

A survey on hygienic and physicochemical properties of Istrian cheese

Višnja Magdić¹, Samir Kalit^{2*}, Mirna Mrkonjić Fuka³,
Andrea Skelin³, Dubravka Samaržija²,
Sulejman Redžepović³, Jasmina Havranek²

¹Croatian extension service, Karlovac county, Branch office Slunj,
Trg Dr. Franje Tuđmana 9, 47240 Slunj, Croatia

²Dairy Science Department, ³Department of Microbiology,
Faculty of Agriculture University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia

Received - Prispjelo: 19.02.2013.

Accepted - Prihvaćeno: 13.05.2013.

Summary

Istrian cheese is the traditional hard cheese produced exclusively from the raw milk of the autochthonous Istrian sheep. The aim of this study was to determine the bacteriological quality, physicochemical properties of the sheep milk and Istrian cheese as well as proteolysis of cheese produced on six family farms during the cheese ripening. The hygienic microbial indicators of the sheep milk were poor in comparison to the other East Adriatic regions. However, the number of bacterial indicators rapidly declined during the ripening and they were under the detection limit for "ready to consume" cheese and therefore indicated their good bacteriological quality. Regression function of the effects of the ripening time on physicochemical properties of Istrian cheese during ripening showed a substantial increase in total solids, in salt, in moisture and in salt content as well as in the lactic acid in the total solids. However, these changes were not significant due to the high variability of the Istrian cheese production. The farm cheese making procedure affected the electrophoretic profile of the primary proteolysis in the cheese samples. Significant ($P < 0.01$) increase of the index beta was found due to the high content of salt in moisture ($> 5\%$). Equal degradation of α_{s1} -casein and β -casein during ripening of Istrian cheese occurred. A low percentage of nitrogen fractions soluble in water and in 12 % trichloroacetic acid were determined.

Key words: sheep milk, Istrian cheese, hygienic quality, proteolysis properties

Introduction

Istrian cheese is a traditional full-fat, hard sheep cheese which has been produced for several hundred years along the Croatian peninsula Istria (Samaržija et al., 2003). The Istrian peninsula is characterised by hilly relief rich with pasture and plenty of aromatic sub-Mediterranean herbs. Many of the local farmers still produce Istrian cheese exclusively from raw ewe's milk by milking the autochthonous Istrian sheep, Pramenka. According to official data only 2,314 head of these Istrian Pramenka breed exist in Istria (Mulc et al., 2011). Istrian sheep produce 200 L of milk per lactation that last on average 198 days.

The Istrian cheese is characterised by a cylindrical shape, 18 to 20 cm in diameter and 7 to 9 cm in height. Only rennet is added without using starters. Therefore, acidification during cheese manufacturing and specific flavour is caused by natural, non starter lactic acid bacteria (Shakeel-Ur-Rahman et al., 2000) that originated from raw ewe's milk and woody tools traditionally used in the Istrian cheese production. Besides the beneficial microorganisms, cheese micro biota may include undesirable species as food-borne pathogens or as spoilage micro flora which activity causes the abnormal ripening, unpleasant tastes and flavours (Ledenbach and Marshall, 2009; Official Gazette, 2008). The higher number of these microorganisms in raw milk is mainly

*Corresponding author/Dopisni autor: E-mail: skalit@agr.hr

reported in countries with poorly controlled hygienic production (de Buyser et al., 2001; Guerra et al., 2001). However, the bacteriological quality of the fermented products obtained from such raw milk is high as a result of antagonistic interaction of the predominant, non starter lactic acid bacteria, on spoilage microorganisms (Fuka et al., 2010; Nero et al., 2008; Samarzija et al., 2007).

During the cheese ripening, intensive proteolysis occurs and there are dramatic changes in the physicochemical properties of the aging cheese (Fox and McSweeney, 1998; Shakeel-Ur-Rahman et al., 2000). However, no physicochemical properties, proteolysis or microbiological hygienic quality of Istrian cheese have been investigated so far. The aim of the present study was therefore to investigate the hygienic quality, chemical composition and physicochemical properties of raw ewe's milk and Istrian cheese during the ripening.

Materials and methods

Farm description

Farms involved in the investigation are characterised by breeding between 50-300 indigenous Istrian Pramenka. Istrian Pramenka sheep are characterised by a convex nasal bone, long legs and black-white wool (Mioč et al., 2012). They are farmed on the natural pastures of the sub-Mediterranean; the pasture contains a whole range of quality species. On these hilly pastures Pramenka have adapted to the local ecological conditions and particularly to the grazing, therefore producing milk of exceptional quality. Hand milking was practiced on the farms. All farms did not have a small dairy plant and therefore the cheese was made in their homes, however not in the houses itself but in separate rooms used especially for cheese making. The cheese was ripened in a ripening chamber with controlled but variable temperature and air humidity (up to 19 °C and up to 90 %, respectively).

Sampling of ewe's milk, curd and cheese

Six batches of representative Istrian cheese (F1-F6) were collected from six different farms. The batch size was about 50 L of ewe's milk per farm. Ewe's milk samples containing a proportional aliquot of milk from the evening and morning milking were collected in 200 mL sterile plastic bottles under sterile conditions

and were transported to the laboratory in temperatures under 4° C. The cheese was ripened in ripening chambers at the family farms. A cheese drill was used to take a sample of about 20 g from the core to the surface. The samples were taken from each cheese after 30, 60, 90 and 120 days of ripening according to the procedure of Licitra et al. (2000).

All of the analyses were performed in the Reference laboratory of the Dairy Science Department of the Faculty of Agriculture, University of Zagreb and Laboratory of the Department of Hygiene and Technology of Animal Products at the Veterinary Faculty, University of Zagreb.

Microbiological analysis

The number of indicator microorganisms was estimated in the samples of milk (M), curd (Cr), young cheese (Ch) and 120 day old ripened Istrian cheese (rCh). Twenty five g of curd or cheese was mixed with 225 mL of sterile Ringer's solution and homogenized in a BagMixer® 400 (Interscience, France) for 3 min. Decimal dilutions of the cheese homogenates were made in sterile, quarter strength Ringer's solution and using the pour plate technique, inoculated on different agars. The decimal dilutions of milk were prepared directly in sterile Ringer's solution and inoculated by pouring the plates with agar. *E. coli* were grown on Coli-ID agar (BioMerieux, France) at 37 °C for 48 hours. *Enterobacteriaceae* were analyzed on Violet Red Bile Glucose agar (Oxoid, England) according to HRN EN ISO 8523:1999 and *Staphylococcus aureus* were grown on Baird-Parker agar (Merck, Germany) at 37 °C for 48 hours (HRN ISO 6888-1:1999). Sulphite reducing clostridia were cultivated under anaerobic conditions on Sulphite Polymyxin Sulphadiazine agar (Merck, Germany) at 37 °C for 72 hours. The presence of *Salmonella* spp. was determinate by HRN ISO 6785:2001 and the presence of *Listeria monocytogenes* was detected according to HRN ISO 10560:2001. *E. coli* O157:H7 were analyzed on O157:H7 ID agar (BioMerieux, France) after growth at 37 °C for 48 hours.

Physicochemical analysis

Milk samples were analyzed in duplicate for the pH value (Mettler Toledo, Seven Multi, according to manufacture's instructions), titratable acidity (°SH) (AOAC 947.05:2000), freezing point (Funke Gerber, Cryostar 1, HRN EN ISO 5764:2003),

somatic cell count (SCC) (Foss Electric, Fosomatic 90, EN ISO 13366-3:1999) and total bacterial count (cfu) (Foss Electric, Bactoscan FC, ISO 21187:2004), as well as for chemical composition of the milk which included determination of the fat, protein, lactose, total solids non-fat content using Foss Electric, MilcoScan FT 120 instrument according to HRN EN ISO 9622:2001 according to HRN EN ISO 9622:2001 method. Casein content in the milk was analyzed according to ISO 17997-1:2004 according to ISO 17997-1:2001 method.

Chemical and physical analyses of the cheese at various stages of ripening were performed using standard methods. Analyses of the cheese included determination of the fat content in the cheese according to the Van Gulik method (HRN EN ISO 3433:1999), protein content according to Kjeldahl method (HRN EN ISO 8968-2:2003), total solids (ISO 5534:2004), pH value (Mettler Toledo, Seven Multi, according to manufacture's instructions), salt according to Møhr method (AOAC 935.43:2000), and the amount of lactic acid (AOAC 920.124:2000). The amount of water soluble nitrogen (WSN (%TN)) and nitrogen soluble in 12 %- trichloroacetic acid (TCA-SN (%TN)) was determined in the cheese according to the Kjeldahl method (Mayer et al., 1998). All analyses were performed in duplicate.

Urea-polyacrylamide-gel electrophoresis and densitometry

The separation of the casein fractions was performed by alkaline urea-PAGE (12 % T; 3.8 % C; pH 8.9; 4.5 M urea), according to the Andrews procedure (1983). Bands were stained with Coomassie brilliant blue G-250 (Blakesley and Boezi, 1977). All electrophoresis chemicals were of electrophoresis purity reagent quality (Biorad, Richmond, CA, USA). Other chemicals were of analytical grade (Merck, Darmstadt, Germany). Urea-PAGE electrophoresis was performed using the Mini Protean III system, supplied by PAC 3000 Power System, BioRad Laboratories, Richmond, CA.

Densitometric evaluation of bands was performed using BioRad Densitometer ChemiDoc XRS System. Two electrophoretic ripening indexes were used: beta index, $\sum \gamma\text{-CN} / \beta\text{-CN}$ and alpha index, $\alpha_{s1}\text{-I-CN} / (\alpha_{s1}\text{-I-CN} + \alpha_{s1}\text{-CN})$ based on the procedure of Kalit et al. (2005).

Statistical analysis

All statistical analyses were performed using SPSS statistical program (SPSS, Version 9.0, USA). Data that was not normally distributed was log-transformed. Effects of ripening time and their interactions on cheese variables were tested using ANOVA analysis and Univariate analysis of variance supplemented in SPSS software. The Post Hoc Tukey-Kramer test was used to determine whether differences existed between ripening stages ($P < 0.05$). Regression analysis was used to predict the changes of the cheese composition with ripening time recommended by Licitra et al. (2000).

Results and discussion

Ewe's milk quality

Means and standard deviations of milk composition, SCC, cfu and physical properties of Istrian ewe's milk from six randomly selected family farms are presented in Table 1. The gross composition of milk showed slightly less values for total solids, fat, protein, casein and casein to the protein ratio in comparison to the ewe's milk from other east Adriatic coastal regions (Antunac et al., 2002; Matutinović et al., 2007; Mikulec et al., 2008). Furthermore, the mean value of SCC after transformation from log value to number of cells per milliliter was $1,096 \times 10^3/\text{mL}$, which were much higher than those observed by Prpić et al. (2003) who determined 407×10^3 SCC/mL or Mikulec et al. (2008) who determined 236×10^3 and 380×10^3 SCC/mL, using the same transformation procedure. A similar result was found comparing a transformed value of bacterial count. Obtained results showed $2,455 \times 10^3$ cfu/mL that it was much higher than the results obtained by Prpić et al. (2003) (750×10^3 cfu/mL) and by Mikulec et al. (2008) (436×10^3 and 478×10^3). Higher SCC and cfu values obtained in the present investigation could be attributed to poor farm management considering problems with hygiene and incidences with mastitis within the dairy herds in comparison to the other east Adriatic coastal regions that produce traditional sheep cheese. The data obtained showed clearly mastitis milk was what may affect the quality of the Istrian cheese.

Table 1. Mean values and standard deviations of milk composition, physical properties, somatic cell count (SCC) and total bacteria count (cfu) of Istrian ewe's milk (n=6)

Measurements	Values
Total solids (g/100 g)	18.04±1.13
Fat (g/100 g)	6.85±0.79
Total solids non-fat (g/100 g)	11.21±0.35
Proteins (g/100 g)	5.82±0.55
Casein (g/100 g)	4.39±0.42
Casein to protein ratio (%)	75.68±6.37
Lactose (g/100 g)	4.51±0.2
pH	6.58±0.07
Acidity (°SH)	10.15±1.37
Freezing point (°C)	-0.57701±0.04
SCC (log ₁₀ cells/mL)	6.04±0.29
cfu (log ₁₀ cells/mL)	6.39±1.31

Bacteriological quality

The results of the microbial analysis are present in Table 2. *E. coli*, *S. aureus* and sulphite reducing clostridia (SRC), these were detected in milk, curd and young cheese. At the early stage of cheese making the fermentation process is weak, therefore not having the visible positive effects considering the number of harmful micro flora. The higher values of all the indicator microorganisms were noticed in curds in comparison to milk samples. There are intensive handling procedures prior to curd formation, therefore it probably influenced the number of respective microbes in investigated curd samples. The number of indicator organisms rapidly declined during the ripening process. They were still detected in young Istrian cheese but the number was lower in comparison to milk or curd. In ripened cheese the number of indicator microbes is under the detection limit probably as a result of the rapid acidification during cheese making and/or negative microbial interaction during the cheese maturation (Donnelly, 2004). Potentially pathogenic *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* were not detected among all the analyzed samples. The absence of spoilage and pathogenic microbes in "ready to consume" ripened Istrian cheese indicates its satisfactory bacteriological quality and safety. The

results are in agreement with the study of Samaržija et al. (2003) who also concluded that traditional Croatian sheep cheese is an unfavorable medium for the growth of most of the food-borne pathogenic microorganisms.

Physicochemical properties of cheese

The regression function of the affects of the ripening time on the physicochemical properties of Istrian cheese during ripening is presented in Table 3. The moisture content was extensively lost during the ripening of Istrian cheese in natural rind. This caused an increase in total solids from 62.69 % to 70.58 %. Consequently, salt in moisture, salt content and lactic acid in the total solids increased, but not significantly, as found in previous investigations for similar cheese (Mikulec et al., 2008). It may have occurred due to the high variability in the handling of milk and in the production of Istrian cheese. The highest standard deviation of ewe's milk was found for fat, protein and casein (g/100 g), as well as for the casein to protein ratio (%) (Table 1). The lower ratio in casein content and higher fat content could be caused by a high SCC that was found in ewe's milk (Table 1) which would concomitant by a inferior protein and fat recovery during cheese making (Kalit et al., 2004).

Table 2. The number of indicator microorganisms in milk (M), curd (Cr), young (Ch) and ripened Istrian cheese (rCh) investigated at 6 different farms (F1-F6). The results are present as cfu/g of curd or cheese and cfu/mL of milk

Farm	Sample	<i>E. coli</i>	<i>S. aureus</i>	SRC	<i>E. coli</i> O157:H7	<i>Salmonella</i> spp.	<i>L. monocytogenes</i>
F1	M	4.0x10 ²	1.5x10 ³	<10	neg	neg	neg
	Cr	1.5x10 ³	6.0x10 ³	<10	neg	neg	neg
	Ch	1.0x10 ³	2.2x10 ³	<10	neg	neg	neg
	rCh	< 0	<10	<10	neg	neg	neg
F2	M2	8.0x10 ³	1.3x10 ³	8.0x10 ²	neg	neg	neg
	Cr1	6.3x10 ⁴	2.3x10 ⁴	2.0x10 ³	neg	neg	neg
	Ch2	1.8x10 ³	1.4x10 ³	<10	neg	neg	neg
	rCh2	<10	<10	<10	neg	neg	neg
F3	M3	3.5x10 ³	2.3x10 ³	<10	neg	neg	neg
	Cr3	1.3x10 ⁵	1.0x10 ⁴	<10	neg	neg	neg
	Ch3	<10	1.0x10 ³	<10	neg	neg	neg
	rCh3	<10	<10	<10	neg	neg	neg
F4	M4	<10	<10	<10	neg	neg	neg
	Cr4	<10	6.0x10 ⁴	<10	neg	neg	neg
	Ch4	<10	4.0x10 ³	<10	neg	neg	neg
	rCh4	<10	<10	<10	neg	neg	neg
F5	M5	1.3x10 ⁴	6.0x10 ³	3.0x10 ²	neg	neg	neg
	Cr5	1.8x10 ⁴	3.7x10 ⁴	6.0x10 ²	neg	neg	neg
	Ch5	4.0x10 ³	2.0x10 ³	<10	neg	neg	neg
	rCh5	<10	<10	<10	neg	neg	neg
F6	M5	<10	2.5x10 ³	<10	neg	neg	neg
	Cr6	<10	1.5x10 ⁴	<10	neg	neg	neg
	Ch6	2.0x10 ²	1.0x10 ³	<10	neg	neg	neg
	rCh6	<10	<10	<10	neg	neg	neg

SRC: sulphite reducing clostridia
neg : no microorganisms detected

Proteolysis of cheese

The electrophoretic profile of ripened Istrian cheese was typical for hard sheep cheese (Fig. 1). The relative quantity of intact casein decreased significantly ($P < 0.01$) with a concomitant increase in degradation products as a consequence of the ripening time. This was in general in agreement with the findings of others (Ferreira et al., 2006; Mikulec et al., 2008). There was extensive chymosin activity during the first 60 days of ripening. The electro-

phoretic ripening index alpha was 0.33 after 30 days and increased significantly to 0.6 after 120 days of ripening (Table 4). The optimal regression function that best explains the total variability of the alpha index showed significant ($P < 0.01$) increase in a quadratic manner because chymosin, and most other milk-clotting enzymes are active when high-cooking temperatures are not practiced during cheese manufacture (Lawrence et al., 1984). It was also found for sheep cheese that has been produced by a similar

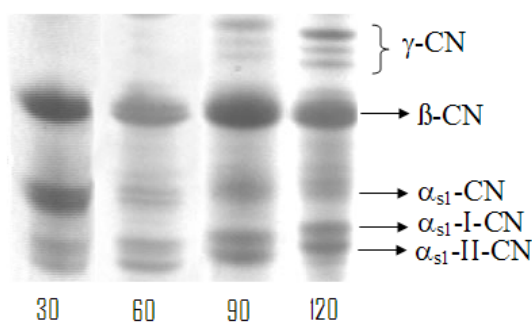


Figure 1. Urea-polyacrylamide gel electrophoresis analysis of a sample of Istrian cheese ripened for 30, 60, 90, 120 days

Table 3. Effects of ripening time on the chemical properties of Istrian cheese (n = 6). Values are given as least square means \pm standard errors

Ripening time (days)	pH	Total solids (g/100 g)	Salt in moisture (g/100 g)	Salt (g/100 g)	Lactic acid/total solids (g/100 g)
30	5.22 \pm 0.30	62.69 \pm 4.44	4.96 \pm 1.30	1.86 \pm 0.50	1.98 \pm 0.59
60	4.99 \pm 0.13	62.03 \pm 5.51	6.53 \pm 2.97	2.37 \pm 0.79	2.14 \pm 0.81
90	5.08 \pm 0.39	66.87 \pm 6.13	7.90 \pm 2.72	2.53 \pm 0.70	2.34 \pm 0.37
120	5.02 \pm 0.59	70.58 \pm 6.78	9.03 \pm 1.07	2.61 \pm 0.40	2.66 \pm 0.29

manufacturing procedure (Mikulec et al., 2008). The relative densitometric intensity of proteolytic degradation products with low electrophoretic mobility of the Istrian cheese, expressed as the beta index had similar intensity like the alpha index (Table 4). It is contrary to the findings of Mikulec et al. (2008) who found β -casein fraction was the most susceptible during Krk cheese ripening and Fallico et al. (2006) who found α_{s1} -casein the most susceptible during the ripening of Piacentinu Ennese cheese. The farm cheese making practice affected the electrophoretic profile of primary proteolysis in the cheese, especially type, application form, and quantity of clotting enzyme applied (Kalit, 2007). A large hydrolysis of α_{s1} -casein fraction occurred in nontraditional cheese compared with traditional cheese, such as Istrian or Krk cheese (Mikulec et al., 2008). Degradation of β -casein was mainly due to the action of plasmin (Kalit et al., 2002a). In our study much more than 5 % content of salt in moisture was found after 30 days of ripening (Table 3). Salt concentration positively influences plasmin

activity on β -casein, especially in cheese produced from raw milk (Somers and Kelly, 2002), therefore explaining the significant ($P < 0.01$) increase of the electrophoretic ripening index beta in our study. Similar intensity of degradation of β -casein was noticed by Mikulec et al. (2008) for Krk sheep cheese therefore confirming that both types of cheese belong to the same group with similar manufacturing procedures. The intensity of proteolysis identified by the electrophoretic ripening index beta and alpha were almost equal for Istrian cheese, however this was not the case for previous studies done on traditional sheep cheese. Degradation of α_s -casein was higher than β -casein for some cheese (Taracchi et al., 2004; Fallico et al., 2006) contra to the higher degradation of β -casein to α_s -casein was noticed for Krk cheese (Mikulec et al., 2008). Equal degradation of α_s -casein and β -casein could be an indicator of the ripening quality of the Istrian cheese. However, the results obtained need to be revised as the SCC of milk used for Istrian cheese production was particularly high. Cheese produced from high

Table 4. Effects of ripening time on the proteolysis of Istrian cheese (n = 6)

	Alpha index	Beta index	WSN (%TN)	TCA-SN (%TN)
Ripening time (T) (days)	**	**	ns	ns
30	0.33±0.13 ^a	0.30±0.08 ^a	17.69±5.80	7.52±2.31
60	0.50±0.10 ^{ab}	0.50±0.14 ^b	17.35±3.90	6.51±3.12
90	0.52±0.08 ^b	0.52±0.10 ^b	22.95±12.82	8.26±3.94
120	0.60±0.09 ^b	0.63±0.10 ^b	27.72±7.61	8.48±4.05
Regression	y= 0.177+0.18x- 0.019x ²	y= 0.117+ 0.237x- 0.03x ²	y= 18.37-2.26x +1.173x ²	y=7.76-0.735x + 0.244x ²
R ²	0.63	0.59	0.29	0.05
F x T	ns	ns	ns	ns

Significance level: *, P<0.05; **, P<0.01

^{a,b,c}: Means within the same column and not sharing the same superscript letter are significantly different (P<0.05)

ns: not significant

SCC milk has a more intense proteolytic activity, especially due to the plasmin activity in milk (Kalit et al., 2002a; Kalit et al., 2002b).

The WSN (%TN) and TCA-SN (%TN) content increased as a consequence of the ripening progression (Table 4) probably due to a high variability in the ripening conditions among different farms and/or different types of coagulant used. Soluble nitrogen (N) components found in this study are formed mainly by the action of coagulation enzymes and to an equal extent by the milk proteinases. Coagulation enzymes are known as the main proteolytic agents responsible for the production of large peptides from casein, whereas bacterial enzymes from cheese starters cause the formation of short chain peptides, amino acids, ammonia and other minor compounds that are soluble in 12 % trichloroacetic acid, especially at the first stage of ripening (Desmazeaud and Gripon, 1977; Pavlinic et al., 2010). In Istrian cheese the low percentage of nitrogen fractions soluble in 12 % trichloroacetic acid was determined. It could be due to the absence of microbial starter cultures during the Istrian cheese production and poor secondary proteolysis. The opposite to a similar type of cheese such as Krk cheese in which a high percentage of nitrogen fraction soluble 12 % trichloroacetic acid was found (20.82 %) after 120 days of ripening in comparison to 8.48 % in Istrian cheese. These differences

could be mainly explained by regular use of starter cultures in the Krk cheese production (Plavljanić et al., 2010).

Conclusion

Considering the physicochemical properties and proteolysis, Istrian cheese belongs to a group of typical non-standardized hard sheep cheese with some specificity. One of them is an equal degradation of α_s -casein and β -casein that could be an indicator of the ripening characteristics of Istrian cheese. However, further investigation is needed to eliminate influences of high SCC on plasmin activity that may increase intensity of β -casein degradation in Istrian cheese. A low percentage of soluble nitrogen fractions were a consequence of not using starter cultures during the Istrian cheese production. Poor secondary proteolysis is a typical characteristic of Istrian cheese in comparison to similar regional cheese produced from raw ewe's milk.

The number of spoilage and pathogenic microorganisms were under the detection limit for the 120 day old ripened cheese. The absence of these microbes in "ready to consume" cheese indicates its bacteriological safety in spite of the poor hygienic quality (high SCC and total bacterial count) of raw milk used for Istrian cheese production.

Pregled higijenskih i fizikalno-kemijskih parametara istarskog sira

Sažetak

Istarski sir je tradicionalni tvrdi sir koji se proizvodi prvenstveno od sirovog mlijeka autohtone istarske ovce. Cilj je ovog rada bio utvrditi bakteriološku kvalitetu i fizikalno-kemijske parametre ovčjeg mlijeka te istarskog sira kao i proteolizu sira proizvedenog na šest obiteljskih gospodarstava tijekom zrenja. Mikrobiološki indikatori higijene ovčjeg mlijeka bili su loši u usporedbi s drugim regijama istočnog Jadrana. Međutim, tijekom zrenja sira broj bakterijskih indikatora higijene drastično je pao, te je njihov broj bio ispod detekcijskog limita u siru "spremnom za konzumaciju" što je bio pokazatelj njegove dobre bakteriološke kvalitete. Regresijska funkcija utjecaja trajanja zrenja na fizikalno-kemijske osobine istarskog sira tijekom zrenja pokazala je osjetno povećanje sadržaja suhe tvari, soli u vodenoj fazi sira i soli u siru kao i sadržaja mliječne kiseline u suhoj tvari sira. Međutim, te promjene nisu bile značajne zbog velike varijabilnosti u proizvodnji istarskog sira. Način proizvodnje sira na gospodarstvima utjecao je na elektroforetski profil primarne proteolize u uzorcima sira. Utvrđeno je signifikantno ($P < 0,01$) povećanje indeksa beta zbog povećanog sadržaja soli u vodenoj fazi sira ($> 5\%$). Tijekom zrenja istarskog sira utvrđena je jednaka razgradnja α_s -kazeina i β -kazeina te niski postotak dušičnih frakcija topljivih u vodi i 12 %-tnoj trikloroctenoj kiselinu.

Ključne riječi: ovčje mlijeko, istarski sir, higijenska kvaliteta, proteolitičke osobine

References

- Andrews, A.T. (1983): Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research* 50, 45-50.
- Antunac, N., Mioc, B., Pavic, V., Lukac Havranek, J., Samaržija, D. (2002): The effect of stage of lactation on the milk quantity and number of somatic cells in sheep milk. *Milchwissenschaft* 6, 310-311.
- AOAC (2000): *Acidity of Cheese, Titrimetric Method. AOAC official method 920.124*. Washington, DC: Association of Official Analytical Chemists.
- AOAC (2000): *Chloride (Total) in Cheese, Volhard Method AOAC official method 935.43*. Washington, DC: Association of Official Analytical Chemists.
- AOAC (2000): *Acidity of Milk, Titrimetric Method AOAC Official Method 947.05*. Washington, DC: Association of Official Analytical Chemists.
- Blakesley, R.W., Boezi, J.A. (1977): A new staining technique for proteins in polyacrilamide gels using Coomassie Brilliant Blue G 250. *Analytical Biochemistry* 82, 580-582.
- de Buyser, M.L., Dufour, B., Maire, M., Lafarge V. (2001): Implication of milk products in foodborne disease in France and in different industrialised countries. *International Journal of Food Microbiology* 67, 1-17.
- Desmazeaud, M.J., Gripon, J.C. (1977): General mechanism of protein breakdown during cheese ripening. *Milchwissenschaft* 32, 731-734.
- Donnelly, C.W. (2004): Growth and survival of microbial pathogens in cheese. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), *Cheese: Chemistry, physics and microbiology, General aspects*. Elsevier Academic Press 541- 557.
- Fallico, V., Tuminello, L., Pediliggieri, C., Horne, J., Carpino, S., Licitra, G. (2006): Proteolysis and microstructure of Piacentinu Ennese cheese made using different farm technology. *Journal of Dairy Science* 89, 37-48.
- Ferreira, I.M.P.L.V.O., Veiros, C., Pinho, O., Veloso, A.C.A., Peres, A.M., Mendonca, A. (2006): Casein breakdown in Terrincho Ovine cheese: Comparison with bovine cheese and with bovine/ovine cheese. *Journal of Dairy Science* 89 2397-2407.
- Fox, P.F., McSweeney, P.L.H. (1998): Enzymology of milk and milk products. In *Dairy Chemistry and Biochemistry*, pp. 317-346. Fox P.L. and McSweeney P.L.H., eds. London, UK: Blackie Academic and Professional.
- Fuka, M.M., Engel, M., Skelin, A., Redzepovic, S., Schloter, M. (2010): Bacterial communities associated with the production of artisanal Istrian cheese. *International Journal of Food Microbiology* 142, 19-24.
- Guerra, M.M., McLauchlin, J.M., Bernardo F. A. (2001): *Listeria* in ready to eat unprocessed foods produced in Portugal. *Food Microbiology* 18, 423-429.
- HRN EN ISO 13366-3 (1999): *Milk-Enumeration of Somatic Cells-Part 3: Fluoro-opto-electronic Method*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
- HRN EN ISO 8523 (1999): *Microbiology - General guidance for the detection of Enterobacteriaceae with pre-enrichment*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
- HRN EN ISO 3433 (1999): *Cheese-Determination of fat content - Van Gulik method*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
- HRN EN ISO 9622 (2001): *Whole Milk - Determination of Milk Fat, Protein, and Lactose Content - Guidance on the Operation of Mid-infrared Instruments*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
- HRN EN ISO 8968-2 (2003): *Milk-Determination of nitrogen content-Part 2: Block digestion method*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).

20. HRN EN ISO/IEC 17025 (2004): *General requirements for the competence of testing and calibration laboratories*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
21. HRN ISO 6888-1 (1999): Microbiology of food and animal feeding stuffs - Horizontal method for enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
22. HRN ISO 6785 (2001): *Milk and milk products - Detection of Salmonella spp.* Zagreb, Croatia: Croatian Standards Institute (in Croatian).
23. HRN ISO 10560 (2001): Milk and milk products - Detection of *Listeria monocytogenes*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
24. HRN ISO 5764 (2003): *Milk - Determination of Freezing point - Thermistor Cryoscope Method*. Zagreb, Croatia: Croatian Standards Institute (in Croatian).
25. ISO 17997-1 (2004): *Milk - Determination of Casein-Nitrogen Content - Part 1: Indirect Method*. Geneva, Switzerland: International Standard Organization.
26. ISO 21187 (2004): *Milk - Quantitative Determination of Bacteriological Quality - Guidance for Establishing and Verifying a Conversion Relationship Between Routine Method and Anchor Method Results*. Geneva, Switzerland: International Standard Organization.
27. ISO 5534 (2004): *Cheese and processed cheese-determination of total solids content (Reference method)*. Geneva, Switzerland: International Standard Organization.
28. Kalit, S., Lukač Havranek, J., Čubrić Ćurik, V. (2002-a): Plasmin: milk indigenous proteinase. *Mljekarstvo* 52, 191-206 (in Croatian).
29. Kalit, S., Lukač Havranek, J., Kapš, M. (2002-b): Plasminogen activation and somatic cell count (SCC) in cheese milk: influence of Podravec cheese ripening. *Milchwissenschaft* 57, 380-382.
30. Kalit, S., Havranek, J., Kaps, M., Perko, B., Cubric Ćurik, V. (2004): Effects of somatic cell counts (SCC) on cheesemilk composition and yield efficiency of artisanal Tounj cheese. *Milchwissenschaft* 11/12, 612-615.
31. Kalit, S., Havranek, J., Kaps, M., Perko, B., Cubric Ćurik, V. (2005) Proteolysis and the optimal ripening optimal ripening time of Tounj cheese. *International Dairy Journal* 15, 619-624.
32. Kalit, S. (2007): Choice and application of clotting enzymes in traditional cheese manufacturing of ewe's and goat's cheese. Ninth Symposium for sheep and goat breeders in the Republic of Croatia, Toplice Sv. Martin, 25. and 26. October, 62-68 (in Croatian).
33. Lawrence, R.C., Heap, H.A., Gilles, J., (1984): A controlled approach to cheese technology. *Journal of Dairy Science* 67, 1632-1645.
34. Ledenbach, L.H., Marshall, R.T. (2009): Microbiological spoilage of dairy products. In: Sperber, W.H., Doyle, M.P. (eds): *Compendium of the Microbiological Spoilage of Foods and Beverages*. Springer Science and Business Media, New York, 41-67.
35. Licitra, G., Campo, P., Manenti, M., Portelli, G., Scuderi, S., Carpino, S. and Barbano, D.M. (2000): Composition of Ragusano cheese during aging. *Journal of Dairy Science* 83, 404-411.
36. Matutinović, S., Rako, A., Kalit, S., Havranek, J. (2007): The importance of traditional cheeses with particular review on Lecevački cheese. *Mljekarstvo* 57, 49-65 (in Croatian).
37. Mayer, H., Rockenbauer, C., Mlcak, H. (1998): Evaluation of proteolysis in Parmesan cheese using electrophoresis and HPLC. *Lait* 78, 425-439.
38. Mikulec, N., Kalit, S., Havranek, J., Antunac, N., Horvat, I., Prpić, Z. (2008): Characteristics of traditional Croatian ewe's cheese from the island of Krk. *International Journal of Dairy Technology* 61, 126-132.
39. Mioč, B., Prpić, Z., Barać, Z., Vnučec, I. (2012): Istrian sheep - Croatian indigenous breed. Croatian association of sheep and goat breeders, Zagreb (in Croatian).
40. Mulc, D., Jurković, D., Duvnjak, G., Sinković, T., Daud, J., Lješić, N., Špehar, M., Dražić, M. (2011): Breed structure of sheep under selection control from 2009 to 2011. In: Annual report 2011, Breeding of Sheep, Goats and Small Animals, 9.
41. Nero, L.A., de Mattos, M.R., de Aguiar Ferreira Barros, M., Ortolani, M.B.T, Beloti, V., de Melo Franco, B.D.G. (2008): *Listeria monocytogenes* and *Salmonella* spp. in Raw Milk Produced in Brazil: Occurrence and Interference of Indigenous Microbiota in their Isolation and Development. *Zoonoses and Public Health* 55, 299-305.
42. Official Gazette (2008): Regulations for microbiology criteria for food (NN 195/08) (in Croatian).
43. Pavlinic, I., Mikulec, N., Kalit, S., Jakasa, I., Havranek, J., Tudor, M., Antunac, N. (2010): Influence of starter culture on peptide profile of Krk cheese. *Milchwissenschaft* 63, 262-266.
44. Prpić, Z., Kalit, S., Lukac Havranek, J., Stimac, M., Jerković, S (2003): Krk Cheese. *Mljekarstvo* 53, 175-194 (in Croatian).
45. Samaržija, D., Antunac, N., Pecina, M., Havranek, J. (2003): Quality of the artisanal hard cheese produced in Mediterranean area of Croatia. *Milchwissenschaft* 58, 43-46.
46. Samaržija, D., Damjanović, S., Pogačić T. (2007): *Staphylococcus aureus* in Cheese. *Mljekarstvo* 57, 31-48. (in Croatian)
47. Shakeel-Ur-Rehman, Fox P.F., McSweeney, P.L.H. (2000): Methods used to study non-starter microorganisms in cheese: a review. *International Journal of Dairy Technology* 53, 113-119.
48. Somers, J.M., Kelly, A.L. (2002): Contribution of plasmin to primary proteolysis during ripening of cheese: effect of milk heat treatment and cheese cooking temperature. *Lait* 82, 181-191.
49. SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.
50. Tarakci, Z., Coskun, H., Tunçturk, Y. (2004): Some properties of fresh and ripened Herby cheese, a traditional variety produced in Turkey. *Food Technology and Biotechnology* 42, 45-50.