Muscle Firmness and Structure of Raw and Cooked Arrow Squid Mantle as Affected by Freshness

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ABSTRACT

Changes in firmness and structure of arrow squid (*Loligo bleekert)* mantle muscle during refrigeration (5°C) were studied. Shear force of raw mantle decreased sharply between 3 and 9 h refrigeration after killing. Light microscopic observations showed many pores between muscle cells in the softened raw samples. Transmission electron microscopic observations suggested that the spaces appeared as a result of detachment of muscle cells from connective tissues. After cooking the stored raw muscle, the shear force and muscle structure were almost the same for samples with different storage times. Freshness had an influence on raw squid muscle firmness and structure but not on cooked muscle.

Key Words: squid, collagen, mantle muscle, shear force, freshness

INTRODUCTION

SQUID MUSCLE SOFTENS AFTER COOKING (Otwell and Hamann, 1979; Stanley and Hultin, 1982; Kolodziejska et al., 1987; Kugino and Kugino, 1994, 1995; Kugino, 1994; Naito et al., 1996; Ando, 1996, 1997). In seafood muscle, firmness has a close relationship with collagen, the major constituent of connective tissues (Ochiai et al., 1985; Sato et al., 1986; Hatae et al., 1986; Olaechea et al., 1993). In cooked muscle, heating decomposes collagen to gelatin and decreases the integrity between muscle cells, leading to muscle softening. In squid muscle, however, the intercellular space is so narrow that collagen fibrils cannot be clearly observed under transmission electron microscopy (Moon and Hulbart, 1975). Therefore, collagen had appeared to have little influence on softening of squid muscle after cooking. The main cause of the softening was reported to be the destruction of muscle cells during cooking (Kugino and Kugino, 1994). However, Ando (1996) reported that there was a considerable amount of collagen in five squid species and that the mantle muscles softened after gelatinization of 30% of total collagen by cooking. In cuttlefish (*Sepia esculenta*), the integrity decreased in proportion to the degree of gelatinization (Ando, 1997). Therefore, according to these reports (Kugino and Kugino, 1994; Ando, 1997), both muscle cells and collagen could be considered to affect squid muscle softening by cooking.

Squid is eaten not only cooked but also raw (*sashimi*) in Japan, and meat texture is very important for its quality. Raw squid muscle softens rapidly during storage leading to quality deterioration. Therefore, resolution of the softening mechanism is very important in fisheries. However, because of difficulty in obtaining live squid for experiments, there have been no reports on the relation between freshness and muscle firmness during short refrigeration.

We had the opportunity to obtain fresh squid samples, and our objective was to histologically observe the structure of raw and cooked squid muscles to clarify the influence of freshness on muscle firmness.

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MATERIALS & METHODS

Sample

Mantle muscle of live arrow squid (*Loligo bleekert*) caught at Yobuko, Saga Pref., Japan, was used. Three individuals were examined. After killing, each one was skinned, packed in a polyethylene bag, and stored at 5°C. The muscle was 5 mm thick.

Measurement of shear force

One muscle strip (10 mm × 40 mm × 5 mm) was excised from the skinned mantle by a cutter blade after 0 h, 3 h, 6 h, 9 h, 12 h, and 24 h storage after death. Each strip was sheared by a rheometer (RT-1002A, Fudoh, Tokyo, Japan) equipped with a cutter blade. The shearing speed was set at 1 mm/s and the shearing direction was set perpendicular to the orientation of muscle cells. A similar sample of the same size was taken at each storage time and cooked in boiling water for 30 min before measurement of shear force.

Histological observations

After measurement of shear force, parts of the raw and cooked samples were fixed in 5% glutaraldehyde (0.1 M phosphate buffer, pH 7.2). Smaller blocks (1 mm × 1 mm × 3 mm) were excised from the fixed samples, and post-fixed in 1% osmic acid medium (0.1 M phosphate buffer, pH 7.2). Dehydration by 50–100% ethanol and substitution with propylene oxide were performed. After embedding in epoxy resin (Epon 812, Ohken Co., Tokyo, Japan), thick sections (1 μm thick) were prepared by an ultramicrotome (MT-6000, Dupont, Wilmington, Del.). They were stained with 0.1% toluidine blue and observed under a light microscope (BX-50, Olympus, Tokyo, Japan). Ultrathin sections (80 nm thick) were prepared from the same blocks by an ultramicrotome. They were stained with 1% uranyl acetate and lead citrate, and observed under a transmission electron microscope (H-800, Hitachi, Tokyo, Japan) with an accelerating voltage of 100 kV.

Estimation for degree of cell detachment

Area of the spaces which were formed between muscle cells was measured with a Digitizer (Nikon, Tokyo, Japan) on the electron micrographs. The area ratio of spaces to whole muscle was calculated.

Statistical analysis

Significance of difference in shear force values and degrees of cell detachment was determined by t-test. Significant was defined at $p < 0.05$.

RESULTS & DISCUSSION

Changes of muscle firmness during storage

Changes in the shear force were compared (Fig. 1) on raw and cooked muscles of refrigerated squid. In the raw muscle, the shear force rapidly decreased from 3–9 h. However, the shear force of the cooked muscle, lower than that of the raw samples remained almost constant throughout the storage period. It has been reported that cooked squid muscle was softer than the raw (Otwell and

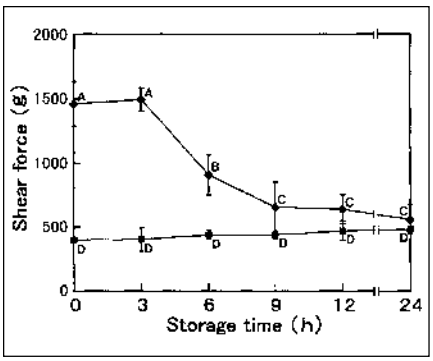

Fig. 1—Changes in shear force of raw and cooked mantle muscles during refrigeration. : raw muscles; : cooked muscles. [ABCD]Different letters with each set of data indicate significant difference ($p < 0.05$).

Hamann, 1979; Stanley and Hultin, 1982; Kolodziejska et al., 1987; Kugino and Kugino, 1994, 1995; Kugino, 1994; Naito et al., 1996; Ando, 1996, 1997). The constancy in firmness of cooked muscle, independent of freshness of original muscle, was demonstrated for the first time in our results.

Light microscopic observation

Light microscopic photographs were compared (Fig. 2) on raw squid muscle stored at 5°C for different times. Just after death, the muscle cells tightly contacted each other with intercellular materials present intact (Fig. 2A). However, at 9 h and 24 h storage, the muscle cells began to detach from each other, and pores were formed between them (Fig. 2B, 2C). The detachment between muscle cells was considered to weaken the connecting forces and soften raw squid muscle during refrigeration. On the contrary, in samples cooked at given storage times, pores observed in the raw muscles could not be found, and there were no structural differences among samples (Fig. 3). These findings account for the absence of changes of shearing force in the cooked samples.

Transmission electron microscopic observation

Raw muscle. Transmission electron micrographs were compared (Fig. 4) on raw squid mantles. There was a unique striped pattern on the transverse section of the muscle, and the pattern became clearer during refrigerated storage. Additionally, mitochondria in the muscle cells became larger (Fig. 4C, arrowheads). Black particles were observed in the muscles of 9 h and 24 h storage (Fig. 4B and C, arrows), but they were hardly observed in fresh muscle (Fig. 4A). Thus, the particles, though not yet identified, were assumed to be some reaction product in the post-mortem changes of muscle. Muscle cells with unknown structure (Co) between them (Fig. 4A) were tightly bound to each other just after death as had been reported by Moon and Hulbart (1975). After 9 h and 24 h refrigeration, spaces began to appear in connective tissue and muscle cells (Fig. 4B). They were considered to weaken the integrity between muscle cells and to result in decreases in shear force. The area ratio of spaces which were formed between muscle cells to whole muscle was compared (Fig. 5). The ratio increased during 9 h storage.

In raw muscle, structural changes of con-

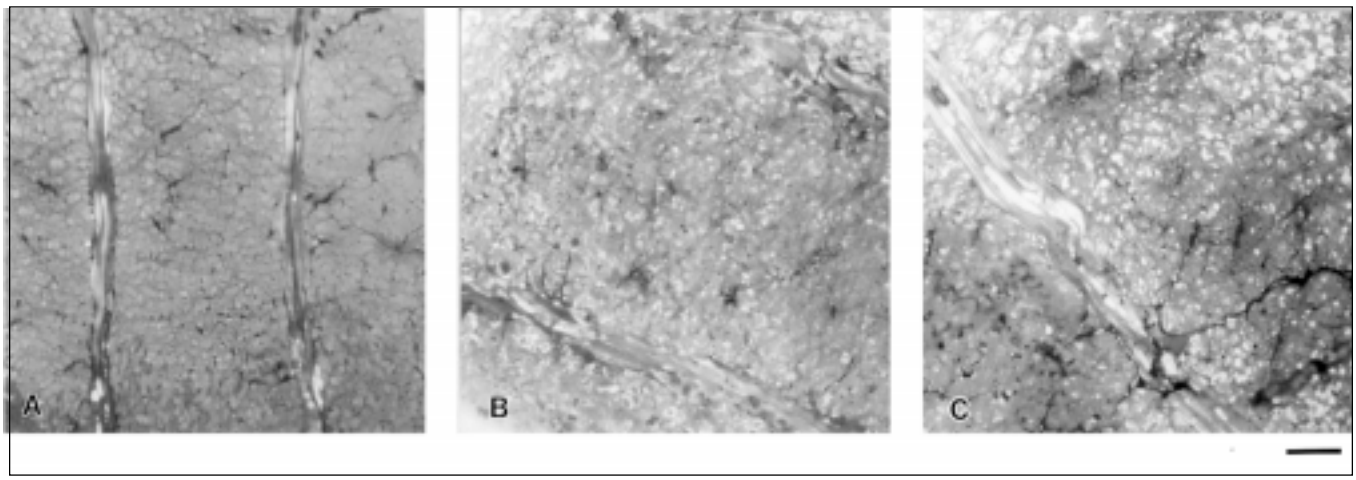

Fig. 2—Light microscopic photographs of raw muscles stored at 5°C. (A) just after killing; (B) 9 h refrigeration; (C) 24 h refrigeration. Bar represents 50 µm.

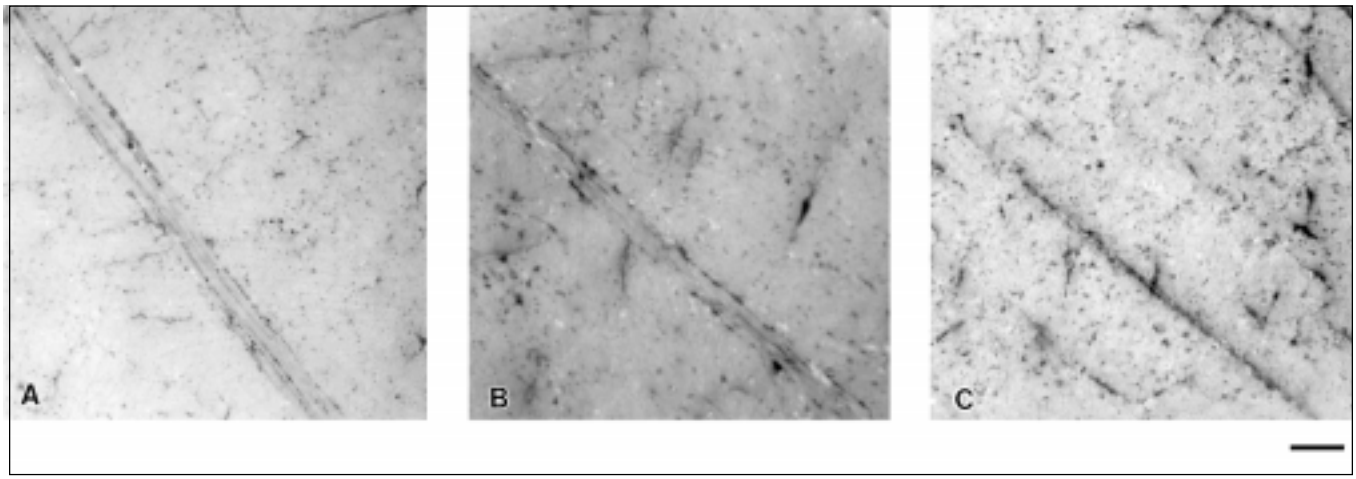

Fig. 3—Light microscopic photographs of cooked muscles. (A) just after killing; (B) 9 h refrigeration; (C) 24 h refrigeration. Bar represents 50 µm.

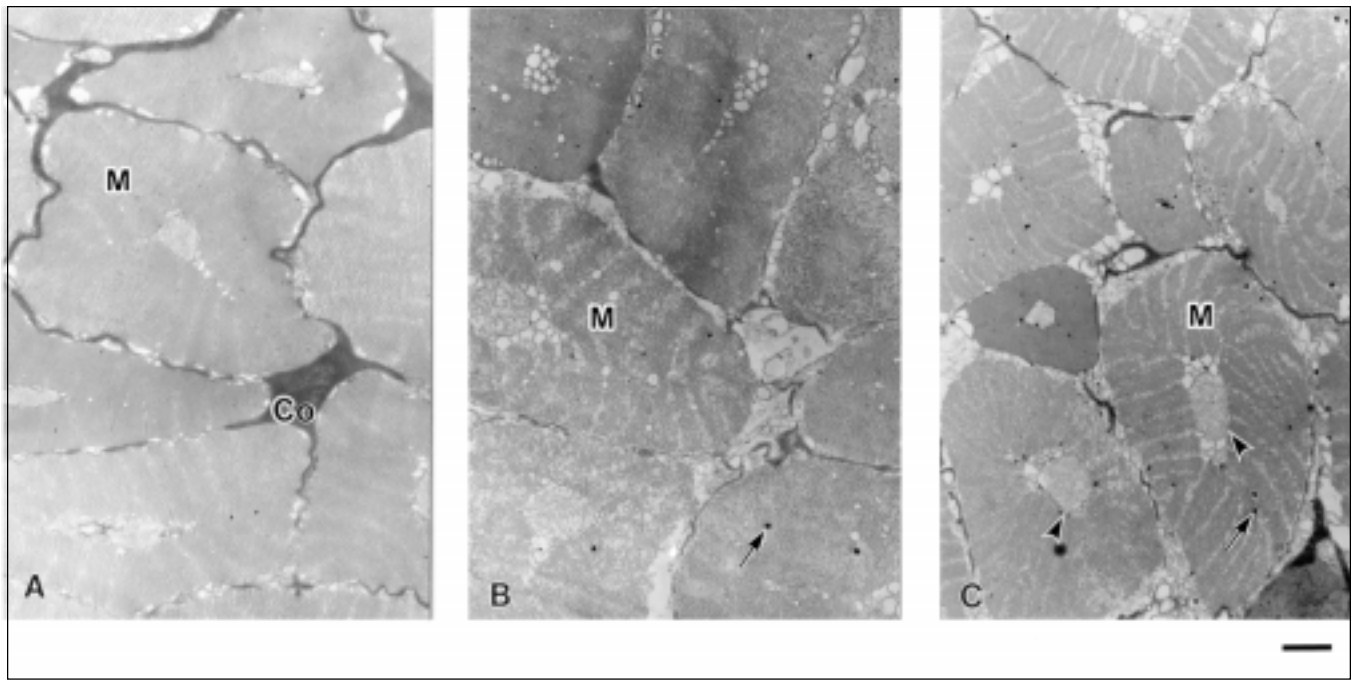

Fig. 4—Transmission electron microscopic photographs of raw muscles. (A) just after killing; (B) 9 h refrigeration; (C) 24 h refrigeration. M = muscle cell; Co = connective tissue-like structure. Arrows = unknown tiny particles; arrowheads = mitochondria. Bar represents 1 µm.

nective tissue and myofibrils during chilled storage have been reported to occur in mammalian and fish muscles (Love et al., 1969; Bremner and Hallet, 1985, 1986; Hallet and Bremner, 1988; Ando et al., 1991, 1992, 1993, 1995; Tachibana et al., 1993; Nishimura et al., 1994; Liu et al., 1994; Sato et al., 1997). Disintegration of collagen fibrils was especially attributed to be the cause of fish muscle softening which occurred within one day of chilled storage (Ando et al., 1992, 1995; Sato et al., 1997). The disjunction among squid muscle cells that we observed suggested the existence of the same softening mechanism in squid muscle as in fish muscle. Conventional transmission electron microscopy did not show the existence of collagen between the muscle cells of squid (Moon and Hulbart, 1975). However, the existence of collagen there was proven (Ando et al., 1998) by the amino acid analysis of intercellular substances left after extraction of cellular components by the method of Ohtani (1987). Three hypotheses on the mechanism of cell detachment in softened squid are possible. First, shrinkage of muscle cells which creates intercellular spaces may soften squid muscle. In mammalian and fish, muscles shrink after death and post-mortem rigor occurs. However, neither muscle shrinkage nor post-mortem rigor occurred in raw squid muscle in our results (data not shown). Therefore, the first hypothesis was excluded in this study.

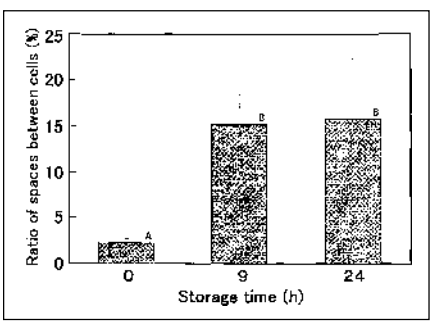

Fig. 5—Estimation of the degree of cell detachment during storage of raw squid muscle. The area of intercellular spaces and whole muscle was measured on electron microphotographs and ratio between them was calculated (means±S.D.). ^{AB}Different letters with each set of data indicate significant difference ($p < 0.01$).

Second, cell detachment may occur as a result of a decrease in the intercellular integrity due to loosening of collagen fibers. Third, it may occur as a result of decrease in the binding force between cell membrane and collagen. The last two hypotheses are related to changes of muscular collagen. In fish muscles, collagen degradation was detected on the first day of chilled storage (Sato et al., 1991, 1997). As squid mantle has high cathepsin B, D, and L activities, the degradation could be brought about by some proteolytic action (Sakai and Matsumoto, 1981; Makinodan et al., 1993). There have been no reported studies on the degradation of squid collagen during refrigeration. Additionally, base membranes and/or integrin, though important in connecting collagen fibers and cell membranes, have not been studied in squid muscle. Therefore, effects of collagen, base membrane, and integrin are still unclear in softening of squid muscle. Further studies are necessary to resolve the mechanism of post-mortem changes in squid muscle.

Cooked muscle. In cooked muscle, the structural features of both cell and connective tissues that were observed in the raw muscle disappeared, and the cell border became unclear (Fig. 6). The spaces observed in the raw muscle were also not present. There were no structural differences in the cooked samples after the different refrigeration periods, which corresponded to the comparable shear forces.

The finding that the firmness of cooked muscle was weak but constant could be explained by considering the occurrence of gelatinization of intercellular collagen. Heating collagen to gelatin is considered to weaken muscle integrity to such a degree that no differences of firmness could be detected in the cooked samples.

Our previous study reported that about 30% of squid collagen was dissolved after 30 min heating while the rest remained insoluble (Ando, 1996). Large masses of collagen were found at intercellular places in squid muscle, and the collagen remained almost intact even after cooking (Ando, 1997). Therefore, the insoluble collagen remaining after 30 min heating may be accounted for by such collagen masses. The solubility differences could be due to the differences in the intermolecular crosslinks of collagen. Pyridinoline (Fujimoto, 1977), a major crosslink of collagen, has a close relationship with heat stability (Horgan et al., 1990; Smith and Judge, 1991; Young et al., 1994)

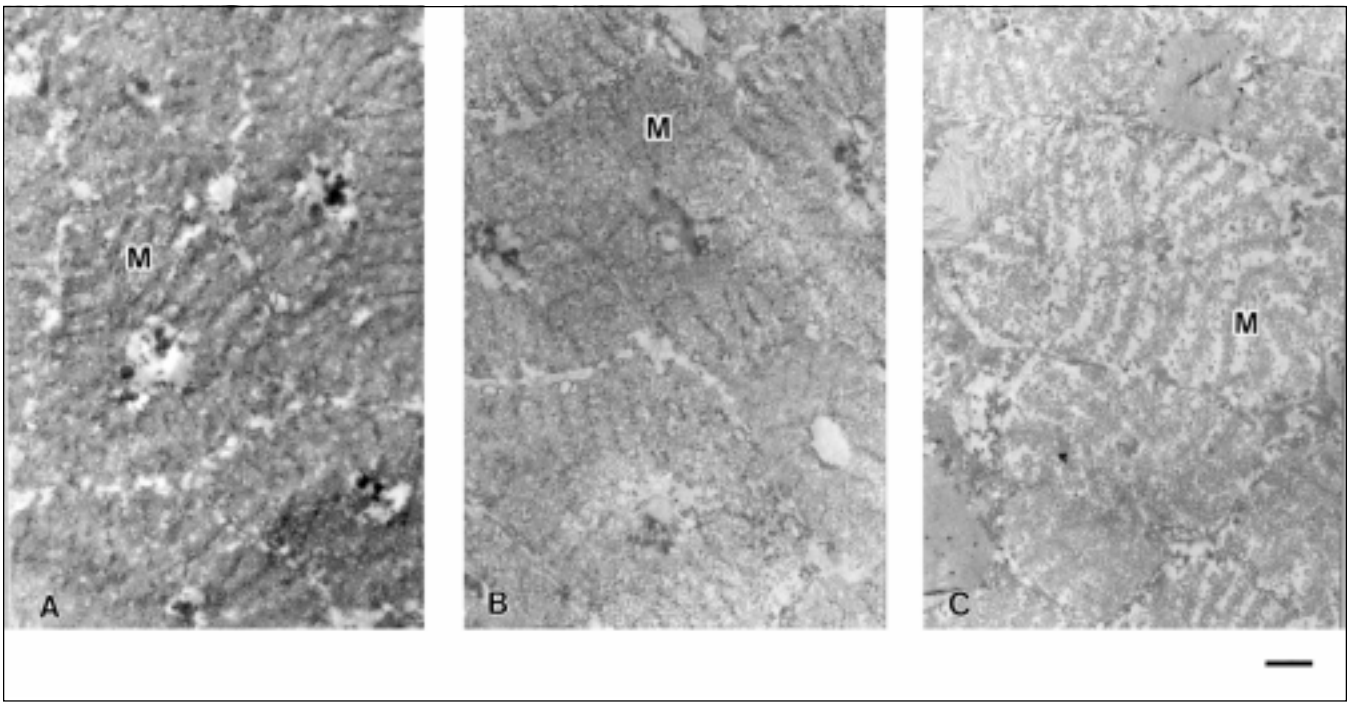

Fig. 6—Transmission electron microscopic photographs of cooked muscles. (A) just after killing; (B) 9 h refrigeration; (C) 24 h refrigeration. M = muscle cell. Bar represents 1 μm

and a capacity to make collagen fibrils in squid less susceptible to proteinase. Therefore, the insoluble collagen in squid was probably more abundant in pyridinoline than soluble collagen would be.

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