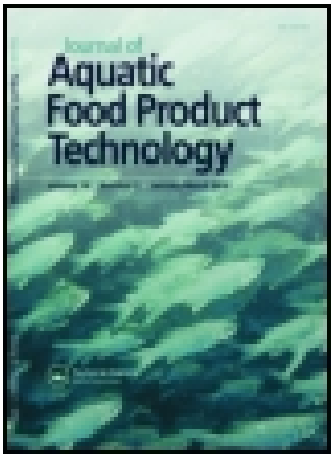

Journal of Aquatic Food Product Technology

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/wafp20

Ultrastructure of Actomyosin in Pre- and Post-Spawning Hake (Merluccius hubbsi Marini) During Frozen Storage

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Published online: 23 Sep 2008.

To cite this article: S. I. Roura , C. L. Montecchia , H. Roldán , O. Pérezborla & M. Crupkin (2000) Ultrastructure of Actomyosin in Pre- and Post-Spawning Hake (Merluccius hubbsi Marini) During Frozen Storage, Journal of Aquatic Food Product Technology, 9:4, 85-94, DOI: 10.1300/J030v09n04_08

To link to this article: http://dx.doi.org/10.1300/J030v09n04_08

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ABSTRACT. The degradation of actomyosin in fillets from pre- and post-spawning hake under frozen storage is studied by electron microscopy and by analysis of changes in protein solubility. The ratio of salt soluble protein did not present significant changes during 240 days of frozen storage for post-spawning hake. Meanwhile the same ratio for pre-spawning hake presented a steady decrease.

In post-spawning hake the proteins retain some of the characteristics of the native structure with some aggregate formation up to 60 days of

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This investigation was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC).

The authors thank Mrs. María E. Almandos for her data analysis assistance.

storage. In pre-spawning hake the formation of aggregates is already extensive after only 15 days of storage.

The solubility of proteins from pre-spawning hake decreased continuously reflecting the changes in the ultrastructure of actomyosin complex. For post-spawning hake, only the formation of soluble aggregates was observed after 240 days of frozen storage. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

KEYWORDS. Hake, actomyosin ultrastructure, frozen storage, reproductive cycle, fish fillet

INTRODUCTION

Changes in quality of fish muscle during frozen storage is attributed to the denaturation of actomyosin. Changes in the actomyosin composition of mature hake are influenced by the metabolic state of the fish and are attributed to its reproductive cycle (Crupkin et al., 1988). The adverse effect of the pre-spawning condition of hake on the chemical, physicochemical and functional properties of actomyosin during frozen storage was reported in previous work (Montecchia et al., 1997). The fact that fillets from pre-spawning hake deteriorate faster than fillets from post-spawning hake during storage at $-20°C$ was indicated by the rate of protein insolubilization, the reduced viscosity and the hydrodynamic properties (Montecchia et al., 1997).

Analysis of changes in protein solubility is the simple and common method for estimating the rate of protein denaturation and its significance for assessing the denaturation mechanism. Electron microscopic observations are frequently used together with the solubility changes of protein to check the protein denaturation during frozen storage (Ohnishi et al., 1978).

In this work, the degradation of actomyosin in fillets from pre- and post-spawning hake under frozen storage is studied by electron microscope observation and by analysis of changes in solubility.

MATERIALS AND METHODS

Fish Samples

Female specimens of hake (*Merluccius hubbsi*) caught in the southwestern Atlantic Ocean between 36°S and 53°S were kept on ice for

48-72 hrs, until they reached the laboratory in an early post-rigor condition. Specimens were 35 to 45 cm long. Quality of the raw material was assessed after the fish had been washed and classified. To determine the maturation stage of each fish the gonadosomatic index (Crupkin et al., 1988) was calculated as follows:

$$\text{GSI (gonadosomatic index)} = \frac{\text{Wet weight of gonads}}{\text{Wet weight (fish} - \text{gonads)}} \times 100$$

Fish were manually filleted and the fillets were interweaved into blocks of approximately 2 kg each. The blocks, wrapped in polyethylene film, were frozen in a commercial plate freezer during 180 min at $-30°C$ and stored in laboratory cabinets at $-20°C$ until they were analyzed. Samples for analysis were made up with three fillets from three different fish. Analyses of protein solubility was performed on the fresh raw material (day 0= assumed fresh + unfrozen sample) and on the frozen samples at 45, 120 and 240 days of storage. Frozen samples were thawed for 8 hr at 5°C. Electron microscopy was performed on samples at 0, 15 and 60 days.

Actomyosin Preparation

Purified natural actomyosin (NAM) was obtained from fillets by the method of Tsuchiya et al. (1975) with the modifications introduced by Crupkin et al. (1982). The myofibrillar protein extract in 0.6 M KCl-0.003 M $NaHCO_3$ (pH 7.0) was centrifuged at 7500 × g. The supernatant was diluted with cold water to a final concentration of 0.2 M KCl. The resulting precipitate was solubilized in 0.6 M KCl. This dilution-solubilization cycle was repeated three times to obtain the purified natural actomyosin.

Protein Solubility

The total myofibrillar extract was obtained by homogenizing 8 g of muscle in 160 mL of a 0.6 M KCl-0.003 M $NaHCO_3$ (pH 7.0) solution for 1 min in a Sorvall Omni-Mixer 17106 (Du Pont Instruments, Newtown, CT). The homogenate was centrifuged for 20 min at 5000 × g in a refrigerated centrifuge Sorwall RC-5B (Du Pont Instruments, Newtown, CT) at 3-5°C. The collected supernatant was de-

fined as the salt-soluble fraction. Results were expressed as percentages of salt-soluble protein to total protein ($S/S_0 \times 100$).

Protein Determination

The Lowry method (Lowry et al., 1951), with bovine serum albumin as standard, was used to determine protein concentration of salt-soluble extracts, total myofibrillar extract and actomyosin solution.

Electron Microscopy

Purified actomyosin was supported on carbon-coated formvar membranes mounted on regular copper grids (300 mesh). Samples were negatively stained applying a 3% uranylacetate solution for 30-60 seconds, followed by several washes with distilled water (Roura et al., 1992). Microscope observations were performed in a JEOL J.S.M. 35 CF microscope, at high magnification, with an accelerator voltage of 80 kV.

Statistical Analysis

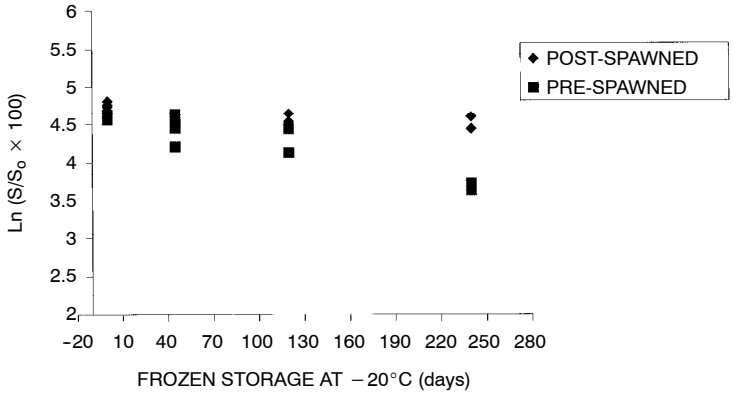
Trends were calculated by the least-square method. The slope for post-spawning solubility values was calculated by the null slope test (Volk, 1969).

RESULTS AND DISCUSSION

The ratio of soluble to total protein during frozen storage is plotted in log scale in Figure 1 for both pre- and post-spawning hake. For post-spawning hake the log of the ratio remained around 4.61 throughout the 240 days storage. The best straight line fit did not explain the results better than the mean value, as tested by the null slope test (Volk, 1969). For pre-spawning hake, however, the ratio decreased continuously and in a semilog plot it can be represented by the straight line

$$\text{Ln}\,(S/S_0 \times 100) = -0.0035\,t + 4.5904\ (R_2 = 0.8414),$$

FIGURE 1. Ratio of salt-soluble to total protein in hake during frozen storage at −20°C



Where S/S_0 is the ratio of salt-soluble protein to total protein and t is the storage time.

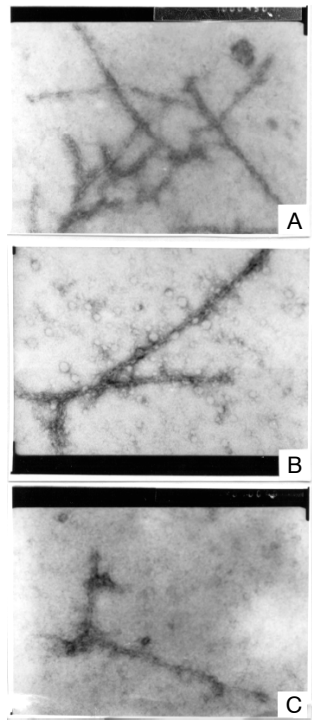
The final soluble protein content in pre-spawning hake is much lower than in post-spawning hake, indicating that the gonadal condition of fish affects the solubility of proteins. These results are in agreement with results reported in a previous work (Montecchia et al., 1997). Castell and Bishop (1973) and Ironside and Love (1958) also reported changes in the salt-extractable proteins related to the physiological condition of fish and to spawning and feeding cycles. Ohnishi et al. (1978) proposed two stages in the solubility of carp actomyosin during frozen storage.

Electron microphotographs of the actomyosin in post-spawning hake are presented in Figure 2 for Day 0 and for frozen samples after 15 and 60 days storage. The corresponding microphotographs of the actomyosin in pre-spawning hake are presented in Figure 3.

The actomyosin of fresh post-spawning hake presented the characteristic arrowhead structure (Figure 2A). After 15 days of frozen storage the actomyosin filaments present little deformation and the arrowhead structure was still discernible (Figure 2B). At day 60 of frozen storage, the arrowhead structure was no longer discernible, filaments were entangled and some aggregate formation was observed (Figure 2C).

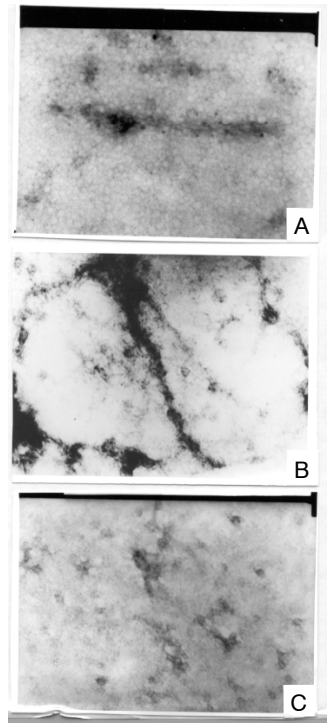
The actomyosin of fresh pre-spawning hake showed a partial loss of

FIGURE 2. Electron micrographs of actomyosin from post-spawning hake muscle during fillets frozen storage at −20°C. A–Fresh actomyosin (magnification: 100,000×) B–After freezing (15 days), (magnification: 100,000×) C–After 60 days, (magnification: 140,000×).



the filament structure; the arrowheads were not discernible and the filaments were shorter, thicker and entangled, with the presence of aggregates (Figure 3A). This loss of the native structure of actomyosin in fresh pre-spawning hake had already been observed in a previous work. It was attributed to a decreased affinity between myosin and actin, probably due to an *in vivo* increase of the proteolitic activity that selectively degrades the heavy myosin chain (Roura et al., 1992). After 15 days of frozen storage, the actomyosin of pre-spawning hake was extensively deformed; the filaments were thick and entangled and

FIGURE 3. Electron micrographs of actomyosin from pre-spawning hake muscle during fillets frozen storage at −20°C. A–Fresh actomyosin (magnification: 100,000×) B–After freezing (15 days), (magnification: 100,000×) C–After 60 days, (magnification: 140,000×).



some aggregates and granules were observed (Figure 3B). At day 60 of frozen storage the actomyosin filaments could not be singled out and were incorporated in aggregates (Figure 3C).

Ohnishi et al. (1978) proposed three types of deformation in the actomyosin filaments from carp during frozen storage: filaments with little or no deformation, filaments deformed to moderate extent, and filaments or objects extensively deformed. Following this classification the actomyosin filaments from post-spawning hake after 60 days storage would be deformed to moderate extent; whereas the actomyo-

sin filaments from pre-spawning hake would be extensively deformed after only 15 days of frozen storage.

The gonadal condition of fish affects the chemical, physicochemical and functional properties of the myofibrillar proteins (Crupkin et al., 1988; Roura et al., 1990; Roura and Crupkin, 1995). The differences in these properties cause different rates of degradation between pre- and post-spawning fillets stored at $-20°C$, as reflected by protein insolubilization, reduced viscosity and hydrodynamic properties (Montecchia et al., 1997). By analyzing the hydrodynamic properties of actomyosin from pre- and post-spawning hake during frozen storage, Montecchia et al. (1997) concluded that after 45 days of storage the actomyosin from pre-spawning hake was more susceptible to aggregation, and its deterioration included loss of extractability and formation of insoluble aggregates. However, in fillets from post-spawning hake, insoluble aggregates were not observed after 240 days of storage.

The solubility of proteins from pre- and post-spawning hake presented different values at time 0. During frozen storage the evolution of the ultrastructure of actomyosin and the solubility of proteins in both samples followed different patterns of degradation. While the actomyosin of post-spawning hake lost its characteristic arrowhead structure only gradually, that of pre-spawning hake presented severe degradation after only 15 days of storage and by day 60 only protein aggregates could be observed. Ohnishi et al. (1978) found that changes in the solubility of proteins do not coincide with ultrastructural changes detected by electron microscopy and, therefore, they proposed that solubility of proteins alone should not be used as an indicator of the status of proteins. According to Sikorski (1978) the loss of protein extractability during frozen storage is caused by aggregate formation. However, Wagner (1986), working with bovine muscle, suggested that during the first stage of protein denaturation soluble aggregates are formed and, only in a second stage, the aggregates become insoluble. Moreover, when working with carp muscle, Oguni et al. (1975) and Tsuchiya et al. (1975) found aggregates of actomyosin filaments in the supernatant actomyosin fraction that was defined as soluble.

Referring to Wagner's suggestion and to the microscopic observations, the different behaviors between pre- and post-spawning hake would indicate that in post-spawning hake the degradation of the

ultrastructure of the actomyosin complex, after 240 days of frozen storage, proceeds only to the point of formation of some soluble aggregates. On the other hand, although some soluble aggregates may form in the actomyosin from pre-spawned hake, they would evolve into insoluble aggregates and the solubility of the protein from pre-spawning hake would decrease continuously (Figure 1).

The changes observed in the microstructure of actomyosin and the results of the solubility of proteins during frozen storage of fillets from pre- and post-spawning hake would constitute further evidence that the gonadal condition of fish affects the properties of myofibrillar proteins.

CONCLUSIONS

The freezing of hake muscle affects the ultrastructure of proteins, both for pre- and post-spawning fish. However these changes are different for both gonadal conditions. In post-spawning hake the proteins retain some of the characteristics of the native structure with some aggregate formation up to 60 days of storage. In pre-spawning hake the formation of aggregates is extensive, even after only 15 days of storage. The solubility of protein from pre-spawning hake decreased continuously reflecting the changes in the ultrastructure of actomyosin from the characteristic arrowhead structure to the formation of insoluble aggregates. For post-spawning hake, only soluble aggregates were observed after 240 days of frozen storage.

Since the actomyosin of fresh post-spawning hake is in its native state and partially retains this structure, resisting the freezing process, it is concluded that this gonadal condition is the best suited for the preparation of frozen products.

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