Shelf-life of Chilled Muscle Tissue of the Common Carp (*Cyprinus carpio* L.) Packaged in Carbon Monoxide Enriched Modified Atmosphere

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Abstract

The aim was to study the effects of modified atmosphere packaging (MAP) consisting of 69% N_2, 25% CO_2, 5% O_2 and 1% CO on sensory and chemical indicators (O_2 in MAP, pH, lipids, total volatile basic nitrogen TVBN, nitrogen-trimethylamine N-TMA, free fatty acids FFA, peroxides PV, malondialdehyde MDA) of carp (*Cyprinus carpio,* L.) fillets and compare them with fillets in simple packaging (control group). A total of 24 carp of 2.17 ± 0.29 kg mean live weight were analyzed. The control fillets were analyzed on days 1, 2, 4, 7, 9 and 11. Fillets in MAP were analyzed on days 1, 4, 7, 9, 11, 14, 16 and 18. Fillets in MAP were pink red and the colour was stable (a carboxy-myoglobin complex), the control fillets were lighter with discolorations (from day 4). A change in the smell and consistency of fillets was observed starting on day 9 (MAP) and 4 (controls). Levels of pH in both types of samples fluctuated and no conclusive results were obtained. TVBN and N-TMA concentrations increased consistently upon fish aging. FFA concentrations on day 1 of monitoring were higher ($p < 0.01$) in MAP samples compared to the controls. Later, the production of these degradation products in the two types of packaging differed. MDA concentrations in MAP samples were lower ($p < 0.01$) throughout the experiment. MDA may be another suitable indicator for the determination of the intensity of fat oxidation.

Fish safety and quality issues, shelf-life, lipid oxidation, chemical properties, carbon monoxide

Fish muscle tissue is one of foodstuffs with very short shelf-life even when the cool chain (+2 ± 2 °C) is maintained. It is subject to irreversible physical and biochemical processes, degenerative autolytic and proteolytic changes catalyzed by the native enzymes, and, later, by the enzymes of the contaminating psychrotrophic micro-organisms (Gould and Abee 1995; Buchtová and Ježek 2006). Proteolytic processes lead to the breakdown of proteins into simpler products (peptides, amino acids, total volatile basic nitrogen, trimethylamine, ammonia) that have an adverse effect on the quality of fish muscle tissue (Ruiz-Capillas and Moral 2001). Increased concentrations of free fatty acids released by lipid hydrolysis are a well-demonstrable post-mortem sign (Ashton 2002). The large amounts of polyunsaturated fatty acids in fish fat trigger oxidation processes whose products include hydroperoxides of fatty acids. The secondary products of oxidation processes could be aldehydes, ketones, carboxylic acids, epoxy acids and alcohols (Huss 1995). During storage, fish muscle tissue is subject to sensory changes in colour, odour and consistency that are very important for the determination of tissue freshness, the shelf-life. The shelf-life of fresh chilled fish is relatively short and at ambient temperatures of +2 ± 2 °C it is about 2 to 3 days. The shelf life of fresh chilled fish can be extended by vacuum packaging or modified atmosphere packaging (MAP) (Özogul et al. 2000).

The gases most frequently used for modified atmosphere packaging are CO_2, N_2 and O_2 applied at different ratios. The packaging gas to the packaged product volume ratio should be from 2:1 to 3:1 (Sivertsvik et al. 2002). CO_2 is used as an antimicrobial component (Debevere and Boskou 1996), N_2 as inert gas retarding the oxidative rancidity (Farber 1991; Church 1998) and O_2 as a prevention of proliferation of anaerobic micro-organisms (*Clostridium* spp.) and red meat colour stabilizer by oxidation of myoglobin and formation

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of oxymyoglobin (Arashisar et al. 2004; Özogul et al. 2004). Another possibility for creating red colour is to add CO to the MAP system. Carbon monoxide, which has 30-50 times greater affinity to myoglobin than oxygen, reacts with oxymyoglobin and produces a carboxy-myoglobin complex of stable bright cherry red colour. This stable red colour may, however, mask microbial spoilage of the meat, which is one of the reasons why CO is not used in EU member states (Smulevich et al. 2007).

The most common freshwater fish retailed in the Czech Republic are carp; placed on the market live, fresh chilled or frozen. The preferred type of fish packaging is vacuum packaging. Modified atmosphere packaging with different gas mixtures is practically not used for fish in the Czech Republic. There are generally very few experimental monitoring data on freshwater fish packaged under modified atmosphere.

The aim of the work was to monitor changes in physical and chemical properties related to shelf-life in common carp (*Cyprinus carpio,* L.) fillet samples packaged under modified atmosphere (MAP) consisting of 69% N_2, 25% CO_2, 5% O_2 and 1% CO, and to compare them to changes of the same indicators in carp fillets packaged in simple packaging (control group).

Materials and Methods

Samples from 24 carp of 2.17 ± 0.29 kg mean live weight were obtained from Rybníkářství Pohořelice Company, and they were processed at the Mušov freshwater fish processing plant using a standard processing procedure (killing by electrocution, descaling, evisceration, filleting and chilling to +2 ± 2 °C). The fillets were then shipped to the Institute of Meat Hygiene and Technology of the University of Veterinary and Pharmaceutical Sciences in Brno without any interruption in the cool chain.

Preparation of experimental samples

Two fillets from each carp were made, and each fillet was divided into 4 segments, i.e. a total of 8 segments were obtained from 1 carp. Of the total of 12 carp, 96 segments were thus made and then sealed into Amilen PA/PE 20/60 protective sheet (VF Verpackungen GmbH, Germany) filled with a mixture of gases (69% N_2, 25% CO_2, 5% O_2 and 1% CO). The sheet permeability declared by the manufacturer at 23 °C is 50 $cm^3 \cdot m^{-2}$ in 24 h.

To prepare control samples, two fillets were collected from 12 carp. Each fillet was cut into 3 segments, i.e. a total of 72 control samples were made.

Carp samples in simple packaging and samples in MAP were stored in a cooling chamber with regulated temperature (+2 ± 2 °C). Experimental samples in MAP were analyzed in the laboratory on days 1, 4, 7, 9, 11, 14, 16 and 18 of storage, and samples in simple packaging on days 1, 2, 4, 7 and 11 of storage.

O_2 concentrations in MAP were measured by means of the Model 2.00 (VEIT Electronics, CR) portable oximeter prior to the unpacking of the samples.

Sensory assessment was made by three trained laboratory assistants. General appearance, muscle colour and odour were examined prior to the chemical analyses.

Muscle pH was measured by a piercing electrode at the dorsal region and the WTW 340-A/SET-2 (WTW GmbH, Germany) digital pH-metre.

The total volatile basic nitrogen (TVBN) and nitrogen-trimethylamine (N-TMA) were determined by direct distillation followed by titration on Kjeltec 2300 (FOSS, Sweden). N-TMA was determined by a modification of the method (with formaldehyde) used for the determination of TVBN. Free fatty acids (FFA) were determined in accordance with CSN ISO 660. Peroxide values (PV) were determined by a modification of the method according to CSN ISO 3960. FFA and PV were determined after lipid extraction with diethyl ether. Oxidation products were determined by the distillation method (Castellini et al. 2002) and quantified as malondialdehyde (MDA) equivalents.

Results (mean ± s.d.) of the analyses were statistically evaluated using one-factor analysis in the ANOVA programme (Microsoft Office EXCEL 2003).

Results

During the first four days of the experiment, O_2 concentrations in MAP gas mixtures (in %) remained practically the same (day 1: 4.86 ± 0.11; day 4: 4.83 ± 0.10). From storage day 4, O_2 concentrations in MAP began to decrease significantly ($p < 0.01$) to reach 0.79 ± 0.58 on day 14 of the experiment. The subsequent decrease in O_2 concentrations to the final level of 0.40 ± 0.44 (18. den) was not significant ($p > 0.05$) (Fig. 1).

From day 1 until almost the end of the experiment, carp fillets in MAP remained pink red and the colour was stable. The first few spots of red and brown discoloration appear only

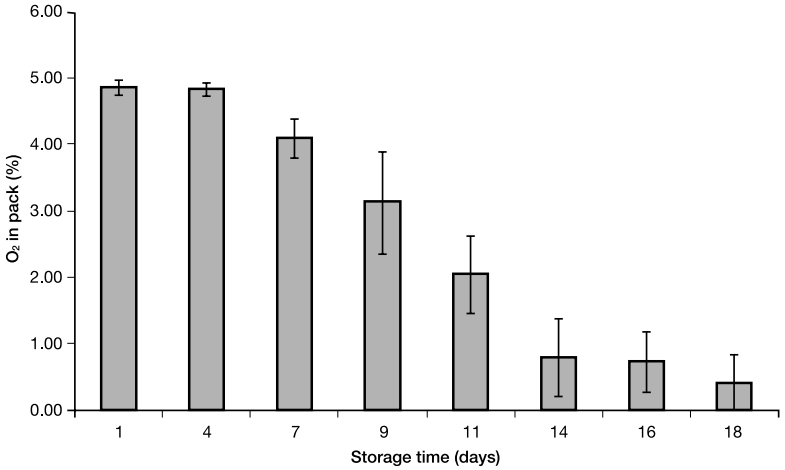

Fig. 1. O_2 concentrations in MAP (69% N_2, 25% CO_2, 5% O_2 and 1% CO) in dependence on storage period at +2 ± 2 °C.

after day 14 of storage. The control fillets were demonstrably lighter in colour from the start of the experiment, and from day 4 onward hints of discolorations were observed. Sensory changes (off-odour, slimy surfaces, softening of consistence) were observed starting on day 4 and day 9 in control samples and experimental fillets in MAP, respectively (Plate II, Fig. 2).

In samples in MAP, a significant ($p < 0.05$) decrease in this indicator was observed between day 1 (pH: 6.59 ± 0.19) and day 4 (pH: 6.38 ± 0.15) of the experiment. In another decrease ($p < 0.01$) after seven days of storage, pH dropped to 6.14 ± 0.11 (day 9), which may be considered the ultimate pH value. The subsequent increase ($p < 0.01$) of pH on day 11 (6.33 ± 0.15) was transitory, and on day 18 of monitoring, fillet pH was again lower (6.09 ± 0.09). Compared to experimental samples, control fillets (in simple packaging) had

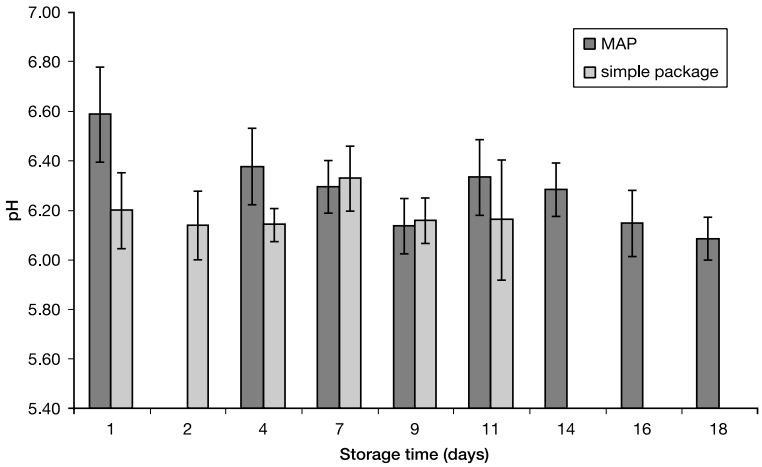

Fig. 3. pH values in carp muscle tissue in dependence on the type of packaging and the length of the storage period at +2 ± 2 °C.

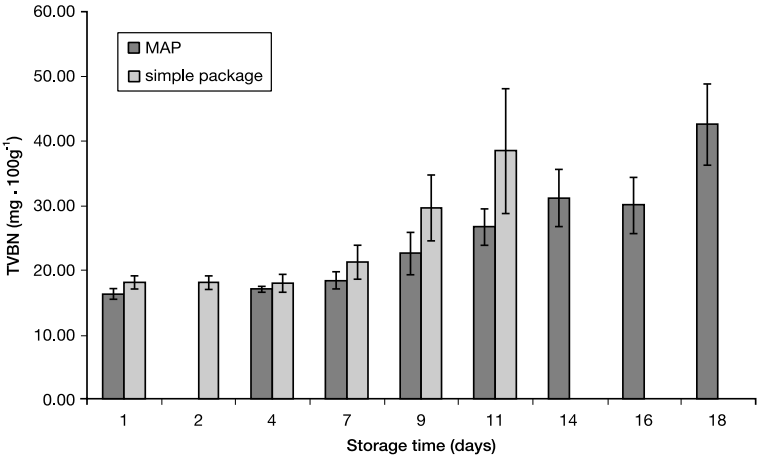

Fig. 4. TVBN concentrations (in mg·100 g^{-1}) in carp muscle tissue in dependence on the type of packaging and the length of the storage period at +2 ± 2 °C.

lower pH (6.20 ± 0.15) on day 1 of the experiment ($p < 0.01$). The ultimate pH value (6.14 ± 0.07) was found already on day 4 of storage. Then there was a temporary increase in pH on day 7 (6.33 ± 0.13) followed by pH decrease to 6.16 ± 0.24 on day 11 (Fig. 3).

Throughout the experiment, carp samples in MAP contained less ($p < 0.01$) TVBN in mg.100 g^{-1} (day 1: 16.25 ± 0.79; day 11: 26.70 ± 2.77) than control fillets (day 1: 18.06 ± 0.98; day 11: 38.41 ± 9.61). After 18 days of storage, i.e. at the end of the experiment, TVBN levels stood at 42.52 ± 6.20 mg.100 g^{-1} (Fig. 4).

Initial values of N-TMA concentrations (in mg.100 g^{-1}) in MAP samples (10.00 ± 1.80) and control samples (9.56 ± 1.02) were the samel. Higher values of N-TMA concentrations in MAP samples were found on days 4 ($p < 0.01$) and 7 ($p < 0.05$) of monitoring, later (on days 9 and 11) non-significantly ($p > 0.05$) higher N-TMA concentrations were found in control samples. Maximum N-TMA concentrations were found at the end of the experiment,

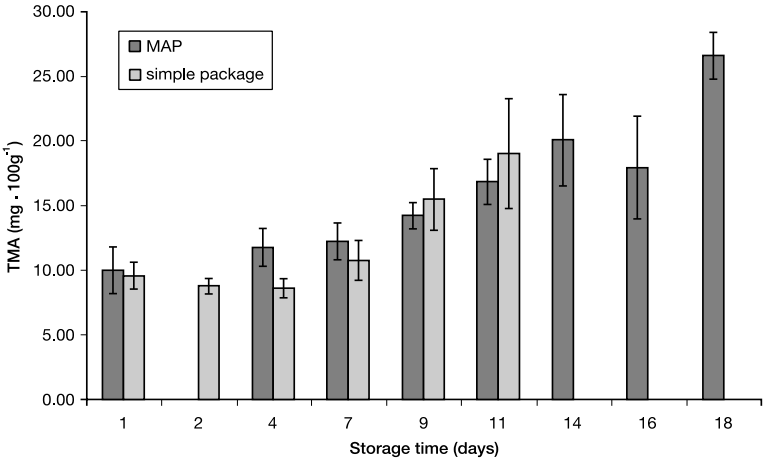

Fig. 5. N-TMA concentrations (in mg·100 g^{-1}) in carp muscle tissue in dependence on the length of the storage period at +2 ± 2 °C.

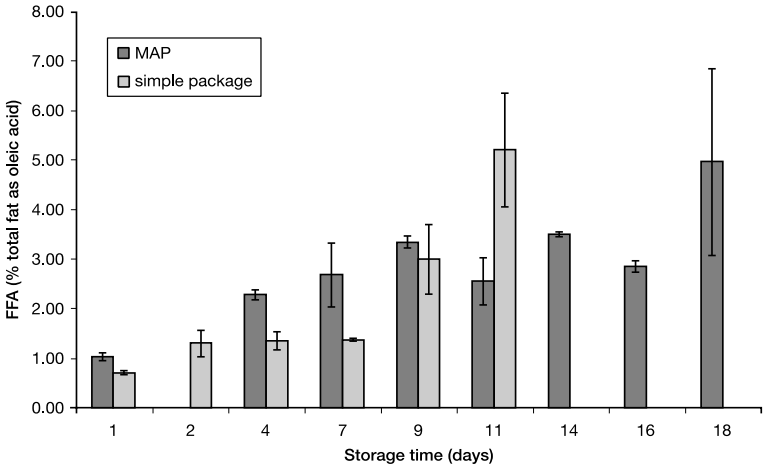

Fig. 6. FFA concentrations (in % total fat as oleic acid) in carp muscle tissue in dependence on the type of packaging and the length of the storage period at +2 ± 2 °C.

i.e. on day 18 in MAP samples (26.55 ± 1.82) and on day 11 in control samples (19.01 ± 4.25) (Fig. 5).

FFA concentrations (in % total fat as oleic acid) on day 1 of monitoring were significantly higher ($p < 0.01$) in MAP samples (1.03 ± 0.08) compared to controls (0.71 ± 0.04). Later, the production of these degradation products in the two types of packaging followed a different course. In MAP samples, a significant ($p < 0.05$) increase in FFA values was observed after day 9 (3.35 ± 0.13) followed by a decrease on day 11 ($p < 0.01$). The highest FFA values in experimental samples were found on day 18 (4.97 ± 1.89). Control fillet segments contained more FFA ($p < 0.01$) already on day 2 (1.30 ± 0.27) of the experiment. Until day 7, FFA levels in control samples of carp fillets remained unchanged. A significant

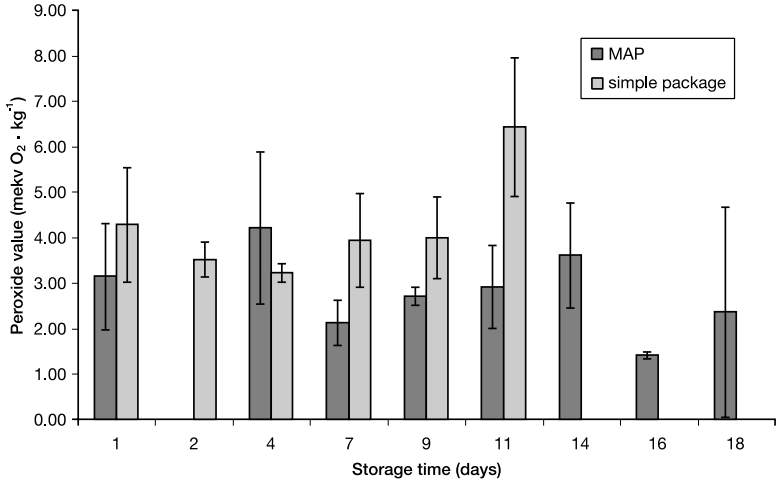

Fig. 7. Peroxide concentrations (in mekv $O_2 \cdot kg^{-1}$) in carp muscle tissue in dependence on the type of packaging and the length of the storage period at +2 ± 2 °C.

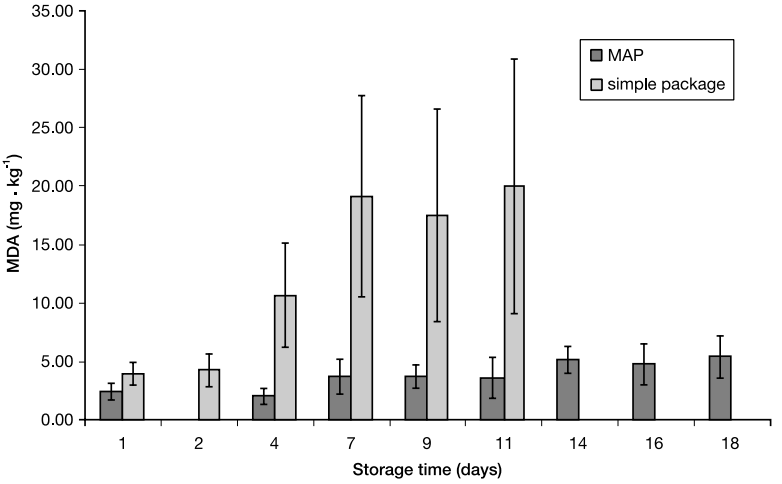

Fig. 8. MDA concentrations (in mg·kg^{-1}) in carp muscle tissue in dependence on the type of packaging and the length of the storage period at +2 ± 2 °C.

($p < 0.01$) increase in FFA concentrations took place between storage days 7 and 9 (3.00 ± 0.70), and days 9 and 11 (5.21 ± 1.15) of monitoring (Fig. 6).

PV values (in mekv O_2·kg^{-1}) on day 1 of the experiment were lower ($p > 0.05$) in MAP samples (3.15 ± 1.17) than in control samples (4.29 ± 1.26). Further development of PV (until day 7 of monitoring), however, was different in the experimental and in the control samples. Between days 1 and 4 peroxide values increased in MAP samples (4.21 ± 1.66) but showed a significant ($p < 0.05$) decrease in control samples (3.23 ± 0.20). Between days 4 and 7 of monitoring, on the contrary, peroxide values showed a decrease in MAP samples ($p < 0.01$) while increasing in control samples ($p < 0.05$). In MAP samples, peroxide values fluctuated until the end of the experiment on day 18 (Fig. 7).

MDA concentrations (in mg·kg^{-1}) in MAP samples were lower ($p < 0.01$) throughout the experiment. A significant increase in MDA concentrations in experimental samples took place between days 4 and 7 ($p < 0.01$) and days 11 and 14 ($p < 0.05$) of monitoring. In control samples, MDA concentrations increased ($p < 0.01$) between days 2 and 7 of monitoring to 19.14 ± 8.61, which is almost 5 times more ($p < 0.01$) that MDA concentration in MAP samples (3.70 ± 1.48) on the same day, i.e. day 7 of the experiment (Fig. 8).

Discussion

The aim of the experimental application of modified atmosphere consisting of 69% N_2, 25% CO_2, 5% O_2 and 1% CO was to obtain information on the effects of this mixture of gases on freshwater fish meat. Although the use of CO for commercial packaging of foods in not permitted in the EU, modified atmospheres including CO (1% CO, 30% CO_2, 5% O_2 and 64% N_2) are commonly used in some countries (Japan, Norway, USA) for fish packaging (John 2005; Eilert 2005).

The advantage of MAP with a low O_2 concentration and enriched with CO lies mainly in the production of carboxy-myoglobine which is associated with stable red colour of meat. The use of CO in meat packaging gases receives mixed reactions. Cornforth and Hunt (2008) believe that foods in MAP with added CO may look fresh although in fact they may contain large quantities of bacteria and may be spoiled. Another disadvantage is the poor reputation CO has in the eyes of consumers who view this gas as a potential health risk. According to Smulevich et al. (2007), CO

helps satisfy the consumer's expectations of stable red colour of tuna fish in modified atmosphere packaging.

In our experiment, carp fillets in MAP with added CO maintained their red colour throughout the monitoring period, i.e. for 18 days (Plate II, Fig. 2). MAP with a high O_2 concentration (80% O_2, 20% CO_2), on the other hand, was only able to maintain the red colour of carp muscle tissues until storage day 7 at most (Ježek and Buchtová 2007).

Fish meat is not acidified to any great extent by the forming lactic acid because fish muscle tissue contains only very little glycogen (0.3%) and a drop in pH below 6.0 is exceptional (Fauconneau et al. 1995). In our experiment, too, pH values never dropped below that limit. Acidulation of fish muscle in MAP may partly be due to CO_2 diffusion to the tissue accompanied by the formation of carbonic acid (Ruiz-Capillas and Moral 2001; Debevere and Boskou 1996). Another factor participating in acidulation of fish meat in MAP mentioned by Stenström (1985) are acid products of micro-organisms present in the meat. According to Ersoy et al. (2008) and Hernández et al. (2009), pH is not a suitable indicator of fish meat freshness. Their opinion is consistent with our observation because pH values fluctuated during our experiment in both types of samples and do not allow any unambiguous results to be drawn (Fig. 3).

The Commission Regulation (EC) 2074/2005 regards as unfit for human consumption the fish (*Sebastes* spp., *Salmo salar*, species belonging to *Pleuronectidae, Merlucidae* and *Gadidae* families) where organoleptic assessment has raised doubts as to their freshness and chemical checks reveal that TVBN levels (25 to 35 mg.100 g^{-1}) have been exceeded. In our experiment, TVBN levels were below 20 mg.100 g^{-1} throughout the sensory acceptability period, i.e. until day 7 and 4 in the case of MAP samples and controls, respectively (Fig. 4). Similar conclusions were also drawn by Hernández et al. (2009) and Ježek and Buchtová (2007), but, for instance, Arashisar et al. (2004) reported a higher (25 mg.100 g^{-1}) acceptable TVBN value for the rainbow trout (*Oncorhynchus mykiss*).

TVBN consists of low-molecular substances of nitrogen character (ammonia, trimethylamine, creatine, purine bases, free amino acids), which are products of microbial catabolism. One of such products associated with fish off-odour is N-TMA produced by trimethylamine oxide (TMAO) reduction. The most active in reducing TMAO are mainly *Aeromonas* spp., *Shewanella putrefaciens*, psychrotolerant enterobacteria and *Vibrio* spp., which are part of spoilage microflora (Hernández et al. 2009; Castro et al. 2006). The amount of N-TMA produced depends on the amount of TMAO in meat and on the composition of spoilage microflora. In our experiment, N-TMA values (in mg.100 g^{-1}) (Fig. 5) at the end of the period of carp fillet sensory acceptability (i.e. 12.24 ± 1.44 on day 7 in MAP samples and 8.63 ± 0.76 on day 4 in the controls) were similar to N-TMA values found by Hernández et al. (2009) in Atlantic shadefish (*Argyrosomus regius*) and Özogul et al. (2006) in turbot (*Scophthalmus maximus*).

The intensity of hydrolytic processes taking place in tissue lipids was assessed according to FFA concentrations in our experiment. FFA concentrations were higher from day 1 in MAP samples ($p < 0.01$) than in controls. Similarly, Fagan et al. (2004) found higher FFA concentrations in fish muscle in MAP at the beginning of experiment compared with air-packaged samples. According to the same authors, the FFA values ascertained had no effect on sensory evaluation and they conclude that the FFA concentration is not a suitable indicator of fish muscle freshness.

Primary products of lipid oxidation are fatty acid peroxides, which may then form substances deleterious to health. Lipid autoxidation in oxygen-rich atmosphere (80% O_2) is faster than in the air. Although low-oxygen MAP should slow oxidation, Ruiz-Capillas and Moral (2001) nevertheless believe that lipid oxidation is affected by the synergic effect of CO_2 and O_2. Lower FFA levels that we found in control samples at the beginning of the experiment (Fig. 6) coincide with higher levels of peroxides (Fig. 7). The possible

explanation in these samples may lie in the more intensive process of FFA autoxidation by the oxygen in the air (21% O_2), which is associated with the formation of primary (hydroperoxides) and secondary (cyclic peroxides, endoperoxides, epoxy acids, aldehydes oxo acids) products. The course of oxidation changes was not, however, unambiguous and this makes the parameter unsuitable as a criterion for the determination of fish muscle shelf-life.

Özogul et al. (2005) and Arashisar et al. (2004) propose malondialdehyde (MDA), a secondary product of lipid oxidation, as a suitable indicator of fish meat freshness. Significantly higher ($p < 0.01$) MDA concentrations ascertained in control fillets during the 11-day long experiment point to a higher intensity of oxidation processes in these samples compared with MAP samples. Hérnandez et al. (2009) mention a positive correlation between MDA concentrations and microbial counts (r = 0.94-0.96) and a participatory role of bacterial enzymes in oxidation. Ersoy et al. (2008) set the suitability limit for consumption at 8 mg·kg^{-1} MDA in fish. In our experiment, that limit was exceeded (10.66 ± 4.44) already on day 4 of monitoring in control fillets, but MDA concentrations in carp samples in MAP remained below that limit for the entire period of monitoring (Fig. 8). According to Ruiz-Capillas and Moral (2001), the monitoring of oxidation processes in samples in MAP is of no major importance.

Our study proved that the most suitable indicator for the assessment of common carp muscle shelf-life is TVBN concentrations. From the results obtained in carp fillets in MAP (69% N_2, 25% CO_2, 5% O_2 and 1% CO), the still acceptable upper limit of TVBN concentration is 20 mg.100 g^{-1}. Another suitable variable for the assessment of the extent of oxidation processes in carp muscle in simple packaging is the MDA concentration. In view of the results obtained, we can recommend 10 mg·kg^{-1} as the maximum limit for carp. In view of sensory changes, the shelf-life of common carp fillets in MAP was set at 7 days in our experiment.

Údržnost chlazené svaloviny kapra obecného (*Cyprinus carpio,* L.) balené do modifikované atmosféry obohacené oxidem uhelnatým

Cílem práce bylo sledovat vliv modifikované atmosféry (MAP) o složení 69 % N_2, 25 % CO_2, 5 % O_2, 1 % CO na senzorické a chemické parametry (O_2 v MAP, pH, tuk, celkové těkavé dusíkaté báze TVBN, dusík-trimetylamin N-TMA, volné mastné kyseliny FFA, peroxidy PV, malondialdehyd MDA) filetů kapra obecného (*Cyprinus carpio,* L.) a porovnat je s filety prostě balenými (kontrolní skupina). Celkem bylo analyzováno 24 kusů kapra o živé hmotnosti 2,17 ± 0,29 g. Kntrolní filety byly analyzovány 1., 2., 4., 7., 9. a 11. den. Filety skladované v MAP byly analyzovány 1., 4., 7., 9., 11., 14., 16. a 18. den. Filety v MAP měly růžověčervenou barvu, která byla stabilní (karboxy-myoglobinový komplex), kontrolní filety byly světlejší s barevnými diskoloracemi (od 4. dne). Změny pachu a konzistence byly zjištěny od 9. dne (MAP) a 4. dne (kontrola). Hodnota pH u obou druhů vzorků kolísala a nedávala jednoznačný výsledek. Obsah TVBN a TMA se zvyšovaly se zráním a stárnutím vzorků. Ve srovnání s kontrolními vzorky byly 1. pokusný den zjištěny u vzorků v MAP vyšší ($p < 0,01$) koncentrace FFA. Později se tvorba těchto degradačních produktů u obou typů balení lišila. Koncentrace MDA u vzorků v MAP byly nižší ($p < 0,01$) během celého experimentu. MDA může být dalším vhodným parametrem pro stanovení intenzity oxidace tuků.

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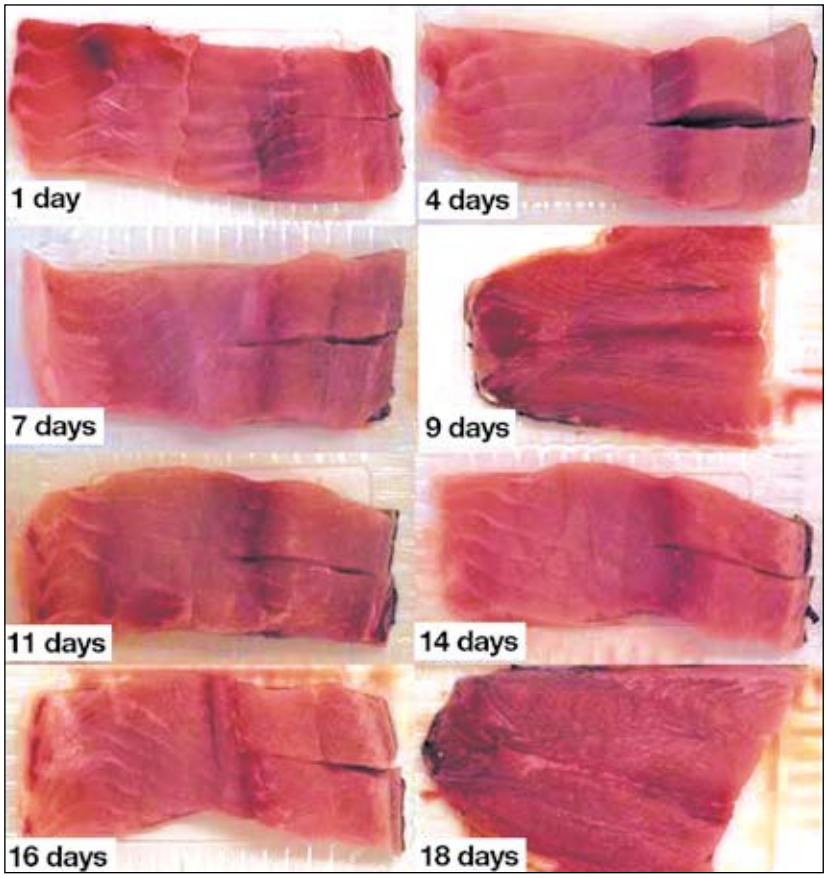

Fig. 2. Carp samples placed on trays and packaged under modified atmosphere (69% N_2, 25% CO_2, 5% O_2 and 1% CO) in dependence on storage period at +2 ± 2 °C.