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Ispitivanje uslova transporta svinja do klanice

Karabasil Neđeljko[1], Vasiljević Milan[2], Dimitrijević Mirjana[1], Vučinić Marijana[1], Đorđević Vesna[3], Ivanović Jelena[1], Kureljušić Jasna[4]

S a d r ž a j: Put životinje od farme do klanice nosi brojne prepreke sa kojima se ona susreće: manipulacija i kontakt sa čovekom/operaterom, transport, drugačiji uslovi i sredina u kojoj se životinja zatekla a na koju nije navikla, uskraćivanje hrane i vode, promena u socijalnoj strukturi, odvajanje i/ili mešanje životinja. Kao posledica svega navedenog, kod životinja se može javiti strah, dehidracija, glad, pojačana fizička napetost i aktivnost, zamor i povrede. Nemogućnost životinje da prevaziđe stresne faktore sredine može dodatno zakomplikovati i naglasiti posledice i imati negativan uticaj na kvalitet mesa. U ovom radu su ispitani uslovi transporta svinja, praćenjem odgovarajućih parametara koji se odnose na prevozno sredstvo i opremu, lice koje upravlja vozilom, postupak istovara životinja i posledice transporta (prelomi, smrtnost i dr.). Transportna sredstva koja su korišćena za prevoz životinja od mesta nabavke do klanice nisu u potpunosti zadovoljavala tražene kriterijume. Kao posledica transporta 3% životinja nije moglo da ustane, 2% životinja je imalo prelome i zabeležen je 1% uginuća.

Ključne reči: *svinje, transport, dobrobit.*

Uvod

Dobrobit životinja je sve više u sferi interesovanja, kako stručne, tako i šire javnosti. Stav potrošača i njihov odnos prema kvalitetu mesa predstavlja važnu informaciju za proizvođače, jer direktno utiče na profit. Kvalitet je teško definisati, ali brojnim istraživanjima iz ove oblasti i adekvatnim obaveštavanjem javnosti, mišljenje potrošača se vremenom može poboljšati, kako u pogledu stava prema kvalitetu mesa, tako i u pogledu načina uzgoja i manipulacije sa životinjama (*Baltić i dr.,* 2002; *Baltić i dr.,* 2010). Životinje su emocionalna bića i mogu da osete bol, patnju, stres, strah i paniku. Samim tim, obaveza čoveka je da, pored očuvanja vrste, brine i o zaštiti života i dobrobiti svake jedinke, naročito domaćih životinja čiji opstanak zavisi od njegove neposredne brige. Dobrobit predstavlja obezbeđivanje uslova u kojima životinja može da ostvaruje svoje fiziološke i druge potrebe svojstvene vrsti, kao što su ishrana i napajanje, prostor za smeštaj, fizička, psihička i toplotna udobnost i sigurnost, ispoljavanje osnovnih oblika ponašanja, socijalni kontakt sa životinjama iste vrste, odsustvo neprijatnih iskustava, kao što su bol, patnja, strah, stres, bolesti i povrede (*Sl. glasnik RS,* 2009). Iako nema jedne jasne definicije, dobrobit životinja možemo pokušati da definišemo kao stanje organizma koje pokazuje kako se životinja prilagodila na uslove života koje joj je obezbedio čovek u skladu sa njenom vrstom. Jedan od važnijih aspekata su zdravlje i kondicija životinje. Drugi aspekt se odnosi na činjenicu da su životinje osećajna bića i pokazuju, kako zadovoljstvo, tako i patnju. Treći aspekt, bila bi mogućnost životinje da ispolji svoju prirodu i živi u skladu sa svojim potrebama i u prirodnom okruženju.

U lancu proizvodnje mesa postoji veliki broj procesnih koraka, a osnovni koraci u proizvodnji životinja za dobijanje mesa su uzgoj životinja na farmi, zatim transport sa farme do stočne pijace ili klanice, pa transport sa stočne pijace do klanice, boravak životinja na klanici i operacije klanja (*Baltić i Karabasil,* 2005). Pored nabrojanih, postoje i brojni međukoraci koji dodatno komplikuju uslove

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Tabela 1. Kontrolna lista za prikupljanje podataka o uslovima transporta svinja
Table 1. Checklist for investigating the condition of pig transport

Nedelja uzorkovanja/Sampling week: Dan u nedelji/Day of the week: Datum/Date:		
PARAMETAR/PARAMETER		**NALAZ/FINDING**
A) PREVOZNO SREDSTVO I OPREMA/TRANSPORTATION VEHICLE AND EQUIPMENT:		
1.	da li ima zaštitu od vremenskih uslova/whether there is protection from the weather conditions;	
2.	površina transportnog sredstva (P), broj životinja i masa živih životinja (M), ujednačenost mase (kategorije: prasad, tovljenici, krmače)/surface of the transportation vehicle (P), the number of animals and live animal weight (M), uniformity of mass (categories: piglets, fatteners, sows)	
3.	da li je omogućen pristup životinjama u transportnom sredstvu/if there is access to animals in the transport vehicle	
4.	program čišćenja i dezinfekcije (procedure, uputstva, zapisi)/cleaning and disinfection program (procedures, instructions, records)	
5.	da li je podna površina od neklizajućeg materijala (da li je hrapava površina ili ne, da li ima fekalnog materijala ili ne, da li je vlažna površina ili ne, da li ima prostirku ili ne)/if the floor area is made of non-slip material (whether rough surface or not, whether there is fecal material or not, whether the surface is wet or not, whether there is a bedding or not)	
6.	da li je obezbeđeno da ne dolazi do izlivanja urina i fecesa/ whether it is provided that there is no leakage of urine and feces	
7.	da li ima odgovarajuću ventilaciju/whether the air ventilation is appropriate	
8.	da li ima odgovarajući svetlosni izvor/whether the light source is appropriate	
9.	da li poseduje protivpožarnu opremu/ whether it has the firefighting equipment	
10.	da li poseduje opremu za hranjenje ako se radi o dužim transportima/ whether it has the feeding equipment in case of longer transports	
11.	ako se radi o dužem transportu (preko osam časova), da li poseduje odgovarajući uređaj za održavanje i praćenje temperature/in the case of longer transport (over eight hours), if there is a suitable device for maintenance and temperature monitoring	
12.	šta čini opremu za utovar/istovar/what makes the equipment for loading / unloading	
13.	obeležavanje vozila/identification of the vehicle	
B) LICE KOJE UPRAVLJA VOZILOM/ THE PERSON DRIVING THE VEHICLE		
14.	koliko ima iskustva u transportu životinja (godine rada na ovim poslovima)/experience in the transportation of animals (how many years on the job)	
15.	da li je prošao obuku vezano za uslove dobrobiti životinja prilikom transporta/whether he/she is trained regarding the requirements of animal welfare during transport	
C) RAMPE ZA ISTOVAR/LIFTOVI/UNLOADING RAMPS-LIFTS		
16.	ako je pod nagibom da li je odgovarajući, ako je nagib preko 10 % da li ima zaštitnu ogradu/ If there is a slope, is it appropriate, if the slope is more than 10% if there is a protective barrier	
17.	da li je rampa zaštićena od vremenskih uslova/whether the unloading ramp is protected from the weather conditions	
D) ŽIVOTINJE/ANIMALS		
18.	period dana kada su životinje transportovane i dužina transporta/period of the day when the animals were transported and transport distances	
19.	spoljašnja temperatura na dan transporta/outside temperature on the day of transport	
20.	kg/m^2 (M/П)?	
21.	da li su životinje/pošiljka sa iste/različite farme ili iz otkupa/whether the animals/shipment originate from the same/different farms or were they purchased	
22.	da li su životinje uznemirene ili se istovaruju u miru (1 – životinje nisu uznemirene: životinje su mirne i uobičajeno se oglašavaju; 2 – životinje su umereno uznemirene: do 50% životinja je uznemireno i intenzivnija je vokalizacija; 3 – životinje su izrazito uznemirene; više od 50% životinja je uznemireno i intenzivnija je vokalizacija)/if the animals are disturbed or are unloaded at rest (1 – animals are not disturbed: the animals are calm and vocalize as usual, 2 – moderately disturbed animals: up to 50% of the animals is disturbed and the vocalization is more intense, 3 – animals are very upset; more than 50% of the animals is upset and there is intense vocalization)	
23.	u kojoj meri se koriste sredstva prinude i koja/the extent to which the means of force are used in animal handling and which means	
24.	broj životinja koje ne mogu da ustanu posle transporta/number of animals that can not stand up after transport	
25.	koliko životinja je sa prelomima/number of animals with fractures	
26.	koliko životinja je sa: A) ranama/ugrizima; ili B) drugim povredama (masnice/ogrebotine)/how many animals are with: A) wounds/bites, or b) other injuries (bruises/scratches)	
27.	da li su životinje čiste (1 – čiste životinje bez vidljivih fekalnih nečistoća; 2 – suva fekalna nečistoća po ekstremitetima, donjim delovima abdomena i sl.; 3 – vlažna fekalna nečistoća po ekstremitetima i donjim delovima abdomena i sl.)/ whether the animals are clean (1 – clean animals with no visible fecal contamination; 2 – dry fecal contamination on the extremities, the lower part of the abdomen, etc.; 3 – wet fecal contamination on the extremities and the lower part of the abdomen, etc.).	
28.	da li ima uginulih životinja i koliko/if there are dead animals and how many	

dobrobiti, tako da je neophodno shvatiti suštinu problema i poželjno je imati što manje manipulativnih koraka sa životinjama (*Karabasil i dr.,* 2011). Široko je prihvaćeno da transport predstavlja stresnu epizodu u životu životinja (*von Borell i Schaffer,* 2005; *EC,* 2004). Prilikom transporta, menja se čitav ambijent na koji je životinja navikla. Transport nije jedan usko definisan stresor nego predstavlja kombinaciju više faktora, jer se životinja sreće sa različitim i eventualno nepoznatim materijalima, mirisima, intenzitetom svetlosti, zvucima i vibracijama, manipulacijom od strane operatera, odvajanjem iz grupe i mešanjem sa nepoznatim jedinkama, promenom temperature i cirkulacijom vazduha, povredama, ograničenim prostorom, uskraćivanjem vode i pića. Kao rezultat toga, neminovno proizilazi činjenica da se prilikom transporta ne mogu, u potpunosti, ispoštovati načela dobrobiti.

Stres predstavlja odgovor organizma na štetne faktore sredine. Ishod stresa može biti adaptacija, ili iscrpljenje adaptacije, što dovodi do kolapsnog stanja. Pod uticajem brojnih stresogenih faktora, kao posledica transporta (ekstremna temperatura, buka, grub postupak, gladovanje i sl.), mogu se javiti i prvi znaci stresa kod svinja u vidu podrhtavanja mišića i repa, kao i nepravilnog i otežanog disanja. Od ostalih znakova stresa javlja se naizmenična pojava belih i zacrvenjenih područja na koži, naglo povećanje telesne temperature, cijanoza, ukočenost mišića i, eventualno, uginuće u kolapsnom stanju. Kao posledica delovanja stresora, organizam teže neutrališe mikroorganizme koji prodiru iz digestivnog trakta i smanjena je otpornost prema infektivnim bolestima. Stres ima i negativan uticaj na senzorna svojstva mesa, konzistenciju, ukus, miris i boju (*Čepin*, 2001; *Barton i dr.,* 2003; *Dokmanović,* 2012). Uginuća tokom transporta predstavljaju objektivan indikator patnje životinja. Prema podacima iz literature, smrtnost svinja kao posledica transporta, u zemljama Evropske unije, nalazi se u opsegu 0,03–0,5%, mada je primetan trend smanjenja (*Christensen i dr.,* 1994; *Barton i dr.,* 2003).

U radu su ispitani parametri transporta životinja koji se odnose na prevozno sredstvo i opremu, rampe za istovar, lice koje upravlja vozilom i same životinje.

Materijal i metode

Ispitivanje uslova transporta svinja sprovedeno je na jednoj klanici u toku pet nedelja, tako da se svake nedelje dan prikupljanja podataka menjao. Sačinjena je kontrolna lista sa definisanim pitanjima za parametre koji su se odnosili na sledeće aspekte uslova transporta: a) ispitivanje uslova prevoznog sredstva i opreme (13 pitanja); b) lice koje upravlja vozilom (dva pitanja); c) rampa za istovar/liftovi (dva pitanja); c) životinje (11 pitanja).

U tabeli 1 prikazana je kontrolna lista sa definisanim pitanjima i parametrima za ocenu uslova dobrobiti i transporta životinja. Ispitivanje je sprovedeno u jesenjem periodu (novembar).

Rezulatati i diskusija

Transport predstavlja jedan od glavnih stresora i može imati negativne posledice po zdravlje, dobrobit životinja i, neminovno, za kvalitet mesa.

Prevozno sredstvo i oprema. Prevoz životinja za potrebe klanice u kojoj je sprovedeno ispitivanje realizovan je sa dva vozila. Oba vozila su imala zaštitu od atmosferskih uslova i odgovarajuću, hrapavu podnu površinu od neklizajućeg materijala. U vozilu A postojala je zaštita od izlivanja urina i fecesa dok je vozilo B nije imalo. Pošto se radilo o klanici malog satnog kapaciteta, koja se životinjima snabdevala iz okruženja, a prevoz nikada nije trajao duže od 8 časova, nije bilo potrebe za opremom za hranjenje. Od mesta nabavke do klanice transport je, najčešće, trajao do 3 časa. S obzirom na specifičnost pošiljke, vozilo bi moralo da bude jasno označeno da je namenjeno transportu životinja, ali ni vozilo A, a ni vozilo B nisu imali adekvatnu oznaku.Vozilo A je imalo unutrašnju površinu od 18 m^2, a vozilo B od 8 m^2. Da ne bi nastao problem sa pretovarenošću vozila, prilikom transporta svinja treba da se vodi računa o dostupnoj podnoj površini u vozilu, kao i o broju i kategoriji životinja. Životinje u transportnom sredstvu treba da imaju mogućnost da legnu i da ustanu kada za to imaju potrebu (*RSPCA,* 2010; *MAF,* 2010; *EC,* 2004). Da bi se zadovoljio ovaj kriterijum, traženi minimum prostora, za tovne svinje (oko 100 kg) ne treba da pređe 235 kg/m^2 (+ 10%), ili da po transportovanom tovljeniku bude obezbeđeno 0,42 m^2 podne površine. Dostupna podna površina (A) se može izračunati iz formule $A = 0{,}0192W^{0.67}$, gde je „W“ masa trupa životinje. Za lakše kategorije svinja (do 30 kg), preporučuje se 169 kg/m^2, ili 0,18 m^2 podne površine. Tokom pet nedelja praćenja uslova transporta svinja, odnosno pet dana sakupljanja podataka, vozilo A je korišćeno za prevoz tri puta, a vozilo B dva puta. Vozilo A, nije bilo ni u jednom slučaju pretovareno, jer je masa po metru kvadratnom iznosila, u prvoj nedelji 183 kg/m^2, u drugoj nedelji 83 kg/m^2 a u trećoj nedelji 217 kg/m^2. Vozilo B, prilikom transporta prasadi, u četvrtoj nedelji eksperimenta nije bilo pretovareno (106 kg/m^2), dok je u petoj nedelji ovo vozilo bilo pretovareno. Prilikom transporta tovljenika i krmača, opterećenje

vozila je bilo 262 kg/m^2, a ne bi smelo da bude više od 258,5 kg/m^2 (235 kg /m^2 + 10%). Prilikom transporta, različite kategorije životinja nisu bile fizički razdvojene, kao ni pošiljka prasadi koja je poticala sa dve farme. Kao posledica, primetan je bio i povećan procenat povreda životinja, pa i uginuća. Jedan od zahteva prilikom transporta svinja jeste da se obezbedi dovoljno prostirke da bi životinje imale odgovarajuće uslove i sprečile eventualne povrede usled klizave i vlažne podne površine. Prilikom osvedočenja u uslove koji se odnose na prostirku, na podnoj površini, ili nije uopšte bila stavljena prostirka, ili je bilo jako malo, što je neopravdano sa aspekta dobrobiti životinja. Iako se po istovaru sprovodilo redovno održavanje vozila, ono se obavljalo rutinski, a radnici nisu imali uputstva, tako da se nisu vodili ni zapisi o održavanju higijene kao ni provera efektivnosti sprovedenog čišćenja, pranja i dezinfekcije.

Lice koje upravlja vozilom. Lice koja upravljaju vozilom treba da ima odgovarajuću obuku iz oblasti dobrobiti životinja (*Sl. glasnik RS,* 14/2010). Takođe, mora da ima dovoljno znanja i veština da, ukoliko ima potrebe, radi i manipuliše sa životinjama. U ispitivanoj klanici, vozilima za transport životinja upravljaju dva lica. Jedan vozač radi na poslovima transporta životinja dve godine, dok drugi četiri meseca. Nijedan od njih nije prošao odgovarajuću obuku iz oblasti dobrobiti životinja, niti ima iskustava u manipulaciji sa životinjama. Obuka osoblja koje vrši prevoz je značajna, da bi mogli da shvate ulogu koju imaju, jer prevoze živa bića i, samim tim, svaki polazak odnosno zaustavljanje vozila mora biti adekvatno kao i sam tok transporta, bez naglih ubrzanja, odnosno kočenja, skretanja i sl.

Rampe za istovar. Osnovni uslov prilikom istovara svinja jeste da prostor ispred životinje bude slobodan i da ima dovoljno prostora za transfer životinja iz vozila u koridor/stočni depo. Prilikom istovara iz vozila A, u kome je postojala ograda, nagib je iznosio 25°. Za vozilo B, rampa je bila pod nagibom od 20°, a vozilo nije imalo odgovarajuću ogradu, tzv. bočnu barijeru. U Standardu za svinje (*RSPCA,* 2010) navodi se da na mestu utovara/istovara svinja, nagib ne bi trebalo da bude veći od 20% u odnosu na horizontalnu površinu, tj. veći od 11°.

Životinje. Ispitivanje uslova transporta svinja sprovedeno je tokom pet nedelja u jesenjem periodu (novembar), s tim što se svake nedelje dan rotirao, da bi se obuhvatili svi radni dani u nedelji (I nedelja – ponedeljak; II nedelja – utorak; III nedelja – sreda; IV nedelja – četvrtak; V nedelja – petak). U pomenutom periodu, prevoz životinja, za potrebe ispitivane klanice, obavljao se u prepodnevnim časovima u intervalu od 07:00 do 12:00 časova. Pošto su se životinje nabavljale iz okruženja, transport je u navedenom periodu ispitivanja najkraće trajao u četvrtoj nedelji (pola sata), a najduže u drugoj nedelji (2 sata i 20 minuta). Dužina trajanja transporta je merena od momenta polaska vozila sa mesta nabavke do momenta prispeća vozila u krug klanice. Životinje su odmah po prispeću istovarane iz vozila u stočni depo. Prilikom istovara, od sredstava prinude radnici su koristili, najčešće, električni gonič. Iako imaju po nekoliko godina iskustva u radu sa životinjama, nijedan od radnika nije imao obuku iz oblasti dobrobiti i ponašanja životinja. Takođe, nije postojalo ni uputstvo za upotrebu električnog goniča, tako da su ga radnici primenjivali relativno često, više puta na istoj životinji, na različitim mestima po telu. Prema osnovnim načelima dobrobiti životinja, sredstva prinude se koriste u krajnjoj nuždi, i to u regiji buta u trajanju od 1 minuta, a ne smeju se koristiti ukoliko životinja nema slobodan prostor ispred sebe (*OIE,* 2011). Radnici, su često i ne vodeći računa, aplikovali električni gonič na životinjama koje su bile poslednje u nizu i, samim tim, stvarali su dodatnu uznemirenost, pošto životinja nije mogla da napravi sledeći korak od životinje koja se nalazila ispred nje. Prema preporuci OIE-a (2011), kao sredstva prinude navode se plastične flaše, zastavice i sl., kao pomoć pri manipulaciji sa životinjama, čija uloga nije u fizičkoj primeni sile i prinude. U petoj nedelji ispitivanja, radnici nisu koristili električni gonič, jer nije bio ispravan, već drveni štap. U ovakvoj situaciji, ne treba kriviti samo radnike, koji, pored toga što nisu imali odgovarajuću obuku, preuzimali su ulogu koju je trebalo da ima rukovodstvo u rešavanju ovako važnog aspekta, a to je korišćenje odgovarajućih i dozvoljenih sredstava prinude pri manipulaciji sa životinjama.

Ocena uslova da li su životinje istovarane u miru ili su uznemirene izvršena je na osnovu skale u okviru liste provere: 1 – životinje nisu uznemirene; životinje su mirne i uobičajeno se oglašavaju; 2 – životinje su umereno uznemirene; do 50% životinja je uznemireno i intenzivnija je vokalizacija; 3 – životinje su izrazito uznemirene; više od 50% životinja je uznemireno i intenzivnija je vokalizacija.

Uznemirenost životinja je samo u jednoj nedelji ocenjena kao „izrazito uznemirene“ (3), dok je za ostale nedelje ispitivanja označena kao „umereno uznemirene“ (2). Jedan od razloga za ovu situaciju je i bučna manipulacija radnika, kao i nekontrolisana upotreba prinudnih sredstava prilikom istovara životinja u, za njih, nepoznatu sredinu, a pri tom ne vodeći računa o obezbeđivanju i dostupnosti slobodnog prostora koji je neophodan da bi životinja mogla da napravi iskorak.

Parametar za ocenu uslova transporta jeste i čistoća samih životinja, koja je posledica uslova u kojima se životinje uzgajaju i transportuju. Za ocenu

čistoće životinja sačinjena je bod skala: 1 – čiste životinje bez vidljivih fekalnih nečistoća; 2 – suva fekalna nečistoća po ekstremitetima, donjim delovima abdomena i sl.; 3 – vlažna fekalna nečistoća po ekstremitetima i donjim delovima abdomena i sl.

U toku prve četiri nedelje čistoća životinja je ocenjena kao „vlažna fekalna nečistoća" (3), dok je u petoj nedelji ocenjena kao „suva fekalna nečistoća" (2). Ovo je, između ostalog, i posledica transporta životinja sa jako malo, ili bez odgovarajuće prostirke, ali, verovatno, i rezultat loših uslova higijene na samoj farmi.

U tabeli 2. prikazan je broj i procenat životinja koje nisu mogle da ustanu, sa prelomima, povredama i uginuća kao posledica transporta. U periodu trajanja ispitivanja, ukupno je prevezeno

Tabela 2. Broj i procenat životinja koje nisu mogle da ustanu, životinje sa prelomima, povredama i uginuća
Table 2. Number and percentage of animals that could not stand up, animals with fractures, injuries and deaths

Nedelja/Week	Kategorija i broj transportovanih životinja/Category and number of transported animals		Broj (procenat) životinja koje nisu mogle da ustanu posle transporta/Number (percentage) of animals that could not stand up after transport	Broj (procenat) životinja sa prelomima/ Number (percentage) of animals with fractures	Broj (procenat) životinja sa ranama/ ugrizima ili drugim povredama/ Number (percentage) of animals with wounds/bites or other injuries	Broj (procenat) uginulih životinja/ Number (percentage) of dead animals
1.	Tovljenici/Fatteners	30	0	0	7 (23%)	0
	Krmače/Sows	–	–	–	–	–
	Prasad/Piglets	–	–	–	–	–
	Ukupno/Total	30	0	0	7 (23%)	0
2.	Tovljenici/fatteners	–	–	–	–	–
	Krmače/Sows	–	–	–	–	–
	Prasad/Piglets	62	2 (3%)	2 (3%)	12 (19%)	0
	Ukupno Total	62	2 (3%)	2 (3%)	12 (19%)	0
3.	Tovljenici/fatteners	21	1 (5%)	1 (5%)	6 (28%)	0
	Krmače/Sows	8	1 (12%)	1 (12%)	0	0
	Prasad/Piglets	26	0	0	4 (15%)	2 (4%)
	Ukupno Total	55	2 (4%)	2 (4%)	10 (18%)	2 (4%)
4.	Tovljenici/Fatteners	–	–	–	–	–
	Krmače/Sows	–	–	–	–	–
	Prasad Piglets	34	1 (3%)	0	16 (47%)	0
	Ukupno Total	34	1 (3%)	0	16 (47%)	0
5.	Tovljenici/Fatteners	11	0	0	5 (45%)	0
	Krmače/Sows	7	0	0	3 (43%)	0
	Prasad/Piglets	–	–	–	–	–
	Ukupno/Total	18	0	0	8 (44%)	0
UKUPNO/TOTAL		**199**	**5 (3%)**	**4 (2%)**	**53 (27%)**	**2 (1%)**

199 životinja. Od tog broja, 3% svinja nakon transporta nije moglo da ustane, dok je 2% životinja imalo prelome. Pošto nije vršeno osvedočenje na mestu nabavke, pretpostavlja se da je jedan od razloga za veliki broj životinja sa povredama (27%) grub postupak sa svinjama prilikom utovara, a najčešće povrede bile su ogrebotine u predelu leđa. Smrt životinja kao posledica transporta predstavlja objektivan indikator patnje. Uginuća su zabeležena jedino u toku treće nedelje ispitivanja, i to dva praseta od 55 životinja koliko je tom prilikom bilo transportovano (26 prasadi, 8 krmača i 21 tovljenik). Procentualno posmatrano, to je 4% za posmatrani dan transporta, a u odnosu na ukupan broj transportovanih životinja obuhvaćenih eksperimentom 1%. U većini zemalja Evrope, prema podacima iz literature (*Christensen i dr.,* 1994; *Barton i dr.,* 2003), uginuća se kreću do 0,5%, i primetan je trend smanjenja.

Zaključak

Na osnovu rezultata i njihovog kritičkog razmatranja može se zaključiti da je neophodno da se unapredi dobrobit životinja sa aspekta uslova transporta. Transportna sredstva koja su korišćena, iako su imala određenu opremu, nisu imala sve uslove za adekvatno sprovođenje transporta. Istovarna rampa nije bila odgovarajuća, jer nije imala zaštitu od atmosferskih prilika. Nagib za istovar za vozilo A nije bio odgovarajući, a za vozilo B rampa nije imala bočne zaštite. Radnici su neadekvatno koristili sredstva prinude. Lica koja su upravljala vozilom nisu imala odgovarajuću obuku kao ni potrebna znanja za transport životinja. Nije se vodilo računa o masi životinja po m^2 raspoloživog prostora u toku transporta, kao ni o razdvajanju različitih kategorija životinja, što je za posledicu imalo veći procenat povreda, pa čak i uginuća.

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The study of transport conditions of pigs to the slaughterhouse

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S u m m a r y: Transport of animals from the farm to the slaughterhouse carries numerous obstacles: manipulation and contact with the man/operator, transport, different conditions and environment which the animal is now facing and is not used to, deprivation of food and water, changes in the social structure, separation and/or mixing of animals. As a consequence, the animals can develop fear, dehydration, hunger, increased physical activity and tension, fatigue and injury. Inability of animals to overcome stress factors may further complicate and highlight the consequences and have a negative impact on the meat quality. In this paper, conditions of transport of pigs were analysed by following the appropriate parameters relating to vehicle and equipment, the person driving the vehicle, the process of unloading animals and effects of transport (fractures, mortality, etc.). The means of transport used to transport animals from the place of procurement to the slaughterhouse did not fully satisfy the required criteria. As a result of transport, 3% of the animals were unable to stand up, 2% of the animals had fractures, and 1% mortality was recorded.

***Key words**: swine, transport, welfare.*

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Investigation of Shiga-like toxigenic *Escherichia coli* in meat products by quantitative PCR

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A b s t r a c t: The aim of this study was to investigate and evaluate effectiveness of quantitative PCR (qPCR) in quantification of Shiga-like toxigenic E. coli (STEC) in artificially contaminated deli meat slices compared to plate count technique. Experiment was designed to investigate capability of qPCR using selected pair of primers amplifying rpoB household gene and serial decimal dilution of an overnight suspension of E. coli O157:H7 culture. Subsequently, slices of smoked pork loin were contaminated by respective decimally diluted dose of STEC. DNA was extracted from the samples and qPCR was run in triplicate. Mean Ct values of amplified rpoB gene were compared to each inoculum dose and standard curves were generated. Results clearly showed that the lowest detectable level of STEC in pork loin slices using qPCR was 6.8×10^1 CFU/g. Further optimization of method should be done in order to resolve, if possible, discrepancies at the levels of contamination less than 100 CFU/g.

Key words: *qPCR, E. coli O157, quantification.*

Introduction

Shiga-like toxigenic E. coli have emerged as important food-borne pathogens, causing hemorrhagic colitis, which is sporadically complicated by hemolytic uremic syndrome. Onset of the illness requires a very low dose of STEC, in between 10-700. Standard or validated alternative methods are available and are recommended to be used for the detection and isolation of STEC O157 from food and animals. For the other serotypes, there are no universally accepted and validated methods, but pragmatic approaches have been produced. Pathogenic *E. coli* strains can be divided into Enteropathogenic *E. coli* (EPEC) strains, which "only" cause A/E (attaching-and-effacing) lesions, Shiga-toxin producing *E. coli* (STEC) strains, which possess and express *stx* genes, and Enterohemorrhagic *E. coli* (EHEC), which constitute a subset of STEC as classical EHEC can cause both hemorrhagic colitis (HC; due to AE lesion) and hemolytic uremic syndrome (HUS; due to the Shiga toxins) (*Bunčić,* 2000). Different virulence genes, such as stx_1 and stx_2 and their variants, which encode Shiga toxins, *eae*, which encodes the bacterial outer-membrane protein intimin, *nle*, which encodes translocated substrates of the type III secretion system, *ehx*A, which encodes the EHEC hemolysin, *iha*, which encodes an adherence-associated protein, *esp*P, which encodes the serine protease, and *hly*A,which encodes enterohemolysin, have been targeted to assess the presence of STEC (*Coombes et al.,* 2008; *Paton and Paton*, 1998; *Pradel et al.,* 2008).

Improved methods for the detection and isolation of STEC non-O157 from foods, animals and the environment should be developed and validated. There is no standard protocol for enumeration of STEC O157 or other STEC serotypes in food or environmental samples and such quantitative methods should be developed. Enumeration of STEC is generally not conducted as part of routine monitoring or testing programs, although quantitative data are essential to better understand the human health risks. Recent advances in molecular detection methods combine the traditional detection methods and target serotype specific genes, *stx*, as well as other virulence genes. However, isolation of STEC, and subsequent strain characterization is still needed in order to ensure that the detected genes are present on the same bacteria.

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Microbial cross-contamination at production site is one of the major factors of contamination of foods with STEC. Good hygiene practices at the abattoirs and at processing plants, including monitoring for microbiological indicators (*Enterobacteriaceae* and in generic *E. coli*), is likely to be the most effective method for reducing the public health risks for STEC infection. However, compliance with the hygiene criteria does not necessarily guarantee the absence of STEC at concentrations sufficient to cause human disease. Application of efficient validated HACCP-procedures for production of raw ready-to-eat meat, meat preparations and other foods is important to reduce the public health risks for STEC infection (EFSA 2008).

Generally, the DNA amplification-based techniques are rapid and will give a result within hours. One of the advantages of DNA-based methods is that it is possible to simultaneously investigate cultures for several genes at the same time. However, when testing mixed cultures the detected genes might not originate from the same STEC strain. By using DNA-based methods it is also possible to differentiate between the different *stx* subtypes. Furthermore, several quantitative PCR methods can be used to assist in subculturing of selected enrichment broths with priority given to the highest target concentration since there is a correlation between the number of *stx* gene copies and the success of isolation of STEC from an enrichment broth. DNA based methods have the disadvantage of being unable to distinguish between DNA from viable and non-viable cells, although this may only be important in specific situations.

In the last 10 years, Real-Time PCR systems based on SYBR Green I and TaqMan technologies have increasingly been used for accurate and reliable detection and quantification of various foodborne pathogens (*Rodríguez-Lázaro et al*., 2004a; *Rawsthorne and Phister*, 2006; *Skånseng et al.,* 2006; *Malorny et al.*, 2007), including STEC in contaminated samples along the food production chain. Compared to conventional PCR-based methods, Real-Time PCR technologies involve a lower risk of cross-contamination because the presence of the target sequence(s) in the sample is indicated by an increase in fluorescence signal, and no post-PCR processing of the sample is required (*Rodríguez-Lázaro et al*., 2004c; *Rossmanith et al*., 2006; *Cocolin and Rantsiou*, 2007).

Materials and methods

E. coli O157:H7 ATCC 35150 strain was used for assessing Real Time PCR efficiency, optimization of amplification conditions and inoculation of pork loin slices.

BHI broth (Oxoid, UK) was used for cultivation of strain preceding DNA extraction and for inoculation of dry pork loin slices. MRD (Merck, Germany) was used for preparation of serial dilutions. ChromID O157:H7 agar (Biomerieux, France) was used for quantification of *E. coli* O157:H7 in both overnight (o/n) culture broth and in inoculated samples. The final concentration of cells in the meat product ranged from 10^8 to 10^1 CFU/g.

Extraction of DNA from cultures and inoculated samples

One mL of an o/n culture was centrifuged at 13.000 rpm for 5 min at 4°C and resuspended in 100 µl of PrepMan Ultra reagent (Applied Biosystems, Foster City CA, USA) placed in a 1.5 mL micro centrifuge tube. The samples were heated in boiling water for 10 minutes, allowed to cool to room temperature and centrifuged at 13.000 rpm for 2 min. The supernatant (containing the DNA) was transferred to a clean 1.5 mL micro centrifuge tube. DNA was quantified by using the UV Biophotometer instrument (Eppendorf, Germany) and diluted to a final concentration of 100 ng/µL.

For loin slices, 10 g of sample was diluted in 90 ml of Maximum Recovery Diluent (MRD) in a stomacher bag and homogenized in a stomacher machine (AES Chemunex, France) for 1 min. The debris was left to deposit for about 5 min. One mL of homogenate was transferred to a 1.5 ml sterile tube

Table 1. List of primers used in experiment
Tabela 1. Spisak prajmera koji su korišćeni u ogledu

Primer name/ Ime prajmera	**Sequence (*5′–3′*)**	**Amplified product size/ Veličina amplifikovanog proizvoda**
rpoB-F	GGTAGTGAATTTCGTCAGTTACA	130 bp
rpoB-R	GTATGTCCAATCGAAACCCCT	

and centrifuged at 13.000 rpm for 5 min. The extraction was further carried as described above.

The oligonucleotides used as PCR primers are shown in Table 1. These amplified a region of *rpoB* housekeeping gene encoding RNA polymerase β subunit.

Amplification conditions

Real Time PCR amplification was performed using a Brilliance III SYBR Green Real Time PCR kit (Agilent, USA) in a total volume of 20 µL containing 10 µL of 2× reaction buffer, 1 µL of each primers, 1 µL of template DNA and 7 µL of PCR water to make up the final volume. Amplification was performed using an Agilent MX3005P thermo cycler (Agilent Technologies, USA). Thermal cycling conditions was as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 10 s and 60°C for 20 s.

Enumeration

An overnight culture of *E. coli* O157:H7 used to contaminate the sliced pork loin was enumerated on ChromID O157:H7 agar to determine the exact count of colony forming unit (CFU) inoculated in the samples. The signals obtained (threshold cycle, Ct) for the serial dilutions of *E. coli* O157:H7 in MRD and in pork loin were plotted against the $\log_{10}$ CFU/mL or CFU/g to construct the calibration curves. Determination coefficients (R^2) and amplification efficiency (AE) were calculated as described previously by *Higuchi et al.* (1993). Due to plating of 0.1 mL volume of each dilution the samples inoculated by <10 CFU/mL were not detectable.

Results and discussion

When cells were diluted in MRD (Figure 1.), the linearity range was from 8.78 $\log_{10}$ CFU/mL to 1.78 $\log_{10}$ CFU/mL, covering 7 orders of magnitude. The efficiency was 99.25% and the correlation coefficient (R^2) was 0.959.

Due to design of experiment, results of 5 levels of inoculation of sliced pork loins with serially diluted *E. coli* O157:H7 cells are displayed (Figure 2). The efficiency in this case was 101.35% and the correlation coefficient (R^2) was 0.972.

Results showed that it was possible to quantify count of *E. coli* O157:H7 in MRD using standard curve down to the level of less than 100 CFU/mL, more precisely at about 60 CFU/mL. At lower dilution levels (from 1 to 10 CFU/mL) Ct signals couldn't be detected. Moreover, even at the 10-fold higher level, discrepancies of the Ct signals were noticed (data not shown) which could be attributed to the very small amount of initial DNA, non-homogenous distribution of bacteria in samples, or variable amplification efficiency during the first several cycles. Regarding quantification of *E. coli* O157:H7 in sliced smoked pork loin, we determined that

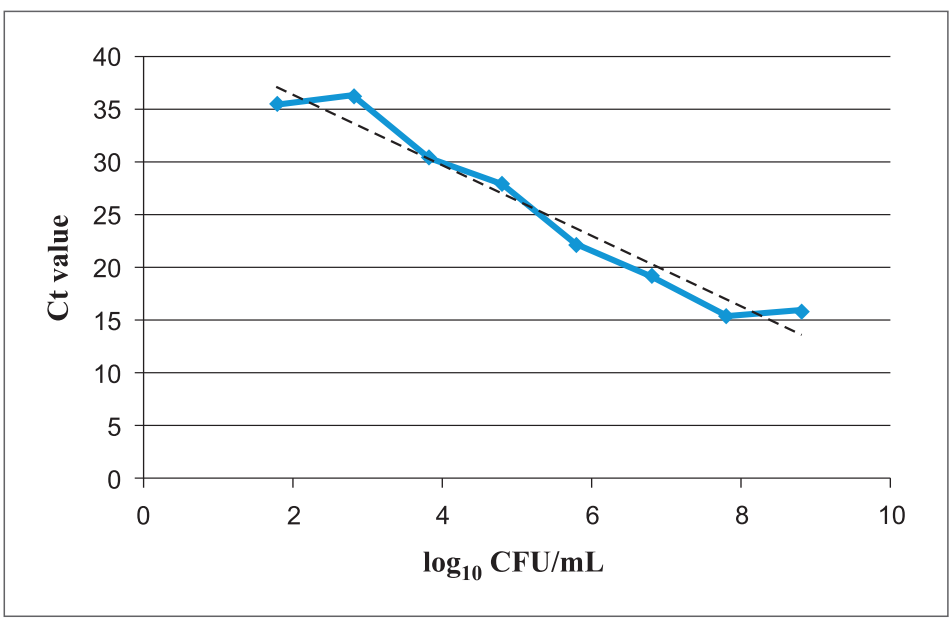

Figure 1. DNA standard curve of E. coli O157:H7; decimal dilutions in MRD
Slika 1. Standardna prava DNK E. coli O157:H7; decimalna razblaženja u MRD

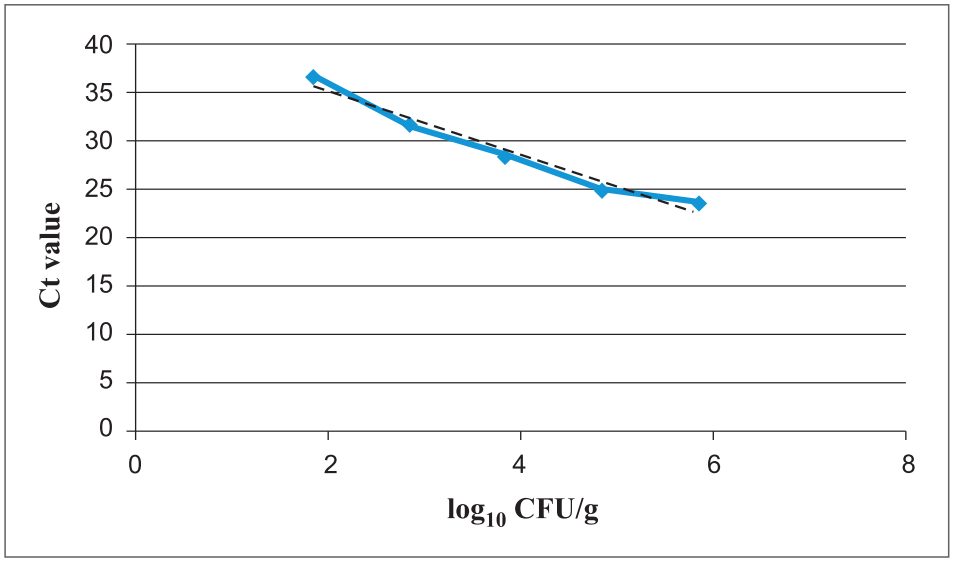

Figure 2. DNA standard curve of E. coli O157:H7 decimal dilutions in slices of smoked pork loin
Slika 2. Standardna prava DNK E. coli O157:H7; decimalna razblaženja u uzorcima svinjske dimljene pečenice

absolute limit of quantification using standard curve was at approximately 70 CFU/g. However, this data should be taken with great care since choice of kit for DNA, possible carryover of impurities or inhibitors, presence of aggregated cells etc., can have a significant effect on quantification results and could lead to misinterpretation.

In our experience which derived from repeated experiments of this type, safety margin (95% CI) in terms of LOQ of *E. coli* O157:H7 in this type of meat product should be in range 100-300 CFU/g.

For the DNA standard curves, the efficiencies were different based on the matrix used, however the R^2 value was always acceptable (≥ 0.930).

Regarding alternative protocols of quantification of food-borne pathogens, *Fukushima et al.* (2007) proposed a buoyant density gradient centrifugation as concentration method for 12 food-borne pathogens. The detection limit of the protocol varied from 10–10^3 CFU/g, presenting favorable applicability for *Salmonella* spp. and *C. jejuni,* for which the detection of 10–10^2 CFU/g in naturally contaminated chicken was obtained in 3 hours.

However, the results in terms of quantification limit obtained here are in agreement with the reports of other authors who developed qPCR protocols to quantify *L. monocytogenes* in meat (*Rodriguez-Lázaro et al*., 2004) and in salmon products (*Rodriguez-Lázaro et al*., 2005).

Conclusion

In this study, we evaluated efficiency of Real-Time PCR quantitative methods against the Plate Count technique for quantification of decimal dilution series of STEC in deli meat matrix. The Real-Time PCR methods showed similar accuracy for quantitative detection of examined samples, but the sensitivity of Plate Count Technique was 1–2 logs lower than the investigated molecular assays. According to the obtained results and with respect to the advantages of the molecular systems, this assay could be considered as a potential alternative to traditional cultural methods used for quantification of food borne pathogens in different foods and culture media matrixes. Care should be taken when calculating counts in the "risky" range of values obtained by using the standard curve, i.e. 100–1000 CFU/g or mL and further studies should be carried out in order to optimize protocol and performance characteristics.

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Ispitivanje Shiga-like toksigenih *Escherichia coli* u proizvodima od mesa pomoću kvantitativnog PCR-a

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R e z i m e : Cilj ovog rada bio je da se ispita, uporedi i oceni efikasnost kvantitativnog PCR-a u kvantifikaciji Shiga-like toksigenih E. coli (STEC) u eksperimentalno kontaminiranim narescima proizvoda od mesa u odnosu na klasičnu tehniku brojanja kolonija. Ogled je osmišljen tako da se primarno ispitaju mogućnosti kvantitativnog PCR-a korišćenjem izabranog para prajmera koji amplifikuje deo rpoB „household" gena u ekstraktima DNK iz serijskih decimalnih razređenja prekonoćne suspenzije kulture E. coli O157:H7. Uzorci svinjske dimljene pečenice kontaminirani su odgovarajućim serijskim razređenjima STEC. Nakon što je ogled ponovljen tri puta, napravljena je standardna prava odnosa srednje Ct vrednosti amplifikacionog signala za gen rpoB i odgovarajućeg razređenja inokuluma. Dobijeni rezultati jasno ukazuju da je limit kvantifikacije STEC u veštački inokulisanoj pečenici koji se može detektovati kvantitativnim PCR-om 68 CFU/g. Neophodna je dalja optimizacija ove metode kako bi se uklonila neslaganja koja se često pojavljuju kod nivoa kontaminacije manjih od 100 CFU/g.

Ključne reči: *qPCR, E. coli O157, kvantifikacija.*

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Pregledni rad

Use of essential oils in order to prevent foodborne illnesses caused by pathogens in meat

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A b s t r a c t: Although food industry has improved production techniques and slaughter hygiene, pathogens found in meat, such as Salmonella spp., Campylobacter spp. and E. coli still cause a number of foodborne illness outbreaks yearly all over the world. The overuse of antibiotics and disinfectants in both veterinary and human medicine practice has led to phenomenon of multi-drug-resistance of bacteria, which highlights the research needs on new antimicrobial agents. One of the alternatives is use of essential oils, which are aromatic oily liquids obtained from plant material by different methods. It has been proved that essential oils exhibit variable antibacterial activity, depending on the type of bacteria as well as on the chemical composition of essential oil being used. Essential oils (Eos) have antioxidant role, and inhibitory role not only to pathogens, but also to the spoilage microorganisms, which subsequently affects quality and extends meat shelf-life in order to produce safer and healthier product.

Key words: *plant essential oils, antibacterial properties, food borne pathogens, meat.*

Introduction

In the recent years, food safety issues have become one of the main public health concerns. In 2005, WHO reported 1.8 million of death caused by diarrheal diseases, mostly associated with contaminated food and drinking water (*Newell et al.*, 2010; *Sofos*, 2008). Before 1970's *Salmonella* spp*., Shigella* spp*., Clostridium botulinum, Staphylococcus aureus, Bacillus cereus* were recognized as the major causes of gastrointestinal disease, and during the 1980's and 1990's *Campylobacter* spp*., Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli O157:H7, Vibrio cholerae non O1, Vibrio vulnificus, Norovirus, Cryptosporidium parvum, Cyclospora cayetanensis, Enterobacter sakazakii* and prions were added on the list of food pathogens, but it is alarming that in about 50% of cases causative agents still remain unknown (*Sofos*, 2008; *Newell et al*., 2010; *Linscott*, 2011). *Salmonella* spp*., Campylobacter* spp., enterohaemorrhagic *E. coli,* including serotype O157:H7, present microbial pathogens of current concern in food, especially in meat, which presents valuable source of proteins, fat, Fe ion and B_{12} vitamin, and has the main role in human diet, while *Listeria monocytogenes* can be found in ready-to-eat meat and poultry products (*Bacon and Sofos*, 2003; *Sofos*, 2008; *Baltić et al*., 2010; *Linscott*, 2011; *Velebit et al*., 2012, *de Castro Cardoso and dos Reis Baltazar Vicente*, 2013). According to farm-to-fork approach in food production, monitoring of foodborne illnesses and pathogens as well as structured approaches to food safety, such as HACCP principles, have been implemented in the food chain (*Newell et al*., 2010). Despite efforts and improvements in slaughter hygiene and food production techniques in food industry, foodborne pathogens found in meat still cause a number of foodborne illness outbreaks yearly all over the world (*Burt*, 2004; *Sofos,* 2008; *Newell et al.,* 2010; *Linscott*, 2011). The overuse of antibiotics in order to reduce pathogens in human medicine, as well as in veterinary practice, has led to phenomenon of multi-drug-resistant bacteria (*Doyle and Erickson*, 2006; *Sofos* 2008; *Tohidpour et al*., 2010). Intestinal infectious diseases and bacterial resistance are not the only problem associated with meat safety. Salt is most common used additive which has been used since

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ancient times for the preservation of meat products and plays great role in sensory and textural properties of meat and meat products. In spite of advantages, use of salt has shortcomings because it is linked to hypertension and consequently increased risk of cardiovascular disease, which is why reducing salt intake presents a new trend, but also a challenge for meat industry (*Desmond*, 2006; *Sofos* 2008; *Weiss et al*., 2010). Reducing NaCl levels below those typically used, without any other preservative measure, reduces product shelf life and allows spoilage flora to grow and render product unsafe for consumption (*Desmond*, 2006; *Weiss et al*., 2010). All of these have led to need for new methods of eliminating or reducing foodborne pathogens and spoilage microorganisms, possibly in combination with the existing and already used methods (*Burt*, 2004). One such possibility is the use of essential oils (EOs), which present a better choice than some synthetic chemical additives, especially for ”organic” and ”natural” food production, which has become popular mostly in the Western society, and is widely accepted by consumers (*Burt*, 2004; *Sofos* 2008; *Gutierrez*, 2009; *Velebit et al*., 2012). Essential oils are aromatic oily liquids obtained from plant material by different methods (*Burt*, 2004). EOs are also known as volatile or ethereal oils, and they have been used since ancient times for their perfume, flavor and preservative properties (*Bauer et al*., 2001; *Burt*, 2004). In adition to antibacterial properties, EOs also possesses antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal properties. Although some of these properties have been recognized long ago, in recent years scientific interest for essential oils and their application in food is renewed (*Burt*, 2004).

Composition of EOs

Essential oils are the low molecular weight liquids, limpid, rarely coloured, volatile mixtures, which are lipid soluble and soluble in organic solvents (*Burt*, 2004; *Bakkali et al*., 2008; *Marković et al.,* 2008; *Tajkarimi et al*., 2010; *Lv et al.,* 2011; *Bajpai et al*., 2012). Essential oils play role in plant defense and some are always present, while others are produced as a response to microbial invasion or physical injury (*Hyldgaard et al*., 2012). They are synthesized by different plant organs, such as flowers, leaves, seeds, fruits, roots, buds, stems, twigs, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes from which they are obtained (*Burt*, 2004; *Bakkali et al*., 2008; *Tajkarimi et al*., 2010; *Lv et al.,* 2011; *Bajpai et al*., 2012). Several methods including steam and hydro distillation, solvent extraction, and expression are used for extracting essential oils (*Burt*, 2004; *Bakkali et al*., 2008; *Tajkarimi et al*., 2010). The most common and the simplest method for producing EOs for commercial purposes is steam distillation. More expensive method is extraction by means of liquid carbon dioxide under low temperature and high pressure produces. EOs produced in this way have more natural organoleptic characteristics, and exhibit greater antimicrobial activity (*Burt*, 2004).

Essential oils may have different properties depending on climate, soil composition, age and vegetative cycle stage, which is why they have to be extracted under the same conditions, from the same organ of the plant which has been growing on the same soil, under the same climate and has been picked in the same season (*Burt*, 2004; *Bakkali et al.,* 2008). Antimicrobial activity also varies, and it is strongest in EOs produced from herbs harvested during or immediately after flowering. Because EOs are volatile, in order to maintain their characteristics after extraction, they need to be stored in airtight containers away from light (*Burt*, 2004).

Essential oils are complex mixtures, and contain between twenty and sixty, and some of them more than sixty individual components, which may be determined by gas chromatography/mass spectrometry. The concentration of components is quite different, and major components can constitute up to 85% of the EO, while other components can be found only in traces. These major components determine the biological properties of the essential oils. However, studies conducted on sage, thyme and oregano have shown that minor components have a critical part to play in antibacterial activity, probably by producing a synergistic effect between major components of EOs. Also, it is proved that oil as a whole possess better antibacterial activity than only a combination of major volatiles of the oil (*Burt*, 2004; *Bakkali et al*., 2008).

The components of essential oils usually are divided into two groups, the main group which is composed of terpenes and terpenoids, and the other composed of aromatic and aliphatic constituents.

Terpenes are made from combinations of several isoprene (5-carbon-base, C5) units. The monoterpenes (C10) and sesquiterpenes (C15) present main classes of terpenes. Monoterpenes constitute 90% of the essential oils and work as carbure, alcohol, aldehyde, ketone, ester, ether, peroxide and phenols. The sesquiterpene compounds contain three isoprene units and the functional properties are very close to monoterpene compounds. Hemiterpenes, diterpenes, triterpenes and tetraterpenes terpenoid (terpene

which contain oxygen) also exist, but in lower concentrations then monoterpenes and sesquiterpenes.

The aromatic compounds are the derivatives of phenylpropane and they consist of aldehydes, alcohols, phenols, methoxy and methylenedioxy in nature. A few nitrogen and sulfur compounds present in EOs are also characterized as plant essential constituents (*Bakkali et al.,* 2008; *Bajpai et al.,* 2012).

Despite a widespread opinion that the phenolic components are responsible for the antibacterial properties of EOs, recent studies showed that non-phenolic compounds of oils extracted from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, purple and bronze muscadine seeds and sage also exhibit antibacterial activity against Gram-positive, as well as against Gram-negative bacteria (*Tajkarimi et al.,* 2010).

Antibacterial and antioxidant properties and mechanism of action of EOs

Before they are added to the meat, antibacterial activity should be tested *in vitro* conditions. The minimum inhibitory concentration (MIC), which is defined by most authors as a measure of the antibacterial performance of EOs, should be determined at first (*Burt* 2004; *Lv et al.*, 2011). Minimum inhibitory concentration of EOs can be detected by diffusion, dilution or bioautographic methods, of which, diffusion method is mostly used in experiments for screening for antibacterial activity, while agar or broth dilution methods are used to determinate strength of antibacterial properties. Scanning electron microscopy is method of choice for observation of physical effects of antibacterial activity (*Burt*, 2004). Although tests for determinations of MIC are not standardized, the NCCLS (*National Committee on Clinical Laboratory Standards, actually CLSI – Clinical and Laboratory Standards)* method for antibacterial susceptibility testing, which is mainly used for the testing of antibiotics, has been modified for testing EOs (*NCCLS*, 2000; *Burt*, 2004). Even so, comparison of published data is complicated because outcome of a test is affected by different factors, such as the method used to extract the EOs from plant material, the volume of inoculum, growth phase, culture medium used, pH of the media incubation time, temperature of incubation and many others, which is why it is preferable for researches to determine MIC by themselves before conducting the experiment (*Burt*, 2004).

Also, it is important to be familiar with the mode of action of EOs, in order to choose the proper one, depending on what the active component of EO is and which bacteria are tested to be inhibited. Since essential oils consist of large number of components, their antibacterial activity is not based on one specific mechanism, but there are several targets in the cell. Interaction and damage of bacterial cell membrane is considered to be the main mode of antibacterial action of EOs. Hydrophobic nature of EOs makes them to interact well with lipid membrane of bacterial cell membrane and mitochondria and cause permeabilization of the membranes. Changes in membrane permeability occur as a result of loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool, which eventually lead to leaking of intracellular constituents, coagulation of cell contents, lysis and cell death. The chemical structure of the individual EO components affects their precise mode of action and antibacterial activity. Generally, the EOs possess the strong antibacterial properties against food borne bacteria because phenolic compounds containing hydroxyl group such as carvacrol, eugenol and thymol, which are responsible for disrupting the cell membrane and inhibiting the functional properties of the cell. It appears that the type of alkyl group has influence on antimicrobial activity of non-phenolic components of EOs (*Burt*, 2004; *Bakkali et al*., 2008; *Lv et al*., 2011; *Bajpai et al*., 2012, *Velebit et al*., 2012). There are some indication that EOs act on the enzymes involved in the energy regulation or synthesis of structural components, which is explained by the fact that some EOs stimulate the growth of pseudomycelia (a series of cells adhering end-to-end as a result of incomplete separation of newly formed cells) in certain yeasts (*Burt*, 2004).

Antibacterial properties of EOs depend not only on EOs chemical characteristics, but also on type of bacteria. Essential oils are more effective against Gram-positive bacteria rather than Gram-negative bacteria. Gram-negative bacteria are less susceptible because their membrane contains hydrophilic lipopolysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds (*Hyldgaard et al*., 2012). Gram-negative bacteria, *Pseudomonas spp.*, in particular *P. aeruginosa*, appear to be least sensitive to the action of EOs, and exception of the rule is *Aeromonas hydrophila*, which appears to be one of the most sensitive species (*Burt*, 2004).

Essential oils have not only antibacterial properties, but their application in meat can affect some meat characteristics as well. Oxidation by free radicals is one of the primary mechanisms of quality deterioration in foods, and especially in meat products. Some secondary products of oxidation, like

short-chain aldehydes, ketones and other oxygenated compounds may adversely affect quality of meat by causing loss of color and nutritive value, limiting shelf-life and making meat potentially dangerous for consumer health (*Simitzis et al.,* 2010). Active essential oil compounds, such as phenolic diterpenes, derivates of hydroxycinnamic acid, flavonoides and triterpenes found in rosemary, oregano, borage and sage have high antioxidant activity (*Sanchez-Escalante et al*., 2003; *Oberdieck,* 2004; *Fasseas et al*., 2007; *Ryan et al.,* 2009; *Weiss et al.,* 2010). Some EOs, for example clove essential oil, have been reported to have higher antioxidant activity than some synthetic antioxidants, like BHT or butylated hydroxyanisole, which is why EOs may present natural alternatives to synthetic antioxidants, without leaving residues in the product or the environment (*Yanishlieva-Maslarova,* 2001; *Simitzis et al*., 2010; *Teixeira et al*., 2013).

Application of EOs in meat

In many studies it has been experimented with essential oils and their effects on meat pathogens as well as on spoilage flora. These studies have shown that efficiency of essential oils depends not only on type, chemical composition and concentration of essential oils, but also on meat characteristics, type of bacteria, mode of application of EOs in meat and some other physical parameters, such as pH values, water activity, oxygen tension, temperature (*Burt*, 2004; *Chouliara et al*., 2007; *Gutierrez et al*., 2008; *Simitzis et al.*, 2008; *Gutierrez et al*., 2009; *Govaris et al.,* 2010, *Emiroğlu et al.*, 2010; *Lv et al.,* 2011; *Hsouna et al.*, 2011; *Karabagias et al.*, 2011; *Bajpai et al*., 2012; *Awaisheh,* 2013; *Khanjari et al*., 2013; *Teixeira et al*., 2013).

Based on antibacterial properties of EOs and type of affected pathogen, some essential oils are better than others in meat applications. Eugenol and coriander, clove, oregano and thyme oils were found to be effective at levels of 5–20 µl/g in inhibiting *L. monocytogenes*, *A. hydrophila* and spoilage flora in meat products, whilst mustard, cilantro, mint and sage oils were less effective or ineffective (*Burt*, 2004). *L. monocytogenes* also exhibited to be sensitive on combination of EOs and nisin (*Tajkarimi et al.,* 2010). Several studies were performed in order to confirm efficacy of EOs against *Salmonella spp*. in food. Results have shown that oregano and thyme EOs, and EOs extracted from *Salvia officinalis* and *Salvia molle* inhibit the growth of *Salmonella* bacteria up to a significant reduction in the CFU levels. However, cinnamon bark EO (7000 mg/kg^{-1}) exerted the strongest antibacterial efficacy against *Salmonella* spp., while rosemary EO showed low antibacterial efficacy (*Hayouni et al*., 2008; *Bajpai et al.,* 2012). Addition of nisin at 500 or 1000 IU/g in minced sheep meat did not show any antibacterial activity against *S.* Enteritidis, but combination with oregano EO at 0.6%, showed to be efficient (*Govaris et al.,* 2010). Experiment in which oregano EOs and sodium nitrite were used against *Clostridium botulinum* spores, has shown that EOs in combination with low concentration of sodium nitrite inhibits or slows growth of bacteria more than the sodium nitrite alone, depending on the number of inoculated spores (*Burt*, 2004; *Tajkarimi et al*., 2010).

Concentration of essential oils, which should be added to meat in order to prevent oxidation, foodborne pathogens, or to extend shelf-life, is usually higher than one used in *in vitro* conditions because of interaction with meat components (*Burt,* 2004; *Hyldgaard et al*., 2012). An exception to this phenomenon is *A. hydrophila* where no higher concentration of EO was needed in experiments to inhibit these bacteria on cooked pork in comparison to tests *in vitro* (*Burt*, 2004).

The high levels of fat or protein in meat and in food generally, appear to reduce the effectiveness of antibacterial EOs. If the EO dissolves in the lipid phase of the food it will be relatively less available to act on bacteria present in the aqueous phase, while the other suggestion is that the lower water content of meat compared to laboratory media may slow down the progress of antibacterial agents to the target site in the bacterial cell. For example, mint and cilantro EOs were not effective in products with a high level of fat, such as pâté and a coating for ham containing canola oil (*Burt*, 2004). However, some studies on beef extract culture medium have shown that efficacy of oregano and thyme oil was greater at higher concentrations of protein, which may have displayed hydrophobic properties with consequent interactions with EOs to facilitate their dissolution in the medium (*Gutierrez et al*., 2008). Also it has been reported that proteins usually possess a high binding capacity for flavor volatile compounds. General opinion is that carbohydrates do not protect bacteria from the action of EOs as much as fat and protein do and some other components of meat, such as water and salt in higher level assist the action of EOs (*Burt*, 2004).

Essential oils can be applied directly in meat or PEO (polyethylene oxide)-based antimicrobial packaging can be used (*Bajpai et al*., 2012; *Hyldgaard et al.,* 2012; *Velebit and Petrović*, 2012). This is one of the many antimicrobial packaging technologies which improve the quality of the

meat products, mainly by reducing spoilage flora and extending shelf life, but also provide information about food quality during storage (*Lončina et al.,* 2013). Essential oils can be encapsulated in polymers of edible and biodegradable coatings or sachets that provide a slow release to the food surface or to the headspace of packages of meat (*Hyldgaard et al.,* 2012). Depending on the concentration, after application in meat, some essential oils may alter the qualitative properties of the product. A way to minimize negative organoleptic effects of essential oils added to the matrix of a meat is to encapsulate essential oils into nanoemulsions. Nanoencapsulation of bioactive compounds represents a viable and efficient approach to increasing the physical stability of the active substances, protecting them from the interactions with the food ingredients and, because of the subcellular size, increasing their bioactivity (*Donsí et al*., 2011; *Hyldgaard et al.,* 2012).

Safety aspect of the use of EOs

Although essential oils possess antibacterial properties and may improve taste and some other characteristics of the meat, they should be used with care, because EOs may cause some side effects (*Burt*, 2004; *Bakkali et al.,* 2008). Some essential oils, such as menthol, eugenol and thymol, depending on concentration, may cause irritation of mucous membranes, probably as a result of membrane lysis and surface activity, while cinnamaldehyde, carvacrol, carvone and thymol *in vitro* appear to have mild to moderate toxic effects at the cellular level (*Burt*, 2004). Some essential oils contain components which cause allergic contact dermatitis in people who use them frequently and the other essential oils contain photoactive molecules like furocoumarins, which cause phototoxic reactions (*Burt*, 2004; *Bakkali et al*., 2008). Several EOs which have been used in phytomedicine and aromatherapy have exhibited spasmolytic or spasmogenic properties, but these effects were not associated with a particular component of EOs (*Burt*, 2004). EOs mostly have no carcinogenic properties, but some of them may be considered as secondary carcinogens after metabolic activation (*Guba*, 2001; *Bakkali et al.,* 2008). For example, some EOs provoke estrogen secretions which can induce estrogen-dependent cancers, while some photosensitizing molecules found in EOs, such as flavins, cyanin, porphyrins can cause cancer (*Bakkali et al*., 2008). However, many studies showed that essential oils have anti-tumoral potentials (*Ferraz et al.,* 2013; *Bostancıoğlu et al.*, 2011; *Sharma et al*., 2009). Because of genotoxicity and other potential sideeffects, use of essential oils as flavorings and their maximum allowed concentration in food products have been controlled by regulations and laws. A number of EO components such as carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol have been registered by the European Commission and considered to present no risk to the health of the consumer. United States Food and Drug Administration (FDA) has classified the substances as generally recognized as safe (GRAS) or as approved food additives, and it is mostly based on the EU registered flavorings list with some modifications (for example, estragole, specifically prohibited as flavoring in the EU, is on the EAFUS list), (*Burt,* 2004; *Bajpai et al.,* 2012).

New flavorings might be considered for registration only after toxicological and metabolic studies proving later not to be dangerous for human health (Commission Decision of 23 February, 1999; Commission Regulation (EC) No 1565/2000 of 18 July, 1565/ 2000; Commission Regulation (EC) No. 622/2002 and Regulation (EC) No 2232/96; *Burt,* 2004).

Conclusion

As the food industry is facing great challenges to produce safe, and at the same time food without synthetic chemical preservatives, essential oils make their way into the scientific focus. Due to their antibacterial, antifungal and antiviral activity, as well as antioxidant properties, they are used to prevent foodborne diseases, to extend shelf-life, and to improve some meat characteristics. Their efficiency against pathogens and spoilage flora depends on many different factors, and their implementation in practice faces some obstacles. Essential oils are recognized to be used, not only as food additives, but also in aromatherapy, antitumor therapy, as potential antimicrobial agents against multi-resistant bacteria, and in other purposes in medical and nonmedical fields. Yet, benefits of their use remain to be confirmed.

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Upotreba eteričnih ulja u cilju prevencije bolesti prenosivih hranom uzrokovane patogenima iz mesa

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R e z i m e: Iako je industrija hrane unapredila način i tehnologiju proizvodnje, kao i higijenu klanja, patogeni mikroorganizmi prenosivi hranom koji se mogu naći u mesu, kao što su Salmonella spp., Campylobacter spp. i E. coli, odgovorni su za milione oboljenja svake godine. Prevelika upotreba antibiotika i dezinficijenasa, kako u veterinarskoj, tako i u humanoj medicini rezultiralo je fenomenom bakterijske rezistencije, zbog čega se javila potreba za novim antimikrobnim sredstvima. Jedna od mogućih alternativa je upotreba eteričnih ulja koja predstavljaju aromatične tečnosti uljane konzistencije, koje se različitim metodama, ekstrahuju iz skoro svih delova biljaka. Dokazano je da eterična ulja, u različitom stepenu, imaju antibakterijsku aktivnost koja zavisi od vrste bakterije kao i vrste i hemijskog sastava ulja koje se koristi. Eterična ulja imaju i antioksidativnu ulogu i inhibiraju rast patogenih, ali i mikroorganizama kvara, utičući na taj način, na kvalitet i održivost mesa u cilju stvaranja bezbednijeg proizvoda.

***Ključne reči**: biljna eterična ulja, antibakterijska svojstva, patogeni prenosivi hranom, meso.*

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Uporedni prikaz rezultata senzorskih i hemijskih i fizičko-hemijskih ispitivanja svežeg ohlađenog junećeg mesa upakovanog u vakuum tokom čuvanja u maloprodajnim uslovima

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S a d r ž a j: Pakovanjem svežeg mesa u vakuumu može se znatno produžiti njegova održivost, a samim tim uticati na usporavanje hemijskih promena i očuvanje poželjnih senzorskih svojstava. Cilj ovog rada bio je uporedno ispitivanje senzorskih svojstava i hemijskih parametara koji se odnose na oksidativne i hidrolitičke promene u uzorcima sveže juneće ruže (m. quadriceps femoris) upakovane u vakuum, kao i ispitivanje međusobne zavisnosti utvrđenih hidrolitičkih i oksidativnih promena i senzorskih svojstava. Za eksperiment je korišćena juneća ruža koja je poticala od tri juneta rase simentalac, prosečne telesne mase 400 kg, koja su zaklana u industrijskoj klanici. Rasecanje mesa i pakovanje uzoraka u vakuum obavljeno je u roku od 40 sati nakon klanja. Uzorci su čuvani u strogo kontrolisanim uslovima, pri temperature 0–2°C (rashladna vitrina, maloprodajni objekat) i 0–4°C (skladište). Dinamika senzorskih i hemijskih ispitivanja je bila: 1. dana (nakon pakovanja),7, 15, 21. i 28. dana u tri odvojena ciklusa. Senzorska svojstva ispitana su pomoću kvantitativno-deskriptivnog testa, na skali intenziteta od 1–5, a ispitane su sledeće osobine: izgled mesa, boja mesa po površini, boja mesa na preseku, struktura, tekstura, miris svežeg mesa, miris posle probe kuvanja, ukus posle probe kuvanja i ukus posle probe pečenja. Hemijsko ispitivanje parametara koji ukazuju na oksidativne i hidrolitičke promene obuhvatalo je kiselinski broj, peroksidni broj, TBK broj (test sa tiobarbiturnom kiselinom kojom se određuje sadržaj MAL–malondialdehida), TVB-N (total volatile basic nitrogen), a_w vrednost i pH. Upakovana juneća ruža u vakuumu je, sa aspekta senzorskih svojstava i utvrđenih hemijskih promena pri datim uslovima skladištenja, bila prihvatljiva u sva tri ciklusa, zaključno sa 21. danom ispitivanja. U prva dva ciklusa ispitivanja, u većini slučajeva, ustanovljena je umerena do jaka negativna korelacija između ispitanih hemijskih parametara i senzorskih svojstava junećeg mesa, dok su u trećem ciklusu uočene slabe negativne korelacije.

***Ključne reči**: sveže juneće meso, vakuum pakovanje, senzorska svojstva, hemijske promene.*

Uvod

Pravilna, kvalitetna i zdravstveno bezbedna ishrana je neophodan preduslov za očuvanje i unapređenje zdravlja stanovništva, kao i za prevenciju mnogih bolesti. Kao dragocen izvor proteina visoke biološke vrednosti, gvožđa, vitamina B_{12} i drugih vitamina B kompleksa, cinka, selena i fosfora, meso je nezamenljiva i najkvalitetnija komponenta zdrave i dobro izbalansirane ishrane (*Biesalski*, 2005).

Pored nutritivne vrednosti, važan aspekt kvaliteta mesa čine i njegova senzorska svojstva (boja, miris, ukus, mekoća, sočnost, konzistencija), koja su, često, odlučujuća za prihvatljivost ove namirnice od strane potrošača (*Parunović i dr.,* 2001). Boja mesa je prvi atribut kvaliteta sa kojim se potrošač sreće i koji za njega predstavlja pokazatelj svežine (*Troy i Kerry*, 2010). U svesti prosečnog potrošača, boja je sinonim za kvalitet svežeg crvenog mesa (*Renerre i Labas*, 1987). Studija kojom su bili obuhvaćeni potrošači u Evropi (*Glitsch,* 2000) je pokazala da je pri kupovini goveđeg mesa boja bila dominantno odlučujuća. U skladu sa ovom studijom su i rezultati odnosa potrošača u Srbiji prema atributima

Napomena: Rezultati su proistekli iz rada na realizaciji Projekta ev. br. III 46009, koji, u okviru Programa istraživanja u oblasti tehnološkog razvoja (2011–2014. godine), finansira Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije.

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kvaliteta goveđeg mesa, u kojoj je oko 43% učesnika ispitivanja navelo boju, kao najvažniji faktor pri kupovini mesa, a ružičastocrvenu boju je ocenilo najpoželjnijom (*Ostojić i dr*., 2006). Brojna ispitivanja percepcije kvaliteta mesa od strane potrošača su pokazala da su tri dominanatna senzorska svojstva kojima potrošači ocenjuju kvalitet mesa pri konzumiranju mekoća, ukus i sočnost, pri čemu se mekoća smatra najvažnijim svojstvom.

Meso je, zbog svog hemijskog sastava, veoma podložno različitim degradativnim procesima (mikrobiološkim i fizičko-hemijskim), koji mogu dovesti i do narušavanja njegove hranljive vrednosti (*Cannarsi i dr.*, 2005). Brojni međusobno povezani faktori utiču na očuvanje svežine i kvaliteta mesa, kao što su temperatura, izloženost svetlu, koncentracija kiseonika, masnokiselinski sastav, odnosno udeo nezasićenih masnih kiselina, endogeni enzimi (*Skibsted i dr*., 1998), a naročito aktivnost mikroorganizama, jer meso prestavlja izuzetan supstrat za rast mikroorganizama kvara i potencijalnih patogena (*Kotula i Kotula*, 2000). Takođe, na dužinu održivosti, odnosno kvar mesa, utiče i fiziološki status životinje pre klanja, kontaminacija za vreme klanja i rasecanja trupova, pH vrednost mesa, način i vrsta pakovanja (*Nychas i dr*., 2008). Svi ovi faktori, samostalno ili u kombinaciji, mogu da dovedu do nepoželjnih promena boje, mirisa, teksture i ukusa mesa (*Zhou i dr.,* 2010).

Oksidacija lipida utiče na pojavu neprijatnog ukusa i mirisa mesa, a započinje na ćelijskim membranama mišićnog tkiva, u delu fosfolipida. Cepanje hidrofobnih veza između masnih kiselina unutar ćelijskih membrana omogućava hidrolizu lipida, oksidaciju slobodnih masnih kiselina i nastanak hidroperoksida (*Enser,* 2001; *Simitzis i Deligeorgis*, 2010). Razgradnja fosfolipida dovodi do nakupljanja sekundarnih produkata, kao što su pentanal, hexanal, 4-hidroksinonenal i malondialdehid (MAL), kao i aldehidi i ketoni (*Fernindez i dr*., 1997). Ovi sekundarni proizvodi mogu dovesti do gubitka boje i nutritivne vrednosti mesa usled reakcija sa mastima, proteinima, ugljenim hidratima i vitaminima (*Bastić i dr.*, 1997) i zajedno sa heterocikličnim aromatičnim aminima direktno su povezani sa nastajanjem kancerogenih, mutagenih i aterogenih procesa (*Skog*, 1993; *Liu i dr*., 1995). Prema *Gray-u* (1978), istovremenim praćenjem oksidativnih promena lipida mesa i senzorskim registrovanjem užeglosti, ustanovljeno je da peroksidi ne prouzrokuju neželjene promene, već da užegao miris i ukus mesa uslovljavaju proizvodi njihove razgradnje, odnosno gore pomenuti sekundarni proizvodi degradacije.

Temperatura je jedan od osnovnih činilaca koji određuje brzinu enzimskih, hemijskih i fizičkih promena u mesu. Hlađenjem svežeg mesa postiže se usporavanje procesa nastanka kvara, s obzirom da snižavanje temperature inhibira rast mikroorganizama (*Cassens,* 1994), a reaktivni molekuli, koji nastaju tokom lipidne oksidacije, su rastvoreni u lipidnoj frakciji i stabilni su na niskim temperaturama (*Zarzycky i Swiniarska*, 1993). Međutim, u uslovima hlađenja, ne inhibira se rast psihrofilnih bakterija, kvasaca i plesni (*Neumeyer i dr*., 1997), tako da se i enzimski i neenzimski procesi nastavljaju, ali mnogo sporije (*Berkel i dr*, 2004). Nastanak isparljivih i neisparljivih amina, TVB-N (ukupno isparljiv azot – total volatile basic nitrogen) i TMA-N (trimetilamin – trimethylamine nitrogen) je, prvenstveno, posledica enzimske dekarboksilacije specifičnih amino-kiselina, odnosno mikrobiološke aktivnosti (*Ruiz-Capillas i Jimenez-Colmenero*, 2004).

Savremeni trendovi u prehrambenoj industriji prate zahteve potrošača za svežim, prirodno očuvanim i kvalitetnim proizvodima koji su što je moguće manje hemijski tretirani. Značajnu ulogu u savremenoj proizvodnji namirnica zauzima primena novih ambalažnih materijala i savremenih uslova i postupaka pakovanja (*Tsigarida i dr.*, 2000). Postoji više faktora koji stimulišu stalno unapređenje načina pakovanja mesa i koji pomažu industriji mesa da zadovolji zahteve i prodavca i potrošača. Među ovim faktorima iz godine u godinu posebno se izdvajaju težnja za smanjenjem radne snage u maloprodaji, sve manje vremena kojim raspolaže današnji potrošač i njegova potreba za svežim, visokokvalitetnim mesom, kao i najveći prioritet industrije mesa da svojim potrošačima isporuči bezbednu hranu (*Belcher,* 2006).

Pakovanje svežeg mesa se sprovodi da bi se sprečila mikrobiološka kontaminacija, odložio kvar, omogućile enzimske aktivnosti u cilju poboljšanja mekoće mesa, smanjio kalo i obezbedila poželjna boja mesa u maloprodaji (*Brody*, 1997). Najrasprostranjeniji načini pakovanja svežeg mesa i proizvoda od mesa na našem tržištu su vakuum pakovanje i pakovanje u modifikovanoj atmosferi (MAP). Vakuum pakovanje podrazumeva uklanjanje vazduha, odnosno kiseonika iz pakovanja, pre njegovog zatvaranja, što se odražava na mikrobiološki profil upakovanog mesa. Ovaj način pakovanja utiče povoljno na senzorska svojstva mesa: sočnost, mekoću, ukus, kao i na zrenje mesa. Za crvena mesa namenjena maloprodaji, vakuum pakovanje se smatra nepodobnim, jer u atmosferi sa smanjenim sadržajem kiseonika nastaje deoksimioglobin, usled čega meso dobija tamnu crvenoljubičastu boju i postaje neprihvatljivo za potrošača (*Gill*, 1996). Uspešnost pakovanja u vakuumu zavisi od početnog mikrobiološkog i tehnološkog kvaliteta proizvoda, kao i

od primene adekvatne temperature skladištenja. Takođe, ambalažni materijal treba da ima dobre fizičko-mehaničke i barijerne karakteristike uz pravilno, hermetično formiranje i zatvaranje ambalaže. Da bi se izbeglo zaostajanje vazduha u pakovanju preporučuje se upotreba termoskupljajućih barijernih folija (*Šakota i dr*., 2002; *Robertson*, 2006).

Cilj ovog rada bio je uporedno ispitivanje senzorskih svojstava i hemijskih parametara koji se odnose na hidrolitičke i oksidativne promene u uzorcima sveže juneće ruže (*m. quadriceps femoris*) upakovane u vakuumu i čuvane u maloprodajnom objektu, pri temperatuti od 0–2°C. Pored toga, cilj je bio da se ispita i međusobna zavisnost utvrđenih hidrolitičkih i oksidativnih promena i senzorskih svojstava.

Materijal i metode

Uzorci svežeg junećeg mesa – ruže (*m. quadriceps femoris*), za potrebe eksperimenta, proizvedeni su u industrijskom objektu za preradu mesa. Juneća ruža je poticala od tri juneta simentalske rase, prosečne mase 400 kg, koja su zaklana u industrijskoj klanici. Rasecanje mesa i pakovanje uzoraka za ispitivanje je obavljeno u roku od 40 sati nakon klanja životinja. Odresci su bili ujednačene debljine 2–3 cm, neto mase između 400–500 g. Uzorci sveže juneće ruže upakovani su pomoću uređaja za vakuumiranje – Webomatic, sa ručnim preklapanjem komore za evakuaciju vazduha. Za pakovanje su korišćene kese S TIP HB-X (Spektar – Gornji Milanovac), od biaksijalno orijentisanog višeslojnog filma (7 slojeva), dimenzija 200×300 mm i debljine 100 mikrona WVTR (Water Vapour Transmission Rate – 6 jedinica, određena prema ASTM E96-00; Oxygen Transmision Rate – 8 jedinica, određena prema ASTM D-3985-95). Temperatura kupatila za potapanje bila je 88°C, a vreme potapanja 2 sekunde. Upakovani uzorci su istog dana, popodne, transportovani vozilom sa termokingom do centralnog magacina, u plastičnim kasetama, a narednog dana ujutru, u hladnom lancu, do maloprodajnog objekta. U maloprodajnom objektu su čuvani u rashladnim vitrinama, sa veštačkim osvetljenjem, pri temperaturi 0–2°C, a po završetku radnog vremena u skladištu, pri temperaturi 0–4°C. Uzorci su u hladnom lancu dostavljani u laboratoriju, gde su obavljena senzorska, hemijska i fizičko-hemijska ispitivanja 1, 8, 15, 21. i 28. dana. U planiranim terminima, po danima ispitivanja gledano od trenutka rasecanja, odnosno pakovanja u industrijskom pogonu, ispitano je po 6 uzoraka upakovane juneće ruže. Navedena uporedna ispitivanja obavljena su u okviru tri testa održivosti, odnosno tri vremenski odvojena ciklusa.

Senzorska ispitivanja rađena su pomoću kvantitativno deskriptivnog testa (SRPS ISO 6658, 2001), na numeričko-deskriptivnoj skali, sa ocenama od 1 do 5, pri čemu je 1 – neprihvatljivo; 2 – na granici prihvatljivosti; 3 – prihvatljivo; 4 – veoma prihvatljivo; 5 – izuzetno prihvatljivo. Ocenjena su sledeća senzorska svojstva: izgled mesa, boja mesa po površini, boja mesa na preseku, struktura, tekstura, miris svežeg mesa, miris posle probe kuvanja, ukus posle probe kuvanja i ukus posle probe pečenja. Grupa od šest ocenjivača činila je panel za ocenu senzorskih svojstava ispitanih uzoraka junećeg mesa upakovanog u vakuumu. Ocenjivačima su prethodno testirana čula pomoću testa za utvrđivanje osećaja ukusa (SRPS ISO 3972, 2001) i testa za obuku ocenjivača u otkrivanju i prepoznavanju mirisa (SRPS ISO 5496, 2001).

Tokom skladištenja u uslovima maloprodaje, u upakovanom junećem mesu, ispitani su i hemijski i fizičko-hemijski parametri koji ukazuju na hidrolitičke i oksidativne promene. Kiselinski broj je određen prema metodi SRPS EN ISO 660/2011, peroksidni broj prema metodi SRPS EN ISO 3960/2011; TBK broj (test sa tiobarbiturnom kiselinom kojom se određuje sadržaj MAL – malondialdehida) prema metodi Tarladgis i dr. (1964) i Holland (1971). pH vrednost uzoraka je merena na laboratorijskom pH-metru, model Cyber Scan, pH 510 Meter (EUTECH Instruments), Holandija, u skladu sa standardnom metodom SRPS ISO 2917/2004, a a_w vrednost je određivana pomoću higrometra (a_w metar FAst/1, proizvođač GBX Scientific Instruments, Francuska) prema standardnoj metodi ISO 21807:2004(E). TVB-N je ispitan primenom metode koja je navedena u Official Journal of the European Union (2005).

Statistička analiza

Statistička obrada rezultata je obavljena korišćenjem softverskog paketa MINITAB, verzija 16.1.0.0, Minitab Inc. © USA. Za svaki ispitani parametar dobijeni podaci su prikazani kroz srednje vrednosti, standardne devijacije, koeficijente varijacija i intervale varijacija. Pre izbora odgovarajućeg statističkog testa određena je najbolja individualna distribucija serije podataka (analizirano je 16 različitih distribucija) bazirana na najnižoj vrednosti Anderson-Darling-ovog koeficijenta i najvišoj p vrednosti (iznad 0,05) u cilju konačnog izbora distribucije koja najbolje prati normalnu raspodelu. Nakon izbora najpogodnije distribucije, urađena je odgovarajuća transformacija podataka. Za određivanje statističke značajnosti razlika između srednjih

vrednosti acuu za ispitana senzorska svojstva, po ciklusima, korišćena je ANOVA sa primenom post hoc Tukey HSD testa. Značajnost korelacionih povezanosti između ispitanih senzorskih i hemijskih i fizičko-hemijskih parametara u okviru pojedinačnih ciklusa ispitivanja, određena je računanjem Pirsonovog (Pearson) korelacionog koeficijenta I. Statistička analiza je sprovedena na nivou značajnosti od 95% ($p < 0,05$).

Rezultati i diskusija

Senzorska ispitivanja

Rezultati senzorskih ispitivanja sveže juneće ruže upakovane u vakuumu prikazani su u tabelama 1 i 2.

Prvog dana, u sva tri ciklusa ispitivanja, kao i osmog dana u trećem ciklusu ispitivanja, sva senzorska svojstva upakovane juneće ruže u vakuumu ocenjena su kao „izuzetno prihvatljiva“ (5,00 ± 0,00). Po površini, kao i na presecima juneće ruže, miris je bio karakterističan za juneće meso, bez stranih primesa. Meso je bilo ružičastocrvene boje, a pripadajuće masno tkivo krembele boje. Posle probe kuvanja i probe pečenja, miris i ukus su bili prijatni, svojstveni za vrstu mesa. U prvom i u drugom ciklusu 8. i 15. dana, kao i 15. dana u trećem ciklusu ispitivanja, senzorska svojstva su takođe ocenjena visokim ocenama koje su se kretale od 4,5 ± 00 do 5,0 ± 00 (izuzetno prihvatljiva). U sva tri ciklusa ispitivanja 21. dana, senzorska svojstva upakovane juneće ruže, iako ocenjena nešto nižim ocenama (od 3,5 ± 00 do 4,2 ± 0,3) bila su „veoma prihvatljiva“. Senzorskim ispitivanjem, 28. dana, u sva tri ciklusa, ustanovljen je kvar mesa i, sa aspekta svih senzorskih svojstava, ono je ocenjeno kao „neprihvatljivo“ (1,0 ± 0,0). U vakuum pakovanju je bila vidljiva izdvojena zamućena tečnost, neprijatnog mirisa. Površina junećeg mesa je bila sluzava i lepljiva, nesvojstvene, sivo–crvene boje, a miris mesa po površini i na presecima izrazito neprijatan. Rezultati senzorskih i hemijskih ispitivanja juneće ruže upakovane u vakuumu, koji se odnose na ispitivanja 28. dana (konstatovan kvar mesa) nisu predstavljeni u ovom radu, niti su uzeti u obzir prilikom obrade podataka. S obzirom da je primenjenom kvantitativno-deskriptivnom skalom kao granica prihvatljivosti definisana ocena 2,0, dobijene vrednosti ocena pokazuju da je upakovana juneća ruža u vakuumu zadržala prihvatljiva senzorska svojstva u sva tri ciklusa ispitivanja zaključno sa 21. danom ispitivanja.

Rezultati senzorskog ispitivanja u ovom radu su u saglasnosti sa rezultatima *Karan i dr*. (neobjavljeni podaci) koji su ispitivali održivost junećeg mesa upakovanog u vakuumu (juneća plećka bez kosti i juneći but bez kosti) pri temperaturi skladištenja +4°C i ustanovili da je juneće meso u vakuum pakovanju zadržalo prihvatljiva senzorska svojstva 20 dana.

Na senzorska svojstva goveđeg mesa utiču brojni faktori koji se mogu podeliti na premortalne (rasa, starost, pol, klanična težina, ishrana, izloženost stresu) i tehnološke (postupci na liniji klanja, hladni lanac, vreme zrenja i dr.), (*Dransfield i dr*., 1992). Sa druge strane, očuvanje kvaliteta svežeg mesa upakovanog u vakuum zavisi od više faktora, pre svega inicijalne mikrobiološke kontaminacije, temperature skladištenja, propustljivosti materijala za pakovanje (*Stiles,* 1991). Shodno tome i rezultati pojedinih ispitivanja održivosti ukazuju na razlike u senzorskim osobinama junećeg mesa upakovanog u vakuumu. Dostupni podaci iz literature o uticaju vakuum pakovanja na senzorska svojstva junećeg mesa su vrlo oskudni i uglavnom su u vezi sa istraživanjima u kojima je uporedo praćen uticaj različitih smeša gasova i vakuuma (uglavnom korišćen kao „kontrola“) na održivost upakovanog mesa.

Ispitivanja *Lagerstedt-a i dr*. (2011), koja su sprovedena na goveđim odrescima *m. longissimus dorsi* upakovanim u vakuumu i smešu gasova (80% O_2 i 20% CO_2), su pokazala da je grupa uzoraka upakovana u vakuumu nakon termičke obrade ocenjena kao sočnija, mekše teksture, sa izraženijim mirisom i ukusom u odnosu na uzorke upakovane u MAP. Prema ispitivanju *Tørngren-a* (2003), mekoća goveđih odrezaka (*m. longissimus dorsi*) upakovanih u smešu gasova (80% O_2 i 20% CO_2) ocenjena je nižom ocenom u poređenju sa mekoćom odrezaka pakovanim u atmosferu sa 50% CO_2 i 50% N_2 i pakovanim u vakuumu, što potvrđuju rezultatima svojih ispitivanja i *Grobbel i dr*. (2008) i *Sørheim i dr.* (2004).

Pojedina istraživanja ukazuju na pozitivan uticaj vakuum pakovanja na stabilnost boje junećeg mesa u odnosu na pakovanje u smeši gasova (MAP). Tako su *Insausti i dr.* (1999) ustanovili veću stabilnost boje goveđeg mesa upakovanog u vakuumu u odnosu na boju mesa upakovanog u smešu gasova (60% O_2 : 30% CO_2 : 10% N_2) tokom petnaest dana skladištenja na temperature 2 ± 1°C. *Canganella i dr*. (1993) su ispitali stabilnost boje goveđeg mesa skladištenjem do 21. dana na 2–4°C u vakuumu i smeši gasova (80% N_2 : 20% CO_2). U pomenutoj studiji, utvrđena je veća stabilnost boje mesa pakovanog u vakuumu, što je u saglasnosti sa rezultatima naših ispitivanja, u kojima je boja mesa upakovanog u vakuumu i 21. dana u sva tri ciklusa ispitivanja bila „veoma prihvatljiva“.

Tabela 1. Senzorska ocena sveže juneće ruže u vakuum pakovanju
Table 1. Sensory evaluation of fresh beef thick flank in vacuum packaging

Osobine/ *Traits*	JUNEĆA RUŽA U VAKUUM PAKOVANJU *BEEF THICK FLANK – VACUUM PACKAGING*											
	CIKLUS I/dan ispitivanja Cycle I/examination day				CIKLUS II/dan ispitivanja Cycle II/examination day				CIKLUS III/dan ispitivanja Cycle III/examination day			
	1	8	15	21	1	8	15	21	1	8	15	21
Izgled mesa/ Appearance of meat	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,8[3]	4,6±0,4[1] 1,0[2] 8,2[3]	3,7±0,5[1] 1,5[2] 14,1[3]	5,0±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	3,7±0,3[1] 0,5[2] 7,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,7±0,5[1] 1,5[2] 14,1[3]
Boja mesa po površini/ Color of meat-surface	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	4,6±0,2[1] 0,5[2] 4,6[3]	3,7±0,3[1] 0,5[2] 7,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	3,7±,0,3[1] 0,5[2] 7,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,7±0,5[1] 1,5[2] 14,1[3]
Boja mesa na preseku/ Color of meat intersection	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	4,6±0,4[1] 1,0[2] 8,2[3]	3,9±0,4[1] 1,0[2] 9,6[3]	5,0±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	3,8±0,3[1] 0,5[2] 6,7[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,2±0,3[1] 0,5[2] 6,2[3]
Struktura/ Structure	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	4,8±0,3[1] 0,5[2] 5,3[3]	4,1±0,2[1] 0,5[2] 5,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	4,8±0,3[1] 0,5[2] 5,8[3]	3,8±0,3[1] 0,5[2] 7,3[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,2±0,3[1] 0,5[2] 6,2[3]
Tekstura/ Texture	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,8[3]	4,6±0,4[1] 1,0[2] 8,2[3]	3,6±0,7[1] 1,5[2] 18,6[3]	5,0±0,0[1] 0[2] 0,0[3]	4,5±0,3[1] 1,0[2] 7,0[3]	4,5±0,3[1] 1,0[2] 7,0[3]	3,5±0,3[1] 1,0[2] 9,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,5±0,6[1] 1,5[2] 18,1[3]
Miris svežeg mesa/ Smell of fresh meat	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	4,8±0,3[1] 0,5[2] 5,8[3]	3,6±0,7[1] 1,5[2] 18,6[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,5±0,3[1] 1,0[2] 9,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	3,5±0,6[1] 1,5[2] 18,1[3]
Miris posle probe kuvanja/ Smell of meat after cooking test	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	4,8±0,3[1] 0,5[2] 5,8[3]	3,8±0,6[1] 1,5[2] 16,3[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,5±0,5[1] 1,0[2] 12,8[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	3,8±0,61[1] 1,5[2] 15,8[3]
Ukus posle probe kuvanja/ Taste of meat after cooking test	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,8[3]	4,6±0,2[1] 0,5[2] 4,5[3]	3,7±0,3[1] 0,5[2] 7,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	4,5±0,0[1] 0[2] 0,0[3]	3,5±0,3[1] 1,0[2] 9,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,7±0,6[1] 1,5[2] 16,5[3]
Ukus posle probe pečenja/ The taste of meat after roasting test	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,8[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,7±0,3[1] 0,5[2] 7,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,7±0,3[1] 0,5[2] 5,5[3]	4,7±0,3[1] 0,5[2] 5,5[3]	3,5±0,3[1] 1,0[2] 9,0[3]	5,0±0,0[1] 0[2] 0,0[3]	5,0±0,0[1] 0[2] 0,0[3]	4,8±0,3[1] 0,5[2] 5,3[3]	3,5±0,6[1] 1,5[2] 18,0[3]

Legenda/Legend: [1]$\bar{x}$ ± Sd (aritmetička sredina ± standardna devijacija/mean value ± standard deviation); [2]Iv (interval varijacije/variation interval); [3]Cv (%) (koeficijent varijacije/variation coefficient)

Na osnovu vrednosti koeficijenta varijacije (Cv%) može se zaključiti da je ocenjivački panel sastavljen od šest ocenjivača bio homogen i da je postignuta konzistentnost u rezultatima tokom sva tri ciklusa ispitivanja. Utvrđeno je da ne postoji statistički značajna razlika ($p > 0,05$) srednjih vrednosti ispitanih senzorskih svojstava juneće ruže upakovane u vakuumu između ciklusa ispitivanja (tabela 2).

Dobijeni rezultati senzorskog ispitivanja mogu se tumačiti ujednačenim inicijalnim kvalitetom junećeg mesa, poštovanjem svih implementiranih procedura u industrijskom objektu na liniji klanja, rasecanja i pakovanja, kao i hladnog lanca tokom skladištenja i dostavljanja uzoraka u laboratoriju na ispitivanje, tokom sva tri ciklusa.

Hemijska i fizičko-hemijska ispitivanja

Rezultati hemijskih i fizičko-hemijskih ispitivanja prikazani su u tabeli 3. Iz tabelarnog prikaza se može videti da su najniže a_w vrednosti izmerene 1. dana ispitivanja u drugom i trećem ciklusu

Tabela 2. Senzorska ocena sveže juneće ruže u vakuum pakovanju – zbirni prikaz po ciklusima
Table 2. Sensory evaluation of fresh beef thick flank – vacuum packaging – summary by cycles

Osobine/*Traits*	JUNEĆA RUŽA U VAKUUM PAKOVANJU *BEEF THICK FLANK – VACUUM PACKAGING*					
	CIKLUS I/ ZBIRNO Cycle I/total		CIKLUS II/ ZBIRNO Cycle II/total		CIKLUS III/ ZBIRNO Cycle III/total	
Izgled mesa/Appearance of meat	4,5±0,6[1]	13,5 [2]	4,4±0,5[1]	11,4[2]	4,6±0,6 [1]	13,5[2]
Boja mesa po površini/Color of meat-surface	4,5±0,5[1]	12,1[2]	4,4±0,5[1]	11,4 [2]	4,6±0,6[1]	13,5[2]
Boja mesa na preseku/Color of meat intersection	4,6±0,5[1]	10,1 [2]	4,5±0,4 [1]	9,9 [2]	4,8±0,4[1]	8,1 [2]
Struktura/Structure	4,7±0,4[1]	8,8[2]	4,6±0,6[1]	11,9[2]	4,8±0,4[1]	8,1[2]
Tekstura/Texture	4,5±0,7[1]	14,9[2]	4,4±0,6[1]	14,0[2]	4,5±0,7[1]	15,5[2]
Miris svežeg mesa/Smell of fresh meat	4,5±0,7[1]	14,9[2]	4,6±0,6[1]	14,0[2]	4,6±0,7[1]	15,7[2]
Miris posle probe kuvanja/Smell of meat after cooking test	4,6±0,6[1]	13,1[2]	4,5±0,6[1]	14,4[2]	4,7±0,6[1]	12,5[2]
Ukus posle probe kuvanja/Taste of meat after cooking test	4,5±0,6[1]	12,3[2]	4,4±0,6[1]	13,2[2]	4,6±0,6[1]	13,9[2]
Ukus posle probe pečenja/The taste of meat after roasting test	4,5±0,6[1]	12,4[2]	4,5±0,6[1]	14,0[2]	4,6±0,7[1]	15,7[2]

Legenda/Legend: [1]$\bar{x}$ ± Sd (aritmetička sredina ± standardna devijacija/mean value ± standard deviation);
[2]Cv (%) (koeficijent varijacije/variation coefficient)

(0,962 ± 0,002 i 0,966 ± 0,002, respektivno), odnosno 8. dana ispitivanja u prvom ciklusu (0,962 ± 0,001), dok su najviše a_w vrednosti zabeležene 21. dana u prvom i trećem ciklusu (0,993 ± 0,002 i 0,990 ± 0,001, respektivno), odnosno 8. dana (0,974 ± 0,002) i 21. dana (0,974 ± 0,002) u drugom ciklusu. S obzirom da sveže meso uglavnom ima a_w vrednost veću od 0,85, potrebno je hlađenjem ili na neki drugi način kontrolisati rast patogena (*Smith i Stratton*, 2006). Vrednosti a_w od 0,980 do 0,995 najviše pogoduju razvoju mikroorganizama.

Kod svih analiziranih uzoraka iz sva tri ciklusa, došlo je do pada pH vrednosti tokom prvih osam dana ispitivanja, nakon čega se pH vrednost postepeno povećavala i 21. dana iznosila 5,93 ± 0,02 (prvi ciklus); 5,97 ± 0,02 (drugi ciklus); 6,13 ± 0,01 (treći ciklus). Prema rezultatima *Russell i dr*. (1996), za rast bakterija kvara mesa najpogodnija je pH vrednost od 5,5 do 7,0. Formiranje sluzi, degradacija strukturnih komponenata, kao i pojava neprijatnog mirisa u mesu, mogu biti posledica rasta mikroorganizama u ovoj oblasti pH vrednosti. Utvrđene pH vrednosti, u našem ispitivanju, za sva tri ciklusa ispitanih uzoraka bile su u opsegu od 5,68 (min.) do 6,13 (max.). Prema *Khaksar i dr.* (2010), sadržaj hidroperoksida kao mera oksidativne razgradnje lipida značajnije raste pri pH 6,8 nego pri pH 3, što dodatno znači i veći sadržaj malondialdehida pri pH 6,8. Ali, nastanak mlečne kiseline, kao rezultat rasta bakterija mlečnokiselinskog vrenja je od najvećeg značaja za opadanje pH vrednosti u upakovanom mesu. Naime, vakuum pakovanje potencira rast fakultativno anaerobne mikroflore, uključujući bakterije mlečne kiseline, bakterije iz familije *Enterobacteriaceae* i *Brochothrix thermosphacta* (*Baltić i dr.,* 2012). U pakovanju se povećava sadržaj CO_2, laktata i drugih kiselih proizvoda, što dovodi do snižavanja pH vrednosti, pri čemu, u ovom procesu ne učestvuju samo mikroorganizmi, već i enzimi samog mesa (*Radetić i dr*., 2007).

Vrednost kiselinskog broja (mg KOH/g), tokom perioda ispitivanja, pokazuje permanentni i brzi porast u sva tri ciklusa (prvi ciklus, 1. dan ispitivanja – 1,08 ± 0,10 i 21. dan – 2,79 ± 0,45; drugi ciklus, 1. dan ispitivanja – 1,24 ± 0,24 i 21. dan – 3,49 ± 0,28; treći ciklus, 1. dan ispitivanja – 1,53 ± 0,34 i 21. dan 5,07 ± 0,44). Drastičniji porast kiselinskog broja, u svim ispitivanim uzorcima, u sva tri ciklusa, utvrđen je u završnoj fazi skladištenja, odnosno u periodu od 15. do 21. dana (tabela 3). Ovaj parametar je pokazatelj početka hidrolitičke degradacije lipida u mesu i njegov porast tokom čuvanja mesa je uobičajena pojava. Vrednost kiselinskog broja je u vezi sa sadržajem vode u mesu, koja doprinosi reakcijama hidrolize (*Naz i dr*., 2005). Takođe, niža pH vrednost utiče na smanjenje lipolize (*Khaksar i dr.*, 2010).

Tabela 3. Hemijski i fizičko-hemijski parametri održivosti sveže juneće ruže u vakuum pakovanju
Table 3. Chemical and physico-chemical parameters of the fresh beef thick flank shelf-life in vacuum packaging

Ispitivani parametar/ Examined parameter	CIKLUS I/dan ispitivanja Cycle I/day of examination				CIKLUS II/dan ispitivanja Cycle II/day of examination				CIKLUS III/dan ispitivanja Cycle III/day of examination			
	1	8	15	21	1	8	15	21	1	8	15	21
Ukupno isparljivi azot TVB-N (mg/100g)/Total volatile basic nitrogen(mg/100)	33,54±0,21[1] 33,22-33,80[2] 0,62[3]	34,91±0,40[1] 34,19-35,30[2] 1,14[3]	30,51±0,18[1] 30,37-30,87[2] 0,59[3]	45,19±0,26[1] 44,79-45,48[2] 0,57[3]	30,23±0,37[1] 29,75-30,76[2] 1,22[3]	31,07±0,13[1] 30,88-31,20[2] 0,42[3]	31,18±0,31[1] 30,80-31,38[2] 0,99[3]	34,04±0,20[1] 34,00-34,28[2] 0,58[3]	30,14±0,28[1] 29,68-30,38[2] 0,92[3]	34,96±0,16[1] 34,71-35,20[2] 0,45[3]	31,08±0,35[1] 30,80-31,64[2] 1,12[3]	35,04±0,48[1] 34,72-35,82[2] 1,73[3]
Kiselinski broj (mg KOH/g)/ Acid value (mg KOH/g)	1,08±0,10[1] 0,96-1,20[2] 9,25[3]	1,65±0,38[1] 1,04-1,95[2] 23,03[3]	2,17±0,14[1] 2,05-2,40[2] 6,45[3]	2,79±0,45[1] 2,23-3,38[2] 16,12[3]	1,24±0,24[1] 0,95-1,50[2] 19,35[3]	2,07±0,14[1] 1,96-2,10[2] 6,76[3]	2,16±0,06[1] 2,08-2,23[2] 2,77[3]	3,49±0,28[1] 2,93-3,61[2] 8,02[3]	1,53±0,34[1] 1,08-1,87[2] 22,22[3]	2,48±0,41[1] 2,17-2,83[2] 16,53[3]	3,10±0,60[1] 2,63-4,00[2] 19,35[3]	5,07±0,44[1] 4,37-5,50[2] 8,67[3]
Peroksidni broj (mmol/kg)/ Peroxide value (mmol/kg)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TBK test/TBA value (mg MAL/kg)	0,00	0,08±0,01[1] 0,07-0,10[2] 12,5[3]	0,16±0,02[1] 0,14-0,18[2] 12,5[3]	0,39±0,01[1] 0,37-0,41[2] 2,56[3]	0,00	0,08±0,02[1] 0,06-0,11[2] 25,00[3]	0,13±0,01[1] 0,13-0,14[2] 7,69[3]	0,14±0,01[1] 0,12-0,15[2] 7,14[3]	0,00	0,05±0,01[1] 0,04-0,05[2] 20,00[3]	0,15±0,01[1] 0,14-0,16[2] 6,66[3]	0,09±0,01[1] 0,08-0,10[2] 11,11[3]
pH vrednost/pH value	6,03±0,03[1] 6,00-6,07[2] 0,50[3]	5,68±0,00[1] 5,68-5,68[2] 0,00[3]	5,87±0,04[1] 5,80-5,93[2] 0,68[3]	5,93±0,02[1] 5,91-5,96[2] 0,33[3]	6,04±0,02[1] 6,02-6,06[2] 0,33[3]	5,96±0,04[1] 5,89-5,98[2] 0,67[3]	6,06±0,02[1] 6,02-6,16[2] 0,33[3]	5,97±0,02[1] 5,95-6,00[2] 0,34[3]	6,05±0,02[1] 6,03-6,07[2] 0,33[3]	5,84±0,01[1] 5,83-5,85[2] 0,17[3]	5,93±0,02[1] 5,91-5,94[2] 0,34[3]	6,13±0,01[1] 6,12-6,14[2] 0,16[3]
a_w aktivnost vode/ a_w water activity	0,964±0,002[1] 0,961-0,965[2] 0,21[3]	0,962±0,001[1] 0,960-0,963[2] 0,10[3]	0,972±0,001[1] 0,991-0,994[2] 0,10[3]	0,993±0,002[1] 0,991-0,996[2] 0,20[3]	0,962±0,002[1] 0,960-0,964[2] 0,20[3]	0,974±0,002[1] 0,961-0,979[2] 0,20[3]	0,964±0,003[1] 0,960-0,966[2] 0,31[3]	0,974±0,002[1] 0,972-0,978[2] 0,20[3]	0,966±0,002[1] 0,962-0,970[2] 0,20[3]	0,983±0,004[1] 0,980-0,989[2] 0,40[3]	0,974±0,001[1] 0,971-0,976[2] 0,10[3]	0,990±0,001[1] 0,988-0,991[2] 0,10[3]

Legenda/Legend: [1]$\bar{x}$ ± Sd (aritmetička sredina ± standardna devijacija/mean value ± standard deviation); [2]Iv (interval varijacije/variation interval); [3]Cv% (koeficijent varijacije/variation coefficient)

U vakuum upakovanoj junećoj ruži, peroksidni broj je, u sva tri ciklusa, u svim ispitivanjima, iznosio 0,00 mmol/kg, što ukazuje da nije utvrđen nastanak primarnih proizvoda oksidacije. Vrednost peroksidnog broja je, uglavnom u vezi sa pH vrednošću mesa. Naime, kada je pH vrednost bliža vrednosti 7, uslovi za oksidaciju su „povoljniji" (*Xie i Wang,* 2007).

U junećoj ruži, upakovanoj u vakuum, sadržaj malondialdehida (MAL) je u sva tri ciklusa 1. dana iznosio 0,00 mg/kg, odnosno nije utvrđeno prisustvo sekundarnih proizvoda oksidacije. U prva dva ciklusa ispitivanja, u periodu od 8. do 21. dana, količina MAL se povećavala, tako da je 21. dana iznosila 0,39 ± 0,01 mg/kg (1. ciklus) i 0,14 ± 0,01 mg/kg (2. ciklus). U trećem ciklusu, 15.dana, utvrđen je porast sadržaja MAL, 0,15 ± 0,01 mg/kg, dok je 21. dana sadržaj MAL pao i iznosio je 0,09 ± 0,01 mg/kg. Prema rezultatima studije *Wong i dr*. (1995), užeglost se može detektovati kada sadržaj malondialdehida dostigne količinu od 3 mg/kg. U svim ispitanim uzorcima mesa utvrđene MAL vrednosti TBK-testom su bile daleko ispod ove kritične vrednosti (max. 0,39 ± 0,01 mg MAL/kg). Prema rezultatima studije *Zhao i dr.,* 1994, lipidna oksidacija se pojavljuje kasnije i sporije napreduje u poređenju sa uočljivijim mikrobiološkim promenama i diskoloracijom tokom skladištenja mesa koje je bilo pakovano u aerobnim uslovima.

Smatra se da je za slab intenzitet oksidacionih procesa odgovorna ishrana goveda od kojih meso potiče. Naime, veći sadržaj prirodnih antioksidanasa u ishrani, kao što je vitamin E na primer, dovodi do povećanja sadržaja vitamina E u mišićnom tkivu goveda i tako sprečava razvoj oksidacionih procesa u mesu (*Yang i dr.*, 2002 i *Gatellier i dr.,* 2005).

U nekoliko studija prikazana je dobra korelacija između izmerenih TBK vrednosti i senzorskih svojstava, kojom je potvrđena užeglost pilećeg (*Salih i dr.,* 1987) i svinjskog mesa (*Tutner i dr.*, 1954), ali, organoleptičke promene su ustanovljene samo u kuvanom, a ne i u salamurenom mesu. *Melton* (1985) je pokazao da se promena mirisa i ukusa može detektovati pri vrednosti TBK od 0,3 do 1,0 mg MAL/kg, u goveđem i svinjskom mesu, i od 1,0 do 2,0 mg MAL/kg, u pilećem mesu. Međutim, ove vrednosti za TBK se ne mogu smatrati „referentnim", jer zavise od ishrane, starosti životinja, kao

od izlaganja/neizlaganja termičkoj obradi. Često se u ohlađenom mesu registruje organoleptički intenzivnija užeglost nego što pokazuju vrednosti za peroksidni i TBK broj, i te razlike između senzorskih zapažanja i hemijskih pokazatelja su verovatno, posledica smanjene mogućnosti ekstrakcije fosfolipida iz ohlađenog mesa i mogućeg gubitka malondialdehida u reakcijama sa sastojcima mesa pri niskim temperaturama (*Butkus*, 1967).

Količine TVB-N su 1. dana ispitivanja u drugom i trećem ciklusu bile slične (30,23 ± 0,37 mg/100g i 30,14 ± 0,28 mg/100g, respektivno), dok je 1. dana ispitivanja u prvom ciklusu utvrđen veći sadržaj ukupnog isparljivog azota (33,54 ± 0,21 mg/100g), (tabela 3). Na osnovu prikazanih rezultata može da se uoči drastičniji porast sadržaja TVB-N sa produženjem vremena skladištenja, što je u skladu sa navodima *Sunki i dr*., 1978. Tako su poslednjeg, 21. dana ispitivanja,

Tabela 4. Vrednosti Pearsovog koeficijenta korelacije (r) između ispitanih senzornih svojstava i hemijskih i fizičko-hemijskih parametara

Table 4. Values for Pearsons correlation coefficient between the examined sensory properties and chemical and physico-chemical parameters

Redni broj ciklusa/ *Cycle number*	Osobine/ *Traits*	JUNEĆA RUŽA – U VAKUUM PAKOVANJU/*BEEF THICK FLANK – VACUUM PACKAGING*								
		Izgled mesa/ *Appearance of meat*	Boja mesa po površini/ *Color of the meat-surface*	Boja mesa na preseku/ *Color of the meat intersection*	Struktura/ *Structure*	Tekstura/ Texture	Miris svežeg mesa/ *Smell of fresh meat*	Miris posle probe kuvanja/ *Smell of meat after cooking test*	Ukus posle probe kuvanja/ *Taste of meat after cooking test*	Ukus posle probe pečenja/ *The taste of meat after roasting test*
I Ciklus/ *Cycle I*	TVB-N	–0,735	–0,814	–0,685	–0,823	–0,713	–0,780	–0,754	–0,812	–0,837
	Kiselinski broj/acid value	–0,816	–0,820	–0,768	–0,698	–0,739	–0,692	–0,743	–0,767	–0,747
	TBK test/ TBA value	–0,814	–0,915	-0,817	–0,841	–0,814	–0,836	–0,814	–0,937	–0,919
	a_w	–0,786	–0,843	–0,800	–0,778	–0,756	–0,761	–0,754	–0,872	–0,841
II Ciklus/ *Cycle II*	TVB-N	–0,943	–0,936	–0,917	–0,881	–0,871	–0,934	–0,910	–0,943	–0,932
	Kiselinski broj/acid value	–0,970	–0,936	–0,949	–0,841	–0,884	–0,880	–0,886	–0,941	–0,882
	TBK test/ TBA value	–0,761	–0,761	–0,798	–0,546	–0,677	-0,611*	–0,595	–0,733	–0,611
	a_w	–0,692	–0,699	–0,693	–0,577	–0,657	–0,679	–0,633	–0,686	–0,690
III Ciklus/ *Cycle III*	TVB-N	–0,452**	–0,469**	–0,557*	–0,543*	–0,456*	–0,476*	–0,465**	–0,448**	–0,513*
	Kiselinski broj/acid value	–0,817	–0,749	–0,803	–0,830	–0,801	–0,834	–0,721	–0,747	–0,789
	TBK test/ TBA value	/	/	/	/	/	/	/	/	/
	a_w	–0,627	–0,619	–0,676	–0,658	–0,628	–0,600*	–0,606*	–0,595*	–0,623

Legenda/Legend : Ukupno isparljivi azot/TVB-N (total volatile basic nitrogen); Test tiobarbiturne kiseline/TBA (thiobarbituric acid number); **a_w** – aktivnost vode/(water activity value);

Napomena: Prikazane vrednosti za koeficijente kolerelacije su na nivou značajnosti $p < 0,001$;

Footnote: Presented correlation coefficients are given at the level of significance $p < 0.001$;

$p < 0,001$; * $p < 0,01$; ** $p < 0,05$;/nisu utvrđene statistički značajne korelacije/correlations were not registered.

količine TVB-N u sva tri ciklusa bile značajno veće u odnosu na vrednosti utvrđene na početku ispitivanja i iznosile su 45,19 ± 0,26 mg/100g (prvi ciklus), 34,04 ± 0,20 mg/100g (drugi ciklus) i 35,04 ± 0,48 mg/100g (treći ciklus). Takođe, *Balamatsia i dr*. (2006), su u studiji u kojoj je praćena održivost neupakovanog pilećeg mesa (kontrolna grupa), pakovanog u vakuum i u MAP, utvrdili porast TVB-N tokom skladištenja, od početne količine od oko 20 mg/100g do 54,5 mg/100g u neupakovanom mesu, 45,8 mg/100 g u vakuumu i 43,12 mg/100 g u MAP upakovanom mesu (15. dan održivosti). Dobijeni rezultati su najverovatnije u vezi sa povećanim stepenom degradacije proteina u završnoj fazi ispitivanja mesa. Količina TVB-N može poslužiti kao indikator svežine i održivosti mesa (*Byun i dr*., 2003). Naime, količine TVB-N od 20 i 30 mg/100g za svinjsko i goveđe meso, respektivno, se smatraju graničnim prihvatljivim vrednostima za procenu svežine mesa (*Connell*, 1990). Međutim, u pomenutoj studiji je prikazano da meso (svinjsko ili goveđe) koje je čuvano na adekvatan način i sa sadržajem ukupnog isparljivog azota bliskim gore navedenim graničnim vrednostima ne mora se nužno smatrati pokvarenim odnosno neupotrebljivim.

U tabeli 4 prikazani su koeficijenti korelacije između ispitanih hemijskih, fizičko-hemijskih i senzorskih parametara. U prva dva ciklusa ispitivanja, u većini slučajeva, utvrđena je umerena do jaka negativna korelacija ispitanih hemijskih parametara i senzorske ocene junećeg mesa, dok je u III ciklusu uočena slaba negativna korelacija.

Koeficijenti korelacije za TVB-N vrednosti u I i II ciklusu ispitivanja su bili od –0,685 do –0,943, a povezanosti su kategorisane kao izrazito negativno korelisane (koeficijenti korelacije između –0,750 i –1,000). U III ciklusu ove korelacije su bile u intervalu –0,448 do –0,543 i klasifikovane kao negativno slabo povezane (koeficijenti korelacije između –0,250 i –0,500). Može se pretpostaviti da je porast sadržaja TVB-N, koji nastaje kao posledica mikrobiološke aktivnosti i povećane degradacije proteina, u vezi sa promenjenim izgledom mesa, bojom mesa po površini i bojom mesa na preseku, kao i mirisom i ukusom posle probe kuvanja.

U pogledu vrednosti kiselinskog broja i jačine njegove veze sa ispitanim senzorskim svojstvima junećeg mesa, uočava se najjača povezanost u II ciklusu ispitivanja. Naime, u II ciklusu uočava se vrlo jaka negativna korelacija između vrednosti kiselinskog broja i izgleda mesa, boje mesa na površini i preseku (tabela 4), u odnosu na I i III ciklus ispitivanja. Pretpostavka je da su početne hidrolitičke promene u lipidima u mesu upakovanom u vakuumu, uslovile slabiju ocenu, pre svega, izgleda mesa, boje na površini i preseku mesa. Iako su utvrđene TBK vrednosti bile daleko manje od 3 mg/kg (vrednost pri kojoj se detektuje užeglost), u I ciklusu se uočava vrlo jaka negativna korelacija između vrednosti ovog parametra i senzorskih svojstava, a naročito u odnosu na senzorsku ocenu ukusa posle probe kuvanja (r = –0,937) i posle probe pečenja (r = –0,919). U II ciklusu, ove korelacije su bile –0,733 (ukus posle probe kuvanja) i –0,611 (ukus posle probe pečenja). U III ciklusu nije utvrđena statistički značajna korelacija između TBK vrednosti i rezultata senzorskih ispitivanja. U pogledu a_w vrednosti i njene korelacije sa ispitanim senzorskim svojstvima junećeg mesa, u II i III ciklusu uočava se prilično ujednačena negativna, umerena povezanost (koeficijenti korelacije između –0,500 i –0,750), dok je u ciklusu ova korelacija klasifikovana kao vrlo jaka negativna.

Zaključak

Na osnovu dobijenih rezultata ispitivanja sveže ohlađene juneće ruže ustanovljeno je da je vakuum pakovanje imalo pozitivan uticaj na usporavanje senzorskih i hemijskih promena i očuvanje poželjnih svojstava. Upakovana juneća ruža u vakuumu je sa aspekta senzorskih osobina i hemijskih promena pri datim uslovima skladištenja i u ambalaži korišćenoj za pakovanje, bila prihvatljiva zaključno sa 21. danom ispitivanja, u sva tri ciklusa. Konzistentnosti rezultata senzorskih ispitivanja i rezultata hemijskih i fizičko-hemijskih ispitivanja, u sva tri ciklusa, značajno je doprineo ujednačeni inicijalni kvalitet junećeg mesa. Na osnovu vrednosti Pirsonovog koeficijenta korelacije I, u I i II ciklusu ispitivanja, u većini slučajeva, utvrđena je umerena do jaka negativna korelacija ispitanih hemijskih parametara i senzorske ocene junećeg mesa, dok je u III ciklusu uočena slaba negativna korelacija. Može se zaključiti da, u uslovima dobre proizvođačke prakse, koja polazi od zdravstvenog stanja i dobrobiti životinja za klanje, preko poštovanja svih procedura na liniji klanja, rasecanja i pakovanja, kao i hladnog lanca i procesne higijene, vakuum pakovanje svežeg, ohlađenog junećeg mesa daje veoma dobre rezultate u pogledu produženja održivosti i očuvanja prihvatljivih senzorskih karakteristika mesa.

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Comparison of results of sensory and chemical and physico-chemical investigations of fresh chilled beef packaged in vacuum during storage in retail conditions

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S u m m a r y: Vacuum packaging of fresh meat can contribute to prolonged shelf-life, slower chemical changes in meat, and maintaining of desirable sensoric properties. The main goal of this work was the comparative examination of sensory and chemical properties relevant for oxidative and hidrolytic changes of cooled fresh beef cuts (m.quadriceps femoris) in vacuum packaging, and the determination of interdependence of hydrolytical and oxidative changes regarding to sensory characteristics. The beef flank cuts taken from primal cuts (quarters) of 3 Simmental steers (average weight of 400 kg; one steer per testing cycle) after slaughtering, dressing and cooling were taken for the experiment. Cutting and packaging in vacuum thermo-shrinkable multilayered bags was performed in the industrial meat establishment, within 40 hours after slaughtering. The vacuum packaged beef cut samples were stored under controlled temperature conditions (0-2°C) in refrigerated retail show case. During the night, samples were removed from show case and stored in cold storage room in the same establishment (0-4°C). This method was repeated as daily routine during examination period. Sensory and chemical examination dynamics was set as: day 1 (immediately after packaging), days 15, 21 and 28 in each particular testing cycle (3). Sensory properties were evaluated by using quantitative-descriptive test, on a scale of 1 to 5. The following properties were evaluated: appearance of meat, the colour of meat surface, the colour of meat intersection, structure, texture, smell of the fresh meat, smell of the meat after cooking, taste of the meat after cooking and roasting.

The examined chemical propertiess indicating oxidative and hydrolytic changes were: acid value, peroxide number, TBA test (test with thiobarbituric acid to determinate malonaldehide content), TVB-N (total volatile basic nitrogen), a_w (water activity value) and pH. Sensory and chemical qualities of vacuum packaged beef flank under established storage conditions were acceptable in all three testing cycles, ending with the 21st day. In the first and second cycle, in most cases, there were confirmed medium to strong correlations of all examined chemical parameters with the sensoric characteristics of beef. In the third cycle, the registered above mentioned correlations between chemical parameters and sensory properties were classified as negative weak correlations.

***Key words**: fresh beef, vacuum packaging, sensory properties, chemical changes.*

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Comparative mycological analysis of spices used in meat industry

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A b s t r a c t: Spices are often considered as one of the possible sources of meat products contamination with toxigenic moulds. Since spices are possible source of contamination of the final product and potential producers of mycotoxins, it is necessary to estimate the degree of moulds contamination. Therefore it is necessary to conduct an adequate and continuous control of spices on presence of the moulds. The choice of a culture media to carry out mycological analysis of food is extremely important to guarantee the reliability of the analysis. This paper presents an overview of the analysis of different spices (n=15) for the presence of xerophilic moulds, by using Dichloran Rose-Bengal Chloramphenicol Agar" (Oxoid, CM 727) and "Dichloran-Glycerol (DG18) Agar Base" (Oxoid, CM 729)., Black and white grounded pepper, were the most contaminated (3.62-3.79 log_{10} cfu/g) of 15 samples tested, while in the remaining samples an average contamination level of 2.7 log_{10} cfu/g was established.

Key words: *a_w, selective medium, xerophilic moulds.*

Introduction

Spices and herbs are valued for their distinctive flavours, colours, and aromas and are among the most versatile and widely used ingredients in food preparation (*Škrinjar et al*., 2012). Modern meat industry cannot be imagined without utilization of spices. However, spices, together with all other dried material of herbal origin, are never sterile. As is the case with many other agricultural products, spices and herbs may be exposed to a wide range of microbial contamination pre – and post – harvest (*Hashem and Alamri,* 2010). In most cases, they contain sporogenic bacteria and moulds. These microorganisms can cause spoilage of the product by their metabolic activity, consequently resulting in significant economic losses.

Presence of moulds in spices and later in sausages or other meat products can result in production of toxic metabolites – mycotoxins independent of contamination degree (*Kocić-Tanackov et al.,* 2007). Mycotoxins are fungal secondary metabolites identified in many agricultural products screened for toxigenic moulds (*Clevsrton and Ljunggren,* 1985). Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, hemorrhagic, and dermatitic to a wide range of organisms, and known to cause hepatic carcinoma in man in humans and animals (*Frisvad et al.,* 2005; *Zinedine et al.,* 2006). Production of toxins primarily depends on genetic factors; however, environmental conditions at the site of mould growth (temperature, water activity, matrix composition, moisture content, pH of the medium, contamination and physical destruction of the substrate, antifungal properties and other factors) are considered highly significant (*Škrinjar et al.,* 2012).

It has been reported that principal contaminants of spices are xerophilic moulds from the genera *Eurotium*, *Aspergillus* and *Penicillium* (*Dimić and Škrinjar,* 1995; *Dimić et al.,* 2000; *Romagnoli et al.,* 2007). Enumeration of yeasts and filamentous fungi usually involves the inoculation of samples in solid culture media through surface or pour plate method (*Beuchat and Hocking,*1990; *Beuchat,* 1998). In general, culture media for fungal evaluation need to be highly selective, suppressing the fast growing bacterial contamination (*Askun et al.,* 2007) and limiting the growth rate and spread of fungal colonies, but should allow the enumeration of theoretically all species present in the samples. More recently, media supplemented with antibiotics, as the dichloran rose of bengal and chloramphenicol (*Škrinjar et al.,* 2012), emerged as more efficient alternative. These media show less toxic effect to the damaged cells,

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they are more effective to inhibit the bacterial development and are used to induce a smaller amount of food particles precipitation due its higher pH (*Medina et al.,* 2005). Routinely, dichloran rose of bengal has been successfully applied to control the rate dissemination of fungal species, limiting the rapid spread of zygomycetes on the plate surface (*Pardo et al.,* 2006). Later, media with reduced activity of water (a_w) were introduced, as the dichloran glycerol agar 18% (a_w 0.95), for a general quantification of moderately xerophilic fungi, those with fastidious growth, which in a medium with traditional activity of water (a_w 0.99) may have their growth inhibited due to the rapid development of other species.

In this paper, the efficacy of these two culture media for quantification of fungi from different spices was evaluated.

Materials and methods

Samples

A total of fifteen (n = 15) samples of commercially available spices were analyzed for mould contamination. The samples were provided from the health food stores in bulk and from supermarkets.

Methods

The samples were processed according to the ISO 21527-1:2008 and ISO 21527-2:2008 methods. All analyses were performed in triplicate under aseptic conditions. Samples were inoculated by surface plate technique according to the dilution performed. For surface plating, 0.1mL aliquots were inoculated in plates with solidified medium, spreading the inoculum on media surface. For the enumeration, two different selective media were used: dichloran 18% glycerol (DG18) agar and dichloran Rose-Bengal chloramphenicol (DRBC) agar. The plates were incubated at 28°C for 5–7 days and examined for presence of moulds. The results were expressed as colony forming unit per sample gram (CFU/g).

Identification of the isolates

For the identification of the cultures, each isolate was inoculated on Czapek-Dox Agar (CZ, Oxoid CM97). Macroscopic and microscopic morphological characteristics were used in the identification process. Colony colour, texture and diameter, the production of diffusible pigments, and exudates were among macroscopic features, while conidia and conidiophore arrangements were the microscopic ones.

Isolates were identified according to standard mycological procedures (*Pitt and Hocking*, 1995; *Samson and Pitt*., 2000).

Results and discussion

Table 1 shows the results of mycological analyses – enumeration of moulds in 15 different spice samples using two different selective media, DG18 and DRBC. These media have different composition of added sugars and water activity (a_w). Numerous xerophilic moulds, which are carriers of spices contamination, have optional a_w below 0.90. Collaborative investigations using those two selective media provide more accurate perspective on micro populations. From fifteen spices, all samples were with growth (Table 1), while samples 5 and 6 had the highest contamination level (3.62-3.79 $\log_{10}$ cfu/g). Generally, the moulds were found with the mean concentration of 2.7 $\log_{10}$ cfu/g.

DG18, of all the media evaluated, showed better efficacy of fungal contamination in spices used in meat industry. DG18 presented the better results in the quantity from the evaluated spices.

Isolated moulds were classified into four genera (*Aspergillus*, *Alternaria*, *Paecylomyces* and *Penicillium*). Considering DG18, mycological research showed better fungal diversity and the dominant presence of genus *Aspregillus* (47.82%). The remaining moulds were from genus *Penicillium* (39.13%)*, Paecylomyces* (8.69%), and *Alternaria* (4.34%). On DRBC, contamination level was the same for *Aspergillus* spp. and *Penicillium* spp. (Table 2). In addition, the number of different genera present was greater on the DG18 agar plates than on the DRBC plates.

Dichloran 18% glycerol (DG18) agar was originally developed to enumerate xerophilic foodborne moulds. However, some laboratories are using DG18 agar as a general medium to enumerate foodborne moulds and yeasts. These results pointed to the fact that media with limited amount of free water suppressed the growth of moulds that are not extremely xerophilic in nature. It is also known that xerophilic moulds can be divided into fast-growing and slow-growing forms (*Kocic-Tanackov et al.,* 2007). Slow-growing moulds, even under optimal conditions can be overgrown by fast-growing xerophiles. The emergence of Aspergilli and Penicillia species on the two different media indicated the presence of these moulds as the dominant mycoflora of different spices. This observation was greatly in agreement with other authors who studied mycoflora of spices

Table 1. Average total count of moulds obtained by different culture media in spices
Tabela 1. Prosečne vrednosti ukupnog broja plesni utvrđene u začinima korišćenjem različitih podloga

Sample	total count (log_{10} cfu/g)	
	DG18 DG18	DRBC
1. Clove/Karanfilić	2.95	2.93
2. Rosemary/Ruzmarin	2.30	2
3. Sesame/Susam	2.20	2
4. Ginger/Đumbir	2.50	2.41
5. White pepper –ground/Mleveni beli biber	3.79	3.77
6. Black pepper – ground/Mleveni crni biber	3.66	3.62
7. White pepper in grain/Beli biber u zrnu	2.51	3.46
8. Black pepper in grain/Crni biber u zrnu	3.62	3.70
9. Oregano/Origano	2	2
10. Caraway/Kim	3.54	3.46
11. Chilli/Čili	2.51	2.49
12. Curry/Kari	3.55	3.49
13. Thyme/Timijan	2.34	2.32
14. Bay leaf/Lovorov list	2	2
15. Sweet basil/Bosiljak	2.07	2

and medicinal herbs (*Dimić et al.,* 2008; *Janković et al.,* 2008; *Škrinjar et al.,* 2012).

The variation in frequency of mycopopulation of spices cultivated on DG18 and DRBC media is most probably related to the strain type within one species. Environmental factors also have significant effect and can induce the growth of mycopopulation on lower a_w values (optimal temperature and type of nutritive components in the medium). Xerophiles, especially selective ones tend to be very sensitive on environmental conditions.

Askun et al. (2007) used Rose-Bengal chloramphenicol Agar (Oxoid, CM 549) and Dichloran-Glycerol (DG18) Agar (Oxoid, CM 729) for determination of xerophilic moulds. Other media can also be used such as Dichloran-Glycerol (DG18) Agar Base (*Pitt and Hocking,* 1985), MY70FG and MY50FG (*Beuchat,* 1998), MY50S and MY40S (*Beuchat and Hocking*, 1990).

Moulds fall into two ecological categories, e.g., field and storage moulds. Field moulds were observed to invade developing or mature seeds while they are on the plant, the major field moulds genera being *Alternaria*, *Fusarium* and *Cladosporium*. On the other hand, storage moulds are those encountered on plants in conditions of moisture commonly found in stored products. These moulds principally belong to species *Aspergillus* and *Penicillium* (*Abou Donia*, 2008). The spices can undergo fungal contamination mainly during spice processing, storage and transport (*Dimić et al.,* 2008).

Table 2. Review of moulds genera isolated from spices
Tabela 2. Pregled izolovanih rodova plesni iz začina

Sample	Genus	
	DG18	DRBC
1. Clove/Karanfilić	*Aspergillus* spp., *Penicillium* spp.	*Aspergillus* spp., *Penicillium* spp.
2. Rosemary/Ruzmarin	*Aspergillus* spp., *Penicillium* spp.	*Penicillium* spp.
3. Sesame/Susam	*Penicillium* spp.	*Penicillium* spp.
4. Ginger/Đumbir	*Aspergillus* spp., *Penicillium* spp.	*Aspergillus* spp., *Penicillium* spp.
5.White pepper ground/Mleveni beli biber	*Aspergillus* spp., *Penicillium* spp.	*Aspergillus* spp., *Penicillium* spp.
6. Black pepper ground/Mleveni crni biber	*Aspergillus* spp., *Paecylomyces* spp.	*Aspergillus* spp.
7. White pepper grain/Beli biber u zrnu	*Aspergillus* spp., *Penicillium* spp.	*Penicillium* spp.
8. Black pepper grain/Crni biber u zrnu	*Aspergillus* spp	*Aspergillus* spp
9. Oregano/Origano	*Aspergillus* spp., *Alternaria* spp.	*Aspergillus* spp.,
10. Caraway/Kim	*Penicillium* spp.	*Penicillium* spp.
11. Chilli/Čili	*Aspergillus* spp	*Aspergillus* spp
12. Curry/Kari	*Aspergillus* spp	*Aspergillus* spp
13. Thyme/Timijan	*Penicillium* spp.	*Penicillium* spp.
14. Bay leaf/Lovorov list	*Aspergillus* spp., *Alternaria* spp.	*Aspergillus* spp., *Alternaria* spp.
15. Sweet basil/Bosiljak	*Penicillium* spp.	*Penicillium* spp.

Conclusion

Based on obtained results, it can be concluded that it is necessary to use different selective media adjusted to specific requirements of xerophiles in order to achieve proper isolation and accurate contamination degree of spices by xerophilic moulds. Utilization of selective media enables acquiring representative insight into spices mycopopulation.

Our investigation showed that DG18, of all the media evaluated, showed better efficacy in determination and enumeration of moulds in spices, fungal diversity and the dominant presence of genus *Aspergillus* (47.82%) in spices used in meat industry.

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Komparativna mikološka analiza začina koji se koriste u industriji mesa

Janković Vesna, Borović Branka, Velebit Branko, Mitrović Radmila, Lakićević Brankica, Spirić Danka, Baltić Tatjana

R e z i m e: Jedan od mogućih izvora kontaminacije proizvoda od mesa su i začini. Kao i svi sušeni biljni materijali, začini nikada nisu sterilni. Sadrže, najčešće, sporogene bakterije i razne vrste plesni. Nezavisno od stepena kontaminacije, prisustvo plesni u začinima, a posledično i u proizvodima od mesa, pod određenim uslovima, može da dovede do sinteze toksičnih metabolita – mikotoksina. Sinteza mikotoksina uslovljena je, pre svega, genetskim faktorima, ali zavisi i od uslova spoljne sredine u kojoj se plesni razmnožavaju (temperatura, sastav supstrata, sadržaj vlage, pH sredine, aktivnost slobodne vode – a_w, prisustvo antifungalnih komponenti itd.).

Kao glavni kontaminenti začina navode se kserofilne vrste plesni, sa rodovima Eurotium, Aspergillus i Penicillium kao predstavnicima. S obzirom na to da začini predstavljaju potencijalni izvor kontaminacije proizvoda od mesa, cilj rada je bio da se utvrdi stepen rasprostranjenosti određenih vrsta plesni korišćenjem različitih selektivnih podloga.

Zbog činjenice da ne postoje odgovarajuće mikrobiološke podloge koje bi bile pogodne, istovremeno, za rast i određivanje „umereno" i „ekstremno" kserofilnih vrsta plesni, neophodno je kombinovanje selektivnih podloga kod kojih je a_w-vrednost znatno redukovana. Korišćenjem dve selektivne podloge DG 18 i DRBC, a u cilju izolovanja i utvrđivanja ukupnog broja plesni u začinima (n = 15), dobijeni su rezultati koji su dali preporuku za njihovu dalju primenu u sličnim ispitivanjima. Izolovane plesni klasifikovane su u četiri roda: Aspergillus, Alternaria, Paecylomyces i Penicillium. Na osnovu mikoloških istraživanja, DG18 podloga pokazala je izraženiji diverzitet i veći stepen efikasnosti u detekciji Aspergillus vrsta (47,82%). Preostale plesni pripadale su rodovima Penicillium (39,13%), Paecylomyces (8.69%) i Alternaria (4,34%). Za DRBC podlogu, utvrđen je isti nivo kontaminacije za Aspergillus spp. i Penicillium spp. Na osnovu dobijenih rezultata, može se zaključiti da je, u cilju izolovanja i utvrđivanja realne mikopopulacije plesni u začinima, neophodna primena selektivnih podloga koje su prilagođene specifičnim svojstvima ispitivanih kserofila.

Ključne reči: *a_w, selektivne podloge, kserofilne plesni.*

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Changes in the proximate and fatty acid composition in carp meat during the semi intensive farming

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S u m m a r y: The aim of this study was to examine and evaluate the proximate composition and fatty acid profiles of carp (Cyprinus carpio) during rearing in the semi-intensive farming conditions, supplementary fed extruded feed. Carp at the age of two years was submitted to trials, from spring to autumn, at the fish farm „Ečka" AD. Samples of carp were collected in April, June, September and October.

The protein content in fish sampled in September was significantly different from the protein content in fish sampled in April, June and October ($p < 0.001$), (17.48%, 17.27%, 18.28% and 17.26%, respectively). The quantities of total lipids slightly increased (2.25%, 2.37%, 3.02% and 4.72%, respectively) with the increase of the fish weight (598 g, 874 g, 1439 g and 1984 g, respectively), but significant increases occurred between September and October ($p < 0.001$). The moisture content decreased (79.55%, 78.86%, 77.46% and 75.72%, respectively). Principal Component Analysis (PCA) and Linear Discrimination Analysis (LDA) indicated that there were significant changes in the fatty acid composition of carp during growth. Starting from April to October the quantities of fatty acids were as follows: SFA (saturated fatty acids) – 28.47%, 28.97%, 24.86% and 23.66%, respectively; MUFA (monounsaturated fatty acids) – 38.57%, 40.52%, 41.68% and 42.43%, respectively; PUFA (polyunsaturated fatty acids) – 32.53%, 30.49%, 31.53% and 32.55%, respectively. The additional feeding of carp with the extruded feed influenced the increase in quantities of MUFA and n-6 PUFA (24.98%, 22.86%, 26.96% and 27.99%, respectively), and the decrease in quantities of the nutritionally important n-3 PUFA (5.13%, 6.59%, 4.57% and 4.57%, respectively). The highest n-3/n-6 ratio was obtained in June (0.30) and the lowest in October (0.16), indicating that the applied extruded feed was rich in n-6 and poor in n-3 PUFA. PCA and LDA have shown that significant changes in the fatty acid composition of carp during the breeding occured. Separation of the carp according to the sampling period was achieved by the LDA analysis, which is consistent with the type of ingested food.

Key words: *carp, semi intensive farming, proximate composition, fatty acids, analysis of variance (ANOVA), Principal component analysis (PCA), Linear discrimination analysis (LDA).*

Introduction

The limited resources of marine fish species and the growing demand for fish for human consumption have led to the expansion of aquaculture in many countries worldwide. Fatty acids (FA) which are provided by water resources play an important role in human nutrition (*Ackman*, 2000; *Hunter and Roberts*, 2000). Long-chain n-3 polyunsaturated fatty acids (PUFA) cannot be synthesized in the human body, and, therefore, they have to be ingested through diet (*Alasalvar et al.*, 2002). There are numerous studies (*Arts et al.,* 2001; *Von Shacky*, 2001; *Mozaffarian et al.,* 2004; *Givens et al.,* 2006; *Sahena et al.,* 2009; *Barcelo-Coblijn and Murphy*, 2009) on the favourable effect of n-3 polyunsaturated fatty acids from fish on human health, confirming that increased fish consumption has a role in the prevention of coronary heart disease, especially myocardial infarction, arteriosclerosis, hypertension and other cardiovascular diseases. In addition to the prevention of coronary heart disease and hypertension reduction, the beneficial effect of n-3 PUFA is reflected in the prevention of the inflammatory (*Moreno and Mitjavila,* 2003) and autoimmune diseases (*Zamaria,* 2004), and cancer (*Terry et al.,* 2004), diabetes (*Nettleton and Katz,* 2005), etc.

Cyprinidae fish family dominates world aquaculture, and the common carp is one of the oldest domesticated fish species for food (*Balon,* 2006). In Europe,

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particularly in the Central and Eastern Europe, cyprinids are one of the most important fish family in aquaculture production, and, among them, the common carp (*Cyprinus carpio)* is the most cultivated species. The dominant form of common carp production is the semi-intensive farming system, where the diet of the fish is based on a combination of natural food and supplementary feed (cereals, such as wheat, maize and barley). To improve and intensify the carp production cereals are replaced by extruded feed (*Steffens and Wirth,* 2007; *Marković et al.,* 2009).

The meat composition and fatty acid profile of farmed carp are, to great extent, influenced by diet (*Caballero et al.,* 2002*; Valente et al.,* 2007; *Ljubojević et al.,* 2012). Generally, under the same farming conditions, feed rich in n-3 fatty acids greatly increases the n-3/n-6 PUFA ratio in the fish tissue (*Robin and Skalli*, 2007; *Al-Souti et al.,* 2012). However, the lipid content and fatty acid composition of fish can differ within species depending on a variety of conditions, including gender, the state of the ecosystem inhabited by the fish, and the environmental conditions (*Źmijewski et al.,* 2006; *Vandeputte et al.,* 2008; *Prato and Biandolino*, 2012). Some other factors such as water temperature and its quality, the type and the availability of food, the season, age, and individual differences can influence these variations, as well (*Rasoarahona et al.,* 2004; *Guler et al.,* 2008; *Trbović et al.,* 2009).

Considering the carp farming, literature data indicate that changes in the muscle mass of fish, which are reflected on its nutritional value, are caused by genetic factors, diet and environmental conditions (*Gery et al.*, 1995; *Fauconneau et al.,* 1995*). It* has been demonstrated providing to carp high-energy feed, in order to stimulate growth and to shorten the breeding time, mostly contributes to the increase in fat content, and protein content remains constant (*Kaushik,* 1995).

Fatty acid composition of farmed fish differs from the fatty acid composition of the fish from open waters, mainly because of diet, and fish from open waters is considered to contain larger amounts of n-3 PUFA. However, some research indicate that farmed fish contain higher amounts of n-3 PUFA, compared to the fish from open waters, when the fatty acids are expressed as mg/100 g of the fish, instead as a weight percentage of the total fatty acids (*Cahu et al*. 2004).

Convenient climate conditions, and numerous rivers and rivers' accumulations in the lowlands of the country, contributed to a long-standing tradition in the cultivation of cyprinids, mainly carp, and in the creation of habits for carp consumption. Thus, freshwater fish belonging to the cyprinid family became economically and nutritionally important for Serbian population, and carp is nowadays one of the most cultivated fish species in the country. The increasing demands for higher productivity of carp farms, and for higher quality of the carp meat are contributing to improving the farming conditions. Carp is cultivated on farms with semi-intensive production systems, in which, except naturally occurring food, fish is additionally fed extruded feed or cereals.

Multivariate data analysis might correlate the fatty acid composition of the fish fed different diets to the fatty acid profiles of the feed *(Barrado et al.,* 2003). The use of multivariate methods, such as principal component analysis (PCA) and linear discrimination analysis (LDA), enables a better understanding of the fatty acid composition of the carp meat according to the fish diet and summarizes the statistical correlation among fatty acids.

The aim of this study was to investigate and evaluate the proximate composition and fatty acid profiles of carp during rearing in semi-intensive farming conditions supplementary fed extruded feed. Data on the effect of supplementary diet on the lipid content and fatty acid composition of carp will be used to improve the nutritional value of carp meat.

Materials and methods

Fish samples

One-year old carp was submitted to trials from spring to autumn at the fish farm „Ečka"AD, a farm with semi-intensive carp breeding system. The conditions on the farm were convenient for carp breeding, since the historical data indicate that an organized carp production started in the year 1891 (www.ribnjakecka.com). Carp samples were collected from spring to fall (April, June, September and October). Except naturally occurring food, according to the breeding season and to the fish farm productivity, fish were additionally fed extruded feed consisting of maize, soybean meal and fish meal. The feed contained 23.81% proteins and 6.97% lipids. Feed provided to the fish was as follows: in April 0.1% to 0.3%, in May 0.3% to 1%, in June 1% to 2%, in July and August 3%, in September 2% to 3%, with respect to fish biomass and depending on the water temperature, its saturation with oxygen and on the amount of accessible natural food. The weight of each fish was determined in the laboratory, on a technical balance. The fish samples were kept at -25ºC until analyses. Before analysis, fish was left at room temperature for an hour to defrost partly, so that the skin, heads, tails, fins and intestines could be removed, and fish afterwards was

filleted. Fish fillets were disintegrated in a CombiMax 600 blender (Braun GmbH, Kronberg, Germany). Determination of proximate composition was performed in triplicate, while fatty acid analyses in duplicate.

Chemicals and standards

The chemicals for proximate composition analysis were of analytical grade purity. The solvents for GC analysis were of GC-grade purity, obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich (Munich, Germany). Following regular cleaning according to the standard laboratory procedure, all glassware was rinsed sequentially with acetone and hexane. Solvent blanks were checked whenever new lots of reagents were used. The reagent for derivatization of fatty acids, 0.25 M TMSH *(*trimethylsulphonium hydroxide) in methanol, grade for GC derivatization, was purchased from Fluka (Buchs, Switzerland). Heneicosanoic acid methyl ester (p.a. ≥99%, Fluka, Buchs, Switzerland) was used as internal standard.

The standards used for determination of fatty acids (Supelco 37 comp. FAME mix, 10 mg mL^{-1} in CH_2Cl_2), analytical standard grade, were purchased from Supelco (Bellefonte, USA). Before gas chromatographic analysis all sample extracts were filtered through a 0.2 µm nylon syringe filters (Nipro Europe N.V., Zaventem, Belgium).

Proximate composition analysis

The proximate composition of fish samples was determined using standard SRPS ISO methods. Protein content in fish filets (N × 6.25) was determined by the Kjeldahl procedure on a Kjeltec Auto 1030 Analyzer (Tecator, Höganäs, Sweden). Moisture content was determined by drying of samples at 103 ± 2°C to constant mass (SRPS ISO 1442:1998). Total fat content was determined by extraction of the weighted amount of fish flesh with petroleum ether (30–50°C b.p.) in a Soxhlet apparatus, after acid hydrolysis of the sample (SRPS ISO 1443:1992). The ash content in the sample was determined by dry ashing at 550 ± 25°C (SRPS ISO 936:1999).

GC analysis of fatty acid

Total lipids were extracted from the fish fillets using accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA, USA), as previously reported (*Spirić et al.,* 2010). Fatty acid methyl esters (FAME) were prepared by transesterification using 0.25M TMSH (EN ISO 5509:2000). Prior to transesterification, 0.05 mL (10 mg/mL) of heneicosanoic acid methyl ester solution was added as an internal standard.

Fatty acid methyl esters were determined by GC Shimadzu 2010 (Kyoto, Japan) equipped with a split/splitless injector, fused silica cyanopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 µm, J&W Scientific, Orangevale, CA, USA), flame ionization detector and work station. The injection volume was 1 µL, in the split ratio of 1:50. Nitrogen was used as carrier gas at flow rate of 1.33 mL min^{-1}. The injector and detector temperatures were 250ºC and 280ºC, respectively. Hydrogen and air were used as flame gases, at flow rates of 40 mL min^{-1} and 400 mL min^{-1}, respectively. Nitrogen was used as a make-up gas at flow rate of 30 mL min^{-1}. The programmed column oven temperature, starting at 125ºC and ending at 230ºC, was applied. More detailed data on the operating conditions have been previously reported (*Trbović et al.,* 2013). Total analysis time was 50.5 min. Chromatographic peaks in the samples were identified by comparing their relative retention times to FAME peaks retention times in the Supelco 37 Component FAME mix standard. Chromatographic peak areas were corrected by response factors. Response factors were calculated by the ratios between the peak area of the individual fatty acid methyl ester and of the internal standard. Relative quantities of fatty acids were expressed as weight% of the total fatty acids. The signal to noise (S/N) ratio was used for the estimation of the limit of detection, LOD (LOD = 3×S/N) and of the limit of quantification, LOQ (LOQ = 10×S/N).

Statistical analysis

Analysis of variance (ANOVA) with Tukey – Kramer test was used to analyze the data at P = 0.05 level. Principal component analysis (PCA) and linear discrimination analysis (LDA) were performed using JMP 8.0.1 software (SAS Institute Inc. NC, USA).

Results and discussion

Data on the water temperature on the farm, and the average carp weight during rearing are presented in Table 1. A significant increase in the fish weight between June and September ($p < 0.001$), and September and October ($p < 0.001$) was established. The significant increase in the carp weight was a consequence of the intensive feeding of fish during summer, when carp consumed large quantities of supplementary feed. The favourable environmental conditions in the aquatic environment contributed to the increase of fish biomass, as well.

Table 1. Water temperature and carp weight during rearing
Tabela 1. Temperatura vode i masa šarana u toku uzgoja

	April (n = 6)	June (n = 7)	September (n = 7)	October (n = 8)
Water temperature, °C/ Temperatura vode, °C	14	22	20	6
Carp weight, g/ Masa šarana, g	598 ± 162^{C}	874 ± 142^{C}	1439 ± 173^{B}	1984 ± 322^{A}

n – number of samples; $^{A, B, C}$ – Values in the same row followed by the same letters do not differ significantly (P>0.05)/
n – broj uzoraka; $^{A, B, C}$ – Vrednosti u istom redu sa istim slovnim oznakama se značajno ne razlikuju (P>0.05)

Data for the proximate composition of carp during rearing are presented in Table 2.

The protein content in fish sampled in September was significantly different from the protein content in fish sampled in April, June and October ($p<0.001$). The total lipids slightly increased with the increasing size of the fish, but a significant increase occurred from September to October ($p<0.001$). On the contrary, the moisture content decreased ($p<0.001$). Generally, the total lipids in the carp meat were in the range from 2.25–4.72%, what classifies the carp from aquaculture in a low fatty fish (*Huss,* 1995). Ash content was significantly different in carp sampled in June from the carp sampled in September ($p<0.01$) and October ($p<0.01$). As ash content is endogenously regulated, this might be a consequence of biological changes during the fish growth (*Shearer,* 1994).

Fatty acid composition (% of total fatty acids) of carp during rearing is presented in Table 3.

From the presented data, it is noticable that the levels of MUFA significantly increased during fish grow, while the levels of SFA decreased. The share of total PUFA in the fillets did not change significantly during carp rearing ($p>0.05$).

ANOVA test indicated that between June and September the content of n-6 PUFA significantly increased ($p<0.01$), while the content of n-3 PUFA decreased ($p<0.01$), what is associated with an increased feed intake during summer period, The increase in n-6 PUFA led to a reduction in the n-3/n-6 ratio, and, thus, to the reduction of the quality of the fish. The n-3/n-6 ratio was the highest in June (0.30), and the lowest in October (0.16), indicating the quality of the carp feed, which was rich in n-6 and poor in n-3 PUFA, in October. *Henderson and Tocher* (1987) have reported n-3/n-6 values of 0.5–3.8 for freshwater fish.

Changes in the fatty acid profiles in carp during rearing are better visualized by PCA (Figure 1 and 2).

Table 2. Proximate composition of carp during rearing
Tabela 2. Hemijski sastav šarana u toku uzgoja

Chemical parameters/ Hemijski parametri	April (n = 6)	June (n = 7)	September (n = 7)	October (n = 8)
Proteins,%/Proteini,%	17.48 ± 0.62^{B}	17.27 ± 0.47^{B}	18.28 ± 0.29^{A}	17.26 ± 0.30^{B}
Moisture,%/Vlaga,%	79.55 ± 1.14^{A}	78.86 ± 0.60^{AB}	77.46 ± 1.22^{B}	75.72 ± 0.93^{C}
Total lipids,%/Ukupni lipidi,%	2.25 ± 0.71^{B}	2.37 ± 0.29^{B}	3.02 ± 1.03^{B}	4.72 ± 0.71^{A}
Ash,%/Pepeo,%	1.17 ± 0.11^{AB}	1.26 ± 0.13^{A}	1.05 ± 0.06^{B}	1.11 ± 0.06^{B}

n – number of samples; $^{A, B, C}$ – Values in the same row followed by the same letters do not differ significantly (p>0.05)
n – broj uzoraka; $^{A, B, C}$ – Vrednosti u istom redu sa istim slovnim oznakama se značajno ne razlikuju (p>0.05)

Table 3. Fatty acid composition (% of total fatty acids) of carp during rearing
Tabela 3. Sastav masnih kiselina (% od ukupnih masnih kiselina) šarana u toku uzgoja

Fatty acids/ Masne kiseline	April (n = 6)	June (n = 7)	September (n = 7)	October (n = 8)
14:0	1.23 ± 0.24^{A}	1.21 ± 0.15^{A}	0.82 ± 0.05^{B}	0.84 ± 0.06^{B}
15:0	0.34 ± 0.22^{A}	0.33 ± 0.16^{A}	0.22 ± 0.03^{A}	0.18 ± 0.04^{A}
16:0	19.89 ± 2.41^{AB}	20.86 ± 1.13^{A}	18.28 ± 0.89^{BC}	17.80 ± 0.76^{C}
16:1	6.32 ± 1.13^{A}	5.43 ± 1.32^{AB}	3.97 ± 0.43^{B}	5.01 ± 0.83^{AB}
17:0	0.64 ± 0.31^{A}	0.61 ± 0.09^{A}	0.39 ± 0.04^{B}	0.34 ± 0.08^{B}
18:0	6.37 ± 1.04^{A}	5.95 ± 0.52^{A}	5.15 ± 0.44^{B}	4.48 ± 0.28^{C}
18:1n-9	26.68 ± 3.20^{B}	30.74 ± 1.58^{AB}	33.55 ± 2.59^{A}	33.09 ± 2.46^{A}
18:1n-7	3.93 ± 1.19^{A}	2.84 ± 0.32^{B}	2.42 ± 0.08^{B}	2.57 ± 0.18^{B}
18:2n-6	22.30 ± 4.19^{B}	21.45 ± 3.24^{B}	25.04 ± 0.62^{AB}	26.09 ± 1.81^{A}
18:3n-6	0 ± 0^{C}	0.12 ± 0.20^{CB}	0.25 ± 0.04^{B}	0.34 ± 0.05^{A}
18:3n-3	2.24 ± 0.52^{B}	3.86 ± 0.54^{A}	2.12 ± 0.34^{B}	2.23 ± 0.27^{B}
20:1	1.74 ± 0.07^{A}	1.51 ± 0.08^{B}	1.70 ± 0.24^{AB}	1.76 ± 0.24^{A}
20:2	1.02 ± 0.13^{A}	0.66 ± 0.12^{B}	0.81 ± 0.13^{B}	0.68 ± 0.09^{B}
20:3n-6	1.41 ± 0.49^{A}	0.64 ± 0.08^{B}	0.86 ± 0.44^{B}	0.85 ± 0.41^{B}
20:3n-3	0.50 ± 0.09^{AB}	0.50 ± 0.10^{B}	0.70 ± 0.22^{A}	0.47 ± 0.11^{B}
22:1+20:4	2.41 ± 0.19^{A}	1.04 ± 0.28^{B}	1.25 ± 0.14^{B}	1.35 ± 0.28^{B}
20:5n-3	0.89 ± 0.26^{A}	0.96 ± 0.26^{A}	0.52 ± 0.16^{B}	0.58 ± 0.13^{B}
22:5n-3	0.52 ± 0.12^{A}	0.46 ± 0.13^{A}	0.29 ± 0.09^{B}	0.28 ± 0.07^{B}
22:6n-3	1.21 ± 0.29^{A}	0.81 ± 0.20^{A}	0.94 ± 0.22^{A}	1.01 ± 0.27^{A}
SFA	28.47 ± 3.92^{A}	28.97 ± 1.25^{A}	24.86 ± 1.03^{B}	23.66 ± 0.80^{B}
MUFA	38.57 ± 2.03^{B}	40.52 ± 2.48^{AB}	41.68 ± 2.59^{A}	42.43 ± 2.93^{A}
PUFA	32.52 ± 3.38^{A}	30.49 ± 3.12^{A}	31.53 ± 1.91^{A}	32.55 ± 2.37^{A}
n-3	5.13 ± 0.90^{B}	6.59 ± 0.89^{A}	4.57 ± 0.59^{B}	4.57 ± 0.66^{B}
n-6	24.98 ± 3.83^{AB}	22.86 ± 3.24^{B}	26.96 ± 1.75^{A}	27.99 ± 1.91^{A}
n-3/n-6	0.21 ± 0.06^{B}	0.29 ± 0.07^{A}	0.17 ± 0.02^{BC}	0.16 ± 0.02^{C}

n – number of samples; $^{A, B, C}$ – Values in the same row followed by the same letters do not differ significantly ($p > 0.05$)
n – broj uzoraka; $^{A, B, C}$ – Vrednosti u istom redu sa istim slovnim oznakama se značajno ne razlikuju ($p > 0.05$)

PCA of the fatty acid profiles, taking carp weight and lipid content as variables, resulted in two principal components model describing 60.3% of the total data variability. In particular, PC1 explained 42.8% of the variability and PC2 explained about 17.5%. The score plot of the first two principal components (Figure 1) indicated to the grouping of carps during growth according to the months of sampling.

Considering groups of FA and the most important fatty acids, such as oleic, 18:1n-9; linoleic, 18:2n-6; linolenic acid, 18:3n-3; EPA, 20:5n-3; DPA, 22:5n-3 and DHA, 22:6n-3, the PCA clearly differentiated carps according to the period of sampling.

As it can be seen from the Figure 2, oleic acid contributed to the great extent to the variability on the positive part of the PC1. High positive correlation of oleic acid with carp weight and total lipids ($r > 0.6$; $p < 0.0001$) indicated that the total lipids and the content of oleic acid increased with the increase of carp weight. Linoleic acid that contributed to the positive part of the PC2 enabled to distinguish carp in September and October with higher amounts of this fatty acid.

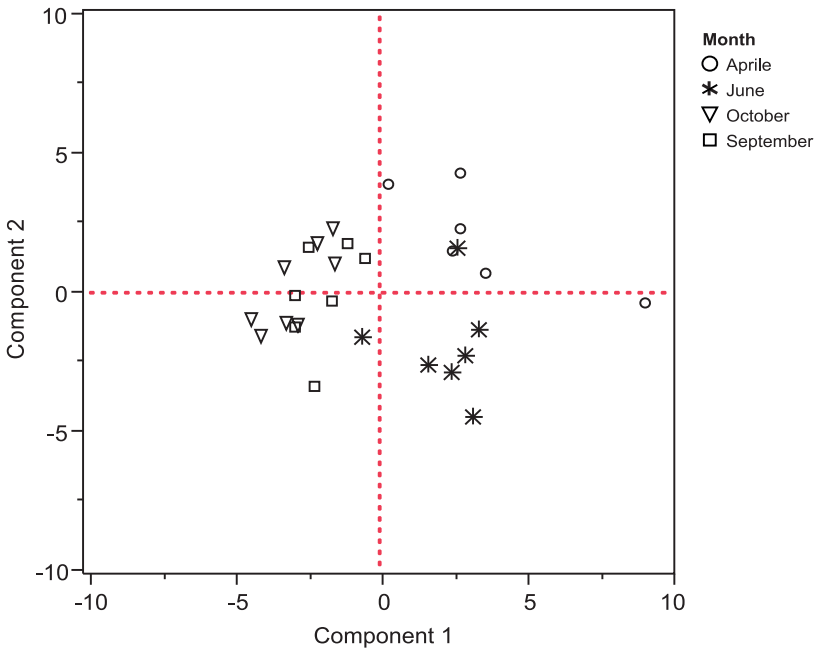

Figure 1. Principal component analysis score plot of the fatty acid profiles of carps during rearing
Slika 1. Vrednosti skorova dobijenih analizom glavnih komponenti za sastav masnih kiselina šarana u toku uzgoja

On the negative part of the PC1, DPA, EPA and linolenic acid correspond to carp which contained higher quantities of these fatty acids in April and June. Carp can adjust the fatty acid metabolism to the prevailing temperature in such a way that increases in the temperature gives rise to the increased metabolism of saturated fatty acids (*Tocher,* 2003). This is, probably, the reason why SFA were presented in June in higher quantities, when temperature was the highest. The presence of the linolenic acid and of the long-chain PUFA (EPA, DPA) in carps in April and June could be a consequence of the higher intake of natural food. The natural food, represented by plankton and benthos, is a source rich in linolenic

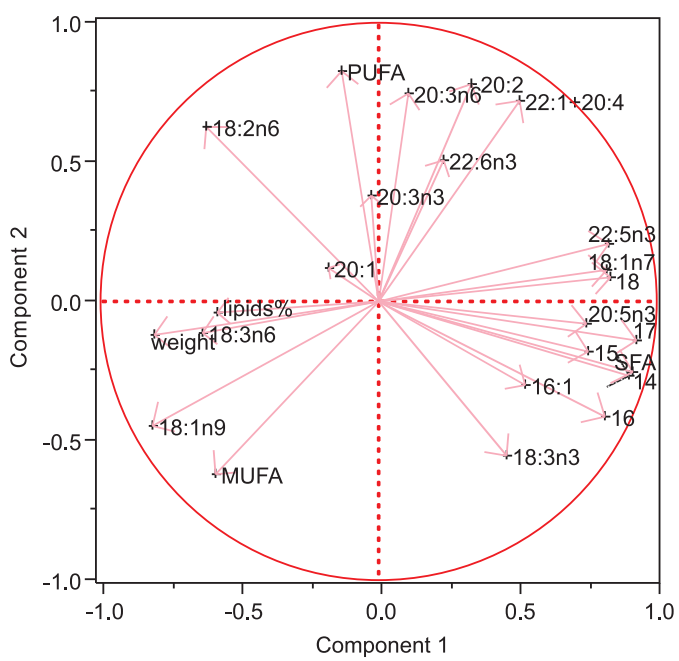

Figure 2. Principal component analysis loading plot of the fatty acid profiles of carps during rearing
Slika 2. Projekcija vektora dobijenih analizom glavnih komponenti za sastav masnih kiselina šarana u toku uzgoja

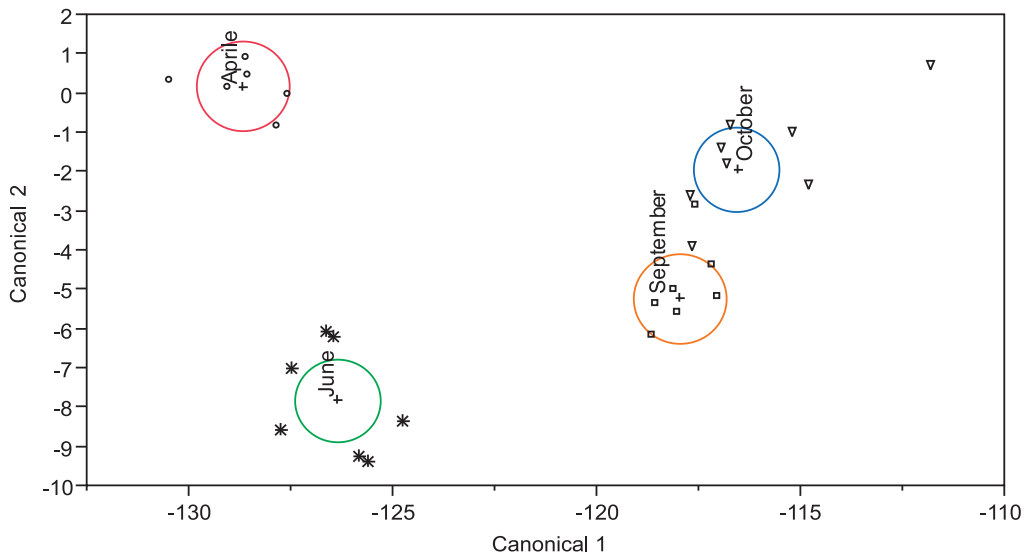

Figure 3. Canonical plot of the fatty acid profiles of carps during rearing
Slika 3. Kanonični prikaz sastava masnih kiselina šarana u toku uzgoja

acid and EPA (*Domaizon et al.,* 2000; *Bogut et al.,* 2007; *Živic et al.,* 2011), but poor in DHA. *Bell et al.* (1994) have reported that DHA in freshwater invertebrates was present in small amounts. The availability of natural food in April and June, probably, caused an increase in the content of n-3 fatty acids in carp, what consequently resulted in a better quality of the fish meat.

The separation of carps during rearing might be improved by the linear discrimination analysis. From the data presented in Figure 3, a clear differentiation of carps in four groups is noticable, according to the months of sampling. The grouping was very satisfactory, and allowed 96% of the fish to be correctly grouped. Out of the 28 tested samples, 27 were classified according to the months of sampling.

LDA demonstrated that the first discriminant eigenvalue (27.7) explained 67% of the total variance and the second eigenvalue (11.7) explained 28% of the total variance. The established Wilks value was equal to 0.0009 ($p<0.0001$). By canonical correlation, the first and the second discriminant functions were established to be 0.982 and 0.960, respectively.

As the distances between the points on the canonical plot are shorter, the differences in the FA profiles of the fish samples are smaller. As it can be seen, fish in April and June are distant one from the other and far from September and October, which is in correlation to the type of the ingested food in that period. The shortest distance, e.g. the greatest similarity in the FA profiles, was observed between carps in September and October, due to the reduction of natural food on the farm and to the higher intake of the supplementary feed.

Conclusion

The obtained data indicate that the protein content in fish sampled in September was significantly different from the protein content in fish sampled in April, June and October ($p<0.001$). The quantities of the total lipids slightly increased with the increase of the fish weight. But, significant increase occurred from September to October ($p<0.001$). On the contrary, the moisture content decreased.

Based on the PCA and LDA, it can be concluded that there were significant changes in the fatty acid composition of carp during the investigating period of growth. Except supplementary feed, the availability of natural food on the carp farm influenced the fatty acid composition of carp during rearing. However, the additional feeding of carp with extruded feed influenced the increase in quantities of MUFA and n-6 PUFA, and the decrease in the quantities of nutritionally important n-3 PUFA. The highest n-3/n-6 ratio was obtained in June (0.30), and the lowest in October (0.16), indicating that the applied extruded feed was rich in n-6 and poor in n-3 PUFA. Analysis of the fatty acid composition in combination with multivariate analysis is a powerful tool in differentiation of carp during rearing according to the food available on the farm, and to the offered supplementary feed, as well. Based on this analysis, it can be concluded that the quality of supplementary feed has to be improved in order to achieve better nutritional quality of the final product.

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Promene hemijskog i masnokiselinskog sastava mesa šarana u toku poluintenzivnog uzgoja

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R e z i m e: Cilj ovog rada bio je ispitivanje osnovnog hemijskog i masnokiselinskog sastava šarana (Cyprinus carpio) u toku uzgoja u poluintenzivnom sistemu uz prihranjivanje ribe ekstrudiranom hranom, kao i statistička evaluacija dobijenih rezultata. Eksperiment je realizovan od proleća do jeseni, na ribarskom gazdinstvu „Ečka" AD, a korišćen je dvogodišnji šaran u nasadu za dvogodišnji. Uzorci šarana su uzimani u toku aprila, juna, septembra i oktobra meseca.

Sadržaj proteina u šaranu koji je uzorkovan u septembru značajno se razlikovao od sadržaja proteina u šaranu koji je uzorkovan u aprilu, junu i oktobru ($p < 0,001$), (17,48%, 17,27%, 18,28% i 17,26%, respektivno). Količine ukupnih lipida su blago rasle (2,25%, 2,37%, 3,02% i 4,72%, respektivno) sa povećanjem mase ribe (598 g, 874 g, 1439 g i 1984 g, respektivno), a između septembra i oktobra došlo je do značajnog povećanja ukupnih lipida ($p < 0,001$). Sadržaj vlage se smanjivao (79,55%, 78,86%, 77,46% i 75,72%, respektivno). Analiza glavnih komponenti (Principal Component Analysis, PCA) i diskriminaciona linearna analiza (Linear Discrimination Analysis, LDA) ukazuju da je u toku perioda rasta ribe došlo do značajnih promena u sastavu masnih kiselina. U periodu istraživanja, od aprila do oktobra, količine masnih kiselina su bile sledeće: ZMK (zasićene masne kiseline) – 28,47%, 28,97%, 24,86% i 23,66%, respektivno; MNMK (mononezasićene masne kiseline) – 38,57%, 40,52%, 41,68% i 42,43%, respektivno, PNMK (polinezasićene masne kiseline) – 32,.53% , 30,49, 31,53% i 32,55%%, respektivno. Prihranjivanje šarana ekstrudiranom hranom uticalo je na porast količina MNMK i n-6 PNMK (24,98%, 22,86%, 26,96% i 27,99%, respektivno), kao i na smanjenje količina nutritivno važnih n-3 PNMK (5,13, 6,59%%, 4,57% i 4,57%, respektivno). Najveći odnos n-3/n-6 masnih kiselina dobijen je u junu (0,30), a najmanji u oktobru (0,16), što ukazuje da je ekstrudirana hrana koja je na ribnjaku korišćena bila bogata sa n-6 i siromašna sa n-3 PNMK. PCA i LDA su pokazale da je došlo do značajnih promena u sastavu masnih kiselina šarana tokom uzgoja. LDA analizom postignuto je razdvajanje šarana prema periodu uzorkovanja, a što je u korelaciji sa vrstom unete hrane.

Ključne reči: *šaran, poluintenzivni uzgoj, osnovni hemijski sastav, masne kiseline, analiza varijansi (ANOVA), analiza glavnih komponenti (PCA), linearna diskriminaciona analiza (LDA).*

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Hemijski sastav, sadržaj holesterola i sastav masnih kiselina šarana (*Cyprinus carpio*) iz slobodnog izlova, poluintenzivnog i kaveznog sistema gajenja

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S a d r ž a j: Poznavanje hemijskog sastava mesa riba je važno, pošto je njegova potrošnja u porastu, na osnovu preporuka da ova vrsta mesa treba da bude bitna komponenta zdrave ishrane. Osnovni cilj istraživanja je bio ispitivanje i poređenje rezultata analiza mesa šarana iz slobodnog izlova (Dunav), poluintenzivnog sistema (RG „Ečka") i kaveznog sistema gajenja (kavezni sistem Vrbas). Rezultati ispitivanja su pokazali da je postojala statistički značajna razlika ($P < 0,05$) u sadržaju vode (73,58; 78,31 i 70,32%, respektivno) i masti (6,95; 3,14; 9,79%, repektivno) u ispitanim uzorcima šarana prema načinu gajenja. Meso šarana sa RG „Ečka" sadržalo je manje masti u poređenju sa mesom šarana koji je izlovljen iz Dunava. Sadržaj proteina se nije značajno razlikovao između ispitanih uzoraka šarana. Sadržaj holesterola je bio značajno veći u uzorcima mesa riba iz Dunava (45,49 mg/100g) i ribnjaka Ečka (49,64 mg/100g), u odnosu na uzorke mesa šarana iz kaveznog sistema (26,53 mg/100g). Palmitinska kiselina je bila najzastupljenija zasićena masna kiselina (polysaturated fatty acids) u mesu sve tri grupe šarana i postojala je statistički značajna razlika ($P < 0,05$), između grupa, pri čemu je najviši sadržaj izmeren kod šarana iz Dunava. Ukupan zbir je bio najveći u šaranima iz Dunava, zatim slede Ečka i Vrbas (27,59; 25,44 i 17,18% respektivno). Sadržaj mononezasićenih masnih kiselina (monosaturated fatty acids), je bio najveći u uzorcima fileta šarana iz Dunava (52,94%), a najmanji u uzorcima mesa ribe iz kaveznog sistema gajenja (37,25%). Sadržaj najdominantnije, oleinske kiseline, bio je približno isti u sve tri grupe šarana. Ukupan sadržaj polinezasićenih masnih kiselina (polyunsaturated fatty acids) je bio najveći u uzorcima šarana iz kaveznog sistema gajenja (45,46%), a najmanji u mesu šarana iz Dunava (19,60%).

Razlog za to je visoki sadržaj linolne kiseline u uzorcima šarana iz kaveznog sistema, koji je bio dva puta veći u odnosu na šarana iz Ečke, a gotovo 5 puta veći u odnosu na šarana iz Dunava. Najpovoljniji ω-3/ω-6 odnos je ustanovljen u filetima dunavskog šarana (0,44%), a najnepovoljniji kod šarana iz kaveznog sistema gajenja (0,10%). U filetima šarana iz ribnjaka AD „Ečka" ustanovljen je ω-3/ω-6 odnos od 0,29. Rezultati ovog istraživanja doprinose boljem poznavanju nutritivnog kvaliteta mesa šarana iz slobodnog izlova i šarana iz akvakulture i mogu biti od značaja prilikom formulisanja kompletnih smeša za ishranu šarana, u cilju postizanja optimalnijih proizvodnih rezultata, poželjnog kvaliteta mesa, a ujedno i ekonomski isplativije proizvodnje.

Ključne reči: *šaran, slobodan izlov, poluintenzivni sistem, kavezni sistem gajenja, hemijski sastav, holesterol, masne kiseline.*

Uvod

Ishrana ribom obezbeđuje ljudskom organizmu dovoljne količine proteina, slobodnih amino-kiselina, minerala i vitamina (*Ackman*, 2000), a, pored toga, i dovoljne količine polinezasićenih masnih kiselina (PUFA, polysaturated fatty acids), a posebno n-3 PUFA (*Kminková i dr*., 2001). Postoje čvrsti dokazi da PUFA snižavaju nivo holesterola u krvi, tako da one mogu biti značajne u prevenciji kardiovaskularnih bolesti (*Conor i Conor*, 2010), a poznato je i da smanjuju mortalitet kod pacijenata sa koronarnim oboljenjima (*Kris-Etherton i dr*., 2002). Pored toga, n-3 PUFA smanjuju sadržaj triacilglicerola, kao i lipoproteina niske gustine u serumu ljudi i inhibiraju agregaciju trombocita, sprečavanju oštećenja krvnih sudova i veoma su važne u prenatalnom razvoju nervnog sistema (*Allen i Harris*, 2001). Dokazana je njihova uloga u poboljšanju sposobnosti za učenje (*Suzuki i dr*., 1998). Takođe, PUFA kako serije n-6, a posebno n-3 učestvuju u prevenciji bolesti nervnog sistema i imaju važnu ulogu u ontogenezi (*Arts i dr*.,

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2001). Poznato je i da ljudski organizam ne može sintetisati masne kiseline dugog lanca (n-3 PUFA) i da se one moraju uneti hranom (*Alasalvar i dr*., 2002). Pošto se n-3 PUFA, kao što su α-linoleinska kiselina (18:3 n-3, ALA), eikozapentaenska (20:5 n-3, EPA) i dokozaheksaenska (22:6 n-3, DHA), efikasno sintetišu samo kod akvatičnih organizama, ljudi mogu uneti u organizam ove esencijalne masne kiseline konzumiranjem morskih i slatkovodnih riba (*Sushchik i dr*., 2007).

Hemijski sastav, vrsta i količina masnih kiselina u mesu riba veoma varira u zavisnosti od načina ishrane (*Ćirković i dr*., 2011; *Ljubojević i dr*., 2013b), veličine i starosti ribe, reproduktivnog statusa, geografskog položaja, godišnjeg doba (*Celik i dr*., 2005; *Guler i dr*., 2008; *Buchtova i dr*., 2010). Hemijski sastav mesa riba iz otvorenih voda je pod velikim uticajem uslova životne sredine, koji određuju dostupnost hranjivih materija (*Izquierdo i dr*., 2003), dok se kod riba iz uzgoja, koje se hrane industrijski proizvedenom hranom, nutritivne materije, obezbeđuju iz te hrane (*Periago i dr*., 2005). Međutim, sadržaj proteina u mesu je pod manjim uticajem ishrane, pošto on najviše zavisi od egzogenih faktora, kao što su vrsta ribe, starost i veličina (*Shearer*, 1994).

Upotreba ribe u ishrani ljudi danas je u porastu, što je u skladu sa preporukama koje ukazuju na to da je ova vrsta mesa bitna komponenta zdrave ishrane. Za potrošače je značajno poznavanje sadržaja holesterola u mesu riba. Postoje studije koje ukazuju na povezanost holesterola unetog hranom, holesterola u krvnoj plazmi i ateroskleroze (*Orban i dr*., 2006). Osim povećanog unosa holesterola hranom, na nivo holesterola u krvi utiču i drugi faktori, kao što su prekomeran unos energije, povećan unos pojedinih zasićenih masnih kiselina (SFA, saturated fatty acids) i trans-izomera nekih nezasićenih masnih kiselina (USFA, unsaturated fatty acids), (*Kris-Etherton i dr*., 2002). Prema podacima iz literature, sadržaj holesterola u mišićnom tkivu šarana značajno varira, u zavisnosti od vrste ribe, starosti, sistema gajenja i godišnjeg doba (*Bieniarz i dr*., 2001; *Ćirković i dr*., 2011). Neki autori (*Kopicova i Vavreinova,* 2007; *Piironen i dr*., 2002) su proučavali sadržaj holesterola u mesu šarana i drugih vrsta riba i ustanovili su isti ili nešto veći nivo holesterola kod većine ispitanih riba u poređenju sa govedim i svinjskim mesom.

Rašireno je verovanje potrošača da je kvalitet mesa riba iz slobodnog izlova bolji od mesa gajenih riba (*Mairesse i dr*., 2005). Ukoliko se sagleda bezbednost potrošača, treba imati u vidu da je, prema dosadašnjim istraživanjima, sadržaj kontaminenata u mesu riba iz ribnjaka daleko ispod maksimalno dozvoljenih granica (*Đinović i dr.,* 2010), što nije bio slučaj sa ribom izlovljenom iz Dunava u okolini Beograda (*Trbović i dr.,* 2011; *Đorđević i dr*., 2013).

U ovom radu je izvršeno ispitivanje, a zatim poređenje hemijskog sastava, uključujući sadržaj holesterola i masnih kiselina, mesa šarana iz slobodnog izlova, šarana gajenog u poluintenzivnom sistemu na najvećem ribnjaku u Republici Srbiji (RG „Ečka“) i šarana gajenog u kaveznom sistemu u Vrbasu, a u cilju boljeg poznavanja nutritivnog kvaliteta mesa šarana iz slobodnog izlova i šarana iz akvakulture. Dobijeni rezultati mogu biti od koristi prilikom formulisanja kompletnih smeša za ishranu šarana, kako bi se postigao što poželjniji kvalitet mesa.

Materijal i metode

Uzorci šarana, približne mase oko 2100 g, uzeti su u jesenjem periodu iz slobodnog izlova (Dunav), sa ribnjaka RG „Ečka“, gde se riba proizvodi u poluintenzivnom sistemu uz dodavanje gotovih smeša, i iz kaveznog sistema u blizini Vrbasa. Po šest uzoraka ribe je uzeto za svaku grupu i, u ručnim frižiderima, otpremljeno u laboratoriju Instituta za higijenu i tehnologiju mesa, iz Beograda. Priprema uzoraka za ispitivanje je izvršena tako što je riba odmrznuta, skinuta je koža, odstranjeni su glava i rep i obavljena je evisceracija. Za analize su korišćeni fileti dobijeni od dorzalnih mišićnih partija.

Analiza osnovnog hemijskog sastava ribe i holesterola

Ispitivanje osnovnog hemijskog sastava mesa ribe je izvršeno prema metodama koje su, u svom radu, naveli *Trbović i dr*. (2009). Sadržaj proteina (N × 6,25) određen je na aparatu Kjeltec Auto 1030 Analyzer (Tecator, Sweden), metodom po Kjeldahlu; sadržaj vode – sušenjem do konstantne mase; sadržaj ukupne masti – upotrebom aparature po Soxletu, a sadržaj pepela žarenjem na temperaturi od 550°C.

Sadržaj holesterola određen je metodom visoko efikasne tečne hromatografije, na aparatu HPLC Waters-2965 Separation modul, sa PDA detektorom (Waters 2996 Photodiodearray detector) prema metodi *Maraschiello i dr*. (1996).

Određivanje sastava masnih kiselina

Ispitivanje masnokiselinskog sastava je izvršeno kapilarnom gasnom hromatografijom, prema metodi koju su opisali *Spirić i dr.* (2010). Nakon ASE ekstrakcije ukupnih masti smešom n-heksana

i 2-propanola i transesterifikacije sa trimetilsulfonijum hidroksidom, masne kiseline su određene kao metilestri masnih kiselina, tehnikom kapilarne gasne hromatografije na apartu GC Shimadazu 2010 (Kyoto, Japan).

Statistička obrada podataka

Dobijeni rezultati su obrađeni u programskom paketu Statistica 10.0. Rezultati su prikazani kao aritmetička sredina ± standardna devijacija. Korišćena je analiza varijanse (ANOVA) na nivou značajnosti $p = 0,05$, a kao post-hoc test je upotrebljen Tukey HSD test.

Rezultati i diskusija

Rezultati ispitivanja hemijskog sastava i sadržaja holesterola u filetima šarana iz sve tri grupe uzoraka su prikazani u tabeli 1. Sadržaj vode i sadržaj masti se statistički značajno razlikovao između ispitanih grupa ($p < 0,05$). Sadržaj proteina je bio najveći kod šarana iz poluintenzivnog sistema gajenja (Ečka), ali nije bilo statistički značajne razlike između grupa ($p \geq 0,05$). *Periago i dr*. (2005), takođe, nisu zapazili značajne razlike kada je u pitanju sadržaj proteina kod lubina iz slobodnog izlova i iz akvakulture. Hemijski parametari za meso šarana iz Dunava bili su približnih vrednosti kao i za meso šarana iz otvorenih voda (*Ljubojević i dr*. 2013a), osim što je prosečan sadržaj masti bio nešto niži u ovom istraživanju (6,95%), u odnosu na prethodno (7,15%). Hemijski sastav mesa šarana koji je uzorkovan u Ečkoj odgovarao je hemijskom sastavu mesa šarana koji je gajen u poluintenzivnim uslovima sa korišćenjem kompletnih smeša za ishranu šarana, osim što je u ovom ispitivanju dokazan nešto niži sadržaja holesterola, u odnosu na rezultate koje su ranije objavili *Ljubojević i dr*. (2013b). U istraživanju *Yeganeh i dr*. (2012) utvrđeno je da je sadržaj proteina bio viši kod riba iz prirodnih staništa u poređenju sa jedinkama gajenim u poluintenzivnom sistemu (oko 17,7% naspram 16,2%). Rezultati iz pomenute studije nisu u saglasnosti sa rezultatima dobijenim u okviru ovog eksperimenta. Naime, sadržaj proteina se nije statistički značajno razlikovao među grupama ispitanih riba. Takođe, u mesu šarana iz poluintenzivnog sistema proizvodnje (RG „Ečka“) sadržaj proteina je bio najveći (17,3%), dok je u mesu šarana iz Dunava bio nešto manji (16,57%), a najniži je bio u filetima šarana iz kaveznog sistema (16,23%). Na osnovu nama dostupne literature, može se reći da do sada nisu objavljeni rezultati ispitivanja hemijskog sastava konzumnog šarana, gajenog u kaveznom sistemu. Sadržaj masti u mesu ribe zavisi od ishrane, godišnjeg doba, geografskog područja, starosti i polne zrelosti (*Buchtova i dr*., 2007; *Guler i dr*., 2008; *Ćirković i dr.*, 2012; *Ljubojević i dr*., 2013b). Meso ribe iz slobodnog izlova, uglavnom, ima visok sadržaj vode i nizak sadržaj masti, dok je za ribu iz uzgoja karakterističan nizak sadržaj vode i visok sadržaj masti (*Makalesi,* 2012). U radu *Yeganeh i dr*. (2012), sadržaj masti u filetima ribe se nije značajno razlikovao između šarana iz prirode i šarana iz poluintenzivnog sistema gajenja ($p > 0,05$). *Periago i dr.* (2005) su, takođe, ustanovili sličan sadržaj masti kod lubina iz izlova i lubina iz akvakulture. Sa druge strane, u najvećem broju istraživanja, sadržaj masti u mesu riba iz akvakulture je veći u odnosu na ribu iz slobodnog izlova (*Alasalvar i dr*., 2002; *Olsson i dr*., 2003; *Periago i dr*., 2005; *Mnari i dr*., 2007). U ovom istraživanju, sadržaj masti u filetima šarana iz ribnjaka Ečka značajno manji je bio u odnosu na sadržaj masti u filetima šarana iz Dunava (3,41% naspram 6,95%). Najveći sadržaj masti je dobijen kod šarana iz kaveznog sistema gajenja, a ovaj sistem ujedno predstavlja i najintenzivniji sistem proizvodnje ribe u Srbiji. Uobičajeno je da gajena riba dobija velike količine masti putem hrane (u slučaju pojedinih vrsta i 20%). Pored toga, u kavezima, riba ima ograničen prostor za fizičke aktivnosti, kao što je plivanje, te je potrošnja energije manja u odnosu na ribu koja živi slobodno, ili koja se gaji u poluintenzivnom sistemu proizvodnje, posebno na velikim površinama, kao što je slučaj na ribnjaku Ečka, a hrana je mnogo dostupnija nego u prirodnim uslovima. Stoga, višak energije može biti deponovan kao mast u mišićnom tkivu, ili oko i u unutrašnjim organima.

Sadržaj holesterola je bio značajno viši u uzorcima fileta riba iz Dunava i iz ribnjaka Ečka, u odnosu na filete šarana iz kaveznog sistema, bez obzira na značajno viši sadržaj masti u poslednjoj grupi. *Ćirković i dr.* (2012) su ustanovili značajne razlike u sadržaja holesterola kod šest vrsta slatkovodnih riba i utvrdili su da se on kretao u opsegu od 34,34 mg/100 g, kod soma do 62,32 mg/100 g kod belog tolstolobika, dok je kod šarana prosečna vrednost iznosila 55,81 mg/100 g. Istraživanje koje su sproveli *Moreira i dr*. (2001) vazano za sadržaj ukupnog holesterola kod više slatkovodnih vrsta riba pokazalo je da se njegove vrednosti kreću od 40,99 do 52,79 mg/100 g. Preporuke vezane za dnevni unos holesterola hranom su da on ne prelazi 300 mg (*James i Ralph*, 2000). Stoga se može reći da meso šarana iz sve tri ispitane grupe u ovom istraživanju predstavlja pogodnu namirnicu, kada se uzme u obzir sadržaj ukupnog holesterola i kada se isti preračuna na porciju ribe.

Tabela 1. Hemijski sastav i sadržaj holesterola u filetima konzumnog šarana iz Dunava, sa ribnjaka RG „Ečka“ i iz kaveznog sistema „Vrbas“ (aritmetička sredina ± standardna devijacija), n = 6

Table 1. Chemical composition and cholesterol content in fillets of marketable size common carp from the Danube river, from the fish farm „Ečka“ and from the cage system „Vrbas“ (mean value ± standard deviation), n = 6

Parametri/Parameters	Dunav/Danube	Ečka/Ečka	Vrbas/Vrbas
Sadržaj vlage%/Moisture content %	73,58 ± 1,21[b]	78,31 ± 1,09[a]	70,32 ± 1,00[c]
Sadržaj proteina %/Protein content %	16,57 ± 0,56	17,30 ± 0,39	16,23 ± 0,54
Sadržaj masti %/Fat content %	6,95 ± 0,89[b]	3,41 ± 1,37[c]	9,79 ± 0,8[a]
Sadržaj pepela %/Ash content %	0,87 ± 0,05	1,04 ± 0,02	0,88 ± 0,01
Sadržaj holesterola mg/100g/ Cholesterol content mg/100g	45,49 ± 7,0[a]	49,64 ± 5,89[a]	26,53 ± 5,56[b]

Legenda/Legend: Vrednosti u istoj koloni označene različitim oznakama u superskriptu značajno se razlikuju ($p<0,05$)/ Values in the same row followed by different letters in superscript are statistically significantly different ($p<0.05$), (JFCA,TM)

U tabeli 2 prikazan je sadržaj pojedinačnih masnih kiselina (kao procentualni udeo u odnosu na sve prisutne masne kiseline), ukupan sadržaj SFA, MUFA, PUFA, serije n-3 i n-6 masnih kiselina, kao i Σn-3/ Σn-6, zatim PUFA/SFA i UFSA/SFA (UFSA, unsaturated fatty acids) odnosi masnih kiselina. Na osnovu prikazanih rezultata može se konstatovati da je palmitinska kiselina bila najzastupljenija SFA u sve tri grupe uzoraka i da je postojala statistički značajna razlika ($P < 0,05$) između grupa. Najveći sadržaj palmitinske kiseline je određen u filetima šarana iz Dunava, što se odrazilo i na ukupan zbir SFA, koji je bio najveći u ovoj grupi. Kod šarana iz kaveznog sistema nije detektovana pentadekanska kiselina, dok kod šarana iz Ečke nije utvrđeno prisustvo arahidske kiseline.

Kada su u pitanju MUFA, njihov ukupan sadržaj je bio najveći u uzorcima mesa šarana iz Dunava, a najmanji u uzorcima mesa šarana iz kaveznog sitema gajenja, iako je sadržaj najdominantnije MUFA, oleinske kiseline, bio približno isti u sve tri grupe. Međutim, vakcenska kiselina nije detektovana u uzorcima fileta šarana iz kaveznog sistema, a njen sadržaj je bio značajno veći u uzorcima ribe iz Dunava. Takođe je i sadržaj palmitooleinske kiseline bio značajno najveći u uzorcima iz slobodnog izlova. Ukupan sadržaj PUFA je bio najveći u filetima šarana iz kaveznog sistema gajenja, a najmanji u uzorcima ribe iz Dunava, najviše zbog visokog sadržaja linolne kiseline, koji je bio dva puta veći u odnosu na meso šarana iz Ečke, a gotovo 5 puta veći u odnosu na meso šarana iz Dunava. Ovo je doprinelo i visokom sadržaju n-6 masnih kiselina u ribi iz kaveznog sistema, kao i veoma nepovoljnom odnosu n-3/n-6 masnih kiselina. Sadržaj EPA, DPA i DHA se nije statistički značajno razlikovao između šarana iz Dunava i Ečke, a bio je značajno niži u uzorcima iz kaveznog sistema. Sa druge strane, odnos PUFA/SFA, koji predstavlja parametar kvaliteta mesa ribe, bio je najpovoljniji u uzorcima šarana iz kaveznog sistema.

Rezultati koji su dobijeni za masnokiselinski sastav mesa šarana uzorkovanog iz Dunava su u saglasnosti sa ranije objavljenim rezultatima *Ljubojević i dr*. (2013a) za meso šarana iz otvorenih voda. Ukupan sadržaj SFA je bio sličan kao i u radu *Ćirkovića i dr*. (2012), kod šarana gajenog u polikulturi u ekstenzivnom sistemu gajenja. Masnokiselinski sastav mesa šarana iz Ečke je, kada su u pitanju ukupne SFA, MUFA i PUFA, bio u saglasnosti sa rezultatima *Ljubojević i dr.* (2013b), za šarana koji je eksperimentalno gajen u poluintenzivnom sistemu sa prihranjivanjem ribe kompletnim smešama.

U istraživanju *Yeganeh i dr*. (2012), masne kiseline koje su detektovane kod šarana iz prirodnih vodenih ekosistema bile su oleinska (21,9%), palmitinska (14,6%), dokozaheksaenska (8%), palmitoleinska (6,5%), stearinska (5,4%), arahidonska (5%), eikozapentaenska (4,4%) i linolna kiselina (3,1%), dok je kod šarana iz poluintenzivnog sistema zastupljenost pojedinačnih masnih kiselina bila nešto drugačija: oleinska (32,1%), palmitinska (17%), linolna (15,3%), stearinska kiselina (5,3%), palmitooleinska (5,2%), arahidonska (3,2%), DHA (2,9%), linoleinska (2,6%) i EPA (2%). U ovom istraživanju, za meso šarana izlovljenog iz Dunava i iz poluintenzivnog sistema proizvodnje može se reći da je zastupljenost individualnih masnih kiselina slična kao i u prethodno navedenom ogledu (*Yeganeh i dr*., 2012), s tim da je sadržaj DHA i EPA, ali i arahidonske kiseline, bio značajno manji u odnosu na prezentovane rezultate. To može

Tabela 2. Sastav masnih kiselina (% od ukupnih masnih kiselina) u filetima konzumnog šarana izlovljenog iz Dunava, RG „Ečka“ i iz kaveznog sistema „Vrbas“ (aritmetička sredina ± standardna devijacija), n = 6

Table 2. Fatty acids composition (% of total fatty acids) in the fillets of marketable size of common carp collected from the Danube river, fish farm „Ečka“ and cage system „Vrbas“ (mean value ± standard deviation), n = 6

Fatty acids, %	Dunav/Danube	Ečka/Ečka	Vrbas/Vrbas
C14:0	2,88 ± 0,11^{a}	0,77 ± 0,1^{b}	0,35 ± 0,06^{c}
C15:0	0,60 ± 0,07^{b}	0,24 ± 0,04^{a}	0,00 ± 0^{c}
C16:0	19,45 ± 0,21^{a}	18,85 ± 0,18^{b}	12,52 ± 0,42^{c}
C16:1	13,82 ± 0,15^{a}	5,22 ± 0,21^{b}	1,94 ± 0,14^{c}
C17:0	0,67 ± 0,13^{a}	0,40 ± 0,08^{b}	0,06 ± 0,02^{c}
C18:0	3,86 ± 0,17^{c}	5,18 ± 0,11^{a}	4,19 ± 0,14^{b}
C18:1cis-9	30,23 ± 0,19^{b}	32,56 ± 2,17^{a}	33,55 ± 0,14^{a}
C18:1cis-11	7,16 ± 0,16^{a}	2,97 ± 0,17^{b}	0,00 ± 0,00^{c}
C18:2, ω-6	8,67 ± 0,28^{c}	19,63 ± 1,58^{b}	38,43 ± 0,61^{a}
C18:3,ω-6	0,26 ± 0,42	0,20 ± 0,06	0,60 ± 0,04
C18:3, ω-3	2,73 ± 0,07^{a}b	2,23 ± 0,32^{b}	3,16 ± 0,17^{a}
C20:0	0,13 ± 0,03^{a}	0,00 ± 0,00^{c}	0,06 ± 0,04^{b}
C20:1	1,73 ± 0,08^{b}	2,48 ± 0,17^{a}	1,76 ± 0,08^{b}
C20:2	1,55 ± 0,1^{a}	0,60 ± 0,11^{b}	0,63 ± 0,05^{b}
C20:3, ω-6	0,75 ± 0,12	0,87 ± 0,15	0,78 ± 0,04
C20:3, ω-3	0,36 ± 0,08^{b}	0,67 ± 0,15^{a}	0,07 ± 0,02^{c}
C20:4 ω-6	2,42 ± 0,1^{a}	1,17 ± 0,16^{b}	1,13 ± 0,16^{b}
C20:5, ω-3	1,33 ± 0,08^{a}	1,17 ± 0,31^{a}	0,20 ± 0,29^{b}
C22:5, ω-3	0,64 ± 0,05^{a}	0,57 ± 0,2^{a}	0,14 ± 0,05^{b}
C22:6, ω-3	0,89 ± 0,08^{a}	1,18 ± 0,29^{a}	0,43 ± 0,52^{b}
SFA	27,59 ± 0,22^{a}	25,44 ± 0,26^{b}	17,18 ± 0,39^{c}
MUFA	52,94 ± 0,19^{a}	43,22 ± 2,11^{b}	37,25 ± 0,17^{c}
PUFA	19,60 ± 0,54^{c}	28,38±0,89^{b}	45,46 ± 0,83^{a}
ω-6	13,64 ± 0,46^{c}	22,48±1,97^{b}	41,56 ± 0,63^{a}
ω-3	5,96 ± 0,16^{a}	5,90 ± 1,16^{a}	4,00 ± 0,88^{b}
ω-3/ω-6	0,44 ± 0,02^{a}	0,29 ± 0,07^{b}	0,10 ± 0,02^{c}
ω-6/ω-3	2,29 ± 0,08^{c}	3,90 ± ,46^{b}	10,79 ± 2,16^{a}
PUFA/SFA	0,71 ± 0,02^{c}	1,12 ± 0,03^{b}	2,65 ± 0,1^{a}
USFA/SFA	2,63 ± 0,03^{b}	2,82 ± 0,12^{b}	4,82 ± 0,14^{a}

Legenda/Legend: Vrednosti u istoj koloni označene različitim oznakama u superskriptu značajno se razlikuju ($p < 0,05$)/Values in the same row followed by different letters in superscript are statistically significantly different ($p < 0.05$), (JFCA,TM)

biti posledica geografskog položaja, pošto se istraživanje *Yeganeha i dr.* (2012) odnosilo na meso riba iz severnih krajeva, gde su prosečne temperature vode značajno niže. Dokazano je da temperatura vode značajno utiče na masnokiselinski sastav mesa šarana (*Guler i dr*., 2008), jer pri nižim temperaturama dolazi do povećanja stepena desaturacije i beta oksidacije masnih kiselina i, na taj način, se udeo nezasićenih masnih kiselina povećava (*Cordier i dr*., 2002; *Tocher i dr*., 2004).

Prema rezultatima *Makalesi* (2012) nivo linolne kiseline kod gofa iz kaveznog sistema je bio skoro deset puta veći u odnosu na istu vrstu ribe iz slobodnog izlova, što je potvrđeno i u ovom istraživanju, kada je šaran u pitanju. Linolna kiselina je najzastupljenija PUFA i veoma je zastupljena u uljima biljnog porekla, kao što su sojino, suncokretovo, kukuruzno i sezamovo ulje (*Chu i Hwang*, 2002), koja su, često, komponente industrijski proizvedene hrane za ishranu riba. U slučaju šarana iz kaveznog sistema u Vrbasu, sojino

ulje je bilo uključeno sa 6% u smešu hrane za ishranu ribe. Povišen nivo linolne kiseline, u odnosu na linoleinsku kiselinu, dovodi do trošenja rezervi dugolančanih n-3 masnih kiselina, uključujući DHA, putem kompeticije za enzime koji su neophodni za desaturaciju i elongaciju (*Horrocks i Yeo*, 1999). Dodavanje ulja biljnog porekla u hranu za ribe, kao izvora energije, je uobičajena praksa prilikom proizvodnje hrane za ribe. Suštinski, sve smeše u kojima se nalaze biljna ulja povećaće sadržaj linolne kiseline u hrani, a samim tim i u mišićnom tkivu riba, u odnosu na smeše koje sadrže riblje ulje, što je i zabeleženo u tkivu ribe, a posebno u jetri lososa (*Pratoomyota i dr*., 2008). Međutim, slična pojava ne zapaža se uvek u slučaju linoleinske kiseline. Naime, povećanje količina linoleinske kiseline u hrani nije dovelo do povećanja sadržaja EPA i DHA u mesa ribe u istoj meri kao kod kod lososa koji je hranjen smešom koja je sadržala riblje ulje (*Pratoomyota i dr*., 2008).

Sadržaj arahidonske kiseline bio je dva puta veći u filetima šarana iz Dunava u odnosu na filete šarana iz druge dve grupe. Veći sadržaj arahidonske kiseline kod riba iz prirode u odnosu na ribe iz uzgoja je, takođe, zabeležen ranije u radu koji su objavili *Fuentes i dr*. (2010). Kod riba u prirodnim staništima je, najverovatnije, veći alimentarni unos ove masne kiseline u odnosu na ribe iz uzgoja, najviše zahvaljujući ingestiji račića i raznih mekušaca. Ljudski organizam sintetiše arahidonsku kiselinu iz esencijalne linolne kiseline, ali sinteza ne zadovoljava uvek potrebe organizma, pa se ova masna kiselina mora uneti hranom, a glavni izvori su riba, meso i jaja, ali i u ovim izvorima je koncentracija arahidonske kiseline relativno niska (*Nisha i dr*., 2009).

Ukupne n-6 polinezasićene masne kiseline su značajno više zastupljene u mesu riba iz uzgoja, pa je tako njihov sadržaj u mesu šarana iz ribnjaka Ečka bio 1,6 puta veći u odnosu na meso šarana iz Dunava, a kod mesa šarana iz kaveza čak 3 puta veći u odnosu na šarana iz Dunava i 1,8 puta veći u odnosu na meso šarana iz Ečke. Ukupne n-3 masne kiseline nisu se značajno razlikovale između fileta šarana iz slobodnog izlova i fileta šarana iz poluintenzivnog sistema gajenja, dok je njihov sadržaj bio nešto niži kod mesa šarana iz kaveznog sistema gajenja.

Generalno, masti riba imaju relativno nizak procenat SFA (<30%), sa izuzetkom nekoliko vrsta (*Guler i dr*., 2008), što odgovara rezultatima prikazanim u ovom radu. Prema rezultatima *Gulera i dr.* (2008) i *Yeganeha i dr*. (2012), oleinska kiselina je bila dominantna MUFA kod šarana, što je u saglasnosti sa rezulatatima prikazanim u ovom radu. Prema navodima iz literature, oleinska kiselina, takođe, ima ulogu u prevenciji kardiovaskularnih bolesti (*Chong i Ng*, 1991; *Peterson i dr*., 1994). *Csengeri i Farkas* (1993), *Kolakowska i dr*. (2000) su dobili slične razultate za sadržaj MUFA u mesu šarana. Visok sadržaj oleinske, palmitooleinske i arahidonske kiseline je karakterističan za masti slatkovodnih riba (*Andrade i dr*., 1995). Prema navodima *Sargent i dr*. (2002), arahidonska kiselina je prekursor prostaglandina i tromboksana, koji ima uticaj na formiranje ugrušaka u krvi i njihovo pričvršćivanje za endotelno tkivo tokom procesa zarastanja rana, a, pored toga ima ulogu i u rastu. *Kmínková i dr.* (2001) su ustanovili da je sadržaj arahidonske kiseline kod šarana 1,3%, a *Guler i dr*. (2008) i do 5,6%, što je u saglasnosti sa prikazanim rezulatatima. *Kmínková i dr*. (2001) su utvrdili da je zbir EPA i DHA kod šarana u opsegu 2,9–6,9%, što je nešto više u odnosu na rezultate dobijene u ovom radu. *Yeganeh i dr.* (2012) su utvrdili odnos n-3/n-6 koji je bio 1,6 u mesu šarana iz slobodnog izlova i 0,4 kod šarana iz poluintenzivnog sistema gajenja, dok u našim istraživanjima ovaj odnos u filetima šarana iz Dunava iznosi 0,44, u filetima šarana iz ribnjaka Ečka 0,29, a u filetima šarana iz kaveznog sistema samo 0,1, zbog veoma visokog sadržaja n-6 PUFA. *Wood i dr*. (2008) su dali preporuku da odnos PUFA/SFA, koji je važan pokazatelj kvaliteta masti treba da bude iznad 0,4 i ovaj zahtev ispunjava meso šarana iz sve tri ispitane grupe.

Odnos USFA/SFA je od velike važnosti za procenu kvaliteta masti kod riba. Pretpostavlja se da je odnos iznad 0,35 povoljan (*Kmínková i dr*., 2001). U ovom istraživanju je odnos USFA/SFA kod šarana iz Dunava 2,63, kod šarana iz poluintenzivnog sistema 2,82, a kod šarana iz kaveznog sistema gajenja 4,82, te je, i sa ove tačke gledišta, meso šarana podesna komponenta u ishrani ljudi. Preporučeni odnos n-6/n-3 trebao bi da bude manji od 4 (*Scollan i dr*., 2006), kao što je slučaj sa šaranom iz Dunava i sa ribnjaka Ečka. S obzirom na nepovoljan odnos n-6/n-3 masnih kiselina ima negativan efekat na zdravlje ljudi. Pojedini autori (*Grigorakis i dr*., 2002) smatraju da u hrani za ribe treba smanjiti količinu n-6 masnih kiselina i time povećati odnos n-3/n-6 najmanje do preporučenih nivoa. Imajući u vidu da hraniva biljnog porekla sadrže relativno visok procenat n-6 PUFA, smatra se da one predstavljaju znaćajnu alternativu hranivima animalnog porekla, čiji su resursi veoma ograničeni.

Zaključak

Značaj prikazanih rezultata leži u činjenici da su rezultati ispitivanja sastava mesa šarana, kao najzastupljenije riblje vrste u ribnjacima u Srbiji, vredna informacija za ekologe, nutricioniste, proizvođače, kao i za širu naučnu javnost. Meso šarana predstavlja važan izvor nutritivnih materija u ishrani ljudi. Hemijski

i masnokiselinski sastav šarana mesa ispitanih grupa šarana je varirao, što je posledica različitih načina ishrane. Ograničene mogućnosti kretanja šarana u kaveznom sistemu proizvodnje, uticali su na visok sadržaj masti i nepovoljni odnos n-3/n-6 masnih kiselina. Odnosi PUFA/SFA i USFA/SFA u mesu svih ispitanih grupa šarana su odgovarali preporučenim vrednostima. Potencijal za povećanje proizvodnje šarana kao visoko vredne namirnice postoji i, zbog toga, postoji i potreba za pouzdanim analitičkim podacima. Buduća istraživanja u akvakulturi, pogotovo kada je gajenje u kaveznom sistemu u pitanju, treba da budu usmerena ka proučavanju potrebnih količina proteinskih i energetskih komponenti u hrani, pronalaženju najoptimalnijeg odnosa proteina i energije u smešama za ishranu u ovakvom načinu gajenja, kao i odgovarajućeg masnokiselinskog sastava smeša koje će doprineti postizanju optimalnih proizvodnih rezultata, poželjnog masnokiselinskog sastava, a ujedno i ekonomski isplativijoj proizvodnji.

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Chemical composition, cholesterol content and fatty acid profiles of common carp (*Cyprinus carpio*) from free-catch, semi-intensive and cage system

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S u m m a r y: Knowledge of the chemical composition of fish meat is important, as its consumption is increasing based on the recommendations that fish meat should be an essential component in a healthy diet. The main goal of this investigation was the determination and the comparison of the results obtained in the analysis of meat of common carp from free catch (the Danube), from the semi-intensive system of rearing (fish farm "Ečka") and from the cage system of rearing (cage system "Vrbas"). The results obtained showed that there was statistically significant difference (P<0.05) in the content of fat (6.95; 3.14 and 9.79% respectively) and water (73.58, 78.31 and 70.32% respectively) in the examined groups of carp. It is important to stress that carp from the fish farm "Ečka" contained less fat compared to carp caught in the Danube. There was no statistically significant difference regarding the protein content between tested groups. The amount of cholesterol in fish fillets was significantly higher in the samples of carp from Danube (45.49 mg/100 g) and "Ečka"(49.64 mg/100 g) in comparison with the carp from cage system of rearing (26.53 mg/100 g), despite the significantly higher content of fat in the last mentioned group. Palmitic acid was the dominant saturated fatty acid (SFA) in all the groups, and there was statistically significant difference (P<0.05) regarding its content between the examined groups. The highest content was established in fillets of common carp from the Danube, which reflected on the total sum of SFA (Danube-27.59; Ečka-25.44 and Vrbas-17.18%), which was the highest in this group. The total amount of monounsaturated fatty acids (MUFA) was the highest in samples from the Danube (52.94%), and the lowest in carp samples from cage system (37.25%), although the content of predominant MUFA, oleic acid, was almost the same in all three groups. Total amount of polyunsaturated fatty acids (PUFA) was the highest in samples obtained from cage system of rearing(45.46%), and the lowest was measured in samples from the Danube (19.60%), mostly because of high level of linoleic acid in the samples from cage system which was twice as high in comparison with the carp from "Ečka", and almost five times in comparison with the carp from the Danube. The best ω-3/ ω-6 ratio was obtained for carps from free catch (the Danube), (0.44), and the worst for carps from cage system "Vrbas" (0.10). This study contributes to a better understanding of the nutritional quality of carp from free catch (wild carp) and carp from aquaculture (cultured carp), and can help in the formulation of industrial feed mixtures for carp in order to achieve the optimum production results, a desirable quality of meat, and also the cost effective production.

Key words: *common carp, chemical composition, cholesterol, fatty acids, free catch, semi-intensive system, cage system*

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Effect of pre-processing of trout by freezing on the characteristics of smoked trout fillets

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A b s t r a c t: In the situation when the capacities of production and further treatment of smoked fish are insufficient, it is necessary to freeze fresh fish during the fishing season and treat it later, when the season is over. That was the reason why the aim of our study was to explore effects of freezing on certain quality parameters of smoked fish. Experimental design included trout separated into two groups: the control group of fresh fish and the experimental group of frozen fish. During production process, total bacterial count was examined, and at the end of the process, chemical composition of the final product (vacuum-packed cold smoked trout) was determined. During the storage of the product at 4°C up to 21 days, microbiological, physicochemical and sensory analyses were performed at regular intervals. Overall, the results of the present study demonstrated that pre-processing freezing of trout is suitable and, in periods of large catches, even recommendable step in smoked trout production, at least for smaller processors.

***Key words**: cold smoking, storage, quality.*

Introduction

Smoked fish is a ready–to–eat product, consumption of which had increased considerably in the last decade in many European countries (*Gallart-Jornet et al.,* 2007). In 2009, more than 121 million tonnes of world fish production was used for direct human consumption. 57 million tonnes, (46.8%), of the fish intended for human consumption was in live and fresh form. 65 million tonnes, (53.2 %), of the world's fish production underwent some form of processing. About 35 million tonnes, (28.6 %) of the total processed fish was used for manufacturing products for direct human consumption in frozen state, followed by canned fish, about 17.5 million tonnes (14.4 %), and smoked fish, about 12.4 million tonnes (10.2 %), (*Anon,* 2009). This was accompanied by a significant increase in production of farmed salmon (*Atlantic salmon*), *(Cardinal et al.,* 2001). At the time, nearly 40-50% of the European farmed salmon was consumed as a cold-smoked product (*Røra et al.,* 1999). For example, each year 45,000 tons of farmed salmon are used in France to produce 18,000 tons of smoked salmon, 15% of which is exported in Italy, Belgium and Germany (*Cardinal et al.,* 2004). Production of smoked fish and especially smoked salmon is one of the most important sectors in European fishery nowadays. Although smoked salmon was earlier considered as a "luxury" food item, it is now more a product of general consumption. In Serbia, within such type of fish products, smoked trout (*Salmo gairdneri)* and smoked carp (*Cyprinus carpio*) are becoming particularly and increasingly popular, so there is a significant market demand for these products.

Production and retailing of cold smoked fish is a complex multi-step process, including salting, smoking, packaging in vacuum or modified atmosphere packaging MAP *(Babić et al.,* 2009; *Milijašević et al.,* 2010), and storage-retail under refrigeration conditions (below 4°C). The shelf-life of the final product depends on numerous and

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interrelated production parameters such as characteristic of raw material, process hygiene, salting method, salt level, smoking conditions, packaging in vacuum or under modified atmosphere, storing conditions, etc. Therefore, it is of great importance to optimize the individual processing steps involved in the production (*Gallart-Jornet et al.,* 2007).

Salting of fish is the first step in the smoked fish production process, which is critical for the shelf life, good quality and yield of the final product. The preservative effect of salting is due to lowering of water activity (a_w), (*Jittinandana et al.*, 2002; *Leroi and Joffraud,* 2000), and thus reducing growth of many spoilage microorganisms (*Horner,* 1997, *Rørvik,* 2000). Dry salting is traditional but still the most common method of fish salting, whilst wet salting is rare in practice. In the latter case, e.g. with the Atlantic salmon fillet, the yield after salting was higher when more diluted brines were used, compared to usage of saturated brine and dry salting (*Gallar-Jornet et al.,* 2007). Nevertheless, it should be noted that, in modern times, fish products are relatively lightly salted. Both the salt content and the level of smoking applied vary among and within European countries (*Cardial et al.,* 2001; *Røra et al.,* 2004; *Gallart-Jornet et al.,* 2007).

Smoking is a subsequent step in the smoked fish production process. The preservative effects of smoking depend also on other factors, including the composition and processing of raw material, and relative humidity (*Kolodziejska et al.,* 2002). Smoke consists of numerous components including aldehydes, ketones, alcoholes, acids, hydrocarbons, esters and phenols (*Doe et al.,* 1998; *Shahidi,* 1998; *Guillèn and Errecalde,* 2002; *Guillén et al.,* 2006). Smoking extends the shelf-life of smoked fish as a result of the combined effects of dehydration, antimicrobial and antioxidant activity of some of smoke constituents (*Doe et al.* 1998; *Horner,* 1997, *Leroy and Joffraud,* 2000; *Rørvik,* 2000). Nevertheless, today, the main reason for smoking is manufacturing of fish products of desirable sensory properties, so "current" smoked fish products typically contain higher moisture and lower salt content (so lower a_w) than traditional, intensively smoked products.

Lightly salted and mildly heated smoked fish may contain bacteria – both spoilage organisms and foodborne pathogens – that, either survive the production process, or are present on the final product during the post-processing handling. Due to the fact that the temperature during the cold smoking never exceeds 28 ºC, it does not have any significant antimicrobial effect on pathogens in cold smoked fish (*Gudbjornsdottir et al.,* 2010). Spoilage of fish, characterised by production of the spoilage odours and flavours, is due to metabolism of the microorganisms and the fish tissue autolytic enzymes having a major impact on textural deterioration (*Hansen et al.,* 1996). In addition to salting and smoking effects, the shelf-life of smoked fish is affected by storage conditions. Differences in packaging– and temperature-related parameters throughout the storage period result in different product shelf-lives (*Church and Parsons* 1995; *Cutter,* 2002; *Bugueño et al.,* 2003).

Decision taken by a producer regarding choice of smoked fish technology and related parameters to be used is influenced by the market demand and by the need for economical profit, as well. Furthermore, the choice of the process control parameters, such as duration of salting, concentration of brine and smoking temperature, is also influenced by specific characteristics of the final product that need to be achieved (*Cardinal et al.,* 2004).

Good knowledge and appropriate selection of raw material is a prerequisite for effective process controls, good yield and required smoked fish quality (*Beltrán and Moral*, 1991; *Røra et al.*, 1999; *Cardinal et al.*, 2001; *Birkeland et al.,* 2004). Frozen fish is frequently used for production of smoked fish products. This is particularly the case during periods when the fish catch exceeds the processing capacities, a situation occurring more often with smaller processors. Therefore, the main objective of this study was to determine the effect of freezing of raw material (fish) on the quality parameters (sensory, chemical and microbiological) of smoked trout fillets.

Material and methods

Fresh whole trout (average mass of 1 kg) were obtained from the fishery Bočac, Banja Luka, Republic of Srpska – Bosnia and Herzegovina, and transported to the fish processing plant in special refrigerated vehicles, at 4°C. The fish was subsequently slaughtered and separated into two groups: fish in the control group, which remained unfrozen, whilst those from the experimental group were frozen at –40°C. Trout from the control group were rinsed in potable water and subsequently wet salted for 24 h in brine solution (9% salt) containing rosemary (*quantum satis),* in a temperature-controlled room (4°C). After salting, before smoking, fish was drained for one hour at 20°C in smoking chambers. The smoking was performed in automated smoke chambers at the temperature of 28°C during the period of 8 hours. The smoke was produced from beech wood sawdust in a generator separated from the smoking chamber. Subsequently, fish was cooled (at 2°C, for 10 h), and then sliced (each slice 0.5 cm thickness;

150 g). During the slicing, both, the skin and the rib bones were removed. Finally, the smoked trout fillets were vacuum packaged. Trout from the experimental group were frozen in a freezing tunnel (air temperature – 40°C), placed individually in plastic bags and stored at –20°C. After 20 days, the fish were thawed by submersion in water (10 °C) for 5 hours and subjected to the processing in the same manner as described for the control group. The vacuum-packed, smoked trout fillets from both, control and experimental, groups were stored for 21 days at 4°C and sampled for analysis at 0, 7th, 14th and 21st days of the storage. Smoked trout fillets from both groups were analysed at 0, 7th, 14th and 21st days of storage for total viable count.

Microbiological analysis

Sampling of fish skin during processing

Skin of both, control and experimental, fish groups was sampled after the fish cleaning, evisceration, and cooling. Skin of the experimental fish was also sampled after thawing. From each group of samples, at each sampling point, a total of ten fish were sampled. Skin sample of 25 cm^2 was excised from the location behind gills of each fish, kept at 4°C, and analysed for the total viable count (TVC) of bacteria within 30 min.

Sampling of fish fillets

From each group, at each sampling point, total of ten vacuum-packages of smoked trout fillets were sampled 0, 7th, 14th and 21st days of storage. Each sample, comprising of 10 g of the product, was kept at 4°C and analysed for the total viable count (TVC) of bacteria within 30 min. Smoked trout fillets from both groups were analysed at 7th, 14th and 21st days of the storage for total lactobacilli count.

Microbiological methods

Total viable count (TVC) of bacteria was determined according to *Roberts et al.* (1995). Briefly, each 10 g sample (skin or fillet) was homogenized in stomacher bag with 90 ml of MRD (Maximum Recovery Diluent, Biolife, Italy) for 2 minutes, and then further decimal dilutions in MRD were prepared. From appropriate dilutions, volumes of 0.1 mL were surface plated on PCA agar plates (Plate Count Agar, Biolife, Italy), the plates were incubated for 72 h at 30°C, and subsequently $\log_{10}$ CFU/cm^2 or g of sample was calculated. Lactobacilli count was determined according to *Cook* (1991). Briefly, samples were homogenized and decimally diluted in MRD in the same manner as described for TVC. Volumes of 0.1 mL were surface plated onto MRS plates (MRS Agar with Tween 80, Biolife, Italy), and each was subsequently overlaid with 10 ml of melted and cooled (to 45°C) PCA agar to obtain microaerophylic conditions for growth of lactobacilli. The inoculated plates were incubated for 72 h at 30°C and subsequently log CFU/g of sample was calculated.

Physicochemical and chemical analysis

Chemical composition of samples was analysed at 0 day of storage according to standard methods. Moisture content was determined in 5 g of fish fillet samples by oven-drying at 105 ± 1°C until a constant weight was obtained (*Anon,* 1998). Fat content was determined using Soxhlet method, by extraction of fat by petrol ether from the dried sample, followed by distillation and drying of sample extract at 105 ± 1°C until constant weight was obtained (*Anon,* 1992a). Protein content was determined using Kjeldalh method with apparatus "Tecator" (*Anon*, 1992b). Ash content was determined by ashing the sample at 550°C until constant mass was obtained (*Anon,* 1999a). Total salt content was determined using Volhard method (*Anon,* 1999b). Salt content in water phase (SWP) was calculated from the corresponding total salt and water contents, using the equation:

SWP= % salt * 100 / % salt + % water

Water activity (a_w value) was calculated from the corresponding SWP value, using the equation (*Gimenéz and Dalgaard,* 2004):

$$a_w = 1 - 0.0052471 \times \mathrm{SWP} - 0.00012206 \times \mathrm{SWP}^2$$

Ethanol content was determined in smoked trout samples (at 0, 7th, 14th and 21st days of storage) according to *Beutler* (1988), using standard enzyme kits based on alcohol dehydrogenase (Megazyme Inter. Ireland Lim).

Sensory analysis

Smoked trout fillets were subjected to the sensory analysis (at 0, 7th, 14th and 21st days of storage) by a panel of 14 trained evaluators. Selection of evaluators was performed according to ISO standard (*Anon,* 2002). Sensory evaluation was performed by quantitative descriptive analysis (*Anon,* 2001). The questionnaire included evaluation of 5 quality parameters. Each property was evaluated using a rating scale from 1 to 5. Marks 1 to 5 indicated level of property expression (sensation of smell and taste to smoke, salinity, and tenderness) as well as

acceptability of property (colour acceptability, total acceptability). The lowest expressed property, or the least acceptable property, was evaluated by 1 on a scale from 1 to 5, and the highest expressed property, or the most acceptable property, was evaluated by 5 on a scale from 1 to 5.

Statistical analysis

For each sample and each parameter tested, at least 6 separate values were obtained. The results were statistically analysed (mean value, standard deviation, standard error, coefficient of variation and confidence interval for a variance), and statistical significance calculated (*t*-test and analysis of variance at 0.01 and 0.05) using Graphpad Prism 4.0 statistical package.

Results and discussion

Total viable count of bacteria on fish skin

It is known that TVC on the fish skin just after the catch is highly variable, between 10^2 and 10^7 (i.e. 2 and 3 log_{10}) CFU/cm^2 (*Liston,* 1980). In the present study, the initial TVC (i.e. after slaughtering) on the skin was 3-4 log_{10} CFU/cm^2. Subsequently, TVC on the skin of the control fish group varied during manufacturing process from 3.53±0.98 log_{10} CFU/cm^2 to 1.64 ± 0.24 log_{10} CFU/cm^2 (Figure 1.), and in the final product it was significantly lower ($p < 0.01$) than after slaughtering and evisceration. Similarly, TVC on the skin of the experimental group of fish varied from 3.79 ± 0.57 log_{10} CFU/cm^2 to 1.07 ± 0.40 log_{10} CFU/cm^2, and was significantly lower ($p < 0.01$) in the final product as compared with initial stages of the processing. It could be presumed that a part of the TVC reductions were due to the removal of bacteria from the skin by washing, normally applied after evisceration. Importance of washing of fish after the first stage of fish processing was stressed by *Kolodziejska et al.* (2002), who observed significant TVC reduction after the washing.

During the later stages of the manufacturing process (i.e. after salting, smoking and cooling), TVC on the trout skin of the experimental group was significantly lower compared to TVC on the trout skin of the control group. After the draining stage there was no statistical sign determined ($p > 0.05$). It is well known that low temperatures inhibit activity of microorganisms and enzymes, but also reduce the count of microorganisms. Decrease of TVC on the trout skin of both the control and experimental group during manufacturing process was predominantly

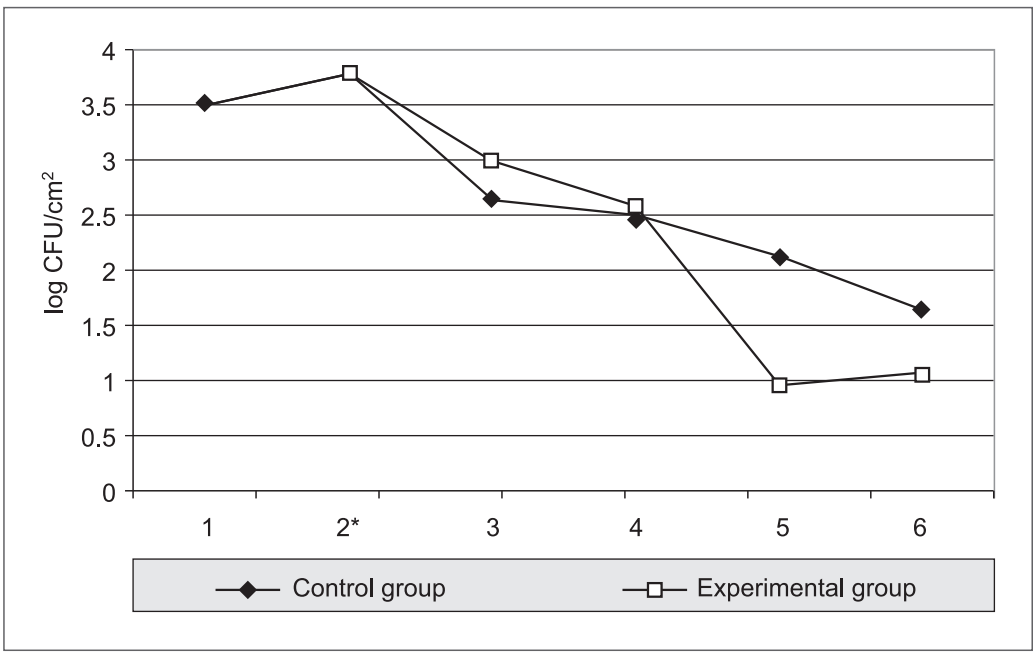


Figure 1. TVC on the skin of trout during manufacturing process (log CFU/cm^2)

Note: Manufacturing phases – TVC on the fish skin **1**– after slaughtering (control group only); **2** –after evisceration; **2***– after thawing; **3** – after salting; **4** – after draining; **5** – after smoking; **6** – after cooling

Slika 1. Ukupan broj bakterija na koži pastrmke u toku procesa proizvodnje

Napomena: faze u toku procesa proizvodnje – ukupan broj bakterija na površini ribe 1 – posle klanja (samo kontrolna grupa); 2 – posle eviseracije; 2* – posle defrostracije; 3 – posle soljenja; 4 – posle ceđenja; 5 – posle dimljenja; 6 – posle hlađenja.

due to the salt effect, both directly (its "toxicity"), (*Goulas and Kontominas*, 2005), and indirectly through decreasing of the water activity in fish meat (a_w value), which caused lowering of water content available to microorganisms. Initial decrease of a_w value progressively inhibits bacterial growth, while further leading to growth cessation, however never to death of bacteria. Sodium chloride is dissolving in water and increases osmotic pressure. Ions also tend to bind with protein molecules. When there are no such bonds enzymes of microorganisms can effectively express their proteolytic activity. Previous reports indicated that, amongst processing-related factors, fish salting caused the largest decrease of TVC on fish skin (*Kolodziejska et al.,* 2002). However, not all of the bacteria are equally affected by salting. Although TVC on the fish is lower after salting, some species can continue to grow even at higher salt concentrations (*Skjervold et al.,* 2001). The results of the present study indicate that TVC on the skin of control group after salting (2.64 ± 0.14 log_{10} CFU/cm^2) was lower compared to TVC on the skin of experimental the group (2.99 ± 0.25 log_{10} CFU/cm^2) after salting. This phenomenon is likely to be due to pre-processing freezing of the experimental group, which probably enhanced the salt penetration into the fish tissues. Previous studies (*Cardinal et al.,* 2001; *Deng,* 1977) demonstrated that salt penetrates better into fish previously frozen, and this is due to freezing-alterations of the tissue cell structure (*Sigurgisladottir et al.,* 2000). After smoking, further decrease of the bacterial counts was observed (Figure 1), probably due to antimicrobial effects of the smoke. This explanation is supported by published data (*Kolodziejska et al.,* 2002) indicating that smoking significantly reduces TVC on fish skin. In this study, TVC after smoking stage was 2.12 ± 0.52 log_{10} CFU/cm^2 (control) and 0.98 ± 0.23 log_{10} CFU/cm^2 (experimental). Comparably slightly higher TVCs (up to 3 log_{10} CFU/cm^2) on fish skin after smoking were reported in other studies *(Hansen et al.,* 1995*; Hansen et al.,* 1996; *Hansen et al.,* 1998; *Leroi et al.,* 1998; *Dondero et al.,* 2004). On the other hand, it should be noted that post-smoking TVC in the experimental group was lower than post-smoking TVC in the control group (Fig. 1). This is likely to be the consequence of pre-processing freezing of the fish from the experimental group, which altered the tissue cell structure (*Sigurgisladottir et al.*, 2000) enabling better penetration of the antimicrobial smoke components.

Chemical composition of the processed and finished smoked trout fillets

Salt content in the experimental fillets was significantly higher than in the control fillets (Table 1). Similar differences in the salt content between salted fish that was fresh or frozen before salting were reported previously (*Sigurgisladottir et al.* 2000, *Cardinal et al.* 2001). These observations were probably due to better salt penetration into freezing-altered fish tissues. Similarly, SWP of the vacuum packaged fillets from the control group (4.58 ± 1.42%) was significantly lower ($p < 0.01$) then the SWP of the vacuum packaged fillets from the experimental group (5.93 ± 0.61%). *Hansen et al.* (1995) showed that increased SWP increases the shelf-life of vacuum packaged fish products. Calculated a_w value in control fillets was 0.98, whilst 0.97 in the experimental fillets. a_w values in both groups, being not lower than 0.97, were insufficient to inhibit growth of microorganisms (*Kolodziejska et al.* 2002).

General microflora of the smoked trout fillets

Shelf-life of vacuum packaged smoked products depends on a number of factors, including initial contamination, manufacturing conditions, antimicrobial factors acting in the product,

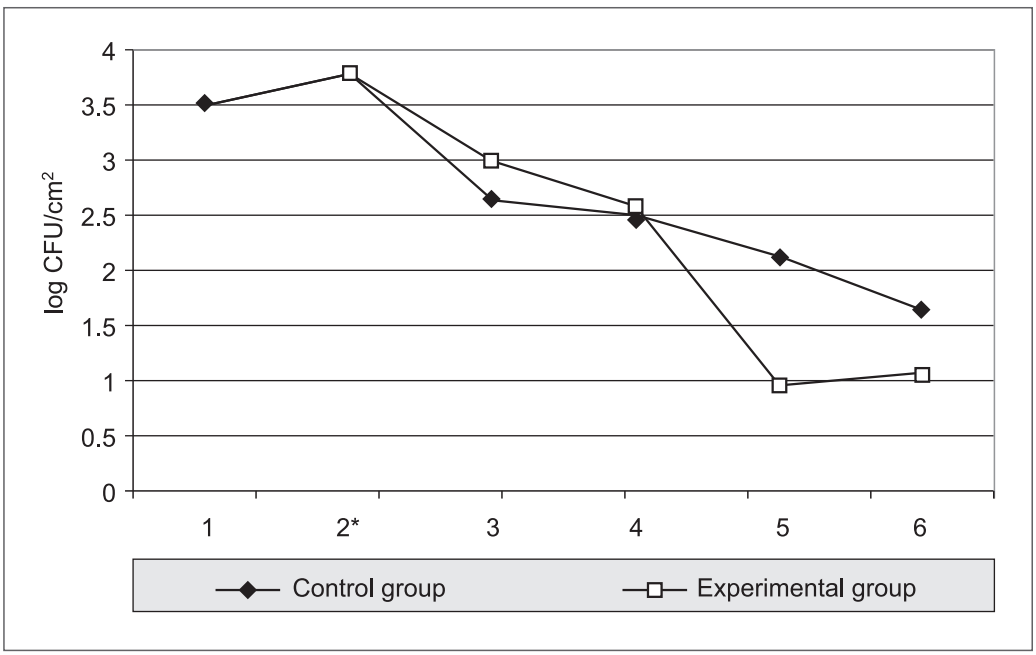
Table 1. Chemical composition of the control and the experimental smoked trout fille
Tabela 1. Hemijski sastav kontrolnih i eksperimentalnih uzoraka fileta dimljene pastrmke

	content of (%)/sadržaj (%)					value of/vrednost	
group/grupa	proteins/ belančevine	lipids/ lipidi	salt/so	moisture/ vlaga	ash/ pepeo	SWP	a_w value/ a_w vrednost
control/kontrolna	21.08^x ± 1.03	2.08^a ± 0.46	3.25^x ± 1.01	71.00^a ± 2.43	4.38^x ± 0.92	4.36^x ± 1.29	0.98
experimental/ ekspeimentalna	19.00^y ± 1.01	1.98^b ± 0.65	4.27^y ± 0.35	72.24^b ± 1.93	5.23^y ± 0.36	5.59^y ± 0.54	0.97

Note: Students' *t*-test – Means with different letter superscripts in the column are significantly different (a, b – $p < 0.05$; x, y – $p < 0.01$)/ Napomena: Studentov *t*-test – srednje vrednosti u koloni, koje su označene različitim slovima u eksperimentu, su signifikantno različite (a, b – $p < 0,05$; x, y – $p < 0,01$)

post-manufacturing handling and storage conditions (*Hansen et al.*, 1995; *Hansen et al.,* 1996; *Leroi et al.*, 1998; *Kolodziejska et al.,* 2002; *Dondero et al.,* 2004).

Total viable count of bacteria in both the control and the experimental group, TVC in vacuum packaged trout fillets stored at 4 °C increased between 0 and 14th day, but subsequently significantly decreased to the end of the 3-week storage (Fig. 2). At the very beginning ("0" sampling time) of the storage, TVC in the experimental fillets was lower than in the control fillets, but later (at 14th and 21st days) TVC differences between the control and the experimental fillets were statistically not significant. Final TVCs (day 21) were approx. 2-2.5 *log* CFU/g in both groups, which could be considered as relatively low. Namely, numerous published data indicate that TVCs in vacuum packaged fish products often reach 10^7-10^8 CFU/g, e.g. *Dondero et al.* (2004) reported TVC as high as 10^6 CFU/g in vacuum packaged trout fillets after 3 weeks of storage at 4°C. Such a large difference in TVCs between different studies is probably caused by large differences in study conditions. It should be noted that in most EU countries related legislation does not limit TVC in vacuum packaged smoked fish products, although French legislation states 10^6 log_{10} CFU/g as maximum permissible TVC in such products (*Cardinal et al.,* 2004). The observed changes of TVC in smoked fillets during storage were probably due to a number of interrelated factors, including microbial competition for nutrients and antagonism amongst the microflora that exists within any specific ecological niche (*Gram*, 1993). Nevertheless, no changes in TVC in vacuum packaged smoked trout were observed during 3-week storage in an earlier study (*Kolodziejska et al.,* 2002), but the storage temperature (2°C) was lower than in the present study (4°C). On the other hand, *Hansen et al.* (1995) has reported comparably much higher TVC (10^8 CFU/g) in vacuum packaged smoked fish products stored for 21 day, but at somewhat higher temperature (5°C) than in the present study. It should be noted that a number of published studies indicated that high TVC did not necessarily lead to spoilage of the product. For the vacuum packaged smoked fish products spoilage, much more relevant is the composition of the microflora and the activity of the specific spoilage microorganisms than TVC (*Hansen et al.,* 1995; *Hansen et al.,* 1996; *Hansen et al.*, 1998; *Leroi et al.,* 1998; *Lyhs et al.,* 1999; *Gonzáles-Rodrígez et al.,* 2002;



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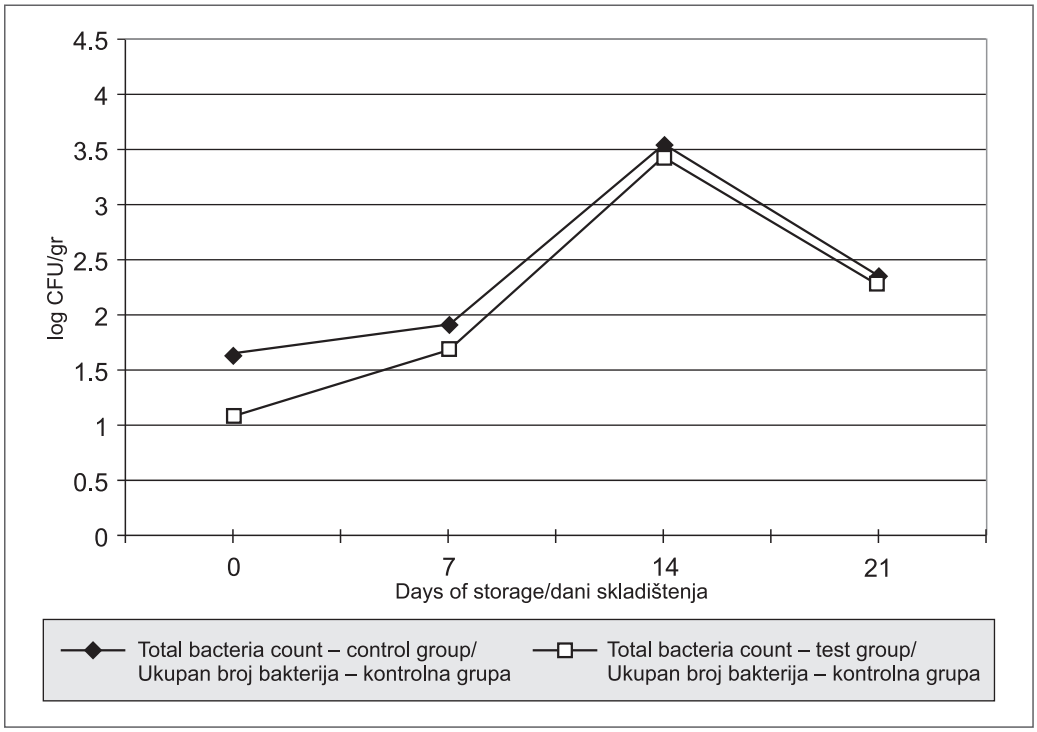

Figure 2. TVC in vacuum packaged smoked trout fillets during storage at 4°C
Slika 2. Ukupan broj bakterija u vakuum pakovanim dimljenim filetima pastrmke u toku skladištenja na 4°C

Cardinal et al., 2004; *Dondero et al.*, 2004; *Espe et al.,* 2004).

Lactobacilli count

Smoked trout fillets from both groups were analysed for total lactobacilli count at 7th, 14th and 21st days of the storage. The results indicated that lactobacilli (LAB) counts in both control and experimental vacuum packaged smoked trout fillets significantly increased between 7th and 14th day of storage at the 4° C, and then decreased (Figure 3). LAB counts in the control fillets were significantly higher ($p < 0.01$) than in the experimental fillets, at both 7th and 14th day, but LAB difference between the control and experimental fillets on 21st day was not significant (approx. between 2.5 and 3 log_{10} CFU/g). In other studies, numerous authors investigated LAB count in vacuum packaged cold-smoked products, and LAB counts varied between 10^3-10^8 CFU/g during 21 day of storage at 4°C (*Hansen et al.*, 1998; *Leroi et al.,* 1998; *Dondero et al.,* 2004). *Lactobacillus* species are considered as the most prominent and the most important microorganisms for shelf-life of cold-smoked vacuum packaged fish products. Lactobacilli can inhibit growth of other bacteria by producing lactic acid and associated pH decrease and/or producing bacteriocins and/or competing for nutrient compounds, so their presence in vacuum packaged products can extend the shelf-life (*Gram and Dalgaard,* 2002). Among lactobacilli, dominant species in vacuum packaged smoked fish products are *L. sakei, L. curvatus, L. homohiochii, L. plantarum, L. delbrueckii, L. casei, L. coryneformis, L. alimentarius* (*Gonzáles-Rodrígez et al.,* 2002). In addition, the second group of microorganisms most frequently isolated from vacuum packaged smoked fish products belong to *Enterobacteriaceae: Proteus mirabilis, Proteus vulgaris* and *Serratia liquefaciens.* The third group of microorganism that can be found in vacuum packaged smoked fish products belong to micrococci, with coagulase-negative staphylococci being predominant. *Frangos et al.* (2010) have observed that Lactobacilli and *Enterobacteriaceae* were also found to be a significant part of the microbial flora of trout fillets, irrespective of packaging and antimicrobial treatment. *Leroi et al.* (1998) have demonstrated that Gram-negative microflora is dominant during the first two weeks of vacuum packaged smoked fish storage, but it is subsequently replaced by Gram-positive microorganisms, predominantly lactobacilli. Overall, the results of this study indicated that hygienically obtained frozen fish is suitable raw material for production of microbiologically high quality smoked trout product. In this study, in the product that was frozen before manufacturing, both TVC and LAB counts during

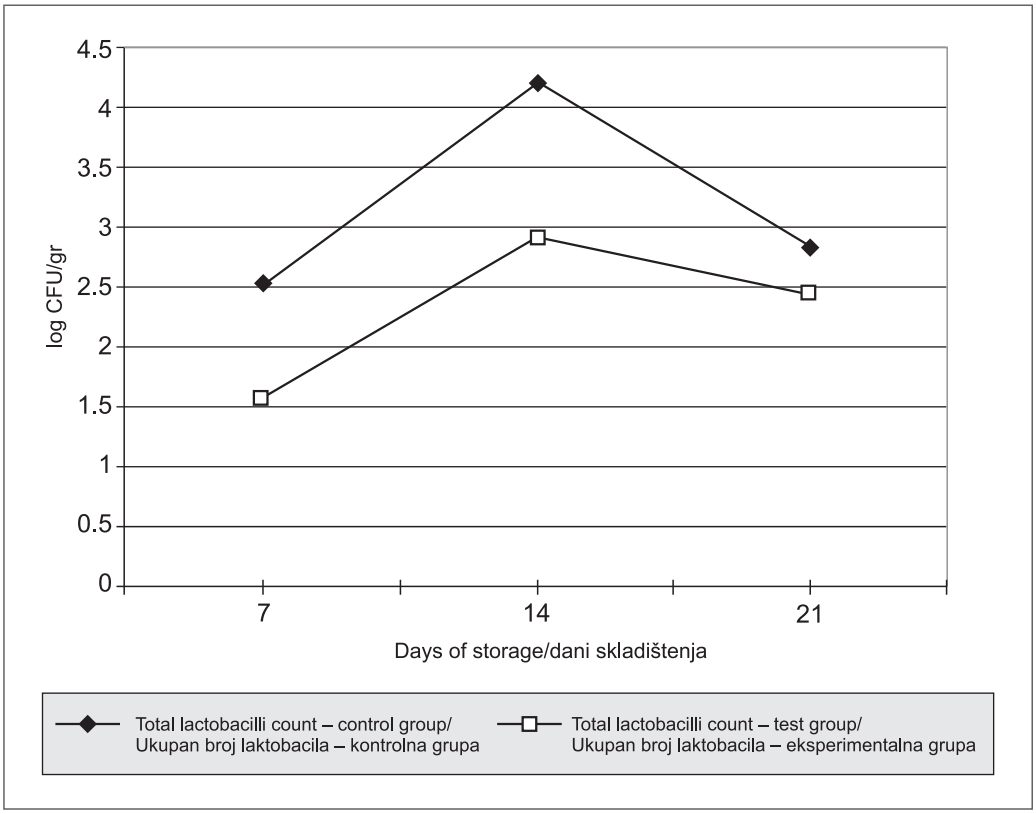

Figure 3. Lactobacilli (LAB) count in vacuum packaged trout fillets during storage at 4°C
Slika 3. Broj laktobacila (LAB) u vakuum pakovanim filetima pastrmke u toku skladištenja na 4°C

21-day chilled storage were lower than in the product produced from fresh fish.

Lactobacilli count versus ethanol content in the smoked trout fillets

Ethanol is a product of bacterial metabolism during anaerobic fermentation (glycolysis) and/or as a product of dezamination and decarboxylation of amino acids such as alanine (*Huss,* 1995). This suggests that ethanol could be used as an indicator of microbiological status of foodstuffs, as well as of their shelf-life. In the present study, the changes in ethanol content (Table 2) reflected the changes in LAB counts (Fig. 3) at corresponding times of the storage at 4°C, in both the control and the experimental smoked fished fillets. This could indicate that ethanol content may be a useful indicator of the dynamics of LAB microflora in the product under given conditions. The association between ethanol content and LAB counts in vacuum packaged cold-smoked fish products was also reported by *Hansen et al.* (1995). However, *Gonzáles-Rodrígez et al.* (2002) claim that no correlation exists between ethanol and LAB counts, on one hand, and sensory assessment of the product, on the other.

Table 2. Ethanol content (mg/kg; mean values) in vacuum packaged smoked trout fillets stored at 4°C for 3 weeks
Tabela 2. Sadržaj etanola (mg/kg; srednje vrednosti) u vakuum pakovanim filetima ribe pastrmke skladištenih na 4°C u toku tri nedelje

group/grupa	Day of storage/dani skladištenja			
	0	7	14	21
control/kontrolna	3.91	4.84	6.32	4.74
experimental/eksperimentalna	4.5	5.61	7.65	5.6

Table 3. Evaluation scores of selected sensory properties of smoked trout
Tabela 3. Rezultati evaluacije odabranih organoleptičkih osobina dimljene pastrmke

	Colour/Boja		*Tenderness/ Nežnost, Mekoća*		*Sensation of smell and taste to smoke/Osećaj mirisa i ukusa na dim*		*Salinity/Slanost*		*Total acceptability/Ukupna prihvatljivost*	
days of storage/dani skladištenja	*Control group/ kontrolna grupa*	*Experimental group/ eksperimentalna grupa*	*Control group/ kontrolna grupa*	*Experimental group/ eksperimentalna grupa*	*Control group/ kontrolna grupa*	*Experimental group/ eksperimentalna grupa*	*Control group/ kontrolna grupa*	*Experimental group/ eksperimentalna grupa*	*Control group/ kontrolna grupa*	*Experimental group/ eksperimentalna grupa*
0	$4.36^{a} \pm 0.40$	$4.04^{b} \pm 0.29$	$4.23^{a} \pm 0.42$	$4.61^{b} \pm 0.49$	4.49 ± 0.47	4.43 ± 0.35	$4.00^{x} \pm 0.29$	$4.86^{y} \pm 0.38$	4.57 ± 0.48	4.27 ± 0.40
7	$4.23^{x} \pm 0.25$	$4.46^{y} \pm 0.11$	4.39 ± 0.42	4.61 ± 0.35	4.68 ± 0.32	4.36 ± 0.50	$3.93^{x} \pm 0.19$	$4.71^{y} \pm 0.49$	4.50 ± 0.48	4.32 ± 0.42
14	$4.43^{x} \pm 0.18$	$4.19^{y} \pm 0.23$	4.37 ± 0.40	4.14 ± 0.29	4.36 ± 0.23	4.21 ± 0.26	$4.07^{x} \pm 0.19$	$4.43^{y} \pm 0.19$	$4.29^{x} \pm 0.24$	$3.99^{y} \pm 0.31$
21	$4.04^{a} \pm 0.24$	$3.62^{b} \pm 0.55$	4.02 ± 0.26	3.80 ± 0.49	4.01 ± 0.26	3.82 ± 0.26	$3.61^{x} \pm 0.20$	$4.21^{y} \pm 0.27$	4.01 ± 0.25	3.77 ± 0.31

Note: Students' *t*-test – Means with different superscript between control and experimental group are significantly different (a, b – $p < 0.05$; x, y – $p < 0.01$)/Napomena: Studentov *t*-test – srednje vrednosti koje su označene različitim slovima, između kolona i eksperimantalnih grupa, su signifikanto različite (a, b – $p < 0,05$; x, y – $p < 0,01$)

Sensory scoring of the smoked trout fillets

Overall, mean scores of selected parameters of sensoriy properties of both control and experimental vacuum packaged smoked trout fillets decreased over the 3-week storage period, and the final scores were significantly lower than the initial ones (Table 3). This was expected, and decrease of sensory qualities of such products over their shelf life have been reported earlier (*Goulas and Kontominas,* 2005). In general, at any given evaluation time, no significant differences in the total acceptability between the control and the experimental fillets were observed. However, although non-significant, somewhat higher total acceptability scores were noted with the control smoked fillets produced from fresh (unfrozen) fish. Many consumers accentuate colour intensity as the main indicator of quality of smoked fish, as reported for German and French consumers (*Torrissen et al.,* 2000). In the present study, mean scores for the colour of control fillets (days 0, 7, 14 and 21) were significantly higher ($p < 0.05$ to $p < 0.01$) than those of the experimental fillets Table 3). Similar results have been reported before (*Cardinal et al.,* 2001), that smoked fish produced from pre-processing frozen fish had less intense colour than in case when the fish was not frozen before processing. In contrast, some other authors (*Leroi et al.,* 2001) have reported no significant colour variations in smoked fish (stored 3 weeks at 4 °C) attributable to pre-processing fish freezing. It should be considered that some other factors could also affect the colour scoring of the smoked fish. For example, a positive correlation between fat content and colour intensity of smoked products has been reported *(Røra et al.,* 1999). Mean scores for tenderness were significantly higher in the control fillets than in the experimental fillets only at day 0 of the storage, but no significant differences were observed on days 7, 14 and 21 (Table 3). It is likely that the initial difference in the tenderness was caused by the corresponding differences in the salt content in the products. Mollifying of fish is a consequence of autolytic processes due to catepsin activity, and increased salt content inhibits activity of catepsins, thus decreasing mollifying (*Reddi et al.,* 1972). On the other hand, some other authors (*Cardinal et al.,* 2001) have reported that smoked fish products obtained from frozen raw material were softer. Mean scores for salinity were significantly higher ($p < 0.01$) in the experimental smoked fillets than in the controls throughout the 3-week storage period at 4°C (Table 3). The main reason for that was better penetration of salt in tissue cells altered by freezing, as indicated before. In some other studies, smoked fish products made from frozen fish also had more intense taste of salinity than the products made from fresh fish (*Cardinal et al.,* 2001). The authors considered that freezing-altered tissue texture stimulated salinity perception causing impression of the salt content being higher than actual.

Conclusions

As far as general microflora (TVC and LAB) is concerned, cold smoked, vacuum-packaged trout fillets produced from pre-processing frozen fish had superior microbiological characteristics compared to those produced from pre-processing unfrozen fish. Freezing-alterations of the fish tissues enabled better penetration of salt leading to higher salt-in-water content and lower water activity which, in turn,

contributed to the extended shelf life of the product. At the same time, pre-processing freezing had no significant detrimental effect on the total sensory acceptability of the smoked vacuum-packaged trout fillets, neither initially or after 3-week cold-storage. Overall, the results of the present study demonstrated that pre-processing freezing of trout is suitable and, in times of large catches, even recommendable step in smoked trout production, at least for smaller processors.

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Uticaj prethodne obrade pastrmke zamrzavanjem na karakteristike fileta dimljene pastrmke

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S a ž e t a k: U situaciji kada su kapaciteti za proizvodnju i dalji tretman dimljene ribe nedovoljni potrebno je da se sveža riba tokom sezone izlova zamrzne i da se preradi kasnije, kada se sezona završi. To je bio razlog zašto je za cilj naših istraživanja postavljeno da se ispitaju efekti zamrzavanja na određene parametre kvaliteta dimljene ribe. Za eksperiment, pastrmke su bile podeljene u dve grupe: kontrolna grupa, koju je sačinjavala sveža riba i eksperimentalna grupa, koju je sačinjavala smrznuta riba. Tokom procesa proizvodnje ispitan je ukupan broj bakterija, a na kraju procesa, u konačnom proizvodu (vakuum-pakovana hladno dimljena pastrmka), određeni su hemijski parametri. U toku skladištenja proizvoda na +4°C tokom 21 dan, u redovnim intervalima su izvršena mikrobiološka, fizičko-hemijska i senzorna ispitivanja. Generalno, rezultati ovih istraživanja su pokazali da je prethodna obrada pastrmke zamrzavanjem pogodna i, u vreme velikih izlova, čak i preporučljiv korak u proizvodnji dimljene pastrmke, bar što se tiče manjih proizvođača.

***Ključne reči**: hladno dimljenje, skladištenje, kvalitet.*

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Distribucija organohlornih pesticida i polihlorovanih bifenila u dve vrste riba iz Dunava

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S a d r ž a j: U ovom radu prikazana je distribucija i sadržaj organohlornih pesticida (organochlorine pesticides, OCP) i polihlorovanih bifenila (polychlorinated biphenyls, PCB) u ribama krupatici (Blicca bjoerkna) i mreni (Barbus barbus) iz Dunava u blizini Batajnice. Ispitano je 16 organohlornih pesticida i 7 kongenera polihlorovanih bifenila. Anlizirana su sledeća jedinjenja: α-heksahlorocikloheksan (α-HCH), β-heksahlorocikloheksan (β-HCH), heksahlorobenzen (HCB), γ-heksahlorocikloheksan (γ-HCH), δ-heksahlorocikloheksan (δ-HCH), heptahlor, aldrin, cis-heptahloroepoksid (cis-HCE), trans-heptahloroepoksid (trans-HCE), γ-hlordan, α-hlordan, p,p'-dihlorodifenildihloroetilen (p,p'-DDE), dieldrin, endrin, p,p'-dihlorodifenildihloroetan (p,p'-DDD) i p,p'-dihlorodifeniltrihloroetan (p,p'-DDT) i PCB kongeneri označeni IUPAC brojevima 28, 52, 101, 138, 153, 180 i 118. Kvalitativna i kvanitativna ispitivanja ovih jedinjenja rađena su GC-ECD metodom. Sadržaj proteina, lipida, vlage i pepela u filetima riba određen je korišćenjem standardnih SRPS ISO metoda. Utvđena je statistički značajna razlika ($p < 0,05$) između sadržaja organohlornih pesticida u krupatici i mreni, kao i između sadržaja polihlorovanih bifenila u ispitanim ribama. Najveći sadržaj među ispitivanim organohlornim pesticidima, u obe vrste ribe utvrđen je za ΣDDT (pp'-DDT + pp'-DDE + pp'-DDD), (13,8 ng/g ribe – krupatica, 2,6 ng/g ribe – mrena). Ukupan sadržaj PCB jedinjenja u filetima krupatice (40,8 ng/g ribe) bio je značajno veći nego u filetima mrene (7,2 ng/g ribe). Na osnovu rezultata Studentovog t-testa ($p = 0,05$) utvđeno je da postoji statistički značajna razlika u sadržaju masti, vlage, pepela i izračunate energetske vrednosti između fileta ispitanih riba. Četiri puta veći sadržaj masti u filetima krupatice (4,25%) u odnosu na filete mrene (1,07%) jedan je od glavih razloga većeg sadržaja organohlornih pesticida i polihlorovanih bifenila u krupatici nego u mreni, koje su uzete sa istog lokaliteta (Dunav, Batajnica).

Ključne reči: *Krupatica, mrena, organohlorni pesticidi, polihlorovani bifenili, hemijski sastav.*

Uvod

Perzistentni organski zagađivači (Persistent Organic Pollutants, POPs) su složena organska jedinjenja velike molekulske mase, koja, često, sadrže halogene elemente, uglavnom hlor. To su jedinjnja koja su rezistentna na fotolitičku, biološku i hemijsku degradaciju. Na osnovu strukture molekula, perzistentni organski zagađivači se mogu podeliti na policiklične aromatične ugljovodonike (polycyclic aromatic hydrocarbons, PAH), tj. PAH jedinjenja i halogenovane ugljovodonike, kao što su pesticidi, polihlorovani bifenili, dioksini itd. POP jedinjenja su slabo rastvorljiva u vodi, a veoma dobro u mastima, tako da se lako transportuju kroz fosfolipidne strukture bioloških membrana, nakon čega se deponuju u masnom tkivu (*Naso i dr.,* 2005).

Organohlorni pesticidi i polihlorovani bifenili dospevaju u životnu sredinu kao posledica primene u poljoprivredi i industriji (*Nie i dr.,* 2012*; Meng i dr.,* 2013). Iako je njhova proizvodnja i upotreba zabranjena, ili ograničena, krajem 80-tih godina prošlog veka, mnoga istraživanja ukazuju da su i OCP i PCB jedinjenja još uvek prisutna u različitim delovima životne sredine, kao što su voda, vazduh i zemljište (*Loganathan i Kannan*, 1994; *Castro-Jimenez i dr.,* 2011; *Barakat i dr.,* 2013). Kao posledica prisustva u životnoj sredini, ova jedinjenja se mogu naći i u hrani (*Babut i dr.,* 2012*; Shoiful i dr.,* 2013). Na taj način dospevaju u lanac ishrane i mogu negativno uticati na zdravlje ljudi (*Baldassari i dr.,* 2007*;*

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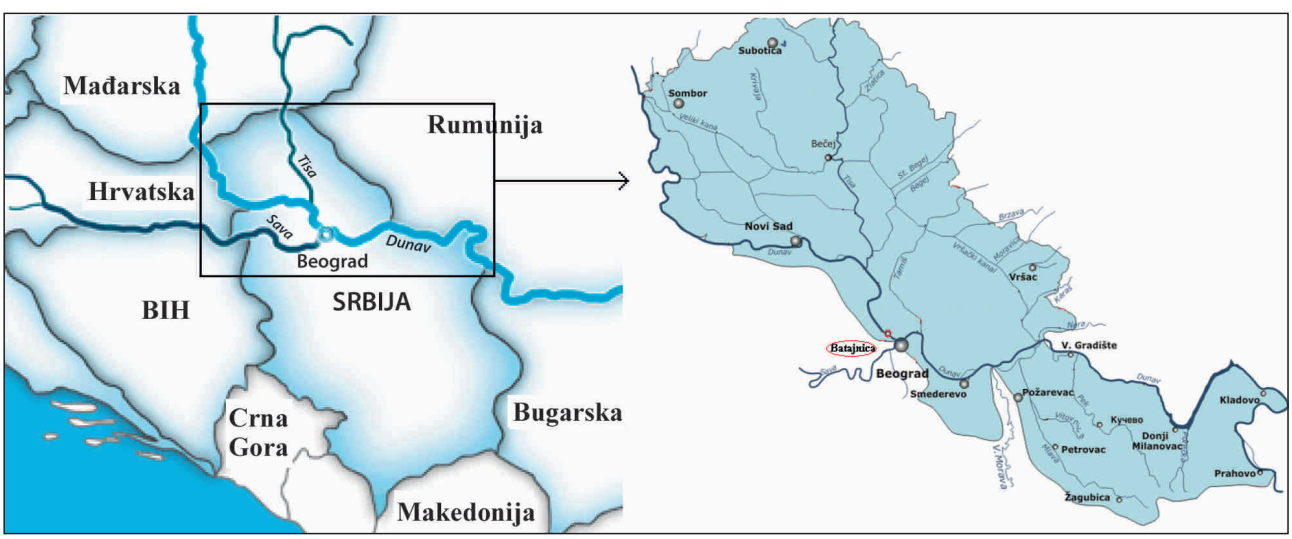
Shi i dr., 2013). Izloženost stanovništva organohlornim zagađivačima je najveća preko hrane, a oko 90% ovih zagađivača se unosi u organizam čoveka konzumiranjem prehrambenih proizvoda životinjskog porekla, pre svega ribe (*Baldassari i dr*., 2007; *Cole i dr*., 2009).

Sadržaji OCP i PCB jedinjenja u ribama mogu da zavise od više faktora, pre svega od zagađenja životne sredine ovim organohlornim jedinjenjima, zatim od vrste ribe, vremenskih prilika u pojedinim geografskim oblastima, itd. (*Eqani i dr.,* 2013). Rezultati istraživanja *Lo Turco i dr*. (2007) pokazuju da kavezno gajeni brancin (*Dicentrarchus labrax*), u Mediteranskom moru u Italiji, ima veći sadržaj DDT (izražen kao suma p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDT i p,p'-DDT) i PCB jedinjenja od brancina iz slobodnog izlova. Rezultati su pokazali da je sadržaj DDT jedinjenja u mišićnom tkivu i jetri kavezno gajenog brancina bio u opsegu 0,2–1,3 μg/kg (mišićno tkivo) i 9,6–48,4 μg/kg (jetra). U ribi iz slobodnog izlova sadržaji DDT jedinjenja je bio znatno manji: 0,1 μg/kg u mišićnom tkivu i 5,1–9,0 μg/kg u jetri. Ukupan sadržaj PCB jedinjenja u mišićnom tkivu i jetri bio je, takođe, veći u gajenom brancinu (5,3–59,7 μg/kg – mišićno tkivo, 74,4–267,4 μg/kg – jetra) u odnosu na istu vrstu ribe iz slobodnog izlova (1,1–1,5 μg/kg – mišićno tkivo, 63,2–109,4 μg/kg – jetra). Ove rezultate autori objašnjavaju prisustvom organohlornih zagađivača u vodama Mediterana, ali i većim sadržajem masti u kavezno gajenoj ribi.

Međutim, kada je reč o šaranu, kao vrsti ribe koja je najzastupljenija na tržištu u Srbiji, razultati ispitivanja zagađivača iz životne sredine u šaranu iz akvakulture (*Đinović i dr.,* 2010; *Trbović i dr.,* 2011; *Milijašević i dr.,* 2012) i šaranu iz slobodnog izlova (*Đinović-Stojanović i dr., neobjavljeni rezultati*) su drugačiji od rezultata italijanskih istraživača (*Lo Turco i dr.,* 2007). Naime, sadržaj DDT (izražen kao suma p,p′-DDE, p,p′-DDD, i p,p′-DDT) u mišićnom tkivu šarana iz akvakulture (28,0 μg/kg) i mišićnom tkivu šarana iz slobodnog izlova (22,0 μg/kg) bio je približno isti, dok je sadržaj polihlorovanih bifenila u mišićnom tkivu šarana iz slobodnog izlova (53,5 μg/kg) bio veći u odnosu na sadržaj PCB jedinjenja u mišićnom tkivu gajenog šarana (30,0 μg/kg).

U mnogim istraživanjima, riba se, često, koristi kao biološki indikator zagađenja životne sredine (*Babut i dr.,* 2012; *Thomas i dr.,* 2012; *Shi i dr.,* 2013). Sadržaji OCP i PCB jedinjenja u različitim vrstama ribe može da ukaže na prisustvo različitih zagađivača, ne samo u vodi, već i u sedimentu i zemljištu. *Harris i dr.* (1999) su pokazali da odnos koncentracija p,p'-DDE i p,p'-DDT u humanom mleku može da posluži kao indikator nedavne upotrebe p,p'-DDT u životnoj sredini. Ako je taj odnos veći od 0,5 može se zaključiti da p,p'-DDT nije bio korišćen, a njegovo prisustvo u ispitanim uzorcima mleka, pre svega, je posledica ishrane, tj. posledica kontamiranja hrane sa reziduama organohlornih jedinjenja. Međutim, kada se govori o biološkim organizmima, kao što je riba, *Eqani i dr.* (2013) ukazuju da odnosi koncentacija p,p'-DDE i p,p'-DDT, kao i odnosi koncentracija p,p'-DDD i p,p'-DDT ne mogu biti siguran indikator nedavne upotrebe p,p'-DDT, jer se degradacija p,p'-DDT dešava i kao posledica metabolizma u živim organizmima (*Muralidharan i dr.,* 2009).



Slika 1. Tok Dunava kroz Srbiju i lokacija uzimanja uzoraka ribe, Batajnica, april 2013.
Picture 1. Danube flow through Serbia and sampling location, Batajnica, april 2013.

Sa druge strane, nutritivne i zdravstvene koristi koje ljudi ostvaruju konzumiranjem ribe jedan su od razloga za povećanom potražnjom ribe na tržištu (*Burger i Gochfeld*, 2009). Sadržaj proteina u ribama kreće se u opsegu od 12 do 24% i veoma je sličan sadržaju proteina u mesu sisara. Riblje meso ima visok sadržaj vode (60 do 80%) i zanemarljivo nizak sadržaj ugljenih hidrata (*Ćirković i dr*., 2002). Nizak sadržaj masti i relativno nizak sadržaj holesterola, kao i značajan sadržaj vitamina i esencijalnih masnih kiselina čine ribu jednom od nutritivno najvrednijih prehrambenih proizvoda.

Cilj ovog rada bio je analiza sadržaja i distribucije organskih zagađivača u ribama, i to organohlornih pesticida i polihlorovanih bifenila u krupatici (*Blicca bjoerkna*) i mreni (*Barbus barbus*) sa lokaliteta Dunav kod Batajnice (slika 1). Takođe je određen i poređen osnovni hemijski sastav ovih riba. Eksperimentalno dobijeni podaci statistički su obrađeni Studentovim t-testom ($p < 0,05$).

Zakonska regulativa u Srbiji

Prema važećim propisima Republike Srbije, koji su u saglasnosti sa legislativom EU, maksimalno dozvoljena količina (MDK) za p,p'-DDT i njegove derivate (p,p'-DDE i p,p'-DDD) u mesu sa sadržajem masti većim od 10% je 1 mg/kg masti, dok za meso sa sadržajem masti manjim od 10% MDK iznosi 0,1 mg/kg jestivog dela (*Sl. glasnik RS,* br. 25/2010, 28/2011 i 20/2013; *Council directive* 86/363/EEC). Navedene vrednosti se u praksi primenjuju i za ribu, s obzirom da ni u Srbiji, a ni u EU ne postoje limiti za p,p′-DDT u ribi i proizvodima od ribe. Komisija Codex Alimentarius propisuje MDK za p,p′-DDT u ribi od 5 mg/kg masti. I u slučaju ostalih organohlornih jedinjenja, njihove MDK vrednosti za meso primenjuju se, u praksi, i za ribu.

Pravilnik o bezbednosti hrane u Srbiji je do polovine 2012. godine propisivao MDK za PCB jedinjenja, ne precizirijući o kojim se kongenerima radi. Propisane vrednosti date su u tabeli 1.

U EU, MDK je propisana samo za dioksine, dibenzofurane i dl-PCB (dioxin like PCB, dl-PCB) i izražava se u WHO-TEQ[1)] (toksični ekvivalent uspostavljen od svetske zdravstvene organizacije). TEQ se bazira na relativnoj toksičnosti svakog kongenera u odnosu na najtoksičniji dioksin, 2,3,7,8-TCDD, čija je toksičnost jednaka jedinici (*Van den Berg i dr.,* 2006*).* Važeći Pravilnik Republike Srbije koji se odnosi na bezbednost hrane (*Sl. glasnik RS,* br. 25/2010, 28/2011 i 20/2013) usklađen je sa EU propisima.

Tabela 1. Maksimalno dozvoljene količine PCB za neke vrste namirnica u Srbiji

Table 1. The maximum residue levels of PCBs in some foods in Serbia

Vrsta namirnice/ Type of food	**MDK, PCB (mg/kg)/ MRL, PCB (mg/kg)**
Mleko i proizvodi od mleka (izraženo na sadržaj masti)/ Milk and dairy products (expressed in relation to fat content)	1
Meso i proizvodi od mesa goveda, ovaca, svinja, konja i živine (izraženo na sadržaj masti)/ Meat and meat products – beef, sheep, pork, horse and poultry meat products (expressed in relation to fat content)	2
Jaja (bez ljuske)	0,3
Ribe, školjke, rakovi i mekušci (izraženo na jestivi deo)/ Eggs (without egg shell) Fish, shellfish, crustaceans and molluscs (expressed in relation to edible part)	3

U pokušajima da se definišu maksimalno dozvoljene količine za ndl-PCB više ekspertskih komisija je, na nivou EU, donosilo radne verzije propisa. Poslednja, iz 2006. god. propisuje količine ndl-PCB u namirnicama na osnovu ALARA (as low as reasonably achievable) principa, a ne na osnovu toksikoloških ispitivanja (*FAO–WHO,* 1997). MDK vrednosti za ndl-PCB u nekim vrstama namirnica su date u tabeli 2.

Materijali i metode

Uzimanje uzoraka

Ribe krupatica i mrena izlovljene su iz Dunava, sa lokaliteta Batajnica (slika 1), u aprilu 2013. godine. Za potrebe ispitivanja uzorkovano je po 6 jedinki svake vrste ribe. Prosečna masa krupatice bila je 174 g, a prosečna dužina 23 cm, dok je mrena imala prosečnu masu 320 g i prosečnu dužinu 31 cm. Uzorci su, do laboratorijskih ispitivanja, čuvani na –18°C. Riba je, pre ispitivanja, ostavljena sat

[1)] WHO-TEQ (World Health Organization, WHO – Toxic Equivalent, TEQ)

Tabela 2. Predložene maksimalno dozvoljene količine za ndl-PCB u EU
Table 2. The proposed maximum residue levels for ndl-PCBs in the EU

Vrsta namirnice/ Type of foods	**MDK, ndl-PCB – suma kongenera/ MRL, ndl-PCB – sum of congeners 28, 52, 101, 138, 153 i 180 (µg/kg)**
Mleko i proizvodi od mleka (izraženo na sadržaj masti)/ ***Milk and dairy products*** (expressed in relation to fat content)	50
Meso (izraženo na sadržaj masti)/ ***Meat*** (expressed in relation to fat content)	50
Preživari/ruminants	50
živina i divljač/poultry and wild game	50
svinje/pigs	
Jetra (izraženo na sadržaj masti)/liver (expressed in relation to fat content)	200
Jaja (izraženo na sadržaj masti)/ ***Eggs*** (expressed in relation to fat content)	50
Ribe i proizvodi od riba (izraženo na jestivi deo)/ Fish and fish products (expressed in relation to edible part)	100
Jegulja (izraženo na jestivi deo)/ Eel (expressed in relation to edible part)	200
Masti i ulja/Fats and oils	
Životinjska/animal	50
Biljna/plant	50
Riblja/fish	200

vremena na sobnoj temperaturi, da bi se delimično odmrzla i lakše skinula koža, odvojila glava i rep i uklonila utroba. Odvojeni fileti ribe su homogenizovani u homogenizatoru Braun CombiMax 600 (Germany).

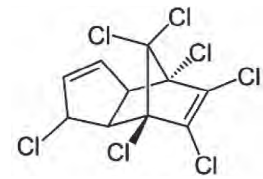
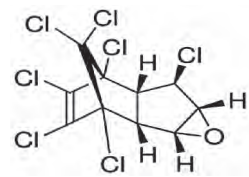
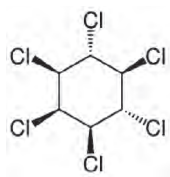
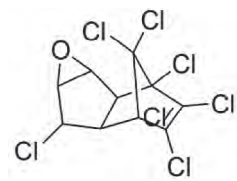
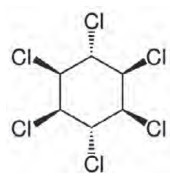
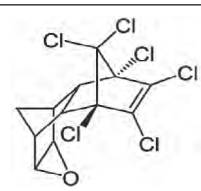
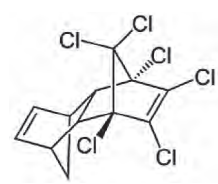
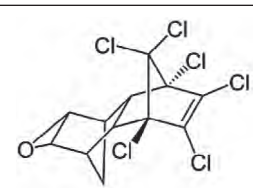
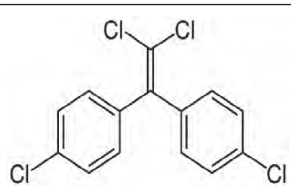
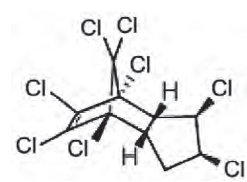
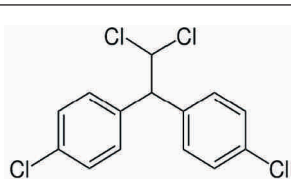
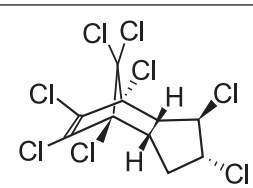
Analiza hemijskog sastava ribe

Hemijski sastav ribe određen je prema standardnim SRPS ISO metodama. Sadržaj proteina (N × 6,25) određen je metodom po Kjeldahlu, na aparatu Kjeltec Auto 1030 Analyzer (Tecator, Sweden), (SPRS ISO 937/1992). Sadržaj vlage određen je sušenjem na temperaturi od 103 ± 2°C, do konstantne mase (SPRS ISO 1442/1998). Ukupna mast određena je ekstrakcijom masti petroletrom, korišćenjem aparature po Soxhletu, nakon kisele hidrolize uzorka (SPRS ISO 1443/1992). Sadržaj pepela je određen merenjem mase ostatka nakon žarenja na temperaturi od 550 ± 25°C (SPRS ISO 936/1999).

Organohlorni pesticidi i polihlorovani bifenili

Ostaci 16 organohlornih pesticida i 7 kongenera polihlorovanih bifenila određeni su u mišićnom tkivu riba. Nazivi i strukturne formule analiziranih organohlornih jedinjenja prikazane su u tabelama 3 i 4. Za potrebe određivanja sadržaja OCP i PCB jedinjenja u ribi, ekstrahuje se mast iz homogenizovanog uzorka ribe pomoću petroletra. Rastvarač se upari na rotacionom vakuum uparivaču, a od ekstrahovane masti odmeri se 0,1 g za dalju analizu. Odmereni ekstrakt masti prečišćava se na koloni sa aluminijum-oksidom, koji je prethodno deaktiviran vodom. Organohlorna jedinjenja se iz ekstrahovane masti, koja je nanesena na kolonu, eluiraju n-heksanom. Eluat se upari do suva, a suvi ostatak se rasvori u 1 ml n-heksana. Organohlorni pesticidi i polihlorovani bifenili su identifikovani i kvantifikovani primenom gasne hromatografije sa ^{63}Ni detektorom sa zahvatom elektrona (GC/ECD). Gasni hromatograf (GC Varian model

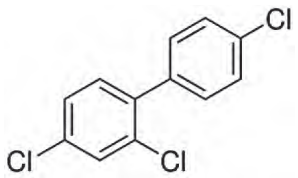
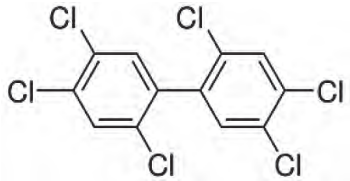
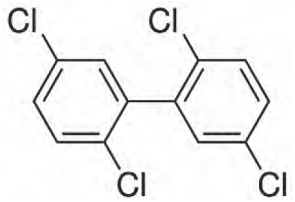
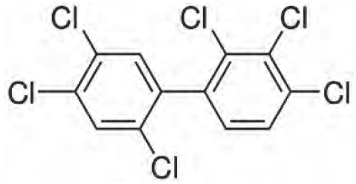
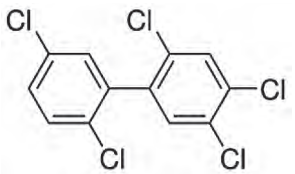
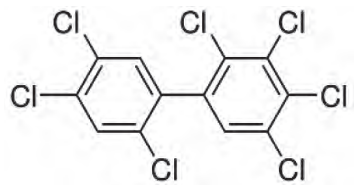
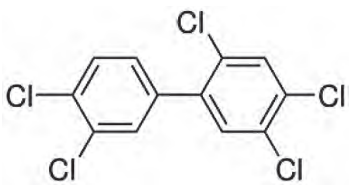
Tabela 3. Nazivi i strukturne formule analiziranih organohlornih pesticida
Table 3. The names and structural formulas of the analyzed organochlorine pesticides

Naziv jedinjenja/ Name of the compound	**Strukturna formula/ Structural formula**	**Naziv jedinjenja/ Name of the compound**	**Strukturna formula/ Structural formula**
α-HCH[a]		Heptahlor/ Heptachlor	
ß-HCH		Heptahlor-endo-epoksid Izomer A (trans) (trans-HCE)/ Heptachlor-endo-epoxide Isomer A (trans) (trans-HCE)	
δ-HCH		Heptahlor-egzo-epoksid Izomer B (cis) (cis-HCE)/ Heptachlor-exo-epoxide Isomer A (trans) (trans-HCE)	
γ-HCH -Lindan		Endrin/ Endrin	
[b] HCB		Aldrin/ Aldrin	
[c] p,p'-DDT		Dieldrin/ Dieldrin	
[d] p,p'-DDE		*cis*-hlordan/ *cis*-chlordane	
[e] p,p'-DDD		*trans* –hlordan *trans*-chlordane	

[a] HCH – heksahlorocikloheksan; [b] HCB – heksahlorobenzen; [c] DDT – p,p'–dihlorodifeniltrihloroetan; [d] DDE – p,p'–dihlorodifenildihloroeten; [e] DDD – p,p'–dihlorodifenildihloroetan

[a] HCH – hexachlorocyclohexane; [b] HCB– hexachlorobenzene [c] DDT – p p'–dichlorodiphenyltrichloroethane; [d] DDE– p p'–dichlorodiphenyldichloroethylene [e] DDD – p p'–dichlorodiphenyldichloroethane

Tabela 4. Nazivi i strukturne formule analiziranih polihlorovanih bifenila[a]
Table 4. The names and structural formulas of analyzed polychlorinated biphenyls[a]

Naziv jedinjenja / Name of the compound	Strukturna formula / Structural formula	Naziv jedinjenja / Name of the compound	Strukturna formula / Structural formula
PCB 28		PCB 153	
PCB 52		PCB 138	
PCB 101		PCB 180	
PCB 118			

[a] PCB–polihlorovanibifenili/[a] PCB – polychlorinated biphenyls:
2,4,4'–trihlorobifenil (PCB -28)/ 2,4,4'–trichlorobiphenyl (PCB -28)
2,2',5,5'–tetrahlorobifenil (PCB-52)/ 2,2',5,5'–tetrachlorobiphenyl (PCB-52)
2,2',4,5,5'–pentahlorobifenil (PCB -101)/ 2,2',4,5,5'–pentachlorobiphenyl (PCB -101)
2,3',4,4',5–pentahlorobifenil (PCB -118)/ 2,3',4,4',5–pentachlorobiphenyl (PCB -118)
2,2',4,4',5,5'–hexahlorobifenil (PCB -153)/ 2,2',4,4',5,5'–hexachlorobiphenyl (PCB -153)
2,2',3,4,4',5'–hexahlorbifenil (PCB 138)/ 2,2',3,4,4',5'–hexachlorbiphenyl (PCB 138)
2,2',3,4,4',5,5'–heptahlorobifenil (PCB -180)/ 2,2',3,4,4',5,5'–heptachlorobiphenyl (PCB -180)

CP-3380) radio je u spilit less modu, primenom odgovarajućeg temperaturnog programa, a korišćena je kolona Zebron ZB-1 (30 m × 0,25 mm i.d. i 0,25 μm debljina filma). Azot visoke čistoće korišćen je kao noseći gas. Podaci su obrađeni korišćenjem Varian Star softvera.

Statistička analiza

Eksperimentalni podaci, prikazani kao srednja vrednost ± standardna devijacija, su statistički obrađeni Studentovim t-testom na nivou značajnosti od 95%. Za statističku obradu rezultata korišćen je softver Microsoft Office Excel 2007 i njegov standardni dodatak Data Analysis ToolPak.

Rezultati i diskusija

Sadržaji OCP i PCB jedinjenja u ispitanim uzorcima riba prikazani u tabeli 5, a osnovni hemijski sastav (sadržaj proteina, lipida, vlage i pepela) i izračunate energetske vrednosti za obe vrste ribe prikazano je u tabeli 6.

Tabela 5. Sadržaj organohlornih pesticida i polihlorovanih bifenila u dve vrste ribe iz Dunava (srednja vrednost ± standardna devijacija, n = 6)

Tabela 5. Content of OCPs and PCBs in two freshwater fish species from the Danube river (mean value ± standard deviation, n = 6)

Vrsta ribe /Fish species / **Jedinjenja / Compounds**	**Krupatica/ (Silver Bream) [ng/g ribe/fish]**	**Mrena/ (Barbel)**
α-HCH	nd*	nd
β-HCH	nd	nd
HCB	0,38 ± 0,07	nd
γ-HCH	0,38 ± 0,08	nd
δ-HCH	nd	nd
Heptahlor	nd	nd
Aldrin	nd	nd
cis-HCE	$0,94^{a}$ ± 0,33	$0,16^{b}$ ± 0,05
trans-HCE	nd	0,24 ± 0,04
γ-Hlordan	nd	nd
α-Hlordan	nd	nd
p,p'-DDE	$9,06^{a}$ ± 1,74	$1,72^{b}$ ± 0,35
Dieldrin	nd	0,28± 0,04
Endrin	nd	nd
p,p'-DDD	$4,74^{a}$ ± 1,56	$0,84^{b}$ ± 0,17
p,p'-DDT	nd	nd
ΣDDT**	$13,80^{a}$ ± 2,59	$2,57^{b}$ ± 0,52
ΣPCB***	$40,91^{a}$ ± 8,44	$7,16^{b}$ ± 0,98

* nd – nije detektovano, ispod limita detekcije (0,001 μg/g masti)/nd – not detected, bellow limit of detection (0,001 μg/g lipid)

** ΣDDT = Σ(p,p'-DDE + p,p'-DDD + p,p'-DDT)

***ΣPCB = Σ(28+52+101+153+138+180)

a,b Vrednosti u istom redu označene različitim slovima se značajno razlikuju (P < 0,05)/Values in the same row followed by different letters are significantly different (P < 0.05)

Tabela 6. Hemijski sastav fileta krupatice i mrene (srednja vrednost ± standardna devijacija, n = 6)

Table 6. Proximate composition of Silver Bream and Barbel fillets (mean value ± standard deviation, n = 6)

Parametri/Parameters	**Krupatica/ (Silver Bream)**	**Mrena/ (Barbel)**
Sadržaj proteina, %/Protein content,%	19,63 ± 0,17	19,38 ± 0,30
Sadržaj masti,%/Fat content,%	$4,25^{a}$ ± 0,05	$1,07^{b}$ ± 0,06
Sadržaj vlage, %/Moisture content,%	$75,14^{a}$ ± 0,23	$78,31^{b}$ ± 0,22
Sadržaj pepela, %/Ash content,%	$0,92^{a}$ ± 0,03	$1,10^{b}$ ± 0,02
Energetska vrednost/Energy value, kcal/100g	$117,01^{a}$ ± 1,06	$87,73^{b}$ ± 0,72
Energetska vrednost/Energy value, kJ/100g	$491,94^{a}$ ± 4,45	$371,49^{b}$ ± 3,09

n – broj uzoraka/number of samples

a,b Vrednosti u istom redu označene različitim slovima se značajno razlikuju (P < 0,05)/Values in the same row followed by different letters are significantly different (P < 0.05)

Od organohlornih pesticida, cis-HCE, p,p'-DDE i p,p'-DDD identifikovani su i kvantifikovani su u filetima obe vrste riba. Njihovi sadržaji u filetima krupatice i mrene su se statistički značajno razlikovali ($P < 0,005$), (tabela 5), a veći sadržaji ovih jedinjenja određeni su u filetima krupatice. U filetima krupatice kvantifikovani su još i HCB i γ-HCH, a u filetima mrene i trans-HCE i dieldrin. Među ispitanim pesticidima u obe vrste ribe najveći sadržaj utvrđen je za p,p'-DDE. Sadržaj p,p'-DDT je bio ispod granice detekcije (0,001 mg/kg) u obe vrste riba. Ovo se može objasniti kratkim vremenom poluraspada p,p'-DDT u ribi (~8 meseci) u poređenju sa vremenom poluraspada DDE i DDD, koji iznosi oko 7 godina (*Binelli i Provini*, 2003). Ako se ukupni DDT (ΣDDT) posmatra kao zbir p,p'-DDT, p,p'-DDE i p,p'-DDD, evidentno je da najveći doprinos ukupnom DDT-u daje metabolit p,p'-DDE, što je u saglasnosti sa istaživanjima drugih autora (*Davodi i dr.,* 2011). p,p'-DDE nastaje razgradnjom p,p'-DDT, veoma je perzistentno u prirodi, i, stoga, može da posluži kao mera ekspozicije p,p'-DDT u prošlosti. U obe vrste riba, α-HCH, β-HCH, δ-HCH, heptahlor, aldrin, γ-hlordan, α-hlordan i endrin nisu detektovani iznad granice detekcije (0,001 mg/kg). Ukupan sadržaj PCB jedinjenja u filetima krupatice (40,9 ng/g ribe) bio je veći nego u filetima mrene (7,2 ng/g ribe) i statistički se značajno razlikovao ($p < 0,05$).

Dobijeni rezultati ispitanih organohlornih zagađivača u uzorcima ribe iz Dunava, Batajnica (tabela 5), u pogledu sadržaja ΣDDT i ΣPCB, mogu se porediti sa sadržajem ovih jedinjenja u istim vrstama riba iz različitih evropskih i svetskih vodenih sredina. Na primer, u mreni koja je uzorkovana u najvećoj i najpoznatijoj močvari u Iranu utvrđen je prosečan sadržaj ΣDDT od 340 ng/g masti (*Davodi i dr.,* 2011), dok je u našem istaživanju ta vrednost za ΣDDT iznosila 240 ng/g masti. Međutim, sadržaj sume 7 kongenera PCB u mreni u našem istaživanju (~670 ng/g masti) je znatno veći od rezultata *Davodi-a i dr.* (2011), (Σ_7PCB = ~215 ng/g masti). Ako dobijene rezultate za mrenu (ΣDDT = 2,6 ng/g ribe; Σ_7PCB = 7,2 ng/g ribe), (tabela 1) poredimo sa rezultatima grčkih istraživača, koji su ispitali mrenu iz reke Nestos (ΣDDT = 0,47-0,25 ng/g ribe; Σ_{36}PCB = 3,6–6,8 ng/g ribe), (*Christoforidis i dr.,* 2008) može se zaključiti da je Dunav u Srbiji zagađeniji organohlornim zagađivačima u poređenju sa rekom Nestos u Grčkoj. Međutim, ako rezultate iz ove studije za sadžaj PCB u mreni (Σ_7PCB = 7,2 ng/g ribe) poredimo sa sadržajem PCB u mreni iz vodotokova na severu Luksemburga (Σ_7PCB = 14,8 ng/g ribe), (*Boscher i dr.,* 2010), može se zaključiti da je sadržaj polihlorovanih bifenila u mreni iz Dunava kod Batajnice dva puta manji.

Prema dostupnoj literaturi koji se odnose na sadržaj organohlornih zagađivača u krupatici iz rečnih vodotokova su veoma oskudni. U krupatici, koja je, takođe, uzimana iz Dunava, ali nizvodno od Pančeva, utvrđeni sadržaj polihlorovanih bifenila (Σ_7PCB = 36,8–40,4 ng/g ribe), (*Jankovic i dr.,* 2011), bio je sličan rezultatima našeg istraživanja (Σ_7PCB = 40,9 ng/g ribe).

Podaci koji se odnose na osnovni hemijski sastav, kako fileta mrene tako i fileta krupatice, su dostupniji u litetaturi i značajni su za poređenje sa dobijenim rezultatima u ovoj studiji (tabela 6). Na osnovu rezultata Studentovog t-testa ($P < 0,05$) utvđeno je da postoji statistički značajna razlika u sadržaju masti, vlage, pepela i izračunate energetske vrednosti između fileta krupatice i mrene. Jedino u slučaju sadražaja proteina nije utvrđena statistički značajna razlika između fileta ispitanih vrsta riba. Sadržaj masti bio je znatno veći (4,25%) u filetima krupatice u odnosu na sadržaj masti u filetima mrene (1,07%). Rezultati istraživanja *Clement-a i dr.* (2012), koji su proučavali ribu iz reke Rone u Francuskoj, takođe, pokazuju da je sadržaj masti u filetima krupatice (16,3%–27,8%) bio veći od saržaja masti u filetima mrene (4,8%–19,9%). Međutim, u poređenju sa rezultatima ovog istraživanja vidi se da je sadržaj masti u istoj vrsti ribe iz reke Rone znatno veći u odnosu na ribu iz Dunava u blizini Batajnice.

Ekotosikološke studije koje koriste ribu kao bioindikatore ističu da biološki faktori, kao što su vrsta ribe, starost, veličina, njihovo fiziološko stanje i dr., mogu uticati na bioakumulaciju organskih zagađivača (*Fisk i dr.*, 2001; *Borga i dr*., 2004). Položaj ribe u lancu ishrane i sadržaj lipida predstavljaju drugi važan faktor u akumulaciji organohlornih jedinjenja (*Roche i dr*., 2000; *Zhou i Wong*, 2004). Rezultati ovog istraživanja mogu se objasniti razlikama u hemijskom sastavu ove dve vrste riba, pre svega u sadržaju ukupnih lipida koji je četiri puta veći u filetima krupatice nego u filetima mrene. Shodno tome, veći sadržaj analiziranih lipofilnih jedinjenja je dokazan u krupatici nego u mreni, izuzev za trans-HCE i dieldrin koji su detektovani samo u mreni.

Zaključak

Rezultati ovog istraživanja su pokazali da postoje statistički značajne razlike (na nivou značajnosti od 95%) između sadržaja organohlornih pesticida i polihlorovanih bifenila u krupatici i mreni iz Dunava. Utvrđeno je da je sadržaj ispitanih organohlornih jedinjenja u krupatici veći nego u mreni, sem za trans-HCE i dieldrin. Statistički značajna razlika utvrđena je između većine parametara osnovnog

hemijskog sastava ispitanih riba (mast, vlaga, pepeo, energetska vrednost), izuzev za sadržaj proteina.

Prisustvo organohlornih pesticida i polihlorovanih bifenila u ribama iz Dunava (Batajnica, Srbija) ukazuje na potrebu za kontinuiranim praćenjem organohlornih, ali i nekih drugih zagađivača u različitim vrstama ribe. Preventivna i efikasna zaštita ekosistema Dunava je neophodna u cilju očuvanja životne sredine, koja može obezbediti direktne i indirektne ekonomske, socijalne i ekološke pogodnosti u čitavom slivu Dunava, odnosno u svih devet zemalja kroz koje Dunav protiče.

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Distribution of organochlorine pesticides and polychlorinated biphenyls in two spices of fish from Danube

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S u m m a r y: The distribution and contents of organochlorine pesticides (OCPs) and congeners of polychlorinated biphenyls (PCBs) were analyzed in Bream (Blicca bjoerkna) and Barbel (Barbus barbus) from the Danube River, near Batajnica. The analysed compounds were 16 OCPs (α-Hexachlorocyclohexane (α-HCH), β-Hexachlorocyclohexane (β-HCH), Hexachlorobenzene (HCB), γ-Hexachlorocyclohexane (γ-HCH), δ-Hexachlorocyclohexane (δ-HCH), Heptachlor, Aldrin, cis-Heptachloroepoxide (cis-HCE), trans-Heptachloroepoxide (trans-HCE), γ-Chlordane, p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE), α-Chlordane, Dieldrin, Endrin, p,p'-Dichlorodiphenyldichloroethane (p,p'-DDD), p,p'-Dichlorodiphenyltri-chloroethane (p,p'-DDT)) and 7 PCB congeners (IUPAC numbers 28, 52, 101, 138, 153, 180, 118). Determination and quantification of OCPs and PCBs were performed by a GC-ECD method. The proximate composition of fish fillets was determined by applying standard SRPS ISO methods. The statistically significant difference ($p < 0.05$) was found between the OCPs content in Bream and Barbel, as well as between PCBs content in the analysed fish filets. Among all analysed pesticides, both fish contained the highest amounts of ΣDDT (expressed as sum of p,p'-DDT + p,p'-DDE + p,p'-DDD), (13.8 ng/g fish – Bream, 2.6 ng/g fish – Barbel). The sum of PCB congeners in Bream filets (40.8 ng/g fish) was significantly higher than in Barbel filets (7.2 ng/g fish). Results of Student's t-test ($p = 0.05$) showed the differences in the content of lipids, moisture, ash and calculated energy value between the analysed fish filets. Four times higher fat content in Bream (4.25%) than in Barbel (1.07%) is one of the main reasons for higher content of OCPs and PCBs in Bream compared to Barbel, which were taken from the same location (The Danube River, Batajnica).

***Key words**: bream, barbel, organochlorine pesticides, polychlorinated bypheils, proximate composition.*

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Morfološke i biohemijske karakteristike prirodnih izolata bakterija mlečne kiseline izolovanih iz Zlatarskog sira

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S a d r ž a j: U radu su izolovani i ispitani sojevi bakterija mlečne kiseline izdvojeni iz Zlatarskog sira, koji je proizveden u skladu sa načelima tradicionalne proizvodnje. Na odgovarajućim selektivnim podlogama (MRS agar, GM17 agar) izolovano je 96 sojeva bakterija mlečne kiseline. Svi izolati su dalje podvrgnuti ispitivanjima koja su obuhvatila Gram pripadnost, ćelijsku morfologiju, katalaza i oksidaza reakciju, stvaranje ugljen-dioksida iz glukoze, rast na različitim temeperaturama (4°, 10°, 15°, 37° i 45°C), različitim vrednostima pH (4,5 i 6,5), u sredinama sa različitim koncentracijama soli (2%, 3%, 4,5% i 6,5%), stvaranje sluzi i ispitivanje acidogene aktivnosti. Izvršeno je preliminarno ispitivanje sposobnosti stvaranja nespecifičnih metabolita – bakteriocina. Determinacijom ovih sojeva pomoću API CHL 50 i Rapid ID 32 Strep testa utvrđeno je da ispitani izolati pripadaju rodovima: Enterococcus, Leuconostoc, Lactobacillus, Lactococcus. Kod 9 izolata utvrđeno je stvaranje antimikrobnih metabolita – bakteriocina.

***Ključne reči**: bakterije mlečne kiseline, prirodni izolati, Zlatarski sir, karakterizacija.*

Uvod

Paralelno sa industrijalizacijom i standardizacijom savremene proizvodnje, proizvodnja sireva bazirana na tradicionalim principima predstavlja značajno obeležje jednog naroda, država i regija. Tradicionalni način prerade mleka u sir još uvek je značajno zastupljen u našoj zemlji, kako kod individualnih proizvođača, tako i u poluindustrijskim pogonima. Ovakav način proizvodnje ima za posledicu širok spektar različitih vrsta sireva na tržištu, kao i sireva sa neujednačenim kvalitetom. Vrlo često se nazivaju *„majstorski"* sirevi, jer su proizvedeni po tradicionalnim tehnologijama koje se prenose sa generacije na generaciju, bez upotrebe komercijalnih starter kultura. Primenom starter kultura, koje su uobičajene u industrijskoj proizvodnji, postigla bi se uniformnost sireva i standardizacija kvaliteta, ali bi se značajno promenila njihova senzorska svojstva koja ih sada čine prepoznatljivim na tržištu.

Zlatarski sir je jedan od najznačajnijih predstavnika domaćih autohtonih belih sireva u salamuri. Proizvodi se od nekuvanog punomasnog kravljeg mleka u okolini Nove Varoši, u podnožju i na obroncima planine Zlatar (*Vesković Moračanin i dr.,* 2012a; *Vesković Moračanin i dr.,* 2012b). Autentičnost sireva zlatarskog podneblja, u odnosu na ostale sireve istog tipa, ali drugih regija, bazirana je na osobenosti autohtone mikroflore, prvenstveno bakterija mlečne kiseline (BMK), koje su nosioci mlečne fermentacije i procesa zrenja sireva. Pored osobenosti, važan element je i njihova raznolikost koja se ogleda u zastupljenosti velikog broja različitih vrsta BMK, ali i sojeva u okviru jedne vrste, koji, kao rezultat svoje metaboličke aktivnosti, dovode do nastanka gotovog proizvoda specifičnog ukusa (*Vesković Moračanin i dr.,* 2013; *Topisirović i dr*., 2007). Dominantnost određene vrste BMK direktno zavisi od vrste mleka, porekla životinje, načina ishrane, vrste i kvaliteta pašnjaka, nadmorske visine i dr. (*Topisirovic i dr.,* 2006). Zbog svega toga, autohtone BMK predstavljaju značajan potencijal u oblasti selekcije tehnološki i protektivno značajnih vrsta ili sojeva koji, primenjeni u domaćoj proizvodnji, mogu dovesti do nastanka zdravstveno bezbednog i proizvoda ujednačenog, standardizovanog kvaliteta (*Vesković Moračanin i Obradović*, 2010, *Radulović i dr.,* 2008; *Mijačević i Bulajić*, 2007; *Barros i dr.,* 2008). Takođe,

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njihova fiziološka, biohemijska, potencijalna protektivna svojstva i molekularno-genetske osobine daju osnovu za selekciju i odabir specifičnih sojeva BMK karakterističnih za određene regione. Proizvodnja sopstvenih (domaćih) starter kultura, sastavljenih od dobro izučenih prirodnih izolata BMK, je osnova za dobijanje proizvoda sa deklaracijom specifičnog geografskog porekla (*Ostojić i Topisirović*, 2006).

Cilj ovoga rada bio je da se ispita i definiše dominantna mikroflora BMK koja je nosilac procesa fermentacije i zrenja Zlatarskog sira; da se izoluju čiste kulture BMK; da se utvrde njihove najvažnije morfološke i biohemijske karakteristike i potencijalna bakteriocinska svojstva kao deo preliminarnih parametara u ispitivanju mogućnosti njihove šire primene u proizvodnji Zlatarskog sira.

Materijal i metode

Sojevi BMK su izolovani iz četiri uzorka Zlatarskog sira koji su imali poželjna senzorska svojstva karakteristična za ovu vrstu sira (prijatan ukus i miris, dobra konzistencija i karakteristična aroma). Osnovna senzorska svojstva sira (spoljašnji izgled, boja, izgled preseka, konzistencija, miris i ukus), bila su visoko ocenjena, sa rasponom ocena od 4,2 do 4,8. Pripadali su I kategoriji sireva sa ocenom „odličan“ i sa % maksimalnog kvaliteta iznad 90.

Izolacija BMK

Nakon uzorkovanja sira (20 g iz unutrašnjosti kriške sira), test uzorak za ispitivanje homogenizovan je sa 180 mL sterilnog 2% natrijum-citrata. Decimalna razblaženja (10^{-1} do 10^{-7}) pripremana su sa sterilnim fiziološkim rastvorom (0,85% NaCl). Po 1 mL svakog decimalnog razblaženja zasejavano je na selektivne podloge i inkubirano na određenim temperaturama karakterističnim za ispitane bakterije mlečne kiseline.

Laktobacili su izolovani metodom razblaženja zasejavanjem na MRS agara (Merck, Nemačka), u mikroaerofilnim uslovima (Anaerocult C mini, Merck, Nemačka) i inkubiranjem tokom 72h i 48h na temperaturama od 30ºC i 37ºC, respektivno. Nakon toga, „pikirane“ su različite kolonije izrasle na MRS agaru i trostruko prečišćavane (nazmenično ploča – bujon) u cilju dobijanja čistih kultura.

Određivanje okruglastih oblika bakterija mlečne kiseline, tj. laktokoka i enterokoka, vršeno je na M17 agaru (Merck, Nemačka) kome je dodato 0.5% glukoze (GM17 agar). Zasejane ploče inkubirane su na 30ºC tokom 48h. Trostruko prečišćavanje izolovanih sojeva vršeno je na isti način kao kod laktobacila.

Dobijeni izolati BMK čuvani su u odgovarajućim medijumima sa 15% glicerola (w/v), na temperaturi od –80ºC. Pre upotrebe, svaki izolat BMK dva puta je supkultivisan u 10 mL odgovarajućeg bujona (MRS ili GM17 sa 1% inokuluma, 24h na 30ºC, odnosno na 37ºC).

Određivanje osnovnih morfoloških i fizioloških svojstava izolata BMK

Karakterizacija izolata BMK, bazirana na njihovim morfološkim i fiziološkim svojstvima, vršena je u skladu sa postupcima i analitičkim koracima koje je postavio Sharpe još 1979 (*Sharpe,* 1979). Naime, izolovane čiste kulture BMK determinisane su pomoću klasičnih mikrobioloških metoda, koje su obuhvatile ispitivanje Gram pripadnosti, ispitivanje ćelijske morfologije, određivanje katalaza i oksidaza reakcije. Sledeća analitička faza bila je primena biohemijskih kitova, API CHL 50 i Rapid ID 32 Strep test (Bio-Mérieux, Francuska), u cilju preliminarne identifikacije, prvenstveno vrsta BMK. Za očitavanje rezultata ovih testova u radu je korišćen softverski paket API Web.

Pripadnost metaboličkoj grupi (homo- ili heterofermentativni put razlaganja šećera) određivana je tako što je u epruvetu sa odgovarajućom tečnom podlogom koja sadrži šećer (glukoza), kojoj je dodata Durhamova cevčica, zasejavano po 0,1 mL ispitivane kulture BMK i, nakon inkubacije (24h, na 37°C tj. na 30ºC), utvrđivano je prisustvo ili odsustvo gasa u cevčici.

Odnos izolata BMK prema soli (halotolerantnost) ispitana je zasejavanjem i inkubacijom u odgovarajućim hranljivim bujonima sa 2%, 3%, 4,5% i 6,5% NaCl. Na isti način određen je rast izolata BMK pri različitim temperaturama sredine (4º, 10º, 15º, 37º i 45°C) i različitim vrednostima pH medijuma (4,5 i 6,5). Navedena ispitivanja ponovljena su tri puta.

Osobina produkcije egzopolisaharida izolata BMK određena je ispitivanjem prisustva sluzi po površini zasejanog bujona, nakon inkubacije od 24–48h, na odgovarajućim temperaturama.

Utvrđivanje potencijalne bakteriocinske aktivnosti izolata BMK

Utvrđivanje potencijalne bakteriocinske aktivnosti izolata BMK vršeno je metodom agar-difuzije („agar well-diffusion“ metod), (*Schillinger i Lücke,* 1989).

Ispitani izolati BMK su preko noćni inkubirani u MRS bujonu na 37ºC, odnosno u GM17 bujonu na 30ºC. Nakon toga, po 1 mL bujonske kulture

prenošen je u mikrokivetu od 2 mL (Sarstedt, Nemačka) i centrifugirano tokom 10 minuta na 10000 obrtaja/min (centrifuga – Genofuge 16M, Techne, USA). Zatim je po 100 µL supernatanta prebacivano u dve mikrokivete; prvoj mikrokiveti je dodavano 50 µL 0,1 M KOH radi neutralisanja nastalih kiselih metabolita, a drugoj 10 µL proteinaze K (AppliChem – 600 m Ansou U/mL). Supernatant sa proteolitičkim enzimom termostatiran je 1 h na 37ºC.

Istovremeno, pripremljene su i ploče sa test--mikroorganizmom *(Listeria monocytogenes* ATCC 19111). U BHI agar ((HiMedia, Indija) sa nižim sadržajem agara (10 g/L), inokulisana je 18-časovna kultura *L. monocytogenes* (0,1 mL kulture koncentracije 10^7–10^8 ćelija/mL dodavano je na 100 mL osnovne podloge). Metalnim diskom, posle hlađenja podloge, pravljena su udubljenja u agaru zapremine, približno, 100 µL. Nakon toga, količina od 100 µL ispitivane kulture BMK je prebacivana u bunarčiće u agaru i posle osnovne difuzije (30 min. na 4ºC) ploče su termostatirane preko noći na 30ºC.

Pored antilisterijske aktivnosti, ispitana je i potencijalna antimikrobna aktivnost izolata BMK u odnosu na *Staphylococcus aureus* ATCC 6538 i *Esherichia coli* ATCC 11303.

Rezultat i diskusija

Na bazi preliminarnih analitičkih koraka (Gram-pripadnost, mikroskopski pregled u cilju utvrđivanja ćelijske morfologije, katalaza i oksidaza reakcija) odabrano je 96, potencijalno, različitih izolata BMK. Rezultati ispitivanja su pokazali dominantno prisustvo okruglih/okruglastih oblika (83), dok je 13 izolata imalo štapićastu formu. Dalja determinacija izolata BMK je nastavljena pomoću biohemijskih kitova, API CHL 50 i Rapid ID 32 Strep, gde je, na osnovu biohemijskih reakcija i fizioloških osobina, utvrđena pripadnost rodu i, potencijalno, vrsti BMK (tabela 1).

Rezultati dobijeni upotrebom softverskog paketa API Web pokazali su da odabrani izolati (46) pripadaju rodu *Lactococcus* (41 pripada izolat *Lactococcus lactis* ssp. *lactis*, 3 izolata pripadaju *Lactococcus garviae* i 2 izolata *Lactococcus* spp.). Rodu *Enterococcus* pripada 34 izolata (svi izolati su determinisani kao *Enterococcus faecalis*), dok je u rod *Lactobacillus* svrstano 13 izolata (11 izolata *Lactobacillus plantarum* i 2 izolata *Lactobacillus sakei*/ *Lactobacillus curvatus*). Kao *Leuconostoc mesenteroides* ssp. *mesenteroides* identifikovano je 3 izolata.

BMK predstavljaju heterogenu grupu Gram--pozitivnih, katalaza i oksidaza-negativnih mikroorganizama. Zahvaljujući svojim metaboličkim svojstvima (stvaraju mlečnu kiselinu iz glukoze) nosioci su mlečnokiselinske fermentacije, procesa zrenja, razvoja konačnog ukusa i nutritivnih karakteristika fermentisanog proizvoda i imaju direktan uticaj na održivost gotovog proizvoda (*Beresford i dr*., 2001; *Leroy i De Vuyst,* 2004). Nastala mlečna kiselina utiče na nekoliko značajnih elemenata u procesu proizvodnje sireva, kao što je aktivnost koagulanata, kvalitet gruša, reološka svojstva sireva, vlažnost i dr. (*Cogan i dr.*, 1997).

Mikroflora sira može se podeliti u dve grupe: starterni BMK i sekundarni mikroorganizmi ili nestarterni BMK (NSBMK) (*Beresford i dr.,* 2001).

Tabela 1. Preliminarna identifikacija BMK izolata na bazi fizioloških osobina i Api testa
Table 1. Preliminary identification of LAB isolates based on physiological properties and Api test

BMK – Rod/ LAB – Genus	**Broj izolata/ Number off isolated**	**BMK – Vrste/ LAB – Species**	**Broj izolata/ Number off isolates**
Lactococcus spp.	46	*Lactococcus lactis* ssp. *lactis* *Lactococcus garviae* *Lactococcus* spp.	41 3 2
Enterococcus spp.	34	*Enterococcus faecalis*	34
Lactobacillus spp.	13	*Lactobacillus plantarum* *Lactobacillus sakei*/ *Lactobacillus curvatus*	11 2
Leuconostoc spp.	3	*Leuconostoc mesenteroides* ssp. *mesenteroides*	3
Ukupan broj izolata BMK/total number of LAB isolates			96

Tabela 2. Potvrdni test potencijalne bakteriocinske aktivnosti izolata BMK
Table 2. Confirmatory test of the potential bacteriocin activity of LAB isolates

Bujonska kultura/ Broth cultures	Neutralisani bujon/ Neutrolized broth	Katalaza test/ Catalase test	Proteinaza test/ Proteinase test
+++	+++	+++	– – –

+++ izražena antimikrobna aktivnost/expressed antimicrobial activity
– – – nema antimikrobne aktivnosti/no antimicrobial activity

NSBMK je termin koji se koristi da bi se opisala sporedna bakterijska flora sposobna da raste pod selektivnim uslovima zrenja sira. Ove bakterije mogu biti rezultat sastava mleka, ili posledica postpasterizacijske kontaminacija iz sirarske opreme ili okolne sredine (*McSweeney i dr.,* 1993). Nalaze se, uglavnom, u tradicionalnim sirevima koji su proizvedeni u specifičnim ekološkim nišama. Ispitivanja su pokazala da su NSBMK od suštinskog značaja za razvoj ukusa, mirisa i ostalih organoleptičkih svojstava tradicionalnih sireva. Veruje se da su razlike u senzorskim svojstvima sireva uslovljene prisustvom različitih vrsta NSBMK koje su karakteristične za svaki region gde se sir proizvodi (*McSweeney i dr.,* 1993). Zbog svega toga, danas je povećan interes za razvojem specifičnih starternih mikroorganizama sačinjenih od prirodnih izolata BMK, čijom primenom bi se omogućila bolja kontrola procesa proizvodnje i nastanak proizvoda ujednačenog kvaliteta. Primena NSBMK našla bi svoj značaj u poluindustrijskim i industrijskim uslovima proizvodnje tradicionalnih sireva.

Relativno visoka učestalost enterokoka u Zlatarskom siru ne iznenađuje, budući da se enterokoke javljaju u različitim tipovima evropskih sireva, naročito u sirevima pripremljenim iz sirovog ili pasterizovanog mleka (*Cogan i dr.,* 1997). Smatra se da je izvor enterokoka u mleku i siru feces, kontaminirana voda, mlekarska oprema, loši higijenski uslovi ili pretrpani prostori za skladištenje (*Gelsomino i dr.,* 2001). Iako je rod *Enterococcus* donedavno smatran indikatorom fekalne kontaminacije, danas se vrste ovog roda smatraju normalnom mikroflorom u hrani koja pozitivno utiče na razvoj specifičnih organoleptičkih karakteristika sira (*Giraffa i dr.,* 1997; *Fuller,* 1989).

Svi izolati BMK (96) rasli su na 37ºC i na 15°C, dok 15 izolata BMK loptastog oblika nije raslo na 45°C. Prisustvo dominantnih mezofilnih BMK je bilo očekivano (*Macedo i dr.,* 1995; *Radulović i dr*., 2004). Mezofilna mikroflora je bila znatno zastupljenija od termofilne, što je u skladu sa tehnologijom izrade Zlatarskog sira (u procesu proizvodnje, pre postupka podsiravanja, ne postoji faza dogrevanja mleka), kao i sa samom klimom zlatarske regije (umerena kontinentalna klima, bez visokih temperatura).

Svi izolati pripadaju grupi katalaza i oksidaza negativnih mikroorganizama.

Testiranjem pripadnosti homo- ili heterofermentativnom tipu razlaganja ugljenih hidrata (glukoze) utvrdjeno je da 5 izolata BMK vrši fermentaciju šećera heterofermentativnim putem, dok ostali 91 izolat BMK fermentiše glukozu bez stvaranja gasa.

Svi izolati BMK rasli su u sredinama sa različitim sadržajem soli (2%, 3%, 4,5% i 6,5%), kao i u sredinama sa kiselošću od 4,5 i 6,5. S obzirom da se tehnologija izrade Zlatarskog sira zasniva na upotrebi relativno velikog procenta soli (aproksimativno šaka soli na red kriški u bačvama), otpornost izolata BMK i njihova sposobnost da rastu u sredinama sa većim % soli može se smatrati jednom od njihovih fizioloških karakteristika (*Radulović i dr*., 2004).

Tri izolata BMK stvarala su sluz na tečnoj podlozi za rast.

Utvrđeno je da od 96 ispitanih izolata BMK poreklom iz Zlatarskog sira, njih 9 pokazuje potencijalnu produkciju antimikrobnih jedinjenja, bakteriocina. Šest izolata *Lc. lactis* ssp. *lactis* i 3 izolata *E. faecalis* imali su tipičan profil u potvrdnom testu koji se koristi za utvrđivanje bakteriocinske aktivnosti, u podlozi sa *L. monocytogenes* (tabela 2).

Izvedeni test sa proteolitičkim enzimom, proteinazom K, (proteinaza test) doveo je do inaktivacije delovanja bakteriocina, čime je na indirektan način potvrđena njegova proteinska priroda (*Joerger i sar.,* 2000).

U odnosu na izabrane test-mikroorganizme, svi izolati BMK pokazuju izraženu antibakterijsku aktivnost u odnosu na *L. monocytogenes*, dok je dejstvo na *S. aureus* i *E. coli* izostalo (tabela 3).

U prilog ovim rezultatima govore i nalazi drugih autora (*Schilinger*, 1990; *Abbe,* 1995) koji ukazuju na činjenicu da je inhibitorna aktivnost bakteriocina BMK dominantna, uglavnom na Gram-pozitivne bakterije. Velika taksonomska bliskost pozicija roda *Listeria* rodu *Lactobacillus* uslovljava visoku osetljivost *Listeria* vrsta prema bakteriocinima produkovanim od strane bakterija *Lactobacillus* spp. (*Ludvig*

Tabela 3. Antimikrobna aktivnost izolata BMK poreklom iz Zlatarskog sira
Table 3. Antimicrobial activity of LAB isolates originating from Zlatar cheese

Test-mikroorganizmi/ Test microorganisms	**Antimikrobna aktivnost izolata BMK/ Antimicrobial activity of LAB isolates *Lc. lactis* ssp. *lactis* (6 izolata/isolates), *E. faecalis* (3 izolata/isolates)**
L. monocytogenes ATCC 19111	+++
S. aureus ATCC 6538	–
E. coli ATCC 11303	–

+++ izražena antimikrobna aktivnost/expressed antimicrobial activity
– nema antimikrobne aktivnosti/no antimicrobial activity

i dr., 1984; *Ruhland i Fiedler,* 1987; *Wilkinson i Jones,* 1977).

Istraživanja iz oblasti antimikrobnog dejstva bakteriocina BMK, uglavnom, se, iz istog razloga, i proveravaju u odnosu na *L. monocytogenes* (*Daeschel,* 1989; *Schillinger i Lücke,* 1989, *Vesković Moračanin*, 2012), kao test mikroorganizam.

Zaključak

Proizvodnja sira, kao i drugih proizvoda od mleka, ima dugu tradiciju izrade na području Balkana. Ovi proizvodi mogu predstavljati vredan izvor bakterija mlečne kiseline s obzirom da naseljavaju različite ekološke niše. Naši rezultati daju značajan doprinos u istraživanju raznolikosti mikrobnih zajednica u Zlatarskom siru. Na temelju ovog istraživanja dobijene su nove informacije o ekologiji BMK i njihovim karakteristikama, ali je dat doprinos istraživanju BMK populacije u sirevima ovoga tipa, u celini. Ostale tehnološke i fiziološke karakteristike izolata koje mogu da utiču na konačan odabir i pripremu startera za proizvodnju sira biće analizirani u daljim eksperimentima. Naravno, neophodno je sprovesti i detaljnu, molekularno-genetsku, analizu izolovanih BMK, kako bi njihova potencijalna tehnološka primena, u proizvodnji Zlatarskog sira, bila u najskorije vreme realizovana.

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Morphological and biochemical characteristics of natural isolates of lactic acid bacteria isolated from Zlatar cheese

Vesković Moračanin Slavica, Borović Branka, Velebit Branko

S u m m a r y: In this research study, lactic acid bacteria (LAB) have been isolated from Zlatar cheese and subsequently scrutinized. The Zlatar cheese has been manufactured as an artisanal product. A total of 96 LAB strains have been isolated using selective media (MRS agar and GM17 agar, respectively). All isolates have been scrutinized using the following: Gram staining, cell morphology, catalase and oxidase reactions, production of carbon-dioxide converted from glucose, growth at the different temperatures (4°, 10°, 15°, 37° and 45°C), at different pH values (4.5 and 6.5) and different salt concentrations (2%, 3%, 4.5% and 6.5%), slime formation and testing of acidogenic activity. Preliminary testing on non-specific metabolites – bacteriocines has been conducted also. Determination of the strains using API CHL 50 and Rapid ID 32 Strep tests indicated that isolates tested belong to the following genera: Enterococcus, Leuconostoc, Lactobacillus and Lactococcus. A total of 9 strains have been confirmed positive on antimicrobial metabolites – bacteriocines synthesis.

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