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Short communication

STRUCTURAL CHANGES IN COMMON CARP (*CYPRINUS CARPIO* L.) FISH MEAT DURING FREEZING

A. PAVLOV, D. DIMITROV, G. PENCHEV & L. GEORGIEV
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

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Histological and morphometrical investigations on dorsal skeletal muscles from fresh and frozen common carp (*Cyprinus carpio* L.) meat were carried out. The light microscopy of samples from fresh meat did not show any microstructural changes. The samples from frozen fish meat showed structural changes in meat. Muscle cells with initial processes of breakdown in the central part prevailed, whereas peripheral changes were not observed. The sarcoplasma of these cells was not with a fibrillar structure, but instead, was a detritus mass with granular composition and a markedly basophilic staining pattern. Morphometric studies of dorsal skeletal muscles showed that muscle bundles in frozen carp meat were larger compared to those of fresh meat. It is concluded that the freezing of carp meat produced various structural changes in dorsal skeletal musculature that could be used in the differentiation of fresh from frozen thawed fish.

Key words: common carp, freezing, histological changes

The freezing of fish and fish products is the best method to prolong their shelf life. Freezing is characterized with easy application and storage conditions, neither adding nor removing any ingredients from meat (Kietzmann *et al.*, 1969).

The effect of low temperatures is due to the fact that the velocity of molecular movement decreases with lowering the temperature and that, as outlined by Reid (1977), slows down all processes in the cell. Second, the formation of ice crystals in the product's tissue reduces the risk of microbial development.

Freezing alters a number of physical and biological parameters of tissues (Rehbein, 1992). Studies on meat from hot-blood animals have demonstrated the effect of freezing on tissue microstructure (Yoon, 2002). The information about the histological alterations occurring in fish meat after freezing is however scarce. There are single reports on changes in trout meat frozen for different periods of time (Foucat *et al.*, 2001). No data are available for other freshwater representatives.

Thus, the aim of the present study was to investigate the histological changes occurring in common carp (*Cyprinus carpio* L.) meat after freezing.

Material from 8 carps, divided into 2 groups: fresh (n=4) and frozen (n=4) meat, was used.

The fish were at the same age and with equal size – weight of 800 g. The fresh carps were provided living and the samples were obtained after stunning. The freezing was performed by putting the fish at a temperature of –18 °C for 14 days.

Ten biopsy specimens were obtained from the dorsal muscles of each fish. They were fixed in Carnoy's and Bouin's fixatives as well as in 10% neutral formalin. After removal of fixatives from tissues using a water bath or ascending ethanol series, the material was cleared in xylene, soaked and embedded in paraffin. Cross sections of 6 μm were stained with haematoxylin-eosin (Ehrlich) and they served for making permanent histological preparations (Pearse, 1960; Vitanov *et al.*, 1995).

Each histological cross section of all biological samples was studied with a light microscope and the microstructure of skeletal dorsal muscles was evaluated. In each specimen, 20 observation fields were studied, quantitating the total number of muscle cells in one muscle bundle. The number of muscle cells with microstructural alterations in each bundle was also determined. Using an eye-piece micrometer (Zeiss, Germany), micromorphometry of skeletal dorsal muscles was performed by the method of Avtandilov (1990). In 20 observation fields from each biological sample, the size of muscle cell bundles, the diameter of each muscle cell within a bundle and the distance between cells in a bundle, were measured. The data were statistically processed by the non-parametric Mann-Whitney test (StatMost for Windows, 1994).

The light microscopy of dorsal skeletal muscles in *fresh carp meat* did not show evidence of any microstructural changes. The connective tissue sheaths of bundles with different location, and sheaths of individual muscle cells (external perimysium, internal perimysium and endomysium) were with intact structural integrity and moderate basophility after haematoxylin/eosin staining. There were no cell elements indicating activated functional state of the sheath tissue. Within bundles, fibres with preserved structural integrity

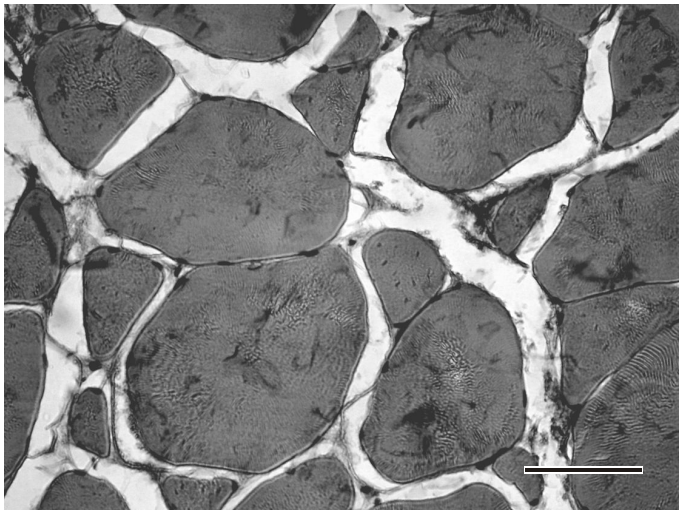

Fig. 1. Area of transversely cut muscle cells from the dorsal musculature of fresh (not frozen) carp meat with relatively intact microstructure. Haematoxylin/eosin staining; bar=5 μm.

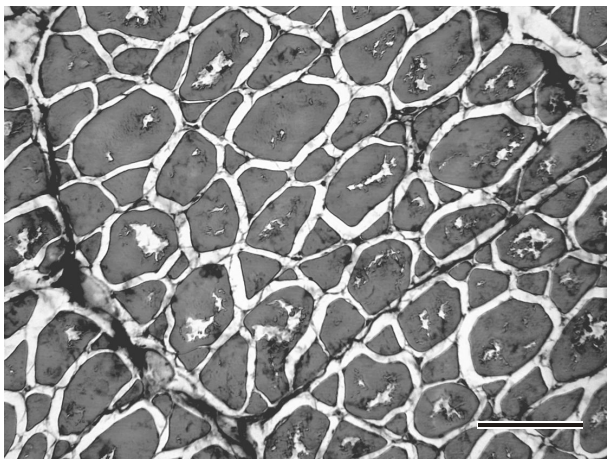

Fig. 2. Area of muscle bundle of fresh carp meat with partial and minor microstructural alterations. Haematoxylin/eosin staining; bar=10 μm.

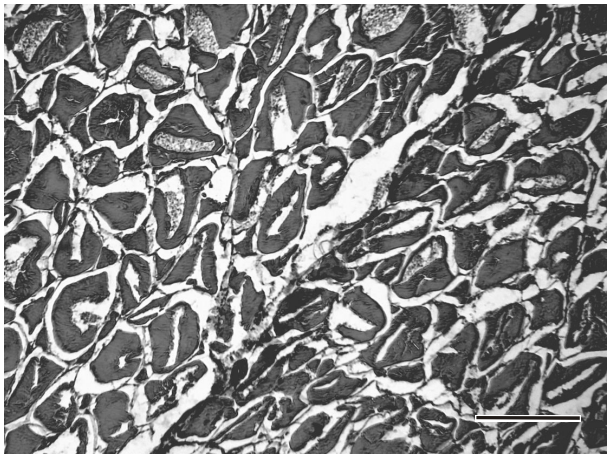

Fig. 3. Musculature of frozen carp meat. Most muscle cells are with central destructive changes while the microstructure of the periphery is preserved. Haematoxylin/eosin staining; bar=10 μm.

and normal staining pattern prevailed (Fig. 1). The enumeration showed that on the average only 10.66±1.63 muscle fibres in a bundle containing 67.36±10.88 fibres demonstrated some structural deviations (Fig. 2). By means of light microscopy it was shown that the structural changes were minor and could be interpreted as artifacts from the histological processing of specimens.

The analysis of histological cross sections obtained from dorsal muscles of *frozen carps*, revealed structural changes. The connective tissue sheaths of bundles exhibited no structural changes that ensured their integrity and the shape of the

cut surface. Only in single muscle cell bundles with most prominent structural changes, there was various degree of destruction of connective tissue sheaths. In a bundle containing 82.20±12.00 muscle cells on the average, 64.80±9.60 cells were with some extent of structural alterations ($P<0.01$ vs the respective number in fresh carp meat). In histological cross sections, muscle cells with initial breakdown processes in the central part and structurally intact periphery prevailed (Fig. 3). Cells with centrally situated foci of early destruction were observed in all studied muscle bundles. In terms of prevalence, then followed cells with breakdown changes on both transverse and longitudinal cross sections. The boundaries and the shape of these cells were well visible and easy to distinguish because of the well preserved connective tissue sheath – the endomysium. Their sarcoplasma had no fibrillar structure, but instead, represented a detritus mass with granular composition and a marked basophilic staining pattern (Fig. 4). In some observation fields, these altered cells occupied up to two thirds of muscle bundles. Areas, with indistinct borders among cells, looking like a common granular basophilically stained detritus mass, were relatively rarely found out.

These results of ours are similar to those reported by Lavety (1991) that freezing of some sea fish species resulted in destruction of connective tissue cohesion, formation of gaps and texture coarseness due to denaturation of myofibril proteins. Similar facts are communicated by Foucat *et al.* (2001) from their studies on fresh and frozen trout meat samples.

The morphometric investigations of dorsal skeletal muscles showed that in frozen carp meat, muscle bundles were larger by 94.79 μm on the average than those in fresh carp meat. The distance between cells within a muscle bundle was by 2.13 μm larger in findings with transversely cut muscle cells in frozen samples and by 8.88 μm in longitudinally cut frozen muscle cells (Table 1). The diameter

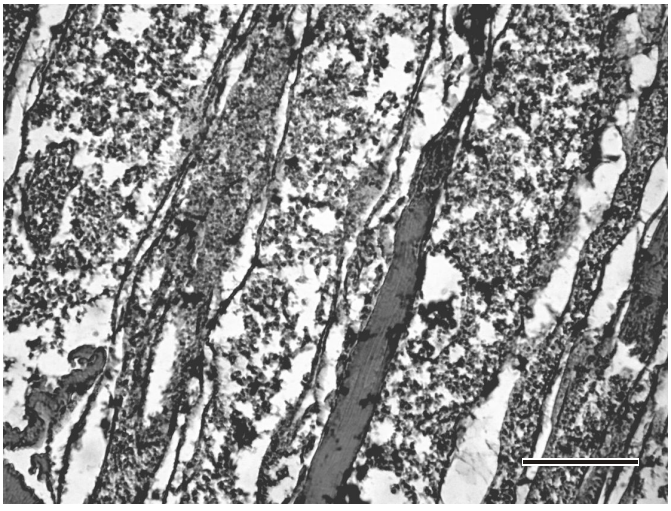

Fig. 4. Musculature of frozen carp meat – area of longitudinally cut muscle fibres whose central part is filled with granular basophilic detritus mass, but the borders of fibres are distinct. Haematoxylin/eosin staining; bar=5 μm.

Table 1. Some morphometric parameters of dorsal muscle from fresh and frozen common carp meat. Values are presented as mean ±SD, n=10

Parameter	Fresh meat	Frozen meat
Size of muscle cell bundle, μm	384.82±24.11	479.61±24.39*
Diameter of a muscle cell, μm		
transverse section	58.58±4.15	60.35±3.36
longitudinal section	43.67±2.14	40.47±3.18
Distance between cells in a muscle bundle, μm		
transverse section	13.14±1.07	15.27±3.36
longitudinal section	12.78±1.42	21.66±3.55
Total number of muscle cells in one bundle	67.36±10.88	82.20±12.00
Number of muscle cells with altered microstructure in one bundle	10.66±1.63	64.80±9.60 **

* $P<0.05$; ** $P< 0.01$ between fresh and frozen meats by the Mann-Whitney test

of muscle cells on transverse sections was almost equal in fresh and frozen carp meat samples. In longitudinal sections however, it was by 3.20 μm longer in fresh meat cells. The statistical analysis of data showed significant changes between fresh and frozen meat with respect to the size of muscle bundle ($P<0.05$).

The results of the present investigations allowed concluding that freezing of carp meat caused structural alterations in dorsal muscles of a various extent. The most extensive microstructural changes possibly resulted in changed ratio of structural elements of musculature whereas the occurring lytic processes led to altered biochemical composition of myofibrils. These changes could be taken into consideration in differentiating fresh from thawed-frozen fish meat.

REFERENCES

Avtandilov, G., 1990. Meditsinskaya Morfometriya [Medical Morphometry], Meditsina, Moskva, pp. 67–73; 191–211 (RU).

Foucat, L., R. Taylor, R. Labas & J. Renou, 2001. Characterization of frozen fish by NMR imaging and histology. *American Laboratory*, **33**, No 16, 38–43.

Kietzmann, U., K. Priebe, D. Rakou & K. Reichstein, 1969. Seefisch als Lebensmittel. Berlin, p. 368.

Lavety, J., 1991. Physico-chemical problems associated with fish freezing. In: *Food Freezing: Today and Tomorrow,* ed. W. B. Bald, Springer-Verlag, London, pp. 123–132.

Pearse, A. G., 1960. Histochemistry Theoretical and Applied, J. & A. Churchill Ltd., London, pp. 53–75.

Rehbein, H., 1992. Physical and biochemical methods for the differentiation between fresh and frozen thawed fish or fillets. *Italian Journal of Food Science*, **2**, 75–86.

Reid, D. S., 1993. Basic physical phenomena in the freezing and thawing of plant and animal tissue. In: *Frozen Food Technology*, ed. C. P. Mallett, Blackie Academic & Professional, Glasgow, pp. 1–19.

Vitanov, S., D. Dimitrov & A. Bochukov, 1995. Rakovodstvo po tsitologiya i histologiya s histologichna tehnika [Manual of Cytology and Histology with Histolo-

gial Techniques], Zemizdat, Sofia, pp. 3–12; 195 (BG).

Yoon, K. S., 2002. Texture and microstructure properties of frozen chicken breasts with salt and phosphate solutions. *Poultry Science*, **81**, 1910–1915.

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Correspondence:

Assoc. Prof. A. Pavlov, DSc
Faculty of Veterinary Medicine,
Trakia University,
Students' Campus
6000 Stara Zagora, Bulgaria