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This year, we continued our emphasis on methods of improving the quality of fish and fishery products. The industry is exhibiting more interest in upgrading quality, and I hope this favorable trend will continue.

Radiation pasteurization continues to show promise as a practical means of doubling or tripling the shelf life of fish and shellfish. The problem of quality control, however, exists no less with irradiated products than with unirradiated ones. It is now clear that irradiated products, too, must be kept at temperatures close to freezing if spoilage is to be delayed and the growth of bacteria prevented.

Groundbreaking ceremonies were held in July 1963 to signal the start of construction of the world's first Marine Products Development Irradiator on our laboratory site at Gloucester. A luncheon of irradiated seafoods, held in conjunction with these ceremonies, gave a vivid idea of the potential of irradiation as a technique in marketing fresh fish. The new facility will be used for the developmental investigations needed to introduce radiopasteurized seafood into the American economy. Large-scale studies on product acceptability will be carried out along with process and economic investigations.

I have observed that quality has different meanings for different people and that, all too often, its interpretation is influenced by economics. Although much is known on how to delay the loss of quality, there are few references on the seriousness of this loss or on the extent to which quality is important to the consumer. This year, we analyzed retail samples of frozen fish fillets to assess the quality of fish available in major cities in the United States. The results, included in this report, indicate that many consumers are purchasing seafood of marginal quality. We cannot define the importance of quality in relation to sales, but we can emphasize that more attention must be given to improving the processing, handling, distribution, and retailing of frozen seafood.

I now feel that we can make some definite statements concerning the value of refrigerated sea water for storing groundfish on the vessel. Our early laboratory studies led to rather optimistic findings--namely, that ocean perch and whiting will keep 3 to 6 days longer in 30° F. refrigerated sea water than in ice. This finding was not confirmed, however, in largescale studies carried out on the vessel and ashore, even though the control of temperature was excellent. We also investigated the use of ultraviolet radiation, which reduced the bacteria in the recirculating sea water, but did not significantly affect the growth of bacteria on the fish. We can, and do, recommend the use of refrigerated sea water for storing groundfish that must be kept for 3 to 5 days. Although 30° F. sea-water facilitates handling and cooling where the application of ice is difficult, no quality benefit is derived during long-term storage.

Our fundamental research on freeze denaturation of fish protein has yielded interesting results. We have additional evidence that fatty acids in fish flesh influence the extent of product susceptibility to actomysin denaturation. Also, we found that the stability of the actomyosin of different species of fish varies with the total lipid content, since the interaction of the fatty acid actomyosin decreases with an increase in lipid content. Our research on proteins was discussed at FAO's Symposium on the Significance of Fundamental Research in the Utilization of fish, which was held in Husum, Germany, May 25-30, 1964. The comments from workers in protein chemistry confirmed many of our laboratory findings.

We were pleased to have the industry evaluate the commercial use of our mobile fish deicing and weighing unit. The unit was favorably received by the fishermen. It gave them a fair weight by eliminating ice, and it did away with the use of forks in handling fish on the dock. We are hopeful that the unit will encourage people in the industry to discard their outmoded forks, which contribute to a substantial lowering in the quality of the product handled.

The Laboratory continued to communicate results of research findings to industry and the scientific community through publications and attendance at local, national, and international meetings. This year 23 technical talks were presented and 16 technical papers were published. We gave increased attention to attendance at international meetings in the field of fishery technology and to cooperative research with laboratories bordering on the North Atlantic, where the fishery problems are very similar to ours.

RESEARCH ON FREEZE DENATURATION OF PROTEINS

by

Maynard A. Steinberg, Assistant Laboratory Director

This year we placed increased emphasis on protein-fatty acid interaction in fish flesh and obtained a better understanding of the many complex factors contributing to protein denaturation. This report discusses highlights of our research on freeze denaturation of proteins.

Recent findings of British workers have led us to extend our hypothesis that interaction with fatty acids renders fish muscle inextractable during frozen storage. Lovern and Olley (1962) have shown that the rate of lipid hydrolysis in cod fillets held initially at -7° C. (19.4° F.) for 1 week is faster in subsequent storage at -7° C. (19.4° F.) then at 0° C. (32° F.) Love (1962a) has shown that the rate of protein denaturation in frozen-stored cod muscle reaches a maximum at a storage temperature of -1.5° C. (29.3° F.)

These findings suggest that the liquid phase of the frozen muscle in which solutes are concentrated as a result of formation of ice crystals serves to peptize proteins and to allow diffusion of lipids, making protein-fatty acid interaction possible. We feel that the rate of the denaturation process may depend largely on the geometry and distribution of the liquid phase and the concentration of solutes in the liquid phase, which is a function of storage temperature.

We reexamined the studies of those who have investigated the effect of freezing rate (Dyer and Dingle, 1961; Love, 1962a), storage temperature (Dyer and Dingle, 1961; Love, 1962a), fluctuations in storage temperature (Dyer, Fraser, Ellis, and MacCallum, 1957; Love, 1962b), thawing (Dyer and Dingle, 1961), preexposure to low temperature (Love and Elerian, 1963), and state of rigor at time of freezing (Love, 1962a). Their results, in terms of the physical effects that these variables have on fish muscle and on the liquid phase in particular, fell nicely into place in a theory of fatty acids reacting with protein in a liquid phase--the geometry, distribution, and ionic strength of which is determined by these variables.

To test the above hypothesis, we used model systems in which salt solutions of varying ionic strength simulated the character of the liquid phase as it was affected by storage temperature. We found that extractability of actomyosin and insolubilization of protein by fatty acid were maximal in extracts of ionic strength 0.5. According to our estimates, this is the same ionic strength that occurs in the liquid phase of cod muscle frozen at -1.5° C (29.3° F.), the temperature at which Love (1962a) reported a maximum rate of denaturation.

As the ionic strength of the extractant increased beyond 0.5, corresponding in frozer muscle to a liquid phase that is decreasing in volume and increasing in solute concentration, a larger portion of the protein extracted was myosin. More fatty acid was required to precipitate protein in these extracts. These results are consistent with the findings of Ellis and Winchester (1959), who have shown that actomyosin is dissociated in salt solutions of increasing ionic strength, and those of Connell¹ and Menzel and Olcott (1964), who found that myosin was aggregated by fatty acid only in concentrations higher than those we have found necessary to insolubilize actomyosin.

These results support our hypothesis that protein-fatty acid interaction causes the denaturation process and that the rate of the process is largely determined by the character of the liquid phase of frozen muscle as influenced by the physical effects of processing and storage variables.

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Figure 1.--Ultracentrifugal analysis of the composition of a protein extract.



Figure 2,--Clarification of a protein extract by preparative ultracentrifugation.



Figure 3.--Viewing the pattern of the protein components of a fish muscle extract as they are separated by the analytical ultracentrifuge.

PRESERVATION AND PROCESSING RESEARCH

by

John A. Peters, Program Leader

We have continued our work on problems of fish quality. As in the previous year, emphasis has been on two major investigations: new refrigeration techniques and time-temperature tolerance of frozen seafoods.

In the work on new refrigeration techniques, we have investigated the effect of ultralow temperature, such as the -320° F. temperature of liquid nitrogen on the quality of fishery products, and the use of sea water refrigerated to 30° F. for the storage of fish both aboard the vessel and at the shore plant.

EFFECT OF ULTRALOW TEMPERATURES ON THE QUALITY OF FISHERY PRODUCTS

The initial experiments performed on freezing fish flesh in liquid nitrogen showed that very rapid freezing resulted in cracking or shattering of the product (Slavin, 1965). To determine optimum conditions of time and temperature for freezing various products in liquid nitrogen, we continued work on the use of strain gages to measure the strains set up in fish flesh under various conditions of freezing; and, in addition, we began a more fundamental analysis of this new freezing technique through obtaining accurate knowledge of the relevant physical properties of fish flesh in the cryogenic (low-temperature) region. The physical properties under investigation are specific heat, density, thermal conductivity, and bound water.

In the strain-gage studies, we worked with experts in strain-gage technology on the problem of firmly attaching the postage-stampsized gages to the fish flesh so that no slippage would occur during freezing. Tests showed that a special contact cement will provide excellent adhesion of the gage to the flesh and will permit reproducible strain readings in the temperature range of interest.

Little information is available on the physical properties of fish flesh, particularly in the cryogenic range of temperatures. This year, we broadened our research to include studies on the measurement of specific heat and thermal conductivity in fish flesh.

Table 1 lists the physical properties found in cod flesh during our preliminary work in the refrigeration range of temperatures. The problems of making accurate measurements, however, are greatly magnified when one is working in the cryogenic range of temperatures (by our definition temperatures below -100° F.). Unless special precautions are taken, heat leakage in the system can account for a very large part of the change in electromotive force produced by the thermocouples (about 20 millionths of a volt per degree F.). We now are constructing specially insulated equipment and are accurately calibrating the thermocouples to give the greatest precision possible in our measurements at low temperatures.

Table 1.--Physical properties of cod flesh in the refrigeration region

Temperature	Specific heat	Density	Thermal conductivity	Bound water
o _F .	<u>B.t.u. lb./F</u> .	Lbs./cu.ft.	$\frac{\text{B.t.u(ft.)/hr.}}{(\text{sq.ft.}(^{\circ}\text{F.}))}$	Percent
40	0.887	61.56		
35	.872	60.93		
30	.817	60.07		
25	.789	57.58		
20	.768	56.08		8.7
15	.743	55.21		4.7
10	1997 Contra 1999		0.570	3.7
5	.648	54.33		.6
0			.690	.4
-10			.789	

STORAGE OF FISH IN REFRIGERATED SEA WATER

Shipboard Tests

Two shipboard trials were conducted on a commercial fishing vessel in which 1,500 and 1,200 pounds of ocean perch were held in a tank of circulating sea water refrigerated at 30° to 33° F. In addition, the sea water was treated with ultraviolet (UV) radiation to kill bacteria.

The equipment used in these tests consisted of (1) an insulated steel tank of about 2,000 pounds capacity; (2) a special high-intensity UV sterilizing unit installed in a storage pen in the vessel's hold; (3) a chiller, built on the design developed at the Vancouver Technological Station of the Fisheries Research Board of Canada (Roach, Harrison, and Tarr, 1961); (4) a pump for the UV unit, a pump for the refrigerated sea-water unit, and a coolant pump; and (5) a refrigeration compressor installed in the engineroom.

The first trial was a failure because the refrigeration equipment broke down. Taste tests on the ocean perch from the second trial showed that the fish had excellent quality after being held for 10 days at 30° to 33° F. in the UV treated refrigerated sea water; however, the fish were not acceptable to a buyer at a processing plant because the normal red color of the skin had been bleached by the sea water.

Laboratory Tests

In studies at the laboratory, 1,000- and 2,000-pound lots of whole whiting or eviscerated pollock were held in circulating sea water

refrigerated to 31° F., both with and without exposure to UV radiation.

For these tests, we used an insulated steel tank of about 5,000 pounds capacity (fig. 4.). When smaller amounts of fish were put into the tank, an expanded metal grid was placed on top of the fish to keep them submerged in the refrigerated sea water. A package chiller provided refrigeration. Methanol, cooled to about 22° to 26° F., was pumped into a 100. gallon plastic tank containing finned copper coils through which the refrigerated sea water was continuously circulated at the rate of 4) gallons per minute. With this system, the temperature of the sea water varied less than \pm 0.5° F.

The UV unit used (fig. 5) was based on the design by Kelly (1962). In operation, the refrigerated sea water was pumped from the bottom of the fish-storage tank (at the rate of 5 gallons per minute) up to the UV unit located above the tank, and then back to the tank.

In addition to periodic visual and taste tests on the fish, we made bacteriological examinations of samples of the refrigerated sea water. The data given in table 2 are typical of the bacterial counts obtained on the recirculated refrigerated sea water used in both the whiting and the pollock tests.

During the winter, rancidity developed very rapidly and unexpectedly in the fatty layer just under the skin of both whiting and pollock held in UV-treated RSW. The rancidity was also found in the iced fish but developed more slowly and to a lesser degree. During the spring additional tests, both with and without UV, showed that, as the season progressed, rancidity ceased to be a problem, appearing only slightly, if at all, in both species of fish. Evidently



DIAGRAM OF REFRIGERATED SEA WATER SYSTEM

Figure 4.--Diagram of laboratory refrigerated sea-water (RSW) system.



LABORATORY ULTRAVIOLET STERILIZING UNIT

Figure 5.--Sketch of laboratory ultraviolet sterilizing unit.

Table 2.--Effect of ultraviolet irradiation on the growth of bacteria in refrigerated sea water in which pollock or whiting were held

	Total pla	te count:
Storage time	Without ultraviolet treatment	With ultraviolet treatment
Days	Count/ml.	Count/ml.
1 2 5 6 7 8 11 12 13	87,000 33,000 100,000 320,000 1,200,000 1,200,000 2,200,000 3,300,000	1,200 1,500 15,000 35,000 69,000 29,000 46,000 46,000 40,000 55,000 83,000

there is a marked seasonal change in fat content, composition, or both that makes the fat more susceptible to rancidity, particularly when the fish are stored in refrigerated sea water.

Since the acceleration of rancidity in fish stored in refrigerated sea water may be caused by (1) salt absorbed through the skin, (2) dissolved oxygen or possibly ozone from the UV radiation, or (3) traces of copper or iron in the refrigerated sea water (resulting in the metal-induced rancidity reported by Castell, Dale, and Dambergs, 1962), we made an additional series of tests in which these variables were strictly controlled. The results of these tests showed that rancidity occurred where oxygen was freely available to the water, and that the rate at which rancidity developed increased as oxygen became more available. The use of UV radiation or the availability of iron, copper, or the salts in sea water did not contribute to the development of rancidity.

Although the use of refrigerated sea water as a storage medium for fish shows some promise, particularly where holding periods are short and little or no ice is currently used, our results to date do not justify the use of this method for long-term storage of the species of fish with which we have worked.

STUDIES ON CANNED RIVER HERRING

Between 1946 and 1963, sales of canned river herring (alewife) decreased by about two-thirds. As a result of this decline, the Middle Atlantic Herring Association requested the Bureau to assist in improving the quality of the present pack and in developing new products with widespread market appeal. In response to this request, a technologist from this laboratory worked with the industry during the 1964 canning season and continued the product-development work at this laboratory during the off season. These efforts have improved the appearance and texture of the customary pack and have resulted in the development of a new product -- river herring in tomato sauce. This new product has met with

favorable response; at least one Association member will produce substantial quanties of it during the 1965 season.

TIME-TEMPERATURE TOLERANCE OF FROZEN FISHERY PRODUCTS

Our time-temperature tolerance studies were continued during the year, with emphasis being placed on determining the effects of preprocessing storage time on the frozen storage life of pollock fillets and on investigating the use of the enzymes in fish flesh as biochemical indices of quality.

EFFECT OF PREPROCESSING STORAGE ON FROZEN STORAGE LIFE

We are attempting to establish a relation between the initial quality of fishery products and their subsequent frozen-storage life at various temperatures. The first species examined was pollock. Commercial-type, 1pound fillet packages and 13.5-pound fillet blocks prepared from fish held for 16 days on ice were stored at 20°, 10°, 0°, -10°, and -20° F. At regular intervals during frozen storage. samples of the 1-pound fillet packages are being removed from storage, and the fillets are steamed and served to the laboratory taste panel for the evaluation of quality. The fillet blocks are cut into portions, battered, breaded, fried in deep fat, and served to the panel. At present, this study is less than half completed; however, already certain trends are of interest. In figure 6, we have extrapolated our data to give an estimate of the storage life of pollock held 1 day and 13 days in ice, then filleted, packed in 1-pound packages, frozen, and stored at +20°, 0°, and -20° F. At high temperatures of frozen storage, preprocessing storage time on ice makes little difference in the rate of quality loss. At 0° and -20° F., however, increasing the iced storage period from 1 to 13



Figure 6.--Probable storage life of pollock fillets cut from fish held 1 and 13 days in ice, then stored at $+20^{\circ}$, 0° , and -20° F.

days about halves the subsequent frozenstorage life.

A very different pattern of quality loss is found when we evaluate the portions prepared from fillets blocks. To date, except for the fish held 16 days in ice, the taste panel has found little change in quality. Evidently the flavors and odors from the breading and the frying oil mask all but the strongest off-flavors and -odors in the fish.

FISH FRESHNESS TESTER

The Laboratory has obtained the newly developed "Interlectron Fish Tester V"¹ for testing fish of commercial importance to this region. This electronic tester was designed to measure the conductivity of fish tissue and thereby to indicate the iced-storage life remaining in a sample of fish. The purpose of our studies is to determine the accuracy and reproductibility of the tester's indications and its usefulness in our U.S. Department of the Interior inspection program for predicting the quality of fish.

Readings have been obtained on cod, pollock, ocean perch, haddock, hake, whiting, dab, yellowtail, and blackback that were held in ice under controlled conditions at the Laboratory. To date, these results are not conclusive but they do indicate that the tester is useful, because, with increasing time of storage in ice, the meter readings decrease almost linearly. Calibrations for "ice-storage life remaining" do not agree with our standards of fish quality, so we are recalibrating the tester to reflect our norms. This is being done by correlating organoleptic evaluations of the cooked fish with readings made with the tester. Typical results are given in figure 7.

BIOCHEMICAL INDICES OF QUALITY

An aim of our work is to evolve an objective test for early quality changes in frozen-stored fish. Our approach to the problem is to examine the fish's own enzyme systems whose kinetic patterns, tested in vitro, alter with freezing or frozen storage. The rationale is that the change in function is more readily detectable than the change in structure. Substrate identification and activity constants are our standard tools; the malic enzyme and alpha-glycerophosphate dehydrogenase are currently of greatest interest, having shown more promise than any other enzyme systems examined thus far.

In our malic enzyme study to date, the following general observations have been made:

1. The cytoplasmic fluid of fresh-fish muscle has a constant level of malic enzyme

¹ Trade names referred to in this publication do not imply endorsement of commercial products.



Figure 7.--Comparison of fish tester-readings and organoleptic evaluation of the cooked fish. The meter readings indicate the days of iced storage life remaining before the fish become inedible.

specific activity that does not vary significantly with season or among specimens within a single species (table 3).

Table 3.--Relation of season to malic enzyme specific activity in fresh haddock muscle

Malic enzyme specific activity
ΔA/ml. CTF/min. ¹
0.35 .39
.42 .43

¹ Change in absorbance at 340 millimicrons per milliliter of centrifuged tissue fluid per minute.

2. The specific activity remains at its initial high level under good frozen-storage conditions (-29° C.) and drops under poor frozen-storage conditions (-7° C.).

3. In all species tested to date--haddock, cod, dab, pollock, striped bass, and ocean pout--the specific activity of malic enzyme rises dramatically after freshfish have been frozen and thawed.

4. This rise in activity does not occur during iced storage. (When low-level gamma radiation was used to prevent bacterial growth, which in itself gives a spurious rise, the malic enzyme specific activity remained at a constant low in iced-stored haddock for 14 days, the duration of the experiment.)

From these observations we conclude that the rise in malic enzyme specific activity is attributable solely to the structural damage in the tissue caused by freeze-thawing and that the subsequent drop in specific activity under poor frozen-storage conditions is attributable to degradation of the enzyme itself.

For assessing functional changes in alphaglycerophosphate dehydrogenase (α GPdH), the variation of reaction rate with substrate concentration was used to derive an activity constant that reflects the enzyme's capacity to function (table 4).

The constant for fresh-frozen fish is the reference standard, against which is measured the degree of change with time of frozen storage. In our pilot storage study with the enzyme system, the activity constant increased appreciably as early as 2 and 4 weeks for haddock stored at -7° C., whereas even a slight increase above the original fresh-frozen value did not appear until 16 weeks in haddock stored at -29° C.

Our reasoning is that the change in activity constant with time of frozen storage is due either to degradation of the enzyme or to the introduction of an isozyme, an enzyme that catalyses the same reaction as another enzyme but has slightly differing electrophoretic mobilities. Our present experimental schedule includes a series of electrophoretic studies in which polyacrylamide gels will be used to determine whether these observed kinetic changes are caused by isozyme intrusion or (as is the case with the malic enzyme) by enzyme degradation.

The assay of enzymic function, under protocols controlled and reproducible in vitro, avoids the interspecimen variations to which quantitative assays are heir. The groundwork for this new approach has been laid over the past year and a half; the purpose of our longrange study is to test its logic.

	GPdH activity	constants for:
Season	Fresh haddock	Fresh-frozen haddock
	ΔA/ml. CTF/min. ¹	$\Delta A/ml. CTF/min.^1$
Fall Winter Summer	0.36, 0.36 .42, .35 .23, .27	0.79, 0.75 .57, .57 .56, .56

Table 4.--Alpha-glycerophosphate dehydrogenase data on flesh of fresh haddock and of fresh-frozen haddock

Note: The summer series was run on commercial l-pound plate-frozen packs, with the dark meat included; previous runs were made on white meat only, vacuum sealed in plastic bags and frozen in still air.

¹ Change in absorbance at 340 millimicrons per milliliter of centrifuged tissue fluid per minute.



Figure 8.--This Warburg manometric apparatus is one of the tools used in the study of fish muscle enzymes, a project designed to find an objective biochemical test of quality change in frozen fish.



Figure 9.--Freezing of fishery products by immersion in liquid nitrogen.

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STANDARDS AND SPECIFICATIONS RESEARCH

by

J. Perry Lane, Program Leader

Of vital concern to the fishing industry and the consuming public is the mechanism whereby high-quality seafoods may be sold and purchased in an orderly and efficient manner. This laboratory has the responsibility for developing buying guides in the form of specifications and quality guidelines, or grade standards, for fishery products. Specifications promote orderly marketing of seafoods by serving as a uniform basis for quality criteria that, in turn, serve as a contractual agreement between a buyer and seller. Grade standards are yardsticks of quality that assist in the production of high-quality seafoods and serve as a recognizable quality level for the consumer.

STANDARDS DEVELOPMENT

Over the past 8 years, grade standards have been developed for 14 frozen fishery products. In keeping with the trend toward increased use of convenience foods, standards for four breaded items were developed or revised and promulgated in fiscal year 1964. These were for frozen-fried and frozen-raw breaded fish sticks and for frozen-fried and raw breaded fish portions.

In addition, the Grade Standard for Frozen Fish Blocks was revised to streamline the requirements and broaden the field of application. If it is to raise the quality of the product effectively, a standard must be based on the highest quality that can be attained by good commercial practice and must not be based simply on idealistic requirements that, for practical economic purposes, are unattainable. One of the integral steps in the development or revision of any grade standard is a test of the practicability of the requirements of the standard. Table 1 illustrates the results of such a test of applicability for the fish-block standard. The quality factors that were considered are given as are also the average number of points that were deducted for each factor because of failure to achieve the required degree of excellence. Information in the lower part of the table indicates that the requirements are realistic and can be met by industry.

Percent

0.8

Table	1Average	effect	of	quality	factors	in	the	grading
		of 12	24 :	fish bloc	eks			

Factor		Average deduction
		Points
Improper fill. Blemishes. Jniformity of size. Angles. Bones. Dehydration. Color. Fexture. Uniformity of weight.		3.1 2.0 1.7 1.2 1.0 .8 .7 .3 .2
Average deductions		10.8
Fish block samples	s graded as being	A, B, or Substandard
Grade A	Grade B	Substandard

Percent

13.7

1

No.

Percent

85.5

106

The consumer is interested in the quality of seafood at the time he purchases it. This time factor means that the cumulative effect of all the stages in the distribution chain, from sea to retail cabinet, must be taken into consideration. To measure the quality of seafoods at the consumer level, we make an annual grading survey on frozen fishery products obtained from retail outlets throughout the country. These products are shipped to the laboratory and graded by USDI inspectors. The results obtained from such surveys enable us to pinpoint those places in the processing and distribution chain that contribute to the loss of quality. During fiscal year 1964, a survey was made on frozen haddock, flounder, and sole fillets, and the findings were reported at an industry meeting.

We obtained samples of frozen fillets from retail outlets in nine metropolitan areas throughout the United States. They were shipped to the Laboratory and held in frozen storage until they were graded in accordance with USDI standards for grades. Trained personnel of our Inspection and Certification Unit did the grading. Table 2 shows the results of this survey. Of the 600 samples of haddock and flounder or sole, about two-thirds were either Grade A or Grade B. The other one-third were Substandard Grade or G.N.C. (Grade Not Certified). This latter category is used for samples that do not meet the product description -- for example, fish portions labeled as "fillets" -- or that are decomposed or otherwise unfit for food purposes.

SPECIFICATIONS DEVELOPMENT

Specifications may be defined as accurate descriptions of the technical requirements for materials, products, or services, including the procedure by which we determine whether the requirements have been met. At the Bureau's Laboratory in Gloucester, both Federal and National Association of State Purchasing Officials (NASPO) Specifications for fishery products are developed and revised as necessary. These specifications serve as a basis for bids by producers on orders from the Department of Defense, the Veterans Administration, various State purchasing agencies, and other institutional food users.

During the past year, we completed developmental work on three Federal specifications: canned clams, natural sponges, and chilled and frozen fish. All these specifications were submitted to the General Services Administration for publication. The task of coordinating specifications with both prospective purchasers and producers of seafoods is in progress for another four Federal specifications--namely, raw clams, raw oysters, canned sardines, and canned tuna. Three NASPO specifications--for canned tuna, canned salmon, and canned sardines--are near completion.

LONG-RANGE RESEARCH PROGRAMS

Research on standards and specifications is a new activity that will enable us to obtain the data needed to improve the quality criteria of standards and specifications or to improve and simplify the application of these documents. Currently, two long-range studies are underway.

Effect of Processing Variables on Flesh Content of Breaded Fishery Products

The first of these is an investigation of the effects of processing variables on the content of fish flesh in breaded fish portions. Preliminary studies have revealed that such processing variables as the temperature of the fish block from which the portions are cut, the manner of preparing the blocks, batter

and dealer by		Distr	ibutio	n of grade	es for	:
Grade	Ha	addock	F1 O	ounder r sole	1	Fotals
	<u>No</u> .	Percent	No.	Percent	<u>No</u> .	Percent
A 3 5/Std 3.N.C	50 132 80 11	18.3 48.4 29.3 4.0	89 132 101 5	27.2 40.4 30.9 1.5	139 264 181 16	23.2 44.0 30.2 2.6
Total	273	100.0	327	100.0	600	100.0

Table 2.--1964 survey of frozen fish fillets by grade

Note: S/Std. = Substandard; G.N.C. = Grade Not Certified.

Measurement or calculation	Result of :	measurement or ca	alculation
Fish weight (g.)		233.7	
Temperature of block	-20° F.	+15° F.	+15-22° F.
Breaded weight (g.)	308.9	304.3	302.2
Percent fish flesh (pickup basis)	75.7	76.8	77.3
Weight after 24 hours (g.)	307.4	302.8	300.7
Debreaded weight of fish (g.)	235.7	229.9	224.9
Percent fish flesh	76.7	75.9	74.8

Table 3.--Effect of block temperature on fish flesh

viscosity, amount of breading, storage time, and temperature of the portions all have an effect on the content of fish flesh. We will investigate each of these factors and determine its effect on fish content by comparing the amount of breading actually placed on the portion with the amount of flesh that can be recovered from the portion by physically removing the coating.

The investigation of the effect of block temperature on the fish content of the breaded portions was completed this year. Table 3 shows that the blocks held at the lowest temperature (-20° F.) picked up more coating material and thus had a lower percent fish flesh than did the portions from blocks at the two higher temperatures. When the portions were stored for 24 hours and then debreaded, the reverse was true--that is, portions from the blocks tempered at from 15° to 22° F. yielded the lowest percent fish flesh, whereas the portions from the -20° F. blocks had the highest. We are trying to determine the reason for this reversal. The indications are that the differences are due to the transfer of moisture from the fish to the breading material.

Species Identification

The second study is concerned with the identification of the species of fish in a processed fishery product. The methods we use involve electrophoresis to separate the different proteins of the fish. Each species has its own characteristic set of proteins. These proteins can be separated by electrical means on a carrying medium such as starch or again gel. The rate at which the proteins separate depends on such factors as the size and shape of the molecules and their net charges. Once separation has taken place, the protein bands can be stained. This staining produces a band pattern that is reproducible and different for each species of fish; thus we can positively identify the species (Figure 1.) even when all the visual means of recognition have been eliminated during processing.

Robert R. Thompson of the U.S. Food & Drug Administration used this principle to develop a starch-gel electrophoretic procedure for the identification of fish species. His method was published in 1960 as a "first action" in the ournal of the Association of Official Agriculral Chemists. To have the method accepted s "official," it must be confirmed by different orkers. Our laboratory did the confirmatory ork, and Thompson's method has now been ecommended for adoption as the official acted. In addition to the work with starch gel, we have been working with a more rapid method of using agar gel as the carrying medium. The aim of the work is to establish a simple, rapid test that untrained persons can use in the field. We are continuing our work with the agar gel and other methods that show promise.



Figure 10--Band pattern for haddock and cod plus the "fingerprint" of an unknown sample, which can be identified as a cod.

RADIATION PASTEURIZATION RESEARCH

by

Louis J. Ronsivalli, Program Leader

Our research on irradiation of fish included studies on: product acceptability and shelflife, packaging, microbiology, and flavor and odors.

PRODUCT ACCEPTABILITY AND SHELF LIFE

Our study of product acceptability and shelf life is an extension of work conducted since fiscal year 1960. Our aims are to determine the optimum dose level for the radiopasteurizing of each species of the commercially important fish of the Northwest Atlantic and to measure the quality and acceptance of the irradiation product (air packed and vacuum packed).

In past work, we found that dose levels of 150,000 to 450,000 rads significantly extended the shelf life of refrigerated clam meats and fillets of haddock, pollock, and ocean perch. A 12-member, trained panel reported that these species were at least moderately acceptable after 20 to 30 days when stored at 33° to 35° F. This period represents two to three times the normal shelf life of fish. But the shelf life of radiopasteurized fish at 42° F. was about one-

half that of samples stored at 33° to 35° F. Also, the shelf-life extension, overall quality, and acceptance were essentially the same for samples whether they were air packed or vacuum packed. For nearly every species, however, vacuum packed samples required lower doses than did air packed samples.

This year, we expanded our studies to include large-scale acceptability tests on irradiated fish fillets. Although a number of trained and consumer-type taste panels had determined the acceptability of irradiated, airpacked, skinless, haddock fillets, the size of each panel was relatively small (12 to 14 judges). With the help of the U.S. Army Research and Engineering Command Field Evaluation Agency, we were able to have a series of large-scale tests at Fort Lee Va. More than 300 troops were fed deep-fat-fried irradiated haddock fillets as well as deep-fat-fried frozen haddock fillets. All fillets were obtained from one batch of good-quality fish and were air packed in No. 10 cans. Half the cans were held frozen; the other half were irradiated at 250,000 rads and held in flake ice at 33° to 35° F. Taste tests were held 14 and 29 days after irradiation. The results of these tests (table 1) indicated that the

Table 1.--Adjusted average hedonic-scale ratings of irradiated and nonirradiated skinless haddock fillets, Fort Lee, Va., 1964

	Adjusted average score	¹ for fillets stored:
Tasters	15 days	29 days
Number	Irradiated fillets sto	red at 33°-35° F.
40 42 66	6.8 5.0 5.4	7.0 5.7 4.7
	Nonirradiated fillets	held frozen at 5° F.
54 35 77	7.0 5.5 5.9	6.9 5.7 5.9

¹ The 9-point scale was used where: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely.

Coolood	Radiati	on levels
Searood	Air-packed	Vacuum-packed
	Rads	Rads
Haddock fillets Clam meats Pollock fillets Ocean perch fillets Cod fillets Mackerel fillets	250,000 450,000 150,000 250,000 150,000 250,000	150,000 350,000 150,000 150,000 150,000

Table 2.--The optimum radiation levels for some canned seafoods

¹ This value is tentative.

oops could not distinguish between the irdiated and the frozen fillets. According to S. Army acceptability levels, these irradied fillets were rated as acceptable as a andard Army ration. Although the overall cores showed that U.S. soldiers have a latively low preference for fish, the results, wertheless, indicate that the organoleptic alities of 1-month-old irradiated haddock lets do not differ significantly from fresh addock fillets stored frozen for the same ngth of time.

The data obtained during fiscal year 1964 proborated earlier findings regarding the accessful application of low-level radiation uses for extending the shelf life of fresh fish roducts to two and three times the normal helf life. We established optimum levels of adiation for cod and mackerel fillets, bringing e number of species studied at this laborary to six.

The optimum doses obtained for clam meats ad the fillets of haddock, pollock, ocean perch, ad, and mackerel indicate that, in general, the acuum packed samples required a lower otimum dose (table 2). We have not determined e reason for this, but it appears that a vacuum ets as a growth inhibitor on the normal bacrial flora, which is predominantly aerobic, ad that this effect adds to the effect of radiaon. No quality difference existed between the r and vacuum packed products except as noted the cod samples.

This year we found, as in earlier work, that e shelf life of the product stored at 42° F. as about one-half that of the product stored 33° to 35° F.

Since the quality of fishused for commercial radiation will vary, we wished to determine by quality in raw fish affects the quality of e irradiated product. Previous studies on addock indicated that the radiation process has ractical application for haddock fillets cut from fish that had been iced as much as 7 to 9 days out of the water.

This year, we expanded the quality studies to include cod. As with the haddock experiments, very fresh cod were stored in ice; and, at periodic intervals of storage, fillets were cut from these cod, air packed in cans, irradiated at the optimum dose, stored at 33° to 35° F., and periodically examined for organoleptic quality. Storage ended when samples received scores of less than fair quality (on the 5-point scale, fair = 2). The results of this experiment are plotted in figure 11 and are comparable with those obtained for haddock, except for the slightly longer shelf life of the cod which may be due to the differing qualities of the fish used.



Figure 11.--The relation between the preirradiation storage time of iced cod and the postirradiation shelf life of the cod fillets held at 33^o F.

PACKAGING RESEARCH

The purpose of our packaging research was to determine if flexible films were suitable for packaging irradiated products. Researchers in the field have thought cans to be unsuitable, because they are closely associated with processed foods, and radiopasteurized fish should be considered more of a fresh product, as is pasteurized milk for example. Furthermore, flexible materials have many advantages--low cost, material availability, freedom in package design, light weight, and transparency.

When haddock fillets packed in polyethylene and polypropylene plastic films were compared with canned fillets, the plastic packs had poorer organoleptic quality and gave higher total plate counts than the canned packs. The plastic packs were first examined for structural inadequacies (such as poor heat seals, pinholes, and low tensile strength) and bacterial permeability. Table 3 lists all the films investigated, their thickness, and comments regarding their suitability.

Our results indicate that the suitability of available plastic films is limited mainly by their rates of oxygen transmission. Products stored in films with high rates of oxygen transmission lost quality more rapidly than did those packed in materials having low rates.

The importance of oxygen within a package of foods has been well defined in relation to the chemistry of oxidative spoilage. The importance of oxidative spoilage in this case is apparently secondary, however, to spoilage by aerobic bacteria.

In general, the plastics tested by this laboratory were impermeable to bacteria and free from pinholes. (Others have reported that films less than 0.5-mil¹ thick may have a high incidence of pinholes.)

MICROBIOLOGY

To complement organoleptic analyses, we had determined the total bacteria plate counts of the fish samples in our studies. Our earlier work was concerned with the determination of total plate counts of irradiated and nonirradiated fish samples and tests on bacterial permeability of plastic films. These data indicated that when haddock fillets were irradiated at 250,000 rads, the number of bacteria was reduced by about 99 percent.

In fiscal year 1964, we made total plate counts for irradiated and nonirradiated samples. During this period, the results of a number of analyses indicated that, from a quality standpoint, irradiated fillets would tolerate higher total plate counts than would nonirradiated fillets. That is, it was not uncommon to find plate counts of as high as 10 million to 1,000 million bacteria per gram in irradiated fillets of fair acceptability in the last stage of storage; but in nonirradiated fillets of similar organoleptic quality, the total plate counts did not exceed 10 million bacteria per gram. This phenomenon has been reported by others. Many theories have been advanced to explainit; perhaps the more acceptable one is that the micro bial flora of the nonirradiated fillets has higher proportion of the common spoilag organisms than does the flora of the irradiate fillets.

Experiments to determine the bacteric permeability of films were continued, and cu results substantiated earlier findings that more of the available plastic films generally are no permeable to bacteria.

We feel that the composition of the micic flora in fish fillets is changed by irradiation Others have shown that irradiation is more ce structive to the predominant, common, spoilage organisms, such as the Pseudomonas group than to other species, thus a shift in microfle; is created. We have considered another in portant factor which may affect the compositio of the microflora. Usually, radiopasteurize fish have been packed in hermetically seale containers, and as oxygen is consumed by sur viving organisms and chemical oxidative re actions, the oxygen tension is lowered to level detrimental to the survival of strict aerobe and some of the facultative species. We ar now studying what effect oxygen availability ha on the composition of the microflora in irradi ated and nonirradiated fish.

FLAVOR AND ODOR RESEARCH

The purposes of this research are to stue the chemistry of fish flavors and odors with th idea of attempting to control factors involvin spoilage and possibly to develop objective test for measuring fish quality. Gas chromate graphic, wet chemistry, and mass spectre metric techniques were used.

This year, we continued gas chromatic graphic analyses of fish volatiles using it tention columns of 2 to 10 percent B,D oxydipropionitrile on Chromosorb-W that we previously acid-washed or treated with n-Hes amethyldisilizane, 25 percent Carbowax 20 on acid-washed Chromosorb W, and 20 perces polymerized ethylene glycol succinate on en bacel, acid-washed Kieselguhr. The volatic compounds in clam meats were studied in tot and by chemical classes.

Carbonyls were extracted from the tot volatiles by a modified version of the Girar T reagent method (Stanley, Ikeda, Vannier, ar Rolle, 1961) and analyzed directly by ga chromatography. A chromatogram of the vola tile carbonyls in untreated 2-day-old clar meats is shown in figure 12. Data obtained thu far indicate that carbonyl measurements ca be used to identify fresh untreated clam meats This idea is based on the following facts: (Carbonyl analyses made of different sample of fresh untreated clam meats, produced simi lar chromatogram as shown in figure 12; (I when clam meats were irradiated and thei volatile carbonyls analyzed, the peaks in th

 $^{11 \}text{ mil} = 0.001 \text{ inch.}$

No.	Plastic	Overall thickness	Suitability	Comments
		(Mils) ¹		
1	Polyethylene	3.4	Unsatisfactory	Gas permeability rate too high
2	Polyethylene	4.0	do	Gas permeability rate too high
3	polypropylene	1.0	do	Gas permeability rate too high
4	Saran-coated cellophane	1.0	·····do·····	Poor sealing characteristics
5	Cellophare	1.0	do	Seals tear with handling
6	Nylon-6	1.0	do	Water transmission rate too high
7	Nylon-11	1.6	Excellent	**********************
	Saran-coated nylon-11	1.6	·····do·····	
6	Polyolefine-coated polyester	2.0	op	**********************
10	Polyethylene-conted polyester	2.0	do	*************************
11	Laminated paper-aluminum-polyethylene	3.0	Good	***********************
12	Laminated paper-aluminum-polyolefine-coated polyster	6.0	Excellent	
υ	Laminated aluminum-paper-polyolefine-coated polyster	6.0	do	
14	Laminated mylon-11 - aluminum	2.0	Good	
25	Leminated saran-polysthylens-nylon	3.5	Excellent	
16	Sarah-coated polystyreme 11ds and trays	{ 7.0 (11ds) { 10.0 (trays)	····do····	
17	Polystyrens lids and trays	(7.0 (lids) (10.0 (trays)	Good to excellent	
18	Rubber hydrochloride	1.2	Unsatisfactory	Seals tear with handling

¹ Mil (a common packaging unit to define thickness) 0.001 inch.



Figure 12.--Gas chromatogram of concentrated volatile carbonyl compounds in raw unirradiated clam meats held for 2 days at 34⁰ F.

resulting chromatogram were much larger and more numerous; (3) when the meats were cooked a pattern similar to that of the irradated sample was obtained. Irradiation or cooking produced a accelerated increases in carbonyls; storage caused slow carbonyl development.

When fresh untreated clams were refrigerated, the concentration of volatile carbonyls increased until about the 15th to 20th day of storage and then decreased until about the 30th day of storage, when we were unable to measure the amount. We are studying this finding, which indicates that the concentration of carbonyls might be used as an index of quality only until spoilage occurs.

We have also studied carbonyls using a precipitation method (Mendelsohn and Steinberg, 1962), and we have studied sulfides using a colorimetric method (American Public Health Association, 1955). Although each method is a quantitative one, neither gives information on individual compounds. These values were used, however, to check the overall results from gas chromatography.

The gas chromatography of sulfides was patterned after that suggested by Bassette, Ozeris, and Whitnah (1962). In our study, we divided the total volatiles into two parts. We reacted one part with mercuric chloride, which served to remove the sulfides; then we chromatographed both aliquots. By the absence of corresponding peaks in the chromatogram of the treated sample, we could detect the sulfides in the other chromatogram.

We have identified more than 20 carbonyls and sulfides, most of them by comparing their retention times with the retention times of purcompounds on at least three separate retention columns. We have also made tentative identifications by using the linear relation between boiling points and retention times of members within homologous series (Bauman and Olund 1962).

A Time of Flight Mass Spectrometer is ex pected to help us accelerate our identificatio of volatile compounds. Since identifications b mass spectrometry are simplified when th fewest number of compounds are analyzed components of a gas chromatograph have bee integrated in the spectrometer so that a mix ture can be separated into individual compound prior to entry in the flight tube. The spectra of standards published by the American Petroleur Institute will be used to confirm identifications

In conclusion, of the gas chromatographi techniques used to study the chemistry of fis volatiles, the analyses of volatile carbonyl appeared to be the most productive. We wer able to show that changes in the carbonyl pattern were a function of storage, heating, an irradiation. Since there were indications tha development and dissipation of carbonyls were both functions of oxygen availability and bacterial activity, these aspects are nowbeing in vestigated. These relations strongly sugges that volatile carbonyls are important spoilagfactors and may be indices of fish quality.

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Figure 13.--Collection of the volatile components from fishery products by a highvacuum, low-temperature distillation technique.





MARINE PRODUCTS DEVELOPMENT IRRADIATOR

by

John D. Kaylor, Program Leader

nvestigations conducted by personnel at this oratory and by others into the bacteriaing effect of low levels of radiation have own that the shelf life of radiopasteurized hery products can be doubled or tripled by ping the pasteurized products at 33° F. The lity of most seafoods to tolerate this cold teurization process has led the U.S. Atomic ergy Commission (AEC) to enter into an reement with the Bureau of Commercial theries (BCF) to convert this phenomenon commercial use.

Funds supplied by AEC have provided for the action of an irradiator at the site of the BCF chnological Laboratory in Gloucester. bundbreaking ceremonies were held in July 3. The facility is expected to be finished loperating by January 1965.

This irradiator will be the only food adiator of this size in the world designed clusively for cold pasteurization of fishery oducts. The facility will contain 250,000 ries of cobalt 60 and will irradiate 1 ton fish per hour at a dose level of 250,000 ds. The design features the simplicity of eration necessary for a commercial facility. The products, which are packaged in regular h fillet tins, will be conveyed into the adiation cell under and over the cobalt 60 source until four complete passes are made. When not in use, the cobalt 60 is lowered into a well of water 15 feet deep.

The irradiator program has several aims, one of which is to provide detailed information on present shipping and marketing practices of fresh fishery products so that the suitability of these practices for the handling of radiopasteurized fishery products can be determined. With this information available, we can recommend whatever new procedures may be necessary to ensure good distribution and marketing of pasteurized seafoods.

Another aim is to carry out large-scale feeding tests to determine the acceptability of marine products pasteurized at those energy levels found most desirable. The fish for such tests will include haddock, cod, ocean perch, flounder, lobster, crabs, and clams.

In addition to providing radiation pasteurization services on large samples for industry evaluation, we aim to make cost studies of the process. Records of labor, raw-material, packaging, variable, fixed, and other costs will be kept so that we can have an accurate actual cost per pound of pasteurized product. We will cooperate closely with industry in order to test every phase of the program for practicability.



Figure 15.--Instrument control panel showing radiation meters, warning-alarm panel lights, closed-circuit television, and motor controls for several safety devices.

by

Philip J. McKay, Regional Supervisory Inspector

he U.S. Department of the Interior's hery Products Inspection Service in the th and Middle Atlantic States has increased number of inspectors from 21 to 24 this r. The increase was required by additional k shifts in several of the plants. The numof inspected plants has remained, however, 2.

ome 103.0 million pounds of fish were cessed in the 12 continuously inspected its, and some 6.3 million pounds were inspected in this geographical region. The inspections of various frozen, salted, med, and smoked fishery products destined distribution to buyers and institutions were made at the requests of vendors and Federal and State procurement offices.

Several problems noted in inspecting imported fishery products, especially fish blocks, were: (1) block cartons did not have enough wax, causing the carton to stick to the fish; (2) foreign material, such as matches, cigarette butts, and rope, were found in the blocks; and (3) flounder and sole fillets were combined within one block.

The headquarters for this unit is at the Bureau's Technological Laboratory, Emerson Avenue, Gloucester, Mass., 01931. It has a suboffice located in the Federal Building, 641 Washington Street, New York, N.Y. 10014.



Figure 16.--Fishery Products Inspector checking raw breaded portions for workmanship, flavor, and odor.

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1964c, Radiation preservation of fisher: products, Presented at a meeting of the Northeastern Resources Committee, Rowe, Mass., April 8-9,

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Joseph W. Slavin, Laboratory Director

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