CHANGE NUMBER OF ENTEROBACTERIA DURING STORAGE OF COLD SMOKED TROUT PACKED IN VACUUM AND MODIFIED ATMOSPHERE

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PROMENA BROJA ENTEROBAKTERIJA U TOKU SKLADIŠTENJA HLADNO DIMLJENE PASTRMKE PAKOVANE U VAKUUMU I MODIFIKOVANOJ ATMOSFERI

Abstrakt

Danas je hladno dimljena riba sastavni deo naše ishrane. Razlog tome treba tražiti kako u povećanju proizvodnje ribe u akvakulturi, koja je samim tim dostupna u većoj količini za preradu, tj. dimljenje, tako i u sve većoj potrebi čoveka da se okrene zdravoj ishrani. Pri tome, hladno dimljena riba, pored toga što zadovoljava visoke nutritivne kriterijume, ona je i atraktivna za potrošača zbog svojih specifičnih senzornih karakteristika. Međutim, i pored toga što je dimljena riba, proizvod poznat vekovima, postojeći problem u proizvodnji dimljene ribe u zemljama Evropske Unije, ali i kod nas, predstavlja nepostojanje unapred utvrđenih kriterijuma koji treba da zadovolje dimljeni proizvodi od ribe. Pri tome se misli na definisanje jedinstvenih kriterijuma kvaliteta dimljene ribe i usaglašavanje pojedinih faktora proizvodnje (soljenje, dimljenje, izbor sirovine, pakovanje), kao i na određivanje održivosti tj. roka trajanja dimljenih proizvoda od ribe, koji se razlikuje od proizvođača do proizvođača. Održivost dimljene ribe, odnosna nastanak kvara, zavisi, od inicijalne kontaminacije, uslova proizvodnje, rukovanja sa proizvodom nakon proizvodnog procesa i temperature skladištenja i načina pakovanja. Iako kvar, hladno dimljenih proizvoda od ribe može nastati delovanjem različitih mehanizma, najvažniji razlog kvara mesa ribe i proizvoda od ribe je rast mikroorganizama i stvaranje produkata, rezultata njihove metaboličke aktivnosti koji dovode do pojave nepoželjnog mirisa i ukusa.

Pakovanje dimljenih proizvoda od mesa ribe u vakuumu, odnosno modifikovanoj atmosferi, može u velikoj meri uticati na održivost proizvoda, ali i udovoljiti zahte-

vima savremenog potrošača koji traži hranu visokog kvaliteta, koja je zadržala senzorne karakteristike i nutritivnu vrednost sirovine od koje je proizvedena, a da je uz to i bezbedna po njegovo zdravlje. Osnovni cilj pakovanja hrane u vakuumu jeste uklanjanje kiseonika iz hrane, s obzirom da on omogućava rast aerobnih mikroorganizama (bakterija, kvasaca i plesni) koji najčešće dovode do kvara mesa ribe i proizvoda, dok se pakovanjem hrane u modifikovanoj atmosferi, konzervišućim delovanjem primenjenih gasova, onemogućava ili usporava rast i razmnožavanja mikroorganizama, odgovornih za nastanak kvara. Zato se prisustvo i promena broja određenih grupa mikroorganizama često uzima kao parametar održivosti ribe i proizvoda od ribe. U literaturi se često kao mikroflora koja je odgovorna za nastanak kvara hladno dimljenih proizvoda, pored ostalih spominju i bakterije iz familije Enterobacteriaceae. Pored značajne uloge ovih bakterija u nastanku kvara hladno dimljenih proizvoda od ribe, postojanje mogućnosti trovanja ljudi, sa pojedinim vrstama bakterija iz ove familije, opredelila nas je da nam cilj istraživanja bude utvrđivanje prisustva i broja bakterije iz familije Enterobacteriaceae u gotovim proizvodima hladno dimljene pastrmke pakovane u vakuumu i modifikovanoj atmosferi, u toku šest nedelja skladištenja pri +3 °C. Za eksperiment su formirane četiri grupe hladno dimljenih fileta pastrki. Prva grupa (I) uzoraka je vakuumirana, a ostale tri su pakovane u tri različite modifikovane atmosfere: druga grupa (II) – 50% CO_2 + 50% N_2, treća grupa (III) - 60% CO_2 + 40% N_2 i četvrta grupa (IV) - 90% CO_2 + 10% N_2, a nultog, a zatim svakih sedam dana, šest nedelja utvrđivan je ukupan broj enterobakterija. Rezultati naših ispitivanja pokazuju da je u uzorcima sve četiri grupe u toku skladištenja došlo do statistički značajnog porasta ukupnog broja enterobakterija. Takođe, rezultati su potvrdili činjenicu da CO_2 deluje inhibitorno pre svega na gram-negativne bakterije, kakvi i jesu mikroorganizmi iz familije Enterobacteriacea, s obzirom da je ukupan broj enterobakterija tokom celog perioda skladištenja bio statistički značajno niži u uzorcima fileta hladno dimljene pastrmke pakovane u smeši gasova. Ujedno, najslabija stopa rasta enterobakterije utvrđena je u filetima hladno dimljene pastrmke IV grupe, tj. grupe u kojoj je procentulno ugljen dioksid bio najzustupljeniiji, tj. da je u njima najviše izraženo antimikrobno dejstvo ugljen dioksida.

S obzirom da kod proizvođača ribe u našoj zemlji postoji interes da prošire asortiman proizvodnje, a u toj mogućnosti proširenja asortimana najinteresantnija je proizvodnja dimljene ribe. Implementacija savremenih načina pakovanja u proizvodnji hladno dimljene pastrmke, ovakav proizvod treba da učine što pristupačnijim i za potrošača. Otuda i u našoj zemlji postoji interes za ispitivanje različitih načina pakovanja koji utiču na kvalitet dimljene pastrmke. A praćenje promena ukupnog broja enterobakterija, kao jednog od najznačajnih indikatora kvaliteta hladno dimljene ribe, u ispitivanim uzorcima pakovanim u vakuumu i modifikovanoj atmosferi, predstavlja samo jedan korak u ka uspostavljanju objektivnih kriterijuma za ocenu kvaliteta, ovog nutritivno vrednog proizvoda od ribe.

Ključne reči: *dimljena riba, vakuum, modifikovana atmosfera, Enterobacteriaceae, kvalitet*

INTRODUCTION

Smoked fish is an integral part of our diet. Smoked fish have lost its image of luxury products, the reason being the steady growth of fish farming in aquaculture in recent years, which contributes to increasing the quantity of fish in the market, and consequently, the amount of fish available for processing, or smoking. This situation, contributes that the world market is regularly supplied by smoked fish, and such uniformity of the market contributes to the price of this product are held for years at the same level, leading to increased demand, consumption, and consequently increasing the production of smoked fish.

The definition of cold-smoked fish, which has been given by the Codex Alimentarius Commission (1979), and The Association of Food and Drug Officials (AFDO 1991) reads: "Cold smoked fish is smoked fish that is produced by exposure to smoke fish and temperature, where there is only a partial coagulation of protein as effect of temperature. Accordingly, such a meager definition of fish products, it is expected that the production of cold-smoked fish, using various parameters of the technological procedure of processing fish. It is therefore to be expected that the quality of cold smoked fish products on the market vary from manufacturer to manufacturer, and is an emerging problem in the production of smoked fish in the European Union and in our country, luck of predetermined criteria that should satisfy the smoked fish products. Defining of uniform criteria for the quality of smoked fish and alignment of certain factors of production (salting, smoking, choice of raw materials, packaging) would contribute to the production of safe products, consistent quality. Second, no exist of uniform, objective criteria for assessing the quality, the producers of smoked fish can have difficulty in monitoring changes in quality and determine the shelf life of products.

The quality of smoked fish is dependent on many factors, those related to fish production (nutrition, environmental conditions, genetic factors, gender and sexual maturity, life cycle) (Hovde et al., 2007) and those that relate the processing and fish processing (slaughter and processing, curing, smoking, packaging) (Sikorski and Kolodziejska, 2002). Special attention was paid to the sustainability of smoked fish that is usually determined with bacteriological status, physical and physic-chemical properties and sensory properties (Ibrahim et al., 2008; Siskos et al., 2007, Cardinal et al., 2004, Rorabaugh et al., 1999).

Shelf life of food, and thus a smoked fish products, can be defined as the time between the packages of food and time in which the product is particularly safe to the health of consumers and in which its sensory characteristics (smell, taste, appearance, texture) and nutritional value unchanged and acceptable to consumers (McMillin, 2008; Sørheim et al. 1997). Shelf life is the time period in which there is no appearance of signs of spoilage. Spoilage of fish and fish products can be defined as any change in fish, which makes the product unusable (unacceptable) for human consumption (Arashisar et al., 2004). The most common reason for spoilage of smoked fish products is microbial activity. Microbial growth and the creation of products, found their metabolic activity (the creation of amines, sulfides, alcohols, primarily ethanol, aldehydes, ketones, organic acids) lead to the undesirable odor and taste and appearance of discoloration (Leroi et al., 2001). What part of the micro flora will grow in the product is determined by the parameters that are related to the production process itself, by storage conditions and packaging, and the presence and change of certain groups of microorganisms is often taken as a parameter for the shelf life of fish and fish products (Muratore and Lic-

ciardello , 2005; Siverstvik et al., 2002, Paludan-Müller et al., 1998). For this reason, the shelf life of fish products depends primarily on the initial contamination, the conditions of production, handling the product after the manufacturing process and storage temperature and the type of packaging (Caglak et al., 2008, Goulas, and Kontominas, 2007; Stamatis and Arkoudelos, 2007; Siverstvik et al., 2002).

Modern consumers demand high quality food that has retained sensory characteristics and nutritive value of raw material from which it is produced, and that it is also safe and on their health. This demand is largely achieved by packaging the products in vacuum or modified atmosphere. In this way the demands of consumers are met, and also manufacturers have everything to gain - not only do they keep, but this way the possibilities to expand the market. Despite the constant evolution in materials and methods of packaging, the basic principle of packaging remained the same. This is to avoid contamination, delay spoilage, permit an enzyme reaction that could improve the softness, reduce the loss of weight and where possible making sure to retain the sensory characteristics of products (Dainelli et al., 2008, Kerry et al., 2006; Hill, 2003, Murcia et al., 2003; Vermeiren et al., 1999). The main objective of food packaging in vacuum and gas mixture is to remove oxygen from the food, since it allows the growth of aerobic microorganisms (bacteria, yeasts and molds) which leads to spoilage of fish and fish products, and to prevent oxidative valence, which deplete vitamins, pigments, lipid components, reducing the quality and nutritive value of the product (Hill, 2003).

Packaging of smoked fish meat products in vacuum are perfect for keeping the product up to three weeks. When packing in a vacuum, eliminating air in packing impermeable to oxygen, forming anaerobic/microaerophilic conditions, increasing the CO_2 content and decreasing pH products thereby inhibiting the growth of aerobic gram-negative bacteria and providing a better shelf life of fish (Soccol and Oetterer, 2003). These changed conditions inhibit the growth of aerobic bacteria and facultative anaerobes allow growth and developing microflora dominated lactic acid bacteria, and a smaller number are present and *Brochotrix thermosphacta, Enterobacteriaceae,* other gram-negative bacteria, micrococcus and yeasts (Leroi et al., 1998; Lyhs et al., 1998, Truelstrup Hansen and Huss, 1998; Truelstrup Hansen et al., 1995).

Packaging of food in the mixture of gases, i.e. modified atmosphere, or MAP (Modified Atmosphere Packaging) packaging technology is leading 21st century, which basically acts as a vacuum packaging, only difference is that the vacuum packing internal conditions that inhibits microorganisms are developed in the package, while in the MAP gas mixtures initiated in order to create the same conditions (Radetić et al. 2007; Goktepe and Moody, 1998). In the last decade of the 21st century, packaging in modified atmosphere gained considerable popularity as a modern, non-thermal method of food preservation (Patsias et al., 2006). There is evidence that the packing of fish and fish products in the mixture of gases to increase their shelf life from 0% to 280% of the fish and fishery products stored in air (Özogul et al., 2004), or about 1.5 to 2 times longer (Erkan et al., 2006; Ward, 2001).

The modified atmosphere includes replacement of air in the packing with a mixture of gases. The most common combination of gases is carbon dioxide, nitrogen and oxygen and their optimal ratio may vary depending on the type of fish (Mullan, 2002). Preserving action of gases, primarily carbon dioxide, used in the packaging of foods based on their ability to preventing or slowing growth and reproduction of microorganisms, influence on stopping or slowing down the degradation caused by microorganisms or natural chemical agents that deeply modify the product making it unfit for consumption

(Masniyom et al., 2002). It was found that the degree of inhibition of microorganisms mainly psychrotrophic, aerobic gram-negative bacteria is proportional to the content of CO_2 (Pantazi et al., 2008). The mechanism by which carbon dioxide slows the growth of bacteria and fungi is complex. It is known that carbon dioxide is causing damage to cell membranes and causes changes in the function of the cell membrane, then reacts with the lipid of cell membrane, thus changing the ability of the membrane transport of certain ions, causing a direct inhibition of the synthesis of certain enzymes or decrease the speed of enzymatic reaction, penetrate the bacterial membranes leading to changes in intracellular pH value (acidification) (Cornforth and Hunt, 2008) and causes direct changes in the physic-chemical properties of proteins (Goulas, 2008; Siverstvik et al., 2002).

There is no doubt that composition of dominant microflora in cold smoked fish products packed in the mixture of gases depends on the mixture of gases used in packaging, and the predominant microflora in cold-smoked fish products packed in modified atmosphere, is the one that is resistant to CO_2 (Siverstvik et al., 2002). In general, gram-negative bacteria are much sensitive to the effect of CO_2, and are also the most inhibited microorganisms. The most sensitive microorganism are *Pseudomonas spp*., then *Enterobacetriacea*, *Acinetobacter spp*., *Achromobacter*, *Flavobacterium*, and *Moraxella spp*. and psychrotrophic bacteria that lead to spoilage of fish. As mentioned most resistant is *Clostridium spp*. (Jay et al., 2005; Devliegher and Debever, 2000). Gram positive bacteria, such as lactic acid bacteria, mainly *Lactobacillus spp*. and *Leuconostoc spp*. and then *Brochothrix thermosphacta*, are not sensitive to the effects of carbon dioxide, and in fish products packed in the mixture of gases they become the dominant flora *(Limbo i sar., 2010*; *McMillin, 2008; Erkan i sar., 2006; Sanjeev i Ramesh, 2006; Siverstvik i sar*. *2003; Mokhele i sar., 1983)*.

As the *Enterobacteriaceae* family of bacteria that is often mentioned in literature as an indicator of spoilage cold smoked fish (Ólafsdóttir et al., 2005), and some of them are pathogenic to humans, the aim of our research was to determine the presence and examine the change in total number of enterobacteria in cold smoked trout packed in two ways, in vacuum and modified atmosphere because there is the lack of such data in this area of obstacles for the establishment of unified criteria for quality and sustainability of cold-smoked fish packed in vacuum and modified atmosphere.

MATERIAL AND METHODS

Salmon trout *(Oncorhynchus mykiss)*, used for experiments weighing about 1 kg. After primary treatment, trout were washed and soaked in vats for curing (wet salting) for 24h and then were pressed, laid on the grid in chambers for over an hour at 20°C. Smoking was performed on the automated smokehouse at a temperature of 28°C for eight hours. The smoking product was used beech sawdust and the smoke was later developed its combustion in the generator, separate from the smoking chamber. Upon completion of the process of smoking, the fish was cooled at 2 ° C for 10 hours. So cold, the fish was threaded with machine to burn ("slicing"), into thin fillets thickness to 0.5 cm. Fillets were then packaged, in about 75 grams. Upon completion of the manufacturing process there were four groups of samples. The first group (I) sample was vacuum packaged, and the other three were packaged in three different modified atmospheres: the second group (II) - 50% CO_2 + 50% N_2, the third group (III) - 60% CO_2 + 40% N_2, and the fourth group (IV) - 90% CO_2 + 10% N_2. Packaging of smoked trout fillets in modified

atmosphere was used Multivac device (Multivac C350, D-87787 Wolfertschwenden, Germany). Packing material was foil OPA / EVOH / PE (oriented polyamide / ethylene vinyl alcohol / polyethylene, UPM - Kymene, Walki Films, Finland) with low gas permeability (permeability of O_2 5 cm^3/m^2/day at 23°C, of N_2 1 cm^3/m^2/day at 23°C, of CO_2 23 cm^3/m^2/day at 23 C and of water vapor 15 g/m^2/day at 38°C). Packages were filled with mixture of gas producer Messer Tehnogas. Ratio of gas/sample in the package was 2:1. After packing the samples of all four groups of trout were kept for six weeks, at a temperature of +3°C. The samples were analyzed by zero, seventh, fourteenth, twenty, twenty-, thirty-fifth and forty-second days of storage in order to determine the number of enterobacteria. Determination of the total number of enterobacteria n cold smoked trout fillets from all four groups was carried out according to the method ISO 21528-2: 2004 (E) - Microbiology of food and animal feeding stuffs - Horizontal Methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count method.

RESULTS AND DISCUSSION

As already stated, Gram negative bacteria are more sensitive to the effects of carbon dioxide. Our results confirm an existing fact. The total number of *Enterobacteriaceae* on the first test in group I was the log CFU / g 1.65 ± 0.20 and was statistically significantly lower than the total number of *Enterobacteriaceae* determined in samples of group II (log CFU / g 2:51 ± 0.24), and samples in Group III (log CFU / y 2:59 ± 0.72), while no statistically significant difference from the total number of *Enterobacteriaceae* in samples of group IV (log CFU / g of 2:03 ± 0.29) (Figure 1 and Figure 2). Statistically significant difference was found between the total number of *Enterobacteriaceae* samples III and IV group (p <0.05), and there were no significant differences between the number of *Enterobacteriaceae* in samples II and III groups. In all groups during storage there was a statistically significant increase in the total number of *Enterobacteriaceae*, which can be seen from the so-called feature. best suited for real, and based on regression equations for each group. Specifically, the positive value of coefficient "b" in the regression equation for all four groups shows a tendency to decrease the total number of *Enterobacteriaceae* in all groups, with what coefficient "b" has the highest value, the regression equation, the fillets of the first group and the lowest fillets in group IV, which indicates that the weakest rate of growth had *Enterobacteriaceae* in frozen group IV, ie. that in their most pronounced antimicrobial effect of carbon dioxide, which is also the highest percentage in the mixture of gases which are packaged fillets Group IV (Figure 2).
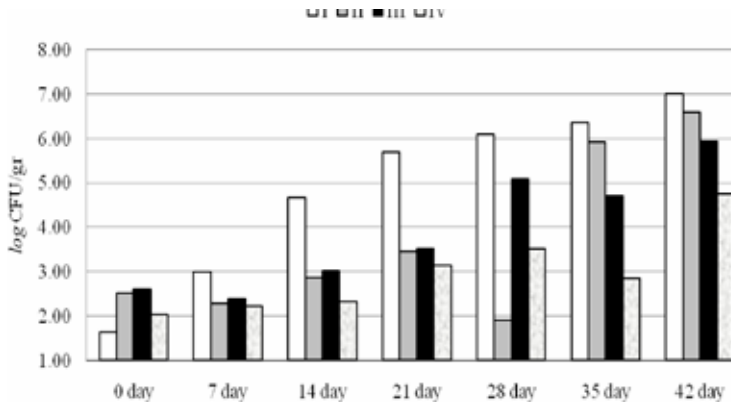
Figure 1. Comparative review the total number of *Enterobacteriaceae* in all four groups of cold smoked trout fillets during six weeks of storage

After six weeks of investigation (after six weeks of storage), it was found that the total number of *Enterobacteriaceae* group IV samples (log CFU/g 4.75 ± 0.16) was lowest and was significantly different from the total number of *Enterobacteriaceae* found in samples of group (log CFU/g $7:01 \pm 0:53$), Group II (log CFU/g 6.60 ± 0.47) and Group III (log CFU/g 5.94 ± 0.34) (Figure 1 and Figure 2). The total number *Enterobacteriaceae* of I group and II group did not differ significantly, but the number of *Enterobacteriaceae* determined in the two groups was statistically significantly higher than the total number of *Enterobacteriaceae* samples of group III. Our results confirm the fact that CO2 has inhibitory effect mainly on gram-negative bacteria, and what are the microorganisms from the family *Enterobacteriacea*. Comparing the results from the literature we can accept claim of Paludan-Müller and et al. (1998) that carbon dioxide has dramatically reduced the growth of gram-negative bacteria, or in such a finding can be restricted, to be more specific and apply only to Group IV samples are packed in the compound where the percentage of CO_2 was the most common (90%), and which, indeed, the total number *Enterobacteriacea* was significantly smaller and the statistical significance level of p <0.001, compared to all groups that were the subject of investigation.
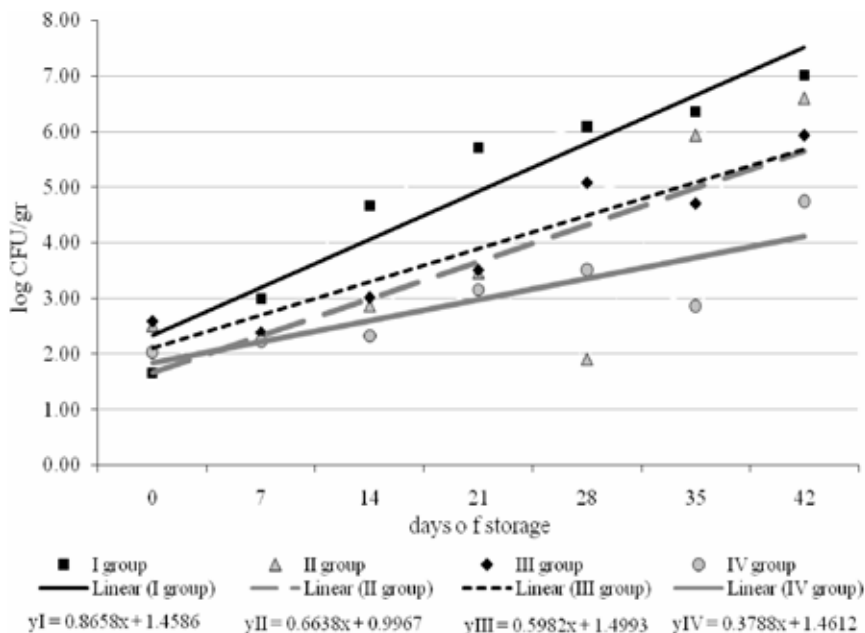
Figure 2. Best custom made and regression equations for the total number of *Enterobacteriacea* in all four groups of cold smoked trout fillets during six weeks of storage.

CONCLUSION

We confirmed in our research that the most sensitive *Enterobacteriaceae* family of bacteria when it comes to food packaging in the mixture of gases containing carbon dioxide. Given that the application of CO_2 successfully reduced the rate of growth of enterobacteria, our results suggest that consumption of cold-smoked fish products packed in the mixture of gases, there is little risk of poisoning people enterobacteria and so it is with food safety, particularly desirable to use food packaging in modified atmosphere. We believe that the perceived benefits of a sound basis for setting a new hypothesis to define the unique quality criteria as cold smoked trout packaged in a mixture of gases as well as those packaged in a vacuum, where it is necessary that special attention be paid to study this type of product safety for human health. Given the very small number of data in the literature pertaining to cold smoked trout packaging in modified atmosphere, further research (with more difference atmosphere) should examine the possibilities of other combinations and relationships of gases in the atmosphere packaging, and find the ideal combination of packaging cold smoked trout fillets in the atmosphere.

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