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SHELF-LIFE OF FREEZE-THAWED FILLETS OF COMMON CARP (*Cyprinus carpio* L.) AND SILVER CARP (*Hypophthalmichthys molitrix* V.) PACKED UNDER AIR

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ABSTRACT

The aim of the study was to evaluate the effect of freezing and thawing on physicochemical changes in fillets of common carp (*Cyprinus carpio,* L.) and silver carp (*Hypophthalmichthys molitrix,* V.). Thawed fillets placed in microtene bags were stored for 11 days at 2 ± 2 °C. The parameters monitored in muscle tissue samples were: water activity, pH value, total volatile basic nitrogen, nitrogen trimethylamine, free fatty acids, peroxide value and thiobarbituric acid assay. Samples were evaluated on days 1, 2, 4, 7, 9 and 11 of cold storage. Results found in thawed fillets were compared with those in fresh fillets, and statistically evaluated. The comparison showed that the course of physicochemical changes in thawed fillets is different from that in fresh fillets.

Key words: freshwater fish / carps / freezing / thawing / cold storage / chemical changes / oxidation / shelf life

1 INTRODUCTION

The most important factors influencing short shelf life include insufficient and only very short-lasting muscle tissue acidification in the early stages of *rigor mortis*, rich sources of microorganisms, specific chemical composition, insufficient barriers in the form of connective tissues and an advantageous ratio between the external and internal surfaces, and the muscle tissue thickness (Pipová *et al.*, 2006). Frozen storage is one of important methods of preserving fish and fish products. Thawing plays an important role in the disintegration of cell membranes, and also affects sensory properties of fish meat (Benjakul and Bauer, 2001; Karoui *et al.,* 2006). Frozen meat, and fish meat in particular, is sometimes passed for fresh meat, which, however, costs more, and that constitutes cheating of customers. In justified cases, the freezing of fish is a necessary veterinary hygiene requirement (Commission Regulation (EC) No. 1276/2011). Frozen fish are usually offered in frozen state to customers, but the sale of thawed raw food is not prohibited. According to the Regulation (EU) No 1169/2011 of the European Parliament and of the Council, food may be thawed prior to sale, but must be labelled as "defrosted" (Buchtová and Ježek, 2012). In some cases, fish muscle tissues are frozen prior to being packed in modified atmosphere (MAP) in order to reduce microbial contamination (Bøknæs *et al.,* 2000).

Post mortem changes in fish muscle are physicochemical, biochemical, microbial and sensory in nature. Proteolytic processes lead to peptide releases and accumulation of low-molecular nitrogenous substances (free amino acids, total volatile basic nitrogen, trimethylamine, ammonia) in the muscle. Demonstrable signs of post mortem processes are quantitative increases in free fatty acids (FFA), fatty acid hydroperoxides and secondary oxidation products (aldehydes, ketones, carboxylic acids, epoxy acids, alcohols) (Huss, 1995; Ruiz-Capillas and Moral, 2001; Ashton, 2002). Shelf life of fresh chilled fish muscle is between 3 to 5 days depending on the type of chilling

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and on storage temperature. If, however, fish muscle has been frozen and then stored as chilled, its shelf life will depend on many factors, such as initial contamination, the interval between killing and freezing, temperature and rate of freezing, length of freezer storage and type of thawing. If freeze-thawed muscle is packed in modified atmosphere, its shelf life can be extended from 11 to 20 or more days (Bøknæs *et al.,* 2000). In the Czech Republic, modified atmosphere packing (MAP) is not the usual practice. At present, no detailed information is available on the shelf life of freeze-thawed fish packed under modified atmosphere and under air.

The aim of our study was to monitor proteolytic, lipolytic and oxidative changes in freeze-thawed fillets of common carp and silver carp packed under air, and to compare them with values published for fresh muscle tissue packed under air of the same fish species (Ježek, 2008; Ježek and Buchtová, 2011).

2 MATERIAL AND METHODS

Common carp and silver carp samples were obtained from Rybníkářství Pohořelice a.s., and they were processed using a standard processing procedure. Fish were stunned with electric current before they were killed, scaled, eviscerated and filleted. Chilled (2 ± 2 °C) unskinned fillets were cut to 3 parts, placed into plastic bags, labelled and frozen at −40 °C to muscle core temperature of −18 °C. Fillet samples were then shipped to the Department of Meat Hygiene and Technology of the University of Veterinary and Pharmaceutical Sciences Brno without a break in the cold chain, and they were stored there at freezer temperature of −18 °C for 7 days. Frozen fish samples were then placed in a cold chamber at 2 ± 2 °C for thawing, and the thawed samples were then kept there at the same temperature for 11 days. A total of 72/72 samples of common carp/silver carp fillets packed under air in microtene bags were examined. The samples were analyzed on days 1, 2, 4, 7, 9 and 11 after thawing. 12 samples were analysed each experimental day. The parameters investigated in the samples included water activity a_w using LabMaster-aw (Novasina Ltd., Switzerland), muscle pH using digital pH-metre inoLab pH 730 (WTW GmbH, Germany), total volatile basic nitrogen (TVBN) and nitrogen trimethylamine (N-TMA) using Kjeltec 2300 (FOSS Analytical AB, Sveden) employing the method of direct distillation and subsequent titration. 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 modified method according to CSN ISO 3960. The thiobarbituric acid assay (TBA) value was determined by the distillation method (Castellini *et al.*, 2002) and oxidation products were quantified as malondialdehyde (MDA) equivalents. Values measured in thawed fillets on individual sampling days were statistically compared using unifactorial analysis of variance in the ANOVA program (Microsoft Office EXCEL 2007) with results obtained from chilled fillets packed under air of the same fish species.72 chilled samples of common carp and 108 chilled samples of silver carp were used.

3 RESULTS AND DISCUSSION

In the first four days, water activity (a_w) in thawed common carp fillets was significantly lower ($P < 0.01$) than in fresh fillets packed under air in plastic bags. The value of a_w in thawed silver carp fillets did not differ from a_w of fresh fillets. The only exception was day 11 when a_w in thawed fillets was higher ($P < 0.01$). During freezing and freezer storage, muscle tissue of unpacked fillets may dry out (sublimation). Because cell structures are damaged, some of their water may be released after thawing. Differences in the chemical composition of muscle tissues between the common carp and the silver carp may affect those processes and thus also the amount of unbound water available for microorganisms (a_w). Thawed common carp fillets had pH values significantly higher ($P < 0.01$) compared with fresh fillets in the first nine days. No difference was found on day 11. This may be due to activities of enzymes and microorganisms that are involved in acidification, and are inactivated by freezing (Bøknæs *et al.,* 2000). In the first 7 days, no differences in pH were found between thawed and fresh silver carp fillets. On days 9 and 11, pH values in thawed fillets were higher ($P < 0.05$) (Table 1). As presumed, pH values during the experiment never fell below 6.00 (Huss, 1995).

In the first 2 days, proteolytic processes (TVBN) in the common carp muscle tissues damaged by freezing were more intensive ($P < 0.01$) than in fresh fillets. The difference was less pronounced on days 4 and 7, and on day 9, a significantly ($P < 0.05$) higher TVBN levels were found in fresh fillets. At the end of the experiment (day 11), however, TVBN levels were again higher ($P < 0.01$) in freeze-thawed fillets. Higher TVBN ($P < 0.01$) values were also found in freeze-thawed fillets of silver carp until day 4, and on day 9, higher TVBN values ($P < 0.05$) were found in fresh

Table 1: *The course of physicochemical processes in freeze-thawed fillets of common carp (Cyprinus carpio) – CC and silver carp (Hypophthalmichthys molitrix) – HM*

Day of experiment		1. mean ± sd	2. mean ± sd	4. mean ± sd	7. mean ± sd	9. mean ± sd	11. mean ± sd
a_w	CC	0.939 ± 0.01 *0.049***	0.939 ± 0.01 *0.058***	0.933 ± 0.00 *0.066***	0.993 ± 0.01 *0.006**	0.999 ± 0.00 *−0.001*	0.999 ± 0.00 *−0.002*
	HM	0.978 ± 0.00 *−0.005*	0.980 ± 0.00 *−0.001*	0.984 ± 0.01 *−0.002*	0.982 ± 0.00 *0.002*	0.982 ± 0.00 *0.002*	0.988 ± 0.00 *−0.004***
pH	CC	6.62 ± 0.19 *−0.42***	6.57 ± 0.22 *−0.43***	6.40 ± 0.18 *−0.26***	6.51 ± 0.15 *−0.18***	6.42 ± 0.14 *−0.26***	6.28 ± 0.23 *−0.12*
	HM	6.31 ± 0.11 *−0.07*	6.23 ± 0.06 *−0.04*	6.24 ± 0.06 *−0.04*	6.26 ± 0.09 *−0.03*	6.16 ± 0.08 *0.10**	6.16 ± 0.05 *0.11**
TVBN mg . 100 g^{-1}	CC	19.44 ± 1.34 *−1.38***	20.05 ± 1.29 *−1.93***	18.68 ± 1.38 *−0.76*	24.52 ± 3.53 *−3.25**	24.77 ± 2.73 *4.85***	76.44 ± 24.09 *−38.03***
	HM	19.45 ± 0.41 *−3.00***	19.32 ± 0.51 *−3.69***	20.14 ± 2.29 *−3.81***	21.22 ± 2.25 *−0.50*	20.93 ± 1.79 *11.44**	28.83 ± 14.54 *5.06*
N-TMA mg . 100 g^{-1}	CC	11.36 ± 1.23 *−1.80***	11.11 ± 0.64 *−2.34***	10.46 ± 1.11 *−1.83***	15.33 ± 2.35 *−4.60***	16.07 ± 2.07 *−0.59*	36.18 ± 7.71 *−17.17***
	HM	10.81 ± 0.24 *−0.64*	10.58 ± 0.53 *−1.66*	12.16 ± 1.69 *−2.86**	12.75 ± 1.62 *−0.26*	12.18 ± 1.70 *6.71**	16.28 ± 6.35 *1.78*
FFA % total lipids of oleic acid	CC	0.95 ± 0.11 *−0.24***	1.77 ± 0.07 *−0.47***	2.11 ± 0.05 *−0.76***	3.59 ± 0.35 *−2.21***	3.94 ± 0.29 *−0.94***	8.69 ± 0.18 *−3.48***
	HM	3.91 ± 0.27 *−2.35***	2.30 ± 0.22 *−1.15***	5.79 ± 0.21 *−4.48***	5.42 ± 0.37 *−3.94***	3.89 ± 0.05 *−1.05**	6.11 ± 1.03 *−2.84***
PV mekv O_2 . kg^{-1}	CC	2.31 ± 0.51 *1.98***	3.53 ± 2.05 *−0.01*	4.48 ± 0.32 *−1.25***	4.43 ± 0.30 *−0.49*	4.39 ± 1.54 *−0.39*	4.27 ± 1.16 *2.16***
	HM	1.73 ± 0.22 *4.80***	5.10 ± 0.70 *1.73*	4.34 ± 0.33 *3.19***	5.47 ± 0.20 *8.36**	9.11 ± 3.09 *0.88*	17.29 ± 2.66 *−4.35*
TBA mg . kg^{-1}	CC	6.87 ± 1.75 *−2.93***	3.68 ± 0.43 *0.58*	8.68 ± 2.75 *1.98*	18.39 ± 7.35 *0.75*	23.33 ± 7.65 *−5.84*	16.26 ± 11.00 *3.76*
	HM	11.11 ± 5.75 *−3.73**	12.57 ± 9.23 *−2.48*	32.60 ± 29.47 *−14.17*	73.39 ± 19.26 *−52.52***	37.10 ± 13.17 *−17.45***	90.59 ± 42.58 *−66.90***

Italics denote the difference obtained by subtracting freeze-thawed fillet values from fresh fillet values of that parameter, with the asterisks identifying statistical significance of that difference (**P < 0.01; *P < 0.05). aw – water activity, TVBN – total volatile basic nitrogen, N-TMA – nitrogen trimethylamine, FFA – free fatty acids, PV – peroxide value

fillets, similarly as in common carp. TVBN is generally considered an indicator of spoilage of fish muscle tissues. Some studies, however, mention a non-significant correlation between TVBN and the storage period, and between TVBN and the sensory quality of fish. The TVBN content may also be influenced by the fish size, species of contaminating microorganisms and the season of the year (Orban *et al.,* 2011). An alternative indicator of freshness is N-TMA. With the exception of day 9, significantly higher N-TMA values (P < 0.01) were found in freeze-thawed common carp fillets than in fresh fillets. In silver carp, on the other hand, higher N-TMA values (P < 0.05) were found in freeze-thawed muscle only on day 4 of the experiment, while N-TMA content in the freeze-thawed fillets on day 9 was lower (P < 0.05) than in fresh fillets (Table 1). Contrary to TVBN, N-TMA is considered a good indicator of quality of some fish species thanks to its high correlation with sensory changes and microbial counts during cold storage. N-TMA concentrations depend on species composition of spoilage microorganisms. Freezing may inactivate trimethylamine-producing microorganisms (such as *Photobacterium phosphoreum*), and other nitrogenous bases are created. That may be the cause of low correlation between TVBN and N-TMA, and, from the N-TMA levels point of view, freeze-thawed fish may have a longer shelf life, and even better odour and taste properties (Orban *et al.,* 2011; Bøknæs *et al.,* 2000).

The intensity of lipid hydrolysis (FFA) in the two carp species (i.e. common carp and silver carp) was higher in freeze-thawed fillets throughout the experiment ($P < 0.01$). It is clear that lipolytic processes are not halted by freezing, and that FFA muscle concentrations increase. Partially hydrolyzed fats are more susceptible to oxidation. The course of primary oxidation (PV) was not uniform in neither common carp nor silver carp, with peroxide values showing considerable variation. PV is not suitable for the monitoring of fish meat freshness (Ježek and Buchtová, 2011). TBA can be considered a more suitable indicator of oxidative processes in fish meat. In common carp, higher TBA values ($P < 0.01$) were found in freeze-thawed fillets on day 1 only; for the rest of the experiment, no differences between fresh and freeze-thawed fillets were found. In the case of silver carp, on the other hand, which is considered a rather high-fat fish, TBA values in freeze-thawed fillets were higher than in fresh fillets throughout the experiment, and the differences were significant ($P < 0.01$) on days 7, 9 and 11. The freezing and thawing accelerates the accumulation of secondary oxidative products, which is caused by the destruction of cell membranes by crystals of ice and the release of pro-oxidants, especially haem iron (Benjakul and Bauer, 2001).

4 CONCLUSION

The study showed that there are differences in biochemical, proteolytic (TVBN, N-TMA) and lipolytic (FFA, PV, TBA) processes between freeze-thawed and fresh fish fillets. The course of the processes is mainly influenced by the species composition of contaminating microorganisms, destruction of the cell structure by ice crystals, and protein denaturation. Because lipid oxidation takes place also in frozen muscle, the course of oxidative processes after thawing is different. The intensity of lipid oxidation is mainly influenced by the chemical composition of fish meat, and especially by its fat content and its composition (spectrum of fatty acids). Fats containing more polyunsaturated fatty acids are more susceptible to oxidative processes. In view of the differences found in post mortem and degradation processes, cold storage of freeze-thawed fillets of common carp and silver carp cannot be recommended.

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6 REFERENCES

Ashton I.P. 2002. Understanding lipid oxidation in fish. In: Safety and quality issues in fish processing. Bremner H.A. (ed.). Cambridge, Woodhead Publishing Limited: 507 p.

Benjakul S., Bauer F. 2001. Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze-thaw cycles. Food Chemistry, 72: 207–217

Bøknæs N., Østerberg C., Nielsen J., Dalgaard P. 2000. Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. Lebensmittel-Wissenschaft und Technologie, 33: 244–248

Buchtová H., Ježek F. 2012. Shelf-life of thawed fillets of common carp (*Cyprinus carpio* L.) and silver carp (*Hypophthalmichthys molitrix* VAL.) (*In Czech*). In: Hygiena Alimentorum XXXIII. Safety and quality of poultry. fish and game products. Proceedings of Lectures and Posters, Štrbské Pleso – High Tatras. Slovakia, 9–11 May 2012. Košice, University of veterinary medicine and pharmacy: 296–298

Castellini C., Mugnai C., Dal Bosco A. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Science, 60: 219–225

Commission Regulation (EU) No 1276/2011 of 8 December 2011 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament an of the Council as regards the treatment to kill viable parasites in fishery products for human consumption. Official Journal of the European Union L327: 39–41

Huss H.H. 1995. Quality and quaity changes in fresh fish. FAO Fisheries Technical Paper-348. Rome, Food and Agriculture Organization of the United Nations:: 202 p.

Ježek F. 2008. Study of selected physicochemical desriptors of post-mortem processes of muscle tissue of common carp (*Cyprinus carpio.* L.) (*In Czech*). PhD thesis. Brno, VFU Brno: 111 p.

Ježek F., Buchtová H. 2011. Physicochemical changes of chilled simple and vacuum packaged muscle tissue of silver carp (*Hypophthalmichthys molitrix.* V.) (*In German*). Fleischwirtschaft, 91,1: 94–98

Karoui R., Thomas E., Dofour E. 2006. Utilisation of a rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen-thawed fish fillets. Food Research International, 39: 349–355

Orban E., Nevigato T., Di Lena G., Masci M., Casini I., Caproni R., Rampacci M. 2011. Total volatile basic nitrogen and trimethylamine nitrogen levels during ice storage of

European hake (*Merluccius merluccius*): A seasonal and size differentiation. Food Chemistry, 128: 679–682

Pipová M., Buchtová H., Cabadaj R., Gima J., Hanzel S., Iglovská N. , Kantíková M., Kohút J., Košuth P., Kozák A., Nagy J., Pliešovský J., Rajský D., Sokol J., Steinhauserová I., Večerek V. 2006. Hygiene and processing technology of freshwater and marine fish (*In Slovak*. *In Czech*). Košice, University of Veterinary Medicine and Pharmacy in Košice:: 417 p.

Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. Official Journal of the European Union L 304: 18–63

Ruiz-Capillas C., Moral A. 2001. Changes in free amino acids during chilled storage of hake (*Merluccius merluccius*. L.) in controlled atmosphere and their use as a quality control index. Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 212: 302–307