksuz u praktikovanju sistema neodržive poljoprivredne proizvodnje, u ovom slučaju sa ekološkog aspekta, postaje nedopustiv.

Savremeni trendovi u vidu rasta broja stanovnika, promene navika u potrošnji hrane i urbanizacija stvaraju veliki pritisak na strani tražnje za hranom. Na strani ponude, proizvodnja hrane je u prethodnim decenijama bila praćena ukrupnjavanjem poljoprivrednih gazdinstava u razvijenim zemljama i sve većom upotrebom onih inputa i metoda proizvodnje koji ostavljaju negativne posledice na životnu sredinu. Razvoj svesti o neodrživosti ovakvog načina proizvodnje hrane u svetu doveo je u poslednjih nekoliko decenija do kreiranja koncepta održivog razvoja, koji je postao vodeća paradigma za kreatore politike i istraživače (Van Passel, 2006). Prema Perman, Ma i McGilvray (pomenuto kod Van Passel, 2008) koncept održivosti je etičke prirode i proističe iz brige za budućnost narednih generacija. Samo pominjanje budućnosti poljoprivredne proizvodnje, a samim tim i budućnosti osnovnih jedinica proizvodnje, poljoprivrednih gazdinstava, uz multidimenzionalnost, dinamičnost i globalni karakter, čini ceo koncept održivosti kompleksnim i teškim za merenje.

Definisanje koncepta održivosti

Najčešće korišćena definicija održivosti je ona koju je Svetska komisija za životnu sredinu i razvoj utvrdila 1987. godine. Prema toj definiciji: „Održivi razvoj je razvoj koji omogućuje sadašnjoj generaciji da zadovolji svoje potrebe bez kompromisa sa mogućnostima budućih generacija da zadovolje svoje sopstvene potrebe“. Iako se ova definicija često koristi u naučnoj literaturi, njeno značenje nije precizno i operativno, pa stoga njeno razumevanje je različito kroz prostor i vreme, kao i među pojedincima (Dillon, Hennessy i Hines, 2009). Osim toga, kompleksnost samog koncepta „potreba“ otežava primenu ekonomske analize.

Pored pomenute definicije postoji i preko 60 manje poznatih definicija održivosti (Kooten, Bulte, 2000). Jasno je, da nema potpune saglasnosti oko koncepta i definicije održivosti. Međutim, jedan ili više elemenata su uvek prisutni u svim pristupima konceptu održivosti. U pitanju su: (i) ograničenost prirodnih resursa, gde postoje limiti u kapacitetima njihove upotrebe u okviru ekosistema planete, (ii) ekonomski, ekološki i društveni ciljevi se moraju ostvarivati u okviru ovih limita, (iii) postoji potreba za unutar i među generacijskim pravom na raspolaganje resursima istog kvantiteta i kvaliteta (Van Passel, 2007).

Održivost poljoprivredne proizvodnje se bazira na tri opšte prihvaćene dimenzije: ekonomskoj, ekološkoj i društvenoj održivosti, kao i na interakciji među njima. Međuzavisnost između dimenzija održive poljoprivredne proizvodnje je visoka. Rezultati dosadašnjih istraživanja su pokazali da su veze između pojedinih dimenzija jake, brojne i kompleksne (EC, 2001).

Ekološka dimenzija obuhvata upravljanje prirodnim resursima, na način koji će obezbediti njihovu jednaku dostupnost u budućnosti, kao i čitav niz drugih elemenata. Prvenstveno se

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misli na: zaštitu pejsaža, životnih staništa, biodiverziteta i obezbeđenje kvaliteta vode za piće i vazduha. Ekonomska dimenzija se odnosi na efikasnu upotrebu resursa, konkurentnost i održivost sektora poljoprivrede i njegov doprinos održivosti ruralnih područja. Efikasne poljoprivredne strukture, adekvatne tehnologije i diverzifikacija izvora dohotka za poljoprivredna domaćinstva, jesu bitni elementi ove dimenzije. Društvena dimenzija obuhvata pitanja o mogućnostima zaposlenja radne snage i pristupa resursima i uslugama poljoprivrednih domaćinstava u poređenju sa drugim ekonomskim jedinicama u ruralnim područjima. Elementi poput jednakosti mogućnosti, kao i društvena briga o etičnosti korišćenih metoda u poljoprivrednoj proizvodnji, takođe pripadaju ovoj dimenziji (EC, 2001).

Iako merenje održivosti nije nimalo lak zadatak, većina autora se slaže da je korak ka merenju održivosti izuzetno bitan, jer predstavlja konkretizaciju i promovisanje koncepta. Nužna posledica toga jeste pojednostavljivanje kako koncepta, tako i karakteristika sistema koji se meri.

Dosadašnji pristupi merenju karakteristika održivosti poljoprivredne proizvodnje mogu se razvrstati u dve grupe. Prvoj grupi pripadaju metode koje su orijentisane ka merenju opterećenosti, tj. merenju troškova negativnog uticaja na životnu sredinu koji nastaju upotrebom resursa u jednoj ekonomskoj aktivnosti u odnosu na drugu. Drugu grupu čine metode usmerene na procenu vrednosti koja je kreirana sa datim resursima i datim uticajem na životnu sredinu u odnosu na vrednost kreiranu sa identičnim resursima u alternativnim proizvodnim procesima (Mondelaers, Van Huylenbroeck, Lauwers, 2011). Za metode iz prve grupe je karakteristično da se ređe primenjuju u kreiranju i merenju efekata agrarne politike, zbog toga što su orijentisani na negativne efekte po životnu sredinu, koji nastaju upotrebom resursa, a ne na vrednost koja je kreirana upotrebom tih resursa. Druga grupa, metoda usmerenih na procenu vrednosti upotrebe resursa, koristi jedan vrednosni pokazatelj za veći skup varijabli jednog proizvodnog procesa (ekonomski, društveni i efekti na životnu sredinu), što im daje izvesnu prednost u praktičnoj primeni.

Postoji više nivoa na kojima se može meriti održivost poljoprivredne proizvodnje: globalni, regionalni, nacionalni i mikro nivo. Prema Bachev (2005) merenje održivosti poljoprivredne proizvodnje na mikro nivou, tj. nivou jednog poljoprivrednog gazdinstva, podrazumeva njegovu sposobnost da se održi tokom više narednih generacija.

Merenje održivosti se operativno sprovodi putem seta odabranih indikatora. Pod indikatorom održivosti podrazumeva se varijabla, koja omogućuje opisivanje, merenje i nadgledanje procesa, stanja i tendencija sistema na više nivoa. Citirajući druge autore Walker (2002) ističe, da treba imati u vidu, da jedan indikator najčešće predstavlja samo jednu od čitavog seta varijabli u okviru svake od tri dimenzije održivosti jednog proizvodnog procesa. To znači, da se odabirom nekoliko indikatora praktično vrši „veliko pojednostavljivanje“ ukupnog obima efekata.

Značaj merenja održivosti proizvodnje mleka

Postoji više razloga zbog kojih je održivost poljoprivrednih gazdinstava koja se bave proizvodnjom mleka u fokusu nauke tokom poslednjih godina. Na prvom mestu je učešće proizvodnje mleka, na globalnom nivou, u emisiji gasova koji doprinose efektu staklene bašte. Zatim, veliki broj stanovnika na planeti živi na gazdinstvima koja proizvode mleko. Procene se kreću čak i do 1 milijarde stanovnika koji direktno zavise od proizvodnje mleka, što utiče na značaj ekonomske i društvene dimenzije održivosti njihovih poljoprivrednih gazdinstava.

Poljoprivreda je jedan od glavnih sektora koji doprinose globalnom zagrevanju, pri čemu proizvodnja mleka ima pojedinačno najveći doprinos tom procesu (Mondelaers, Van Huylenbroeck, Lauwers, 2011). Štetni gasovi koji se emituju iz proizvodnje mleka su: metan (CH_4), azotsuboksid (N_2O) i ugljendioksid (CO_2). Da bi podaci o emisiji pomenutih gasova bili uporedivi, u praksi se najčešće koristi metod obračuna emisije metana i azotsuboksida u ekvivalentne količine ugljendioksida. Metan ima najveći udeo koji se kreće od 55 do 69% (Hagemann i drugi, 2011), pri čemu najvećim delom nastaje u procesu fermentacije u buragu krava, a manjim delom iz stajnjaka. Na drugom mestu se nalazi azotsuboksid sa udeom koji se kreće u rasponu od 22 do 32%.

Naravno, osim emisije štetnih gasova proizvodnja mleka potencijalno može imati još negativnih uticaja na životnu sredinu. Neki od mogućih negativnih uticaja su: neizbalansiran odnos dodatih nutrijenata (N, P i K) poljoprivrednom zemljištu putem stajnjaka i osoke i potreba uzgajanih useva za nutrijentima, zatim, efekti na kvalitet vode za piće i vazduha, erozija zemljišta, narušavanje biodiverziteta, itd. Svi navedeni negativni uticaji posebno dobijaju na značaju zbog prisutnog procesa restrukturiranja poljoprivrednih gazdinstava u većem broju zemalja. Restrukturiranje podrazumeva smanjenje broja gazdinstava koja se bave proizvodnjom mleka, povećanje broja krava po jednom gazdinstvu i izbor načina obavljanja proizvodnje. U nekim zemljama, poput SAD, Novog Zelanda, Brazila i Japana, prisutan je rapidan rast upravo onih gazdinstava koja imaju najveći broj krava i čiji kapaciteti se mere u hiljadama grla.

Proizvodnja kravljeg mleka je visoko značajna ukoliko se posmatra sa stanovišta ekonomske održivosti gazdinstava. Na primeru Republike Srbije, to znači da je oko ¼ ruralnog stanovništva direktno zavisno od ekonomskih rezultata koje postižu proizvođači mleka na svojim gazdinstvima. Procena je zasnovana na 269.000 poljoprivrednih gazdinstava (Goss i drugi, 2010) koja se bave proizvodnjom kravljeg mleka, što je oko 50% svih poljoprivrednih gazdinstava, i prosečnom broju članova domaćinstva u ruralnim regionnima (RZS, Popis 2011.).

Ranija istraživanja su u većem obimu bila fokusirana na ekonomsku i ekološku održivost, dok je društvena održivost manje razvijana. Društvena održivost se može dvostrano posmatrati. S jedne strane, postoji odgovornost društva prema proizvođačima mleka (mere agrarne politike), koja se ogleda u kreiranju jednakosti mogućnosti sa drugim subjektima u ruralnim područjima i ostvarivanje zadovoljavajućeg životnog standarda. Tu spada i spremnost potrošača da plate više cene za proizvode iz održive proizvodnje (Aistars, 1999). S druge strane, na nivou poljoprivednih gazdinstava često postoje etički aspekti u odabiru metoda proizvodnje mleka (npr. Upotreba hormona, blagostanje životinja, radni uslovi poljoprivrednika i sl.). Dimenzija društvene održivosti obuhvata i sposobnost očuvanja postojećih i kreiranje novih radnih mesta u proizvodnji mleka.

Tabela 1. IFCN (INTERNATIONAL FARM COMPARISON NETWORK) PRISTUP MERENJU ODRŽIVOSTI POLJOPRIVREDNIH GAZDINSTAVA KOJA SE BAVE PROIZVODNJOM MLEKA.

Table 1. IFCN APPROACH TO SUSTAINABILITY IN DAIRY FARMS.

Dimenzija održivosti	Oblast održivosti	Indikator	Granična vrednost održivosti
Ekonomska	Ekonomske performanse	Preduzetnički profit	-1 ≥ 1$/100kg mleka ECM*
	Rizik	Marža operativnog profita	Od 12% do 15%
	Konkurentnost resursa	Prinosa na radnu snagu/prosečna zarada	Od 90% do 110%
Ekološka	Emisija štetnih gasova	Emisija ugljen dioksida	130 do 140 g CO_2 ekv./100 kg mleka ECM
	Upotreba resursa	Direktna i indirektna potrošnja vode	1.800 do 2.000 l vode/kg mleka ECM
	Intenzivnost proizvodnje	Broj grla/ha	1 do 1,2 grla/ha
Društvena	Politička podrška	Udeo subvencija u dohotku polj. Gazdinstva	25% do 30%
	Radni uslovi	Godišnji broj radnih sati	2.000 do 2.200 sati godišnje
	Kreiranje radnih mesta	Broj radnika/100t proizvedenog mleka	0,9 ≥ 1,1 Uslovnih radnika

*ECM – Energy corrected milk

Jedan, od brojnih pristupa merenju održivosti proizvodnja mleka na nivou poljoprivrednog gazdinstva definisali su Assah, Steglich i Hemme (2011). U pitanju je trostepeni postupak. Prvo se određuju oblasti održivosti koje će se meriti. Drugi korak podrazumeva odabir adekvatnih i merljivih indikatora za svaku od odabranih oblasti. Na kraju, neophodno je odrediti granice za svaki indikator, na osnovu kojih je moguće jasno razlikovati održiva od neodrživih poljoprivrednih gazdinstava. U tabeli 1 dat je prikaz ovog pristupa s merljivim indikatorima. Granični opsezi vrednosti indikatora su postavljeni tako da omogućavaju merenje na globalnom nivou održivosti gazdinstava koja se bave proizvodnjom kravljeg mleka. Ukoliko su izmerene vrednosti svih indikatora nekog gazdinstva bolje od graničnih, to ukazuju na viši stepen održivosti poljoprivrednog gazdinstva. Dodatno kalibriranje dobijenih rezultata se obavlja sa skalom ocenjivanja od -5 do 5, pri čemu se vrednost 0 dodeljuje graničnom opsegu. Autori naglašavaju da je u nekim slučajevima bolje koristiti lokalno definisane granične vrednosti indikatora od globalnih.

Metod održive vrednosti koji su izgradili Figge i Hahn (2004), dodatno su razradili sa inkorporisanjem proizvodne funkcije Mondelaers, Van Huylenbroeck, Lauwers (2011). Metod pripada savremenijem pristupu, a podrazumeva ocenu performansi održivosti jednog poljoprivrednog gazdinstva na bazi vrednosti koju je moguće kreirati upotrebom istih resursa u alternativnom proizvodnom procesu. Dve nove dimenzije su karakteristične za ovaj metod. Na prvom mestu, metod pripada grupi pristupa koji su orijentisani na procenu krirane vrednosti. Drugo, integriše principe finansijske ekonomije i procenjuje upotrebu resursa sa stanovišta investitora.

Primenom metoda održive vrednosti utvrđuje se prosečna produktivnost kao količnik godišnjeg prihoda poljoprivrednog gazdinstva i svakog pojedinačnog indikatora (B), koja se zatim poredi sa standardom (C). Ukoliko je produktivnost pojedinačnog indikatora viša od standardne produktivnosti, ta-

Tabela 2. PRIMENA METODA ODRŽIVE VREDNOSTI U PROIZVODNJI MLEKA

Table 2. ELEMENTS OF SUSTAINABLE VALUE METHOD IN DAIRY FARM

Tip kapitala	Vrsta kapitala	Jedinica mere (j.m.)	Poljoprivr. gazdinstvo	Granična produktivnost (Prihod / j.m.)		Kreirana vrednost (€)	Održiva vrednost
				Polj. gazd.	Benčmark		
			A	B	C	D=(B-C)*A	E=∑D/7
Ekološki	Voda	m^3					
	Emisija CO_2	tona CO2 ekv.					
	Izlučivanje N	kg/ha					
Društveni	Zemljište	ha					
	Radna snaga	radni časovi					
Proizvedeni	Kapital polj. gazdinstva	€					
	Koncentrat	tona					
							+ / -

Izvor: Mondelaers, Van Huylenbroeck, Lauwers (2011)

da gazdinstvo kreira održivu vrednost i doprinosi održivosti. U obrnutom slučaju niža produktivnost nekog indikatora od standardne doprinosi narušavanju održive vrednosti.

Merenje održivosti poljoprivredne proizvodnje na nivou gazdinstva i na nivou jedinice proizvoda može imati kontradiktorne rezultate. Usled rasta veličine specijalizovanih poljoprivrednih gazdinstava u nekoj od linija stočarske proizvodnje dolazi do njihovog sve većeg negativnog uticaja na životnu sredinu, zbog koncentrisanog uticaja na manje područje. Međutim, istraživanje koje je sproveo Hagemann i drugi, 2011. godine pokazalo je da intenzivna proizvodnja mleka ima manje negativne uticaje na životnu sredinu ukoliko se nivo uticaja meri po litri proizvedenog mleka.

U jednom broju istraživanja (Dillon i ostali, 2009; Van Calker, 2005) pokušano je da se izvrši integrisanje odabrane grupe indikatora u jedan zajednički (kompozitni) indikator, ali se taj pristup nije pokazao korisnim. Razlozi za to su ležali u međusobnom potiranju indikatora iz pojedinih dimenzija održivosti. Kao primer može poslužiti odnos indikatora održivosti životne sredine i ekonomske održivosti, koji su visoko negativno korelisani, pa se njihovi rezultati potiru u kompozitnom indikatoru.

ZAKLJUČAK

Istraživanja sprovedena tokom prethodne decenije označila su između ostalih i poljoprivredu, a u okviru nje proizvodnju mleka kao ključni izvor emisije gasova koji doprinose globalnom zagrevanju. Zbog toga je proizvodnja mleka i njena održivost na nivou poljoprivrednih gazdinstava često predmet istraživanja u mnogim zemljama.

Težnja poljoprivrednika da ostvare viši nivo dohotka, koji im omogućuje životni standard sličan domaćinstvima u nepoljoprivrednim sektorima, glavna je pokretačka snaga za rast prosečne veličine poljoprivrednih gazdinstava. Sa druge strane trend rasta cena hrane utiče i na zainteresovanost velikog broja investitora za pokretanje proizvodnje mleka sa većim kapacitetima. U našem okruženju postoji nekoliko takvih primera poput: BD Agro (2.100 grla), kao i veći broj mlekara srednjeg i većeg kapaciteta, čiji kapaciteti proizvodnje se mere od nekoliko stotina do nekoliko hiljada grla. Povećanje kapaciteta proizvodnje mleka neminovno ističe u prvi plan i potencijalne negativne ekološke posledice. Naravno, u ovakvim uslovima i dimenzija društvene održivosti trpi izvesne promene.

Koncept održivosti poljoprivredne proizvodnje nije definitivan i njegov dalji razvoj se očekuje u narednim godinama. Metode koje su do sada primenjivane mogu se grupisati u dva polja: metode orijentisane ka merenju opterećenja i metode usmerene na procenu kreirane vrednosti. Drugoj grupi metoda se daje operativna prednost jer se omogućavaju sumiranje vrednosti odabranih indikatora u jedinstvenu vrednosnu kategoriju. Osim razvoja metoda, jedno od ključnih polja u primeni koncepta održivosti, jeste pravilan odabir kvalitetnih indikatora. Pri tome je neophodna sistematska analiza kvaliteta pojedinih indikatora.

Merenje i monitoring održivosti proizvodnje mleka od posebne je važnosti za poljoprivrednike i kreatore mera agrarne politike. Rast svesti potrošača, dodatno utiče na prethodno pomenute dve grupe da doprinesu poboljšanju održivosti proizvodnje mleka.

ZAHVALNICA

Rad je deo istraživanja u okviru projekta „Unapređenje i razvoj higijenskih i tehnoloških postupaka u proizvodnji namirnica životinjskog porekla u cilju dobijanja kvalitetnih i bezbednih proizvoda konkurentnih na svetskom tržištu“, evidencioni broj III-46009, koji finansira Ministarstvo prosvete i nauke Republike Srbije.

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SUMMARY

APPROACHES TO SUSTAINABILITY OF MILK PRODUCTION

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Approaches, methods and indicators of dairy farm sustainability were examined in this paper. The concept of sustainable development is not definitive and it will develop in future period. As a consequence, used methods for sustainability assessment are based on simplification of theory. Quality selection of indicators of economic, environmental and social dimension of sustainability, as well as application of contemporary methods, enable better measurement and monitoring of dairy farm sustainability.

Key words: farm • milk production • sustainability

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SCIENTIFIC PAPER

UDK: 637.1:339.13

PRODUCTION OF SJENICA CHEESE TYPE IN INDUSTRIAL CONDITIONS

Traditionally, almost all white brined cheeses are made from raw sheep's milk or mixed sheep and goat, or cow and sheep milk. Today there is a tendency to change this and to produce cheese from pasteurized and standardized cow's milk and to add culture of lactic acid bacteria, except to indigenous cheeses, which require the use of sheep milk and indigenous culture. Given that the most indigenous cheeses have a problem of uneven texture and quality, the paper describes the type of Sjenica cheese where all operations also aimed to get a standard feature of cheese in dairy industry. Technology is specific compared to other cheeses: soft curd, formation of curd are not the same as for other cheeses, slowly and gradually separation of the whey, there is no temperature increase. In the first stage of whey draining selfpressing occurs and in the second phase there is slightly pressing forces (shaking curd) or slightly load. Salting has multiple roles in this group of cheeses, and it consists of two parts: the first salting is always dry salting, ripening in a brine, whose properties and concentrations of salt depend on the quality of cheese.

It was manufactured soft, full fat cheese (with a water content in fat-free matter from 70,17 to 71,07%, and fat in dry matter 49,32 to 51,38%), the standard form (triangular) and size (15x15x2-3 cm) and the weight of the slices (300-330 g), color white with a yellowish hue, closed texture with small holes, typical mild sour-salty taste with lactic acid flavour. Sensory evaluation placed cheese to the first and the extra class, which also shows high quality of cheese.

Key words: white cheese • Sjenica • standardized milk

INTRODUCTION

At the Sjenica-Pešter plateau is organized production of Sjenica cheese from sheep's milk, Sjenica cheese type from cow milk and mixed sheep and cow milk. These cheeses are declared as "Sjenica sheep cheese", "Sjenica cow cheese" and " Sjenica mixed cheese."

Sheep cheese is mainly produced on farms. The geographical area in which the production of Sjenica cheese is organized (the territory of the Municipality Sjenica and part of the territory of the Municipality of Tutin) grow from about 35.000 to 40.000 sheep. Often this number is significantly higher in summer period. Of the total number of sheep only a small number can not be milked (10-15%), so the estimation of number of sheep for effective production in this area is from around 30.000 to 35.000 sheep.

Cheese production per sheep in this area is in range from 10 to 12 kg, which is about 300 to 350 tons of Sjenica sheep cheese per year.

Mixed cheese is produced in much smaller quantities. It is estimated that the production is about 50 to 70 tons per year, mostly produced for a known customer. Mixed cheese production is mainly organized in farms.

Cow's milk cheese is produced in small plants (dairies), registered dairy workshops, as well as in farms. Cheese is produced from raw milk from distinctive geographical origin and by traditional technology.

In small dairies in Sjenica, cheese is made in five facilities: "Sandžak-komerc" about 120 t/year; "Beni-komerc" about 150 t/year, "Turković" Sjenica about 100 t/year; "Sjeničanka" about 80 t/year and "Fas" Sjenica about 50 t/year, which is in total about 500 t/year. From workshops and registrated farms, cheese production is organized in: SZR "Šanac" Sjenica about 15 t/year; SZR" Sjenički delikatesi" about 5 t/year, Radoslav Vranić farm about 5 t/year and Jakup Selmanović farm about 5 t/year, which is in total about 30 tons/year.

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Registered farms, which were not included in the purchase of milk because of bed roads and small processing capacities, also produce cheese. It is estimated that the annual production of these farms is about 400 to 450 tons of Sjenica cow cheese.

Therefore, it follows that the current annual production of Sjenica type cow´s cheese in the territory of Sjenica-Pešter plateau is about 900 to 1.000 tons, which is not a small amount in terms of financial effects.

About Sjenica cheese, as "the most famous Serbian white cheese", Zdanovski wrote in 1947. Since then, many studies about Sjenica cheese has been made by authors: Dozet et. al. (1996, 2004, 2006), Miočinović et. al. (1982), Jovanović et. al. (2004), Radulović (2004, 2005), Maćej et. al. (2006), Bogdanović et. al. (2004) and others.

Sjenica cheese type has been produced from cow's milk, The Serbian Pied races and Simmental cows, that have long been kept on pasture (which consists of plants with a special composition and flavor). With regard to cows feeding (greater part of pasture and hay) and the environment with special climate and other natural conditions, the milk is of specially good quality and has a great nutritional value. The cheese produced from such milk in the future will certainly have a high reputation and will have the increased demand on the market. Given that for satisfying dairy product placements, standard quality is especially important, our research aim was to ensure standardization of milk and cheese production and maturation in industrial conditions, which provides a more rational production and greater economic gains.

MATERIALS AND METHODS

Research included the recording and slight modification of industrial technology of Sjenica cheese in plant Beni Komerc in the area of Sjenica. The samples were collected from standardized and pasturized milk during 10 days of production. After the ripening of cheese, sensory evaluation were performed by five-member commission in terms of appearance, colour, taste, smell and texture, according to Havranek et al. 2003. Physicochemical properties of milk prior to cheese making and cheese after ripening (30 days) included the following methods and analyses (Carić et al., 2000):

- Determination of dry matter in milk and cheese using the standard method of drying at 102 ± 1 ºC;
- Determination of milk fat in cheese according to Van Gulik and in raw milk by Gerber;
- Determination of total nitrogen according to Kjeldahl method;
- Determination of casein content in milk by precipitation with acetic acid and sodium acetate;
- Determination of lactose content (gravimetrically);
- Determination of milk density with lactodensimeter;
- Determination of NaCl in the cheese, according to Mohr;
- Determination of titratable acidity according to Soxhlet-Henkel method;
- Determination of pH values using a pH meter, model SensIon+pH1 Portable pH Meter, Hach Company USA.

Figure 1. KEEPING ANIMALS ON PASTURE

Slika 1. DRŽANJE ŽIVOTINJA NA PAŠI

RESULTS AND DISCUSSION

Main direction of livestock production in Sjenica-Pešter plateau is the production of milk in order to improve processing in cheese. Cattle are held throughout the year in the stalls, during the growing season are put out to pasture every day. Keeping animals on pasture is not only important in terms of nutrition but also their health, as reflected in the quality of milk (Popović-Vranješ et al., 2011)

Table 1. PHYSICAL AND CHEMICAL PROPERTIES OF PASTEURIZED MILK FOR PRODUCTION OF SJENICA CHEESE TYPE (n = 10)

Tabela 1. FIZIČKE I HEMIJSKE OSOBINE PASTERIZOVANOG MLEKA ZA PROIZVODNJU SIRA U TIPU SJENIČKOG (n = 10)

Parameters / Parametri	Min.	Max.	Average / Prosečno
pH value / pH	6.68	6.76	6.72
Acidity (ºSH) / Kiselost (ºSH)	7.60	7.80	7.70
Density / Gustina	1.03	1.03	1.03
Fat (%) / Mlečna mast (%)	3.00	3.30	3.15
Dry matter (%) / Suva materija (%)	11.76	11.89	11.83
Fat-free milk solids (%)	8.49	8.83	8.66
Protein (%) / Proteini (%)	3.36	3.42	3.39
Lactose (%) / Laktoza (%)	4.56	4.73	4.64
Casein (%) / Kazein (%)	2.81	2.89	2.85
Fat/Casein / Mlečna mast/Kazein	1.07	1.14	1.10
Casein/Fat / Kazein/Mlečna mast	0.94	0.87	0.90
Ash (%) / Pepeo (%)	0.73	0.72	0.73
Ca (%)	0.10	0.11	0.108

Table 2. THE TECHNOLOGY OF MAKING SJENICA CHEESE TYPE

Tabela 2. TEHNOLOGIJA PROIZVODNJE SIRA U TIPU SJENIČKOG

<table>
<tr><td>Milk</td><td>More important technological operations</td></tr>
<tr><td>1. Coagulation of milk
 
Figure 2.1. Coagulation of milk</td><td>Fresh milk is regularly received (filtering, aeration, measuring and cooling) and stored. After completed analyses and confirmation that the milk is suitable for making cheese, milk is sent to the line of standardization and pasteurization, after which milk with the temperature of 30-32°C is sent to cheese vat. Starter cultures for white cheese and calcium chloride are added with mixing. After that milk is left to mature for the biological acidity of 7.8 °SH. Then, rennet is added (liquid microbial rennet, 4-7 ml/l). Coagulation time is about 40-60 minutes.</td></tr>
<tr><td>2. Selfpressing and pressing
 
Figure 2.2. Selfpressing in cloths and molding</td><td rowspan="2">After coagulation, cheese curd without processing is put in cloths (cheese making cloth) which form a clump during 0.5 to 1 h. This is a variant that is used in some dairies.
In other dairies, for selfpressing cloths are placed in perforated molds (plastic) and in such a complex are placed the curd. Then, cheese curd is made by shaking with hands, with a little whey separation.
Finally, in both cases, selfpressing and curd binding is performed without load. Pressing takes 1.5 to 3 h. In some dairies the load is not used but in some slight load is used (0.5-2 kg/kg).
 
Figure 2.4. Selfpressing and forming clumps in bind cloths</td></tr>
<tr><td>3. Selfpressing and pressing
 
Figure 2.3. Selfpressing of cheese only in hanging cloths</td></tr>
<tr><td>4. Cutting
 
Figure 2.5. Curd is cut into triangular slices 15x15x5 cm</td><td>5. Salting
 
Figure 2.6. Salting is done with dry sea salt in the containers (a line of salt, a line of cheese)</td></tr>
<tr><td>6. Ripening
 
Figure 2.7. Left to mature for 20 to 40 days in the brine (salt whey) and a cool (14-18°C).</td><td>7. Transport package
 
Figure 2.8. Transport package</td></tr>
</table>

Milk and cheese have certain characteristics that are influenced by natural conditions and practices in breeding of domestic animals in this area. Physicochemical and chemical properties of the standardized cow's cheese milk are shown in table 1.

For good quality of most cheeses, milk must not have an elevated level of acidity. Standardization of fat in soft and hard cheeses is common at the 2.8 to 3.2% and for full-fat cheese with a minimum of 45% fat in dry matter should be around 3.0% (Popović-Vranješ A., 2009). The ratio of casein to fat is very important to maintain the quality of cheese. When this ratio is out of balance, the body of cheese is too soft or too hard. Determined fat/casein and casein/fat ratio in milk and cheese was proper (1.07 and 0.94 respectivly).

Lactose does not contribute much to cheese yield. As fermented sugar it regulates the pH of milk and curd. Minerals play an important role in milk for cheese, especially Ca and Mg salts, and phosphoric and citric acids. Calcium (as phosphate) entrance into the structure of the casein complex. The established physical and chemical parameters show that cow's milk has all factors suited for production of Sjenica cheese type.

Table 3. CHEMICAL COMPOSITION OF SJENICA CHEESE TYPE

Tabela 3. HEMIJSKI SASTAV SIRA U TIPU SJENIČKOG

Parameters / Parametri	Statistical parameters / Statistički parametri		
	X_{min}	X_{max}	$X_{aver.}$ (n=10)
Fat (%) / Mlečna mast (%)	21.97	23.97	22.97
Proteins (%) / Proteini (%)	10.15	11.99	11.07
Dry matter (%) / Suva materija (%)	44.54	46.65	45.59
NaCl (%) / NaCl (%)	2.5	3.1	2.8
Moisture (%) / Voda (%)	55.46	53.35	54.40
FDM (%) / Mlečna mast u suvoj materiji (%)	49.32	51.38	50.38
MFFB (%) / Voda u bezmasnoj materiji sira(%)	71.07	70.17	70.62
pH value / pH	4.63	4.76	4.69
Acidity (ºSH) / Kiselost (ºSH)	58.32	60.05	59.18

FDM- fat in dry matter; MFFB- moisture on a fat free basis

Technology and characteristics of milk processing in Sjenica area as in many mountainous regions, is a technology in which are highlighted the similarities, but also the specific production of white brined cheeses.

Based on the recording that was made in our research of production technology in industrial conditions, in table 2 are presented the characteristics of production.

Sjenica cheese produced in industrial conditions has a high moisture content (54.40%). The water content in the fat-free matter ranged from 70.11 to 71.07% with average 70.62% on the basis of which can be classified into a group of soft cheeses.

According to the average fat content in dry matter (50.38%), Sjenica cheese produced in industrial conditions can be classified into a group of full fat cheeses.

Acidity and pH value of the cheese was in a narrow range (4.63-4.6), and it is characteristic of soft white cheese in brine.

Cheese produced in industrial conditions after ripening was bacteriologically safe and in accordance with the Regulations given in „Official Gazette RS.", No 72/10).

Based on the sensory assessment and received scores it was concluded that Sjenica cheese type had a typical sensory characteristics. On the basis of points scored, the cheese is placed in the I (16.5-18.25 points) and Extra Class (18.5-20.0 points).

Sjenica cheese type is characterized by flavour richness, sour, milky and moderately salted taste, the appearance of mature slice of cheese is a typical (triangular, height 1-2 cm), cross section without holes (Figure 3).

Table 4. SENSORY ASSESSMENT OF SJENICA CHEESES TYPE PRODUCED IN INDUSTRIAL CONDITIONS, n = 10

Tabela 4. SENZORNA OCENA SIRA U TIPU SJENIČKOG PROIZVEDENOG U INDUSTRIJSKIM USLOVIMA, n = 10

Characteristics/ Karakteristike	Max. Score / Max. rezultat	Min.	Max.	Average / Prosečno
Appearance / Izgled	2	1.75	2	1.875
Colour / Boja	1	0.75	1	0.98
Cheese structure/ Struktura sira	2	1.5	2	1.70
Cut / Presek	3	2.5	2.75	2.69
Odour / Miris	2	1.5	2	1.77
Taste / Ukus	10	7.75	9.5	8.73
Total / Ukupno	**20**	**15.75**	**19.25**	**17.75**

Figure 3. THE APPEARANCE OF THE SJENICA CHEESE TYPE FOR SENSORY ASSESSMENT

Slika 3. IZGLED SIRA U TIPU SJENIČKOG PRIPREMLJENOG ZA SENZORNO OCENJIVANJE

CONCLUSION

Standardization of cow's milk (heat treatment and adjusting the fat content) and applying of some slightly modified operations of cheese production, get a good quality of Sjenica cheese type from cow's milk, with standard properties. In this way it solves the problems of continuous presence of a large deviation of cheese properties, which are present in the traditional production in the households.

The application of these technologies in the industrial conditions of Sjenica cheese type production has resulted in better utilization of raw milk, much safer health products, and higher economic return.

ACKNOWLEDGEMENT

The research was carried out within the project: „Production of hard cheese with added value of milk produced in organic and self-sustaining systems" financed by the Ministry of Science and Technological Development of RS, TR-31095.

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IZVOD

PROIZVODNJA SJENIČKOG SIRA U INDUSTRIJSKIM USLOVIMA

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Tradicionalno, skoro svi beli salamureni sirevi se proizvode od sirovog ovčijeg mleka ili od mešanog ovčijeg i kozjeg, ili ovčijeg i kravljeg mleka. Danas je tendencija da se to promeni, u potpunosti ili delimično, i da se sirevi proizvode od pasterizovanog standardizovanog kravljeg mleka te da se dodaju kulture kiselo mlečnih bakterija, osim za autohtone sireve, za koje je obavezno korišćenje ovčijeg mleka i autohtone kulture. S obzirom da je kod većene autohtonih sireva prisutan problem neujednačenog sastava i kvaliteta, u radu je prikazana proizvodnja sira u tipu sjeničkog gde su sve tehnološke operacije tako usmerene da se dobije sir standardnih osobina u industrijskim uslovima u mlekarama na Sjenici. Tehnologija izrade je specifična u odnosu na druge sireve: nežan gruš, ne vrši se formiranje zrna kao kod drugih sireva, lagano i postepeno odvajanje surutke, nema povećanja temperature sirne mase, u prvoj fazi ceđenja vrši se samopresovanje a u drugoj fazi se forsira blago presovanje (protresanjem gruša) ili blago opterećenje. Solenje ima višestruku ulogu u ovoj grupi sireva i ono se sastoji iz dva dela: prvo solenje je uvek suvo solenje a zrenje je u salamuri ili slanoj surutki od čijih osobina i koncentracije soli zavisi kvalitet sira. Proizveden je meki, punomasni sir (sa sadržajem vode u bezmasnoj materiji 70,17-71,07%, a masti u suvoj materiji 49,32-51,38%), standardnog oblika (trouglast), dimenzija (15x15x2-3 cm) i težine kriške (300-330g), bele boje sa žućkastom nijansom, zatvorene teksture sa malim rupicama, tipičnog blago mlečno kiselo-slanog ukusa. Na osnovu postignute ocene senzornih osobina, sirevi su svrstani u I i ekstra klasu, što je takođe jedan od pokazatelja visokog kvaliteta ovog sira.

Ključne reči: beli sir • Sjenica • standarizovano mleko

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NAUČNI RAD

NALAZ I AKTIVNOST BAKTERIJA MLEČNE KISELINE U AUTOHTONOM SJENIČKOM SIRU

UDK: 637.354.62:547.472.3 (497.11)

U radu je prikazana prevalenca bakterija mlečne kiseline tokom procesa proizvodnje i zrenja autohtonog sjeničkog sira. Aktivnost bakterija mlečne kiseline je procenjena na osnovu promene pH vrednosti i koeficijenta zrelosti tokom perioda zrenja od 90 dana.

Ključne reči: autohtoni sjenički sir • bakterije mlečne kiseline • pH i koeficijent zrelosti

UVOD

Jedan od najznačajnijih predstavnika autohtonih belih sireva u salamuri je sjenički sir koji se po tradicionalnoj tehnologiji proizvodi isključivo na planinskim visoravnima koji okružuju Sjenicu (Sjeničko-Pešterska visoravan), kao i na područjima opština Novi Pazar, Tutin i Prijepolje. Autohtono se proizvodi od sirovog ovčijeg mleka, a u tipu sjeničkog sira od mešanog (ovčijeg i kravljeg) i kravljeg mleka.

Sjenički sir ima dugogodišnju tradiciju. Po autohtonoj tehnologiji proizvodi se od ovčijeg mleka u katunima ili stanovima daleko od naseljenih područja za vreme ispaše ovaca, od sredine maja do kraja septembra. Mleko se posle muže cedi i podsirava. Podsiravanje traje 2-3 časa, a potom se prebacuje u cedila i cedi bez opterećenja. Ceđenje traje 3-4 časa, sir se postavi na sirarsku dasku, opetereti drvenim krugom i ceđenje traje dodatna 3-4 časa. Sirna pogača (gruda) se reže na četvrtaste komade, soli i slaže u kace. Sir zri u slanoj surutki, a tokom zrenja je neophodno dopunjavanje kace 2-3 puta. Tokom zrenja sir se sleže i formira kompaktno testo u kome nema jasno odvojenih kriški. Zrenje sira je minimum 90 dana.

Kao i kod ostalih sireva proizvedenih od sirovog mleka, kvalitet i senzorni profil sjeničkog sira je prvenstveno uslovljen specifičnošću geografskog područja sa koga potiče, odnosno pedioklimatskim karakteristikama okruženja, ali i tradicionalnom tehnikom proizvodnje, koja isključuje primenu termičkog tretmana. Kako je supstrat isključivo sirovo mleko, to su proces fermentacije i zrenja kod sjeničkog sira uslovljeni isključivo dinamikom razvoja i aktivnošću autohtone populacije bakterija mlečne kiseline (BMK). Time se zajednica indogene mikroflore BMK, koja se selekcioniše iz prirodne mikroflore sirovog mleka u datim uslovima mikrosredine, proizvodnog okruženja, i specifičnom tehnologijom, može smatrati jednim od glavnih faktora u određivanju autohtonog karaktera sjeničkog sira (Bulajić i Mijačević, 2007).

Sjenički autohtoni sir se, s obzirom na regionalno poreklo i specifičnost tradicionalne tehnologije može smatrati zasebnim ekološkim entitetom (Garabal, 2007) i veoma dinamičnim biohemijskim supstratom, koji tokom proizvodnje, a posebno perioda zrenja prolazi kroz značajne promene, pre svega kroz sukcesiju glavnih mikrobnih grupa bakterija mlečne kiseline, a na osnovu njihove metaboličke aktivnosti i evoluciju fizičko-hemijskih parametara kao što su pH, količina ukupnog azota i rastvorljivih azotnih materija, čiji odnos karakteriše obim proteolize.

Cilj ovog rada jeste da se ispita zastupljenost i dinamika razvoja autohtone populacije bakterija mlečne kiseline, a u odnosu na njihovu metaboličku aktivnost, kao rezultat interakcije supstrata, mikroklimatskih faktora, specifičnosti tehnologije izrade i fiziološkog potencijala prisutne populacije BMK i dinamika promene pH i koeficijenta zrelosti tokom perioda zrenja autohtonog sjeničkog sira. Preliminarna karakterizacija autohtonog sjeničkog sira u odnosu na navedene zadate parametre daje rezultate neophodne u sprovođenju standardizacije tehnološkog postupka u cilju dobijanja tradicionalnog proizvoda ujednačenog kvaliteta uz očuvanje biodiverziteta bakterijskih vrsta povezanih sa ovim specifičnim proizvodom.

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MATERIJAL I METODI

Materijal ispitivanja su predstavljali uzorci sirovog mleka i sjeničkog sira tokom procesa proizvodnje i perioda zrenja (7., 15., 30., 60. i 90.-ti dan zrenja).

Za mikrobiološka ispitivanja 20 ml (g) mleka, odnosno sira u različitim fazama proizvodnje i perioda zrenja homogenizovano je u 180 ml 2% rastvora Na-citrata primenom Stomahera (Bag Mixer, Interscience), a potom su pripremljena serijska decimalna razblaženja sa puferisanom peptonskom vodom. Odgovarajuće razblaženje je preneseno na specifične podloge za dokazivanje broja: termofilni laktobacili na MRS agar (Merck) pri 37^{o}C tokom 48h inkubacije u anaerobnim uslovima; mezofilne laktoke na M17 Agar (Merck) pri 30^{o}C tokom 48h i enterokoke na Kanamycin Aesculin Azide Agar (KAA agar) pri 37^{o}C tokom 24h inkubacije.

Za praćenje promene azotnih materija tokom zrenja sira primenjene su sledeće metode:

- Ukupne azotne materije po metodi Kjeldahl-u pomoću Kjeltec sistema (IDF standard 20B:1993)
- Rastvorljive azotne materije metodom po van Slyke-u i Hart-u (Pejić i Đorđević, 1963).

Koeficijent zrelosti, kao pravi pokazatelj zrenja, odnosno stepena zrelosti sira i intenzifikacije proteolitičkih promena u matriksu sira, određen je računskim putem kao odnos rastvorljivog i ukupnog azota.

REZULTATI I DISKUSIJA

Distribucija identifikovanih bakterija mlečne kiseline tokom procesa proizvodnje autohtonog sjeničkog sira i perioda zrenja od 90 dana prikazana je u tabelama 1 i 2.

Prema rezultatima prikazanim u tabeli 1 laktokoke predstavljaju najzastupljeniju grupu bakterija mlečne kiseline, potom slede laktobacili i enterokoke. Povećanje njihova broja tokom procesa proizvodnje, pre svega u fazi formiranja grude nije neočekivano. Rezultat je prirodnog fenomena prisutnog kao posledica intenzifikacije njihova umnožavanja usled toga što temperatura podsiravanja pogoduje njihovom rastu, ali i kao rezultat fizičke retencije mikroorganizama u grudi prilikom odstranjivanja surutke. Sličnu prevalencu laktokoka laktobacila i enterokoka utvrđuju Mijačević i Bulajić (2008) prilikom mikrobiološke karakterizacije autohtonog Somborskog sira (tabela 2).

Tabela 1. BROJ (LOG_{10} CFU/ML/G) GLAVNIH MIKROBNIH GRUPA (*LACTOCOCCUS* SPP., *LACTOBACILLUS* SPP. I *ENTEROCOCCUS* SPP.) BAKTERIJA MLEČNE KISELINE TOKOM PROIZVODNJE AUTOHTONOG SJENIČKOG SIRA

Table 1. MICROBIAL SUCCESSION - CHANGES IN THE COUNTS (LOG CFU/ML/G) OF THE MAIN MICROBIAL GROUPS OF LAB DURING MANUFACTURING OF AUTOCHTHONOUS SJENICA CHEESE

Supstrat Substrate	**Bakterije mlečne kiseline Lactic Acid Bacteria**		
	***Lactococcus* spp.**	***Lactobacillus* spp.**	***Enterococcus* spp**
Mleko Milk	7,54±1,16	4,93±0,47	3,65±0,77
Gruš Crude	6,83±0,91	4,93±0,76	3,79±1,10
Gruda	8,37±0,18	6,86±0,68	4,51±0,27

Tokom perioda zrenja laktokoke se u najvećem broju održavaju tokom prvih 7 dana, kada je u matriksu sira i najintenzivniji proces acidifikacije. Do kraja perioda zrenja laktokoke se održavaju u prilično visokom broju, mada je prisutan trend smanjenja njihova broja kao posledica permanentnog snižavanja pH vrednosti sira i primenjene procedure soljenja, što se negativno odražava na stopu rasta laktokoka. U prvih 30 dana zrenja, populacija laktobacila je prisutna u prilično konstantnom broju, a objašnjenje ovoga leži u činjenici da je ova grupa mikroorganizama, u odnosu na laktokoke, daleko otpornija na nepovoljne uslove sredine kao što su nizak pH, visoka koncentracija soli, anaerobni uslovi, i nedostatak hranjivih materija. Enterokoke svoj maksimum postižu 30.-og dana zrenja, a populacija se neznatno smanjuje do kraja perioda zrenja. Karakteristično za mnoge sireve proizvedene od sirovog mleka jeste da su enterokoke u praktično konstantnom broju prisutne tokom celog perioda zrenja (Mas i sar., 2002; Bulajić, 2007; Litopoulou-Tzanetaki i Tzanetakis, 1992), što reflektuje njihovu rezistenciju na nepovoljne uslove (nizak pH, niska aw, visoka koncentracija soli) ostvarene napredovanjem procesa zrenja.

Aktivnost bakterija mlečne kiseline predstavljena je kroz promenu pH i koeficijenta zrelosti u matriksu sira tokom perioda zrenja od 90 dana. (tabela 3). U sjeničkom siru, kao predstavniku belih sireva u salamurim koji po teksturi pripadaju mekim sirevima, odvijaju se intenzivni procesi fermentacije kao posledica metaboličke aktivnost bakterija mlečne kiseline što dovodi do acidifikacije supstrata i promene pH vrednosti. Tokom zrenja proteini u siru se transformišu do produkata nižih molekulskih masa koje u velikoj meri definišu senzorni profil i reološke karakteristike sira, a proteoliza jeste jedan od najznačajnih biohemijskih procesa u supstratu sira. Kao pokaza-

Tabela 2. BROJ (LOG_{10} CFU/G) GLAVNIH MIKROBNIH GRUPA (*LACTOCOCCUS* SPP., *LACTOBACILLUS* SPP. I *ENTEROCOCCUS* SPP.) BAKTERIJA MLEČNE KISELINE TOKOM PERIODA ZRENJA AUTOHTONOG SJENIČKOG SIRA

Table 2. MICROBIAL SUCCESSION - CHANGES IN THE COUNTS (LOG CFU/ML/G) OF THE MAIN MICROBIAL GROUPS OF LAB DURING RIPENING OF AUTOCHTHONOUS SJENICA CHEESE

Period zrenja Ripening period (in days)	**Bakterije mlečne kiseline Lactic Acid Bacteria**		
	***Lactococcus* spp.**	***Lactobacillus* spp.**	***Enterococcus* spp.**
7	8,76±0,66	7,86±0,68	5,15±0,63
15	7,91±0,39	7,86±0,75	4,64±0,65
30	7,47±0,80	7,53±0,28	5,47±0,36
60	7,96±0,33	6,02±0,57	4,55±0,54
90	7,21±0,03	6,30±0,30	4,93±0,48

telj stepena zrelosti, najčešće se koristi odnos sadržaja rastvorljivog azota i ukupnog azota, parametar poznat u literaturi kao koeficijent zrelosti.

Iz rezultata prikazanih u tabeli 3. zapaža se intenzivna aktivnost bakterija mlečne kiseline u prvih 30 dana zrenja, što je praćeno značajnom promenom pH vrednosti u matriksu sira. Do kraja perioda zrenja, pH vrednost se ne menja značajno, dok se promene na proteinima intenziviraju, čime se udeo rastvorljivih proteina povećava i kao posledica toga imamo i povećanje koeficijenta zrelosti sira. Prema literaturnim podacima, grupa mekih sireva na kraju perioda zrenja pokazuje koeficijent zrelosti od 13 do 20 (Jovanović, 1994; Petrović, 1986; Maćej 1989; Mijačević i Bulajić, 2008), što je u dobroj korelaciji sa rezultatima prikazanim u radu.

Tabela 3. POKAZATELJ AKTIVNOSTI BAKTERIJA MLEČNE KISELINE KROZ KOEFICIJENT ZRELOSTI I PH TOKOM PERIODA ZRENJA AUTOHTONOG SJENIČKOG SIRA

Table 3. THE ACTIVITY OF LACTIC ACID BACTERIA REPRESENTED AS RIPENING COEFFICIENT AND PH VALUES DYNAMICS DURING RIPENING OF AUTOCHTHONOUS SJENICA CHEESE

Period zrenja Ripening period (in days)	Koeficijent zrelosti Ripening coefficient	pH pH value
7	11,05±0,01	5,55±0,09
15	13,10±0,13	5,38±0,03
30	14,20±0,38	4,71±0,08
60	16,20±0,63	4,56±0,06
90	17,68±0,08	4,54±0,03

ZAKLJUČAK

Tokom proizvodnje autohtonog sjeničkog sira zaključno sa formiranjem grude, utvrđeno je povećanje populacije laktobacila za 2 $\log_{10}$, a laktokoka i enterokoka za 1 $\log_{10}$.

Tokom perioda zrenja, laktokoke su najbrojnije u prvih 7 dana, populacija laktobacila se održava na visokom broju od 7 $\log_{10}$ prvih 30, a potom njihov broj neznatno opada, dok su enterokoke, kao rezistentni mikroorganizmi u praktično konstantnom broju prisutne kroz čitav proces zrenja.

Aktivnost bakterija mlečne kiseline u odnosu na acidifikaciju supstrata najviše je izražena u prvih 30 dana zrenja, dok se proteolitičke promene intenziviraju posle 30.-og dana zrenja, što je iskazano kroz povećanje koeficijenta zrelosti.

ZAHVALNICA

Rad je deo istraživanja na projektu III 46009 finansiranom od strane Ministarstva prosvete i nauke Republike Srbije.

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SUMMARY

PREVALENCE AND ACTIVITY OF LACTIC ACID BACTERIA IN AUTOCHTHONOUS SJENICA CHEESE

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In this article the prevalence of lactic acid bacteria through the production and ripening period of autochthonous Sjenica cheese was reviewed. The activity of main LAB group was evaluated by pH changes and evolution of ripening coefficient.

Key words: autochthonous Sjenica cheese • lactic acid bacteria • pH and ripening coefficient

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UDK: 637.13:637.055:579.8

UTICAJ PRIMARNE OBRADE NA LIPOLITIČKE PROMENE U SIROVOM MLEKU

Mleko, kao jedna od osnovnih životnih namirnica u ishrani ljudi, mora da bude proizvedeno po najvišim standardima kako bi očuvalo prirodne karakteristike. U tom smislu, od velikog značaja je higijena proizvodnje mleka i njegove obrade posle muže. Efikasnost higijenskih postupaka pozitivno utiče na bakteriološku ispravnost mleka, čime je moguće izbeći neželjene posledice organoleptičkih promena mleka.

U ovom radu prikazani su rezultati ispitivanja uticaja primenjenih tehnoloških operacija obrade na kvalitet mleka. Na farmama različite veličine ispitivane su promene hemijskog sastava i fizičkih osobina mleka tokom hlađenja. Bakteriološka ispravnost mleka ispitana je utvrđivanjem ukupnog broja mikroorganizama, sa posebnim osvrtom na psihrofilne i lipolitičke mikroorganizme.

Ključne reči: sirovo mleko • lipolitičke promene • higijenski kvalitet

UVOD

Po svom sastavu mleko predstavlja kompleksan fizičko-hemijski sistem u kome se sastojci nalaze u ravnoteži. Svaka promena pojedinih sastojaka mleka utiče na kvalitet, tehnološku i prehrambenu vrednost proizvoda.

Kvalitet mleka može da se tretira sa tehnološkog stanovišta, u pogledu organoleptičkih svojstava i mikrobiološke ispravnosti. Tehnološka vrednost i organoleptička svojstva kvaliteta neposredno su vezana za hemijski sastav mleka.

Ističući značaj odnosa pojedinih sastojaka u mleku ne sme se zanemariti potreba izbora sirovine u pogledu njegove mikrobiološke ispravnosti; u suprotnom je ona ozbiljan nedostatak i najčešći uzrok promena pojedinih sastojaka mleka. Naročito pri nesmetanom razviću mikroorganizama u mleku transformišu se pojedini sastojci, kao što su laktoza, proteini i masti. Pri tome nastaju nova jedinjenja, koja mogu da promene hemijske, fizičke i senzorne osobine mleka.

Promene mogu da se jave i kod hlađenog mleka koje se duže vreme drži na nižim temperaturama. U tom slučaju stepen hlađenja mleka deluje selektivno na mikrofloru, jer se vremenom razvijaju samo određene vrste psihrofilnih mikroorganizama. Ovi mikroorganizmi skoro uopšte ne transformišu laktozu, nego prvenstveno za svoj metabolizam koriste proteine i masti iz mleka, što dovodi do promena ukusa i mirisa mleka.

Veliki broj masnih kiselina, koje ulaze u sastav mlečne masti, daje joj visoku biološku vrednost. Međutim, zbog heterogenog sastava dolazi do različitih promena na mlečnoj masti. Na primer, karakteristično je za maslačnu kiselinu da se odlikuje neprijatnim i vrlo prodornim mirisom i ukusom. Prisustvo ove kiseline u izuzetno malim količinama dovoljno je da mleko poprimi miris i ukus užeglosti. Kod mleka i mlečnih proizvoda ova pojava poznata je kao hidrolitička užeglost mlečne masti. Stepen užeglosti može biti slabije ili jače izražen, što zavisi od koncentracije i dužine delovanja enzima (Deeth i Fitz-Gerald, 2006).

Predmet ovih istraživanja bio je proučavanje promena kvaliteta mleka posle muže u zavisnosti od primenjenih postupaka obrade. Posebna pažnja je posvećena lipolitičkim promenama kvaliteta sirovog mleka.

MATERIJAL I METODI

Terenski deo istraživanja je obavljen na malim, srednjim i velikim farmama, gde se mleko hladi i skladišti na temperaturama od 0 do 4^{o}C. Hlađenje mleka je praćeno promenama temperature posle muže, pre i posle postizanja željene temperature konzervacije.

Na velikim farmama mleko je ispitivano ujutru odmah posle muže i hlađenja, a mleko dobijeno večernjom mužom sledećeg dana ujutru. Na srednjim i malim farmama ohlađeno mleko je uzorkovano u jutarnjim i večernjim časovima i analizirano u roku od 24 časa.

U ukupno 30 uzoraka ispitivane su hemijske, fizičke, mikrobiološke i biohemijske promene u sirovom mleku. Hemijske promene mleka su praćene utvrđivanjem količine mlečne masti, suve materije i proteina standardnim

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metodama (Ostojić i sar., 2008). Fizičke promene gustine, titracione kiselosti i potenciometrijske kiselosti su utvrđivane standardnim metodama (Carić i sar., 2000). Mikrobiološke promene sirovog mleka su praćene utvrđivanjem ukupnog broja i broja psihrofilnih mikroorganizama, prisustva *E.coli* i broja lipolitičkih bakterija (Anon, 1980). Biohemijske promene sirovog mleka su indirektno praćene lipolitičkom aktivnošću enzima preko kiselosti mlečne masti (Đorđević, 1982).

REZULTATI I DISKUSIJA

Od proizvodnje do finalne potrošnje mleka potrebno je ograničiti degradaciju hrane kroz održavanje "hladnog lanca". Prema DEBD-u (2008) razlikuje se pet faza u hladnom lancu, i to:

- faza hlađenja u proizvodnji (hlađenje mleka na farmi),
- faza hlađenja posle obrade mleka (hlađenje mleka u mlekari),
- faza hlađenja u transportu (kontejner za prevoz ohlađenog mleka do trgovine),
- faza hlađenja u distribuciji (očuvanje proizvoda u vitrinama),
- faza hlađenja u domaćinstvu do konzumiranja (hlađenje u frižideru).

Faza koja je bila predmet našeg interesovanja je hlađenje u proizvodnji mleka. Neadekvatni postupci sa mlekom posle muže utiču na njegov kvalitet, ali njihov efekat je znatno nepovoljniji ukoliko uslovi pod kojima se izvodi muža nisu odgovarajući (higijena vimena, muzača, pribora i opreme, i slično). Higijena muže primarno utiče na njegov kvalitet i dalje promene, usled brojnih mogućnosti kontaminacije mleka mikroorganizmima iz okoline Vuković i sar., 2007; Relić i Vuković, 2008; Relić i sar., 2009; Relić i Jež, 2010). Posle muže, postupci ceđenja, mešanja i skladištenja mleka do predaje mlekari često se nestručno izvode što to dovodi do dodatnih oblika pogoršanja kvaliteta ili pospešivanja promena kvaliteta izazvanih postupcima muže (FAO, 1985; Ostojić, 2007).

Metodi hlađenja mleka posle muže su poznati u teoriji i praksi kao dobar način očuvanja kvaliteta sirovog mleka, za šta je organizovanje sabiranja mleka jedan od važnih uslova. U tabeli 1 prikazan je hemijski sastav uzoraka mleka sa sabirnih mesta.

Tabela 1. HEMIJSKI SASTAV SIROVOG MLEKA

Table 1. CHEMICAL COMPOSITION OF RAW MILK

Vreme sabiranja/ analize ***Time of collecting/ analyses***	**Broj uzoraka** ***Number of samples***	**Mlečna mast** ***Milk fat*** **(ml/100 ml)**	**Suva materija** ***Dry matter*** **(ml/100 ml)**	**SMBM** ***Non fat dry matter*** **(ml/100 ml)**	**Proteini** ***Proteins*** **(ml/100 ml)**
Jutro/*Morning*	30	4,00	12,63	8,63	3,30
Veče/*Evening*	30	4,10	12,91	8,81	3,40
Prosek/*Average*	30	4,05	12,77	8,72	3,35

Iz tabele 1 uočava se da su prosečne vrednosti svih ispitivanih parametara u uzorcima mleka imale nešto veće vrednosti u mleku iz večernje muže, u odnosu na uzorke iz jutarnje muže. Vrednosti koje se odnose na mlečnu mast mogu se pripisati delimičnom raslojavanju masti pri mešanju jutarnjeg i večernjeg mleka.

Primenom savremenih načina hlađenja mleka može se uspešno sačuvati njegov kvalitet pre prerade. Ovo je posebno značajno u cilju sprečavanja raslojavanja mlečne masti.

Tabela 2. FIZIČKE OSOBINE SIROVOG MLEKA

Table 2. PHYSICAL PROPERTIES OF RAW MILK

Vreme sabiranja/ analize *Time of collecting/ analyses*	Broj uzoraka *Number of samples*	Zapreminska masa *Volumetric mass*	Viskozitet *Viscosity* (cP)	pH	Kiselost *Acidity* (^oSH)	Kiselost masti *Fat acidity* (^oK)
Jutro/*Morning*	30	1,031	2,373	6,83	7,00	0,364
Veče/*Evening*	30	1,031	2,051	6,87	6,60	0,281
Prosek/*Average*	30	1,030	2,120	6,85	6,80	0,323

Fizičke osobine mleka u ispitivanim uzorcima prikazane su u tabeli 2.

Prema podacima iz tabele 2, fizičke osobine mleka bile su u skladu sa uslovima proizvodnje i potrebnom tehnološkom podesnosti za njegovu preradu. Ispitivanje kiselosti masti ukazalo je na moguće lipolitičke promene osobina mleka. Prema dobijenim rezultatima može se konstatovati da je kiselost mlečne masti na nivou koji ne ukazuje na lipolitičke promene u mleku. To je u saglasnosti sa podacima Đorđevića (1982).

Rezultati ukazuju da, bez obzira na veličinu farme, postupci obrade mleka bili su pravilno primenjeni i očuvan je kompozitni kvalitet.

U tabeli 3 prikazani su rezultati mikrobiološkog ispitivanja uzoraka mleka.

Podaci iz tabele 3 ukazuju na povećanje ukupnog broja i broja lipolitičkih bakterija posle mešanja mleka jutarnje i večernje muže. Ukupan broj mikroorganizama ukazuje na izvesne slabosti u održavanju higijenskih uslova, koje se u najvećoj meri odnose na male farme. Uprkos izvesnom povećanju broja lipolitičkih bakterija, prema podacima iz tabele 2 može se konstatovati da se njihova aktivnost nije značajno odrazila na povećanje slobodnih masnih kiselina. Interesantno je napomenuti da se, nasuprot tome, broj psihrofilnih bakterija smanjio. Mišljenja smo da je to posledica pravilnih postupaka sa mlekom posle mu-

Tabela 3. BAKTERIOLOŠKA ISPRAVNOST SIROVOG MLEKA

Table 3. BACTERIOLOGICAL SAFETY OF RAW MILK

Vreme sabiranja/ analize *Time of collecting/ analyses*	Broj uzoraka *Number of samples*	*E.coli*	UBB *Total bacteria count*	Lipolitičke bakterije *Lipolytic bacteria*	Psihrotrofne bakterije *Psychrophile bacteria*
Jutro/*Morning*	30	-	86.00	7.500	33.000
Veče/ *Evening*	30	-	123.500	8.000	28.000
Prosek/*Average*	30	-	104.750	7.250	30.500

že i posebno brzine njegovog hlađenja.

Psihrofilni mikroorganizmi široko su rasprostranjeni u prirodi i nalaze se u vodi i zemljištu, zbog čega postoje mnogobrojne i vrlo različite mogućnosti za kontaminaciju mleka ovim mikroorganizmima. Psihrofilni mikroorganizmi u mleku prvenstveno razlažu proteine i masti, dok mlečni šećer gotovo uopšte ne transformišu. Zbog toga se reakcija mleka ne menja ili jedva primetno postaje alkalna. Međutim, usled nastalih proteolitičkih i lipolitičkih promena često dolazi do promene ukusa mleka.

Zbog svoje slabe termorezistentnosti psihrofilne mikroorganizme uništava temperatura pasterizacije. Mali broj fakultativnih psihrofila, odnosno psihrotrofa može da preživi temperaturu pasterizacije i da kasnije u mlečnim proizvodima dovede do neželjenih promena. Promenjen ukus (gorak, zagoreo, užegao) ne nestaje pasterizacijom.

Lipoliza sirovog mleka je uzrokovana delovanjem lipaze mleka na mlečne masti koja je omogućena dostupnim mehaničkim i/ili temperaturnim dejstvom na svojstvenu osetljivost mleka (Deeth i Fitz-Gerald, 2006). Iako brzo hlađenje mleka nakon muže teži da spreči lipolizu, dogrevanje ili ponovno hlađenje može da proizvede ozbiljne reakcije (Slaghuis i sar., 2007). Efekat mehaničke aktivacije zavisi od prethodne temperature mleka, ali i od temperature u toku mehaničke obrade, prirode mehaničkih postupaka i različitih karakteristika mleka. Međutim, mešanje mleka nakon perioda skladištenja (4°C) može da proizvede povišenu aktivaciju, zbog povećane aktivnosti lipaze na mlečnoj masti pod takvim uslovima. Generalno gledano, to mogu da budu značajne posledice mašinske muže i delimično neadekvatnog protoka mleka kroz mlekovode. Zato je veoma važno da projektovane i postavljene instalacije imaju radne karakteristike koje izbegavaju ovakve negativne efekte. Mašine za mužu koje stvaraju turbulenciju mleka sa prisutnim vazduhom, generalno dovode do stvaranje pene i posebno su štetni. Ovi uslovi mogu biti u velikoj meri eliminisati dobrim mašinskim instalacijama, pravilnim radom i redovnim održavanjem. Tako se lipoliza može izbeći.

Razumno je pretpostaviti da do aktivacije lipolitičkih procesa može doći tokom produženih perioda hlađenja. To je posledica oštrog ili preteranog mešanja mleka ili velikih temperaturnih oscilacija. Međutim, uslovi za ozbiljnu aktivaciju lipolize zavise i od brzine rada pumpi, dugih mlekovoda i vremena skladištenja mleka pre njegove dalje obrade. Praksa je pokazala dobre rezultate kod nekih mlekara koje su primenile termizaciju mleka na prijemu na temperaturi od 63° C za 15 sekundi (Couteauh, 2009). Tako je došlo do inaktivacije indukovane lipolize, što je potvrdila i mikrobiološka kontrola. Pored toga, poboljšana je stabilnost sirovine, koje proističu iz ovog tretmana, a to nudi veću fleksibilnost u obradi i preradi.

Veliki broj masnih kiselina koji ulazi u sastav mlečne masti, daje joj visoku biološku vrednost. Međutim, zbog heterogenog sastava dolazi do različitih promena na mlečnoj masti. Posebno treba pomenuti prisustvo masnih kiselina s nezasićenim hemijskim vezama, koje su pogodne za adiranje kiseonika, usled čega dolazi do razlaganja i oslobađanja nekih masnih kiselina.

Naročito u toku stajanja i lagerovanja mleka pod dejstvom fermenta lipaze, koji proističe delom od mikroorganizama, a delom od mlečne ćelije, razlaže se mlečna mast na glicerin i masne kiseline. Treba istaći da lipoliza mlečne masti ne ide nikada do kraja, usled nastajanja maslačne kiseline i nekih nižih masnih kiselina koje su rastvorljive u vodi i sprečavaju dalje delovanje lipaze.

Pod dejstvom hlađenja mleka može doći do kristalizacije globule masti, što može dovesti i do pucanja membrane i oslobađanja tečne faze mlečne masti. Oslobođena mast postaje hidrofobna i tezi da se aglomerira i odvaja od vodene faze. Iz ovoga se može izvući zaključak da neophodni mehanički i termički tretmani mleka mogu da imaju negativne posledice aglomerirane mlečne masti na površini mleka (Sharma i Rathore, 2010). Najozbiljnija posledica promene masne globule je mogućnost delovanja lipaze, čija aktivnost je moguća i na temperaturama blizu 0^{o} C.

Promene na membrani masne globule omogućavaju kontakt između masti i lipaze. To se može objasniti mehanizmima delovanja. Prvi mehanizam odvija se pod dejstvom hlađenja mleka na temperaturu ispod 10°C, a naročito kada se ono brzo obavlja između 0 i 5°C, kada je lipaza sa adsorbcionog sloja masne globule neaktivna. Zato se ova aktivnost često naziva spontana lipoliza. Obim eventualne degradacije je uslovljen značajnijim promenama membrane masne globule. U normalnom mleku, rizik od ove lipoliza je ograničen, jer je lipaza praktično inaktivirana. Kod mleka nekih muznih životinja, kao kod neuhranjenih krava ili hranjenih hranivima prebogatih koncentratima, kod mleka staromuznih krava ili obolelih od mastitisa, delatnost lipaze je visoka i hlađenje može da dovede do značajnih lipolitičkih promena (Chazal i sar., 1987).

Stepen lipolize je veoma nizak kada je hlađenje brzo (15 sekundi), a veći sa prosečnim vremenom hlađenja od oko 25 minuta. Iz ovog razloga potrebno je da se izabere najbolja temperature u datim uslovima hlađenja. Takođe, sukcesivno dodavanje toplog mleka u mleko koje se već hladi mora biti propraćeno odgovarajućim načinima njegovog mešanja, čime se maksimalno izbegava aktivnost lipaze.

Drugi mehanizam je više značajan za preradu mleka, ali se pod određenim uslovima može aktivirati i u obradi sirovog mleka. Pod uticajem mehaničke aktivnosti obrade (homogenizacija) a zatim hlađenja mleka, membrana masne globule biva mehanički razorena i mast se razliva po plazmi mleka. Tada dolazi do formiranja nove membrane uz sadejstvo proteina koji se uključuju u kazeinski matriks. Pored toga, usled povećanja broja masnih globula, dolazi i do povećanja njihove ukupne površine pa je moguća povećana agitacija lipazne aktivnosti. Ovo poslednje je pospešeno kada je omogućen ulazak vazduha u mleko i stvorena njegova pena. Osetljivost lipaze na termičke tretmane je iskorišćena radi njene inaktivacije ili razaranja. Ona se uništava zagrevanjem 30 sekundi na 80°C. To znači da primenjena pasterizacija ne mora uvek da ima potpune inhibitorne efekte.

Hlađenjem mleka deo kalcijumfosfata koji je vezan za kazein se rastvara. To dovodi do povećanja kalcijuma i neorganskih fosfata u vodenoj fazi na račun koloidne faze mleka. Konzerviranjem mleka na 3-4°C 48 sati, ovo povećanje može dostići 10 do 20% za kalcijum i 8 do 10% za fosfate (Coulon i sar., 1991).

Ove fizičko-hemijske promene mogu da imaju ozbiljne posledice u preradi mleka, posebno u sirarstvu. Najčešće je to uzrok produženoj koagulaciji mleka pod dejstvom sirišnog enzima. Posle toga se mogu primetiti promene reoloških karakteristika koaguluma. Gruš je mekši i krhkiji, a to

otežava njegovu mehaničku obradu. Direktna posledica je zadržavanje veće količine vode od željene posle presovanja sira. Takođe su uočene i posledice uslova skladištenja na proizvodnju UHT mleka (Panfil-Kuncewicz i sar., 2005).

ZAKLJUČAK

Pored osnovnih uslova, kao što su odabir rase, uslovi smeštaja, načini ishrane, zdravstvena zaštita mlečnih grla i drugo, u proizvodnji mleka veoma su važni način muže i postupak sa mlekom posle muže. Na osnovu naših ispitivanja obrade mleka posle muže na malim, srednjim i velikim farmama može se zaključiti sledeće:

- Posle muže, pa sve do prerade, mleko je permenentno izloženo rizicima promene kvaliteta.
- Promene kvaliteta mleka mogu da budu hemijske, fizičke i mikrobiološke. U našim ispitivanjima nije konstatovana bitna promena kvaliteta mleka.
- Organizacija sabiranja mleka podrazumeva očuvanje njegovog kvaliteta.Ovo se najbolje pokazalo kod velikih farmi, dobro kod srednjih farmi, a sa delimičnim propustima kod malih farmi.
- Hlađenje mleka odmah posle muže je bilo kod velikih i srednjih farmi i to na temperaturama ispod 4^{o}C uz odgovarajuće mešanje.

Mišljenja smo da se kod malih farmi nedovoljno posvećuje pažnja tehnološkim operacijama obrade mleka, što bi u narednom periodu trebalo korigovati. Mnogo više se posvećuje pažnja uštedi sredstava (neadekvatne pokretne mašine za mužu, loše hlađenje mleka, neodgovarajuća higijena i slično) nego očuvanju kvaliteta mleka.

ZAHVALNICA

Rad je deo istraživanja u okviru projekata TR 31095 i TR 31086 koje finansira Ministarstvo za obrazovanje i nauku Republike Srbije

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SUMMARY

THE EFFECT OF PRIMARY TREATMENT ON LIPOLYTIC CHANGES IN RAW MILK

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Milk, as one of the basic food in the human diet has to be produced according to the highest standards in order to preserve all its properties. In this context, hygiene of milk production and its processing after milking are of the great importance. The effecttiveness of hygienic procedures has a positive influence on the bacteriological safety of milk, making it possible to avoid adverse effects of milk sensory characteristics.

In this paper the results of the impact of applied processing technological operations to the quality of milk are presented. On different size farms, the changes of the chemical composition and physical properties of milk during cooling were investigated. Bacteriological milk accuracy is investigated by total microorganisms' number determination, with special reference to psychrophilic and lipolytic microorganisms.

Key words: raw milk • lipolytic changes • hygienic quality

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