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TECHNOLOGICAL CHARACTERISTICS OF LACTIC ACID BACTERIA AND ENTEROCOCCI ISOLATED PROM KACHKAVAL ORIGINATED FROM STARA PLANINA 1

Zora Mijačević, Snežana Bulajić, Tatjana Božić 2

**Stract: Hard cheese - kachkaval originated from Stara Planina Mount has been structured in artisan dairies according to traditional technology. The production have been varied only in ambiental conditions, while the technological production has been very similar through centuries. Cow, sheep and goat milk the production of kachkaval also originated from Stara Planina Mount.

By the analyse of sensory characteristics of 7 samples of cheese the differences the size of cake, texture and salinity of cheese were established, and a specific of ripened kachkaval was noticed.

In the samples of kachkaval from Stara Planina Mount we have isolated 16 strains according to the strains of lactobacilles and 5 strains of enterococci. Biochemical activities of isolated strains of lactic acid bacteria show that they belong to the mesophilic bacteria, can grow in conditions with 2% NaCl and at pH 9.2, have acity to coagulate milk and possess proteolitic enzymes.

Enterococci proliferate in the conditions of higher concentration of salt (6% NaCl), intensively at temperatures of 15, 30 and 45°C and at pH 9.2. Observing the known that enterococci are often multiple resistent we have investigated their resistence to

Key words: kackhaval, sensory characteristics, lactic acid bacteria, enterococci

Introduction

Kachkaval is manufactured in the region of the Mediterranean, Adriatic Sea and Black Sea, in the areas once occupied by the Old Greece, Roman Empire and their eastern and southern colonies. From written historical monuments it can be learned that manufacturing of kachkaval has a very old history. Columella, Roman writer from 68 AD. described manufacturing of cheese »manum pressum«, which almost completely correspond the manufacturing of cheese.

The region where kachkaval was manufactured in the past and where it is manufactured today is in the fact the region where during the II century before Christ and all up to the VII century of the new era there were great migrations of nomadic tribes, whose major work was cattle and sheep breeding.

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On Balkan Peninsula, and especially in Romania, Bulgaria, Greece, Serbia, cattle breeding was mostly in the hands of Tzintzars, who were manufacturing especially hard cheese – kachkaval. According to historical data Tzintzars were natives of Balkan Pensisula, who settled there before Romans, and even perhaps before the ancient Greeks. According to the historical material it can be most certainly presumed that the manufacturing of cheese was brought to Balkan Peninsula by the nomadic tribes from the East. Later the manufacturing of this cheese was brought to Italy, and from Italy to Britain during the reign of Caesar. In Britan the technology of making this cheese had been adapted to climatic conditions and in this way the technology of Čedar cheese developed.

The technology of making cheese is mostly spread in Balkan Peninsula and in Italy, and besides that it is present in southern regions of Russia (the Crimea, Besarabia, south Ukraine), then in Turkey, Algeria, Tunisia, Egypt and Morocco, that is in the regions

with warm and dry climate, hilly relief and developed cattle breeding.

In each of these countries with developed manufacturing of hard cheese during the time some sorts or types of hard cheese distinguished themselves according to their properties and quality, such as hard cheese from Sarplanina Mount, Pirot hard cheese in Serbia, Pirdop cheese from Bulgaria, Penlu from Romania, Tesalia and Epiria cheese from Greece.

The origin of the name kachkaval can be found in the language of the people who most probably brought kachkaval to Balkan Peninsula from where it further spread

around. In Tzintzar language the word "kač" means cheese.

Talking about Dojkinački cheese it is impossible not to mention nomadic cattle breeders – sheep breeders, who are known as "Crnovunci" (Blackwool people). They got such a name because of the black colour of the wool of the sheep which they raised. In the period from the end of XIX century up to the third decade of the XX century Crnovunci populated the pastures of Stara Planina Mount with their flocks of 500-1000 sheep. Great quantities of sheep milk which gave their flocks, Crnovunci processed into white cheese and kept it in bellows. In order to prolong the durability of this cheese, it was soaked into hot water, mixed and salted, that is, in other words, the processing of milk into hard cheese – kachkaval had started. The skill of making kachkaval Crnovunci passed on to the people of Dojkinci village, who improved it to the perfection and kept untill today.

The analysis of today's manufacturing of kachkaval in Dojkinci dairy plant

Daily quantity of milk of 800 l is being collected from 100 producers of milk from Dojkinci village, who bring the milk to the dairy plant. After that the remaining 1200 l is being collected from the producers of milk from surrounding villages and transported to the dairy plant in plastic containers on the tractor trailer. The milk collecting in the village starts at 6 a.m. and lasts to 8 a.m. The farmers pour the milk into milk gauge, and after the measuring the milk is through drainer transferred to the curdling bath. Majority of producers bring milk in aluminium cans, while some of them bring it in alluminum pails. The temperature of milk is 21°C - 23°C, external temperature is 16°C at 6h a.m. and 20°C in 7h a.m. Prepared milk (800 l) is then poured into the boiler for whey and is being warmed to the temperature of 65-70°C. This temperature is reached within 2 hours, and about 10 a.m. there arrives the milk from surrounding villages. Milk in plastic cans has a temperature of 22,2°C, acidity 8,6°SH. This milk is being taken out from the cans by a pump and pipe and transferred to the curdling bath. It is then being mixtured

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the warmed milk from the boiler and the curdling temperature of 30°C is acheived. De curdling temperature does not change during the whole phase of curdling which 60 minutes. The phase of processing curd started by curd chopping up by means the cross and lasted for 5 minutes, the temperature of separated whey was 29.6°C and 6.6°SH, while the temperature of the curd was 29°C and acidity 5,2°SH. After separating the part of whey the curd is again intensively mixed to separate the grains and the part of whey is being separated, with the acidity of 6,4°SH, and temperature 39°C, while the curd had acidity of 10.8°SH and temperature of 29,5°C. After that the water from the bolier is added for cheese fuming and the temperature of curd is to 34,9°C. The duration of phase of drying grain is 10 min, until the required seenght of the grains has been reached. The acidity of whey is 9.6 °SH. The curd is then in the bath, pressed for 10 min to divide whey and then cut, taken out from the bath transferred to the drainer on to the cheese-making table, is being pulverized, alloted and pressed for 20 minutes, the pressing being achieved by screwing the press according the experience. After the pressing the lump is cut into pieces, taken out from the drainer and left on the draining table until tomorrow. The temperature of the room is 20.7°C, and the period of ripening of baskia is 19-20h. The room temperature during the might is 15-16°C. After the acidity has been inspected by spreading, baskia is being chopped on the cutter like that for cabbage and vapoured in the water whose temperature > 75°C. Fuming is performed in weaved baskets containing about 6 kg of baskia, sevaporated and put onto the cheese-making table, on which it is being spread and salted, formed into the ball and put into casts. The cheese remains in the casts until tomorrow, then is taken out, measured and transferred into the room for drying in which temperature is 20°C. After drying of 1-2 days, it is being transferred into the room for cheese ripening. The temperature in the room for ripening is 20°C, and the cakes of cheese are put one onto the other and salted with dry salt. Finally, the ripened kachkaval is being packed into vaccum bags.

Major characteristics of chemical composition of hard cheese from Stara Planina

Mount are shown in Table 1.

Table 1. Chemical composition of kachkaval from Stara Planina Mount
Tabela 1. Hemijski sastav kačkavalja sa Stare planine

Parametri Parametres	n	X±s	min	max
% sm % dry matter	7	60,79 ± 3,37	54,55	64,05
% masti % fat	7	29,50 ± 2,25	0 ± 2,25 25,00	
% vbm % water without fat	7	55,57 ± 3,55	50,07	60,60
% msm % fat in dry matter	7	48,52 ± 2,41	45,83	53,03
% belančevina % protein	7	24,99 ± 1,60	25,07	27,38
% rast. belanč % dissolved protein	7	5,72 ± 0,22	5,42	6,02
% NaCl	7	$3,80 \pm 2,57$	1,66	7,67
рН	7	$5,62 \pm 0,09$	5,50	5,75
°SH	7	37,82 ± 8,41	23,20	51,20

In regard to average values of chemical composition of kachkaval from Stara Planina, the kachkaval from Dojkinci had 62,17% dry matter, 30,00% fat, 54,04% water without fat, 48,25% fat in dry matter, 25,43% protein, 6,02% dissolved protein, 3,01%NaCl, pH 5.75 and acidity of 38,24°SH.

The analysis of sensory characteristics of kachkaval from Stara Planina Mount

Sensory analysis of 7 samples of cheese (2 made of sheep milk and 5 mixtures of cow and sheep milk) was performed. Major characteristics of evaluated cheese samples in relation to cutting, structure, consistency, colour, flavour, smell are shown tabulary and selectively with regards to the groups of cheese of the same age and origin.

Poreklo i starost sira Origin and the age of cheese	Senzorne karakteristike / Sensory characteristics				
mešano mleko mixture of cow and sheep milk	the control of the co				
do mesec dana starosti 3 uzorka / a month old 3 samples	On the cross-section the flakiness is observed, technological holes consistency from very elastic to completely brittle, non-typical smell flavour of young cheese with greater salt quantity. Most often is observed: bad consistency. Na preseku se zapaža lisnatost, često tehnološke šupljike konzistencije od jako elastične do potpuno krte, miris netipičan, ukus mladog sira sa većom količinom soli. Najčešća primedba: loša konzistencija				
do 2 meseca starosti 2 uzorka / two months old 2 samples	Fine, formed crust of cheese is observed, of light yellow colour, on the cross-section the technological holes are observed, flakiness, often crudely expressed, light yellow to yellow colour, often unequlized, typical smell, but not distinct, flavour acceptable although covered by greater quantity of salt, and the taste of goat milk is observed Zapaža se fina, formirana kora sira, svetložute boje, na preseku su vidljive tehnološke šupljike, lisnatost, često grubo izražena, boja svetložuta do žuta, često neujednačena, miris tipičan, ali neizražen, ukus prihvatljiv iako ga prekriva veća količina soli, a pored toga zapaža se i ukus kozijeg mleka				
<u>Ovčiji</u> Sheep	The state of the s				
do 2 meseca starosti 1 uzorak / two months old 1 sample	Typical gold yellow colour of crust, at the cross-section technological holes are observed, crude to very fine flakiness, soft-elastic consistency corresponding to ripened cheese, smell of sheep milk, salty, typical taste Karakteristična zlatnožuta boja kore, na preseku se zapažaju tehnološke šupljike, na preseku gruba do vrlo fina lisnatost, mekoelastične konzistencije koja odgovara zrelom siru, mirisa na ovčije mleko, slan, tipičan ukus				
do 4 meseca starosti 1 uzorak four months old 1 sample	Yellow crust, typical, technological holes are observed on cross- section, good flakiness, hard consistency, mouth-melting, flavour of sheep milk, typical smell, without developing subsequent taste Boja kore žuta, karakteristična, na preseku tehnološke šupljike, lisnatost dobra, tvrde konzistencije, topiv u ustima, mirisa na ovčije mleko, svojstven ukus, bez razvijanja naknadno ukusa				

Within the reduced in Doj meter, weight pearance of crossistency brittle pening for one mening period.

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Within the range of inspected sensory characteristics two kachkavals of cow milk modeced in Dojkinci dairy plant were examined. The size of cake was 15 cm in Therefore, weighing 2 kg, what is typical for hard cheese from this region. The appearance of crust is typical, yellow, colour of dough is uniformed, flakiness rough, existency brittle, salty taste. The flavour of Dojkinci hard cheese which had been spening for one month also has distinct bitterness, which is lost after 2 months of mening period .

On the basis of the results obtained in sensory analysis, major conclusions in brief

be the following:

- 1. Cross-section of hard cheese has certain shortcomings, what is manifested by the pearance of grains of non-paired baskia. Technological holes are allowed. Technological process should be directed in order to achieve good flakiness.
- 2. For this type of cheese firm consistency is typical. Often happens that we have elastic untypical consistency, what should be avoided by introducing corective heasures.
- 3. The smell of cheese should be like the smell of milk from which it is made. The mesence of goat milk should be avoided.
- 4. The taste of cheese should be pleasant, with the characteristic of melting in the Certain salinity is allowed, but not bitternes or sour taste. The taste of mestigated cheese is often non-typical, bitter, salty, sour.

Technological characteristics of microorganisms of the strains Lactococcus, Lactobacillus i Enterococcus isolated from the kachkaval originated from Stara Planina Mount

In examined cheese samples the presence of Lactococcus was observed in the count = 2.7-3,0x10⁷CFU/g, while the Lactobacillus was established in the count of 3x10⁷-15x108 CFU/g. Enterococcuses in the examined samples of cheese were present in the of 2,2-2,3x10⁷CFU/g. Major characterisities of bacteria of lactic acid and Emerococcus isolated from cheese are shown in Table 2.

Table 2. Major characteristics of lactic acid bacteria and Enterococcus isolated from kachkaval from Stara Planina Mount Tabela 2. Osnovne karakteristike bakterija mlečne kiseline i enterokoka izolovanih iz kačkavalja sa Stare planine

Mikroorganizmi Microorganims	Osnovne biohemijske karakteristike Major biochemical characteristics										
	Razlaganje Degradation	Razlaganje Rast u prisustvu					Rast pri temperaturama Growth at temperatures				
	Eskulina Esculin		4%NaCl	2%NaCl	pH 9,2	15°C	30°C	45°C			
Lactococcus	-	10.5	+(1)	+ (12)	+(10)	+(16)	+(16)	+(16)			
Lactobacillus	m still still i	o 52	+(2)	+(5)	+(5)	+(6)	(10)	+(10)			
Enterococcus	+(5)	+(5)	+(5)	+(5)	+(5)	+(5)	+(5)	+(5)			

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Major characteristics of Lactococcuses are that their optimal growth temperature is 30°C, what indicates their mesophiles character, then that they cannot stand greater quantities of salt, but are very resistant to the variations of pH environment. Lactobacilluses isolated from kachkaval have optimal temperature of growth at 30°C and are differently sensitive to the salt quantity and pH value. Enterococcuses have good proliferation in the conditions of various salt concentrations, at high pH and at investigated temperatures. By the use of disc-diffusive dough the resistence of isolated strains of Enterococcus to tetracycline, lincomycine and neomycine was established.

Proteolitic activity of lactic acid bacteria and Enterococci was determined according to the quantity of dissolved nitrogen in trichlor acetic acid, after the incubation of milk with certain strains during 72h/25±1°C. The quantity of dissolved nitrogen in control sample of milk (no inoculation) in the period of incubation of 72h/25±1°C is 0,63%, while in milk samples incubated with strains of Enterococci the quantity of dissolved nitrogen was from 0,73-1,04%, for Lactococci 0,53-0,83% and for Lactobacilles 0,76%.

Conclusion

Kackhaval declaration from Stara Planina Mount is unsuitable like for example "Old mountain hard cheese made of sheep milk", while according to the type of cheese it belongs to semi-hard fat cheese, then "Old mountain Senokočki hard cheese made of sheep milk". Also it is declared as semi-hard fat cheese with designation of a traditional manual workmanshop. Dojkinački hard cheese also has a designation "Old Serbian cheese from Stara Planina Mount" and a typical content composition. Cheese declarations should be complete and in accordance with existing regulations (Regulation Book on quality and other requirements for milk, milk products, composite dairy products and starter cultures, Yugoslav Official Register 26/02), whereas the traditional workmanship should be especially emphasized. Since the tradition of making cheese still exists on Stara Planina Mount and during a very long time a different types of cheese like Senokoški, Dojkinački and Pirot kachkaval have been known, it seems to be fully justified that hard cheese-kachkaval from this region should be protected on the grounds of a geographic origin. The preserving the tradition of manufacturing kachkaval means also the preserving cultural identity of the region.

TEHNOLOŠKE KARAKTERISTIKE BAKTERIJA MLEČNE KISELINE I ENTEROKOKA IZOLOVANIH IZ KAČKAVALJA POREKLOM SA STARE PLANINE

Zora Mijačević, Snežana Bulajić, Tatjana Božić

Rezime

Kačkavalj poreklom sa Stare Planine izrađivao se u zanatskim mlekarama prema tradicionalnoj tehnologiji. Proizvodni uslovi su se promenili samo po pitanju ambijentalnih parametara, dok je tehnološki proces ostao isti. Kravlje, ovčije i kozje

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karama prema o po pitanju ovčije i kozie

mleko predstavljaju sirovine za proizvodni proces izrade kačkavalja. Senzornom analizom 7 uzoraka sira utvrđene su razlike u veličini pogače, teksturi i slanosti sira i primećen je specifičan miris prezrelog sira. Iz uzoraka kačkavalja sa Stare Planine zolovano je 16 sojeva laktokoka, 10 sojeva laktobacila i 5 sojeva enterokoka. . Biohemijske karakteristike izolovanih sojeva bakterija mlečne kiseline pokazuju da pripadaju grupi mezofilnih bakterija, da mogu rasti u uslovima sa 2% NaCl i pri pH 9,2, koagulišu mleko i poseduju proteolitičke enzime. Prisutne enterokoke se azmnožavaju i u uslovima veće koncentracije soli (6% NaCl), intenzivno rastu na emperaturama 15, 30 i 45°C i pri pH 9,2. Polazeći od poznate činjenice da su enterokoke često multipno rezistentne ispitana je njihova rezistentnost prema bemoterapeuticima.

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