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STRUCTURAL BINDING PROPERTIES OF SILVERCARP (HYPOPTALAMICTHYS MOLITRIX) MUSCLE AFFECTED BY NaCl AND CaCl₂ TREATMENTS

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Abstract

The texture of restructured muscle products is well known to be strongly affected by various salts. In the present work the effects of NaCl and CaCl₂ on the ultrastructure of both fresh and cooking salted silvercarp (Hyophtalmichthys molitrix) muscle were examined, in order to elucidate the heat-initiated binding phenomenon. Sodium chloride at 0.3 and 1.5% caused swelling and fusion of the myofibrils and loss of arrayed structure. At 12% NaCl there was a compaction of myofibrillar structure. Calcium chloride at all the tested concentrations resulted in shrinkage of myofibrils. The present study demonstrated two main effects of salts on the ultrastructure of fish muscles: (i) swelling of myofibrils which, at relatively high NaCl concentrations (1.5%) resulted in conversion of the arrayed structure of the myofibriles into a homogeneous and amorphous mass, and (ii) shrinkage of myofibrils, which may result in compact and denser appearance of filaments within myofibrils and their shortening. The textural changes which resulted from the different salt treatments are explained by these findings.

Introduction

Sodium chloride and phosphates are commonly used in muscle food systems in order to reduce drip and to improve functional properties such as binding and emulsification.

Hamm (1960) stated that the effect of salts on muscle tissue may be understood by considering both the cations and the anions. Salts such as NaCl, the ions of which contribute electrical charges to the peptide chains, result in repulsion or attraction between the protein molecules and this brings about increased or decreased hydration. Divalent ions such as Ca²⁺ and Mg²⁺ are believed to cross-link protein chains and this narrows the intermolecular spaces available for water and results in decreased hydration. At high concentrations there is a decrease in water holding capacity for most salts, which is explained by cleavage of hydrogen bonds and excessive folding of the peptide chains ("salting out"). Various ions have been arranged in the so-called ''Hofmeister" series which ranks them according to their effectiveness in salting out proteins. With this regard, increased conformational stability was associated with loss of solubility (Haschemeyer and Haschemeyer, 1973). Weinberg et al. (1984a) found a high correlation between the expressible moisture in cold muscle as affected by various salts and the texture of loaves that were prepared from ground cod. In that study 0.25M NaCl resulted in a cohesive and elastic texture in the cooked loaves; 2M NaCl (12%) resulted in compact and grainy texture; CaCl₂ at ionic strength range between 0.085 and 0.5M resulted in soft and crumbling loaves which expressed a great volume of moisture upon cooking. Similar results were also obtained with silvercarp (Weinberg and Angel, 1984; 1985).

The formation of a cohesive and elastic muscle matrix from small pieces is achieved by mixing small muscle cubes with NaCl and phosphates. A tacky and swollen batter is then obtained, and when heat is applied the proteins coagulate and binding between the separate muscle pieces occurs. This phenomenon is referred to as 'heat initiated binding' (Vadehra and Baker, 1970). The mechanism through which the heat initiated binding is achieved is believed to be
due to the solubilization and extraction of the myofibrillar proteins by the salts. These proteins then serve as binders between the muscle particles (Vadehra et al., 1981; Macfarlane et al., 1977; Siegel et al., 1978).

Offer and Trinick (1983) used light microscopy as well as transmission electron microscopy (TEM) to study the swelling behavior of isolated myofibrils from psoas rabbit muscle as affected by various NaCl and pyrophosphate solutions. They related the swelling of the myofibrils to the water retention properties of the muscle, and proposed a mechanism by which NaCl and phosphate resulted in swelling by adding electrical charges (Hemm, 1960), and by detachment of transverse cross bridges from the actin filaments. When Voyle et al. (1984) studied the effect of salt and phosphate bringing on the ultrastructure of pig muscle by TEM, they found swelling, extraction of A-band proteins and Z-line break-up; these results depended upon muscle age post mortem, pH, and duration that muscle samples were soaked in salt and phosphate solutions. In addition, loss of structure was attributed to either the extraction or detachment of thin or thick filaments from the myofibrils. In Leander et al. (1980) a study of structural changes in heated bovine muscle by SEM and TEM showed that heating muscle samples to temperatures between 63°C and 70°C caused sarcosome contraction and loss of the I-band. Some Z-line breakage was also noticed.

In the present work an attempt was made to shed light on the understanding of the heat initiated binding phenomenon by studying the ultrastructure of fresh and heated silver carp muscle tissue treated with NaCl and CaCl₂.

**Materials and Methods**

**Preparation of the fish muscle**

Fresh silver carp (Bryophthalmichthys molitrix) was purchased from a local dealer, filleted and ground through a 5mm end plate. Seventy five g of chilled (2°C) ground fish was mixed thoroughly by hand for 3 min, with or without various salts. The treatments were: control (no salt added), 0.3% NaCl (W/W), 1.5% NaCl, 12% NaCl, 0.55% CaCl₂, 1.25% CaCl₂, and 2.5% CaCl₂. Care was taken to avoid the formation of air pockets. After a homogeneous mass was achieved, a mixed sample from each treatment was taken for ultrastructural examination. The rest of the fish was stuffed into standard 100 ml Pyrex beakers covered with aluminum foil punctured with several small holes, and placed in an oven at 170°C for 20 min, to reach an internal temperature of 75°C. After these samples were cooled to room temperature, portions from the internal zone were also taken for ultrastructural examination.

**Electron microscopy**

Muscle samples were fixed for 2 h in 3.5% glutaraldehyde in 0.1M sodium cacodylate, pH 7.0, cooled within a water-ice bath, and postfixed for 2h in 2% OsO₄ in 0.1M phosphate buffer. The fixed samples were dehydrated with increased concentrations (20, 40, 60 and 100%) of ethanol, each step lasting ca. 30 min, and this was followed by several rinsings with absolute acetone, for 1 h each. Then, the samples were embedded within Araldite (Agar Aid) at room temperature, and left overnight at 70°C for polymerization. Ultrathin sections were prepared with an LKB ultramicrotome with a diamond knife of Diatom, Switzerland, stained with uranyl acetate and lead citrate, and examined with a TEM (JEOL 100CX II-2000) at 80 kV.

**Results and Discussion**

Sodium and calcium chloride at the various concentrations were chosen for the present ultrastructural study because of two main reasons: 1. these salts have been found to exert very different textural and water holding effects on fish muscle tissue (Weinberg et al., 1984a; Weinberg and Angel, 1984, 1985). 2. differential scanning calorimetry studies on the effects of various salts on cod muscle revealed very different thermal transition curves of NaCl and CaCl₂, treatments (Weinberg et al., 1984b) which probably indicated different effects on the conformation of the proteins.

Both textural and thermodynamical analyses might indicate possible ultrastructural differences which may explain the physical properties of the treated muscle.

The myofibrillar arrangement of the silver carp is typical for muscle and characterized by distinct sarcomeres (Figs. 1a, 1b). In Fig. 1c the array structure is evident (Fig. 1c), as are the details at higher magnification (Fig. 1d). Heat treatment resulted in shrinkage of the sarcomere length accompanied by disintegration of both the I-band (including the Z-line) and the H-zone, as indicated by areas of very low densities (Fig. 2a). Higher magnification reveals residues of filamentous elements in the I and H zones which might have served as binding structures between adjacent sarcomeric elements.

Based on the present study it seems that the disintegration of the myofibrils into separate sarcomeric elements might explain the softening of the fish muscle obtained by cooking. It also appears that the heat treatment resulted in disappearance and fusion of the thick and thin filaments of the myofibrils (Figs. 1b, 1d) and converted them into a homogeneous matrix of amorphous pattern (Fig. 2b).

Johnson et al. (1981) discussed the role of textural elements in a rheological model of fish flesh. They envisioned fracture of textural elements upon heating, and the above mentioned

**Figures 1a, 1b. Longitudinal ultrasection of fresh silver carp muscle. The typical sarcomeric zones (A, H, I, M, Z) are indicated. 1a - bar = 800 nm; 1b - bar = 30 nm.**

**Figures 1c, 1d. Cross ultrasections of the muscle described in Fig. 1a. 1c - bar = 230 nm; 1d - bar = 30 nm.**

**Figures 2a, 2b. Longitudinal ultrasection of heat treated muscle. Remnants of the typical sarcomeric zones (H, I, Z) are indicated. 2a - bar = 700 nm; 2b - bar = 35 nm.**
disappearance of filaments from the myofibrils could support such ideas.

Myofibrillar proteins can be solubilized and extracted from muscle tissue at relatively high NaCl concentrations (0.25 M and above). However, in the present study NaCl concentrations as low as 0.3% resulted in swelling of the I-band (Fig. 3a). This significant change is illustrated by comparing Figures 1b and 3b. It is interesting to reveal that with 0.3% NaCl the A-band is composed of several bands of various densities (Fig. 3a). Figure 3c demonstrates a high magnification of the area around the H-zone, Fig. 3d shows the muscle array in cross section in which several densities may be observed. Higher magnification of the myofibrillar cross-section shows diverse densities within the myofibrils (Fig. 3e). Figures 4a, 4b show a typical pattern of the 0.3% NaCl treatment upon heating. The sarcomeric arrangement is still retained, however there is some fusion of adjacent sarcomeric elements which is apparent (Fig. 4b).

At 1.5% NaCl treatment both the myofibrillar and sarcomeric arrangement disappear in the fresh muscle (Fig. 5a), and a filamentous pattern was not observed (Fig. 5b). However, remnants of intermyofibrillar regions can be identified in the homogeneous mass of the both fresh and heat treated samples (Figs. 5a, 5b, 6). It appears that this ultrastructure is a result of marked swelling of the myofibrils upon salting. Swelling of the myofibrils resulted in the loss of the array structure, fusion of the myofibrillar proteins into an amorphous mass, and might explain the resulting tacky muscle batter which leads to a cohesive and elastic texture upon heating (Valdehra and Baker, 1970; Weinberg and Angel, 1984).

Upon heating, the 1.5% NaCl-treated samples exhibited two main patterns: 1. homogeneous amorphous pattern, and 2. residues of sarcomeric elements (Fig. 6). It is possible that where the salt has completely reacted with the muscle proteins a complete homogeneous mass was obtained. When the mixing with salt was incomplete some residual sarcomeric elements can still be identified with the disintegrated I-bands (Fig. 6). Voyle et al. (1984) suggested an accumulation of protein globules on the muscle fibers membranes of both control and salted samples. However, in the present study no such material was identified in the homogeneous amorphous mass. Fig. 7a shows the pattern of the 12% NaCl treated samples in which the borders between adjacent myofibrils may be identified. However, higher magnification of these samples (Fig. 7b) reveals a filamentous pattern (which was undetectable at 1.5% NaCl, as seen in Fig. 5b) in the Z-zones. The pattern of the filamentous material is very dense and compact. Indeed the texture of the fish loaves prepared with 12% NaCl was very compact, grainy and inelastic (Weinberg and Angel, 1984).

Calcium treatment of 0.55% resulted in some initial shrinkage of the I-band and H-zones (Fig. 8). The I-bands were more susceptible to shrinkage and this formed a segmental structure of the myofibrils. The heat treated samples of 0.55% CaCl2 showed a narrow pattern of the myofibrils, with distinct z-lines and "empty" I-bands. In addition, the H-zones shrunk considerably (Fig. 9). This might explain the large amount of drip that resulted from the calcium addition. At higher calcium concentrations the sarcomeric pattern of the shrunken myofibrils is almost lost (Fig. 10a). At higher magnification the various sarcomeric zones can still be identified (Fig. 10b). A significant shrinkage is observed with the heat treated samples in which the sarcomeric pattern is lost (Fig. 11). At 2.5% CaCl2, the myofibrillar structure is distorted (Fig. 12).

**Summary and Conclusions**

The present study demonstrated two main structural effects of salts on the ultrastructure of fish muscles: 1. swelling of the myofibrils at 0.3% NaCl treatment which increased at 1.5% NaCl treatment resulting in fusion and conversion of the myofibrillar arrayed structure into an amorphous pattern. 2. shrinkage of the myofibrils which occurred with the CaCl2 treatment (at all the treated concentrations) which resulted in a compact and denser appearance of the filaments within the myofibrils and their shortening. Particularly dense structure have been observed at 12% NaCl.

Based on the present study, it is suggested that the first effect is a result of conformational instability of proteins which leads to fusion of the myofibrils and to a loss of their arrayed structure. Such fusion might be related to the binding and cohesive properties of these proteins. On the other hand, the effect of shrinkage is a result of cross linking of the relatively open and solubilized protein structure which leads to deformation of the myofibrils which are protected from denaturation. It is interesting to note that CaCl2 affects only shrinkage, while NaCl brings about both swelling and fusion at relatively low concentrations and compactness and shrinkage at high concentrations.

**Acknowledgment**

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References


Discussion with Reviewers

G.R. Schmidt: In figures 1c and 1d, what causes the unusual banding patterns in these cross-sections?

Authors: These patterns are dependent on the sectioning plane in relation to the longitudinal direction of the myofibrils. An explanation for this is found in a diagrammatic illustration in "The Science of Meat and Meat Products" (1970), pp. 35 (by Price J.F. and Schweigert, B.S., FNF, Westport, CT.)

G.R. Schmidt: Can the authors explain how the striated banding pattern shown in figure 7b "returned"? Could this be the result of a non-homogeneous distribution of salt in the mixed fish muscle? Was there any attempt to check for homogeneous salt distribution? Would it have been better to hold the raw mixture for 24 h equilibration period prior to sampling and cooking?

Authors: The various treatments with dry salts exerted immediate and obvious effects on the texture of the fish muscle after mixing. The purpose of the present study was to follow the structural properties of these changes. The word "return" used in the original manuscript to describe the effect of 12% NaCl is not quite adequate. It was actually related to compare the 12% NaCl and the 1.5% NaCl treatments. Anyhow the striated pattern was obtained upon treatment with 12% NaCl. We did not follow here changes that might occur upon gradual increase of the salt concentration in the same sample.

G.R. Schmidt: Why is no data presented on drip since reference to this is made in the text?

Authors: Visual impression of the drip volumes was very obvious; we did not record the amount of drip.

Reviewer II: Overall, the manuscript does not elaborate "binding" property, instead, it describes some simple salting-in and salting-out phenomena in the presence of various levels of salt.

Authors: The description of heat initiated binding is given in the Introduction, and it explains how the present study is related to this phenomena. Indeed it is related to salting in, salting-cut and water retention properties of the muscle proteins, as emphasized in the Introduction.

Reviewer II: Why did the authors use dry salt instead of brine? For uniform distribution of as low as 0.3% salt, a brine would seem to be more reasonable.
Authors: We used dry salts and not brine for the following reasons: 1) Dry salts exerted immediate and obvious effects on the fish muscle. 2) Dry salts are used in the industry to prepare ham and loaf type products. 3) Additional water of the brine might have changed the structure as a result of dilution effect, or washing out of various components.

Reviewer II: A 12% salt is not practical for a restructured muscle food; the phenomenon described is nothing but a dehydration (cosmetic drying) rather than a protein solubilization.

Authors: Previous studies examined the effect of a 12% NaCl and found it to strongly affect the texture (Weinberg et al., 1984a; Weinberg and Angel, 1984). Therefore we examined in the present study the ultrastructure of the muscle tissue also under such extreme conditions. The purpose of this study was to follow the ultrastructure of fish muscle with various textural properties, rather than for practical implications. As mentioned in the introduction, textural properties of restructured muscle products is highly correlated with water holding properties (Weinberg et al., 1984a). This phenomenon involves also protein solubilization (Vadera and Baker, 1970; Macfarlane et al., 1977; Siegel et al., 1970). However, it could well be that a 12% NaCl treatment involves also dehydration besides conformational changes in the proteins.

D.F. Lewis: Swelling without dispersion is unlikely to cause fusion - indeed, swelling would be expected to move structures further apart. According to the present study, the amorphous ultrastructure is a result of fusion.

Authors: The same salt treatment resulted in different textures in a cooked product prepared from different fish and avian muscles. The fish product was significantly more tender than the avian products. Biochemical tests also revealed differences between the fish and the avian salt-soluble proteins (Weinberg and Angel, submitted). The comparison of different muscles treated with salts (including mammalian muscles) should be made now under the microscope as well.

D.F. Lewis: Why did the authors use minced fish which adds the difficulties of the interpretation?

Authors: Mincing through 5 mm grinder does not affect the sarcomeric ultrastructure as obvious from the ultrastructure of the unsalted muscle.

Figure 8. Longitudinal ultrasection of 0.55% NaCl treated muscle. Shrunken regions (A, I) are marked. Bar=800 nm.

Figure 9. Longitudinal ultrasection of the heat treated muscle described in Fig. 8. Remnants of sarcomeric regions (H, I) are clearly identifiable. Bar=800 nm.

Figures 10a, 10b. Longitudinal ultrasection of 1.25% NaCl, treated muscle. Shrunken sarcomeric zones H and I (A, I) can still be identified. 10a - bar=800 nm; 10b - bar=230 nm.

Figure 11. Ultrathin section of heat-treated muscle described in Fig 10a. Remnants of the various sarcomeric zones can be identified (were not marked). Bar=800 nm.

Figure 12. Ultrathin section of 2.5% NaCl treated muscle. Sarcomeric patterns are distorted, but the filamentous structure is observed. Zone A and the inter myofibrillar region (IMR) can be identified. Bar=60 nm.