#### Review - Pregledni rad

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# An overview of researches on cheeses ripening in animal skin

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#### Summary

The aim of this paper is to review the existing knowledge of manufacturing procedures, chemical composition, sensory attributes, biochemistry and microbiology of different kind of cheeses which ripen in the animal skin. These kinds of cheeses are produced in Croatia, Bosnia and Herzegovina, Montenegro and Turkey and there are few differences in the manufacturing procedure between them. The main specificity of these cheeses is ripening in a sack made of the whole lamb or goat body skin. Two or three months of anaerobic ripening in the skin gives the cheese markedly piquant taste and flavour. These cheese characteristics originate from intensive lipolysis and proteolysis. According to the content of moisture in the non fat solids and fat in total solids, cheeses in a sack belong to the group of hard or semi hard, full-fat or low-fat cheeses.

Key words: cheese in a sack, properties, technology, animal skin, ripening

#### Introduction

A few types of cheeses ripened in animal skin are produced in specific areas of the world and known as cheese in a sack. There are few differences in the manufacturing procedure of those types of cheeses which are produced in Croatia, Bosnia and Herzegovina, Montenegro and Turkey. In Croatia it is produced in the Dinara mountain area, in Turkey in the mountain regions of East and Central Anatolia and in Bosnia and Herzegovina in several mountain regions. Probably, very early in the past, nomadic sheep-breeders started to use a lamb skin for cheese storage and transportation from mountain to valleys due to the lack of wood for production of storage and transportation equipment. This cheese is produced from ewe, cow, goat and buffalo milk and their mixtures. The main specificity of this cheese type is ripening in a lamb skin which can have different names regionally and locally: mišina (Croatia), mjeh (Bosnia and Herzegovina), tulum (Turkey) and is produced by unique processes. It can be described as a bag or sack made of the whole lamb or goat body skin. Probably, two or three months of anaerobic ripening in the skin gives the cheese unique sensory attributes: markedly piquant taste and odour.

The aim of this research is to review the existing knowledge of manufacturing procedures, chemical composition, sensory attributes, biochemistry and microbiology during ripening of different kind of cheeses which ripen in the animal skin.

#### The manufacturing procedure of cheese in a sack

According to the literature data, the production of cheese in sack in Croatia started in the period of Illyrians and Thracians when they grazed their sheep on the pastures on mountain Dinara. The cheese in a sack is traditionally produced on family farms in relatively small amounts in the hinterland of the town Šibenik. It is produced from full-fat raw ewe or cow milk or their mixture without using starter cultures according to the manufacturing procedure of semi-hard cheeses. Table 1 shows the average composition of ewe milk used for the manufacturing of cheese in a sack (Tudor et al., 2008).

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Parameter/Parametar	$\overline{x} \pm SD$
Total solids (g/100 g)/Suha tvar (g/100 g)	19.65±1.84
Milk fat (g/100 g)/Mliječna mast (g/100 g)	8.22±1.50
Proteins (g/100 g)/Proteini (g/100 g)	6.37±0.74
Lactose (%)/Laktoza (%)	4.23±0.29
Solids-nonfat (%)/Suha tvar bez masti (%)	11.40±0.46
pH	6.26±0.33
Freezing point (°C)/Točka ledišta (°C)	-0.597±0.05
SD - standard derivation /standard derivation	

Table 1. Composition of ewe milk used for production of cheese in a sack (Tudor et al., 2008) Table 1. Sastav ovčjeg mlijeka za proizvodnju sira iz mišine (Tudor i sur., 2008.)

SD = standard deviation/standardna devijacija

Due to non standardized manufacturing procedures, there are some differences between family farms in some particular stages of cheeses production. Natural or commercial rennet is used at renneting temperatures of milk between 32-37 °C. After finished coagulation, curd is cut by scoop into irregular shape small pieces or by knife into cube shape pieces approximately sized 3x3 cm. Curd grains are heated and stirred by hand or scoop up to temperature that can vary between 34 °C and 40 °C. Curd grains after draining have size of nuts or walnuts. Rough curd is shaped by hand, enveloped and drained in cloth by self pressing. After draining of whey, cheese is cut into pieces approximately 10x10x5 cm in size, it is salted by large grain sea salt and then put into skin sack. If there is not enough cheese and the sack is not filled in one day, half filled sack will be full filled the next day. Both sides of the skin can be used: outer layer of the skin (wool side) is in the contact with cheese, or cheese is in contact with the inner side of skin (the part of skin near the meat). Ripening in the skin sack is carried out at a temperature of 18-20 °C and relative air humidity of 65-80 % (Tudor et al., 2009).

There are two varieties of Herzegovinian cheese in a sack. One and the most often is made of skim milk after manufacturing of cream (kajmak) which has very high nutritional value - the high content of proteins and the low content of fat. The second option is using full fat or semi skimmed milk. Another specific product known in Herzegovina is kajmak in a sack. After manufacturing, kajmak first ripens in wooden vat (kačice) for 1-2 months and then in skin sack till the time of consumption (Dozet and Stanišić, 1962; Dozet et al., 1963a; Dozet et al., 1980; Bijeljac, 2004; Jovanović et al., 2007; Sarić et al., 2008). The manufacturing procedure of autochthonous Herzegovinian cheese in a sack is similar to the Croatian way of production. The main difference is seen in temporary storage of salted cheese in a wooden vat (usually under pressure) after draining, to wait for enough amount of cheese for filling the skin sack. This is aerobic phase when lactic acid bacteria intensively convert lactose in the lactic acid. Another difference is the form of cheese in the skin sack. It is broken by hand in smaller irregular shaped pieces, salted again if needed and put into skin sack. Therefore the texture of this cheese is crumble in opposite to Croatian type which is compact and easy to cut. The ripening period varies between two and three months at temperatures of 12-15 °C (Zdanovski, 1947; Dozet et al., 1963b; Dozet et al., 1996; Sarić and Bijeljac, 2003; Bijeljac and Sarić, 2005; Samardžić, 2009).

In Turkey there are many types of cheeses which ripen in skin sack that are produced using different manufacturing procedures. Some of them are Izmir Brined Tulum, Divle, Karin Kaymagi, Antalya Cimi, Selcuklu, Tomas (Hayaloglu et al., 2007b). Cheese with the most similar procedure of production to Croatian and Herzegovinian cheeses from a sack is Tulum cheese. It is produced using ewe and cow milk, but it can also be produced using goat and buffalo milk and their mixture. Traditionally it is produced from skimmed milk after production of butter. Crumble curd is dry salted and stored in a goat skin in which it ripens for three to six months at a temperature of 6-10 °C and relative air humidity of 65-85 % (Yilmaz et al., 2005).

#### Preparing of skin for cheese ripening

Much attention is paid to the preparing of animal skin which is the medium for cheese ripening. Preparation of the sack requires a particular technique to avoid any damage of skin because incoming air into the skin sack can cause spoiling of cheese. After scalping of skin, the wool and tallow are removed, after which skin is washed in water several times. It is dried in the sun and wind and sometimes smoked. Before filling with cheese, skin is soaked in warm water and/or whey to soften. Holes from the feet and the neck are tied and tested by filling with air to check if there are cracks in the skin. To disinfect all openings of skin, they are smeared with vinegar or traditional alcoholic drink called rakija. During the period of ripening the skin sack requires special care. In the first days of ripening skin sack is turned every day, and later twice a week. It is washed with a wet cloth to remove mould (Bijeljac and Sarić, 2005; Tudor et al., 2009). Proper preparation of the skin is of crucial importance because it gives cheese specific taste. Although ripening in skin sack is a traditional procedure, in industrial conditions skin is sometimes replaced by wooden or plastic barrels. However, cheese which ripens in skin develops stronger flavour and gets higher consumer grades in comparison with cheese which ripens in plastic. The possible reason for the higher quality of cheese in animal skin sack, is piercing the bottom of plastic barrels (5-6 holes with 3 mm diameter) to drain excess whey. This also creates different ripening conditions than those in fully closed skin sack (Hayaloglu et al., 2007b).

# Physical and chemical properties of cheese in a sack

There are just a few publications on physical and chemical properties of cheese in a sack. An average physico-chemical composition of cheeses in a sack produced in different countries is shown in Table 2. The first research of chemical composition of cheese in a sack from the Knin region in Croatia was carried out by Milin in 1969. He classified it as a soft, full-fat cheese. Because of different age of analyzed cheeses, results of above mentioned data are slightly different from new one. According to Tudor et al. (2009) concerning the content of fat in total solids, cheese in a sack after 75 days of ripening belongs to the group of full-fat cheese. Although it is produced according to the manufacturing procedure of semihard cheeses, taking into account the size of curd grain, temperature of heating curd grain and content of fat in total solids, it belongs to the group of hard cheeses. The permeability of the skin allows the loss of moisture from cheese which leads to the relative increase of the content of total solids in cheese. Full ripened cheese in a sack from Herzegovina has the higher percentage of total solids in comparison with the other cheeses ripened in a sack (Dozet et al., 1996).

Similar research was conducted on Tulum cheese which showed that cheese after 60 days of ripening according to the content of moisture in the non-fat solids belongs to the group of semi-hard cheeses (Yilmaz et al., 2005). Increase of the total solids content during ripening is faster in the skin than in plastic due to the porous structure of skin which allows the loss of water. After 60 days of ripening, cheese which ripens in skin approximately has 56 % of total solids while cheese which ripens in plastic barrels has a lower percentage of total solids, 49 % (Hayaloglu et al., 2007a).

Tudor et al. (2008) analysed the physicochemical parameters of cheese in a sack throughout ripening period of 75 days, in two weeks intervals. Linear regression of the time of ripening on total solids content, water in the non-fat solids, fat content, protein content and NaCl is recorded. Dry matter content increases by 0.15 %, fat by 0.087 %, protein by 0.0597 %, salt by 0.027 % and water in the non-fat solids decreases by 0.148 % during every day of ripening. The average pH value of curd (5.33) decreases in the first 15 days of ripening to the value of 4.79. Then it increases by 0.0111 in every day of ripening till the end of ripening. This data show that physico-chemical modifications during ripening progress slowly and the possible reason is a protective effect of the skin sack in which cheese ripens (Tudor et al., 2008). The research about the influence of skin sack on the speed of ripening is underway.

Authors/Autori	Milin, 1969	Dozet et al., 1980	Dozet et al., 1996	Yilmaz et al., 2005	Jovanović et al., 2007	Tudor et al., 2009
Maturity of cheese (days) Zrelost sira (dani)	Different Različita	-	Different Različita	60	Different Različita	75
Total solids (%) Suha tvar (%)	62.96	48.10	78.98	53.04	52.78	66.52
Proteins (%) Proteini (%)	24.8 33.35		31.17	-	20.33	24.92
Fat (%) Mast (%)	32.92	9.26	39.40	29.5	25.40	36.3
Fat in total solids (%) Mast u suhoj tvari (%)	52.5	18.26	49.54	-	48.08	54.30
Moisture in solids-nonfat (%) Voda u bezmasnoj suhoj tvari (%)	-	-	-	-	63.26	52.08
NaCl (%)	4.29	3.02	-	4.16	3.13	4.29
pН	-	-		5.03	5.06	5.35
Lactic acid (%) Mliječna kiselina (%)	-	0.80	0.85	-	-	1.25
Ash (%) Pepeo (%)	-	4.44	7.08	-	-	-
Phosphorus (%) Fosfor (%)	-	0.349	0.85	-	-	-
Calcium (%) Kalcij (%)	-	0.201	0.27	-	-	-

Table 2. An average physico-chemical composition of cheeses which ripen in an animal skin Tablica 2. Prosječni fizikalno-kemijski sastav sireva koji zriju u životinjskoj koži

#### Sensory properties of cheese in a sack

Independently on geographic origin and manufacture process, cheese in a sack is white to yellowish colour, of hard texture, while in Herzegovina and Turkey its texture is crumbly. It has strong and piquant flavour which is attributed to anaerobic ripening conditions in animal skin and possible natural presence of moulds inside the sack (Bijeljac and Sarić, 2005; Yilmaz et al., 2005). The average consumers quality assessment score was 16.06, while group of experts grade it with 17.13 out of maximal 20 scores for sensory properties. This points out the need of raising the level of quality of this cheese (Tudor et al., 2009).

#### **Biochemical changes during ripening**

Among biochemical reactions occurring during ripening, the proteolysis and lipolysis are the most

significant due to primary milk microflora, starter cultures, indigenous enzymes and rennet (Urbach, 1997). Those processes are researched only in Tulum cheese while in Croatian cheese in a sack are currently underway.

The strong taste, flavour and aroma of Tulum cheese originate from intensive lipolysis and proteolysis. Levels of water-soluble nitrogen (WSN) and nitrogen fraction soluble in triacetic acid (TCA-SN), increase continuously during ripening. The average degree of proteolysis measured by share of WSN in TN is 26 % (Hayaloglu et al., 2007b). The material for ripening (skin or plastic) does not affect the level of WSN in cheese. The concentration of total free amino acids (FAA) increased continuously during ripening. There is no recorded difference in the concentration of total FAA between cheeses which ripen in animal skin and those which ripen in plastic barrels, but there is a difference in the concentrations of single FAA. The concentration of single FAA is higher during the whole period of ripening in cheeses which ripen in animal skin while the only exception are amino acids Asp, Ser, Glu, Tyr, His i Lys which concentrations are higher in cheeses which ripen in plastic. Proteolysis is followed by two methods: urea-polyacrylamide gel electrophoresis (urea-PAGE) and reversed-phase high performance liquid chromatography (RP-HPLC). Urea-PAGE electrophoretograms are similar for all cheeses independently on ripening media. The hydrolysis of casein is the same for all cheese samples due to the main role of rennet and plasmin in the hydrolysis of casein. Much of the  $\alpha_{_{\rm Sl}}\text{-}{\rm casein}$  is hydrolyzed after 120 days of ripening but ß-casein remain intact at the end of ripening. In general, ripening in either animal skin or plastic barrels did not have effect on peptides profiles but the duration of ripening had (Hayaloglu et al., 2007a).

Milk fat has significant effect on cheese flavour and aroma, but also on its texture (Akin et al., 2003). Free fatty acids (FFA) liberated during ripening directly affect cheese flavour and aroma, but they are also precursors of catabolic reactions wherein aroma and flavour compounds such as methyl ketones, lactones, esters and secondary alcohols are produced (Collins et al., 2003). In cheeses which ripen in animal skin, intensive lipolysis occurs which is observed by high levels of FFA and acid degree value. Güler and Uraz (2003) explained rancid, strong and bitter flavour of Tulum cheese by the high level of lipolysis. Mentioned cheese characteristics are intensified with the increase of the FFA level (especially short-chain) and with acid degree level which amounts 4,4-8 mg KOH/ g of fat in cheese ripened for 90 days (Koçak et al., 1995; Güler and Uraz, 2003; Yilmaz et al., 2005). By the addition of microbial lipase in the manufacture of Tulum cheese, the FFA content increases, the typical flavour and aroma for this cheese variety also increases. But increase of the concentration of added lipase does not increase sensory scores of cheese (Yilmaz et al., 2005). The research of lipolysis in Tulum cheese showed that the most abundant FFAs are C16:0 (50.86 mg/100g of cheese) and C18:1 (49.30 mg/100 g of cheese) which is shown in Table 3 (Yilmaz et al., 2005). Higher levels are found in 20 samples of Tulum cheese purchased from Turkish supermarkets: C16:0 (56.08 mg/100 g of cheese) and C18:1 (49.99 mg/100 g of cheese). The possible reason is different manufacturing and ripening period (Güler and Uraz, 2003). Although short-chain fatty acids constitute the small proportion of total FFAs, they are major contributors to typical flavour and aroma of Tulum cheese (Yilmaz et al., 2005).

#### Microbiological characteristics of cheese in a sack

Due to the fact that cheeses in a sack are produced from raw milk, requirements for its hygienic quality are high. However, the research showed that most of the milk for the production of Croatian

Table 3. Average values of the content of some FFA during ripening of Tulum cheese Tablica 3. Srednje vrijednosti pojedinih slobodinih masnih kiselina tijekom zrenja Tulum sira

5	5 1 5					5	5			
Authors/Autori	Maturity of cheese (days) Zrelost sira (dani)	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1
Yilmaz et al., 2005 (mg/100 g of cheese) Yilmaz i sur., 2005. (mg/100 g sira)	1	1.07	1.12	1.92	2.96	4.79	9.04	30.74	7.14	24.74
	15	2.96	1.52	1.7	4.01	5.76	13.69	37.83	10.48	36.71
	30	3.87	2.00	1.91	5.86	7.84	16.35	41.11	11.64	39.11
	60	4.56	2.52	2.94	6.01	9.95	20.49	47.77	15.25	460.86
	90	8.56	3.96	4.12	8.01	10.56	26.15	50.86	18.15	49.30
Güler and Uraz, 2003 (mg/100 g of cheese) Güler i Uraz, 2003. (mg/100 g sira)	Different Različita	75	62.50	73.30	184.60	153.60	271.70	560.80	221.00	499,90

cheese in a sack is not satisfactory considering total bacterial counts. The analyses of sheep milk showed that only 5 out of 16 analyzed samples, are acceptable according to the Regulation of hygiene of animal origin food (NN 99/07), which means that 31 % of sheep milk samples contain the number of microorganisms under 500000 in 1 mL, while 69 % of them exceed this allowed amount (Kaić et al., 2008). In Tulum cheese during ripening, the majority of bacteria are mesophilic whose number decreases in the first 120 days of ripening and after that its number increases. In Tulum cheese after 150 days of ripening there is 6.15-6.74 log cfu/g of cheese. Yeasts and moulds are present in Tulum cheese in high number, with an average value of 7 log cfu/g of cheese. Cheese which ripens in the animal skin has higher number of yeasts and moulds in comparison with cheese which ripens in plastic barrels. The possible reason for that is higher permeability of skin for the air. Coliform bacteria were also identified in cheese, but their number decreases during ripening and at the end of ripening it amounts  $<1 \log$ cfu/g of cheese. The coliform bacteria counts can be attributed to the low pH, low content of water, high salt content, presence of higher counts of moulds and mesophilic bacteria (Hayaloglu et al., 2007a).

## Conclusion

Today cheeses which ripen in the animal skin are rarely produced. Due to its rarity and specificity there are just a few researches of this cheese variety. The most researches of this cheese are conducted in Turkey, while cheeses from Bosnia and Herzegovina and Croatia are analyzed for basic chemical composition and physical properties. According to the content of fat in total solids and moisture in the non fat solids, cheeses in a sack could be classified as hard or semi hard, full-fat or low fat cheeses depending on manufacturing technology and region. The manufacture procedures for all cheeses that are ripened in skin sack are described. Changes in proteolysis and lypolisis are published for Turkish cheese in a sack. The strong taste, flavour and aroma of cheese originate from intensive lipolysis and proteolysis. Levels of water-soluble nitrogen (WSN) and nitrogen fraction soluble in triacetic acid (TCA-SN), increase continuously during ripening. The most abundant FFAs are C16:0 and C18:1. Furthermore, for better understanding the biochemical changes during ripening of cheese in skin several researches are underway.

# Pregled istraživanja sireva koji zriju u životinjskoj koži

## Sažetak

U radu je dan pregled dosadašnjih istraživanja o tehnologiji proizvodnje, fizikalno-kemijskom sastavu, mikrobiološkim karakteristikama, senzorskim svojstvima, biokemijskim promjenama sireva koji zriju u životinjskoj koži. Sirevi koji zriju u životinjskoj koži uz određene razlike u tehnologiji proizvode se u Hrvatskoj, Bosni i Hercegovini, Crnoj Gori i Turskoj. Glavna karakteristika ovih sireva je zrenje u vreći načinjenoj od janjeće ili kozje kože. Upravo takav način zrenja u trajanju od 2 do 3 mjeseca daje siru izrazito pikantan okus i miris. Te karakteristike sira potječu od intenzivnih procesa lipolize i proteolize tijekom zrenja. S obzirom na udio vode u bezmasnoj suhoj tvari i udio masti sirevi koji zriju u životinjskoj koži pripadaju skupini polutvrdih ili tvrdih masnih sireva ili sireva sa sniženim udjelom masti.

*Ključne riječi:* sir, mišina, mijeh, sastav, tehnologija, zrenje, životinjska koža

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