ISSN 2334-9883

UNIVERSITY OF KRAGUJEVAC
FACULTY OF AGRONOMY ČAČAK

PROCEEDINGS
CONFERENCE OF AGRONOMY STUDENTS
with international participation

Vol. 10, Issue 10, 2017

Čačak, 23-25 August 2017

CONFERENCE OF AGRONOMY STUDENTS

ORGANISER AND PUBLISHER

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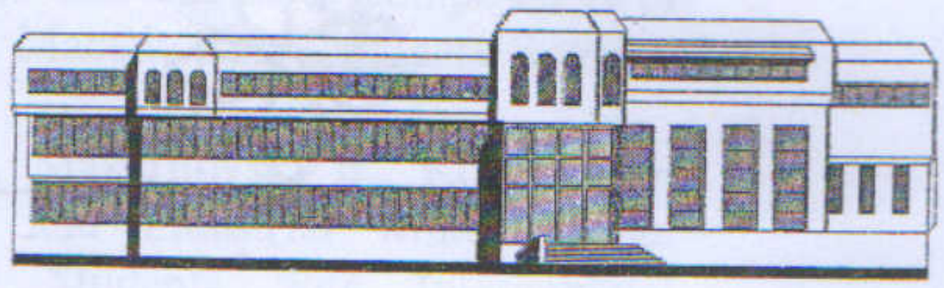
www.kg.ac.rs



For the Publisher:
Prof. Dr. Vladeta Stevović

Computer editing and typesetting: Dalibor Tomić, B. Sc., Dušan Marković, B. Sc.
Cover page: Dr. Snežana Tanasković

Supported by: Ministry of Education, Science and Technological Development, Republic of Serbia and City of Čačak

Printed and bound by: "Studio za dizajn", Vukašina Ignjatovića br. 12, Čačak
Number of copies: 120

Physico-chemical and microbiological changes during manufacture of white brined cheese

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Abstract: The aim of research was to evaluate changes of physico-chemical characteristics, somatic cell count and microbiological changes during ripening of white brined cheese. During the process of ripening from one bathes was taken sample from curd and cheese samples at 1-st, 5-th and 10-th day. From each cheese two samples were taken, one from the middle and one from the cheese rind. The Escherichia coli count was reduced during the ripening process. It was found negative coefficients of correlation between content of NaCl, pH and Escherichia coli counts ($r=-0.86$ $p<0.001$; $r=-0.98$ $p<0,001$, respectively).

Key words: cheese, Escherichia coli, ripening

Introduction

Cheese is the generic name for a group of fermented milk-based food products, delivered in an extensive variety of flavors and forms all through the world (Fox, F.P., et al., 2000). The cheese itself is regarded as a nearly complete source of valuable nutrients, especially conjugated linoleic acid, calcium, phosphorus and high – quality protein (Fox, P. and McSweeney P, 2004; Ercan D., 2009; Waleed A. M., 2013). Cheese is a biochemically dynamic product and undergoes significant changes during ripening (Fox, F.P., et al., 2000), they may be grouped into primary (lipolysis, proteolysis and the metabolism of residual lactose and of lactate and citrate) or secondary (metabolism of fatty acids and of amino acids) biochemical changes (McSweeney P., 2004).

White brined cheeses are the most widely produced and consumed cheeses in Macedonia. Currently, white brined cheeses in Macedonia are produced by both traditional and industrial methods. Traditionally, white brined cheeses have usually been made from unpasteurized or medium heat-treated milk, without starter cultures and in small dairy plants and households using simple

equipment. Macedonian white brined cheese might be characterized as a soft (50 - 60% moisture), high fat cheese (25-30%), with protein content of 12–21% and high salty (3–5%) with a pH range of 4.20 – 5.05 (Mojsova S., 2013).

The microbiological quality of raw milk can be affected by several factors, such as milking, housing, farming system (organic, conventional), and the season of the year (Bogdanovicova K, et al., 2016). E. coli bacteria are considered an important hygiene indicator throughout the process of raw milk obtaining, storage, transport, and sale. E. coli is commonly found in the intestinal microflora of humans and warm-blooded animals, but it may become a pathogenic organism (Costa et al. 2009). In heat untreated raw materials of animal origin, such as raw milk, Escherichia coli occurs quite frequently (Badri et al. 2009).

It is evident that the rate of survival and/or growth of pathogenic bacteria in cheeses depends on the ecological conditions (Aw (water activity), pH, salt content, temperature of maturation) within the cheese and/or brine. Although storage in brine is thought to cause a decrease in the populations of undesirable contaminants, there is great concern that the brine can also serve as a reservoir of certain salt-tolerant pathogens (Bintsis T., and Papademas P., 2002). Generally, bacteria belonging to the family Enterobacteriaceae, such as E. coli, do not tolerate high salt levels (Fatimah A.B., and Anderson J.G., 2009)

Material and methods

Cheese manufacture and sampling

Sample analyses were conducted on traditionally produced cheese in laboratory conditions at Faculty of Biotechnical sciences - Bitola. Raw cow's milk was used to manufacture the cheese. Samples of milk, curd, cheese over dry salting process (1st day of ripening), and 5-th and 10-th days of brine ripening were analyzed. The curdling was performed with liquid commercial rennet for 45-50 min without heating the milk. Further on, the curd was cut in cubes of 2 cm^3, resting for 5 minutes and afterwards pressed in cheese mold for 24 hours. Than the curd was cut into four parts and salted on the surface with coarse-grained salt. Cheese blocks were placed in plastic cans filled with brine solution of 15 g NaCl/100g. During the ripening period of first 10 days the cheese was held at 15-17°C (Figure 1 White-brined cheese traditional technology).

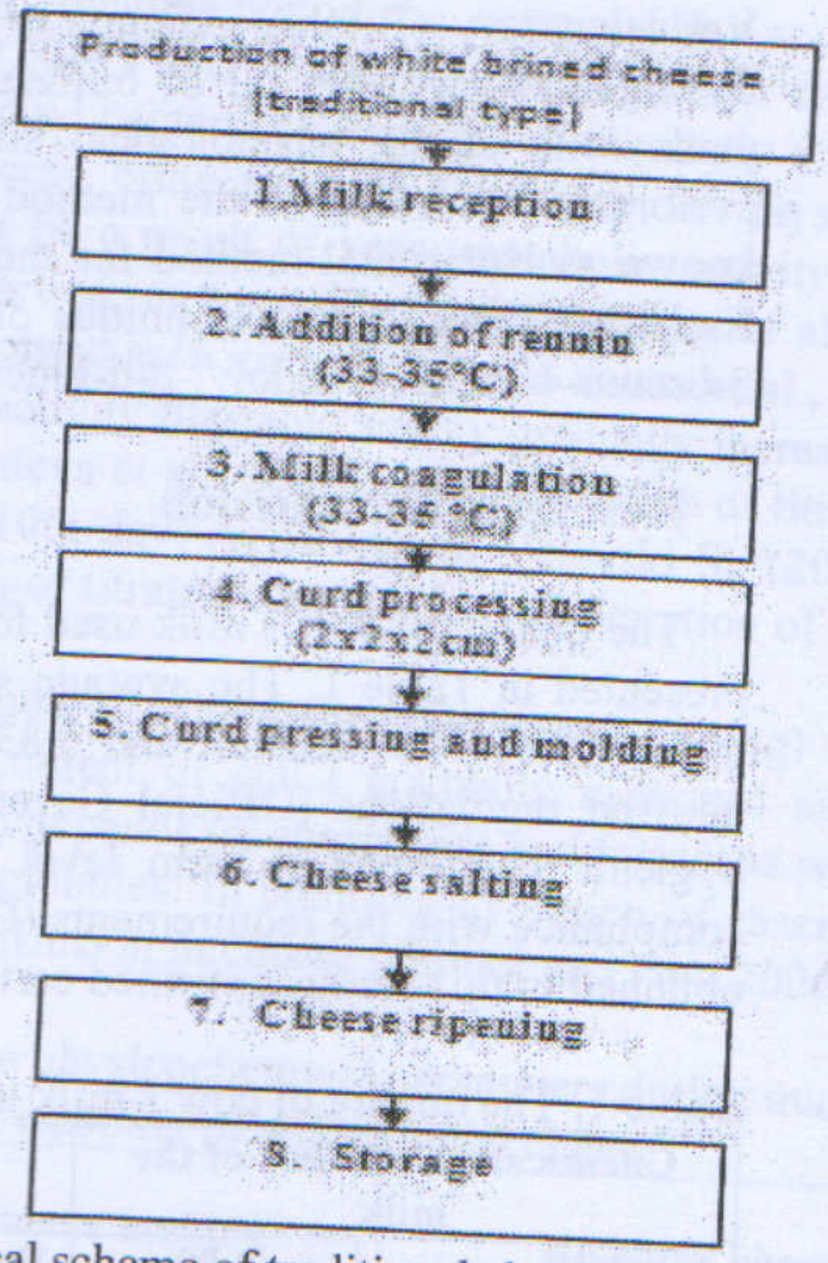

Figure 1. Technological scheme of traditional cheese (Popovski N. et al., 2015)

Microbiological and physicochemical analysis of the cheese

Physicochemical and microbiological analysis was examined on milk, curd and cheese samples on the 1st day, 5th day and 10th day of manufacture. Lactoscan MCC was used for physicochemical analysis of milk. The determination of fat content, pH and ^{0}SH, dry matter (%), water content (%), NaCl (%) was done on curd and cheese samples. The determination of the content of milk fat in cheese is determined by the Gerber method (Caric at al. 2002). The pH of milk and cheese samples was measured using a digital pH meter (model MP120FK Mettler Toledo, Greifensee, Switzerland). Titratable activity (TA) was determined by titration using Soxlet-Henkel method. Dry matter and water content was determined by MJ33 Mettler Toledo. The NaCl contents in curd and cheese during ripening were determined by the Mohr method. All analyses were performed in triplicate Milk sample examined by Beta star screening kit (Neogen, USA) which is used for rapid detection of the betalactam antibiotics such as penicillin, ampicillin, amoxicillin, cloxacillin, and cephapirin.

Samples for microbiological analysis were taken under sterile conditions, placed into plastic sterile dishes and kept under refrigeration until analysis. Microbiological analyses were preformed within 2 h after the sampling at Anima

Vet laboratory (Bitola). Aliquot of 10 mL of milk or 10 g of cheese was homogenized with 90 ml of buffered peptone solution. Decimal dilution was made with sterile physiological solution. The enumeration of E. coli was performed according to the method defined by MKC EN ISO 16649-2:2008, known as Horizontal method for the determination of β-glucuronidase-positive Escherichia coli, by the technique of counting colonies cultured at 44°C, using 5-bromo-4-chloro-3 indolyl-β-d-glucuronide.

Results and discussion

The quality of cow's milk used for the manufacture of white brined cheese is presented in Table 1. The average somatic cell and total bacterial count in the milk was $9,8*10^5$ cells/ml and $5,836*10^6$ cells/ml which is much higher than allowed regulations (Official Gazette no. 197/2016). This confirms the poor hygienic conditions at farm level. The chemical parameters in milk are in compliance with the requirements (Official Gazette no. 96/2011) also the results obtained in this study confirmed earlier findings of Mojsova S., et al., (2013).

Table 1. The quality of cow's milk used for manufacture of white brined cheeses

Chemical parameters of the milk		**Microbiological parameters of the milk (CFU/ml)**	
Fat (%)	3,80	Enterobacteriaceae	$0,033*10^6$
Solids non fat SNF (%)	8,30	Coliform bacteria	$5,1*10^6$
Density (%)	27,99	Staphylococcus	$0,42*10^6$
Lactose (%)	4,55	Eschericia coli	$0,0024*10^6$
Solids (%)	0.68	Total bacterial count	$5,836*10^6$
Protein (%)	3,04		
^{0}SH (%)			
Condictivity (%)	3,43		
Freezing point	-0,528		
Antibiotic residues	No positive detection		
SCC (cells/ml)	$0,98*10^6$		

The physico-chemical characteristics of white brined cheese during manufacturing and ripening are presented in Table 2. Acidity is one of the most important properties of cheese which influences the structure, rheological, sensory characteristics and overall quality of the cheese (Savic Z., 2015). During

the first 10th days of ripening period the average pH was 5,34. According to Levkov V., et al., (2014) decreasing of the pH was a result of lactic acid production by lactic acid bacteria (LAB). But in our study a gradual increase of pH value during cheese brine ripening (1, 5 and 10 days of ripening) was observed. This might be a result of yeast metabolic activity which uses lactic acid as a source of carbon, or a result of great amounts of alkaline compounds released during proteolytic activities (Volken de Souza et al., 2003). As a result of diverse metabolic activity titratable acidity gradually increased during cheese ripening (Dubrova Mateva et al., 2008), but lower value of titratable acidity was recorded on 5th and 10th day. According to Velevski S., (2015) lack of lactic acid leads to reduction of titratable acidity and adulteration of cheese during the ripening time.

The combined method of salting (dry salting and brining) was used. During cheese ripening, the content of NaCl gradually increased as a result of salt diffusion, which in turn depends on cheese size and format as well as on size and quantity of cheese fat globules. In addition, hydration of casein micelles might interfere with the salt diffusion in cheese (Guinee and Fox, 2004).

Table 2. Changes in the physicochemical parameters during manufacturing and ripening of white brined cheese

Parameters	**Cheese making**		**Ripening cheese (days)**		
	Milk	**Curd**	**1**	**5**	**10**
pH	6,67	5,30	5,29	5,29	5,44
Titratable acidity (^{0}SH)	7,2	72	80,8	42	42,4
Dry matter (%)	12,08	48,94	49,29	48,82	47,02
Water content (%)	/	51.06	50,71	51,18	52,98
Fat (%)	3,9	27	27	27	27
NaCl (%)	/	/	1,60	7,68	8,17

The traditional cheeses contain original microflora (Beresford et al., 2001) which evolved during ripening as a result of nutritive and environmental changes in cheese (Williams et al., 2002). The evolution of Escherichia coli counts during the manufacturing and ripening of white brined cheese are shown in Table 3 and

Figure 2, and the maximal average values of Escherichia coli were attained in the curd ($1,76*10^6$). The counts of Escherichia coli are reduced constantly during the ripening period. The negative coefficients of correlation between content of NaCl and Escherichia coli counts ($r=-0.86$ $p<0.001$) suggest the inhibitory effect of high salt content. The negative coefficient of correlation between pH and the investigated group of bacteria ($r=-0.98$ $p<0,001$) might indicate the possible adaptation of microorganisms to lower values of pH in cheese.

Table 3. Changes in counts of Escherichia coli (CFU/ml) during manufacturing and ripening of laboratory produced white brined cheese

Parameters	Cheese making		Ripening cheese (days)		
	Milk	Curd	1	5	10
Escherichia coli (CFU/ml)	$0,0024 *10^6$	$1,76 * 10^6$	$0,2 *10^6$	$0,11 * 10^6$	$0,011 * 10^6$

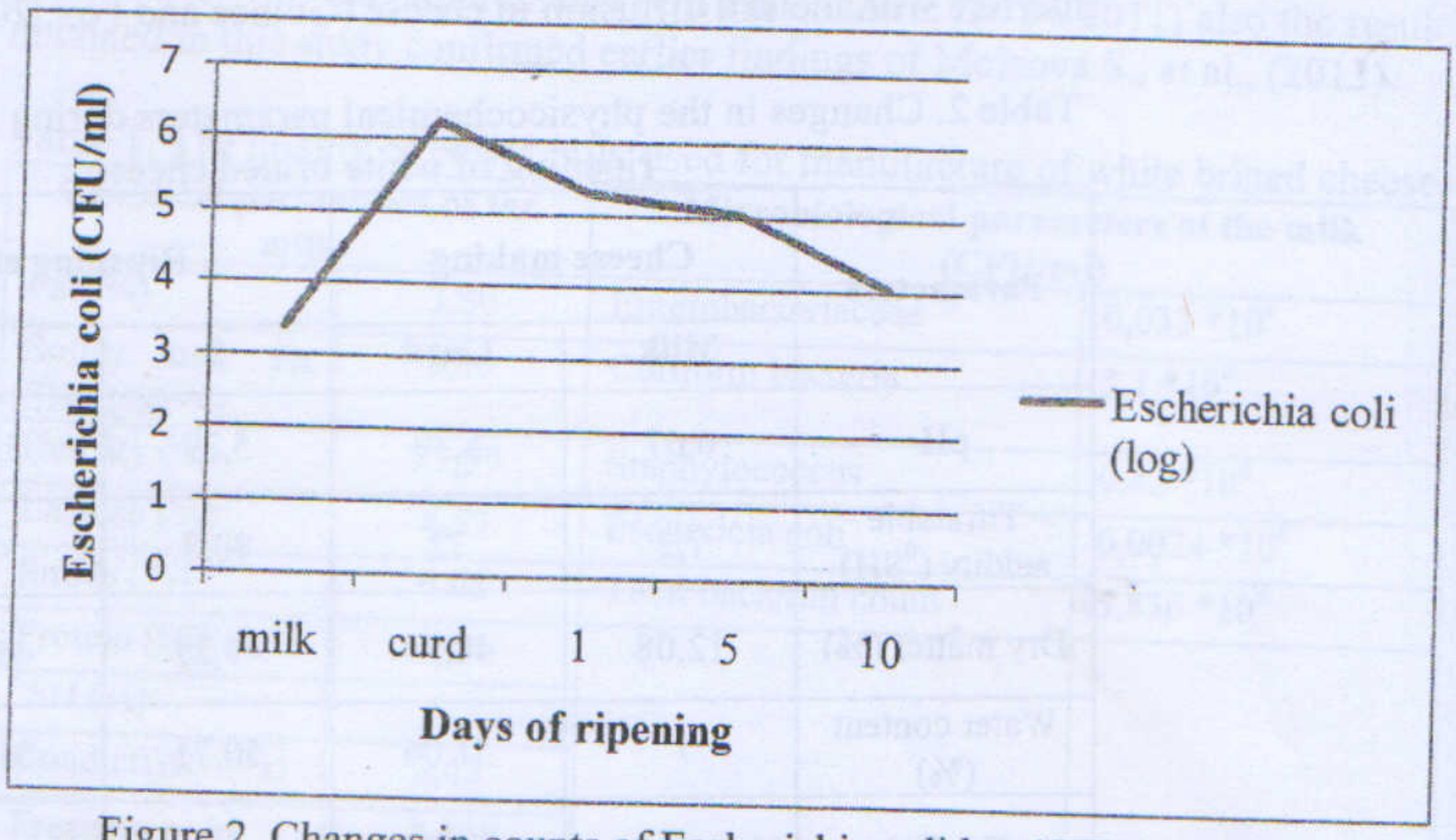

Figure 2. Changes in counts of Escherichia coli log (CFU/ml) during manufacturing and ripening of laboratory produced white brined cheese

Conclusion

The obtained results indicated a high number of all investigated groups of microorganisms in all stages of production, starting from the raw milk. The presence of high counts of Escherichia coli demonstrated the poor sanitary and

hygienic conditions at raw milk. Reduction of Escherichia coli content during the first 10 days of cheese ripening is influenced by salt content in cheese.

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