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Abstract. Because of the importance of different packaging methods for the extension of fish shelf life, as a highly perishable food, the aim of the present study was to examine the effect of vacuum and modified atmosphere packaging on the total *Enterobacteriaceae* and lactic acid bacteria counts of cold-smoked Salmon trout (*Oncorhynchus mykiss*) stored at 3°C during six weeks. Trout fillets were vacuumed packaged (VP) or packaged in one of two different modified atmospheres, with gas ratio of 50%CO_2/50%N_2 (MAP1) and 90%CO_2/10%N_2 (MAP2) and analysed on days 0, 7, 14, 21, 28, 35 and 42. Both the total *Enterobacteriaceae* and total lactic acid bacteria counts increased in the trout fillets in all packaging types during storage. A significantly lower total *Enterobacteriaceae* count was determined in the MAP fish compared to the VP fish, with the weakest growth rate and lowest numbers attained in MAP2 fillets. The lactic acid bacteria count was higher in trout packaged in MAP compared to VP, with the highest number in the MAP with 90% CO_2 (MAP2).

1. Introduction

Fish are gradually becoming the favoured food in many countries because of their favourable content of proteins, minerals, vitamins and essential fatty acids, as well as their positive ratio of n-6/n-3 fatty acids [1]. However, fish is highly perishable. Quality loss and subsequent spoilage of fish and fish products are mainly the result of microbial spoilage [2]. Fish preservation, including smoking as one of the oldest preservation methods, dates from prehistory, and is also used in today's production process [3]. Because the modern consumer is looking for safe, high-quality food, with retained sensory characteristics and nutritional value of the raw material, as well as longer shelf life, various new packaging systems to delay the spoilage and extend the shelf life of fish and meat and their products were developed in the last decade [4].

In vacuum packaging (VP), air removal ensures anaerobic/microaerophilic conditions, increases the CO_2 and reduces pH of the product. O_2 in the packaging is converted into CO_2 due to respiration of meat tissue and bacterial activity. This prevents the growth of aerobic bacteria and allows the growth of facultative anaerobes. Regular vacuuming extends shelf life up to three weeks, but the food can become dry [5]. Therefore, food packaging in a gas mixture, i.e. modified atmosphere packaging (MAP), became the leading packaging technology in the 21st century. MAP basically acts similarly to VP, with the difference that in VP, the gas composition that inhibits microorganisms develops in the package during storage, while in MAP, the gas mixture in the package is modified in order to create the same conditions. MAP can contain a mixture of different gases (O_2, CO_2, N_2, CO, etc.), is very popular for fresh meats and is frequently used because it suppresses bacterial spoilage. Depending on the type of meat or meat product, the gases in the packaging can be used individually or can be combined in different proportions in order to achieve the desirable effect. The most frequently used gas in MAP is CO_2, which possesses strong antimicrobial activity, especially on Gram negative

bacteria, resulting in extended bacterial lag phase and generation time**.** Its inhibitory effect on the growth of microorganisms is concentration-dependent, and the higher the concentration of CO_2 in the system, the higher is the inhibitory effect observed [6]. For fish and fishery products, most gas mixtures do not include O_2 because high-O_2 systems, in most cases, provide limited benefit to shelf life extension of fishery products. The reduction of O_2 slows down lipid oxidation and the development of rancidity [2]. The role of N_2, also used in MAP systems, is to prevent fat oxidation and package collapse [7,8].

Because of the importance of the different packaging methods for extension of fish shelf life as well different amounts and types of gases in MAP, the aim of the present study was to investigate the effect of VP and MAP with two different gases on microbiological properties (total *Enterobacteriaceae* and lactic acid bacteria counts) of cold-smoked trout.

2. Materials and methods

2.1. Cold-smoked trout preparation

Salmon trout (*Oncorhynchus mykiss*) used in the present study originated from the Bočac fish farm (Tropic, Banja Luka, Republika Srpska, Bosnia and Herzegovina), where all fish were grown and kept under the same conditions. Live fish were transported from the fish farm to the processing plant in specialised vehicles and placed into the open pools at the processing plant yard, with necessary conditions for proper accommodation of the fish (constant water flow, controlled oxygen and temperature), without feed. Trout were primarily processed, stunned, bleed and eviscerated in the manner usual for industrial plant. Fish weighed about 1 kg. After primarily processing, the spine was removed by cutting parallel to it, whereby the left and right halves were obtained. Trout halves were rinsed with water and immersed in barrels with 9% salt brine (wet salting) and rosemary (*Quantum satis*) during 24 hours at 4°C. After brining, fish were drained for one hour at 20°C and then smoked for eight hours in an automatic smoked chamber at 28°C. Smoke was produced by combustion of beech sawdust in a generator separated from the smoking chamber. After smoking, fish were cooled at 2°C for ten hours, then residual parts of the ribs were removed and fish halves were sliced from the medial side, into thin fillets, up to 0.5cm thick, each weighing about 75g.

2.2 Cold-smoked trout fillet packaging

Cold-smoked fillets were split into three groups. The first group was vacuum packaged (VP), the second was packaged in modified atmosphere with 50% CO_2 and 50% N_2 (MAP1) and the third group was packaged in modified atmosphere with 90% CO_2 and 10% N_2 (MAP2). MAP was conducted using a Multivac machine (Multivac C350, D-87787 Wolfertschwenden, Germany). The packaging foil was OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene, UPM-Kymmene, Walki Films, Finland) with low gas permeability (degree of permeability to oxygen 5cm^3 m^{-2} day^{-1} at 23°C, nitrogen 1cm^3 m^{-2} day^{-1} at 23°C and water vapour 15g m^{-2} day^{-1} at 38°C). Packages were filled with commercial gas mixture (Messer Tehnogas, Serbia). The ratio of gas:fish in the packages was 2:1. After packaging, all groups of the fish fillets were stored at 3°C for six weeks.

2.3. Microbiological analyses

Cold-smoked trout packaged in VP or MAP was analysed for total *Enterobacteriaceae* count and lactic acid bacteria (LAB) count on day 0 and on days 7, 14, 21, 28, 35 and 42 of storage. For bacterial enumeration, 10g of fish meat was weighed out aseptically after package opening, transferred into sterile Stomacher bags and 90 ml of Maximum Recovery Diluent (MRD; Merck, Germany) was added to each sample. Samples were homogenized in a Stomacher blender (Stomacher 400 Circulator, Seward, UK) for 2 min. Serial decimal dilutions were prepared and 0.1 ml of appropriately diluted suspension was inoculated directly on the surface of appropriate media. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose Agar (VRBGA, Merck, Germany) after incubation at 37°C for 24h according to ISO 21528-2:2004 [9], while LAB were enumerated on MRS (Merck, Germany)

following incubation at 30°C for 72h according to ISO 15214:1998 [10]. After an appropriate period of incubation for each type of bacteria, plates were examined visually for typical colony types and morphological characteristics associated with each growth medium, number of colonies was counted, and results were recorded as colony forming units per g (CFU/g).

2.4. Statistical analyses

The experiment was conducted in a completely randomized design, six repetitions were carried out for each treatment and the treatments were arranged in a 3 x 7 factorial design (3 treatments, 7 storage periods). Numbers of microorganism were transformed into logarithms (log). Statistical analyses of the results were conducted using the software GraphPad Prism version 6.00 (GraphPad Software, San Diego, California USA, www.graphpad.com). The results were expressed as the mean±standard deviation. The effects of different treatments during storage period were appraised by one-factor analysis of variance-ANOVA with Tukey's multiple comparison test at 95% confidence level (difference considered significant if $p<0.05$). Linear regression was used to establish the statistical relationship between the number of microorganisms in different packaging and storage period.

3. Results and Discussion

The total *Enterobacteriaceae* and LAB counts and significant differences between all groups of cold-smoked trout fillets during six weeks of storage are shown in Table 1.

Table 1. Total *Enterobacteriaceae* count and lactic acid bacteria (LAB) count (log CFU/g) in smoked trout fillets during six weeks of storage (mean±SD)

Day of Storage	Total *Enterobacteriaceae* count			LAB count		
	VP	MAP1	MAP2	VP	MAP1	MAP2
0	1.65^{A}±0.20	2.51^{A}±0.24	2.03±0.29	1.03^{A}±0.25	1.38^{A}±0.29	1.28±0.21
7	2.99^{AB}±0.82	2.29^{A}±0.41	2.23^{B}±0.49	2.17^{A} ±0.32	1.77±0.47	1.57^{A}±0.39
14	4.67^{AB}±0.19	2.87^{A}±0.80	2.33^{B}±0.15	2.47^{AB}±0.42	1.64^{A}±0.46	1.50^{B}±0.40
21	5.70^{AB}±0.49	3.46^{A}±0.78	3.15^{B}±0.50	3.58^{AB}±0.22	4.40^{AC}±0.14	4.59^{BC}±0.10
28	6.08^{AB}±0.15	1.91^{AC}±0.30	3.51^{BC}±0.56	5.03^{A}±0.26	4.88^{B}±0.10	6.01^{AB}±0.56
35	6.36^{A}±0.31	5.93^{B}±0.37	2.86^{AB}±0.20	5.09^{AB}±0.45	7.02^{A}±0.19	7.11^{B}±0.16
42	7.01^{A}±0.53	6.60^{B}±0.47	4.75^{AB}±0.17	6.20^{AB}±0.30	6.88^{AC}±0.38	7.32^{BC}±0.19

$^{A-C}$ same uppercase letter within rows within the same bacterial group indicates significant difference $p<0.05$ between different packaging types

In all packaging types, significant increases of the total *Enterobacteriaceae* count were noted, which can also be seen based on the regression equation of each packaging type (Figure 1). The positive values of coefficient b in the regression equations for all packaging types indicate the trend of constantly increasing total *Enterobacteriaceae* counts. Coefficient b had the highest value in the regression equation for the VP fillets, and the lowest for the MAP2 fillets. This indicated the weakest growth rate of *Enterobacteriaceae* occurred in MAP2 fillets, so this type of packaging provided the most pronounced antimicrobial effect of CO_2.

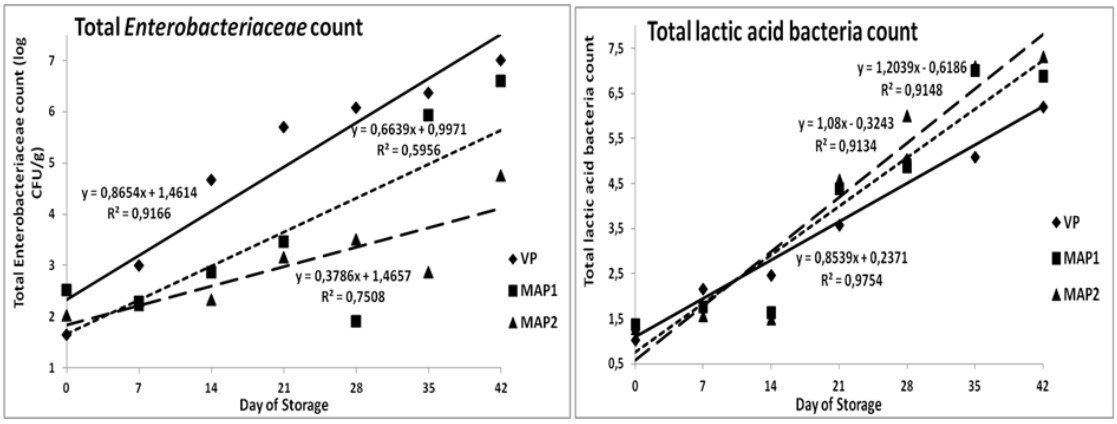

Figure 1. Regression equations for total *Enterobacteriaceae* and lactic acid bacteria counts (log CFU/g) in smoked trout fillets during six weeks of storage

On the one hand, some authors claim that the spoilage of fish meat is correlated with the *Enterobacteriaceae* count and that the *Enterobacteriaceae* are the main cause of spoilage of fish meat and cold-smoked fish meat products, because of the ability of this family of microorganisms to grow at low temperatures [11,12]. On the other hand, some other authors consider that *Enterobacteriaceae* count cannot be the only indicator of spoilage, and that it can only be used as an indicator of the quality of fish and cold-smoked fish products [3]. The initial number of *Enterobacteriaceae* which will be present in meat is mostly affected by hygiene during the production process, but also the types and forms of meat. In spoilage, these organisms degrade amino acids to sulphur compounds and diamines (volatile, malodorous components), which affect sensory properties of the meat, on the basis of which the final estimate of the spoilage is made [12,13]. The results of the present study showed total *Enterobacteriaceae* counts were between 6 and 7 log CFU/g in the fish from VP and MAP1, while in the fish from MAP2, numbers were lower (below 5 log CFU/g) at the moment when the trout fillets were considered unacceptable on the basis of sensory analysis (data not shown; [14]). Although the total *Enterobacteriaceae* count found in the present study was far more than 10^3 log CFU/g, which according to the Dutch standard was considered as limit value for the total number of *Enterobacteriaceae* in fresh fish [15,16], results are in accordance with the fact that CO_2, primarily in high concentrations, acts inhibitively on Gram negative bacteria, including *Enterobacteriaceae*. In accordance with the present study are results of others [17,18,19,20,21], in which the influence of MAP on fresh fish was examined, and which came to the conclusion that high levels of CO_2 in the package (>50%) have a protective effect on the product and consequently, reduce both the total *Enterobacteriaceae* count as well as the total number of bacteria.

On the basis of the positive values of the coefficient b in the regression equations, it can be seen that during the storage, the total number of LAB showed a tendency to increase in fish fillets in all packaging types (Figure 1). These results are in accordance with research of Arkoudelos *et al.* [19], Dondero *et al.* [22] and Masniyom *et al.* [23].

The dominant microbiota of fish cold-smoked products packaged in a mixture of gases undoubtedly depends on the gas composition. The dominant microbiota in cold-smoked fish products packaged in a modified atmosphere with CO_2 is CO_2-resistant microbiota [6]. Gram positive bacteria such as LAB, primarily *Lactobacillus* spp., *Leuconostoc* spp. and *Brochothrix thermosphacta*, are not sensitive to CO_2, which is why they become dominant in MAP [24-27]. Also, the fact that LAB adapt very quickly to the environmental conditions (salt content, pH value, cold storage regime) enable these bacteria to become the dominant microbiota in cold-smoked fish products during storage [3]. Although LAB are not typical causative agents of spoilage of cold-smoked fish products, when they are in greater numbers they can affect the sensory properties by creating volatile components, and

consequently create typical odour defects. Even so, Patsias *et al*. [28] consider that the spoilage that occurs due to an increase in LAB numbers is less offensive than in the case of spoilage caused by the growth of aerobic bacteria. The results of the present study showed higher LAB counts in fish packaged in MAP compared to VP, with the highest number in the trout packed in MAP with 90% CO_2. The explanation may be the fact that CO_2 inhibited the activity of some groups of potentially competitive microorganisms, which led to uninterrupted growth of LAB. Competition for substrates and microbial antagonism are considered to be the most important factors in the survival of specific microorganisms/microbiota in a particular ecological niche [29]. The lack of competition among the various bacterial species, due to the environment conditions, likely promoted undisturbed growth of LAB on fillets packaged in MAP, which is the case in the present study [3].

Acknowledgment

This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, through the funding of the Project – Selected biological hazards to the safety/quality of food of animal origin and the control measures from farm to consumer (No 31011).

References

[1] Baltic ZM, Kilibarda N, Teodorovic V, Dimitrijevic M., Karabasil N and Djuric J 2010 Značaj ribe u ishrani ljudi. *XXX seminar inovacije znanja veterinara, Fakultet veterinarske medicine, Beograd, Zbornik predavanja* pp. 39-58

[2] DeWitt CAM and Oliveira A 2016 Modified atmosphere systems and shelf life extension of fish and fishery products *Foods* **5**(3) 48

[3] Gómez-Guillén MC, Gómez-Estaca J, Giménez B and Montero P 2009 Alternative fish species for cold-smoking process *Int. J. Food Sci. Technol.* **44**(8) 1525-1535

[4] Bøknæs N, Jensen KN, Guldager HS, Østerberg C, Nielsen J and Dalgaard P 2002 Thawed chilled Barents Sea cod fillets in modified atmosphere packaging-application of multivariate data analysis to select key parameters in good manufacturing practice. *LWT-Food Sci. Technol.* **35**(5) 436-443

[5] Radetić P, Milijašević M, Jovanović J and Velebit B 2007 Pakovanje svežeg mesa u modifikovanoj atmosferi-trend koji traje *Tehnologija mesa* **48**(1-2) 99-108

[6] Sivertsvik M, Jeksrud WK and Rosnes JT 2002 A review of modified atmosphere packaging of fish and fishery products–significance of microbial growth, activities and safety. *Int. J. Food Sci. & Technol.* **37**(2) 107-127

[7] Skandamis PN and Nychas GJE 2002 Preservation of fresh meat with active and modified atmosphere packaging conditions. *Int. J. Food Microbiol.* **79**(1) 35-45

[8] Singh P, Wani AA, Saengerlaub S and Langowski HC 2011 Understanding critical factors for the quality and shelf-life of MAP fresh meat: a review *Crit. Rev. Food Sci. Nutr.* **51**(2) 146-177

[9] ISO 21528-2:2004 – Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 2: Colony count method.

[10] ISO 15214:1998 – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30 degrees C.

[11] Ólafsdóttir G, Chanie E, Westad F, Jonsdottir R, Thalmann CR, Bazzo S, Labreche S, Marcq P, Lundby F and Haugen JE 2005 Prediction of microbial and sensory quality of cold smoked Atlantic salmon (*Salmo salar*) by electric nose *J. Food Sci.* **70**(9) 563-574

[12] Säde, E, Murros, A and Björkroth J 2013 Predominant enterobacteria on modified-atmosphere packaged meat and poultry *Food Microbiol.* **34**(2) 252-258

[13] El-Gendy NM, Ibrahim HA, Al-Shabasy NA and Samaha IA 2014 *Enterobacteriaceae* in beef products (Luncheon, Pasterma, Frankfurter and Minced meat) from Alexandria retail outlets *Alexandria J. Vet. Sci.* **41**(1) 80-86

[14] Djordjevic J, Pavlićevic N, Boškovic M, Baltic T, Ivanovic J, Glamoclija N and Baltic ZM 2016 Sensory evaluation of cold-smoked trout packaged in vacuum and modified atmosphere. *Meat Technol.* **57**(2) 141-149
[15] Lyhs U, Lahtinen, J and Schelvis-Smit R 2007 Microbiological quality of maatjes herring stored in air and under modified atmosphere at 4 and 10 C *Food Microbiol.* **24**(5) 508-516
[16] Vaz-Pires P and Barbosa A 2004 Sensory, microbiological, physical and nutritional properties of iced whole common octopus (*Octopus vulgaris*) *LWT - Food Sci. Technol.* **37** (1) 105-114
[17] Torrieri E, Cavella S, Villani F and Masi P 2006 Influence of modified atmosphere packaging on the chilled shelf life of gutted farmed bass (*Dicentrarchus labrax*) *J. Food Eng.* **77**(4) 1078-1086
[18] Ježek F and Buchtová H 2007 Physical and chemical changes in fresh chilled muscle tissue of common carp (*Cyprinus carpio L.*) packed in a modified atmosphere *Acta Vet. Brno* **76**(8) 83-92
[19] Arkoudelos J, Stamatis N and Samaras F 2007 Quality attributes of farmed eel (*Anguilla anguilla*) stored under air, vacuum and modified atmosphere packaging at 0 degrees C *Food Microbiol.* **24**(7) 728-735
[20] Pantazi D, Papavergou A, Pournis N, Kontominas MG and Savvaidis IN 2008 Shelf-life of chilled fresh Mediterranean swordfish (*Xiphias gladius*) stored under various packaging conditions: Microbiological, biochemical and sensory attributes *Food Microbiol* **25**(1) 136-143
[21] Stamatis N and Arkoudelos J 2007 Quality assessment of *Scomber colias japonicus* under modified atmosphere and vacuum packaging *Food Control* **18**(4) 292-300
[22] Dondero M, Cisternas F, Carvajal L, Simpson R 2004 Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature *Food Chem.* **87** 543-550
[23] Masniyom P, Benjakul S and Visessanguan W 2002 Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging *J. Sci. Food Agric.* **82** 873-880
[24] Limbo S, Torri L, Sinelli N, Franzetti L and Casiraghi E 2010 Evaluation and predictive modelling of shelf life of minced beef stored in high-oxygen modified atmosphere packaging at different temperatures *Meat Sci.* **84** 129-136
[25] McMillin KW 2008 Where is MAP going? A review and future potential of modified atmosphere packaging for meat *Meat Sci.* **80** 43-65
[26] Sanjeev K and Ramesh MN 2006 Low oxygen and inert gas processing of foods *Crit. Rev. Food Sci. Nutr.* **46**(5) 423-451
[27] Sivertsvik M, Rosnes TJ and Kleiberg GH 2003 Effect of Modified Atmosphere Packaging and Superchilled Storage on the Microbial and Sensory Quality of Atlantic Salmon (Salmo salar) Fillet *Food Microbiol. Safety* **68**(4) 1467-1472
[28] Patsias A,Chouliara I, Badeka A, Savvaidis IN and Kontominas MG 2006 Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes *Food Microbiol.* **23** 423–429
[29] Gram L and Huss HH 1996 Microbiological spoilage of fish and fish products *Int. J. Food Microbiol.* **33**(1) 121-137