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Effect of Modified Atmosphere Packaging on the Shelf Life of Chilled Common Carp (*Cyprinus carpio*) Steaks: Chemical and Sensory Attributes

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Abstract

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The effect of modified atmosphere packaging (MAP 1 – 80% O_2 + 20% CO_2 and MAP 2 – 90% CO_2 + 10% N_2) on selected chemical and sensory parameters of common carp steaks stored at 3 ± 0.5°C, and determination of the shelf life of the products was studied in this research. Samples were analysed on day 1, 3, 5, 7, 9, 11, 13, 15, and 17. An increase in TVB-N values in carp steaks followed the order: MAP 2 < control < MAP 1. From day 9 of storage FFA concentrations were higher ($P < 0.01$) in MAP 2 samples compared to control and MAP 1 samples. The presence of oxygen (MAP 1 and control) resulted in an increase in TBA values in comparison with samples packaged in the absence of oxygen. According to sensory evaluation, it was concluded that carp steaks packaged in modified atmosphere with 80% O_2 + 20% CO_2 remained acceptable up to 15 days of storage, whereas samples packaged under 90% CO_2 + 10% N_2 as well as samples kept on flaked ice remained unchanged until the end of the experiment.

Keywords: FFA; freshness; freshwater fish; sensory assessment; TBA; TVB-N

Fish belong among the most perishable foods. The high water and free amino acid content, and the lower content of connective tissue as compared to other flesh foods lead to the more rapid spoilage of fish. Deterioration of fish mainly occurs as a result of bacteriological activity leading to loss of quality and subsequent spoilage (Liston 1980). Endogenous proteases also play an important role in the post mortem degradation of fish muscle protein. The disintegration of proteins yields peptides and amino acids, which are susceptible to further decay resulting in a high content of biogenic amines (Križek *et al*. 2004). These processes lead to a change in the textural and sensory characteristics of fish muscle. The quality of fish can be estimated by sensory tests, microbial methods or by chemical methods such as measuring volatile compounds, lipid oxidation, determination of ATP breakdown products, and the formation of biogenic amines (Gulsun *et al*. 2009).

The combined total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) and other basic nitrogenous compounds volatile under the analysis conditions in fish is called the Total Volatile Basic Nitrogen (TVB-N); its content in the fish meat is commonly used as a parameter for spoilage estimation, and as an index for freshness of fish. It is produced during the degradation of protein and non-protein nitrogen components, caused mainly by metabolic activity of fish spoilage bacteria and endogenous enzyme action (Connell 1990). The changes in fish lipids are responsible for the quality deterioration with extended storage, especially under

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inappropriate conditions. They involve lipolysis, lipid oxidation, and the interaction of the products of these processes with non-lipid components such as protein. The formation of peroxides is considered an indicator of the rate of primary lipid oxidation, while the thiobarbituric acid value is an indicator of secondary lipid oxidation (Ježek & Buchtová 2012). Lipid oxidation in fish depends on several factors, such as fish species, storage temperature, lipid composition etc. It is often the main reason for a shorter shelf life of fish and fish products.

The shelf life of fresh chilled fish is relatively short and at ambient temperatures of 2 ± 2°C it is about 2–3 days. It has been confirmed that fish packaging in modified atmosphere significantly extends the shelf life of the product (Masniyom *et al.* 2002; Stamatis & Arkoudelos 2007; Provincial *et al.* 2010). The most common freshwater fish retailed in the Serbia are carp. Fish are sold live, fresh chilled unpacked (with shelf life from 2 to 3 days), vacuum packed (with shelf life from 5 to 7 days) or frozen. The preferred type of fish packaging is vacuum packaging. Modified atmosphere packaging (MAP) with different gas mixtures is not practically used for fish in Serbia.

In this research were edchanges were monitored in selected chemical and sensory parameters of common carp (*Cyprinus carpio*) steaks packaged in modified atmosphere during storage at 3 ± 0.5°C, and the shelf life of the products was determined.

MATERIAL AND METHODS

Sampling. Samples from fourteen common carps (*Cyprinus carpio*) of average body weight of 2.5 ± 0.3 kg were obtained from a fishpond where a semi-intensive rearing system was used. Fish were transported live to the fish slaughtering and processing facility, where they were stunned, slaughtered, scale cleared, and the carcass was cut into steaks 2 cm thick and of average weight 220 g. The eighty-one carp steaks were divided into three groups.

The first sample group was placed on top of flaked ice in polystyrene boxes with outlets for water drainage. The ice/fish ratio was 3 : 1 and it was maintained constant throughout the experiment. The first experimental group was used as the control one. Other two sample groups of carp steaks were packaged in modified atmosphere with different gas ratios: MAP 1 – 80% O_2 + 20% CO_2 and MAP 2 – 90% CO_2 + 10% N_2. The machine used for packaging of samples was Variovac Primus (Zarrentin, Germany), and the material used for packaging was the foil OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene) (Dynopack; Polimoon, Norway) with low gas permeability (degree of permeability for O_2 – 3.2 cm^3/m^2/day at 23°C, N_2 – 1 cm^3/m^2/day at 23°C, 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 the gas and sample in the package was 2 : 1. All samples were stored in the same conditions at the temperature of 3 ± 0.5°C and on day 1, 3, 5, 7, 9, 11, 13, 15, and 17 of storage chemical and sensory testing was performed.

Chemical analysis. The total volatile basic nitrogen (TVB-N) was determined by using the official steam distillation method according to Commission Regulation (EC) 2074/2005 and it was expressed as mg TVB-N/100 g.

Free fatty acid (FFA) content, expressed as percentage of oleic acid, was determined in accordance with EN ISO 660:2009.

Peroxide value (PV), expressed in milliequivalents of peroxide oxygen per kilogram of fat, was determined by the EN ISO3960:2009 method.

Thiobarbituric acid (TBA) value was determined by the distillation method (Tarladgis *et al*. 1964; Holland 1971) and oxidation products were quantified as malondialdehyde (MDA) (mg/kg).

Sensory evaluation. The sensory evaluation was performed by six trained panellists prior to the physicochemical analyses. The samples were evaluated for overall acceptability, with regard to odour, flesh colour and texture using 1–5 intensity scale, with 5 corresponding to the most liked sample and 1 corresponding to the least liked sample. The product was defined as unacceptable with score less than 2 points recorded by at least of 50% of the judges.

Statistics. The mean values and standard deviations were calculated by using column statistics with the processing of 6 values for each analysed group. Significant differences between groups were calculated by using one-way ANOVA and Tukey's comparative test (Microsoft Office Excel 2010). Differences were considered as significant when $P < 0.05$.

RESULTS AND DISCUSSION

As shown in Figure 1A, TVB-N values of carp steaks were strongly affected by used atmosphere. An increase in TVB-N values followed the order: MAP 2 < control < MAP 1 and ranged from 12.35 ± 0.46 to

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18.31 ± 0.48 mg N/100 g in MAP 2 samples, in control samples from 12.38 ± 0.25 to 20.82 ± 1.45 mg N/100 g and from 12.4 ± 1.48 mg N/100 g to 23.77 ± 0.84 mg N/100 g in MAP 1 samples during the 17-day period of storage. TVB-N levels in samples packaged under MAP 2 changed to a lesser extent compared to those levels in samples packaged under MAP 1 and control samples.

Starting from day 7 of the experiment, the composition of MAP 2 significantly ($P < 0.01$) slowed down TVB-N formation compared to MAP 1. These differences in TVB-N values may be attributed to the higher CO_2 content in MAP 2 in comparison with MAP 1 (90 vs. 20%). According to Masniyom *et al.* (2013) the higher CO_2 concentration potentially inhibited the growth of mainly Gram-negative microorganisms and decreased the deamination capacity of bacteria, resulting in lower volatile compound production. The same observations were reported by Milijašević *et al.* (2010) and Babić *et al.* (2014) for carp samples stored under MAP, which support the results of the present study. In our research, control samples had a lower TVB-N value compared to samples packaged in MAP 1. This could be explained by the presence of a high concentration of oxygen (80%) in MAP 1 packaging, which could promote aerobic bacterial growth and subsequent increase of TVB-N caused by the bacterial decomposition of fish flesh.

The TVB-N limit from 25 to 35 mg N/100 g has been recommended by some researchers as an indicator for rejecting commercial fresh whole fish and processed fish products (Connell 1990). However, no limit for acceptability of common carp has been laid down by Commission Regulation (EC) 2074/2005. In their research, Ježek and Buchtová (2010) recommended 20 mg N/100 g in carp meat as the highest acceptable limit for TVB-N. In comparison with our study, TVB-N values in MAP 2 samples remained below this limit of acceptability throughout the entire storage period. However, this limit was exceeded in control samples (day 17) and MAP 1 samples (day 15).

During the entire storage period control samples had a lower FFA value compared to samples packaged in MAP (Figure 1B). At the same time, the FFA value growth in this group of samples was less distinctive (1.63 ± 0.18 on day 17). From storage day 1 to 7, there were no significant ($P > 0.05$) differences between FFA values of samples packaged in MAP 1 and

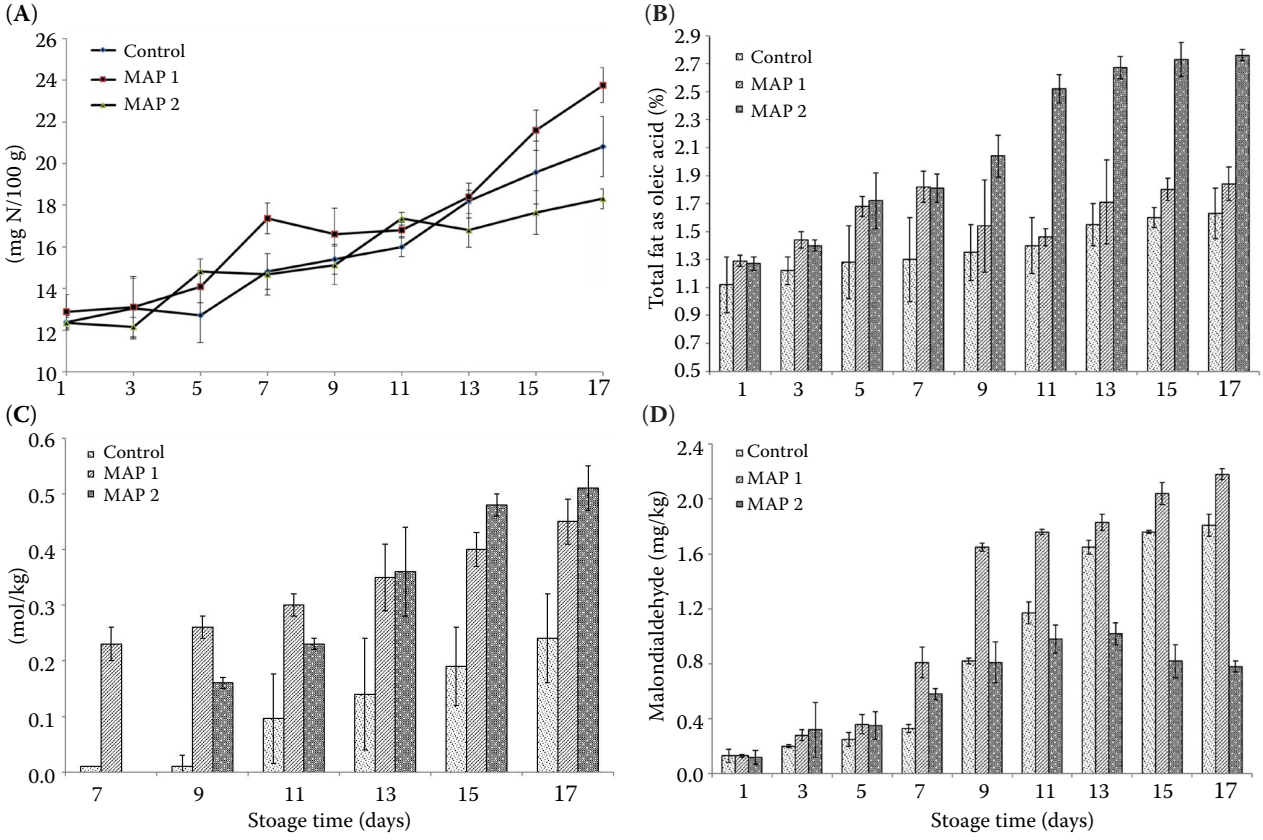

Figure 1. Changes in the (**A**) TVB-N value, (**B**) FFA concentration, (**C**) PV, and (**D**) TBA values of common carp steaks packaged under different conditions during the storage period

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MAP 2. Later on, the production of these degradation products in the two types of packaging followed a different course. In MAP 2 samples a highly significant ($P < 0.01$) increase of FFA was observed from day 9 (2.04 ± 0.5) until the end of the experiment (2.76 ± 0.04). In MAP 1 samples a significant decrease of FFA was determined between storage day 7 (1.82 ± 0.11) and day 11 (1.46 ± 0.06) of experiment. From that day onward the FFA value increased until the end of the experiment (1.84 ± 0.12).

The process of lipid hydrolysis is accompanied by the release of free fatty acids. In our research, keeping carp steaks on flaked ice had the smallest impact on generating FFA. A significantly lower FFA level in MAP 1 samples ($P < 0.01$) compared to MAP 2 starting from storage day 9 can be explained by the rapid FFA conversion to oxidation products due to the presence of O_2 in MAP 1. The same observations were reported by Ježek and Buchtová (2012) for silver carp samples stored under MAP.

In our research, during the first 5 days of storage, no PV was detected in either unpackaged or packaged fish meat (Figure 1C). Later during the experiment PV was lower in control samples in comparison with PV values of samples packaged in MAP. From day 7 to day 13 PV values were at the highest level in samples kept in oxygen-rich atmosphere (80%). At the end of the experiment (day 15 and 17) PV values were higher in samples packaged in atmosphere without oxygen. As reported by Jayasingh *et al*. (2002), lipid oxidation was higher in samples packaged in MAP with 80% of O_2 compared to control samples exposed to ambient air, which is in agreement with the results of the present study. As concluded in Ruiz-Capillas and Moral (2001) research, lipid oxidation depends on the synergy effect between CO_2 and O_2. For that reason, lipid oxidation in the atmosphere with 40% of O_2 could be more intensive compared with the atmosphere with 60% of O_2. Fluctuations in PV values that have been recorded in our research are in line with the results of other authors (Ježek & Buchtová 2010), pointing out the fact that the PV cannot be considered as a suitable indicator of fish muscle freshness.

As can be seen from the results of the present study (Figure 1D), an increase in TBA was observed in all samples during the storage period. At the end of the experiment a decrease of these values was detected (MAP 2 samples), and a lower increase rate in control samples. According to Connell (1990), TBA values of 1–2 mg MDA/kg of fish muscle are usually regarded as the limit beyond which fish will normally develop an offensive odour or taste. The TBA values of the present study for MAP2 samples did not exceed the value of 1 mg MDA/kg throughout the storage period (0.78 mg MDA/kg after 17 days of storage), while the TBA values of control samples exceeded this value (1.17 mg MDA/kg) on day 11 of storage. In contrast, the TBA values of MAP 1 samples exceeded the limit of 2 mg MDA/kg (2.04 and 2.18 mg MDA/kg) on day 15 and 17 of storage, respectively. Since rancid odour was detected in MAP1 samples on day 17 of our research, it can be concluded that the value of 2 mg MDA/kg represents the limit for carp fish above which offensive odour is generated.

Table 1. Sensory evaluation of carp steaks packaged under different conditions during the storage period

Sensory parameter	Packaging conditions	Storage time (days)								
		1	3	5	7	9	11	13	15	17
Odour	Control	5.0 ± 0.0^a	5.0 ± 0.0^a	4.8 ± 0.3^a	4.7 ± 0.2^a	4.5 ± 0.1^a	4.2 ± 0.9^a	3.6 ± 0.5^b	3.4 ± 0.8^b	3.2 ± 0.6^b
	MAP 1	5.0 ± 0.0^a	5.0 ± 0.0^a	4.8 ± 0.3^a	4.1 ± 0.4^b	3.6 ± 0.3^b	3.5 ± 0.0^b	3.3 ± 0.2^b	2.6 ± 0.4^c	1.2 ± 0.2^d
	MAP 2	5.0 ± 0.0^a	5.0 ± 0.0^a	4.9 ± 0.2^a	4.8 ± 0.2^a	4.6 ± 0.3^a	4.0 ± 0.4^b	3.8 ± 0.5^b	3.5 ± 0.4^b	2.8 ± 0.5^c
Flesh texture	Control	5.0 ± 0.0^a	5.0 ± 0.0^a	4.8 ± 0.2^a	4.6 ± 0.5^a	4.4 ± 0.4^a	3.7 ± 0.5^b	3.6 ± 0.8^b	3.2 ± 0.5^b	3.1 ± 0.2^b
	MAP 1	4.9 ± 0.5^a	4.9 ± 0.5^a	4.8 ± 0.7^a	4.5 ± 0.4^a	4.0 ± 0.0^a	3.8 ± 0.8^a	3.5 ± 0.2^a	2.7 ± 0.4^b	2.5 ± 0.3^b
	MAP 2	4.8 ± 0.2^a	4.1 ± 0.0^b	4.0 ± 0.0^b	3.7 ± 0.2^b	3.6 ± 0.7^b	3.5 ± 0.9^b	3.2 ± 0.6^b	2.9 ± 0.6^b	2.7 ± 0.4^b
Flesh colour	Control	5.0 ± 0.0^a	5.0 ± 0.0^a	4.9 ± 0.1^a	4.8 ± 0.2^a	4.7 ± 0.3^a	4.6 ± 0.3^a	4.3 ± 0.3^a	3.7 ± 0.2^b	3.5 ± 0.7^b
	MAP 1	5.0 ± 0.0^a	4.2 ± 0.3^b	3.7 ± 0.4^b	3.7 ± 0.4^b	3.6 ± 0.4^b	3.3 ± 0.4^b	3.4 ± 0.3^b	2.5 ± 0.1^c	1.8 ± 0.6^d
	MAP 2	5.0 ± 0.0^a	5.0 ± 0.0^a	4.8 ± 0.2^a	4.6 ± 0.6^a	4.1 ± 0.6^a	3.6 ± 0.7^b	3.6 ± 0.5^b	3.4 ± 0.6^b	2.6 ± 0.4^c
Overall acceptability	Control	5.0 ± 0.0^a	4.9 ± 0.7^a	4.8 ± 0.7^a	4.8 ± 0.4^a	4.5 ± 0.6^a	4.4 ± 0.6^a	4.2 ± 0.8^a	3.8 ± 0.7^a	3.3 ± 0.5^a
	MAP 1	4.9 ± 0.3^a	4.6 ± 0.7^a	4.2 ± 0.3^a	3.7 ± 0.4^a	3.6 ± 0.4^a	3.5 ± 0.2^a	3.5 ± 0.4^a	2.6 ± 0.4^b	1.2 ± 0.2^c
	MAP 2	5.0 ± 0.0^a	5.0 ± 0.0^a	4.8 ± 0.8^a	4.5 ± 0.3^a	4.7 ± 0.5^a	4.2 ± 0.6^b	4.1 ± 0.6^b	3.6 ± 0.4^b	2.7 ± 0.6^c

The same lowercase letters in a row indicate no significant differences ($P > 0.05$)

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In our research, TBA values of the samples packaged in the absence of oxygen (MAP 2) were significantly lower ($P < 0.05$) than the TBA values of the other two groups of samples (control and MAP 1). It is important to note that MAP 2 samples produced significant quantities of thiobarbituric acid reactive substances although these samples did not contain any oxygen. These results indicate that MDA production depends not only on the amount of oxygen in the package but also on other factors, i.e. the type of microbial flora present (Ruiz-Capillas & Moral 2001), probable inactivation of antioxidative enzymes as a result of carbonic acid production in fish muscle packaged in high CO_2 concentration (Masniyom *et al*. 2002), and CO_2 dissolution in the tissue, which intensify the autoxidation of polyunsaturated fatty acids (Ruiz-Capillas & Moral 2001). According to Auburg (1993), TBA values may not reveal the actual degree of lipid oxidation since MDA can interact with other components of the fish body. Such components may be amines, nucleosides and nucleic acid, proteins, phospholipids, and other aldehydes that are end products of lipid oxidation.

The results of the sensory evaluation of carp steaks are presented in Table 1. As the results show, all estimated sensory characteristics of carp steaks packaged in MAP 1 received significantly lower ($P < 0.05$) scores on day 15. 'Rancid' odour of MAP 1 samples detected on day 17 caused that the odour score was below the acceptability limit of 2. On the last day of the experiment, a reduced intensity of pink cream colouring of carp muscle was observed together with surface slime.

A decrease in scores of the sensory attributes of control and MAP 2 samples was observed throughout the storage period. However, all estimated sensory characteristics were at the acceptability level. It is significant to note, although being acceptable, the texture of MAP 2 samples was still rated with lower marks. The reason was the softened texture of samples from day 3 of the experiment.

CONCLUSIONS

Packaging of common carp under 90% CO_2 + 10% N_2 slowed down the proteolytic reaction as well as secondary lipid oxidation. According to those indicators, packaging of common carp in 90% CO_2 + 10% N_2 is more suitable compared to packaging in 80% O_2 + 20% CO_2 gas mixture. Based primarily on odour scores, it was concluded that common carp samples packaged in modified atmosphere with 80% O_2 + 20% CO_2 remained acceptable for up to 15 days of storage period, whereas samples packaged under 90% CO_2 + 10% N_2 as well as samples kept on flaked ice remained unchanged until the end of the experiment.

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