

## VARIATION IN ENTEROBACTERIACEAE COUNT DETERMINED IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND CARP (*CYPRINUS CARPIO*) STEAKS PACKED IN VACUUM AND MODIFIED ATMOSPHERE

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### PRAĆENJE PROMENE BROJA BAKTERIJA FAMILIJE ENTEROBACTERIACEAE U PASTRMCI I ODRESCIMA ŠARANA UPAKOVANIM U VAKUUM I MODIFIKOVANU ATMOSFERU

#### *Abstrakt*

Kvar ribe može se definisati kao bilo koja promena u mesu ribe koja proizvod čini neprihvatljivim za ljudsku ishranu i najčešće nastaje kao rezultat rasta mikroorganizama. Od toga koji su mikroorganizmi najzastupljeniji u ribi u trenutku ulova u velikoj meri zavisi i kolika će biti njena održivost tokom skladištenja. Bakterije koje pripadaju familiji Enterobacteriaceae su fakultativni anaerobi, gram negativni i katalaza pozitivni mikroorganizmi. Ovoj familiji pripadaju sledeći rodovi: Salmonelle, Escherichia, Proteus, Shigella, Serratia, Citrobacter, Klebsiella, Enterobacter i Erwinia, a neki od njih su izraziti patogeni i izazivači velikog broja različitih oboljenja kod ljudi, kao što su septikemija, pneumonija, meningitis, infekcije urinarnog trakta, bolesti organa za varenje i mnogih drugih bolesti. Široko su rasprostranjeni u spoljašnjoj sredini, a neki su saprofitski stanovnici digestivnog trakta.

Cilj ovog eksperimenta je bio da se ispita rast bakterija familije Enterobacteriaceae u svežoj pastrmci i odrescima šarana upakovanim u vakuum i modifikovanu atmosferu. Uzorci su podeljeni u tri grupe. Grupa I je upakovana u modifikovanu atmosferu sa odnosom gasova 60%CO<sub>2</sub> i 40%N<sub>2</sub>, grupa II je upakovana u modifikovanu atmosferu sa 40%CO<sub>2</sub> i 60%N<sub>2</sub> dok je III grupa upakovana u vakuum. Odnos gas/uzorak u pakovanju bio je 2:1. Svi uzorci su skladišteni pri istovetnim uslovima na temperaturi od +3°C. Određivanje broja bakterija familije Enterobacteriaceae prema ISO 21528 -2:2004 obavljeno je 0, 7. i 14. dana skladištenja. U pastrmci i odrescima šarana upakovanim u

modifikovanu atmosferu sa 60%CO<sub>2</sub> i 40% N<sub>2</sub> (I grupa), rast ukupnog broja enterobakterija bio je sporiji nego u pastrmci i odrescima šarana upakovanim u modifikovanu atmosferu sa 40%CO<sub>2</sub> i 60%N<sub>2</sub> (II grupa) ili pak u vakuum (III grupa). Ovo se može objasniti antimikrobnim dejstvom ugljen-dioksida, koji je procentualno najzastupljeniji u smeši gasova u koju su upakovani uzorci I grupe kao i činjenicom da ugljen-dioksid deluje inhibitorno pre svega na gram-negativne bakterije, kakvi i jesu mikroorganizmi iz familije Enterobacteriaceae. Najveću stopu rasta imale su enterobakterije u pastrmci i odrescima šarana III grupe tj. u uzorcima upakovanim u vakuum. Ovi rezultati ukazuju na sposobnost Enterobacteriaceae da rastu u anaerobnim uslovima, na temperaturi frižidera u proizvodima upakovanim u vakuum.

Modifikovana atmosfera smanjuje ukupan broj enterobakterija, a najniža vrednost je dobijena kod odrezaka šarana upakovanih u modifikovanu atmosferu sa 60%CO<sub>2</sub> i 40%N<sub>2</sub>.

**Ključne reči:** *Enterobacteriaceae, pastrmka, odresci šarana, modifikovana atmosfera, vakuum*

## INTRODUCTION

Spoilage of fish can be defined as any change in fish meat rendering the product unfit for human consumption, and often is result of bacterial growth. Initial contamination by microorganisms depends on numerous factors: type of fish habitat (sea or fresh water, tropical or cold water, open water fish, or fish from coastal area or seabed fish), and on level of water contamination. Type of microflora growing in the product is determined by intrinsic (poikilotherm nature of the fish, pH post mortem, presence of trimethylamine oxide and other non-protein nitrogen compounds, content of fat) and anthropogenic parameters (storage temperature, procedures during production, packaging, etc.) (Siverstvik et al., 2002).

Bacterial flora of fish that has just been caught is very diverse. The following Gram negative species are present: *Pseudomonas*, *Moraxella*, *Actinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Aeromonas*. The major Gram-positive species are *Micrococcus* and coryneform bacteria.

Bacteria belonging to the family *Enterobacteriaceae* are facultative anaerobes, Gram negative and catalase positive microorganisms. The following genera belong to this family: *Salmonella*, *Escherichia*, *Proteus*, *Shigella*, *Serratia*, *Citrobacter*, *Klebsiella*, *Enterobacter* and *Erwinia*, and some of them are major pathogens and causes of numerous diseases in humans, such as septicemia, pneumonia, meningitis, urinary tract infections, diseases of the digestive organs and many other diseases (Govedarica, 1995). They are widespread in the environment, and some are saprophyte inhabitants of the digestive tract.

In 31% of tested samples of fresh water fish, stored at +7°C in retail stores Lindberg et al. (1998) proved the presence of *Enterobacteriaceae*. Addition of the conservants, vacuum packaging and packaging in modified atmosphere influence the microbial population of foodstuffs (Vasilopoulos et al., 2010). Vacuum packaging supresses the growth of *Pseudomonas-Actinetobacter-Moraxella* association, while in contrast, microaerophilic *Enterobacteriaceae* are growing (Radetić et al., 2007). Gram negative bacteria are more sensitive to the effect of CO<sub>2</sub> mainly used in concentrations of 40-60%

for packaging of foodstuffs in modified atmosphere. CO<sub>2</sub> inhibits the growth of microorganisms of spoilage, especially *Pseudomonas spp.*, *Enterobacteriaceae*, *Acinetobacter spp.*, *Achromobacter*, *Flavobacterium* and *Moraxella spp.*, as well as psychotrophic bacteria which cause meat spoilage. Arashisar et al. (2004) established that the growth rate of aerobic mesophilic and psychotrophic bacteria, as well as enterobacteria during whole storage period was the lowest in trout fillets packaged in atmosphere with 100%CO<sub>2</sub>. Results obtained by Milijašević et al. (2010) show antimicrobial effect of CO<sub>2</sub>, where significantly lower count of aerobic mesophilic bacteria and enterobacteria was determined in carp steaks packaged in atmosphere with 100%CO<sub>2</sub> compared to steaks packaged in the gas mixture of 40%CO<sub>2</sub> and 60%N<sub>2</sub>.

## MATERIAL AND METHODS

Rainbow trout (*Oncorhynchus mykiss*) used in the experiment was farmed in the same conditions and comes from trout pool located on the slopes of mountain Zlatibor. Fish was transported live from the fish farm to the slaughtering and processing facility, where it was placed in reception pool, and subsequently stunned by using electricity. Slaughtering and evisceration of fish was performed on an automatic apparatus, and carcasses were washed manually, under the stream of water. Marketable carp (*Cyprinus carpio*) originated from the fishpond located in the low land region of Serbia, where semi intensive farming was used. In this experiment, two year old carps of average body weight of 2,5 kg were used. Carps were transported live to the fish slaughtering and processing facility, where they were stunned, slaughtered, scale cleared, and carcass was cut in steaks 2 cm thick. Three sample groups of cleaned trout and carp steaks were formed. First two groups were packaged in modified atmosphere with different gas ratios: 60%CO<sub>2</sub>+40%N<sub>2</sub> (I group) and 40%CO<sub>2</sub>+60%N<sub>2</sub>(II group), whereas the third group of samples were vacuum packaged. The machine used for packaging of samples was Variovac (Variovac Primus, Zarrentin, Germany), and material used for packaging was foil OPA/EVOH/PE (oriented polyamide/etilene vinyl alcohol/polyetilene, Dynopack, Polimoon, Kristiansand, Norway) with low gas permeability (degree of permeability for O<sub>2</sub> – 3,2 cm<sup>3</sup>/m<sup>2</sup>/day at 23°C, for N<sub>2</sub> - 1 cm<sup>3</sup>/m<sup>2</sup>/day at 23°C, for CO<sub>2</sub> – 14 cm<sup>3</sup>/m<sup>2</sup>/day at 23°C and for steam 15 g/m<sup>2</sup>/day at 38°C). Ratio gas : sample in the package was 2:1. All samples were stored in the same conditions at the temperature of +3°C and on 0, 7. and 14. day of storage, microbiological testing was performed.

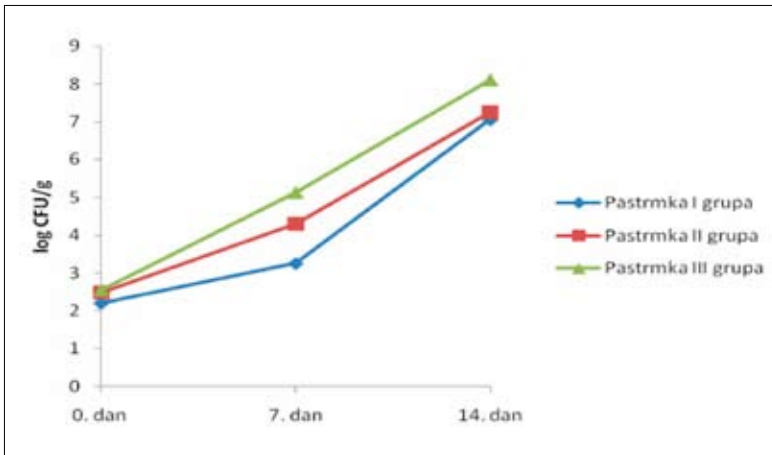
### Microbiological testing

Number of bacteria of the family *Enterobacteriaceae* was determined according to ISO 21528 -2:2004. Medium used for detection and enumeration of bacteria of the family *Enterobacteriaceae* was VRBD (violet red bile glucose agar).

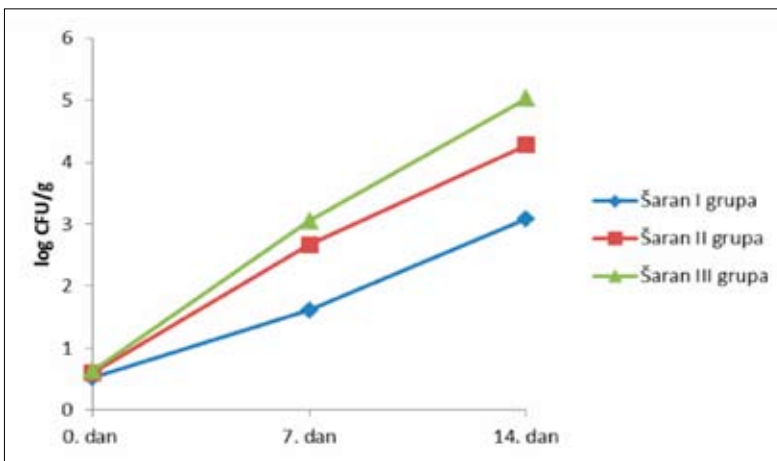
## RESULTS AND DISCUSSION

During storing of packaged carp steaks and trout at the temperature of +3°C, in duration of fourteen days, there was a statistically significant increase of total enterobacteriaceae count in all tested sample groups. In trout and carp steaks packaged in modified atmosphere with 60%CO<sub>2</sub> and 40% N<sub>2</sub> (I group), growth of total enterobacteriaceae count was slower than in trout and carp steaks packaged in the modified atmosphere with 40%CO<sub>2</sub> and 60%N<sub>2</sub> (II group) or vacuum packaged samples (III group) (Graph 1. and 2.). This can be explained by antimicrobial effect of carbon dioxide which was most

present in percentages in gas mixture used for samples of group I, as well as the fact that carbon dioxide has inhibitory effect primarily on Gram negative bacteria, such as the microorganisms of the family *Enterobacteriaceae*. The highest growth rate was established for enterobacteriaceae in trout and carp steaks of group III, i.e. vacuum packaged samples. These results show the ability of *Enterobacteriaceae* to grow in anaerobic conditions, at fridge temperature in the vacuum packaged products. Researches by other authors relating to the testing of the enterobacteriaceae count in fresh fish packaged in the modified atmosphere (Arashisar et al., 2004; Milijašević et al., 2010; Torrieri et al., 2006.) are in accordance with our results.



**Graph 1.** Change of the total enterobacteriaceae count I, II and III group of trout samples



**Graph 2.** Change of the total enterobacteriaceae count I, II and III group of carp steak samples

## CONCLUSION

Based on research results it was concluded that total enterobacteriaceae count in all three tested groups of trout was statistically significantly higher compared to enterobacteriaceae count recorded in carp steaks packaged and stored in same conditions. The lowest growth rate was established in case of enterobacteriaceae in trout and carp steaks of the group I, i.e. samples packaged in the modified atmosphere with 60%CO<sub>2</sub> and 40% N<sub>2</sub>.

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