

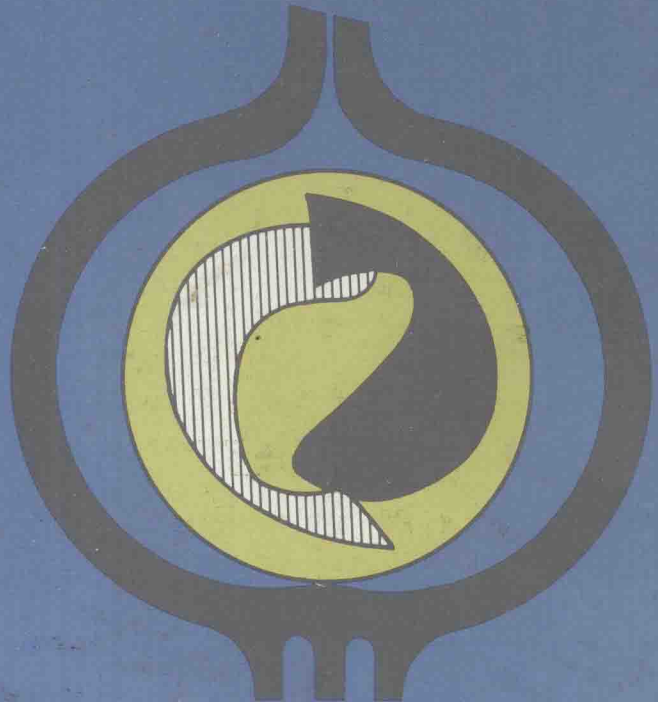
Developments in Food Science

15

**SEAFOOD QUALITY
DETERMINATION**

Edited by

**DONALD E. KRAMER
JOHN LISTON**



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SEAFOOD QUALITY DETERMINATION

**Proceedings of the International
Symposium on Seafood Quality
Determination, Coordinated by the
University of Alaska Sea Grant
College Program, Anchorage, Alaska,
U.S.A., 10-14 November 1986**

Edited by

DONALD E. KRAMER

*Marine Advisory Program, University of Alaska,
P.O. Box 103160, Anchorage, AK 99501, U.S.A.*

JOHN LISTON

*Institute for Food Science & Technology, University of Washington,
HF-10, Seattle, WA 98195, U.S.A.*

With the assistance of

T. Frady (Managing Editor) and **Brenda R. Melteff** (Symposium Coordinator)
University of Alaska Sea Grant College Program



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SEAFOOD QUALITY DETERMINATION

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FOREWORD

The University of Alaska Sea Grant College Program was very pleased to host the International Symposium on Seafood Quality Determination in Anchorage, Alaska, November 10-14, 1986. I would especially like to commend the symposium coordinator Brenda Melteff for an outstanding effort in putting this conference together.

The symposium brought researchers and industry representatives together to form an international forum to discuss what constitutes quality in seafood, how quality can be measured, and what standards are available for fishery products. The nutritional aspects of seafood products as well as public health concerns were included.

The papers in this book are organized under the same nine topic areas used to structure sessions at the meeting: instrumental measurements of seafood quality, sensory evaluation of seafood quality, chemical and biochemical indices of seafoods, microbiological evaluation of seafood quality, public health hazards associated with seafood, shelf life estimation for seafood, nutritional aspects of seafood quality, consumer perception of seafood quality, and seafood quality standards.

The symposium began with welcoming remarks by Marvin O. Looney, chancellor of the Community Colleges, Rural Education, and Extension Division of the University of Alaska. Roy E. Martin, vice president for science and technology at the National Fisheries Institute, gave the introductory address in which he discussed different aspects of quality: wholesomeness, integrity, and freshness. His remarks were referred to many times by other symposium speakers.

In addition to the papers published in this book, the symposium included a sensory evaluation workshop with both lecture and a hands-on demonstration coordinated by H. Richard Throm of the U.S. Food and Drug Administration (FDA).

In the lecture portion, Throm introduced the participants to the FDA classification system of evaluating seafood products. At the hands-on session, representative samples of three of the most popular seafood products (canned tuna, fish fillets, and shrimp) were examined and discussed. Agency representatives then explained where the FDA's minimum acceptable level would be. In general, there was close agreement between judgments by participants and the FDA guidelines, but occasionally participants were more critical than present FDA guidelines would allow.

The accent of the meeting was, of course, on standards that can be used internationally and the measurements available to make the standards usable. It is my hope that the papers given at this symposium will stimulate further study and will move us closer to quality measurements that will be accepted and used in the international trade of seafood products.

Donald E. Kramer
University of Alaska

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Tom Bellamore, A.D. Chandler III, Douglas Donegan, Marie Fried, Donald E. Kramer, Jong S. Lee, Peter A. Lerke, John Liston, Robert J. Price, John Spinelli, George A. Strasdine, Marleen M. Wekell, Ronald Wrolstad.

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Symposium Session Chairs

James R. Brooker, John Farquhar, Donald E. Kramer, Lucina E. Lampila, Jong S. Lee, John Liston, George A. Miller, John Spinelli, George A. Strasdine, H.R. Throm, Marleen M. Wekell.

Sensory Evaluation Workshop

Coleen Grupe, Jack LaRose, and George A. Miller of the U.S. Food and Drug Administration in Seattle; Anthony F. LaTerza of the U.S. Food and Drug Administration in Boston; and Don Cosgrove of the National Food Processors Association served as instructors for this workshop. Facilities for holding this workshop were provided by the John Cabot Trading Company and we thank Jim Wiitala for making these arrangements.

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CHANGES IN THE QUALITY AND MICROSTRUCTURE OF FROZEN ROCKFISH

D.S. REID, N.F. DOONG, M. SNIDER, and A. FOIN

Department of Food Science and Technology, University of California, Davis, CA 95616 (USA)

SUMMARY

Rockfish have been slow and fast frozen, and maintained in frozen storage at -5° , -12° , and -20°C . The chemical/biochemical status of the fish has been assessed by measuring protein solubility, myofibrillar fraction ATPase activity, and differential scanning calorimetry (DSC) denaturation profiles. The frozen muscle was isothermally freeze-fixed to enable investigation of the frozen structure. Ice crystal sizes have been estimated. There are parallels between the rates of structural change and the rates of biochemical change. The composition of the frozen matrix is an important determinant of the rate of change in frozen systems, and the frozen structure also appears to play a role in the rate of some deterioration processes.

INTRODUCTION

The quality of fish maintained in frozen storage deteriorates at a steady rate. This loss in quality is a significant factor in the utilization of fish. It is expedient upon the fish industry to minimize this loss. A readily available solution to most quality deterioration is using very low storage temperatures. Such storage, though, is costly. There is, therefore, always a tendency to store frozen fish at the highest practical temperature, in an attempt to maximize the cost-benefit relationship.

We do not have, as yet, a complete understanding of the processes of deterioration in frozen fish during frozen storage. Indeed, we have as yet an imperfect understanding of the processes that take place consequent to the freezing process itself. Changes are known to occur in the chemical/biochemical composition of the fish, [refs. 1-3]. These are species dependent. Changes also take place in the structure of the tissue as a consequence of freezing, [refs. 3-5]. These may be freezing rate dependent. Further changes then occur in the structure of the frozen tissues during frozen storage. There is little information available on correlations between structural change and chemical/biochemical change, although intuitively it would appear that such correlations might exist.

In this study we have been using special microscopic procedures to follow the structural changes that take place in frozen stored fish. In order to see the frozen tissues with a minimum of distortion, we use isothermal freeze fixation (IFF), in which a fixative is employed that has been adjusted by colligative means to be in thermodynamic equilibrium with ice at the temperature of frozen storage [refs. 6,7]. In this way the chemical cross-

linking of the non-ice matrix can occur without the ice crystals in the matrix being disturbed. The structure of the frozen tissue can then be visualized. Cavities will exist in the fixed tissue that correspond to the size and location of the ice crystals present in the original material. The tissues, appropriately fixed, can be prepared for observation by light microscopy, or by electron microscopic methods such as scanning electron microscopy, and transmission electron microscopy.

Each of these techniques has its own particular advantages and disadvantages. In order to study the size, number, and distribution of ice crystals within the frozen tissue, light microscopic examination of the fixed tissues is the most appropriate tool. This is because the size of the ice crystals is such that the range of magnifications available to light microscopy is appropriate.

Direct observation of the freezing of small tissue samples on a specially constructed cryomicroscope stage is also a useful probe of the structural aspects of freezing. In this paper we report data on the structural aspects of the freezing and frozen storage of fish obtained by optical microscope studies of IFF samples of fish frozen either slowly or rapidly, and maintained in storage at one of three temperatures. Some supplementary data relating to the mechanics of the freezing process are derived from a cryomicroscopic study of fish tissue freezing, using a specially designed controlled-temperature microscope that allows for the control of freezing conditions. The chemical/biochemical status of the fish is monitored by measuring such parameters as water holding capacity, myofibrillar protein solubility, protein ATPase activity, and tissue homogenate pH. In addition, the protein functionality has been assessed by monitoring the denaturation profiles using differential scanning calorimetry.

EXPERIMENTAL METHODS

The fish used in these studies are *Sebastes* species, caught in the Pacific Ocean off the San Francisco to Fort Bragg coastal region in California. The fish were processed in our pilot plant as soon after catching as was practical. Fish were stored on ice prior to processing, and were filleted on arrival at Davis. The fillets were washed, obvious red muscle was removed, and approximately 0.5 to 1 lb of fish was placed in a deep aluminum tray and wrapped to minimize moisture loss. The trays of fish were frozen in a Conrad air blast freezer operating at -69°C . The temperature change in selected samples was monitored by a thermocouple located close to the thermal center. After extraction of an appropriate amount of heat, the fish was removed. The frozen fish was then sealed in a freezer bag to prevent moisture loss during frozen storage, and transferred to the appropriate storage environment. Slow-frozen fish was packaged in a tray as described above, but instead of freezing in the blast freezer, it was placed directly into the appropriate storage environment.

Isothermal freeze fixation

By employing colligative freezing point lowering, the fixatives for isothermal freeze fixation are formulated to be slushy at the temperature of fixation. Their melting point is only slightly above the temperature of fixation. Cacodylate at 0.1 M is used as a buffer to maintain a pH close to 6.3. This is an appropriate buffer, and no evidence of its crystallizing in the frozen system has been obtained. Phosphate buffers may crystallize, with consequent pH shifts. The fixative is glutaraldehyde at about 3 percent concentration. Sufficient dimethyl sulfoxide (DMSO) is added to adjust the melting point of the fixative solution to about one to two degrees above the fixation temperature, so the fixative has a small amount of ice present at its working temperature. Washing solutions, in which the glutaraldehyde is replaced by glycerol, are used when fixative has to be removed prior to thawing. Formulations of typical solutions are listed in Table 1.

TABLE 1

Composition of isothermal freeze fixatives using DMSO cosolute

| Temperature ($^{\circ}$ C) | DMSO (wt %) | Cacodylate (wt %) | Glutaraldehyde (wt %) |
|-----------------------------|-------------|-------------------|-----------------------|
| -20 | 34 | 2.4 | 3 |
| -12 | 24 | 2.4 | 3 |
| -5 | 7 | 2.4 | 3 |

To fix frozen tissue, cores are removed from the frozen material and placed, still frozen, in vials of fixative at the same temperature. The vials are maintained in storage at this temperature until fixation is complete. The cores, now isothermally freeze-fixed, are removed, washed, and prepared for examination by the standard microscopic techniques. In this study, the tissues have been prepared for examination by optical microscopy. Tissues have been embedded in glycol methacrylate, following the procedures of Cole and Sykes [ref. 8].

Water holding capacity

The centrifugal method of Eide *et al.* [ref. 9] was employed. Two grams of homogenized flesh were placed in a tared, stoppered holder and weighed. The sample holder, a tube with a mesh bottom, was then placed in a centrifuge tube half filled with glass beads. The assembly was spun for 5 min at 1,500 x *g*. The sample weight loss was determined immediately after the centrifugation.

Homogenate pH

The pH of the flesh was determined after homogenizing in 4 volumes of water.

Myofibrillar protein solubility

The myofibrillar protein solubility of homogenized light muscle was measured by the method of Hashimoto *et al.* [ref. 10]. All operations were carried out at 3⁰ to 4⁰C. A 20 g sample of light muscle was homogenized in 200 ml of 0.05 M phosphate buffer, with the pH adjusted to 7.5. This mixture was then centrifuged at 5,000 x *g* for 15 min. The liquid fraction was removed, and the extraction repeated on the residue. The residual material from the second extraction was homogenized yet again, this time in 10 volumes of a KCl/phosphate buffer with an ionic strength of 0.5, and a pH of 7.5. After centrifugation and separation of the supernatant, this procedure was repeated on the residue. The combined supernatant solutions from the extractions with the I = 0.5 buffer make up the myofibrillar protein fraction. The protein content of the fraction is estimated by the biuret method [ref. 11].

ATPase activity

ATPase activity was determined on a myofibrillar fraction, which was assayed for ATPase activity by adding ATP as substrate [ref. 12]. Ground fish was homogenized with 3 volumes of a 0.1 M KCl/40 mM borate buffer at pH 7. Care was taken to ensure adequate homogenization. The homogenate was diluted into 17 volumes of 0.1 M KCl, then centrifuged at 1,300 x *g* for 10 min. The pellet resulting from this procedure was purified by repeated cycles of suspension in 0.1 M KCl and centrifugation. The final pellet was resuspended in 0.1 M KCl. The protein content was determined by the biuret method. ATPase assays were performed in duplicate at 25⁰C. Five ml of the protein preparation, adjusted to a protein concentration of 0.25 mg per ml was used. The reaction mixture also contained 0.1 M KCl, 20 mM tris maleate (pH 7), and 1 mM ATP. Either 5 mM CaCl₂ or 1 mM MgCl₂ was also present. The reaction was initiated by adding the ATP to the previously prepared mixture of the other components. After 5 min, the reaction was stopped by adding 1 ml of cold, 15 percent trichloroacetic acid. This precipitates the protein. After immediate chilling and centrifugation to remove the precipitated protein, the supernatant was assayed for liberated phosphate. A protein-free blank was performed, using all other reagents in the appropriate amounts.

Differential scanning calorimetry

Differential scanning calorimetry was performed using a Perkin-Elmer DSC-2C differential scanning calorimeter equipped with the TADS computerized data acquisition system. Samples of muscle tissue weighing approximately 10 to 15 mg were sealed in Perkin-Elmer aluminum "volatile sample pans." The reference pan was empty. Samples were heated 10 K per min over the temperature range 290 K to 370 K. Prior to calorimetric study, care was taken to ensure that sample temperatures would not exceed 300 K, to prevent unwanted thermally induced changes from occurring. This temperature is chosen because it is near the maximum

temperature that might be experienced by the live fish. Also, a preliminary study showed that there were no major thermal events at less than 300 K on the DSC trace for rockfish muscle.

A common problem in storage studies of fish is the inherent variability of the raw material. In an attempt to minimize the effect of this variability individual fish were maintained in storage and subjected to the same assay at intervals during the storage period. Each fish was also assayed immediately prior to freezing and soon after freezing. Samples of tissue from each fish have also been fixed at each time so that, should it be required, a microscopic examination is possible. Each time, the sample for microscopic examination was removed from the same area as the sample that was taken for biochemical assessment. In this way it is hoped that more meaningful correlations between structure and chemical change might be possible, since the structural data and the chemical data will relate to the same material, which will have experienced the same storage history.

RESULTS

Figure 1 illustrates the freezing profiles obtained for a sample of fish frozen under the described conditions in the blast freezer. What we have termed fast freezing is essentially complete in 2 hr. In contrast, a fish sample frozen slowly took more than 24 hr to completely freeze.

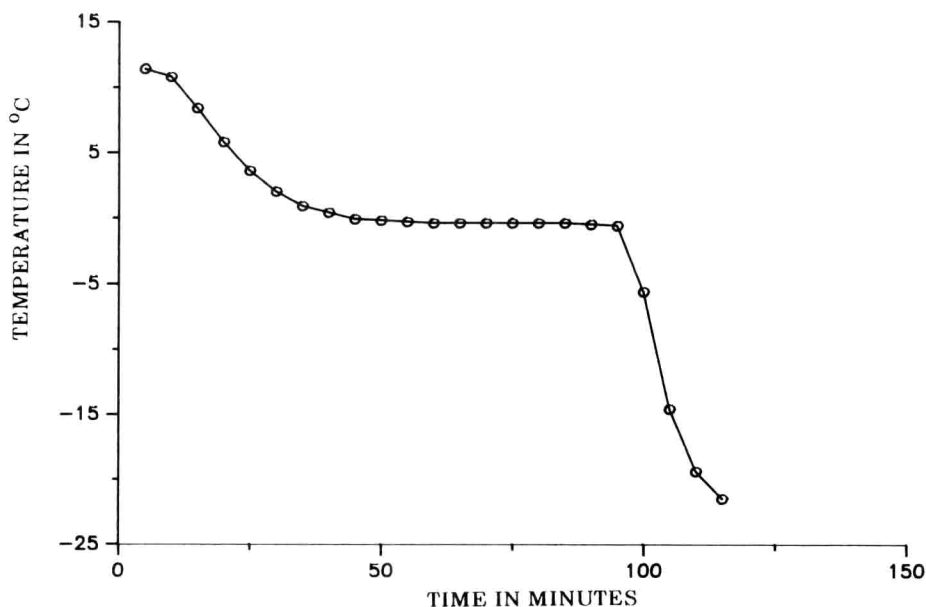


Fig. 1. Freezing rate of fish sample in Conrad blast freezer.