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Influence of modified atmosphere packaging on the shelf life and quality of chilled common carp (*Cyprinus carpio*) steaks

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ABSTRACT

The objective of this study was to investigate the impact of modified atmosphere packaging (MAP1: 80% O_2 + 20% CO_2 and MAP2: 90% CO_2 + 10% N_2) on selected chemical and sensory attributes of common carp (*Cyprinus carpio*) steaks stored at 3 ± 0.5 °C, and to establish the shelf life of the products. Samples were assessed on days 1, 3, 5, 7, 9, 11, 13, 15 and 17. Carp steaks stored in a CO_2-enriched atmosphere exhibited lower pH values throughout the entire storage period than steaks in the other atmospheres. The increase in TVB-N values followed this order: MAP2 < control < MAP1. From day 9 of storage, FFA contents were significantly higher ($p < 0.01$) in MAP2 fish compared to control and MAP1 fish. The presence of oxygen (in MAP1 and control fish) led to an elevation in total volatile basic nitrogen (TVB-N) compared to fish packaged in the absence of oxygen. Based primarily on sensory, but also chemical parameters, it was determined that carp steaks packaged in modified atmosphere with 80% O_2 + 20% CO_2 remained acceptable for up to 15 days of storage, whereas carp steaks packaged under 90% CO_2 + 10% N_2, as well as carp steaks stored on flaked ice in air, remained unchanged until the end of the study (17 days) .

1. Introduction

Fish, owing to its nutritional richness, plays a pivotal role in human diets. What makes fish especially appealing to consumers is its abundance of proteins, minerals and vitamins, alongside it being a notable source of essential fatty acids crucial for averting various human ailments. With such attributes, fish stands out as one of the most nutritionally significant food sources. In recent times, there has been a global surge in consumer preference for fresh fish over frozen or processed fish. This shift has induced the advancement of modified atmosphere packaging (MAP) for fish and fish products, ensuring prolonged shelf life and the preservation of key freshness indicators (*Gimenéz et al.*, 2002).

The shelf life of any food product, including fresh fish, is characterized by the post-packaging duration within which the product remains safe for consumption. During this period, the sensory characteristics (colour, odour, flavour and texture) and nutritional quality of the product must remain consistent and acceptable to consumers (*Huss*, 1995).

The assessment of fish quality can be conducted through sensory evaluations, microbial analyses, or chemical techniques, such as the measurement

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of volatile compounds, lipid oxidation, determination of ATP breakdown products and the presence of biogenic amines (*Gulsun et al*., 2009). The collective quantity of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is termed total volatile base nitrogen (TVB-N). Its level in fish flesh is commonly used as a parameter for estimating spoilage and as an indicator of fish freshness. These compounds are generated during the degradation of proteins and non-protein nitrogen components, primarily due to the metabolic activity of spoilage bacteria in fish and the action of endogenous enzymes (*Connell*, 1990). These processes contribute to alterations in the textural and sensory properties of fish muscle.

Hydrolytic changes in lipids result in the release of free fatty acids (FFA), which are highly susceptible to oxidative processes. Fish oil contains significant quantities of polyunsaturated fatty acids, leading to the initiation of oxidation reactions and the formation of hydroperoxides and other potentially detrimental secondary oxidation by-products. The peroxide value (PV) is considered an indicator of the primary oxidation rate, while the thiobarbituric acid (TBA) value serves as an indicator of secondary oxidation (*Ježek and Buchtová*, 2012). The alterations in lipid composition in fish and shellfish contribute to the deterioration of quality during prolonged storage, especially under unfavourable conditions.

The shelf life of fresh chilled fish is relatively short, typically lasting about 2 to 3 days at ambient temperatures of 2 ± 2 °C. It has been demonstrated that packaging fish in a modified atmosphere significantly prolongs the product's shelf life.

In Serbia, carp is the most commonly retailed freshwater fish. Fish are typically sold live, fresh chilled and unpacked (with a shelf life of 2 to 3 days), vacuum-packed (with a shelf life of 5 to 7 days), or frozen. Vacuum packaging is the preferred method of fish packaging. Modified atmosphere packaging with various gas mixtures is rarely used for fish in Serbia. Experimental monitoring data on freshwater fish packaged under modified atmosphere are generally limited.

The objective of this research was to observe changes in selected chemical and sensory parameters of common carp (*Cyprinus carpio*) steaks packaged in a modified atmosphere during storage at 3 ± 0.5 °C and to determine the shelf life of the products.

2. Materials and Methods

2.1. Sampling

Fourteen common carp (*Cyprinus carpio*) of average body weight of 2.50 ± 0.30 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, scaled, and the carcasses were cut into steaks 2 cm thick and of 220 g average weight. The 81 carp steaks were divided into three groups.

One group of fish was placed on top of flaked ice placed in polystyrene boxes with outlets for water drainage. The ice:fish ratio was 3:1 and was maintained constantly throughout the experiment. The flaked ice was changed daily. This experimental group, fish on ice, was used as control. The other two groups of carp steaks were both packaged in modified atmospheres, but with different gas ratios: MAP1: 80% O_2 + 20% CO_2 and MAP2: 90% CO_2 + 10% N_2. The packaging machine used was a Variovac (Variovac Primus, Zarrentin, Germany), and the packaging material was foil OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene, 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:fish in the package was 2:1. All carp steaks were stored in the same conditions at 3 ± 0.5 °C and on 1, 3, 5, 7, 9, 11, 13, 15 and 17 days of storage, chemical and sensory testing was performed.

2.2. Chemical analyses

Muscle pH was measured by Cyber Scan pH-510 digital pH-meter (EUTECH Instruments, Netherland).

The TVB-N was determined in triplicate by using the official steam distillation method according to Commission Regulation (EC) 2074/2005 and was expressed as mg TVB-N/100 g.

The FFA content, expressed as % of oleic acid, was determined in accordance with EN ISO 660:2020.

The PV, expressed in milliequivalents of peroxide oxygen per kg of fat, was determined by the EN ISO 3960:2017 method.

2.3. Sensory evaluation

Sensory analysis was conducted by six experienced panellists from our laboratory staff, in the sensory evaluation laboratory, at room temperature (20 °C) and with adequate lighting. Each piece of fish was removed from the packaging 10 min before the evaluation and presented on a tray. These trays were coded by randomly chosen 3-digit numbers. Each panellist analysed the fish steaks individually for overall acceptability, with regard to odour, flesh colour and texture, using a 1–5 intensity scale, with 5 corresponding to the most liked sample and 1 corresponding to least liked sample. Fish was defined as unacceptable when a score of <2 points was recorded by at least of 50% of the panellists. Fish steaks from all three fish groups were evaluated throughout the 17-day storage period on each sampling day.

2.4. Statistics

The mean values and standard deviations for chemical and sensory data were calculated by using column statistics with the processing of six values for each analysed group. Significant differences between groups were calculated by using one-way ANOVA. When a significant F was found, additional post-hoc tests with Tukey's adjustment were performed. Differences were considered as significant when the p-value was ≤ 0.05. All analyses were performed using the program Microsoft Office Excel (2016).

3. Results and Discussion

Figure 1 shows the pH of common carp steaks packaged in different atmospheres. In MAP1 fish, a significant ($p < 0.01$) increase in pH was noted between day 5 (pH: 6.49 ± 0.03) and day 9 (pH: 6.63 ± 0.06) of the study. Afterwards, the pH began to decrease, reaching 6.30 ± 0.04 by day 17. Conversely, a decrease in pH was observed in MAP2 fish throughout the entire storage period, with the lowest pH of 6.19 ± 0.02 recorded on day 15. During the storage period, the pH of control (iced and in air) fish fluctuated and ranged from 6.51 ± 0.09 to 6.63 ± 0.03. Compared to control fish, those packaged in MAP containing 90% CO_2 + 10% N_2 exhibited lower pH throughout the storage period, while the pH in MAP1 fish was significantly lower ($p < 0.01$) after 9 days of storage. The mean pH values for

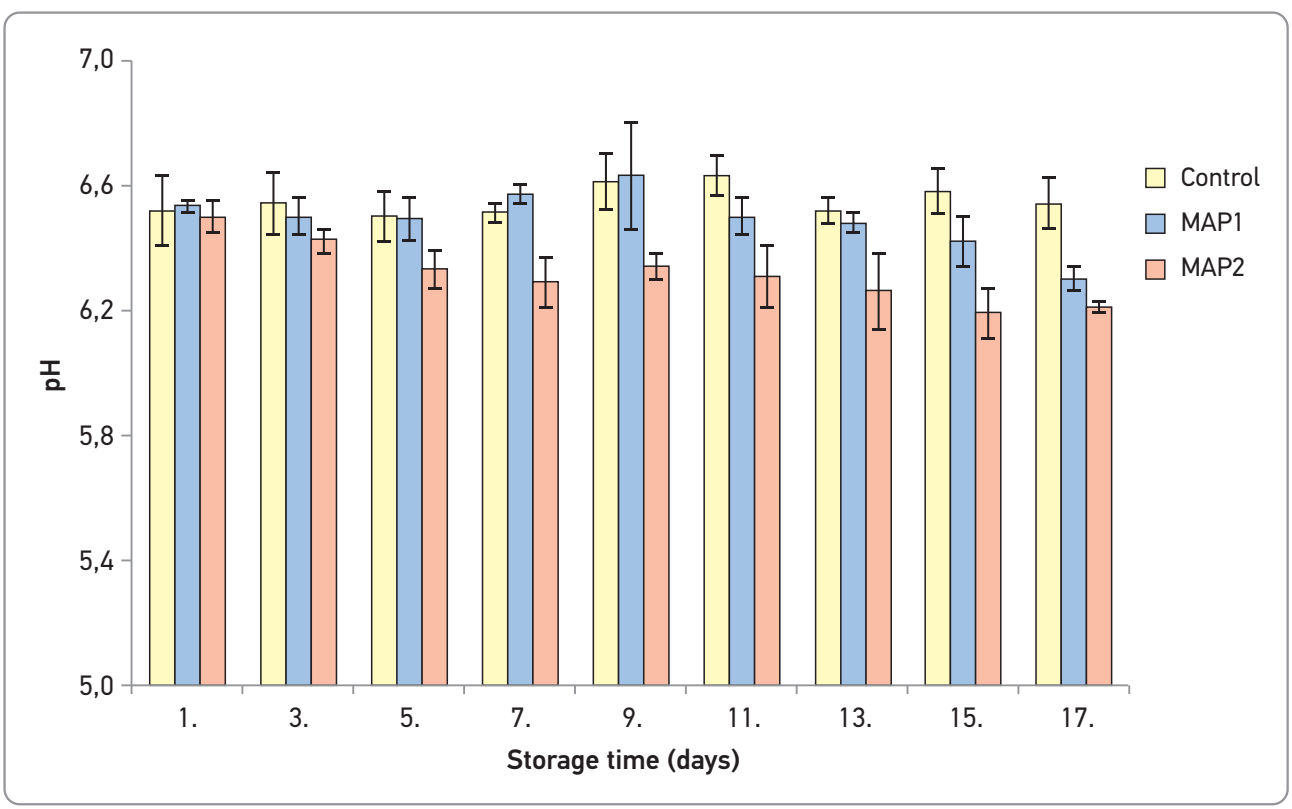

Figure 1. pH of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

control carp steaks and carp steaks packaged in MAP1 and MAP2 during storage were 6.55 ± 0.08, 6.49 ± 0.06, and 6.32 ± 0.07, respectively.

The pH of live fish muscle tissue typically hovers around 7.0, but *post-mortem* pH generally ranges from 6.0 to 7.1, depending on factors such as the season, fish species and other variables. The increase in lactic acid production during glycolysis under anaerobic conditions causes a decrease in the *post-mortem* pH of fish muscle, influencing the quality of fish meat (*Ashie et al*., 1996).

As shown in Figure 1, the lowest pH was recorded in carp steaks packaged in the atmosphere with 90% CO_2. Some other studies (*Milijašević et al*., 2010; *Provincial et al*., 2010, *Babić Milijašević et al*., 2023) have also reported significantly lower pH in fish packaged in modified atmospheres with a higher percentage of CO_2, attributed to the dissolution of CO_2 in fish muscle and leading to an increase in carbonic acid production. However, *Stenstrom* (1995) concluded that decrease of pH can be caused by acidic metabolic products produced by various bacteria, particularly lactobacilli. The moderate increase in pH of MAP1 fish after five days of storage may be caused by the higher quantity of basic compounds produced by the activity of fish spoilage bacteria (*Ruiz-Capillas* and *Moral*, 2001), which had favourable growth conditions provided by the high concentration of O_2 in this gas mixture (80% O_2).

The pH of common carp muscle and its variations under different experimental conditions in our study are in accordance with the findings of other studies (*Masniyom et al*., 2002; *Goulas and Kontominas*, 2007; *Babić et al*., 2014). In contrast, *Arashisar et al*. (2004) did not find significant differences among pH of rainbow trout fillets packaged in different atmospheres.

Figure 2 gives TVB-N values (mg/100 g) for the common carp steaks packaged in different atmospheres. The levels of TVB-N in carp steaks were practically indistinguishable ($P > 0.05$) at the beginning of the study. However, as the storage period progressed, there was an observable increase in TVB-N values in all experimental groups. Figure 2 illustrates how TVB-N values in carp steaks were significantly influenced by the atmospheric conditions used. The increase in TVB-N values followed this order: MAP2 < control < MAP1, with levels ranging from 12.35 ± 0.46 to 18.31 ± 0.48 mg N/100 g in MAP2 fish, from 12.38 ± 0.25 to 20.82 ± 1.45 mg N/100 g

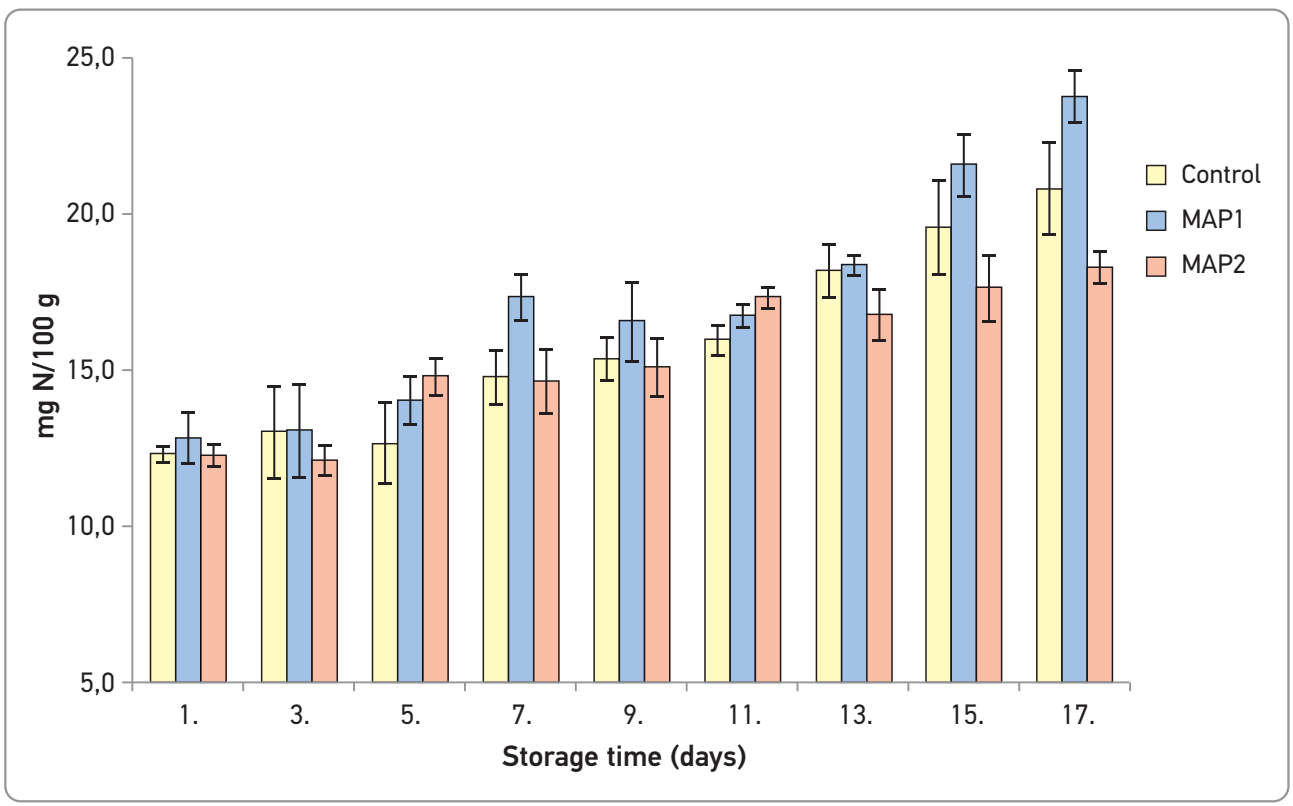

Figure 2. Total volatile base nitrogen content (mg/100 g) of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

in control fish, and from 12.40 ± 1.48 mg N/100 g to 23.77 ± 0.84 mg N/100 g in MAP1 fish during the 17-day storage period. Notably, TVB-N levels in MAP2 fish changed to lesser extent than did those in MAP1 and control fish. From day 11 onward, TVB-N values in MAP2 fish were significantly lower ($p < 0.01$) than in control fish, and compared to MAP1 fish, values were lower ($p< 0.01$) starting from day 7 of storage until the end of the study.

From day 7 onwards, the gas composition of MAP2 significantly ($p < 0.01$) delayed the formation of TVB-N compared to MAP1 fish. These discrepancies in TVB-N values may be attributed to the higher CO_2 content in MAP2 compared to MAP1 (90% versus 20%). Previous studies by *Masniyom et al*. (2013) suggested that higher CO_2 concentrations potentially inhibit the growth of predominantly Gram-negative microorganisms and reduce bacterial deamination capacity, thereby leading to a decrease in volatile compound production. Similar findings were reported by *Milijašević et al*. (2010) and *Babić et al*. (2014) for carp steaks stored under MAP, corroborating the results of the present study. Sea bass samples kept under higher CO_2 concentrations also exhibited lower TVB-N values (*Masniyom et al*., 2002). Generally, control sea bass showed higher TVB-N values compared to samples stored in CO_2-enriched atmospheres throughout the storage period (*Masniyom et al*., 2002). In our research, control fish (iced and in air) exhibited lower TVB-N values than fish packaged in MAP1. This could be explained by the presence of a high concentration of oxygen (80%) in our MAP1 packaging, which could have facilitated aerobic bacterial growth and subsequent increases in TVB-N due to bacterial decomposition of fish flesh.

While some researchers have recommended the TVB-N limit of 25 to 35 mg N/100g as an indicator for rejecting commercial fresh whole fish and processed fish products (*Connell*, 1990), no specified limit for acceptability of common carp has been established by Commission Regulation (EC) 2074/2005. In their study, *Ježek and Buhtova* (2010) proposed 20 mg N/100g in carp meat as the highest acceptable limit for TVB-N. In comparison with our study, TVB-N levels in MAP2 fish consistently remained below this limit specified by *Ježek and Buhtova* (2010) throughout the entire storage period. However, this limit was exceeded in our study by control fish (day 17) and MAP1 fish (day 15).

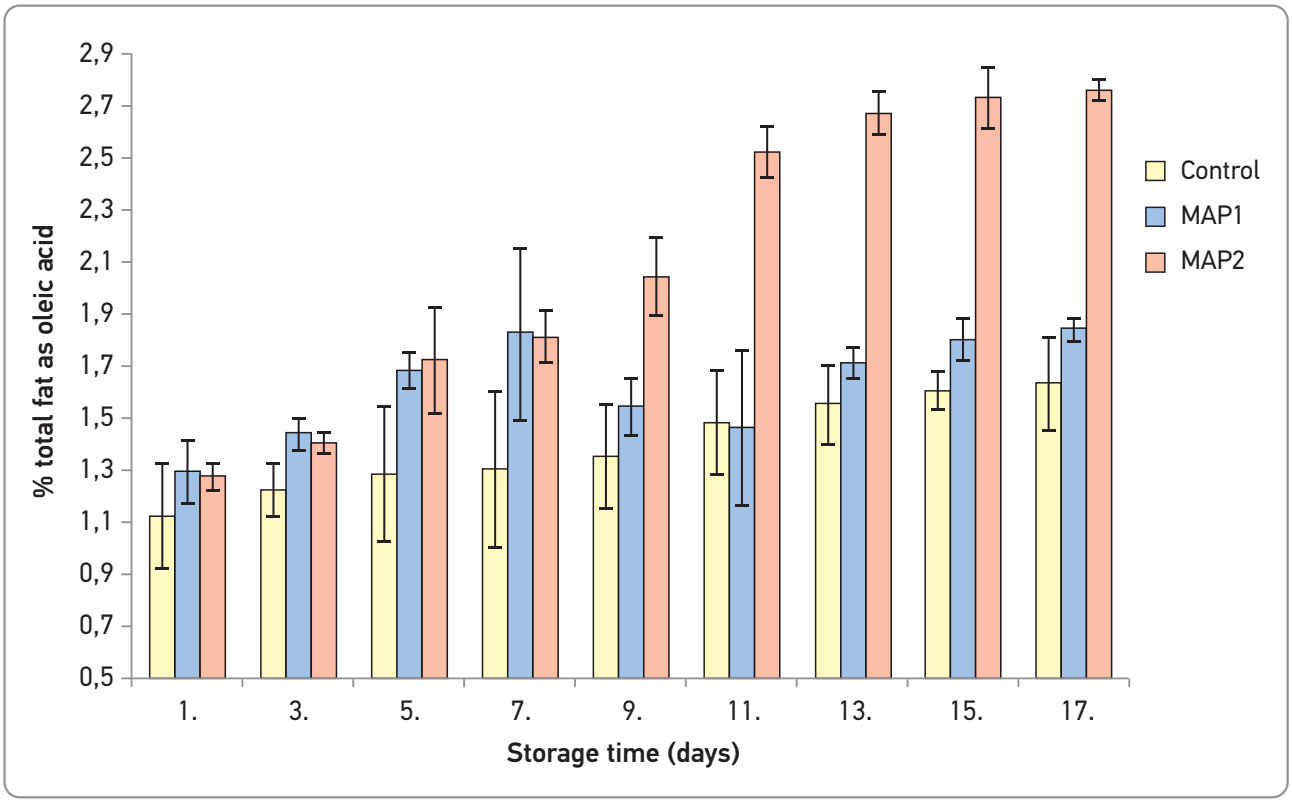

Figure 3. Free fatty acid content of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

Figure 3 shows the FFA contents (in % total fat as oleic acid) in common carp steaks packaged in the different atmospheres. Throughout the entire storage period, control fish had lower FFA contents compared to fish packaged in MAP. Simultaneously, the growth of FFA values in control fish was less pronounced (1.63 ± 0.18 on day 17) than in the MAP fish. From day 1 to day 7, there were no significant ($p > 0.05$) differences between the FFA contents of fish packaged in MAP1 and MAP2. However, the production of these degradation products followed a different line in the two types of packaging. In MAP2 fish, a highly significant ($p \leq 0.01$) increase in FFA was observed from day 9 (2.04 ± 0.1) until the end of the study (2.76 ± 0.04). On the other hand, in MAP1 fish, a significant decrease in FFA was determined between storage day 7 (1.82 ± 0.11) and day 11 (1.46 ± 0.06). From that day onward, the FFA content increased in the MAP1 fish until the end of the study (1.84 ± 0.12).

The process of lipid hydrolysis is followed by the release of FFAs. In our study, storage of carp steaks on flaked ice and in air had the smallest impact on FFA production. The significantly lower FFA level in MAP1 fish ($p \leq 0.01$) compared to MAP2 fish starting from day 9 can be attributed to the rapid conversion of FFA to oxidation products, due to the presence of O_2 in MAP1. Similar results were reported by *Ježek and Buhtova* (2012) for silver carp stored under MAP, supporting the results of our study. According to *Ozyurt et al.*, (2009) FFAs interact with myofibrillar proteins and negatively affect muscle texture. This author found a good correlation between FFA formation and the loss of freshness in fish. Otherwise, *Fagan et al*. (2004) pointed out that FFA levels have no effect on the sensory quality of fish.

Figure 4 shows the PV of common carp steaks packaged in the different atmospheres. Lipid oxidation in fish depends on several factors, such as fish species, storage temperature and lipid composition. This oxidation is often main reason for the short shelf life of fish and fish products. The PV was used to determine primary products of lipid oxidation, mainly hydroperoxides.

During the first five days of storage, PV was not detected in both, unpackaged and packaged fish. Later on, PV was lower in the control fish than in fish packaged in MAP. PV in fish packaged in the oxygen-rich atmosphere (80%) produced the highest PV from day 7 to day 13. At the end of the study (days 15 and 17), PVs were higher in fish packaged

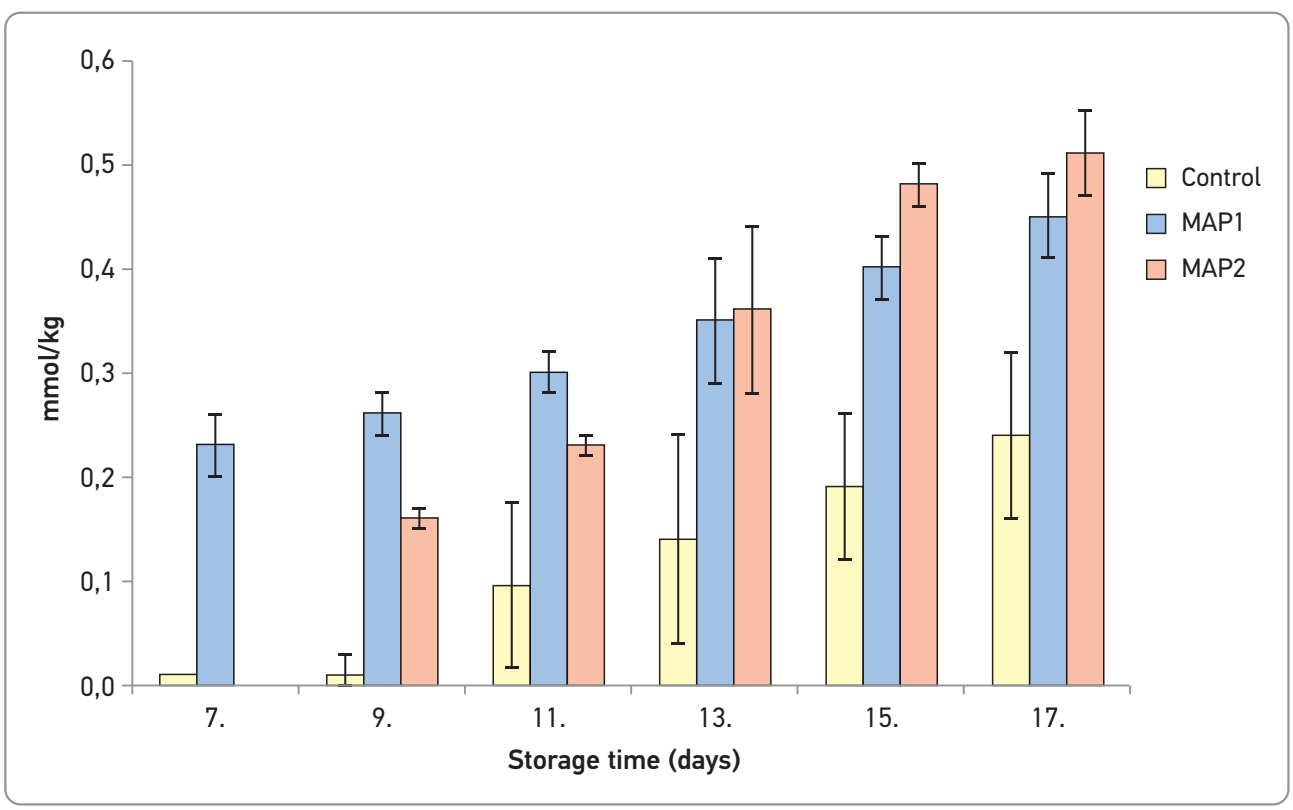

Figure 4. Peroxide value of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

Table 1. Sensory evaluation of carp steaks packaged under different conditions during storage at 3˚C

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}
	MAP2	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}
	MAP1	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}
	MAP2	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}

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2. Same lowercase letters in a row indicate no significant differences ($p > 0.05$).

in the MAP2 atmosphere without oxygen. In their research, *Ruiz-Capillas and Moral* (2001) suggested that lipid oxidation depends on the synergy effect between CO_2 and O_2. For that reason, lipid oxidation in the atmosphere with 40% O_2 could be more intensive than in the atmosphere with 60% O_2. The fluctuations in PV that were recorded in our research are in line with the results of *Ježek and Buhtova* (2007), and they indicate the fact that PV cannot be considered as a suitable indicator of fish freshness.

The sensory evaluation results for carp steaks are outlined in Table 1. It is evident that carp steaks packaged in MAP1 received significantly lower scores ($P < 0.05$) for all sensory attributes by day 15 than the fish stored under other conditions. On day 17, the rancid odour detected in MAP1 fish caused the odour score to fall below the acceptability threshold of 2. On the last day of the study, a diminished intensity of the pink cream colour of carp muscle was observed, alongside surface slime.

Throughout the storage period, both control and MAP2 fish showed a decrease in sensory attribute scores, yet they remained within acceptable levels. Notably, despite being deemed acceptable, the texture of MAP2 fish consistently received lower ratings. This was attributed to a softened texture observed from day 3 onwards. This soft texture could be ascribed to the CO_2 percentage in the MAP2 fish. Dissolution of CO_2 in the fish muscle's aqueous phase led to a decrease in pH and subsequent loss of meat juice, adversely affecting product consistency. Furthermore, the absence of O_2 and the higher CO_2 percentage in the MAP2 gas mixture resulted in a greyish hue, which received relatively low scores from our panellists. According to the odour scores, common carp steaks packaged under 80% O_2 + 20% CO_2 would likely have a shelf life of up to 15 days at 3 °C. Common carp steaks packaged in 90% CO_2 + 10% N_2 and those kept on flaked ice in air would likely be acceptable (from an odour perspective) for 17 days.

4. Conclusion

In conclusion, packaging common carp steaks in a 90% CO_2 + 10% N_2 atmosphere slowed down both proteolytic reactions and secondary lipid oxidation compared to packaging in an 80% O_2 + 20% CO_2 gas mixture. Based primarily on odour scores, it was concluded that common carp steaks packaged in a modified atmosphere with 80% O_2 + 20% CO_2 remained acceptable for up to 15 days of storage at 3 °C. In contrast, common carp steaks packaged in 90% CO_2 + 10% N_2 and those kept on flaked ice in air remained unchanged until the end of the study (17 days).

Uticaj pakovanja u modifikovanu atmosferu na održivost i odabrane parametre kvaliteta ohlađenih odrezaka šarana (*Cyprinus carpio*)

Jelena Babić Milijašević, Vesna Đorđević, Jasna Đinović-Stojanović, Srđan Stefanović, Zoran Petrović i Milan Milijašević

INFORMACIJE O RADU

Ključne reči:
Šaran
Cyprinus carpio
FFA
TVB-N
Senzorna svojstva
Održivost

APSTRAKT

Cilj ovog rada bio je da se ispita uticaj pakovanja u modifikovanu atmosferu (MAP1: 80%O2 + 20%CO2 i MAP2: 90%CO2 + 10%N2) na odabrane hemijske i senzorske parametre kvaliteta odrezaka šarana (Cyprinus carpio) i da se odredi njihova održivost. Uzorci su ispitivani 1, 3, 5, 7, 9, 11, 13, 15 i 17 dana eksperimenta. Odresci šarana upakovani u modifikovanu atmosferu sa većim procentom ugljen-dioksida imali su nižu pH vrednost tokom eksperimenta. TVB-N vrednosti su se povećavale sledećim redosledom: MAP2 < kontrola < MAP1. Od devetog dana eksperimenta vrednosti FFA bile su značajno veće ($p < 0,01$) u MAP 2 uzorcima u poređenju sa kontrolnim i uzorcima upakovanim u MAP1. Prisustvo kiseonika u MAP1 uzorcima i kod uzoraka čuvanih na ledu dovelo je do povećanja TBA vrednosti.

Rezultati senzorskih i hemijskih ispitivanja su pokazali da odresci šarana pakovani u atmosferu sa 80 posto kiseonika i 20 posto ugljen-dioksida ostaju nepromenjeni do petnaestog dana, a uzorci pakovani u atmosferu sa 90 posto ugljen-dioksida, kao i uzorci čuvani na ledu do sedamnaestog dana skladištenja.

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