FEDERAL SPECIFICATIONS FOR FISHERY PRODUCTS

The Service's Technological Section is responsible, as a service to other Federal agencies, for the development of Federal specifications for fishery food products. This assignment includes responsibility for (1) development of the specification and coordination with Federal agencies and with industry and (2) amendment, revision, and further development as found to be necessary. This work is now coordinated at the Fishery Technological Laboratory, East Boston, Mass., as part of the program on the development for fishery products of standards for quality. The other Service laboratories in College Park, Md., Seattle, Wash., and Ketchikan, Alaska, cooperate in the work by developing the requirements for the particular products common to their areas and by reviewing the individual specifications.

By definition, a specification is an accurate description of the technical requirements for a material, a product, or service, including the procedure by which it will be determined that the requirements have been met. Specifications are prepared as needed to meet the requirements of Federal agencies for the purchase of fishery food items.

The following specifications have been revised during the past several years:

PP-0-956, Oysters, raw, shucked (fresh or frozen), Issued July 27, 1950.

PP-C-401, Clams, raw, shucked; fresh (chilled) and frozen, Issued April 25, 1952.

PP-F-381, Fish; fresh (chilled) and frozen, Issued Sept. 3, 1954.

PP-S-31, Salmon, canned, Issued February 3, 1954.

The following specifications have been revised and issued as Interim specifications (coordination with other Federal agencies and with industry has been completed and they will soon be issued as Federal specifications):

PP-S-00311a, Shrimp, Canned, Interim specification issued November 18, 1954.

PP-S-00316a, Shrimp, raw and cooked, chilled and frozen, Interim specification issued November 18, 1954.

PP-C-00656a, Crab Meat, cooked; chilled and frozen, Interim specification issued May 2, 1955.

The following specifications have been revised and are out for review by the industry:

PP-C-651, (Revision "a") Crab Meat; Canned, Revised June 9, 1955.

PP-S-51, Sardines, Canned (Revised July 1955 for preliminary industry review and coordination with other Federal agencies).

The following specifications have been cancelled:

PP-F-371, Fish; Flaked, Canned, March 31, 1931.

PP-F-401, Fish; Salted or Smoked, March 31, 1931.

The following specification is being revised:

PP-0-951, Oysters, Canned, Issued March 3, 1931.

No action has been taken on the specification for Tuna Fish, Canned, PP-T-771, March 31, 1931, pending development of standards of identity and fill by the Department of Health, Education, and Welfare.

(Single copies of Federal fishery specifications and other product specifications required by activities outside the Federal government for bidding purposes are available without charge at the General Services Administration Regional Offices in Boston, New York, Atlanta, Chicago, Kansas City, Mo., Dallas, Denver, San Francisco, Los Angeles, Seattle, and Washington, D. C.)

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CHEMICAL CHANGES IN FISH PROTEIN DURING FREEZING AND STORAGE

One of the factors limiting the sale of frozen fishery products is the tendency for fish and shellfish to become tough during frozen storage. Although this problem has been recognized for many years, progress toward its solution has been slow. One reason for this delay has been the lack of a basic understanding of the causes of toughening. The Fishery Products Laboratory at Ketchikan has therefore undertaken a fundamental study of this problem.

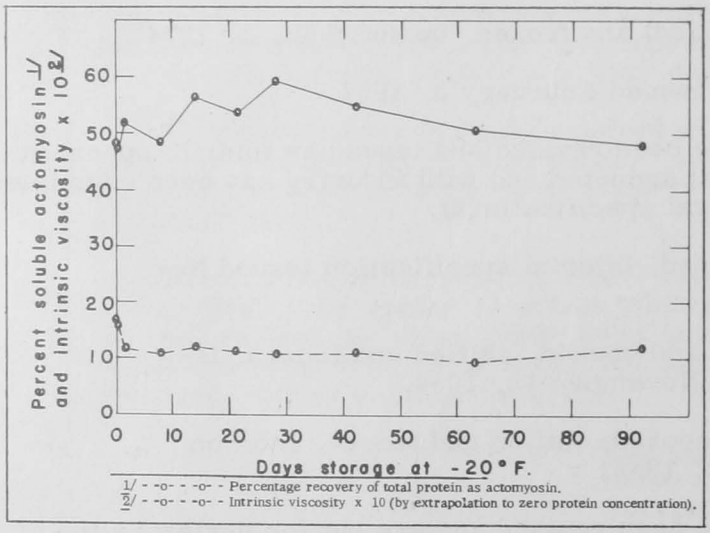

Fig. 1 - Viscosity and solubility properties of actomyosin isolated from frozen-thawed yellow-striped rockfish.

There is evidence to indicate that toughening is related to structural changes in the fish protein. Since such changes would probably alter the shape or size of the protein molecules, a clue to the extent of these changes is the absolute viscosity of a solution of the thawed protein. A study of the viscosity of protein solutions is thus the current phase of investigations at the Ketchikan Laboratory.

A method has been developed whereby the protein fraction called actomyosin is isolated from fish muscle and subjected to viscosity measurement, yielding a value

which is a function of the asymmetry of the protein molecule. Yellow-striped rockfish (Sebastodes nebulosus) meat that was held in frozen storage (-20° F.) up to 92 days was subjected to evaluation by this method at intervals during this storage period. An estimation of residual soluble actomyosin in dilute salt solution was also carried out at these same intervals for comparative purposes. The results of these tests are briefly presented in figure 1.

The soluble actomyosin values increased toward a maximum value after about one month's storage. They subsequently declined to approximately the initial value. A sharp drop in viscosity occurred within $1\frac{1}{2}$ days of storage; thereafter the viscosity values remained quite constant. Other isolated experiments indicate, however, a significant decrease in viscosity and solubility resulting from either long storage periods (approximately one year) at relatively low temperature (-20° F.) or short storage periods (approximately two months) at relatively high temperature (10° F.). Apparently the change of state of the actomyosin follows a time-temperature relationship, at least as indicated by the results of these viscosity and solubility measurements.

Using refined isolation and viscosity techniques, it is planned to continue this study on the rate and extent of "freeze-denaturation" of actomyosin in fish muscle. The effect of the freezing process itself and the "time-temperature" factor will be more closely studied.

The changes in the protein of a species of fish known to be quite resistant to toughening in frozen storage will be compared to those of a species very susceptible to these undesirable changes. If it can be demonstrated that the solubility and viscosity changes of the protein fraction actomyosin correlate closely with texture changes in the frozen stored fish meat, a useful tool will have been found for improvement in freezing and frozen storage procedures. The effects of the initial condition of the raw material and of processing techniques such as the rate, temperature, and time of freezing, and the temperature and time of frozen storage could then be measured objectively and at each stage.

UNIDENTIFIED GROWTH FACTORS IN FISH BYPRODUCTS

SUMMARY

Concentrated fractions of the growth factor(s) in tuna livers, salmon eggs, and menhaden meals were prepared by solvent extraction, dialysis, and final purification--either by 12-stage countercurrent distribution or by passage through ion-exchange resins. The maximum growth response produced by the various fractions were measured by means of a microbiological assay in which L. casei was used.

A 35- to 45-percent maximum growth response was obtained when the crude fraction prepared by extraction and dialysis was assayed. All the raw materials--tuna livers, salmon eggs, and menhaden meals--gave about the same maximum growth response.

The fraction adsorbed on the anion-exchange resin produced a significant growth response, but there was no adsorption of growth factors on the cation column.

Efforts to prepare fish-byproduct fractions that will yield greater information as to the number of growth factors present or as to the number of modifications in chemical structure of the growth factor(s) are now in progress.

BACKGROUND

Studies on unidentified growth factor(s) in fish byproducts have been in progress at the Service's Seattle Technological Laboratory since July 1951. This work has had two main objectives: (1) to establish a chemical fractionation procedure by which the growth factor(s) could be concentrated, and (2) to develop a microbiological assay that could take the place of the expensive and time-consuming animal assays.

Concurrent with the work in Seattle, a study has been under way in the Service's College Park Laboratory on the animal assay of the unidentified growth factor(s). Since this study has included assays on the fractions resulting from the chemical procedures at Seattle, it will show whether correlation exists between the results obtained by the microbiological and the animal assays. These findings will be described subsequently in a separate report.

CHEMICAL FRACTIONATION PROCEDURES

In the fractionation work, three types of raw material have been used: tuna livers, salmon eggs, and--to a lesser extent--menhaden meals. The procedures that have been used to concentrate the growth factors in these raw materials are similar to those used by Menge, Denton, Sizemore, Lillie, and Bird (1953) to fractionate fish solubles and by Menge and Combs (1952) to fractionate liver extracts.

EXTRACTION OF THE GROWTH FACTOR(S): In the fractionation of tuna livers and salmon eggs, a sample of approximately 25 pounds was ground to a homogeneous slurry. Ninety-five percent ethanol was than stirred in at a ratio of 1 gallon of ethanol to 5 pounds of sample. The resulting mixture was allowed to stand, with occasional stirring, in a cold room at 34° F. for 24 hours. The alcoholic solution was then removed from the precipitated tissue protein by means of a basket centrifuge, and the residue of protein was given one extraction with 80-percent ethanol, at a ration of about 2 liters of ethanol to 1 pound of residue. Finally, the two alcoholic extracts were combined and then concentrated, under reduced pressure, to about $\frac{1}{10}$ the original volume. The resulting crude concentrate was called fraction I.

REMOVAL OF LIQUID MATERIAL AND DIALYSIS: Fraction I was extracted repeatedly with chloroform to remove lipid material. The aqueous concentrate remaining after chloroform extraction was then further concentrated to approximately 1 liter. This concentrate was transferred to a cellophane dialysis tube ($3\frac{1}{2}$-inches in diameter) and was dialyzed against distilled water at 34° F. for 3 weeks, with a change of dialysate water every third day. The collected dialysates were finally concentrated to approximately 500 milliliters. This final concentrate was called fraction II.

Glacial acetic acid was now added to the concentrated dialysate (fraction II) to make a 2-percent (v/v) acetic acid solution. This solution was extracted three times with 300-milliliter volumes of either para cresol or water-saturated phenol. (The growth factor went into the phenolic layer.) The para cresol or phenol extracts were combined and then diluted with 5 volumes of acetone, and this mixture was stored overnight at 34° F. The brown amorphous precipitate that formed was collected in 250-milliliter bottles by centrifugation. Finally, the precipitate was washed in the bottles--first with acetone and second with ether--and was then dried in a vacuum oven for 8 hours at 50° C. The resulting concentrate was called fraction III. This concentrate (fraction III) was then divided into two parts, the one being called fraction IIIA and the other, fraction IIIB.

PURIFICATION BY COUNTERCURRENT DISTRIBUTION: Fraction IIIA was purified by countercurrent distribution (CCD) in a two-phase system, comprising a phenolic phase and a water phase. The phenolic phase was made up of 70

percent phenol and 30 percent secondary butyl alcohol (w/w). The water phase was a solution of 3-percent acetic acid (v/v) adjusted to pH 2.0 with hydrochloric acid.

The distribution was carried out in twelve 1-liter separatory funnels numbered from 0 to 11. One gram of sample was dissolved in 350 milliliters of the aqueous layer in the initial stage of the distribution. Each of 12 fractions (2 phases present) obtained by counter-current distribution was transferred to a 3-liter separatory funnel, and 1 liter of ether was added to extract phenol and secondary butyl alcohol (the growth factor went into the aqueous phase). The 12 aqueous phases remaining after each of the ether layers was removed were each concentrated under reduced pressure to a volume of approximately 15 to 25 milliliters. Each concentrated phase was then diluted with 5 volumes of acetone and stored overnight at 0° F. The precipitates that formed were each collected in small centrifuge tubes and were washed first with acetone and second with ether. Finally, the precipitates were dried in a vacuum desiccator over silica gel.

These dried precipitates were numbered 0 to 11, according to the separatory funnel from which each was obtained (CCD fractions 0 to 11).

PURIFICATION BY ION-EXCHANGE: Fraction IIIB was purified by passing weighed samples, dissolved in water, through an anion-exchange resin (Amberlite IRA--400) and a cation-exchange resin (Amberlite IR-120). The material absorbed on the anion column was removed by washing with 5-percent sodium bicarbonate adjusted to pH 8.5, whereas the material absorbed on the cation column was removed by washing with 3-percent acetic acid adjusted to pH 2.0. This treatment gave fractions A-A (adsorbed) and A-U (unadsorbed), from the anion column, and fractions C-A (adsorbed) and C-U (unadsorbed), from the cation column.

EXTRACTION OF MENHADEN MEALS: The menhaden meal was extracted as follows:

A mixture was formed by the addition of 3-percent acetic acid to the dry meal at a ratio of 2 liters of acid solution to 1 kilogram of meal. This mixture was autoclaved at a pressure of 15 pounds and was then allowed to cool to room temperature. Finally, the supernatant liquid was decanted and filtered.

The remaining solid material was extracted two more times, with the same procedure being used as with the dry meal, and was then discarded.

The resulting three aqueous extracts were combined and then concentrated under reduced pressure. The concentrate thus formed was diluted with 95-percent ethanol to the lower concentration of 80-percent ethanol. Finally, the alcohol-soluble portion was filtered off.

The procedure from there on was the same as with the tuna livers and salmon eggs.

MICROBIOLOGICAL ASSAY

A convenient synthetic medium for lactic acid bacteria was described by Flynn, Williams, O'Dell, and Hogan (1951). By the use of this medium, it was found that the addition of fraction I consistently produced a 40- to 45-percent growth increase with L. casei (ATCC - 7469), as measured by the increase in acid produced in comparison with controls containing no added fraction. Since the growth response obtained with fraction I was good, it was decided to determine the L. casei growth response produced by the other fractions.

It was found that fractions II and III produced a maximum growth response of 35 to 45 percent, indicating little or no loss of growth-factor(s) as a result of the concentration procedures used.

All the raw materials--tuna livers, salmon eggs, and menhaden meals--gave about the same maximum growth response.

ASSAY OF COUNTERCURRENT-DISTRIBUTION FRACTIONS: The microbiological assay of fractions obtained by countercurrent distribution (CCD fractions 0 to 11) showed that fractions 5 and 6 each produced a maximum growth response of 7 to 12 percent and that fraction 11 produced a slight growth response, which was of questionable significance. Fraction 0 produced a maximum growth response of 10 to 20 percent, indicating that some of the growth factor(s) in fraction III did not migrate in this solvent system.

These results obtained on CCD fractions by L. casei assay are similar to those obtained by Menge and Combs (1952) using chicks to assay CCD liver fractions.

ASSAY OF ION-EXCHANGE FRACTIONS: The use of ion-exchange columns with fraction IIIB resulted in considerable loss of growth-factor activity as measured by the L. casei assay. The growth factor(s) appeared to be adsorbed on the anion resin, since very little growth response was obtained from the unadsorbed material. The material obtained from the washings of the anion column gave a 5-to 10-percent maximum growth response. No material was adsorbed by the cation column.

ADDITIONAL STUDIES: Efforts to prepare fish-byproduct fractions that will yield greater information as to the number of growth factors present or as to the number of modifications in chemical structure of the growth factor(s) are now in progress.

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FISH OIL AND MEAL SAMPLES COLLECTED FOR EVALUATION

The U. S. Fish and Wildlife Service has completed arrangements with the menhaden plant operators for the collection at weekly intervals of fish-oil samples throughout the season. The purpose of this sampling program is to provide oils of known history as to season, locality of fish capture, and size of fish processed. Scientists at North Carolina State College in Raleigh are evaluating these oils for the principal physical and chemical constants including iodine value, free fatty acids, saponification value, unsaponifiable matter, refractive index, and color index.

The Service's College Park Fishery Technological Laboratory has obtained and packaged for distribution a large lot of fish meal and fish oil from a menhaden processing plant. These samples from the same lot of fish will be used as positive controls in the extensive chicken and swine feeding tests to be conducted at the University of Oregon at Corvallis, University of Delaware at Newark, and University of Connecticut at Storrs. They will also serve as common reference samples for the work on meal and oil that other contractors active in the newly inaugurated research program now have under way.

These projects are being financed by Saltonstall-Kennedy Act funds.