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Contemporary Agriculture, Vol. 72, No. 4 (2023): 230-234
DOI: 10.2478/contagri-2023-0031
UDC: 639.3

Original scientific paper

PROTEOLYTIC CHANGES DURING RIPENING OF KUČKI CHEESE

ISSN 2466-4774
https://www.contagri.info/

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Submitted: 11.10.2023.
Accepted: 29.11.2023.

SUMMARY

Production of various traditional dairy products, particularly cheese, has a long history in Montenegro. White-brined cheeses are the most well-known. One of them is Kučki cheese, with distinct flavor and higher level of proteolysis. The aim of this research was to analyze the proteolysis process during ripening of Kučki cheese. The cheese is produced using traditional technology. FTIR Spectrophotometry was used to calculate the amount of total protein in cheese (IDF141C: 2000). The degree of proteolysis was determined by SDS PAGE (Laemmli, 1970). The cheese was examined for their water-soluble nitrogen (WSN) content using Kuchroo and Fox's method from 1982, as well as their 5% phosphotungstic acid soluble nitrogen (PTAN) content using Stadhouser's method from 1960. The results were expressed as percentages of WSN and PTAN of the total nitrogen matter (WSN/TN and PTAN/TN), as well as PTAN as a percentage of WSN (PTAN/WSN). All analyses were done on 10th, 20th and 30th day of cheese ripening. Four samples of cheese were analyzed for each ripening period. The content of proteins increased during the ripening period. The parameters that determine the process of proteolysis increased during the first 30 days of ripening. The WSN/TN ranged from 13.33 to 44.32%. Also, PTAN/WSN varied from 3.79 to 21.57%. The initial results show that uneven ripening conditions have a direct impact on how proteolytic changes develop throughout ripening. Due to the absence of established cheese-making procedures, Kučki cheese has a considerable degree of heterogeneity in their protein content and proteolysis parameters. However, it is not possible to strictly define the optimal values of the degree of ripening for Kučki cheese. In order to reach certain conclusions, it is necessary to carry out further studies focusing on analyzing a larger number of samples.

Key words:
cheese, proteins, proteolysis, electrophoresis

INTRODUCTION

The term "white cheeses" refers to a large group of cheeses that mature and are stored in brine. They appear by the names "сыр в рассоле" in Russian literature, "pickled cheese" in England, and "saumurés fromage" in the Codex. One of the most popular cheeses around the world is white-brine cheese. White-brined cheeses, including popular ones like Feta in Greece, Domiati in Egypt, Bayaz peynir in Turkey, and Halloumi in Cyprus, are the most popular varieties of cheese made in the North-Eastern Mediterranean region and the Balkans (Bintsis & Papademas, 2002). Cheese frequently carries the name of the region, place, and municipality where it is primarily made.

One of the most well-known white-brined cheeses from Montenegro is Kučki cheese. It is full-fat, soft to semi-hard cheese that is typically made from raw cow's milk and mixed milk (cow's + sheep's), though it is occasionally made only from sheep's milk, and it is characterized by a strong taste and aroma (Dozet et al., 1996).

Cheese production is an unavoidable segment of Kuči history and culture. Kučki cheese is produced in Montenegro according to traditional technology. The cheese is made from raw milk (mainly cow's). After cooling milk to 28-32 °C, 1.3 g 100 L^{-1} commercial rennet is added. Milk begins to coagulate after 20 to 30 minutes, and curd is formed after 60 minutes. The curd is split into 3 to 5 cm cubes, gently mixed, and rested for 5 to 6 minutes while being heated to 35 °C. Drained and shaped in cheesecloth, the curd is placed under a wooden plank that is further burdened by a stone. About six hours after pressing, the curd is removed from the cheesecloth and placed in wooden vats. The curd is then poured over the brine, additionally covered by a wooden plank burdened with a heavy stone, which ensures that all the curd is below the surface of the brine. Depending on the desired level of flavor, aroma, and texture, the curd matures under these conditions for 4 weeks on average, but it may take longer. During this period, strong proteolytic changes occur in the cheese, which are reflected in the sensory properties of the cheese. During the ripening, the cheese goes through numerous chemical and biochemical, physical, and microbiological changes, resulting in formation of their distinctive aroma, taste, and consistency. Proteolysis in cheese during ripening plays a vital role in development of texture and flavor (Lavasani et al., 2011; Galiou et al., 2013). Because of high degree of proteolysis, some consumers find the taste of mature Kučki cheese too strong, even unpleasant.

The aim of this study was to analyze the process of proteolysis during the cheese ripening, considering that the proteolysis of Kučki cheese was not the subject of other research in Montenegro. Data can be used to provide the preconditions for standardization of the quality of Kučki cheese.

MATERIAL AND METHODS

At the time when the experiment was conducted, the animals were exclusively fed with grass from natural pasture. Pastures and farms were located on the slopes of the Kuči Mountains – northeast of Podgorica. The animals grazed from 6 am to 6 pm during the day, while during the night they were kept inside barns. All the time, the animals had free access to water.

The samples of cheese for the experiment were supplied from 12 producers that produce cheese by traditional technology. Households that participated in the experiment were selected by survey, and more than 50 households were surveyed. The chemical quality of cheese, i.e. total protein content in cheese, was determined using the instrument MilkoScan ™ FT 120 by FTIR spectrophotometry (IDF141C: 2000).

The degree of proteolysis was determined using SDS PAGE electrophoresis (Polyacrylamide electrophoresis), performed according to the method of Laemmli (1970).

In addition, the cheese samples were analyzed for their water-soluble nitrogen (WSN) content using Kuchroo & Fox's method from 1982, as well as their 5% phosphotungstic acid soluble nitrogen (PTAN) content (Stadhousers, 1960). The results were expressed as percentages of WSN and PTAN of the total nitrogen matter (WSN/TN and PTAN/TN), as well as PTAN as a percentage of WSN (PTAN/WSN). All analyses were done on 10^{th}, 20^{th}, and 30^{th} day of cheese ripening.

Four samples of cheese were analyzed for each ripening period. The data were processed using the computer program STATISTICA 12. Analysis of variance was performed – one-factor ANOVA for the effect of days of ripening on protein content and primary and secondary proteolysis indicators. Means and variability measures were determined. The Duncan test (Duncan Multiple Range Test) was used to test statistical significance of mean differences at the $P \leq 0.05$ probability level.

The densitometric analysis included 9 samples of cheese with ripening lasting 30 days or more according to data from households.

RESULTS AND DISCUSSION

The chemical quality of cheese (total protein content), WSN – water soluble nitrogen, PTAN – phosphotungstic acid soluble nitrogen, TN – total nitrogen were used to analyze the process of proteolysis during ripening in the samples of Kučki cheese. Proteolytic changes after 10, 20, and 30 days of the cheese ripening are presented in Table 1.

The content of protein increased during the ripening period. The protein content on 10^{th} day of ripening was 15.39-18.90%, on 20^{th} day it was 14.88-18.97%, and on 30^{th} day it was 14.35-19.49%. Statistical analysis revealed that differences in the protein content were not statistically significant ($P>0.05$). Our results are in compliance with the results on the protein content of Feta and Domiati cheese (Tratnik et al., 2000) and Homoljski cheese (Sikimić et al., 2006).

Table 1. Proteolytic changes after 10, 20 and 30 days of ripening

Days of ripening	Proteins (%)	WSN (%)	PTAN (%)	TN (%)	WSN/TN	PTAN/TN	PTAN/WSN
10	15.39	0.340	0.013	2.41	**14.10**	0.53	**3.79**
	16.36	0.439	0.052	2.56	**17.11**	2.02	**11.83**
	17.42	0.401	0.082	2.73	**14.69**	3.01	**20.47**
	18.90	0.395	0.085	2.96	**13.33**	2.88	**21.57**
$\bar{x}$ ± **SD**	**17.01±1.50**			2.665	**14.81^{a}±1.63**		**14.41±8.31**
20	16.83	0.558	0.022	2.64	**21.15**	0.82	**3.87**
	14.88	0.474	0.021	2.33	**20.31**	0.90	**4.43**
	17.53	0.060	0.068	2.75	**21.98**	2.48	**11.28**
	18.97	0.614	0.055	2.97	**20.66**	1.86	**8.99**
$\bar{x}$ ± **SD**	**17.05±1.70**			2.67	**21.02^{a}±0.72**		**7.14±3.59**
30	17.65	0.585	0.071	2.77	**21.15**	2.57	**12.15**
	14.35	0.535	0.034	2.25	**23.80**	1.52	**6.37**
	19.49	1.082	0.065	3.05	**35.41**	2.13	**6.01**
	17.72	1.231	0.042	2.78	**44.32**	1.53	**3.44**
$\bar{x}$ ± **SD**	**17.30±2.14**			2.71	**31.17^{b}±10.73**		**6.99±3.68**

Legend: WSN – water soluble nitrogen; PTAN – phosphotungstic acid soluble nitrogen; TN – total nitrogen; WSN/TN – ripening index; PTAN/TN and PTAN/WSN secondary proteolysis indicator; a,b,c different letters indicate significant difference between the means in columns ($P<0.05$)

The contents of TN in Kučki cheese in brine varied in the range of 2.25-3.05%, which is in agreement with the literature data on the TN content of Beli cheese (Đerovski et al., 2006), and Homoljski cheese (Jovanović et al., 2004). The average TN in Kučki cheese ranged from 2.665 (10 days) to 2.71 (30 days). Živković (1964) showed that the TN content in total solid during ripening of white cheeses in brine was reduced, which indicates that the amount of TN in brine constantly increases. Also, the decrease in the content of total nitrogen in the dry matter of Lighvan cheese from the first to 90th day is due to diffusion of nitrogen substances in the whey as a result of proteolysis, as reported by Mehrnaz et al. (2014).

The water soluble nitrogen (WSN), expressed as percentages of the total nitrogen matter (ripening index), is an indicator of primary proteolysis. It differs significantly depending on the type of cheese, but in all of them it increases as ripening progresses. The results presented in Table 1 point to great variability regarding the parameters of primary and secondary proteolysis in different cheese samples. This indicates uneven ripening conditions and course of proteolytic changes during ripening. The ripening index in the samples of Kučki cheese showed a wide interval of variations, which is a consequence of different composition of cheese, production process parameters and ripening process of the cheese. Abd-El Salam et al. (1993) found that the ripening index in white cheeses in brine mostly ranged between 12-20%, while the maximum reached a value of 25%.

As expected, the parameters that determine the process of proteolysis increased during the first 30 days of ripening. At the beginning of ripening, the amount of water soluble nitrogen was low, but it increased as ripening progressed. The percentage of water-soluble matter (WSN) in total nitrogen (WSN/TN), as a primary proteolysis parameter, increased from 13.33% to 44.32% during 30 days of ripening. This parameter represents the ripening index and it is an indicator of primary proteolytic changes. Miočinović et al. (2016), who studied Zlatarski cheese, determined that the ripening index of cheese sampled directly at households was 37.49%, while it ranged from 7.85 to 24.57% in the samples taken from markets. The authentic Zlatarski cheese has a higher ripening index compared to other samples taken from the market, i.e. from cheese that certain producers place on the market before it reaches the required maturity. Compared to the results cited by Miočinović (2016), the maturity coefficient of Kučki cheese samples ranged from 13.33 to 44.32%, which indicates quite variable conditions and course of ripening changes.

Secondary proteolysis parameter (PTAN/WSN) varied from 3.44 to 21.57%. More intense changes occur in the initial ripening period, which is in agreement with the results by Ostojić (2010) for Golijski cheese. Statistical analysis revealed significant differences only in WSN/TN content, while the differences in other parameters were not statistically significant.

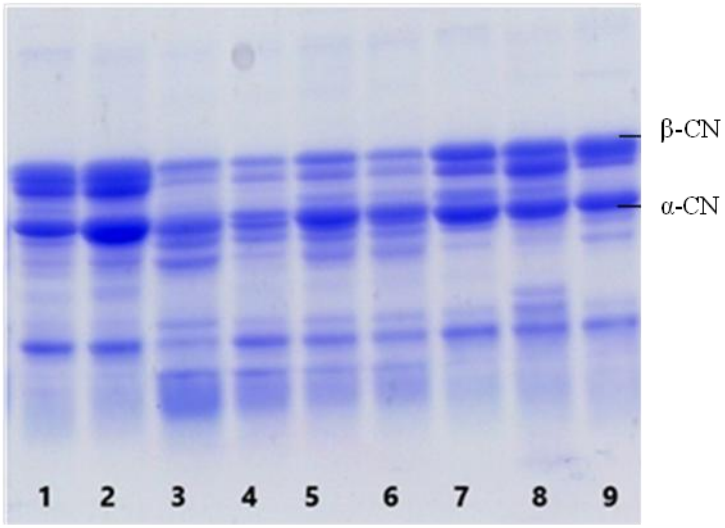

Figure 1. SDS electrophoretogram of Kučki cheese

The changes in the protein profile during the first month of ripening were similar to the changes in Lighvan cheese (Mehrnaz, 2014). During the first month, β and αS-casein were degraded significantly and a weaker intensity of their connections was noticed. The decomposition of β and αS casein is usually dependent on the action of residual enzymes in the curd, originating from rennet and plasmin, as well as the presence of proteolytic enzymes.
Changes in ripening were noticed on the electrophoretogram and were not uniform (Fig. 1). In fact, for some samples, there was an intensive decomposition of αS and β casein (samples 3, 4, 5, 6), while in the other parts degradation of αS and β casein was poorly expressed. Changes in the electrophoretic patterns of proteins in the cheese during pickling indicated that both α_s - and β-caseins were attacked. β-Casein, however, was less affected.

CONCLUSION

These initial results show that uneven ripening conditions have direct impact on how proteolytic changes are developed throughout ripening. The absence of standardized cheese-making processes caused great heterogeneity of the protein content and proteolysis characteristics of Kučki cheese. Considering the scope of the research, it is challenging to strictly define the optimal values of the degree of ripening for Kučki cheese. In order to reach certain conclusions, it is necessary to carry out further studies with a focus on analyzing a larger number of samples.

***Acknowledgements*:** This publication is the result of work on a scientific research project: „Valorization of Montenegrin katuns through sustainable development of agriculture and tourism – KATUN", which was funded by the Ministry of Science of Montenegro through the INVO-HERIC program of the World bank.

Conflict of interest: The authors declare that they have no conflict of interest.

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