Monitoring of Physicochemical Changes in Frozen Fish Muscle Tissue

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Summary

The aim of the study was to monitor physicochemical parameters (pH, nitrogen trimethylamine N-TMA, total volatile basic nitrogen TVBN, free fatty acids FFA, peroxide value and thiobarbituric acid assay TBA) of postmortal changes in muscle tissue of silver carp (*Hypophthalmichthys molitrix*) during a period of storage at -18°C. Fresh silver carp samples and samples after three, six, nine and 12 months of storage were tested. The degree of acidification during the experiment was insignificant ($P > 0.05$). Proteolytic changes were almost stopped and TVBN levels remained unchanged ($P > 0.05$), while N-TMA levels fluctuated significantly ($P < 0.01$) between months 3 and 12. The essential were lipid hydrolysis and oxidation, which caused a significant increase in FFA values (5.89 ± 0.99% total lipids as oleic acid), peroxides (9.90 ± 2.83 mekv $O_2.kg^{-1}$) and TBA values (50.76 ± 31.52 mg MDA.kg^{-1}). The shelf life recommended for silver carp was set at three months.

Key words

fish, storage, rancidity, shelf life

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Aim

The aim of the study was to monitor selected physicochemical descriptors (pH, N-TMA, TVBN, peroxide values, TBA) of postmortal processes in frozen muscle tissue of silver carp (*Hypophthalmichthys molitrix*) during a 12-month storage period. Individual descriptors were then processed and evaluated as indicators of freshness, and compared with values ascertained in fresh samples in order to determine a suitable shelf life period for frozen fish stored at -18°C.

Material and methods

Samples of silver carp (*Hypophthalmichthys molitrix*) were obtained from Rybníkářství Pohořelice Company, and were processed at the Mušov freshwater fish processing plant using a standard processing procedure. Fish were stunned with electric current before they were killed, scaled and eviscerated. Then they were filleted and cut up. Samples were then vacuum-packed and blast-frozen using -40°C air flow, and stored in a freezing facility of the Institute of Meat Hygiene and Technology of the University of Veterinary and Pharmaceutical Sciences Brno for 12 months at -18°C. A total of 60 samples from 12 fish were examined. The samples were analyzed as fresh samples at the beginning of the experiment and then after three, six, nine and 12 months of freezer storage. The pH value of the samples monitored was measured using the inoLab pH 730 digital pH-metre (WTW GmbH, Germany). The total volatile basic nitrogen (TVBN) was determined by direct distillation followed by titration on Kjeltec 2300 (FOSS, Sweden). The same method as for TVBN determination was used to determine nitrogen trimethylamine (N-TMA) after formaldehyde was added to samples to unbind primary and secondary amines. Free fatty acids (FFA) and peroxide values (PV) were determined after fat extraction with diethyl ether. FFA were determined in accordance with CSN ISO 660. Peroxide values were determined by a modification of the method according to CSN ISO 3960. The thiobarbituric acid assay (TBA) was determined by the distillation method (Castellini et al., 2002) and oxidation products were quantified as malondialdehyde (MDA, $mg.kg^{-1}$) equivalents. Results of the analyses were statistically evaluated using a one-factor analysis of the ANOVA programme (Microsoft Office EXCEL 2007).

Results and discussion

Long-term fish consumption in the Czech Republic is relatively low. In recent years, the average annual per capita consumption of fish meat has been 5.8 kg, of which only 1.4 kg was freshwater fish. In the Czech Republic, most of the freshwater fish sold is live fish with only a relatively small percentage of the fish (about 8.8% of total production) that is being processed into fish products (Abrahamová, 2011). One of possible options for fish processing and long-term storage is freezing. During freezer storage, however, the quality of fish meat deteriorates; protein denaturation processes take place as well as discolouration, decrease in weight, lipid oxidation and texture changes. These changes can be analyzed from changes in physicochemical parameters of freshness (Ersoy et al., 2008).

Fresh fish muscle pH is most frequently in the 6.0 to 6.5 range (Fan et al., 2009; Ersoy et al., 2008). The pH of fresh silver carp

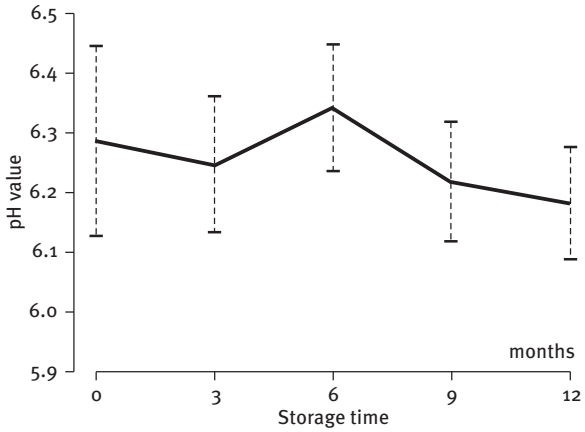

Figure 1. Changes in pH in silver carp muscle tissue during freezer storage

samples was calculated at 6.29 ± 0.16. In the first three months of storage, pH decreased insignificantly to 6.25 ± 0.12 and increased again to reach 6.34 ± 0.11 by month six. Between months and nine, a significant ($P < 0.05$) decrease in pH to 6.22 ± 0.10 was observed (Fig. 1).

The initial decrease in pH values may be due to the dissolution of CO_2 in fish muscle, and the subsequent increase in pH values depends on the production of volatile nitrogen bases (ammonia, trimethylamine and others) in dependence of enzymatic activity (Fan et al., 2009; Ruiz-Capillas and Moral, 2001).

Frozen fish quality can also be determined from the amounts of TVBN, which is a product of proteolytic changes in fish meat. TVBN concentrations are affected by fish species, when and where it was caught, its age and sex. The upper limit when a fish can be considered spoiled lies between 35 and 40 $mg.100\ g^{-1}$ (Fan et al., 2009; Ersoy et al., 2008). The TVBN value of fresh fish samples was 15.99 ± 2.38 $mg.100\ g^{-1}$. Throughout the storage period, no significant ($P > 0.05$) differences in TVBN concentrations were found, and the TVBN concentration ascertained after 12 months of freezer storage was 15.73 ± 1.13 (Fig. 2). It is therefore clear that fish freezing is a reliable prevention of changes in fish meat caused by proteolysis.

A large percentage of TVBN consists of products of microbial catabolism, for instance trimethylamine (TMA) produced by reduction of trimethylamine oxide (TMAO). The reduction of TMAO to TMO is typical for certain bacteria (*Aeromonas* spp., psychrotolerant enterobacteria, *Shewanella putrefaciens* and *Vibrio* spp.) that are present in spoiling fish (Huss, 1995). The level of trimethylamine in muscle depends on the species of fish, climatic and storage conditions, as well as on the harvest area (Robertson, 2010). In fresh fish, N-TMA concentration of 9.32 ± 2.15 $mg.100\ g^{-1}$ has been reported. N-TMA values show a higher variability during the storage period than TVBN values. N-TMA values decreased significantly between storage month three and six to 7.32 ± 1.34 $mg.100\ g^{-1}$ ($P < 0.01$). By month nine, however, N-TMA concentrations increased significantly to 11.55 ± 0.83 $mg.100\ g^{-1}$ ($P < 0.01$) only to decrease significantly again by storage month 12 to 8.12 ± 1.28 $mg.100\ g^{-1}$ ($P < 0.01$) (Fig. 2).

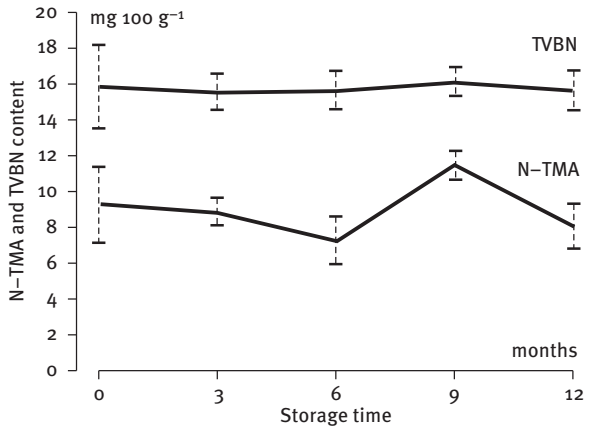

Figure 2. TVBN and N-TMA concentrations in frozen muscle tissue of silver carp

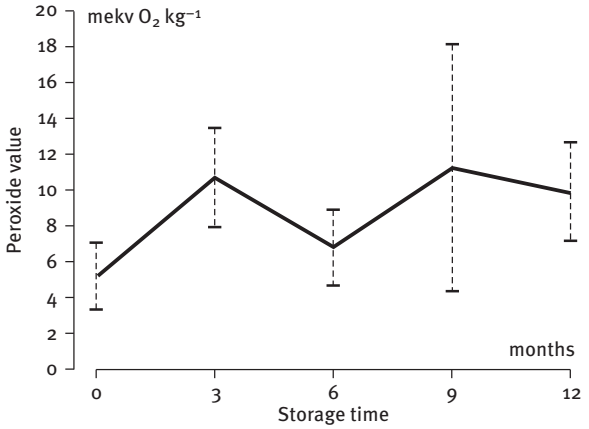

Figure 4. Peroxide concentrations in frozen muscle tissue of silver carp

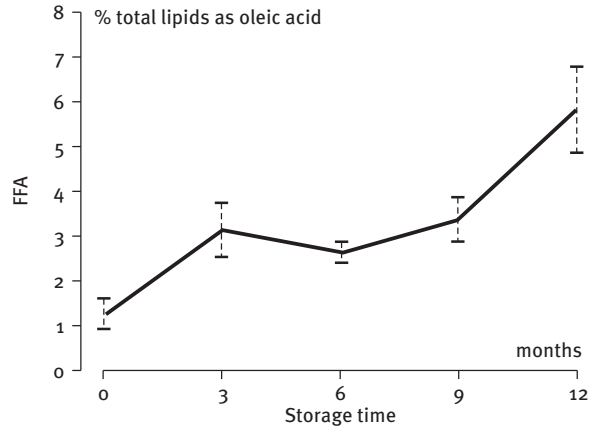

Figure 3. Formation of FFA in frozen muscle tissue of silver carp

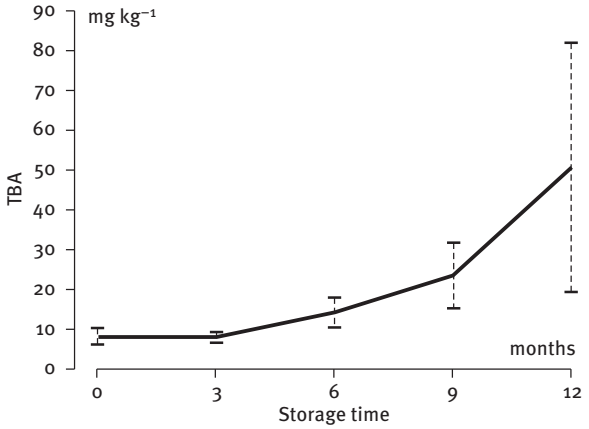

Figure 5. Secondary oxidation processes in frozen muscle tissue of silver carp

N-TMA concentrations could be used as a spoilage indicator but are not suitable as a freshness index (Hernández et al., 2009).

Lipid hydrolysis can be assessed according to FFA concentrations in fish muscle. Free fatty acids (FFA) are known to be products of enzymatic hydrolysis of lipids. A significant increase in FFA ($P < 0.01$) during freezer storage indicates that hydrolytic changes take place even at low temperatures (Fig. 3). FFA are particularly rapidly formed in the initial period (0-4 months) of storage. This finding is usually explained as a result of a maximum release of lipase from liposomes in the first month of storage (Rodríguez et al., 2007). A significant ($P < 0.01$) increase in FFA in the first period (0-3 months) of storage from 1.28 ± 0.36 % total lipids as oleic acid to 3.21 ± 0.60 % total lipids as oleic was also recorded in silver carp. Between months three and six, FFA concentrations decreased to 2.66 ± 0.23 % total lipids as oleic acid ($P < 0.01$). By the end of the experiment (month 12), FFA concentrations in frozen muscle tissues of silver carp increased significantly ($P < 0.01$) to 5.89 ± 0.99 % total lipids as oleic acid (Fig. 3) According to the Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin, the maximum permitted level of FFA in edible fat of other animals is 1.25 % total lipids as oleic acid. Compared with that value, FFA concentrations in frozen muscle of silver carp is high, and it correlates positive with the length of the storage period ($r^2 = 0.81$) (Rodríguez et al., 2007).

Pro-oxidative effects of FFA on the lipidic component were demonstrated and explained on the basis of catalytic effects of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides (Losada et al., 2007). Thus, partially hydrolyzed fats are more sensitive to oxidation. Primary oxidation can be monitored from peroxide values. The course of primary oxidation is affected by the dynamics of the formation and transformation of hydroperoxides to secondary oxidation products (ketones, aldehydes, hydroxides, epoxides, dimers, oligomers) (Sikorski and Kołakowska, 2003).

The peroxide value at the beginning of the experiment was 5.18 ± 1.87 mekv O_2.kg^{-1}. Peroxide formation during the stor-

age period was not uniform and the values fluctuated considerably ($P > 0.01$). At the end of the experiment, however, peroxide concentrations in frozen silver carp muscle were significantly higher (9.90 ± 2.83 mekv O_2.kg^{-1}; $P < 0.01$) than at the beginning (Fig. 4).

Secondary oxidation products have considerable influence on sensory characteristics of meat. Huss (1995) found significant positive correlations between TBA values and sensory analysis ($r = 0.82$-0.98). From the point of view of carcinogenic and toxic effects on the organism, malondialdehyde ranks among the most important products of secondary lipid oxidation (Fernández et al., 1997). A good technique for the monitoring of oxidation processes in meat is use of the thiobarbituric acid assay (TBA) after convertion to malondialdehyde equivalents (Fan et al., 2009). While there was no increase in TBA values in silver carp muscle in the first three months of storage, from month three to the end of the experiment TBA values increased significantly from 8.32 ± 1.06 mg MDA.kg^{-1} (month 3) to 50.76 ± 31.52 mg MDA.kg^{-1} (month 12) (Fig. 5).

Conclusions

There was only a slight muscle tissue acidification during freezer storage of silver carp. Proteolytic processes were almost halted by low temperatures. On the other hand, lipolytic processes are not suppressed by freezer storage temperatures, and enzyme activity persists. Free fatty acids are being formed even at temperatures below the freezing point. They have pro-oxidative effects and accelerate the formation of peroxides often harmful to human health and of secondary oxidation products that then decrease the sensory value of fish meat. For freezer storage of silver carp, a period of three months is recommended when there is still no accumulation of secondary oxidation products.

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