

## INFLUENCE OF STARTER CULTURE, TEMPERATURE AND PROCESSING TECHNOLOGY ON THE QUALITY OF MACEDONIAN WHITE BRINED CHEESE

E. Sulejmani<sup>1</sup>, Z. H. Musliu<sup>2</sup>, S. Srbinovska<sup>3</sup>

<sup>1</sup> Faculty of Food Technology and Nutrition, Tetovo, Macedonia

<sup>2</sup> Faculty of Veterinary Medicine, Skopje, Macedonia

<sup>3</sup> Faculty of Agriculture Science and Food, Skopje, Macedonia

Corresponding author: erhan.sulejmani@unite.edu.mk

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**Abstract:** The effect of the starter culture, temperature of curdling and processing technology on the composition, cheese yield and process optimization of Macedonian White cheese (MWC) was studied during 60 days of ripening in brine. Three treatments of cheese were made using current technological process and yogurt as starter–culture gained along processing of previous day (MWCK), freeze dried culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* 3:1; F–DVS YF–3331 Yo-Flex version: 2 PI-EU-EN (MWCB1), and (MWCB2) with same starter culture as previous but with 5 minute earlier processing of curd and temperature of curdling at 39°C. As ripening progressed, titratable acidity (°SH), salt and protein contents of the (MWCB2) treatment continuously increased, whereas their fat-in-dry-matter and lactose contents decreased. In same production conditions depending on the used temperature. Way of processing and starter cultures the cheese from (MWCB2) treatment was with highest acidity of  $66.63 \pm 2.73^{\circ}\text{SH}$  until the end of ripening of the cheese. Moisture of cheeses remained stable during ripening. The pH of cheese at the 1 day of ripening, which decreased by increasing the temperature of curdling (5.03, 5.11 and 5.00 for MWCK, MWCB1 and MWCB2, respectively), significantly ( $P < 0.05$ ) affected most of the chemical characteristics of cheese. The content of salt at the end of storage at (B1) and (B2) variant is  $5.23 \pm 0.31$  and  $5.52 \pm 0.31$  respectively. Higher temperature of curdling decreased moisture and pH, whereas cheese protein content increased. The consumption of milk for production of a 1 kilogram of cheese ranged from 7.8 to 8.3 liters of milk. It was concluded that starter cultures have positively influenced and improved the quality of white cheese.

**Key words:** Lactose, starter – culture, white cheese

## Introduction

The use of starter cultures containing lactic acid bacteria is an essential requirement in the manufacture of most cheeses (*Cogan and Hill, 1993*) including Macedonian White cheese (MWC). Their major function is to produce lactic acid and in some cases, flavour compounds (*Fox et al., 2000*). It is well known that reduction in milk pH due to acidification by starter cells at the appropriate rate and time is the key step in the manufacture of a good quality cheese (*Bintsis and Papademas, 2002*). For this reason, the changes of acidity of different treatments were studied separately during ripening and ripening. The basic composition and structure of cheeses are determined by the manufacturing operations like pH at renneting, but it is during ripening that the individual and unique characteristics of each cheese variety develop (*Fox et al., 1993*). The ripening cheeses do not have typical sensory properties immediately after hooping and salting. These are developed only during the cheese ripening. One of the most important biochemical processes determining the taste and texture of a cheese is proteolysis which includes microbiological enzymatic and physico-chemical processes (*Fox and Law, 1991*). The production of Macedonia White brined cheese depends on the hydrolysis of lactose by lactic acid bacteria to produce lactic acid (*Sulejmani, 2010*). The breakdown of the degradation of lactose in the curd has a major effect on the quality of the ripened cheese; for example, excessive lactic acid in cheese curd leads to a low pH, strong, acidic, harsh taste, and a brittle structure. The selection, maintenance and use of starter cultures are perhaps, the most important aspects of cheese making, particularly in the context of a modern mechanized process for which predictability and consistency are essential (*Bintsis and Papademas, 2002; Özer, 1999*).

Macedonian White cheese (Belo Sirenje) is brine-salted cheese variety with salty, acid taste and close texture resembling Beyaz Peynir (Turkish White cheese) and Feta but differs from Feta in the way it is made (*Sulejmani et al., 2011*). It is for example manufactured without dry salting of curd and slime formation on the curd surface before brining which are essential for the development of the characteristic Feta flavour during ripening. At the industrial level, the ripening period is 20 to 60 day.

The fact that classical White cheese is often produced traditionally without the addition of starter cultures frequently leads to indifferent quality of the product. This study was aimed in optimizing the manufacture process of Macedonian White brined cheese in which case was determined influence of the starter culture, optimal temperature of milk curdling and optimum time of curd cutting on the quality and yield of the cheese.

## Materials and methods

Macedonian white-brined cheese was manufactured in triplicate from pasteurized cow's milk in a local dairy plant (Mlekara Tetovo, Tetovo, the Republic of Macedonia). Raw cow's milk supplied from the whole Tetova region in Macedonia was pasteurized (75°C for 15 second using a plate pasteurizer) and cooled at 5°C. The chemical composition of the milk used in the manufacture of white cheese was 12.68% total solids, 4.28% fat, 3.28% protein, 0.61% ash, and 4.41% lactose. The pH of the milk was 6.49. Cheese making procedures were described in a previous paper (*Sulejmani et al., 2011*). Briefly 3 treatment of the cheese were made where at the first control cheese (MWCK) yogurt as starter culture was added. The second and third treatment was inoculated with freeze dried culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* 3:1; F-DVS YF-3331 Yo-Flex version: 2 PI-EU-EN and processing of curd were applied 5 minute earlier from current. The temperature of curdling at MWCK and MWCB1 cheeses was 37°C while at MWCB2 cheese was 39°C. Cow milk was coagulated with chymosin rennet (Chy Max™ Liquid Plus derived by fermentation of *Aspergillus niger* var. *awamori* Christian Hansen Inc Danish, 200 IU) in 30 min. The curd was cut in cubes of 1 cm<sup>3</sup> and left of 5 minutes. Cheese of square shape 12 cm (length) × 10 cm (width) × 10 cm (height), were pressed for 3 hour than brine salted for 18 hour and ripened at 17°C for 20 days and hold afterword at 6°C.

Titratable acidity of cow milk was determined by the Soxhlet-Henkel method, and its total solids were determined Milkoscan 4000 (Foss Electric, Hillerød, Denmark) (*IDF, 2000*). The pH of milk and cheese samples was measured using a digital pH meter (digital pH meter, model MP120FK Mettler Toledo, Greifensee, Switzerland). Cheese was analyzed for lactose content by Gravimetric method (*AOAC, 2005*). Cheese samples were analysed for moisture by the oven-drying method at 102°C (*IDF, 1982*), fat and salt by the methods described by Ardö and Polychroniadou (1999), and total nitrogen by the micro-Kjeldahl method (*IDF, 1993*). All chemical measurements were done in triplicate or more. Cheese samples were chemically analyzed at d 1, 10, 20 and 40 of ripening. Apparent yield was calculated as the weight of cheese before brining (after 19 to 20 h ripening at 23 to 25°C) divided by the weight of the milk used.

The cheeses were evaluated at 60 d of ripening by 7 trained panelist's familiar whith the cheese according to the described procedure for Belo cheese (*Sulejmani, 2010*). The samples were evaluated by criteria appearance (scale 0-5), odour (scale 0-5), texture (0-10), and flavour (scale 0-15). Water and bread were provided to panelist to rinse their mouths between samples. Sensory analysis was conducted in 3 replicate trials and cheeses were evaluated in duplicate by each panelist.

The experiment was replicated 3 times in a randomized complete block design, which incorporated 3 treatments (MWCK, MWCB1 and MWCB2), 4 ripening periods (10, 20, 40, and 60 d) was used to analyze the response variables related to cheese composition and yield. The ANOVA was performed using a general linear model procedure (SAS Institute, 1995), where the effect of treatment and replicates were estimated for response variables. The Tukey multiple-comparison test was used as a guide for pair comparisons of treatment means. The level of significance of differences between treatments was determined at  $P < 0.05$ .

## Results and Discussion

The composition of cow's milk cheeses and the differences in chemical composition between the cheese treatments are summarized in Table 1.

**Table 1.** Mean ( $\pm$  SE) of chemical composition of cheeses made with different starter<sup>1</sup> (g 100 g<sup>-1</sup>) ( $n = 3$ )

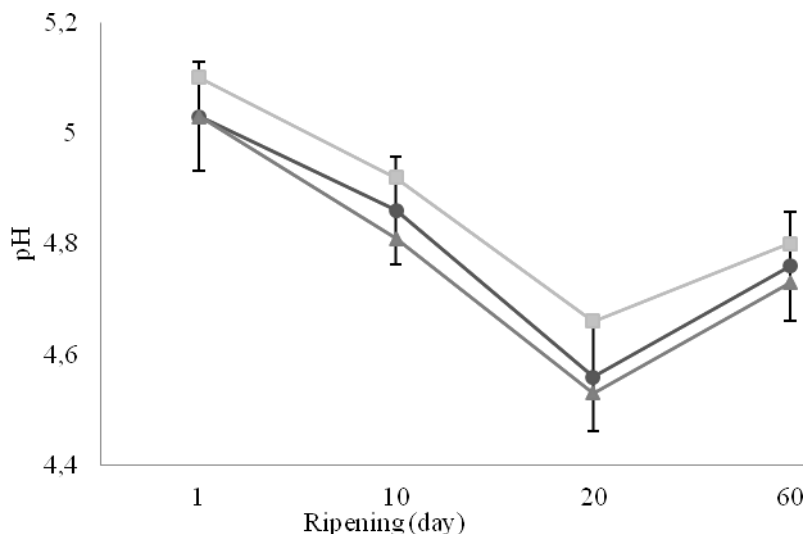
Cheeses	Age (day)	Acidity, (°SH)	Moisture, %	Salt, %	Protein, %	Fat-in-dry matter, %	Lactose, %
MWCK	1	20.81 $\pm$ 2.72 <sup>a</sup>	53.28 $\pm$ 0.67 <sup>a</sup>	3.98 $\pm$ 0.25 <sup>a</sup>	14.43 $\pm$ 0.23 <sup>a</sup>	53.44 $\pm$ 2.14 <sup>a</sup>	2.68 $\pm$ 0.44 <sup>a</sup>
	10	27.21 $\pm$ 2.93 <sup>b</sup>	55.12 $\pm$ 2.15 <sup>b</sup>	4.48 $\pm$ 0.16 <sup>a</sup>	14.25 $\pm$ 0.86 <sup>a</sup>	53.24 $\pm$ 2.43 <sup>a</sup>	1.96 $\pm$ 0.05 <sup>b</sup>
	20	43.73 $\pm$ 2.21 <sup>c</sup>	53.46 $\pm$ 0.48 <sup>a</sup>	4.36 $\pm$ 0.48 <sup>a</sup>	14.03 $\pm$ 0.41 <sup>a</sup>	55.83 $\pm$ 1.23 <sup>b</sup>	1.86 $\pm$ 0.05 <sup>b</sup>
	60	47.86 $\pm$ 3.83 <sup>d</sup>	53.32 $\pm$ 0.48 <sup>a</sup>	5.43 $\pm$ 0.23 <sup>b</sup>	13.41 $\pm$ 0.24 <sup>a</sup>	49.31 $\pm$ 3.25 <sup>c</sup>	1.30 $\pm$ 0.08 <sup>b</sup>
MWCB1	1	19.86 $\pm$ 1.44 <sup>a</sup>	53.06 $\pm$ 0.52 <sup>a</sup>	3.63 $\pm$ 0.18 <sup>a</sup>	13.52 $\pm$ 0.45 <sup>b</sup>	51.42 $\pm$ 3.33 <sup>c</sup>	2.56 $\pm$ 0.38 <sup>a</sup>
	10	27.73 $\pm$ 6.10 <sup>b</sup>	52.91 $\pm$ 0.40 <sup>c</sup>	4.36 $\pm$ 0.23 <sup>a</sup>	14.17 $\pm$ 0.54 <sup>a</sup>	54.21 $\pm$ 3.15 <sup>b</sup>	1.96 $\pm$ 0.03 <sup>b</sup>
	20	41.86 $\pm$ 2.39 <sup>c</sup>	48.05 $\pm$ 0.40 <sup>c</sup>	4.55 $\pm$ 0.44 <sup>a</sup>	15.45 $\pm$ 1.19 <sup>b</sup>	54.56 $\pm$ 1.69 <sup>b</sup>	1.91 $\pm$ 0.03 <sup>b</sup>
	60	54.35 $\pm$ 7.13 <sup>d</sup>	49.15 $\pm$ 0.35 <sup>c</sup>	5.23 $\pm$ 0.31 <sup>b</sup>	14.70 $\pm$ 0.24 <sup>a</sup>	46.21 $\pm$ 3.05 <sup>f</sup>	1.06 $\pm$ 0.26 <sup>b</sup>
MWCB2	1	24.13 $\pm$ 1.26 <sup>a</sup>	51.85 $\pm$ 0.39 <sup>c</sup>	3.82 $\pm$ 0.48 <sup>a</sup>	14.56 $\pm$ 1.02 <sup>a</sup>	53.32 $\pm$ 1.68 <sup>a</sup>	2.63 $\pm$ 0.40 <sup>a</sup>
	10	31.24 $\pm$ 5.73 <sup>b</sup>	53.94 $\pm$ 0.50 <sup>d</sup>	4.71 $\pm$ 0.35 <sup>a</sup>	14.13 $\pm$ 0.95 <sup>a</sup>	52.43 $\pm$ 3.21 <sup>e</sup>	2.03 $\pm$ 0.07 <sup>a</sup>
	20	54.82 $\pm$ 1.15 <sup>c</sup>	51.51 $\pm$ 1.13 <sup>d</sup>	4.31 $\pm$ 0.46 <sup>a</sup>	14.86 $\pm$ 1.08 <sup>a</sup>	57.04 $\pm$ 0.44 <sup>d</sup>	1.86 $\pm$ 0.05 <sup>b</sup>
	60	66.63 $\pm$ 2.73 <sup>d</sup>	51.21 $\pm$ 0.91 <sup>d</sup>	5.52 $\pm$ 0.31 <sup>b</sup>	14.83 $\pm$ 0.20 <sup>a</sup>	48.14 $\pm$ 2.70 <sup>f</sup>	1.01 $\pm$ 0.33 <sup>b</sup>

<sup>a-1</sup>Means within the same column with different superscripts differ significantly ( $P < 0.05$ ). MWCK= Macedonian cheese made using yogurt as starter – culture, MWCB1= Macedonian cheese made using comercial freeze dried culture, curdling at 37°C, MWCB2= Macedonian cheese made using comercial freeze dried culture, curdling at 39°C.

The highest level of acidity (°SH), protein and fat at day 1 of ripening were (mean  $\pm$  SE) observed at the MWCB2 cheeses containing 24.13, 14.56 and 25.68% respectively. MWCB2 treatment has higher titratable acidity value than others during the ripening (Table 1), ranging from 24.13 to 66.63°SH.

The influence of different starter culture and processing technology on the pH values of the cheeses during ripening is shown in Figure 1. The starter culture and temperature of curdling had no significant effect on the pH values of cheeses;

however the lowest and highest level of pH values during ripening was observed in the MWCB2 or MWCB1 cheeses respectively.



**Figure 1.** Mean values of pH in Macedonian white cheese made using yogurt as starter – culture MWCK (circles), cheese made using freeze dried culture F–DVS YF–3331 curdling at 37 °C MWCB1 (squares), cheese made using freeze dried culture F–DVS YF–3331 curdling at 39 °C MWCB2 (triangles).

The protein content except MWCB1 treatment decreased in the first 10 days of ripening. The results showed that protein of White brined cheese with different starter culture ranged from 14.43 to 13.41% for MWCK, 13.52 to 14.70% for MWCB1 and 14.56 to 14.83% for MWCB2 cheeses. From the statistical analysis it is observed that the lactose content of White brined cheese was not significantly ( $P > 0.05$ ) decreased during ripening. An increased reduction of lactose in all treatments has been shown during the first 10 days of ripening. At the day 60 of ripening White cheese is containing traces of lactose with a level ranging from 1.01 to 1.30 in MWCB2 or MWCK cheese treatment, respectively.

The salt content, expressed as a percentage (Table 1), ranging from 3.98% to 5.43% for MWCK cheeses produced with yogurt as starter culture, while results obtained from MWCB2 cheeses at the end of ripening were slightly higher.

The chemical composition of all cheese treatments was generally within the range typical for white brined cheese. Macedonian white brined cheese may be characterized as soft (50–60%) moisture, high fat cheese (25–30%), protein (12–21%) high salt (3–5%) content and the final pH range of 4.20 – 5.05 (Mojsova *et al.*, 2013). Similarly to our results, it is reported a decrease in pH during ripening

of Iranian White cheese in brine (10%, pH = 7.4), mainly because of completion of lactose fermentation and the liberation of amino and free fatty acids (Azarnia et al., 1997). The results for protein content of Macedonian White cheese are in agreement with the studies reported for Turkish White cheese (Hayaloglu et al., 2005). Differences in term of acidity ( $^{\circ}\text{SH}$ ) among the cheeses were significant ( $P < 0.05$ ) during ripening. The moisture content decreased during ripening in all cheeses (Table 1) which is in accordance with results of semihard ewe cheeses (Juan et al., 2007). The protein of cheese increased with use of commercial starter culture and with increase of clotting temperature of milk. Thermophilic starters induce a dynamic which increasing of protein concentration in MWCB2 cheese. Also with increasing of clotting temperature of milk in concert with thermophilic starter culture especially in MWCB2 treatment has capability to keep the protein concentration durable.

The differences in the protein content of cheeses during ripening are due to hydrolysis of proteins to water soluble nitrogenous compounds and to the diffusion of these products into the brine. The parameters for proteins, moisture content and pH are similar with the parameters determined in Feta cheese (Abd El-Salam and Alichanidis, 2004). Fermentation of lactose takes place from the very beginning the process of cheese manufacturing (Shakeel-Ur-Rehman et al., 2004). The results of lactose in cheese from the first day in all treatments were greater than the results reported for Feta - cheese (Bintsis, 2001). The same author, for the same cheese, on the 60 day of cheese, obtained smaller value of the concentration of lactose, ie from 0.2 to 0.6% compared with our results.

During production of cheese large amounts of lactose from the milk is lost in the whey, a small degree of lactose that remains in coagulum rapidly metabolized by the activity of starters or non starter cultures present in raw milk or environment (McSweeney, 2004). Lactate produced by the activity of starter-cultures is an important starting point for a series of roadsigns that contribute positively or negatively the development of aroma in cheeses. The concentration of salt in cheese depends on the initial condition of the cheese, the percentage of salt in brine, the type of salt, temperature and pH of the cheese (Pavia et al., 2000). The results of Macedonian white cheese yield (Figure 2) are in correspondence with results for white brined cheese Minas Padra (Anonymous, 2004).

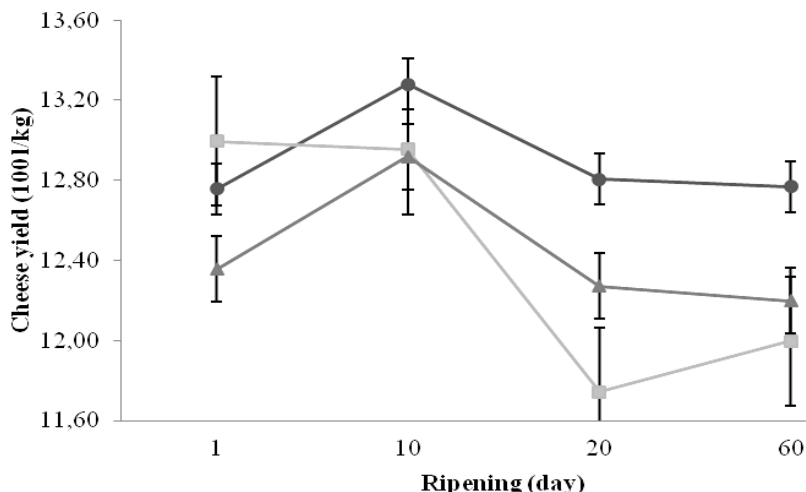


Figure 2. Mean values of yield of Macedonian white cheese made using yogurt as starter – culture MWCK (circles), cheese made using freeze dried culture F–DVS YF–3331 curdling at 37 °C MWCBC1 (squares), cheese made using freeze dried culture F–DVS YF–3331 curdling at 39 °C MWCBC2 (triangles).

The sensory evaluation results of the 60 days old cow cheeses are shown in Figure 3.

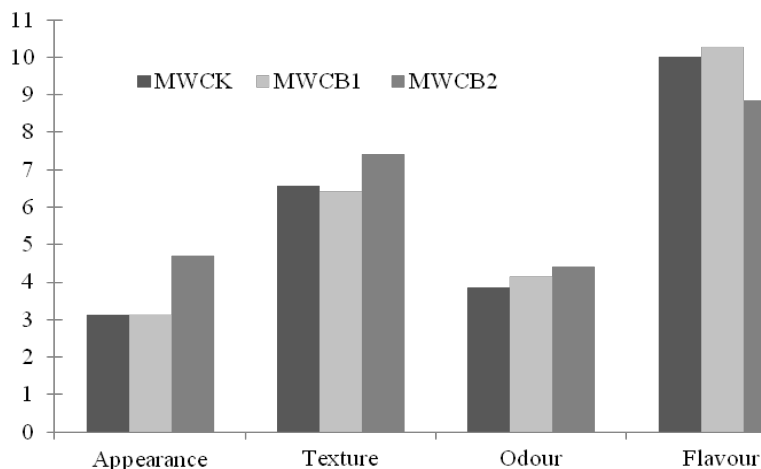


Figure 3. Mean values of sensory evaluation of Macedonian white cheese made using yogurt as starter – culture MWCK, cheese made using freeze dried culture F–DVS YF–3331 curdling at 37 °C MWCBC1, cheese made using freeze dried culture F–DVS YF–3331 curdling at 39 °C MWCBC2.

Overall cheeses were characterized by a salty and acidic taste and had a semi-hard texture except controll cheeses (MWCK). Flavour and total sensory scores were significantly influenced by the use of starter culture, processing technology and temperature of curdling. Cheeses made using freeze dried culture F-DVS YF-3331 curdling at 39°C MWCB2 had a more satisfactory appearance, texture and odour and quality than others Macedonian White cheese treatments (Sulejmani et al., 2011). Use of a thermophilic starter culture resulted in higher quality scores than did use of yogurt as starter culture.

## Conclusion

The results obtained from this study indicate that a commercial starter culture was suitable for Macedonian white cheese manufacture. Differences in chemical and sensory attributes were correlated with differences in the starter culture and manufacture protocols used in the cheese treatments. The titratable acidity and dry matter content was significantly higher in the cheese manufactured with commercial starter culture. The ripening of cheeses produced with traditional technology (without added commercial starter culture) is longer while the cheeses with commercial starter culture had the best organoleptic scores due to the fact that the aroma principles were fully expressed. Further studies with SPME-GC-MC and olfactometry techniques may be useful to establish key odorants and their role in Macedonian white cheese flavour characteristics.

## Uticaj starter kulture, temperature i obrade na kvalitet makedonskog belog salamurenog sira

E. Sulejmani, Z. H. Musliu, S. Srbinovska

## Rezime

Uticaj starter kultura, temperature koagulacije i tehnologije prerade na sastav, prinos sira i proces optimizacije makedonske belog sira (MWC) je ispitivan tokom 60 dana zrenja. U okviru tri tretmana, sir je napravljen korišćenjem tekućeg tehnološkog procesa i jogurt kao starter kulture dobijen u obradi prethodnog dana (MWCK), zamrznuto osušenih kultura *Lactobacillus delbrueckii subsp. bulgaricus* i *Streptococcus thermophilus* 3:1; F - DVS IF -3331 Jo - Flek Verzija: 2 PI - ES - RU MWCB1) i ( MWCB2) sa istim starter kulturama kao i prethodni, ali je pet minuta ranije tretman i temperatura koagulacije na 39°C. Kao sazrevanja napreduje, titraciona kiselosti (°SH), soli i sadržaj proteina (MWCB2) raste, dok se



masti u suvoj materiji i sadržaj laktoze smanjuje, u istim proizvodnim uslovima zavisno od upotrebljene temperature. Način obrade i starter kultura sira (MWCB2) varijante je sa najvećom kiselosti  $66.63 + 2.73^{\circ}\text{SH}$  do kraja zrenja sira. Vlažnost sira je ostala stabilna tokom zrenja. pH sira na prvi dan zrenja, koji je smanjen povećanjem temperature koagulacije (5.03, 5.11 i 5.00 za MWCK, MWCB1 i MWCB2), značajno ( $p < 0.05$ ), koje su uticale na hemijske karakteristike sira. Sadržaj soli na kraju skladištenja u (MWCB1) i (MWCB2) varijante je  $5.23 \pm 0.31$  i  $5.52 \pm 0.31$ , respektivno. Viša temperatura zgrušavanja smanjuje vlagu i pH, dok se sadržaj proteina povećao. Potrošnja mleka za proizvodnju 1 kilograma sira kretala se od 7.8 do 8.3 litara. Zaključeno je da je korišćenje starter kultura uticalo na poboljšanje kvaliteta belog sira.

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