A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part II — Physiology Studies

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October 1986

Canadian Industry Report of Fisheries and Aquatic Sciences

No. 177

Fisheries and Oceans Pêches et Océans

Canada

Canadian Industry Report of Fisheries and Aquatic Sciences

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Canadian Industry Report of
Fisheries and Aquatic Sciences No. 177

October 1986

A STUDY OF THE USE OF COD, COD BY-PRODUCTS AND CRUSTACEANS BY-PRODUCTS FOR SURIMI AND SURIMI-BASED PRODUCTS: PART II - PHYSIOLOGY STUDIES

by

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Cat. No. Fs 97-14/177 ISSN 0704-3694

Correct citation for this publication:

Haard, N. 1986. A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part II - Physiology Studies. Can. Ind. Rep. Fish. Aquat. Sci. 177 Viii + 34 p.

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High grade surimi can be produced from bled cod held on ice for up to 7 days and bled winter cod iced for 12 days. Better quality surimi can be made from cod fillet held at 0 ± 0.2°C than from cod held at -3 ± 1°C. Bled summer cod retains its quality for surimi production 50% longer than unbled cod. The apparent viscosity of a simple actomyosin extract from muscle is a sensitive indicator of kamaboko making potential of cod surimi. At this time Ca^{+2} ATPase does not appear to be a reliable indicator of gel strength potential. Proteolytic hydrolysis does not appear to play a significant direct role in the loss of gel strength potential. However, it may play an indirect role in the loss of gel strength potential. However, it may play an indirect role in the loss of actomyosin viscosity, which is associated with loss in gel strength potential.

RÉSUMÉ

Haard, N. October 1986. A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part II - Physiology Studies. Can. Ind. Rep. Fish. Aquat. Sci. viii + 34 p.

On peut obtenir du surimi de qualité supérieure à partir de morue saignée gardée en glace jusgu'à sept jours et de morue d'hiver saignée gardée en glace pendant 12 jours. Des filets de morue gardée à 0 x 0,2°C fournissent une meilleure qualité de surimi que la morue gardée à -3 + 1°C. La morue d'été saignée conserve sa qualité pour la production de surimi 50% plus longtemps que la morue non-saignée. La viscosité apparente d'un extrait simple d'actomyosine musculaire est un indicateur sensible du potentiel de production de kamaboko à partir de surimi de morue. Actuellement, la Ca^{+2}ATPase ne semble pas être un indicateur fiable du potentiel de résistance du gel. L'hydrolyse protéolytique ne semble pas jouer un rôle direct important dans la perte de potentiel de résistance du gel mais elle peut toutefois y contribuer indirectement. De plus, cette hydrolyse peut être impliquée indirectement dans la réduction de la viscosité de l'actomyosine qui découle de la perte de potentiel de la résistance du gel.

PREFACE

A contract was awarded to a research team from the Newfoundland and Labrador Institute of Fisheries and Marine Technology (The Marine Institute) and Memorial University to study the potential of selected Canadian Atlantic fish species and shellfish processing wastes to produce surimi and shellfish flavours. Research to develop methodology for rapidly determining the potential of raw materials to produce surimi was initiated, objective laboratory techniques for evaluating the quality of surimi were established, a pilot line for producing surimi was installed, surimi was produced from cod and other groundfish. Some biochemical indices of cod were analysed under various conditions of storage and methods were established which may assist as predictors of surimi potential from cod and possibly other species. Crab process wastes and scallop process wastes were analysed for their contents of flavour-active components. Methods of concentrating flavours were identified.

Three separate publications from this contract were issued. Correct citation for these are:

Chandra, C.V. 1986. A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part 1 - Raw Material Assessment.

Haard, N. 1986. A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part 11 - Physiology Studies.

Haard, N. 1986. A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part 11 - Physiology Studies.

Voigt, M.N. 1986. A Study of the Use of Cod, Cod By-Products and Crustacean By-Products for Surimi and Surimi-Based Products: Part 111 - Shellfish Flavour Extraction Studies.

The opinions and interpretations expressed in the report are those of the author, and do not necessarily reflect those of the Department of Fisheries and Oceans.

This report was produced under DFO/DSS Contract: OºSC.FP001-5-2092.

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INTRODUCTION

The myosin molecule comprises about 40% of the total protein in fillets from Atlantic cod (Gadus morhua). Myosin and associated contractile proteins are concentrated during the manufacture of surimi such that more than 60% of the protein in surimi is myosin. The gel-forming property of surimi is directly related to its content of native myosin. The mechanism by which myosin and related proteins form a gel as a consequence of heat treatment is not fully understood at this time.

Myosin is a large rod shaped molecule having a molecular weight of approximately 500,000 daltons. It contains two identical heavy chains which are wound around each other to form a lollypop shaped structure. Associated with the globular head of myosin are additional proteins called light chains. There are two classes of light chain, one of which is called "alkali light chain" and is essential for myosin ATPase (Adenosine Triphosphatase) activity. The globular head of myosin is responsible for the ATPase activity of the molecule and its ability to interact with actin.

Actin is the second most abundant muscle protein. It comprises 15-30% of surimi protein and probably exists in muscle as fibrous actin (F-actin). The latter is made up of monomeric units of globular actin (G-actin) which has a molecular weight of about 40,000 daltons.

When purified actin and myosin are mixed in vitro a complex called actomyosin is formed. The protein interactions that occur during the onset of rigor appear to involve a similar interaction of actin and myosin. The consequence of actin and myosin interactions, e.g. during rigor, on the

functional characteristics of surimi is not fully understood at this time. For meat products, the protein interactions that occur during rigor hinders the ability of salt to improve the water binding and swelling of meat paste. It is likely that a similar type of combination between actin and myosin molecules occurs in surimi and therein has importance to its functional properties. Overnight extraction of muscle of surimi with a buffer of ionic strength about 0.6 yields a crude actomyosin solution, sometimes called "myosin B". Actomyosin has a high viscosity and like myosin has ATPase activity. The high viscosity of actomyosin appears to be the result of its long rod like structure.

Myosin appears to be the most important component of muscle tissue or surimi with respect to gel-forming ability. Actin assists in this process by forming F-actomyosin which in turn interacts with myosin. The head portion of myosin undergoes irreversible aggregation (involving disulfide interaction) during heating of meat paste and this appears to contribute to formation of the three dimensional network of the gel. The elongated tail portion of the myosin molecule undergoes a partial unravelling during heating of meat paste and this also contributes to the formation of a three dimensional network. Hence, gel formation occurs when myosin is denatured under strictly controlled conditions. However, denaturation of myosin prior to heating under strictly controlled conditions can result in a loss of gel-forming ability.

Fish myofibrillar protein, notably myosin, is much more sensitive to denaturation than that from land animals. The mechanism by which fish proteins denature is not completely understood. However, it involves

aggregation of myosin and/or actomyosin initially by non-covalent bonds (weak ionic interactions between protein molecules themselves or hydrophobic interactions with the aid of free fatty acids formed as a result of lipid hydrolysis) and in some systems secondarily by covalent crosslinks (brought about by disulfide linkages and aldehydes formed by lipid oxidation or trimethylamine oxide catabolism). Water washing minced fish muscle and addition of cryoprotectants to the washed product serves to markedly improve the stability of myofibrillar proteins during frozen storage.

Proteolytic enzymes which hydrolyze myosin or associated myofibrillar proteins may also decrease the gel-forming ability of fish paste. Proteolytic enzymes may decrease the gel-forming ability of fish proteins during postmortem storage of muscle prior to the manufacture of surimi or may act during the time the fish paste is heated to form a gel. An example of proteolytic action during postmortem storage is the rapid loss in gel strength of parasitized Peruvian hake muscle due to proteases associated with protozoan infection. Protein hydrolysis during the heating of fish paste is exemplified by alkaline proteases in croaker (Lin, T.S. and T.C. Lanier. 1980) which appear to hydrolyze proteins during intermediate temperatures (e.g. 60°C) of heating during gel-formation.

The key mechanistic factor(s) which causes the loss in ability of myosin from cod and other raw materials in Newfoundland to form a three dimensional network or gel during heating is not known at this time. For

several reasons, understanding these factors is necessary for the prudent development of a surimi industry. One reason is that understanding these factors may provide insight into the best choice of raw material for use in surimi manufacture. Intraspecific factors, such as water temperature, migratory history, spawning, year-class, feeding history, etc. as well as harvest and postharvest catching and handling methods are probably as important as or more important than species differences with respect to suitability of the raw material. A second reason is that such knowledge will provide a scientific rather than an empirical basis for trouble shooting unexpected problems which will arise in the industry from time to time. For example, knowing that the thermal instability of myosin from aged fish is lower than that of myosin from very fresh fish indicates that the surimi processor must exercise more stringent temperature control over the process when the raw material is not fresh. A third reason is that such knowledge can form the basis of a method to judge or grade raw material intended for use in surimi manufacture. Grading systems currently employed in the fish processing industry are not well suited for the surimi industry because they are more or less intended to assess the progress of bacterial spoilage. This type of grading system may bear little relation to the suitability of the raw material for surimi manufacture.

MATERIALS AND METHODS

1. BIOLOGICAL MATERIAL -

Atlantic cod (Gadus morhua) purchased from local fishermen were used for preliminary studies. The majority of studies were conducted with fish harvested during the trap season and maintained in a 181,840 litre (40,000

gallon aquarium at Marine Science Research Laboratory Memorial University. Fish were normally approximately 55cm in length.

2. HANDLING -

Specimens were removed from the holding tank, stunned with a blow to the head, and bled by cutting the jugular and submerging in sea water for approximately 5 minutes. In certain studies, fish were not bled after stunning. All fish were immediately packed in ice and after approximately 2 hours were filleted. Fillets were vacuum packed and stored at 0 ± 0.2°C or in some studies at -3 ± 1°C for up to 21 days.

3. LABORATORY SURIMI -

Surimi was prepared by hand mincing a 1 kg fillet; washing the mince with 8 L tap water (approximately 8°C) for 5 minutes; decanting the slurry through a China cap strainer (1mm bores), repeating the water wash three additional times; washing the mince with 8 L 0.14% NaCl (4°C), and dewatering the washed mince in a muslin bag with a Carver press at 69,000 KPA, (10,000 psi) for 5 minutes.

4. KAMABOKO -

Kamaboko was prepared by blending 4% sucrose, 4% sorbitol, 0.3% sodium tripolyphosphate with the dewatered mince. To this was added 3% NaCl with chopping and blending to form a paste. The resulting dough was steamed with a Rival automatic steamer using 250 ml water.

A fold test to measure elasticity was performed on samples of the Kamaboko 18 hours after cooking. "AA" indicates no cracks or breaks when 5 x 35 mm discs are folded in quarters.

5. ACTOMYOSIN -

Actomyosin was prepared from fillets or from surimi by the following procedure at 0-2°C. Raw material (10g) was suspended in 30 ml 0.05 N imidazole - HCl, pH 7.0, centrifuged at 15,00 x g for 20 minutes and the supernatant discarded. This step was repeated twice more. The residue was suspended in 60 ml .05N imidazole - HCl containing 0.6N KCl and stirred for 18 hours at 4°C. the actomyosin was recovered as a supernatant after centrifugation at 15,000 x g for 20 minutes. This type of actomyosin preparation is sometimes called "Myosin B" by other workers.

6. APPARENT VISCOSITY -

Actomyosin was adjusted to 2.5 mg/ml with KCL buffer and held at 5°C for 30 minutes. Samples (1.8 ml) were centrifuged at 12,000 rpm at 4°C and the viscosity of the supernatant was determined at 5°C with a Wells - Brookfield microviscometer model LVT at a spindle speed of 6 rpm. For certain studies the samples were preincubated at temperatures higher than 5°C in order to determine thermal stability of actomyosin.

7. ATPASE -

The protein adjusted actomyosin (2.5 mg/ml) was dialyzed against 0.6N KCl, 4°C to remove buffer. A 1 ml aliquot as assayed in 9.6 ml of either activating or relaxing reaction mixtures. Activating reaction mixture contained 0.05N KCl, 4 mM $MgCl_2$, and 0.25 M $CaCl_2$, pH 7.4. Relaxing reaction mixture contained 0.05N KCl, 4 mM $MgCl_2$, and 1 mM EGTA, pH 7.4 The reaction was started by adding 0.4 ml 100 mMATP - $MgCl_2$. The raction mixture was titrated with 0.01 N KOH to maintain the pH at 7.4 using a Metrohm Herisau Dosimat and Impulsomat at 5°C. From the KOH titer, the equivalents of protons formed from ATP (Adenosine Triphosphate) hydrolysis were calculated. The same procedure was used to measure muscle ATPase, where approximately 0.2 g muscle replaced the actomyosin solution.

Samples of muscle (1 g), free of mycommata connective tissue, were mixed with 9 ml sodium dodecylsulfate, polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, boiled for 15 minutes and homogenized to extract protein. The protein was separated from insoluble matter by centrifugation of the mixture at 5,000 x g for 15 minutes. Electrophoresis was performed on Laemmli slab gel samples (18 x 20 cm) for 6 hours at 35 ma per plate using a BioRad Protean II unit and manufacturers instructions.

RESULTS

Influence of storage temperature on actomyosin denaturation - A faster rate of actomyosin denaturation occurred during storage of cod fillets at -3 ± 1.0°C than during storage at 0 ± 0.2°C (Figure 1, commercial fish; Figure 2, tank fish). Surimi prepared from fish held on ice for several days had lower gel strength than that from fish which was freshly harvested. Measurement of the apparent viscosity of actomyosin from surimi indicated the actomyosin fraction from surimi with low gel strength was denatured (see below). These results differ from those of a previous study which showed that a lower rate of actomyosin denaturation occurred during storage of cod muscle at -3 ± 0.1°C compared to 0 ± 0.2°C (Simpson and Haard 1986). Hence it appears that the benefit of partial freezing, with respect to minimizing protein denaturation, is dependent on ability to stringently control the temperature of the storage environment, e.g. ±0.1°C. Due to the high cost of currently available freezers which can meet this rigorous temperature control, the use of partial freezing to extend the useful storage life of cod for surimi manufacture would not appear to be practical.

Influence of sea water temperature and season of catch on actomyosin denaturation during storage on ice - A faster rate of actomyosin denaturation occurred during ice storage of fish harvested in July than in fish harvested in February. When fish which were harvested in July were held live in an aquarium at 0°C for 3 - 4 weeks prior to sacrifice, the rate of post-mortem actomyosin denaturation was slower and like that of fish harvested in February (Figure 3A). For bled cod harvested from a tank in July - August and held on ice for more than 5 - 7 days the resulting surimi did not exhibit an "AA" fold test; whereas a high surimi gel strength was obtained with cod harvested from tanks at ambient sea water temperature in February and held on ice for as long as 12 days (Figure 4A). These results indicate that cod harvested from grounds which have a low water temperature have a longer acceptable ice time for use in surimi than cod harvested from fishing areas which have a higher water temperature. Cold acclimated cod also appear to have a higher concentration of extractable actomyosin prior to post-mortem storage (Table 1) and would therefore be expected to yield more surimi per mass of mince or fillet.

Influence of bleeding cod at harvest time on actomyosin denaturation during ice storage - A slower rate of actomyosin denaturation occurred during storage of cod fillet (fish harvested in July - August) on ice when they were bled at the time of sacrifice (Figure 5). Again, slower rate of actomyosin denaturation was associated with a slower rate of loss of surimi gel strength. Similar results were also obtained with fish harvested from tanks held at ambient sea water temperature in February (Figure 4B). Graduate student (PHD., Memorial University of Newfoundland) Mike Hawco has

shown that in vitro mixing of cod fish blood with extracted cod fish actomyosin results in rapid denaturation of the actomyosin solution (M. Hawco PERS. COMM). Therefore it appears that blood may act to directly accelerate protein denaturation in post-mortem cod. The results indicate that the useful ice storage life of cod used for surimi manufacture may be extended by as much as 50 to 100 per cent by bleeding fish at harvest. Additional work is needed to verify these results with fish harvested and bled by fishermen and processed with the pilot surimi line at Newfoundland and Labrador Institute of Fisheries and marine Technology.

Molecular basis of actomyosin denaturation during ice storage of post-mortem cod - Extractability of actomyosin from cod muscle decreased gradually as a function of ice time. This appears to be associated with non-covalent aggregation of the actomyosin since the protein which becomes insoluble in buffered salt solution is readily solubilized in solvent containing detergent and is apparently not different from native cod actomyosin. A more sensitive indication of actomyosin denaturation than loss of salt extractable protein is the apparent viscosity of the extractable actomyosin. Extractable actomyosin was prepared from either stored cod fillet or its surimi product. Loss in surimi gel strength was highly correlated with a decline in the apparent viscosity of extractable actomyosin (Figures 6 - 8). Actomyosin viscosity appears to be a much more sensitive indicator of post-mortem denaturation of actomyosin than is the fold test.

The molecular size of cod myosin appears to increase slightly during storage of fish on ice as evidenced by the broader, somewhat faster moving band representing cod myosin (Figures 9 and 10). This may indicate limited proteolytic hydrolysis of myosin occurs during ice storage. The average molecular size of myosin decreased from approximately 185,000 daltons to approximately 165,000 daltons during 11 days storage. The second major protein in cod surimi, actin, does not appear to change during ice storage. Other changes in muscle protein were noted (see bands 1, 2 and 3, Figures 9 and 10). A 68,000 dalton protein (band #1) and a 35,000 dalton protein (band #3) gradually disappeared during 11 days storage on ice. A 37,000 dalton protein (band #2) gradually increased during this same storage time. This experiment indicates that the decline in actomyosin viscosity is not directly caused by the gross hydrolysis of myosin or by the formation of myosin or actomyosin aggregates stabilized by covalent cross linkages. It is possible that the relatively minor hydrolysis of proteins may be directly related to the denaturation of myosin in muscle. Denaturation, (unfolding or aggregation), would not be detected by gel electrophoresis of muscle proteins because the detergents employed denature the protein. However, sedimentation velocity of denatured actomyosin (low viscosity) showed that the apparent molecular size of denatured actomyosin is greater than that of native actomyosin. From these experiments we can conclude that the decline in viscosity and gel-forming ability of cod actomyosin, that occurs during storage of the fish on ice, is directly related to denaturation - aggregation phenomena which involve non-covalent interactions. In other words, the loss in actomyosin viscosity does not

appear to involve covalent cross linkage (e.g. from formation of formaldehyde from trimethylamine oxidate reduction) nor does it appear to be the direct result of protein hydrolysis leading to a large change in the molecular size of heavy chain myosin.

Relationship between ATPase activity and actomyosin denaturation during storage of cod in ice - Skeletal muscle contains a myriad of different enzymes which catalyze hydrolysis of adenosine triphosphate (ATP). Muscle ATPase activity includes enzymes involved with the transport of ions and other solute molecules across cell membranes, notably those of the sarcoplasmic reticulum and mitochondrion which function in the transport of calcium ions. Pure myosin is also an ATPase and its activity requires Ca^{+2} and is inhibited by Mg^{+2}. The myosin of actomyosin also has ATPase activity, however its activity is normally stimulated by Mg^{+2}. Protein denaturation is normally accompanied by loss in biochemical function, i.e. loss of enzyme activity. Previous studies in Japan have shown that myosin or actomyosin ATPase activity of Alaska pollack or Peruvian hake appears to be a useful indicator of the gel-forming ability of surimi. We have studied the activity of various ATPase fractions isolated from cod muscle as a function of ice storage time to get insight into whether or not the ATPase activity of intact muscle could be used as a means of evaluating the extent of actomyosin denaturation. Our main objectives were to develop a kinetic method to measure ATPase activity of intact muscle tissue and to determine whether muscle ATPase activity is an indicator of actomyosin denaturation.

A pH stat method was found to be a simple kinetic method for measuring ATPase activity of intact cod muscle. The reaction of the hydrolysis of ATP is as follows:

$$ATP + H_2O \text{ ---- } ADP + Pi + H^+$$

ADP = ADENOSINE DIPHOSPHATE

Pi = INORGANIC PHOSPHATE

The principle of this assay system is that the formation of protons during ATP hydrolysis can be measured from the equivalents of base required to maintain the pH. An advantage of this method over other end point methods, i.e. which measure Pi formed, is that the reaction can be monitored directly and continuously and is therefore amenable to analysis of ATPase by tissue surface sensor techniques. A limitation of this method is the marked decrease in sensitivity below pH 7 because of the buffering capacity of proteins in this pH range.

Myosin was purified from prerigor cod muscle by a modification of Connell's method. The Ca^{+2} activated ATPase activity of the purified myosin decreased by about 90% over 18 days storage at 4°C (Figure 11). The Mg^{+2} activated activity of this enzyme was approximately 0.25 that of the Ca^{+2} activated activity and decreased at a slower rate during storage.

Sarcoplasmic ATPase, soluble in low ionic strength buffer, was isolated from cod which had been glycerinated prior to analysis. The major component of sarcoplasmic ATPase is probably from the sarcoplasmic reticulum. Sarcoplasmic ATPase activity from cod held on ice increased

approximately five fold during the first week of storage and thereafter decreased (Figure 12). The Ca^{+2} activated and Mg^{+2} activated ATPase activity of this fraction did not change in parallel with loss in gel strength of surimi prepared from the same lots of fish.

Ca^{+2} - ATPase activity of actomyosin extracted from cod held on ice for up to 21 days declined rapdily during the late stages of storage but did not appear to be a sensitive indicator of actomyosin denaturation as judged by viscosity (Figures 13B and 14B). The data indicate that Ca^{+2} - ATPase of extracted actomyosin is not a sensitive indicator of gel strength potential.

The Ca^{+2} activated and Mg^{+2} - ATPase activity of glycerinated cod muscle was also measured and these results suggested a close parallel between actomyosin viscosity and the ratio of Ca/Mg ATPase activity during 8 days storage of cod on ice (Figure 15B).

The Ca^{+2} activated ATPase activity of untreated and intact muscle sections from bled cod stored for up to 9 days on ice showed a high correlation with actomyosin viscosity (r^2 = 0.995). During storage of bled cod for up to 9 days the viscosity of actomyosin declined gradually from about 24 cps to about 16 cps and Ca^{+2} activated ATPase activity declined from 9.52 and 2.32 umoles Pi/min - g tissue (Figure 13A). However, during subsequent storage of cod from 9 to 21 days, the apparent viscosity of actomyosin decreased more rapidly, from 16 to 3 cps while the Ca^{+2} activated ATPase activity increased from 2.32 to 7.83 umoles Pi/min - g tissue. Regression analysis of apparent viscosity as a function of ATPase over 21 days storage gave an r^2 = 0.002.

The pattern of whole tissue Mg^{+2} - ATPase over 21 days storage was similar to that of Ca^{+2} - ATPase, although it did not correlate with viscosity as well as Ca^{+2} - ATPase during 9 days storage (r^2 = 0.504) it correlated better than 21 days storage (r^2 = 0.327).

Multiple regression analysis with viscosity as the dependent variable and Ca^{+2} - ATPase and Mg^{+2} - ATPase as independent variables for 9 days or 21 days gave better fits, r^2 = 0.998 and 0.422 respectively.

These data indicate Ca^{+2} - ATPase activity may be a sensitive early indicator of myosin denaturation during the storage of bled cod on ice.

A study to determine the relationship between whole tissue ATPase and actomyosin viscosity during storage of unbled cod on ice is summarized in Figure 14A. The results indicate a similar, although more stable, pattern of ATPase activity change during storage. The earlier decline in actomyosin viscosity seen with bled cod (after 6 days, Figure 14) in contrast to unbled cod (after 13 days, Figure 13) did not appear to be associated with a more rapid initial decline in ATPase activity. As a result, the good correlation between actomyosin viscosity and muscle ATPase observed for bled cod may be more coincidental rather than based on a direct relationship to actomyosin denaturation.

A preliminary study was determined to test an ATPase method with a surface electrode on an intact fillet by pH shift. The results indicate a time dependent decline in pH after addition of ATP (Figure 16). A method like this may be amenable to dockside grading.

Thermal stability of cod actomyosin - Preincubation of cod actomyosin at various temperatures prior to measurement of viscosity at 5°C indicated the temperature at which denaturation occurs, as evidenced by a marked decline in viscosity, decreased with post-mortem storage time of the raw material. For example, for actomyosin from fresh cod, the denaturation temperature is about 20°C while it is about 10°C for actomyosin from cod held on ice for 8 days (Figure 17). This finding may indicate that temperature control during surimi processing of raw material which is less than very fresh may be very important.

CONCLUSIONS

1. Atlantic cod is a very durable raw material for surimi processing. It is possible to prepare a good yield of "AA" kamaboko from surimi prepared by laboratory procedures from bled summer cod held on ice for up to 7 days and from bled winter cod held on ice for up to 12 days. it will be important to verify these results with pilot scale lots of cod since the ability to dewater and the yield of surimi from aged raw material may be much lower under commercial or pilot scale conditions of dewatering.

2. Cod fillet held at 0 ± 0.2°C makes better quality surimi than cod held at -3 ± 1°C. It is likely the oppposite results would be obtained with better temperature control at -3°C using partial freezing chambers now commercially available in Japan.

3. Cod which were bled prior to storage of their fillets at 0°C maintain better quality (approximately 50% longer) for surimi production than cod which were not bled. These observations are interesting and may be of practical importance, but they will need to be investigated by holding bled and unbled round or dressed cod on ice for various times prior to preparation of surimi with the pilot line.

4. The apparent viscosity of a simple actomyosin extract from cod muscle or surimi is a sensitive indicator of kamaboko making potential of cod surimi. While there is some indication that Ca^{+2} - ATPase of whole muscle is an indicator of surimi gel strength potential the data are quite complex and, at this time, ATPase does not appear to be a promising predictor of gel strength potential. Additional research may show that under appropriate conditions of assay, ATPase can be used as a predictor of surimi quality.

5. Proteolytic hydrolysis of cod muscle proteins does not appear to play a significant direct role in loss in gel strength potential. There appears to be a decrease in the molecular size of myosin and major changes occur in other minor muscle proteins. The possibility that proteolysis is indirectly involved with the loss in gel strength potential cannot be ruled out at this time. However, the loss in actomyosin viscosity which is associated with the loss in gel strength potential appears to be directly related to aggregation of these proteins.

TABLE 1. Influence of Acclimation Temperature Prior to Sacrifice of the Chemical Composition of Muscle.

Tank Temp.	Moisture	Ash	Crude Prot	Extract Prot	Free A.A.	TMAO
°C	%	%	%	%	mg%	mg%
10	81.36±.57 [a]	1.26±.09 [a]	16.20±.79 [a]	9.71±.21 [a]	340 [a]	416 [a]
0	81.03±.22 [b]	1.40±.03 [a]	18.70±.62 [a]	12.70±.45 [a]	500 [a]	415 [b]
12(July) [2]	–	–	16.88±.59 [b]	9.87±.46 [b]	–	–
2(Feb) [2]	–	–	18.25±.86 [b]	13.20±.58 [b]	–	–

[1] Average of four determinations, except TMAO is average of two determinations. Values in column followed by same letter are significantly different($P<0.05$)

[2] Ambient sea water temperature: 12°C in July and −2°C in February

FIGURE 1. **Fillet Storage Time vs Fold Test**

"Commercial" Atlantic Cod

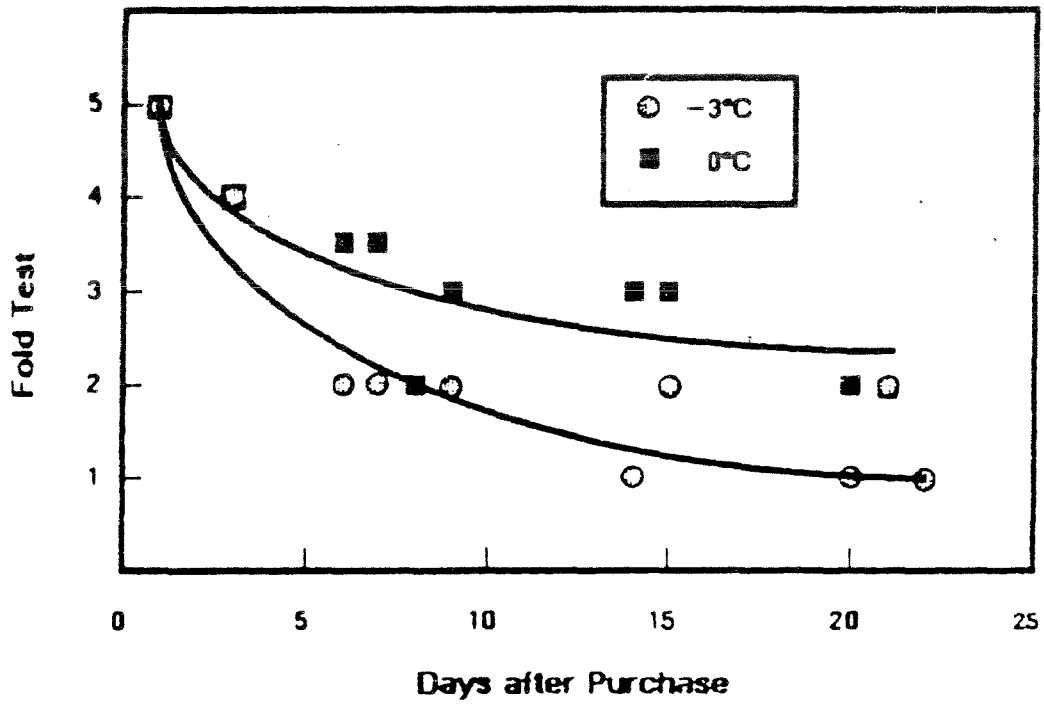
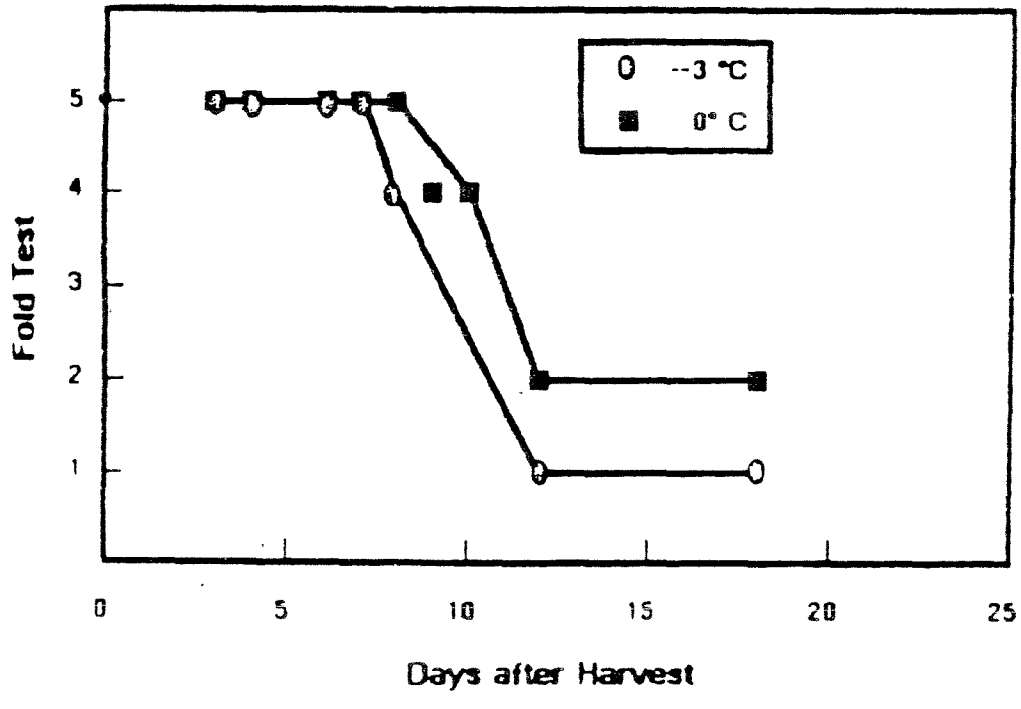


FIGURE 2. **Fillet Storage Time vs Fold Test**

Tank Fish, Bled & Iced at Harvest



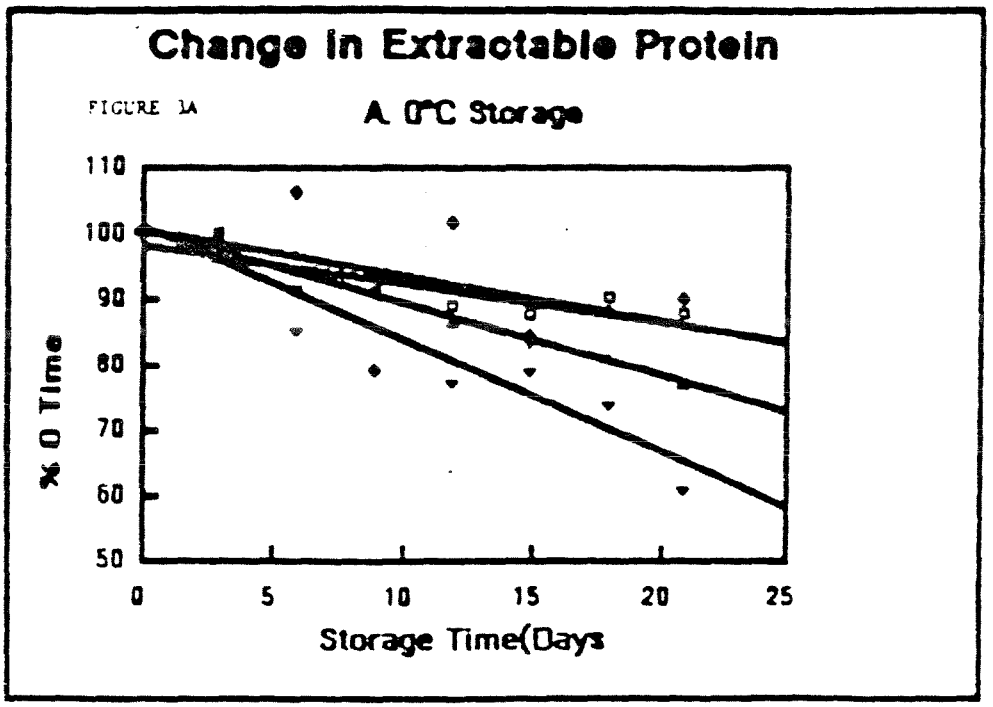


FIGURE 1A

10°C Acclimated
Sarcoplasmic
Myofibril

0°C Acclimated
Sarcoplasmic
Myofibril

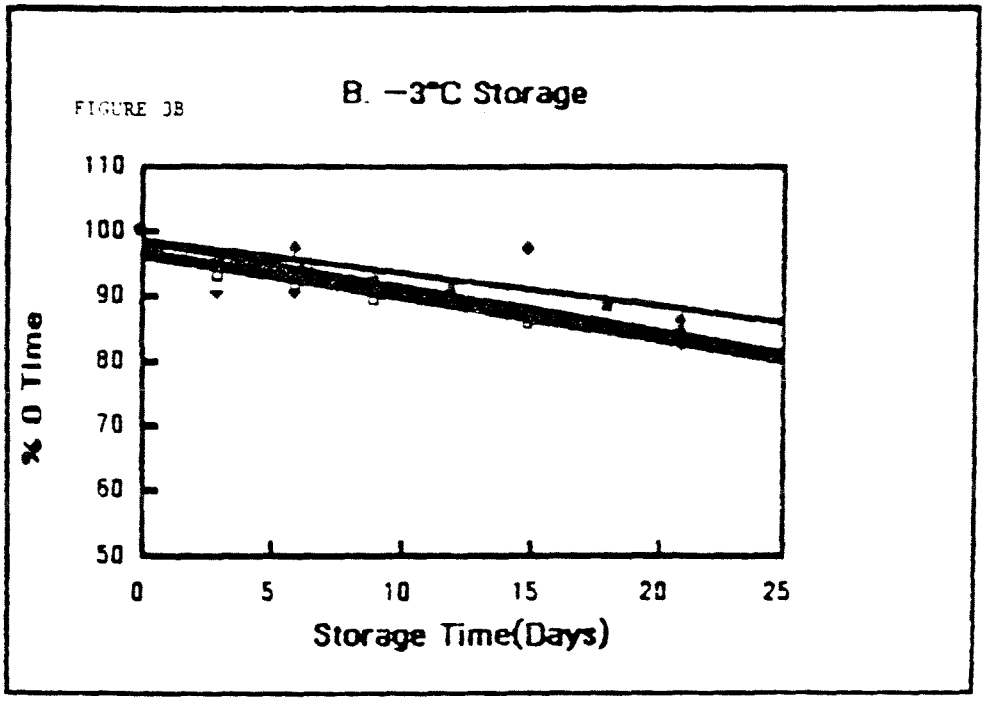


FIGURE 1B

FIGURE 4A.

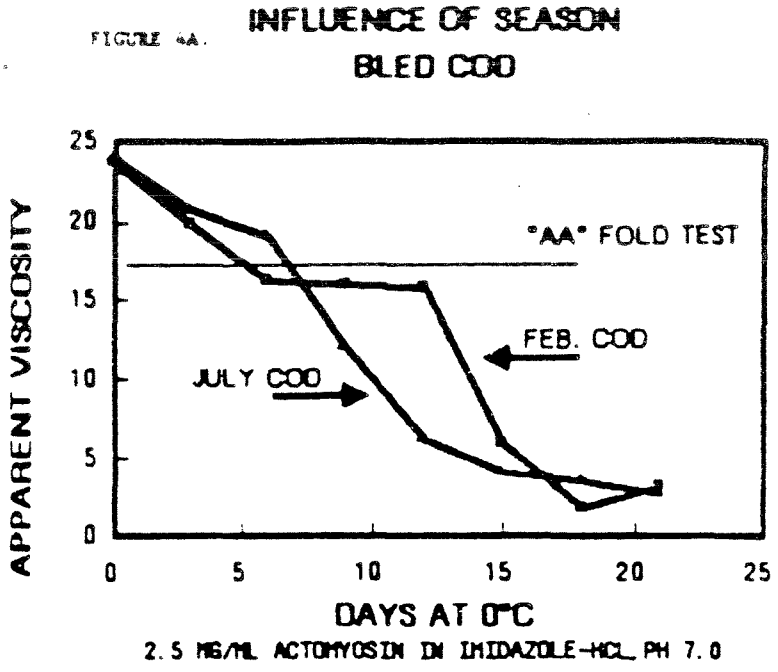


FIGURE 4B.

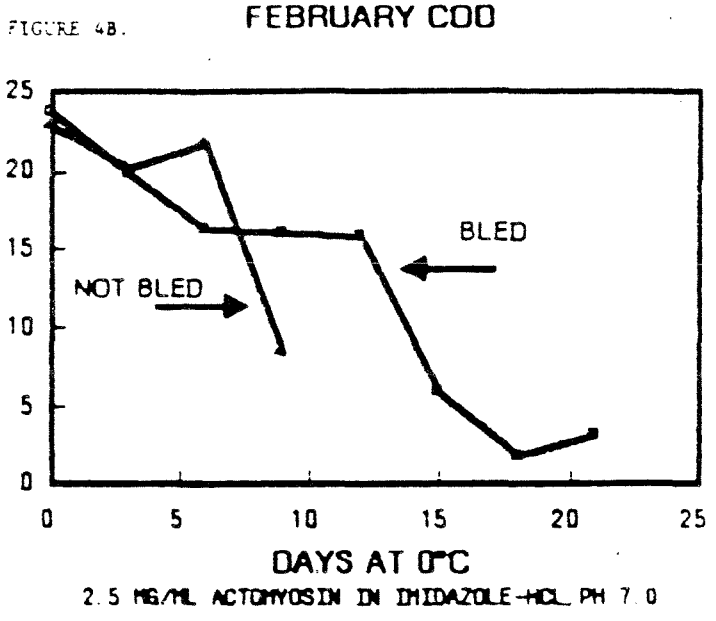


FIGURE 5. **Fillet Storage Time vs Fold Test**

Tank Fish, Bled vs Not Bled

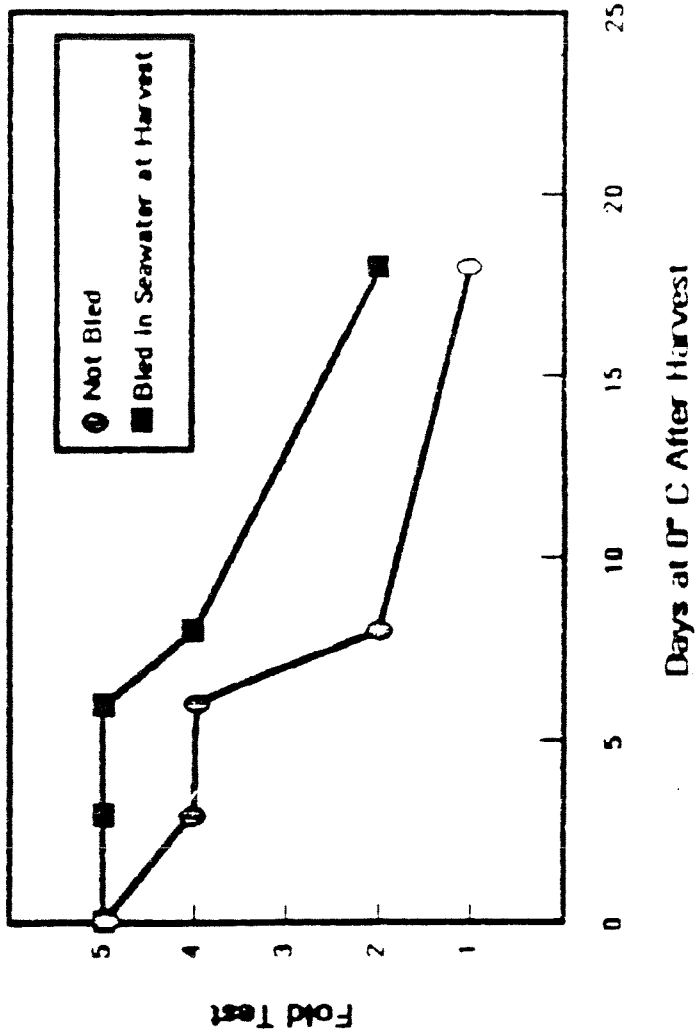


FIGURE 6. **Actomyosin Viscosity vs Fold Test**

Surimi from Atlantic Cod of Varying Postmortem Age

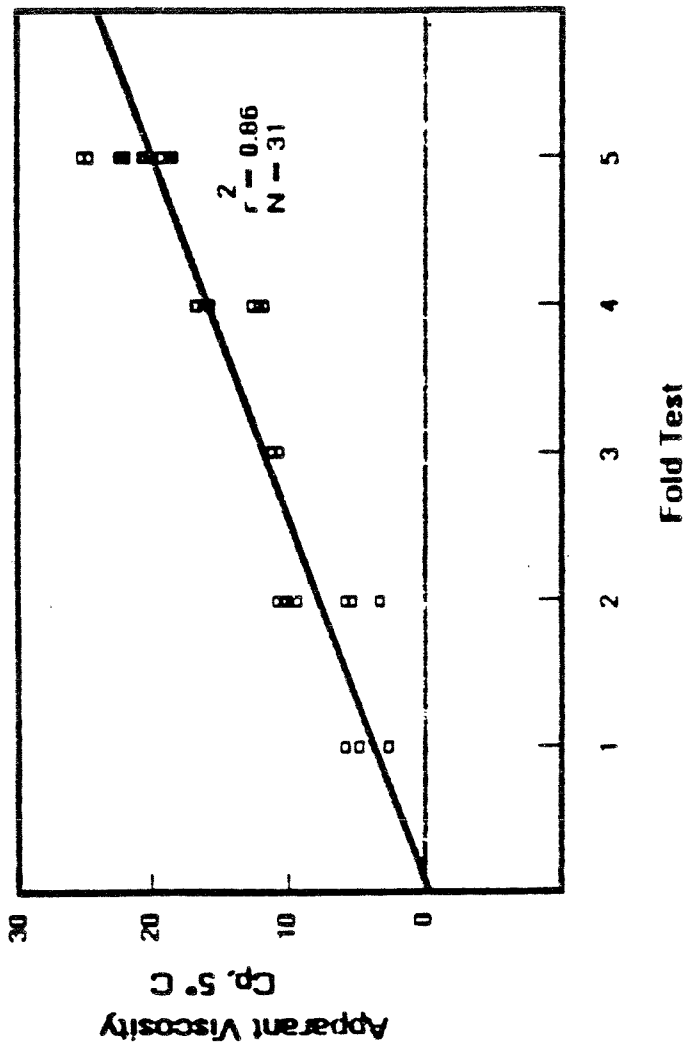


FIGURE 7.

Actomyosin Viscosity & Fold Test vs Fillet Storage

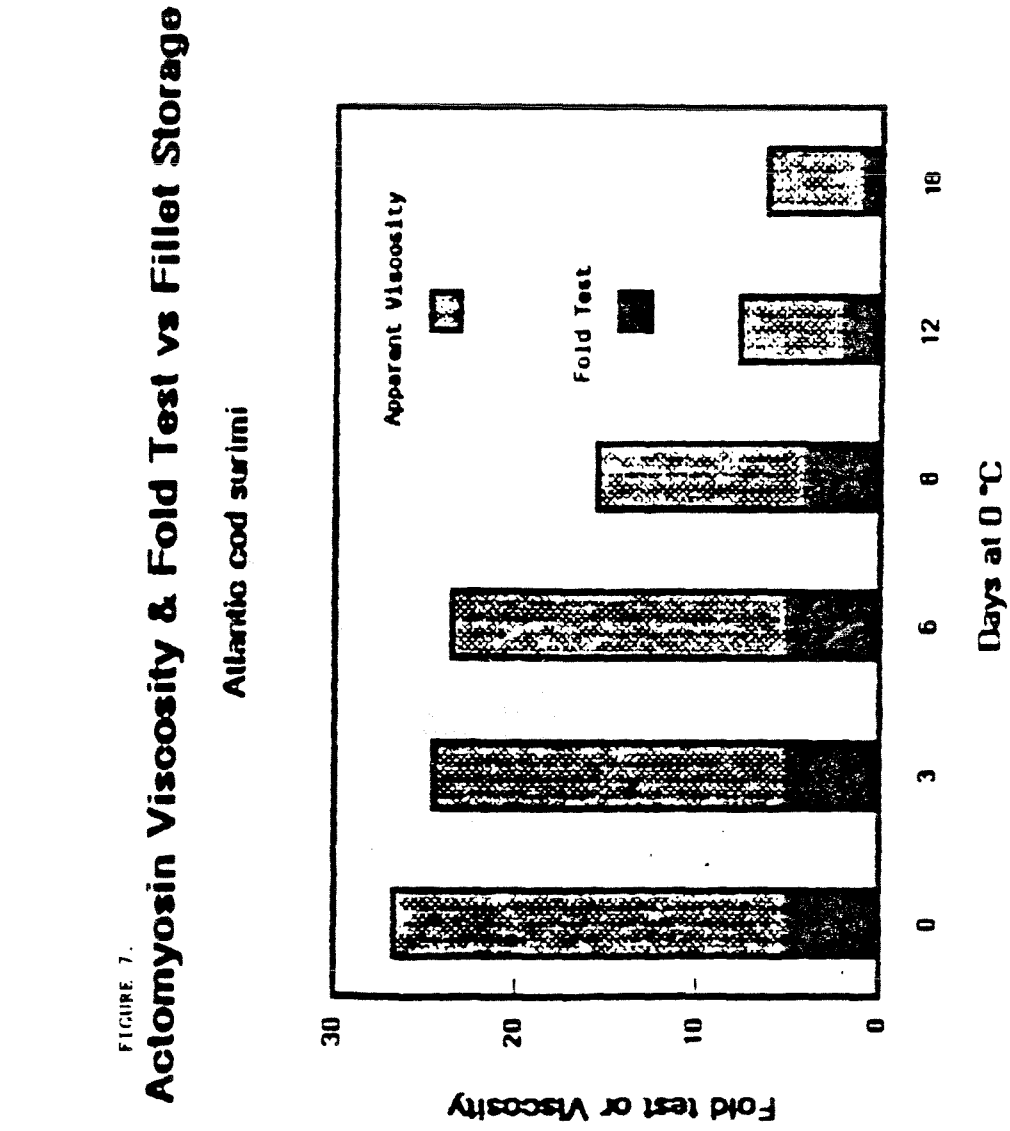
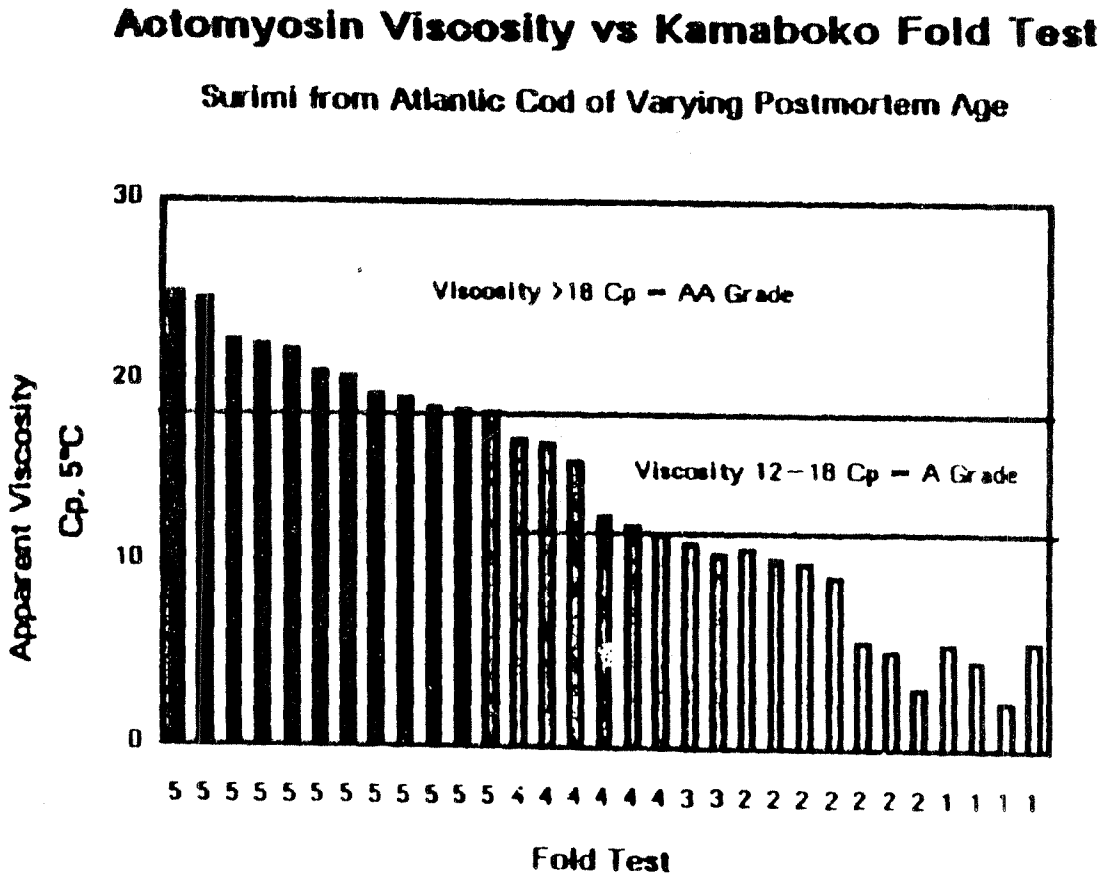


FIGURE 8.


Actomyosin Viscosity vs Kamaboko Fold Test
Surimi from Atlantic Cod of Varying Postmortem Age
Apparent Viscosity
Cp, 5°C
30
20
10
0
Viscosity >18 Cp = AA Grade
Viscosity 12-18 Cp = A Grade
5 5 5 5 5 5 5 5 5 5 5 5 4 4 4 4 4 4 3 3 2 2 2 2 2 2 2 1 1 1 1
Fold Test

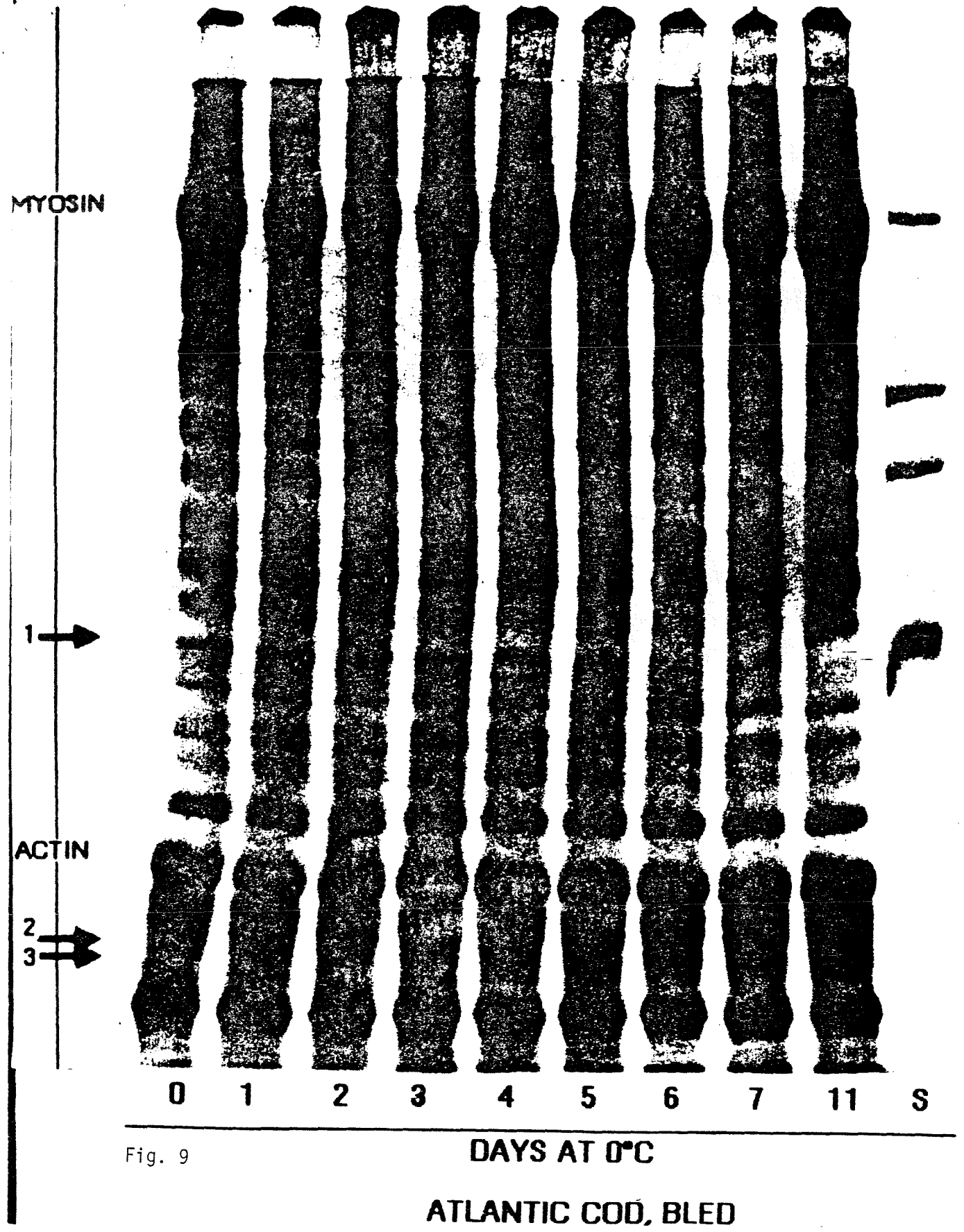


Fig. 9

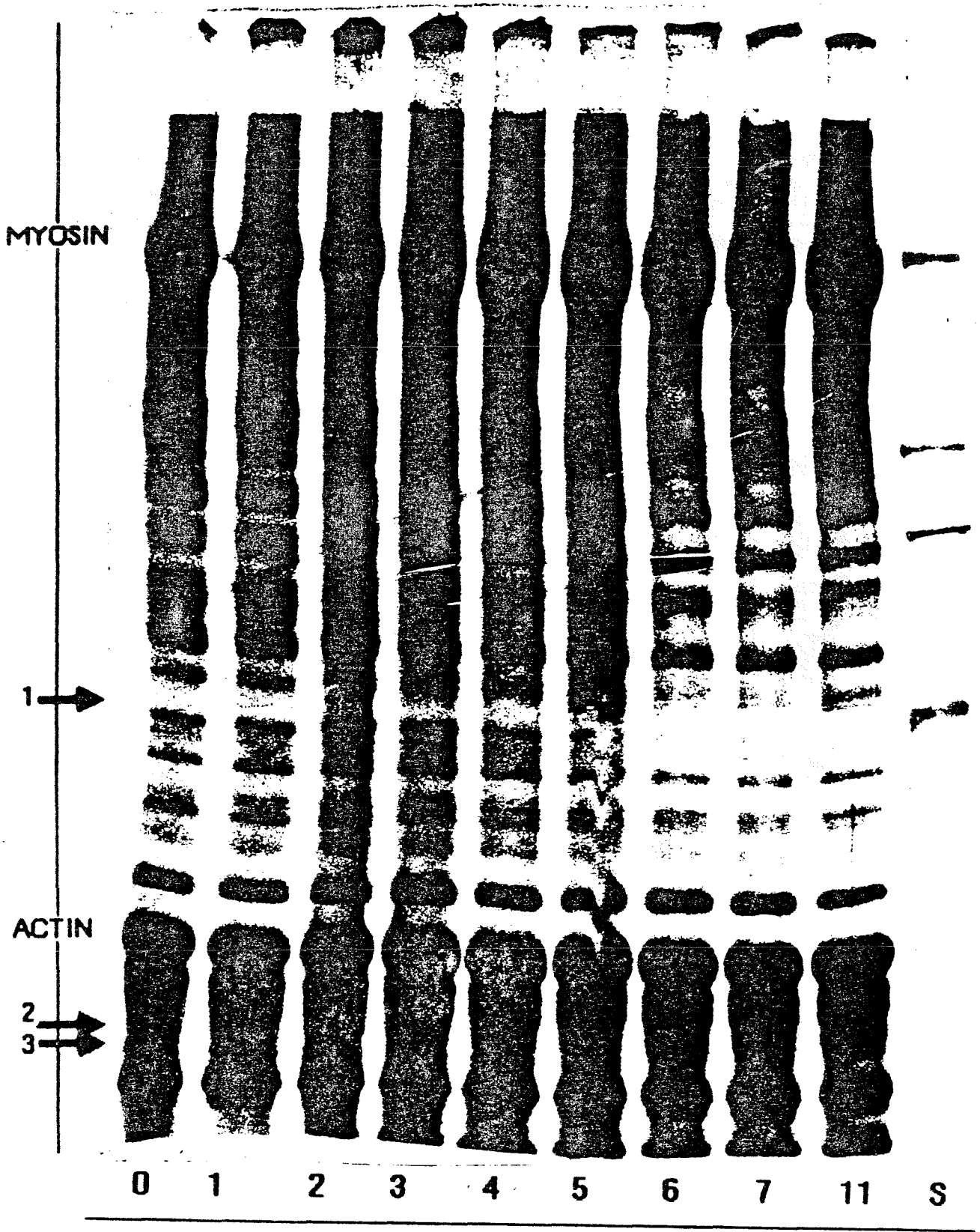
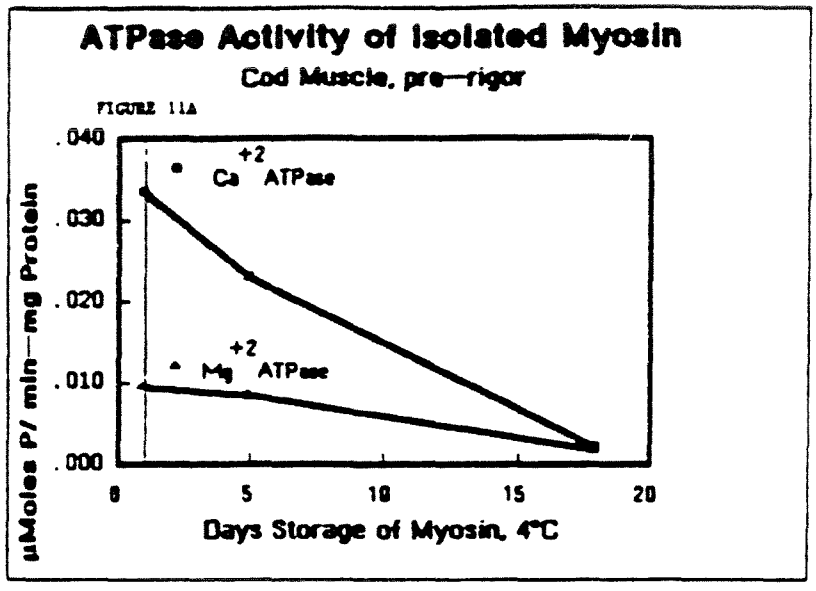


Fig. 10

ATLANTIC COD, NOT BLED


ATPase Activity of Isolated Myosin
Cod Muscle, pre—rigor
FIGURE 11A
.040
.030
.020
.010
.000
+2
Ca ATPase
+2
Mg ATPase
µMoles P/ min—mg Protein
0
5
10
15
20
Days Storage of Myosin, 4°C

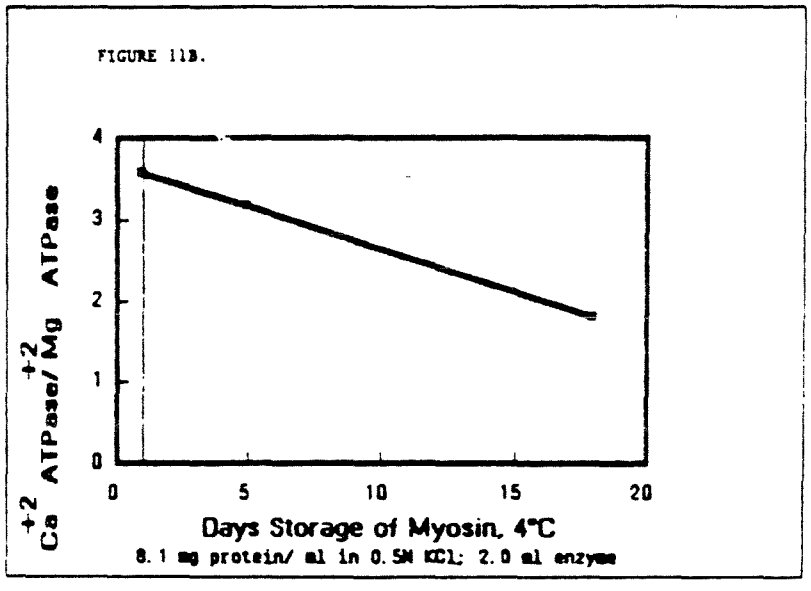
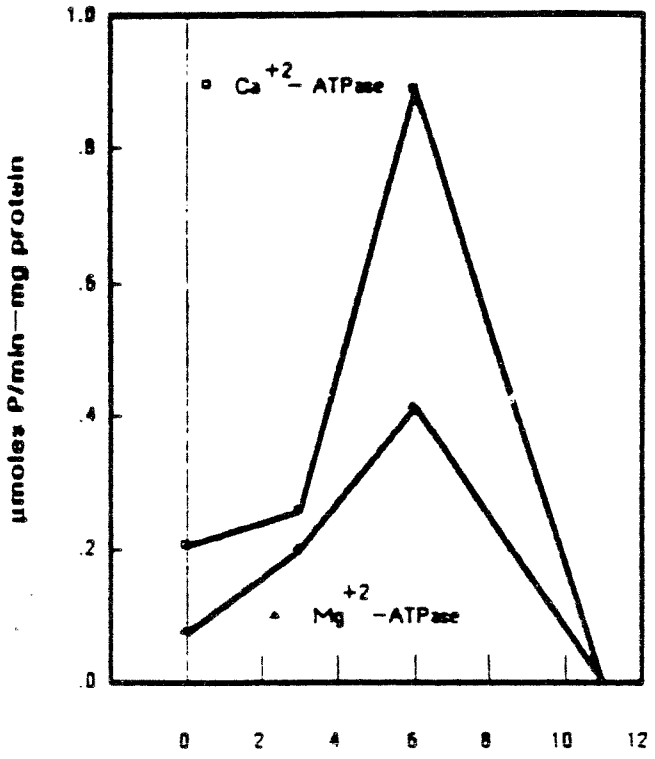


FIGURE 11B.
4
3
2
1
0
+2 +2
Ca ATPase/ Mg ATPase
0
5
10
15
20
Days Storage of Myosin, 4°C
8.1 mg protein/ ml in 0.5M KCl; 2.0 ml enzyme

FIGURE 12. **Sarcoplasmic ATPase**

Glycerinated Muscle

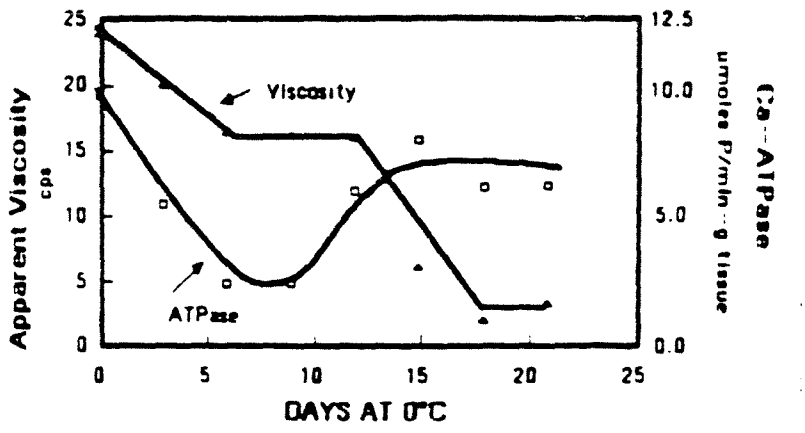


Storage(Days) on Ice prior to glycerination

Bled Cod

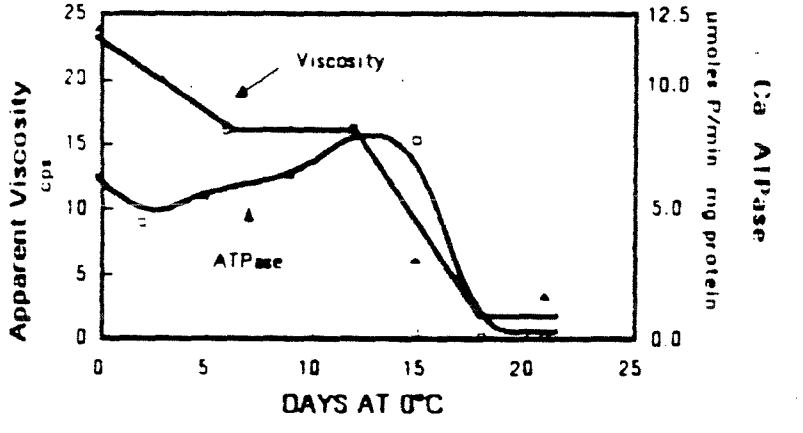
Actomyosin Viscosity vs Muscle ATPase

FIGURE 13A. BLED COD



Actomyosin Viscosity vs Actomyosin ATPase

FIGURE 13B. BLED COD



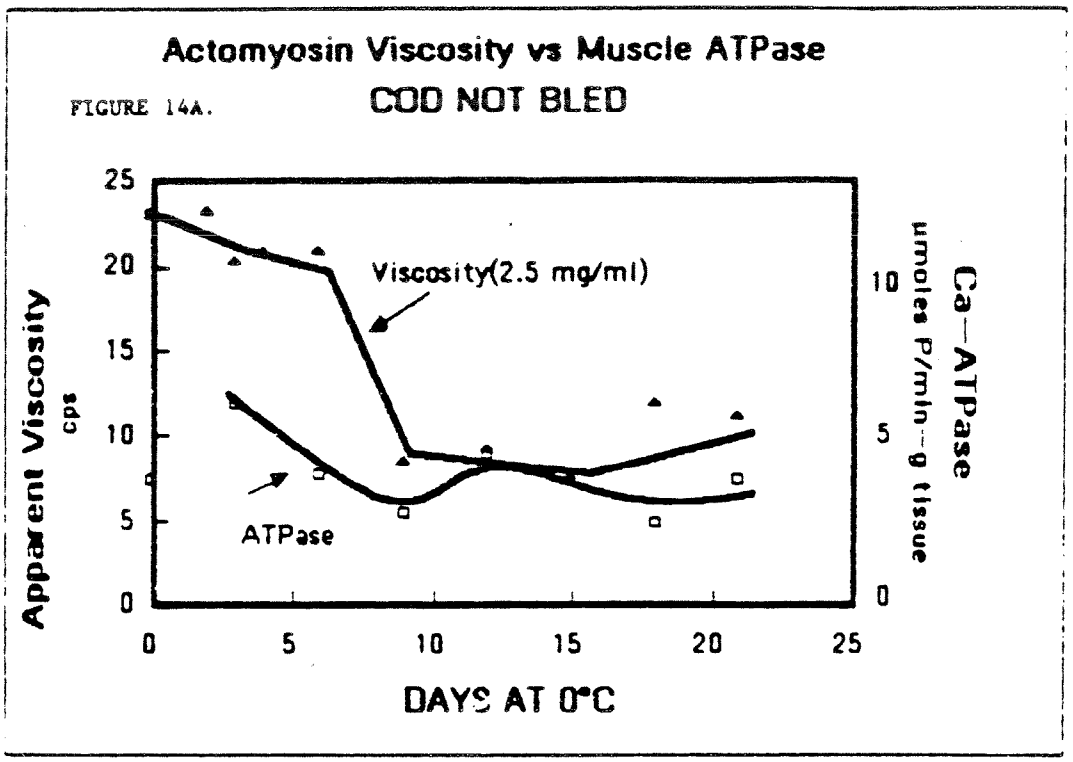


FIGURE 14A.

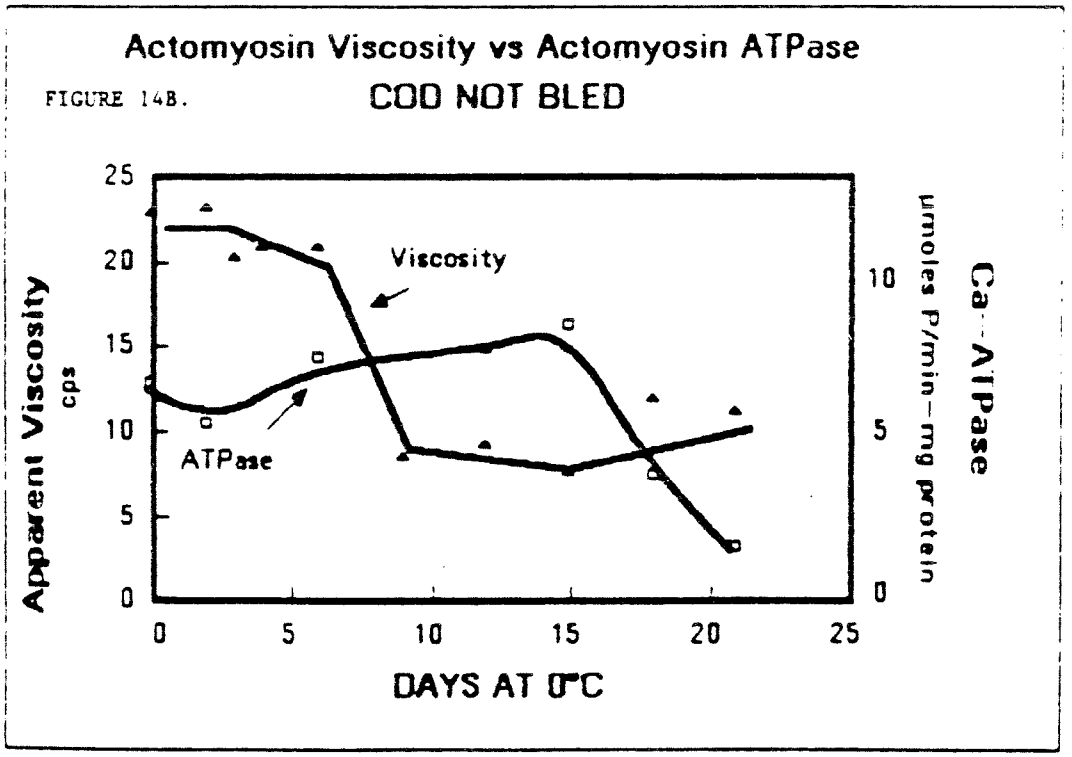


FIGURE 14B.

FIGURE 15A.

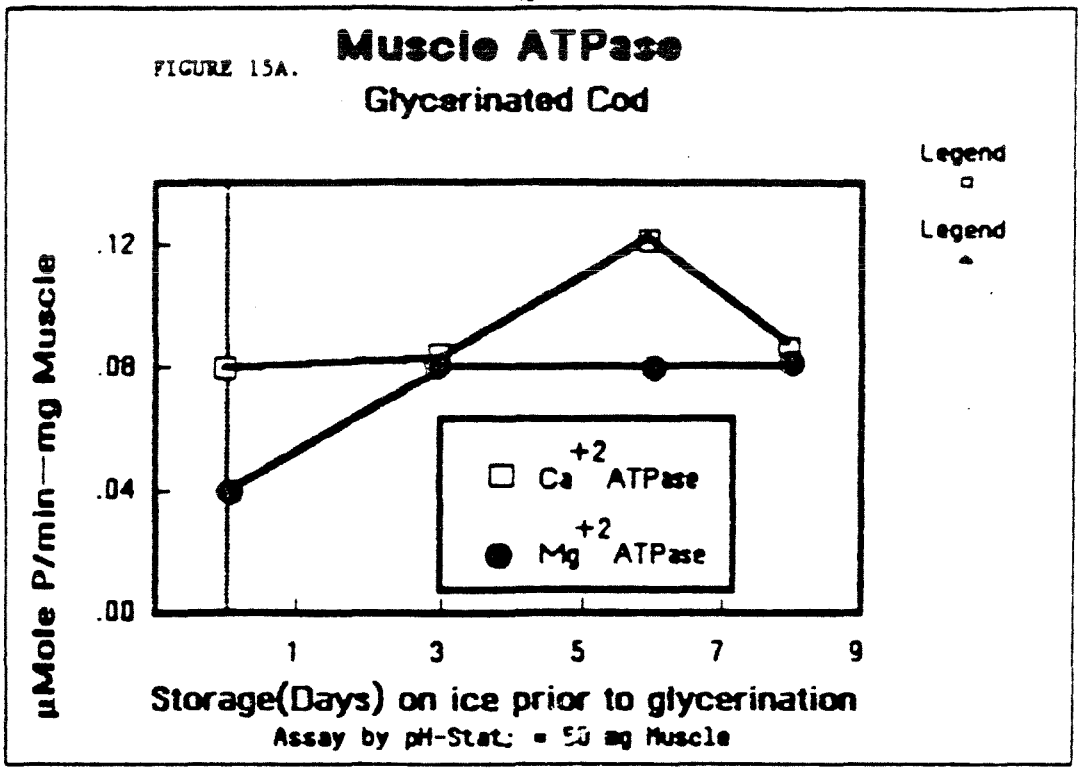
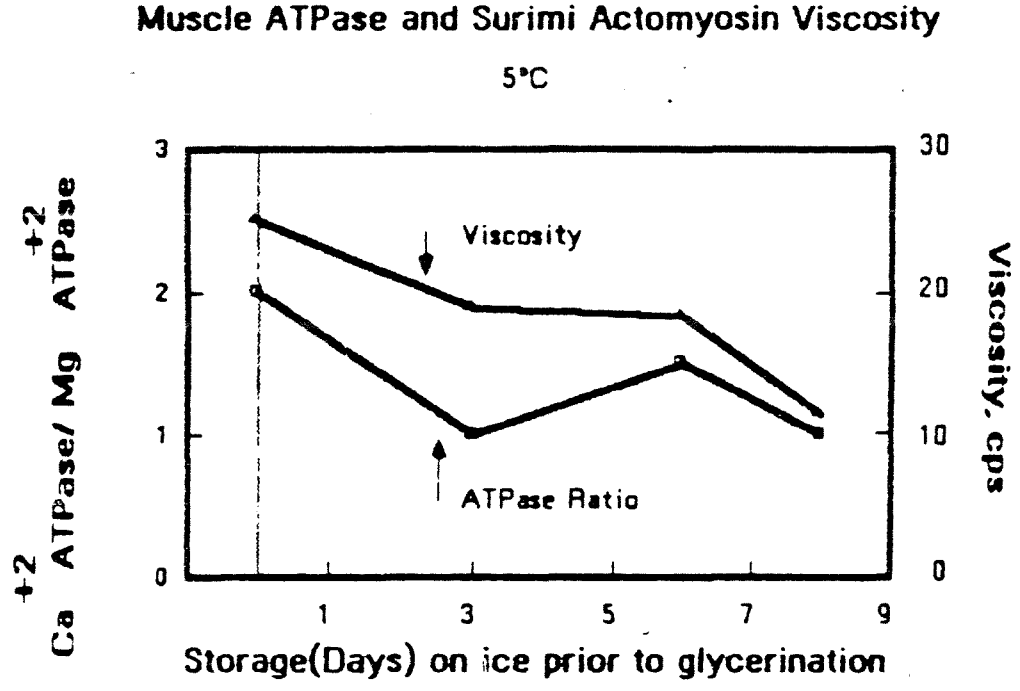


FIGURE 15B.



Assay of Muscle ATPase with a Surface Electrode

FIGURE 16.

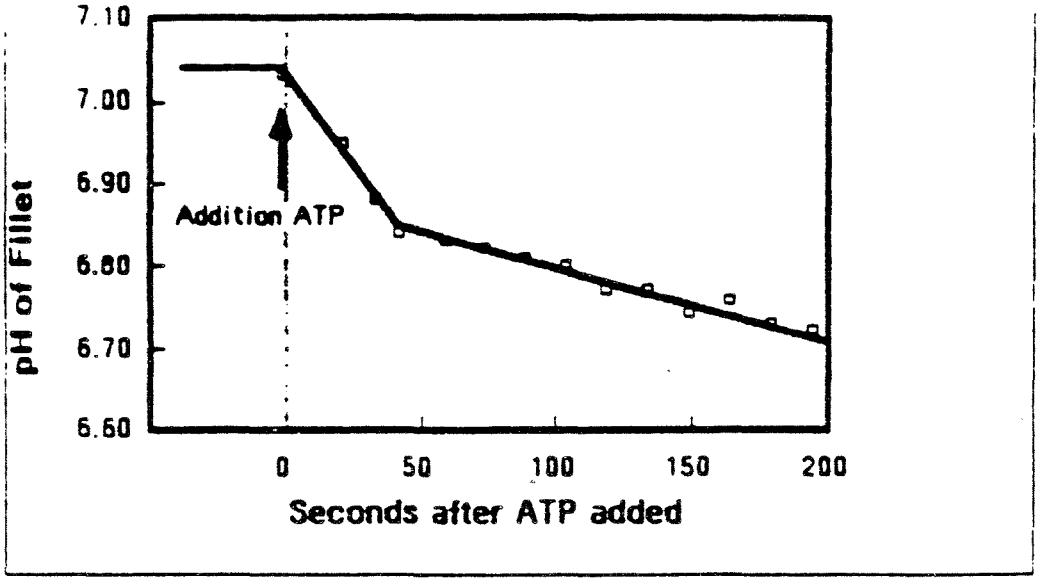
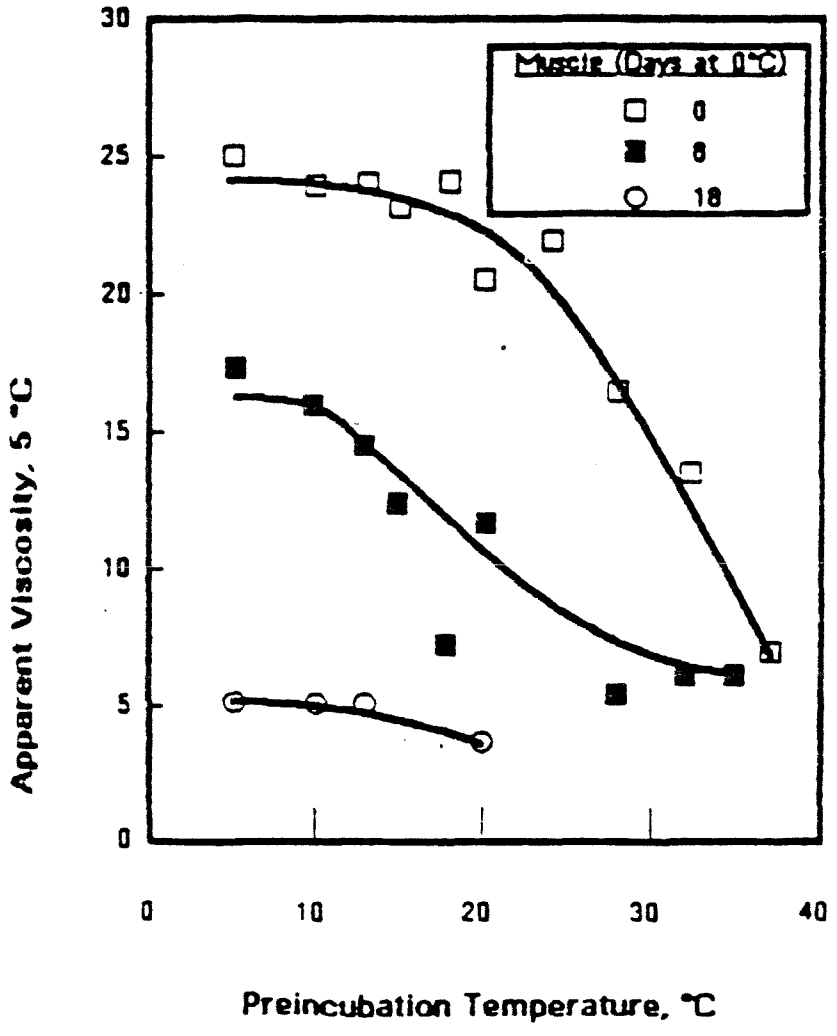


FIGURE 17.

Thermal Instability of Surimi Actomyosin



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Simpson, M. and N.F. Haard. 1986. J. Food Biochem., submitted.