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CHANGES IN THE MICROSTRUCTURE OF SKIPJACK TUNA DURING FROZEN STORAGE AND HEAT TREATMENT

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Abstract

Samples of fresh, frozen and heat-treated skipjack tuna muscle were observed by scanning electron microscopy. The photomicrographs were used to assess changes in the microstructure of fish muscle during frozen storage and thermal processing. Differences noted in frozen tissue could be related to the formation of gaps between fibers and the deformation of muscle fibers. No freeze mediated damage to the cell wall was observed at the lower limits, 20,000x, of high resolution scanning electron microscopy. The degree of muscle fiber shrinkage and erosion and the behavior of certain protein fractions was found to be affected by thermal processing. Granular material presumed to be primarily sarcoplasmic proteins aggregated at the surface of the muscle fiber and in the interstices between the sarcolemma and the muscle fiber. The sarcomere was disrupted upon precooking. The I-band and the A-band have congealed to form a single band which constitutes the majority of the sarcomere.

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Introduction

Post-mortem fish muscle is susceptible to many physicochemical changes. Freezing and frozen storage of such tissue can have more profound effects. These changes can be broadly classified in the areas of texture, protein functionality or organoleptic quality. A slow rate of freezing and higher storage temperatures (-5 to -10°C) can distort cells and crush myofibrils (Bello et al., 1982). Freezing can cause deformation of the sarcoplasmic reticulum (Jarenback and Liljemark, 1975a,b,c; Liljemark, 1969). Further, to a limited extent, large ice crystals can rupture cell membranes thus initiating the outward movement of cellular constituents (Giddings and Hill, 1979; Hamm, 1960; Deatherage and Hamm, 1960). The investigation of structural damage has been accomplished more often with transmission than with scanning electron microscopy.

Scanning electron micrographs of rainbow trout muscle held under either chilled storage (Schaller and Powrie, 1971) or given thermal treatments (Schaller and Powrie, 1972) have been studied. Under refrigeration temperatures (3°C), for 6 days, the trout muscle showed a shortening of the transverse elements and a perforation of the sarcolemma (Schaller and Powrie, 1971). The majority of published work relating scanning electron microscopy (SEM) to muscle has generally involved heated beef muscle.

Hoestetler and Landmann (1968) studied photomicrographs of beef longissimus dorsi during heating of muscle fiber fragments from 29 to 80°C. The results indicated that there was a decrease in muscle fiber width (23 to 27%) at temperatures from 53 to 77°C and that this change occurred within five minutes. Further, it was found that during the denaturation of myofilaments, soluble sarcoplasmic proteins were visible as amorphous particles around the muscle fiber. With this, the fiber became narrower, or shrank. The process was thought to be complete when the temperature reached 53°C. Schaller and Powrie (1972) observed a shrinkage of the muscle fiber and an accumulation of granular material between the muscle fiber and the sarcolemma in beef and chicken muscle heated to 97°C. This occurrence was not detected in trout dorsi heated to the

same end point. Granulation of the sarcolemma of bovine semitendinosus was found to occur at 60°C and edomysial collagen became nonfibrous at 60°C (Jones et al., 1977). Perimysial collagen was found to degrade at 70°C (Cheng and Parrish, 1976). Fish collagen deteriorates at lower heating temperatures.

In trout muscle heated to 97°C, a gap appeared at the H-zone and breaks across the fibers occurred at the Z-disc (Schaller and Powrie, 1972). Heating beef muscle to greater than 60°C also showed breaks to occur at the Z-disc and a wider or separated I-band region resulting (Jones et al., 1977; Cheng and Parrish, 1976).

The objective of this work was to assess changes in the microstructure of skipjack tuna during frozen storage and thermal processing. Attention was given more to the phenomena rather than to the biochemical mechanisms involved. The post-mortem biochemical changes and canning yields as affected by freezing and frozen storage are the subject of a separate paper (Lampila et al., manuscript submitted).

Materials and Methods

Brine-frozen raw, precooked and canned chunk style skipjack tuna (Euthynnus pelamis) was obtained from a commercial tuna processor. Tuna was received over a period of eighteen months from approximately fifteen different ships. Two to three skipjack tuna were evaluated from each ship. It was reported that the tuna were frozen according to commercially acceptable practices. At the time of microscopic examination, tuna samples had been frozen for ca. three months. Commercially frozen tuna (frozen at sea) was compared with fresh skipjack tuna obtained from Southern California and Hawaii.

A 2.5 cm thick steak was removed from behind the dorsal fin with the aid of a band saw. From this section a 2 cm^2 specimen was removed from the upper left quadrant of white loin with a clean, sharp scalpel. The cube of meat was placed into a clean petri dish and was sliced into 3 x 5 mm sections with an oil-free, sharp razor blade. Specimen sections were immediately immersed into fixative. The tissue, raw and precooked, in a frozen state was easier to slice into the appropriate size sections for fixation. Similar sampling procedures were followed for precooked and canned tuna.

Determination of Collagen

Samples of raw tuna were immersed into either a phosphate buffer alone or phosphate buffer plus collagenase (Sigma, Type VII). Specimens were incubated at 37°C for 4 h, using procedures as described by Eino and Stanley (1973).

Sarcoplasmic Proteins

Sarcoplasmic proteins were isolated as described by Hashimoto et al. (1979). These proteins were resolubilized and subsequently heat denatured by immersing specimens into a water bath at 100°C for 5 min.

Fiber Diameter

The diameters of muscle bundles were measured from photomicrographs. The mean of between sixty and ninety fiber bundles was calculated. Measurements were determined from the center one-third of photomicrographs taken at low magnification, with minimal tilt (less than 4°). Data were analyzed using the Analysis of Variance system of Madigan and Lawrence (1982) and Duncan's Multiple Range test described by O'Mahony (1986). Formulas for unequal sample sizes were used.

Specimen Preparation

Specimens were fixed by a modification of the methods described by Sabatini et al. (1963). Fixation was conducted in a 0.1 M cacodylate buffer plus 2% glutaraldehyde (pH 5.8) for 16 h at 22°C. After washing in 0.1 M cacodylate buffer plus 5% sucrose, the samples were cryofractured. This step was followed by serial dehydration in ethanol and substitution with amyl acetate. Specimens were dried to critical point, mounted onto aluminum stubs and pulse-sputter-coated with gold. Specimens were viewed with either the Philips PSEM Model 501 equipped with a tungsten filament or the ISI DS 130 High Resolution Research Microscope equipped with a LaB_6 (lanthanum hexaboride) cathode.

Results and Discussion

Frozen Storage

As a rule, tuna destined for commercial canneries are frozen on board ship in the round in a concentrated (23%) NaCl brine to -10 to -12°C and may be held as such for as long as three months before further processing. In order to conserve energy and maximize brine freezing efficiency, the catch may be held in refrigerated sea water until the vessel's holding wells are filled. The actual on board freezing rate depends on the following factors: temperature of the fish and of the brine; the quantity of fish packed into a well; the circulation of the brine; and on the refrigeration systems and their efficient use. Both freezing time and the storage temperature affect the rate of growth and the final size of ice crystals. According to Stansby (1956) and Dyer (1971) the critical freezing range in which most of the water is frozen out is between -1 to -5°C. For an allied study in this laboratory, fresh skipjack tuna (1.5 to 2.5 kg) were frozen in a concentrated (23%) NaCl brine to simulate on board handling. The freezing rate was monitored and is presented in Figure 1 (Lampila et al., manuscript submitted). The length of time for the backbone temperature to decrease to -5°C and then to -12°C was 36 and 84 hrs, respectively (Figure 1). This is a relatively slow rate of freezing. Evidence of and conclusions regarding the effects of freezing rate and ice crystal damage on muscle structure are controversial.

Fresh, never frozen, tuna muscle fibers appear to be uniform in shape and fully contracted (Figure 2). Individual myofibrils and their transverse striations which delineate sarcomeres are well detailed. The sarcomeres measure ca. 2.2 µm in length. In contrast, after freezing to ca. - 12°C, some deformation of tuna muscle fibers can be noted, a consequence presumably of ice crystal formation during freezing (Figure 3). Large spaces were noted between these muscle

fibers, probably the site of ice crystal deposition. These findings are supported by published transmission electron micrographs of Bello et al. (1982); Jarenback and Liljemark (1975a,b,c) and Liljemark (1969). More recent scanning electron micrographs of rockfish isothermally freeze-fixed at either -5° or -20°C present additional confirmatory evidence of the distortion of the fascicular grouping and a separation of the basal lamina from the muscle cell during freezing and frozen storage (Lampila et al., 1985). The deformation of the skipjack tuna tissue was however, not consistent. It is presumed that migration of sarcoplasmic fluid back into the cell upon thawing resulted in some cells returning to their native conformation. The consistency with which a uniform cellular shape is restored may be attributed to the original handling of the tuna; as practices of engineers on different ships may vary more than is reported.

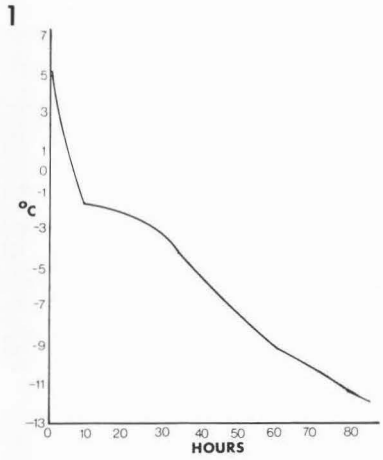
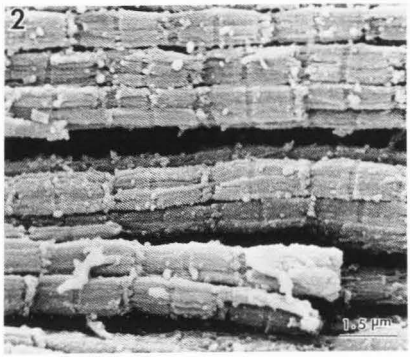


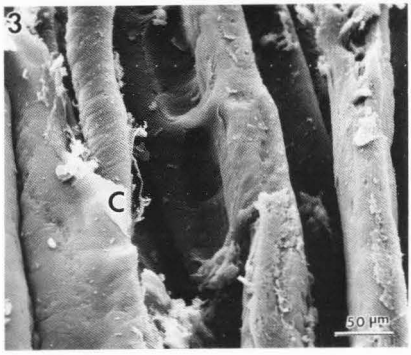
Figure 1. This represents the freezing rate of tuna in a concentrated (23%) NaCl brine.

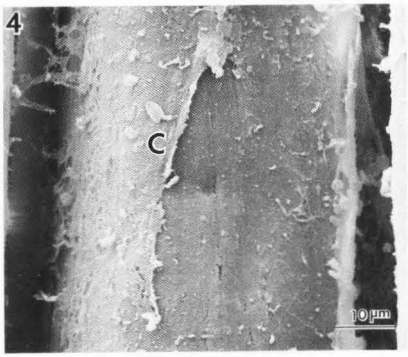
Figure 2. Longitudinal view of fresh, never frozen tuna.

Figure 3. The structural fibers of raw, previously frozen tuna muscle show deformation, a consequence presumed to be caused by ice crystal damage. Connective tissue (C) is indicated.

Figure 4. The connective tissue (C) or sarcolemma is shown surrounding a single muscle fiber from tuna frozen aboard ship.







The connective tissue surrounding a single tuna muscle fiber is shown in Figure 4. Presence of collagen was verified by treating tuna muscle samples with collagenase and establishing the disappearance of the tissues in question (Figure 5). At low magnification, the transverse striations characteristic of muscle tissue were evident. No ice crystal damage was evident to the structure below the surface of the cell membrane. The tissue was however fixed after being thawed, and, as previously suggested, the cells may have returned either to or very close to their native conformation.

Use of the SEM is limited to surface examination; therefore the condition of the material at the time of fixation is important. Due to the migration of fluids in and out of the cell during thawing, changes mediated by freezing and frozen storage may not be accurately preserved when the tissue is chemically fixed. As a consequence, some structural changes, such as a weakened sarcolemma, may not be detected. Further, the pliable nature of collagen may provide the extensibility needed for ice crystal growth, without showing direct evidence of cellular rupture.

Thermal processing

In the United States, tuna destined for human consumption is most commonly canned. Before canning, tuna is normally precooked to facilitate removal of the skin, bones and red meat. The precooking of tuna involves heating to a backbone temperature of about 60°C. In the standard, retail size (No. 307x113) can, tuna is retorted at temperatures exceeding 111°C for periods of two to two and one-half hours.

Thermally processed (precooked and canned) tuna muscle underwent some expected changes in the structure. A granulation of the sarcolemma (Figure 6) proposed to be, on beef muscle proteins, a coagulation of collagenous and sarcoplasmic proteins (Cheng and Parrish, 1976; Schaller and Powrie, 1972), appears to have resulted from thermal processing as it was not detected on chemically fixed fresh or frozen tissue. Rupture of cellular membranes, or a weakening of the same, may have facilitated the outward movement of intracellular constituents and enhanced, to some extent, the degree of granulation noted. In cross-section, sarcoplasmic proteins, probable collagenous materials and other constituents were present in the interstices between the shrunken muscle fiber and the endomysium (Figure 7). This material also appears to be granular in nature. Schaller and Powrie (1972) isolated this granular material by centrifuging isolated sarcoplasmic proteins and observing the same under the SEM. In this laboratory, sarcoplasmic proteins were isolated from raw tuna muscle by methods described by Hashimoto et al. (1979). The sarcoplasmic isolate was heat coagulated, fixed and observed under the SEM. The results concurred with those of Schaller and Powrie (1972) that the granular material formed between fibers during cooking could be, in part, precipitated sarcoplasmic proteins from fluids that collect at the interface of the sarcolemma during fiber shrinkage. Machlik and Draudt (1963) noted that beef collagen shrinkage is essentially complete between 57 to 59°C. Transformation of beef collagen into gelatin begins around 63°C (Hamm, 1966). The conversion of beef collagen into gelatin occurs at a temperature higher than for fish. It is therefore likely that the granular material observed may be a combination of heat coagulated sarcoplasmic proteins and gelatin.

After tuna was precooked and canned, there was noticeable erosion of the muscle fiber (Figure 8). Erosion can be defined as the rupture of the cell wall and a discharge of the heat coagulated sarcoplasmic constituents, resulting in more granular material in the interstices between muscle bundles. Doty and Pierce (1961) found that erosion of the muscle fibers increased with the duration of heat treatment. Further, Hearne et al. (1978) studied the effects of the rate of heating of bovine semitendinosus to 40, 50, 60 and 70°C. The results indicated that rapid heating caused an acceleration and increase of myofibrillar fragmentation. The precooked and canned tuna specimens studied here however evidenced minimal apparent fragmentation when rapidly heated to ca. 60°C (precooking) and ca. 111°C (canning), respectively; the fibers appeared to be congealed and homogenous.

Muscle Structure

The size of the sarcomere (1.5 μm) indicates that the sarcomere is fully contracted. SEM of cryofractured myofibrils revealed the presence of Z-lines, A-bands and H-zones (Figure 9). There was disruption of the sarcomere after precooking and canning. The I-band and A-band have congealed into a solid block of actomyosin which constitutes the majority of the sarcomere (Figure 10). These findings were in agreement with those described in beef muscle (Locker and Wild, 1982; Leander et al., 1980; Carroll and Jones, 1979; Schaller and Powrie, 1972). There has been

Figure 5. Verification of collagen from frozen samples was obtained by treating tuna muscle with collagenase. The sarcolemma has disappeared thus exposing the characteristic striations.

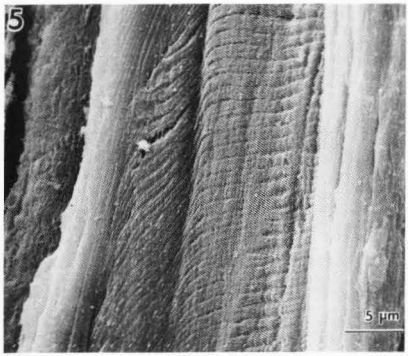
Figure 6. Precooked tuna has undergone some muscle fiber erosion. Granular materials, presumed to include sarcoplasmic proteins (S), have aggregated at the eroded surface.

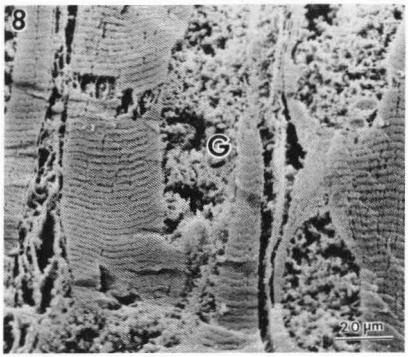
Figure 7. In cross-section, the muscle fibers of precooked tuna have shrunken. Granular material (G) has aggregated between the muscle fiber and the sarcolemma (S).

Figure 8. Longitudinal view of canned tuna. Granular material (G) has accumulated between muscle fibers.

Figure 9. After cryofracture through a raw, previously frozen tuna muscle fiber, Z-lines (Z), A-bands (A) and H-zones (H) were evident.

Figure 10. The sarcomere was disrupted upon precooking. The I-band and A-band have merged together and the congealed A-band (A) constitutes the majority of the sarcomere. Remnants of the Z-lines (Z) and H-zone (H) were noticeable.


5
5 µm


8
G
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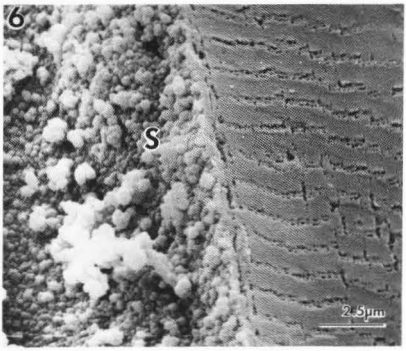
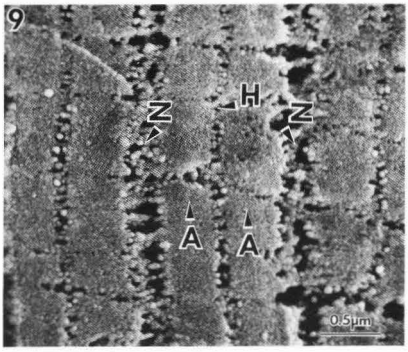
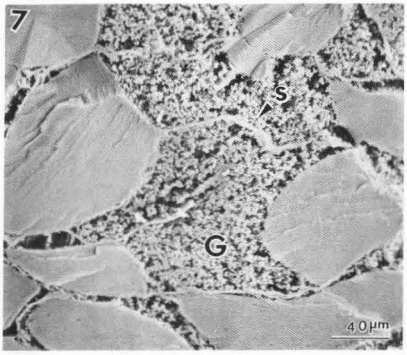
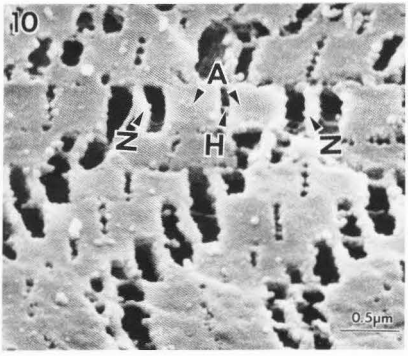

6
S
2.5µm


9
Z
H
Z
A
A
0.5µm


7
S
G
4.0µm


10
A
Z
H
Z
0.5µm

some further distortion of the sarcomere during thermal processing. The damage is evident in this figure due to greater magnification and high resolution. The sarcomere has been extremely shortened and large spaces have developed between each structural unit. This may thus indicate the combined effects of freezing and thermal processing on the muscle structure.

Fiber Diameter

The mean fiber bundle diameters are presented in Table 1. The difference between the diameter of raw and either precooked or canned fibers was statistically significant ($P< 0.05$). There was, however, no difference between the diameters of precooked and canned muscle fibers. This does indicate that shrinkage of the fibers did occur upon the first thermal treatment, and can be attributed to altered hydration or cook-cool loss. Hatae et al. (1984), measured the diameters of heat-set muscle fibers from five different fish species. Their results indicated that the diameters varied between species and that the diameters correlated with drip loss and firmness. These researchers did indicate that distinguishing textural differences of different species was possible via discriminant analysis but not through individual parameters.

Table 1. Mean Fiber Diameter of Raw, Precooked and Canned Skipjack Tuna Muscle.

Raw	Precooked (μm)	Canned
52.0±18.0[a]	45.6±14.8[b]	46.3±15.6[b]

[a,b]Means within each row represented by different letters indicate significant differences ($P<0.05$). Data are presented as mean ± standard deviation of 62, 71 and 76 fibers.

Conclusions

In tuna, freezing caused more of either a shifting or a distorting of muscle fibers than damage at the cell wall level observable at the lower limits, 20,000x, of high resolution scanning electron microscopy. During thermal processing of tuna, changes occurred manifested as a granulation of the sarcolemma. Also structural bands were altered and the migration of cellular constituents into the interstices of the muscle fiber and cell wall were detected. Further work is planned to investigate the effects of freezing and frozen storage on the muscle structure with the aid of isothermal freeze-fixation.

Acknowledgements

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Discussion with Reviewers

R.J. Carroll: The authors state the muscle fibers in Fig. 2 are fully contracted and the sarcomeres measured 2.2 μm. The authors also state a fully contracted sarcomere is 1.5 μm. Can you explain this discrepancy?
Authors: The muscle fiber shown in Figure 2 is of fresh fish that was never frozen. The sarcomere did measure 2.2 μm in length. The fully contracted sarcomere measuring 1.5 μm in length refers to Figure 9. The muscle shown in Figure 9 had been frozen. Further, the 2.2 μm measure relates to the exterior of the sarcomere. The 1.5 μm measurement refers indeed to the cryofractured specimen and a measure of the interior of the sarcomere.

R.J. Carroll: Is the change in appearance of the sarcomeres in Fig. 9 compared to those in Fig. 2 due to the freezing of the muscle?
Authors: Freezing may in part have caused some shrinkage due to drip loss upon thawing. Also, we reiterate that the two different figures represent surface versus interior views of the muscle sample.

G.M. Pigott: This paper could be improved if the damage or non-damage to cell walls, as assessed by the photomicrographs, could be correlated with water loss during processing and storage.
Authors: To some extent, "drip" data are available, as indicated by Table 1 and the text section entitled Fiber Diameter. Further, more detailed information is from allied studies which involved the handling, freezing and frozen storage of fresh skipjack tuna. The screening of tuna from commercial fishing vessels (described herein) and later, simulating on board conditions in the laboratory (a separate manuscript which is in review for publication) have provided some insight into the handling of tuna and subsequently the impact on the biochemistry and yield parameters. This screening work was preliminary in nature and provided the basis for the later work involving the handling of fresh fish.