CHEMICAL COMPOUNDS FORMED DURING THE SPOILAGE OF FISH

ABSTRACT

A progress report on a chromatographic study of the protein-breakdown products appearing in the meat of fish during spoilage. Free ninhydrin-reactive components such as the amino acids were studied on homogenates of iced round fish and of minced fish throughout a 15-day storage period. No ninhydrin-reactive compounds other than those reported were observed. No significant increases in free amino acids were noted though free lysine apparently decreased in quantity during storage.

INTRODUCTION

Different types of spoilage occur in fish of different species and even in fish of the same species, depending upon such factors as the variations in the chemical constituents of the muscle of different species, the conditions of handling during storage, and the kinds of enzymes and bacteria involved. A knowledge of why different types of spoilage occur under various handling conditions and of what types of chemical compounds are formed would be of great value in preservation studies and in the development of objective tests for determining the freshness of fish. Consequently the Seattle Fishery Technological Laboratory of the U. S. Fish and Wildlife Service began a study of the various types of chemical compounds that are formed in the meat of fish when spoilage occurs.

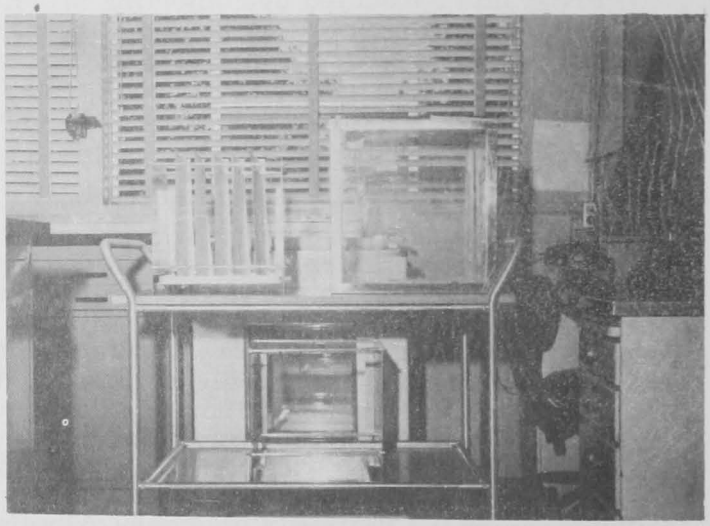

Fig. 1 - Chromatographic equipment.

Work to date has included a preliminary investigation of protein-breakdown products such as amino acids and amines. Qualitative (identifying) and semi-quantitative (not wholly precise measurement of amounts) determination of these substances have been made by means of filter-paper chromatography. This technique is well adapted for making preliminary separations and identifications of the wide range of substances that may form in small quantities in fish meat during spoilage.

In paper chromatography, a small drop of the solution containing the substances to be separated is applied to a strip or a sheet of filter paper at a short distance from one end of the paper. The drop is allowed to dry. The end of the paper nearest to the spot is placed in the "developing" solvent, which usually is water con-

taining an organic solvent. The developing solvent flows by capillary action past the original spot and up the length of the paper. Because the various substances in the original spot have different rates of flow in this solvent, they are separated and may be identified by position, shape, and color of the spots that form subsequently. Although much work has been done in recent years in the field of paper chromatography, in any particular application much additional research work often is necessary on the use of various combinations of solvents, filter papers, and methods of detecting spots before the compounds in any particular product can be separated and identified satisfactorily.

The free amino acid content in fish of varying degrees of freshness has been investigated by several workers. Amano and Bito (1950) found glycine, alanine, and glutamic acid in all species examined and also found that other amino acids appeared during the decomposition of fish muscle. Jones (1954) found that the free amino acid composition of North Sea codling muscle varied with the season and changed considerably when the muscle was stored in ice, owing (1) to leaching by the melting ice and to the action of autolytic enzymes during the earlier period of storage and (2) to the added effects of spoilage micro-organisms during the later period of storage.

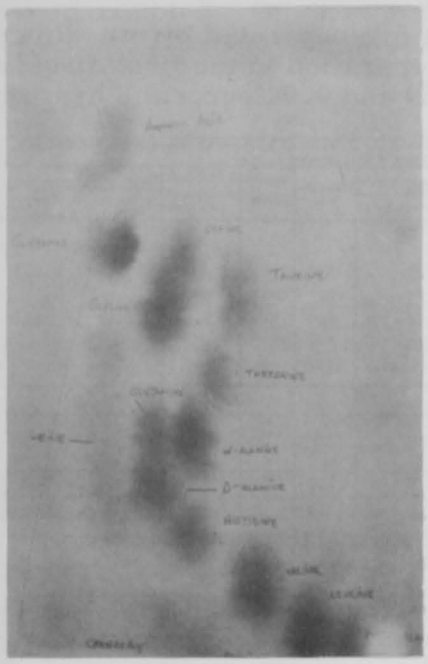

Fig. 2 - Standard chromatogram.

Pacific cod (Gadus Macrocephalus) was chosen for the studies reported in the present paper because it is a fish that is available throughout the year in relatively large quantities in Seattle. The cod were stored in two different forms: whole fish and ground fish muscle. Often in experimental work it is desirable to grind and mix all of the fish being studied in order that the samples will be homogeneous and representative of the entire lot. Grinding the fish, however, may introduce variables that are not present in work with the whole fish. The data presented here include results both from whole fish and from ground fish.

EXPERIMENTAL PROCEDURE

The reaction of ninhydrin with compounds containing the amine groups--such as amino acids and amines--to give distinctive colored compounds is used as the basis for studying protein-breakdown products during the spoilage of fish. In the present work, the free amino acids and other ninhydrin-reactive substances in cod muscle were studied by two-dimensional paper chromatography to determine what changes in the ninhydrin-reactive substances occur as the muscle deteriorates during refrigerated storage.

Two groups of fish were studied. The first group, called lot 1, consisted of Pacific cod that had been stored aboard the vessel in the round in ice for 4 or 5 days. The second group, lot 2, was not iced but was stored for 2 days aboard the vessel on top of other iced fish. The second lot of cod was used to check the results obtained with the first one. The difference in treatment of the cod aboard the fishing vessel was not part of the experimental design and is reported only to make the history of the samples complete. The exact treatment given each lot of fish is shown in table 1.

In the analysis of the samples for free amino acids and other ninhydrin reactive substances, the aqueous fraction was extracted from cod muscle by the method outlined by Block, Durrum, and Zweig (1955). The procedure used is as follows:

1. Mix 25 grams of cod meat with 80 milliliters of absolute ethanol in an electric blendor for 5 minutes.
2. Separate out the solid material by suction filtration, wash the solids with 80-percent ethanol, add the washings to the filtrate, and then discard the solids.
3. Transfer the filtrate to a separatory funnel, add three volumes of chloroform to each volume of the filtrate, and shake the mixture thoroughly.
4. Allow the two layers in the funnel to separate, then discard the bottom layer.
5. Concentrate the aqueous (upper) layer to a volume of 5 to 10 milliliters (accurately measured).

The ninhydrin-reactive constituents of the extract are separated by two-dimensional paper chromatography. The solvent used for separation in the first direction consists of liquid phenol and metal-free water (4:1 v/v) and 0.04 percent 8 hydroxyquinoline; the solvent used for separation in the second direction consists of 2,6-lutidine, 2, 4, 6-collidine, water, and diethylamine (100:100:100:3 v/v) (Block et al. 1955). The various compounds that separate are detected by dipping the chromatograms in 0.25 percent (w/v) ninhydrin in acetone (Toennies 1951). After the chromatogram is dried in a hood, the color is developed by placing the chromatogram in a warm oven at about 85° C. for 5 to 10 minutes. Spots of varying colors and shapes appear as a result of the reaction between ninhydrin and the various separated compounds.

Table 1 - Changes in Free Amino Acid Content (As Determined by Paper Chromatography) of Cod with Length of Time of Refrigerated Storage

Lot	History of Samples: Aboard Vessel	History of Samples: At the Laboratory: Treatment	History of Samples: At the Laboratory: Storage Time	Sample No.	Free Amino Acids Content 1/: Lysine	Free Amino Acids Content 1/: Valine and Leucines	Free Amino Acids Content 1/: Glutamic Acid, Glycine, Taurine, Proline, α- and β-Alanine
			Days				
1	Pacific cod, in the round, stored in ice for 4 or 5 days	Fillets cut into small particles in meat chopper, mixed thoroughly, filled into jars with snap-on plastic covers, and stored at 34° F.	0	7M	+	+	+
			12	8F	Trace	Trace	+
			15	9E	-	Trace	+
		Round fish stored in ice	15	9D	-	+	+
2	Pacific cod, not iced but stored on top of other iced fish for 2 days; the internal temperature of the cod was 42° to 46° F. when they were obtained.	Fillets cut into small particles in meat chopper, mixed thoroughly, filled into jars with snap-on plastic covers, and stored at 34° F.	0	10B	+	+	+
				10C	+	+	+
				10D	+	+	+
				10F	+	+	+
			6	11A	Trace	Trace	+
				11B	+	Trace	+
			10	12A	-	+	+
				12C	-	+	+
				12B	Trace	+	+
			27	12G	-	+	+
		Round fish stored in ice	6	11C	+	+	+
				11D	+	+	+
			10	12D	Trace	+	+
				12E	Trace	+	+

1/ A plus indicates that a definite chromatographic spot was found, and a minus indicates that no spot was found.

A series of standard solutions containing various combinations of pure amino acids and amines are also chromatographed, and each of the various compounds is ultimately identified on the "standard" chromatogram. The spots appearing on the chromatograms of the various cod samples then are identified by comparing them with the known spots on the standard chromatogram.

RESULTS

The data obtained with lot 1 (table 1) indicated the following: (1) free lysine, valine, leucines, glutamic acid, glycine, taurine, proline, and α-and β-alanine were present in the fresh samples; (2) no changes were detected in the amount of glutamic acid, glycine, taurine, proline, and α-and β-alanine as the samples were stored; (3) the amounts of valine and of leucines decreased in the ground samples stored in jars at 34° F. but not in the whole fish stored in ice; (4) the amount of lysine decreased both in the ground sample stored at 34° F. and in the whole fish stored in ice.

The results obtained with lot 2 are in essential agreement with those obtained with lot 1, except that the amounts of valine and of leucines decreased only in the ground samples stored for 6 days but not in any of the other ground samples.

Except for the amino acids shown in table 1, no other ninhydrin-reactive compounds were found by the experimental procedure used.

SUMMARY

In a study of the various types of chemical compounds that are found in fish meat when spoilage occurs, Pacific cod (Gadus macrocephalus) were stored in the round in ice, and samples of the ground cod were stored in jars at 34° F. Paper chromatography was used to detect the ninhydrin-reactive compounds. Free lysine, valine, leucines, glutamic acid, glycine, taurine, proline, and α- and ζ-alanine were detected in the fresh samples. Lysine decreased in quantity in the samples during storage. No changes in the quantities of glutamic acid, glycine, taurine, proline, and α- and ζ-alanine were detected. The results obtained to date are not sufficiently conclusive to establish a definite difference between whole samples and ground samples. Other than the compounds already reported in this summary, no other ninhydrin-reactive substances were found by the experimental procedure used.

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"GRAYING" IN CANNED SHRIMP

Research on the most prevalent form of discoloration--"graying"--in canned wet pack shrimp has shown that the discoloration is dependent upon the age or condition of the shrimp prior to canning. That is, as the age of the shrimp in ice increased, both the pH and the amount of "graying" increased in the canned product. It was also noted that black iron sulphide container discoloration was more likely to occur with an elevated product pH (Food Technology, May 1956).

TECHNICAL NOTE NO.36 - DETERMINING FISH CONTENT OF FROZEN FRIED FISH STICKS

ABSTRACT

This report describes the basic procedure for determining the content of the meat of fish in frozen fried fish sticks. The procedure offers packers a simple and rapid method of analysis that can be used conveniently as a tool in the control of quality. The procedure is useful also in determining compliance with the proposed standards for grades of frozen fried fish sticks.

INTRODUCTION

The voluntary Federal Standards for Grades of Frozen Fried Fish Sticks (Federal Register 1956) provide that frozen fried fish sticks contain not less than 60 percent, by weight, of fish meat. The Technological Laboratory of the U. S. Fish and Wildlife Service at East Boston, Mass., developed a method of analysis capable of determining compliance with this requirement.

The method that was developed is simple and rapid. It consists, essentially, of the following steps: (1) weigh the whole frozen fried fish stick, (2) dip the fish stick in a colored solution (copper sulfate) at room temperature for a specified time, (3) remove the coating on the fish stick by scraping the stick with a spatula, (4) weigh the remaining fish meat, and (5) calculate the content of fish meat in the fish stick by use of the formula:

$$\text{Percent fish meat} \quad \frac{(\text{wt. of fish meat after removal of coating})\ 100}{(\text{wt. of whole frozen-fried fish stick})}$$

PROCEDURE

APPARATUS AND MATERIALS:

1. Triple-beam or similar rapid-weighing balance that is sensitive to $\pm$ 0.05 gram.
2. Two-liter beaker.
3. Copper-sulfate solution.
4. Immersion thermometer.
5. Clip tongs of wire, plastic, or glass.
6. Stop watch or regular watch with second hand.
7. Ordinary paper towels.
8. Spatula, 4-inch blade with rounded tip.
9. Container for waste breading and fish.
10. Glass funnel, medium size.
11. Glass wool, coarse filter paper, or paper towel.

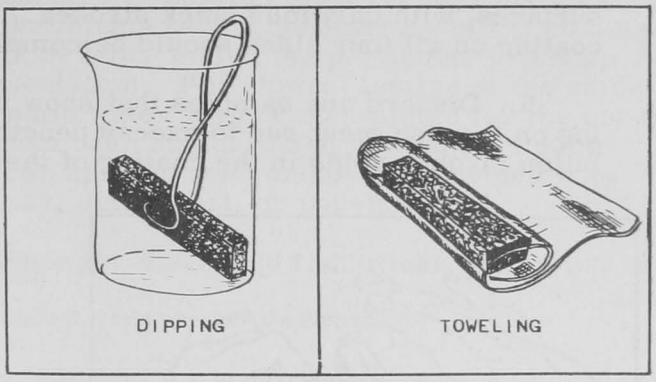
PREPARATION OF THE DIPPING SOLUTION: Dissolve approximately 250 grams of copper sulfate crystals ($CuSO_4.5H_2O$) in a small quantity of hot water. Add cold water to bring to 1 liter. Cool to room temperature.

PREPARATION OF SAMPLE: Maintain samples in refrigerated storage at temperatures of 0° F. or lower. Remove individual fish-stick samples only as immediately needed for the analysis.

Determination:

1. Remove the frozen fish stick from the refrigerated storage and weigh the fish stick to the nearest 0.1 gram immediately (within 20 seconds). Do not use broken or damaged fish sticks or those with large voids in the coating.

2. Employing the clip tongs, promptly immerse the fish stick into the copper-sulfate solution for the appropriate time specified (table 1). Allow the tongs to remain attached to the fish stick (fig. 1) to avoid delay in the subsequent removal of the fish stick from the solution.



3. At the end of the interval specified in table 1, promptly remove the fish stick from the solution, roll the stick lightly in a paper towel, press the towel lightly against the stick on all sides to absorb excess moisture, and then unroll the towel. The rolling, pressing, and unrolling should be completed in not more than 7 seconds.

Table 1 - Dipping-Time Guide

Temperature of the Copper-Sulfate Solution	Temperature of the Frozen Fried Fish Sticks		Dipping Time	
			Fish Sticks Fried in Nonhydrogenated Oils	Fish Sticks Fried in Hydrogenated or Hardened Oils 2/
	Degree F.	Degree C.	(Seconds)	(Seconds)
Room Temperature 1/ (65° to 85° F.)	0 to -10	-18 to -23	20 to 25	25 to 30
	-10 to -15	-23 to -26	20 to 25	30 to 40
	-15 to -20	-26 to -29	25 to 30	35 to 45
	-20 to -30	-29 to -34	30 to 35	45 to 60

1/ The exact temperature, within this range, was found not to be critical.

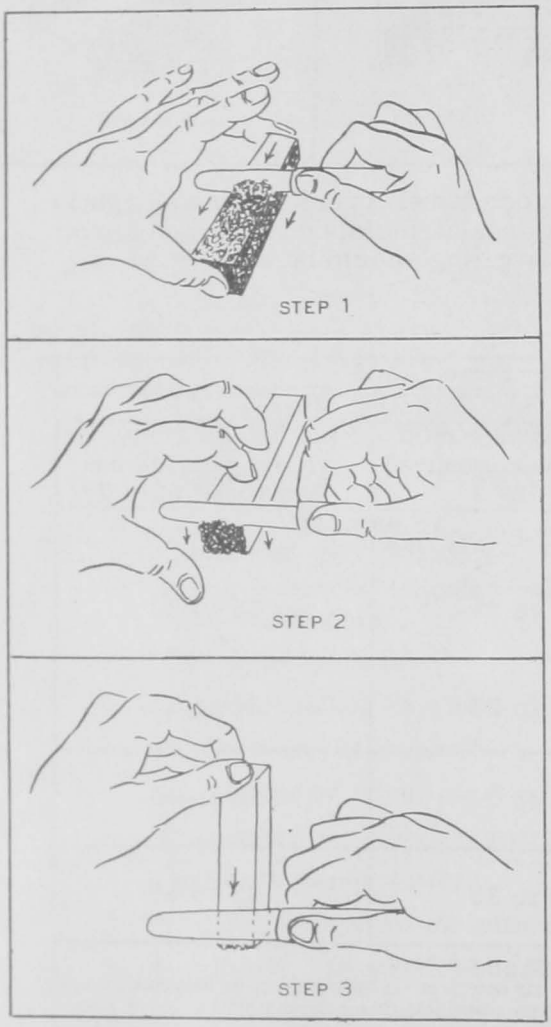
2/ A crude method for determining whether a fish stick has been fried in hydrogenated or hardened fats is as follows: Remove a small portion of the coating from one stick. Place the material in a test tube, add several ml. of ether or acetone, and shake lightly. Decant a portion of the solvent solution onto a piece of paper towel. Hydrogenated or hardened fats leave a white solid spot on the paper after the solvent has evaporated.

4. Under adequate lighting, remove the coating from the top side of the fish stick. To do this (a) grasp the fish stick (which is lying flat on the towel) by the ends (fig. 2, step 1); (b) place the spatula on the top surface of the fish stick at the end that is away from the operator and press the spatula into the coating perpendicular to the surface, being careful not to penetrate the meat of the fish stick; and (c) quickly draw the spatula blade

over the fish stick in one long continuous motion, removing the coating. The fibrous-appearing fish meat should remain, whereas the yellow coating should roll off.

5. Quickly repeat operation 4 on the three remaining sides. These operations remove the major potion of the coating from the four principal surfaces, with only four quick strokes. This phase of the removal of the coating on all four sides should be completed within 20 seconds.

6. Discard any samples that show more than a trace of blue coloring on the fish meat due to excess penetration of the copper sulfate solution through voids in the coating of the fish stick.



7. Remove the coating from the two ends of the fish stick. To do this, hold the blade of the spatula at and parallel to the end, and cut away the coating (fig. 2, step 2).

8. Remove from the still-frozen fish stick any remaining coating, which appears as a yellow film, by use of the rounded tip of the spatula. Scrape the fish lightly, taking care to scrape only those surfaces where the film remains.

9. Remove any coating on the edges by holding the fish stick upright and cutting away the coating with the blade of the spatula being held almost parallel to the edge of the fish stick (fig. 2, step 3).

10. Weigh the fish meat remaining.

11. Calculate the amount of fish meat by use of the formula:

$$\text{Percent fish meat} = \frac{(\text{weight of fish meat})\ 100}{(\text{weight of whole stick})}$$

DISCUSSION

The weighings and the removal of the coating should be carried out as rapidly as is compatible with good care. The following precautions should be observed:

1. The total elapsed time for analysis of each fish stick should not exceed $3\frac{1}{2}$ minutes. A period of time greater than this may (a) permit errors resulting from the condensation of moisture on the fish stick or loss of water from the fish into the coating and (b) by allowing the fish stick to thaw or soften, make it difficult to scrape off the coating without removing some of the fish meat.

2. Care should be taken to scape only those portions of the fish stick where the coating remains and not to rescrape any exposed portion of the fish meat.

3. Any samples showing excessive penetration of the dipping solution (more than a trace of blue coloring on the fish meat) should be discarded. The speed of penetration through the coating to the surface of the fish meat depends upon (a) the temperature of the fish stick and (b) the thickness and consistency of the coating. The exact dipping time therefore should be adjusted for the particular product, within the ranges specified, by experimentation. For control testing of the same commercial production of fish sticks, plain water may be substituted for the copper-sulfate solution, once the dipping time has been standardized for the particular product. The copper-sulfate solution can be used over and over, provided it is clarified by filtration through filter paper, glass wool, or paper towel.

4. Copper sulfate is a poison. Hence the discarded fish sticks should not be recoated or be used to feed animals.

Note: The author gratefully acknowledges the aid of Mr. Boris O. Knake who drew the illustrations.

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RAISING OF SHRIMP

The success of an enterprise to raise shrimp would depend, among other things, upon proper preparation of nursery and rearing ponds. One kind of shrimp known scientifically as Penaeus monodon Fabricus, has been cultivated in Philippine estuarine ponds with an average production of 280 pounds per acre over a period of six months.

The shrimp are caught by dip nets when about one half inch long, and then placed in the nursery ponds about 20 square yards in size. These nurseries must be cleaned of predators, such as fish and crabs, and have a flow of tidal water. The water depth is kept at about $1\frac{1}{2}$ to 4 inches. Screens prevent escape of the small shrimp and the entrance of predators.

The larger rearing ponds are drained and cleaned before planting the shrimp from the nursery ponds. They are usually about 1 to 3 acres in size.

To raise shrimp in Florida would meet with specific problems. Conditions are such that while possible it is not believed to be generally practical to attempt such an enterprise.

--Sea Secrets, The Marine Laboratory, University of Marine, Coral Gables, Fla.