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Effect of vacuum and modified atmosphere on *Enterobacteriaceae* count determined in rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) steaks

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Abstract

The aim of our research was to determine the presence and examine the change in total number of *Enterobacteriaceae* in trout and carp cuts packed in vacuum and modified atmosphere. To conduct the study, three sample groups of cleaned trout and carp cuts were formed. The first two groups were packaged in modified atmospheres with different gas ratios: 60%CO_2+40%N_2 (I group) and 40%CO_2+60%N_2 (II group), whereas the third group of fish cuts were vacuum packaged. Our results suggest that modified atmosphere packaging of fresh fish with an appropriate percentage of CO_2 might reduce the risk of poisoning people with *Enterobacteriaceae*.

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Keywords: carp cut; *Enterobacteriaceae;* modified atmosphere; vacuum; trout

1. Introduction

Due to its biological composition, fresh fish is classified into easily perishable foodstuffs. Basic reasons for faster spoilage processes in fish compared to meat originating from homeothermic organisms are lower content of

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connective tissue in fish muscle structure, increased water content in fish, higher pH, overall chemical composition, as well as specificities in microbiota and enzymes that can be found in aquatic organisms[1]. Initial changes in fish muscle occur at the moment of death, or even earlier, during fish harvesting, and are a consequence of enzymatic activity, microorganisms' metabolism and lipid oxidation[2]. Microbial growth is the most common cause of sensoric changes that make fish product unacceptable for human consumption[3]. The dominant type of microbiota developing in the product is determined by intristic factors (poikilothermic nature of fish, postmortem pH value, presence of trimethylamine oxide (TMAO) and other non-protein nitrogen-based compounds, fat content) and anthropogenic factors (storage temperature, operating procedures during production, types of packaging)[4]. Mesophilic species of *Enterobacteriaceae* contaminate foodstuffs to a lesser degree, while psychrotrophic microorganisms can grow at temperatures below 5°C and can be responsible for spoilage of foodstuffs that are stored within the cold chain[5].

Addition of preservation agents and packaging in vacuum or modified atmosphere have large influenes on the microbial status of foodstuffs. Radetić *et* al[6] concluded that vacuum packaging inhibits growth of *Psudomonas-Actinetobacter-Moraxella* association, while at the same time, microaerophilic *Enterobacteriaceae* show increased growth. Unlike the vacuum packaging of fish, which stimulates growth of microorganisms that use TMAO as a terminal electron acceptor, modified atmosphere packaging inhibits the growth of trimethylamine-producing microorganisms, as well as microorganisms that produce H_2S[7]. Modified atmosphere packaging replaces air with a single gas or gas mixture. Mixtures mainly consist of carbon-monoxide (CO_2), nitrogen (N_2) and oxygen (O_2) in different concentrations. Carbon dioxide is the principal antimicrobial gas, usually used at concentrations of 40-60% in modified atmosphere packaging of foodstuffs. Antimicrobial potential of CO_2 has been also confirmed by the results of Milijašević *et al*.[8] who found significantly lower counts of aerobic mesophilic bacteria and *Enterobacteriaceae* in carp cuts packaged in atmosphere containing 100% of CO_2 compared to cuts packaged in gas mixture (40% CO_2 and 60% N_2). The aim of our research was to determine the presence and examine the change in total number of *Enterobacteriaceae* in trout and carp cuts packed in two ways, in vacuum and modified atmosphere.

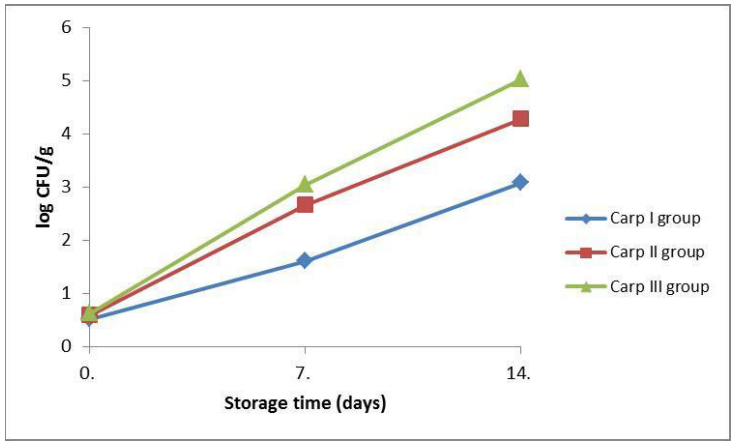
2. Materials and methods

Rainbow trout (*Oncorhynchus mykiss*) used in the study were farmed in the same conditions and came from a trout pool located on the slopes of Zlatibor Mountain. Marketable carp (*Cyprinus carpio*) originated from a fishpond located in the lowland region of Serbia, where semi intensive farming was used. In this study, two year old carp of average body weight of 2.5 kg were used. Three sample groups of cleaned trout and carp cuts were formed. The first two groups were packaged in modified atmosphere with different gas ratios: 60%CO_2+40%N_2 (I group) and 40%CO_2+60%N_2 (II group), whereas the third group of cuts were vacuum packaged. The machine used for packaging the cuts 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_2 - 3.2 cm^3/m^2/day at 23°C, for N_2 - 1 cm^3/m^2/day at 23°C, for CO_2 - 14 cm^3/m^2/day at 23°C and for steam 15 g/m^2/day at 38°C). The ratio of gas:sample in the packages was 2:1. All samples were stored in the same conditions at the temperature of +3 °C and on days 0, 7 and 14 of storage, microbiological testing was performed. *Enterobacteriaceae* numbers were determined according to ISO 21528-2:2004 (E) using violet red bile glucose agar. Characteristic colonies were from pink to red or violet color, or without halo precipitation rings.

3. Results and discussion

During storing of packaged carp steaks and trout at +3˚C, over the duration of fourteen days, there was a statistically significant increase of total *Enterobacteriaceae* count in all tested fish cut groups. In trout and carp steaks packaged in modified atmosphere with 60%CO_2 and 40% N_2 (I group), growth of total *Enterobacteriaceae* count was slower than in trout and carp steaks packaged in the modified atmosphere with 40%CO_2 and 60%N_2 (II group) or vacuum packaged samples (III group) (Figs. 1 and 2). This can be explained by the antimicrobial effect of carbon dioxide, the concentration of which was highest in gas mixture used for group I samples, as well as the fact that carbon dioxide has inhibitory effects 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 refrigeration temperature in the vacuum packaged fish. Investigations by other authors on the *Enterobacteriaceae* counts in fresh fish packaged in modified atmospheres[3,8,9] are in accordance with our results.



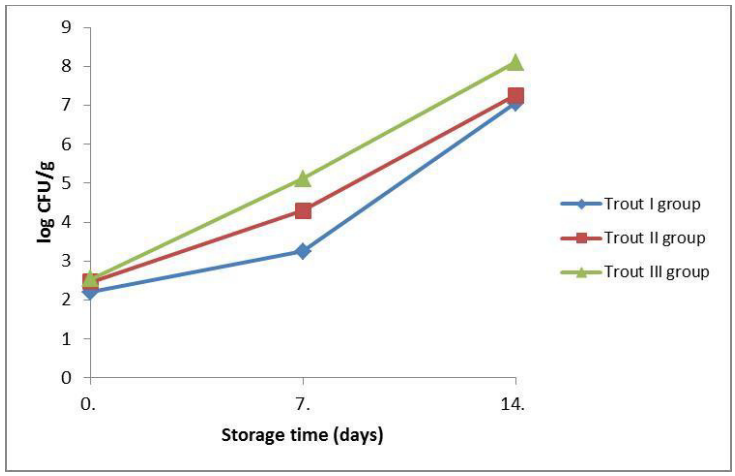


Fig.1. Change of the total *Enterobacteriaceae* count in I, II and III group of carp steaks.

Fig. 2. Change of the total *Enterobacteriaceae* count in I, II and III group trout steaks.

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