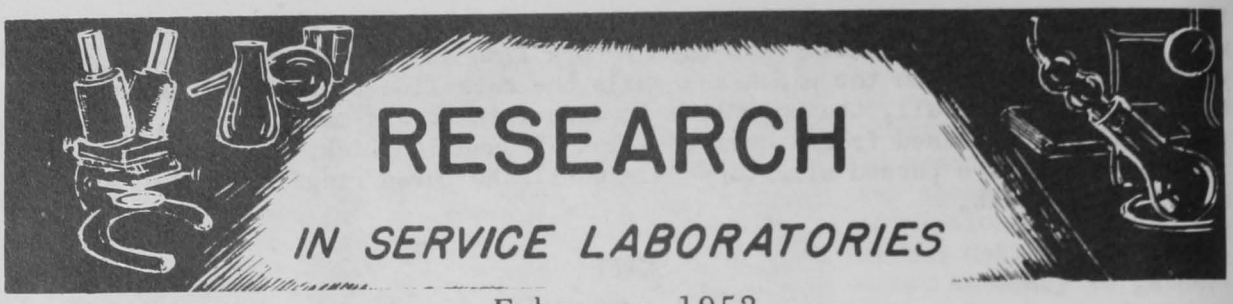

February 1952

REFRIGERATION: Freezing Fish at Sea, Defrosting, Filleting, and Refreezing the Fillets: Shipyard work was completed on repairs to the main engine and the auxiliary equipment on the trawler Delaware. The vessel will be dry-docked shortly for hull and underwater repairs. Further work will include expansion of the frozen-fish storage area and improvements in the fish-freezing equipment.

(Boston)

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NUTRITION: Chemical and Physical Properties of Fish and Shellfish Proteins: During the last month work was initiated on the development of a method for the determination of drip in fish. Factors affecting the quantity of drip are being studied. The work should provide much needed information on the water retentivity of fish proteins and should serve as a basis for more fundamental studies.

Several procedures for determining drip in frozen fish have been proposed. One method is to thaw the fishery product in the open air and collect the liquid which remains. The amount of the liquid or the loss in weight of the fish represents the drip. There are many variations to this general method. Another procedure is to expose under pressure a uniform cut section of the fish. The water is pressed out and discarded. The loss in weight of the fish section is considered the drip. All present methods are rather empirical and are apparently only of value for control work.

It is our purpose in this work to develop a method for determining drip which will not alter the physical condition of the fish and which will approach conditions similar to those that occur in the normal handling of the fish. The pressure method for drip is, therefore, automatically eliminated from consideration.

Experience gained so far has indicated that under similar thawing conditions: (1) the amount of water released from fillets of the same species of fish varies from fillet to fillet; (2) spoiled fillets release more water than fresh ones; (3) the amount of water released will vary from one species of fish to another; (4) there is little difference in the amount of water released from large and from small Pacific oysters; and (5) within certain limits, there is little difference in the amount of water released from thawed oysters which were frozen at different rates. (These are preliminary results and, of course, must await further verification.)

Further studies on the factors affecting drip in fish indicate that the amount of water released under similar controlled conditions from the thawed head portions of frozen fillets was not significantly different from the thawed tail portion of the same frozen fillets. The results were an average of 2.8 percent water from the head portion and 3.3 percent water (or "drip") from the tail portion.

Additional studies are being carried out on the factors affecting the quantity of "drip" in an effort to provide basic information which will lead to a standard method for the drip determination. (Seattle).

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BYPRODUCTS: Vitamin Content and Nutritive Value of Fishery Byproducts: Studies on the unknown growth factors in fishery byproducts were continued. Tests on the rockfish liver dialysate were made to determine whether it contained growth factors measured by the microbiological method. The cultural medium used was that described by Flynn, Williams, O'Dell, and Hogan, in the journal Analytical Chemistry 23, 180 (1951). The test organism was Lactobacillus casei.

When this medium was supplemented with the rockfish liver dialysate at concentrations ranging from 1 to 300,000 parts per million, a considerable increase in the growth rate of L. casei was noted. The maximum increase in growth was between 45-50 percent over the unsupplemented medium. The next step will be to determine whether this growth factor also applies to tests with chicks. A large batch of fish livers is being processed and it is expected that the finished dialysate will soon be ready for chick tests at Washington State College.

This demonstration that fish-liver dialysate contains a factor or factors which greatly stimulate the growth of Lactobacillus casei makes it possible to begin further fractionation experiments on the dialysate. These experiments will be directed toward the purification and eventual isolation of the unknown factor which shall be referred to in these reports as the L. casei fish factor. Ultimately, it is hoped that the L. casei fish factor will be shown to be the same factor as the one in fish meal which stimulates chick growth.

There are in general three techniques which can be used to purify and isolate naturally-occurring compounds on a micro scale. These techniques are: fractional precipitation, absorption chromatography, and partition chromatography. The most common is fractional precipitation. If it is possible to find a precipitant for the L. casei fish factor, then considerable purification of the factor can be made rather easily. A solvent which has been used extensively to precipitate the vitamins belonging to the water-soluble class is acetone. Consequently, an experiment was carried out to determine if the L. casei fish factor could be precipitated with acetone. Ten ml. volumes of a fish liver dialysate were placed in each of two 100-ml. centrifuge tubes which had been previously weighed. Eight volumes (80 ml.) of acetone were slowly added with stirring. The covered tubes were stored in a refrigerator for a week. The tubes were centrifuged for 10 minutes at 2,000 rpm. and the supernatant liquid was decanted off. The residual acetone was removed with a slow stream of filtered air. The tubes were placed in a vacuum desiccator for two weeks. They were then weighed.

The precipitate in each centrifuge tube was dissolved in water and quantitatively transferred to a 10 ml. volumetric flask. The flasks were diluted to the mark with distilled water. The L. casei assay procedure was then carried out with aliquots of the acetone-insoluble fraction added to the basal medium. The assay was made in triplicate.

The results show that the L. casei fish factor is present in the acetone-insoluble fraction and that maximum growth is obtained with approximately 0.02 g. of the acetone-insoluble material.

* * * * *

Riboflavin and niacin assays were completed on products from one pilchard meal plant which used an air-lift drier. The results are as follows:

Sample	C O M P O S I T I O N				
	Moisture	Oil	Solids	Riboflavin	Niacin
	Percent	Percent	Percent	Micrograms Per Gram	Micrograms Per Gram
Air-Lift Drier Reduction Plant					
Raw Sardines	69.9	12.5	22.6	5.3	97
Press Cake	49.5	4.1	46.4	2.8	39
Stickwater	86.1	7.0	6.9	8.0	245
Meal	13.3	7.0	80.0	2.6	42

(Seattle)

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ANALYSIS AND COMPOSITION: Composition and Cold-Storage Life of Fresh-Water Fish. Additional data on the composition of sheepshead and blue pike were obtained and are presented in the following tables.

Composition of Sheepshead (Aplodinotus grunniens)

Sample Number	Length of Fish in Centimeters	Weight of Fish in Grams	Fillet Yield in Percent	Proximate Composition in Percent			
				Moisture	Fat	Protein	Ash
7	32	480	35.4	76.2	6.1	17.4	0.98
8	33	550	36.4	74.4	8.6	18.5	1.10
9	35.5	580	35.3	74.7	8.3	19.1	1.15
10	35	565	38.9	74.2	8.2	17.7	1.02
11	36	645	34.4	75.3	6.5	17.8	1.07
12	36	690	31.9	75.9	7.0	17.6	1.04
13	37.5	740	33.1	72.2	8.1	19.1	0.98
14	38.5	760	35.5	75.1	5.6	18.8	1.00
15	45	1160	26.7	76.6	2.7	19.0	1.16
16	54	2545	31.4	75.0	7.3	18.8	1.03

Composition of Blue Pike (Stizostedion vitreum glaucum)

Sample Number	Length of Fish in Centimeters	Weight of Fish in Grams	Fillet Yield in Percent	Proximate Composition in Percent			
				Moisture	Fat	Protein	Ash
7	28	165	43.6	79.4	0.75	19.1	1.24
8	30	195	42.0	80.2	0.91	19.3	1.26
9	27	155	43.2	79.4	0.95	19.5	1.31
10	28	175	44.0	80.0	0.73	19.3	1.25
11	30	220	45.4	79.6	0.81	19.1	1.18
12	29.5	220	45.4	79.9	0.90	19.4	1.15
13	32	245	45.7	80.1	0.78	19.3	1.38
14	30	200	45.0	80.3	0.90	18.9	1.20
15	30.5	217	41.5	79.7	1.00	19.3	1.28
16	28.5	192	46.9	79.6	1.19	19.4	1.18

(Seattle)

TECHNICAL NOTE NO. 18 - PROXIMATE COMPOSITION OF THE CLASSIFIED TRIMMINGS FROM PINK SALMON

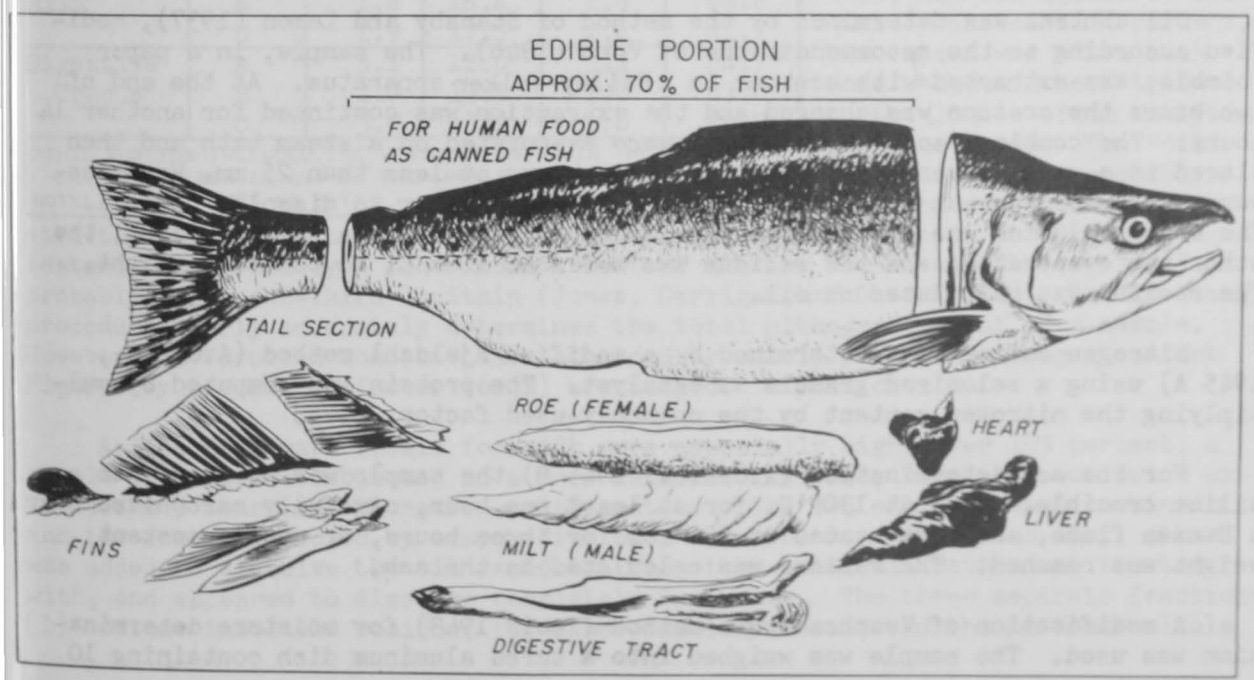
ABSTRACT

SAMPLES OF TRIMMINGS FROM PINK SALMON WERE SECURED FROM A KETCHIKAN, ALASKA, CANNERY DURING EACH OF THREE CANNING SEASONS. THE TRIMMINGS WERE SEPARATED INTO EIGHT COMPONENTS: HEADS, COLLARS, FINS, TAILS, LIVERS, EGGS, MILT, AND DIGESTIVE TRACTS. THE PROXIMATE COMPOSITION - OIL, PROTEIN, ASH, AND MOISTURE - WAS DETERMINED FOR EACH CLASSIFIED PORTION.

INTRODUCTION

Progress toward the goal of complete utilization of the wastes from Alaskan salmon canneries has been slow, partly because the problems involved and partly because the prospects were not fully known. Therefore, an important phase of the Ketchikan Fishery Products Laboratory program has been research to secure basic technical data concerning the raw material. Data on the available quantities of each of the separated parts of the salmon cannery trimmings have been reported (Magnusson and Hagevig 1950).

The present paper reports on an incidental analytical study of trimmings from the most abundant salmon, the pink or humpback (Oncorhynchus gorbuscha). The pink salmon raw materials had been collected at a Ketchikan cannery during three seasons in connection with other research projects. It was believed that it would be of interest to determine the proximate composition of these lots of trimmings even though it was realized that the batches of trimmings available for analysis were not necessarily representative of all types of pink salmon cannery waste available in southeastern Alaska. The proximate composition - oil, protein, ash, and moisture contents - was determined for each of eight sorted components of the trimmings: heads, collars, fins, tails, livers, eggs, milt, and digestive tracts.



SOURCE OF SAMPLES

During 1946, 1947, and 1948 several representative samples of trimmings were secured at a Ketchikan, Alaska, salmon cannery which handled only trap-caught fish. The collections were made from the flumes that carry the trimmings away from the butchering and cleaning equipment--the "header" and "Iron Chink." The trimmings were allowed to drain free of excess water and were transported immediately to the laboratory, where they were carefully sorted. The "header" section of the waste was hand-butchered to separate the head from the collar portion. That part of the liver, if any, in the collar was removed and added to the liver portion of the Iron Chink trimmings. The portion "fins" included the ventral fins of the fish with the adjacent skin and flesh as cut by the Iron Chink. The collar portion included the pectoral fins. Each season's composite sample represented trimmings from a minimum of 100 fish. The separated trimmings collected in 1946 were finely ground and well mixed, and then representative samples were sealed in half-pound cans. Some of the canned samples were preserved by processing for 90 minutes at 242° F., and the remaining samples were preserved by storing at 0° F. Since the processed samples were packed in hermetically-sealed containers, it is unlikely that processing would affect the proximate composition of the material in the cans; therefore, no differentiation was indicated in the data for the processed and the frozen samples. Trimmings collected in 1947 and 1948 were frozen and stored in five-gallon cans at 0° F. Later the material was partially thawed and then ground and mixed. Representative samples were taken for immediate analysis or saved in sealed one-pound cans stored at 0° F. All analyses were carried out during the summer and fall of 1948.

ANALYTICAL METHODS

The analytical methods employed were based on those in the Methods of Analysis of the Association of Official Agricultural Chemists and the Journal of the Association of Official Agricultural Chemists. Each sample to be analyzed was transferred, as completely as possible, from the can to a blendor jar and thoroughly blended to a creamy fluid or a uniform paste. Portions of this blended sample were weighed out for the subsequent analyses.

Oil content was determined by the method of Stansby and Lemon (1937), modified according to the recommendations of Voth (1946). The sample, in a paper thimble, was extracted with acetone in a Bailey-Walker apparatus. At the end of two hours the acetone was changed and the extraction was continued for another 14 hours. The combined acetone solutions were evaporated on a steam bath and then placed in a vacuum oven at 100° C. for three hours at less than 25 mm. Hg. pressure. The residue was treated with anhydrous ethyl ether to dissolve the oil. The ether solution was filtered through sintered glass into a weighed flask; the ether was evaporated; and the residue was dried at 100° C. to constant weight. The residue was calculated as oil.

Nitrogen content was determined by a modified Kjeldahl method (A.O.A.C., 1945 A) using a selenized granule as catalyst. The protein was computed by multiplying the nitrogen content by the commonly-used factor, 6.25.

For the ash determination (A.O.A.C., 1945 B) the sample was weighed into a silica crucible, dried at 130° C. for at least one hour, carefully carbonized over a Bunsen flame, and then heated at 550° C. for three hours, or until constant weight was reached. The residue was calculated as the ash.

A modification of Veschezerov's method (Tubis 1943) for moisture determination was used. The sample was weighed into a tared aluminum dish containing 10

grams of washed and muffled sand. The sample and the sand were well mixed. After a preliminary drying in an air oven at 100° C. for 1.5 hours, the drying was completed at 135° C. for one hour. The loss in weight was calculated as moisture.

DISCUSSION OF PROCEDURES AND RESULTS

Table 1 - Proximate Composition of Classified Pink Salmon Trimmings from Trap-Caught Fish at Ketchikan, Alaska

Parts of the Trimmings	Season Lot Number	Proximate Composition			
		Oil	Protein	Ash	Moisture
		Percent	Percent	Percent	Percent
Heads	A	15.9	12.5	3.7	68.2
	B	11.8	13.1	3.3	71.6
	C	12.4	13.0	3.9	70.9
Collars	A	10.5	17.5	2.6	71.2
	B	9.8	17.2	2.4	70.9
	C	11.3	17.1	2.9	70.3
Fins*	A	-	-	-	-
	B	10.5	16.7	3.6	71.4
	C	13.7	17.7	3.7	66.8
Tails	A	6.7	18.4	3.3	73.6
	B	6.0	19.2	4.7	70.3
	C	7.3	19.4	5.1	68.8
Livers	A	4.6	16.5	1.7	79.1
	B	3.7	16.7	1.6	78.7
	C	3.8	16.2	1.6	80.6
Eggs	A	12.1	26.3	1.9	60.0
	B	10.9	24.8	1.9	62.1
	C	10.2	23.2	1.7	65.2
Milt	A	1.7	17.1	2.2	82.1
	B	1.8	18.4	2.6	80.6
	C	1.9	17.5	2.4	81.7
Digestive Tracts	A	3.1	12.6	1.1	84.7
	B	2.8	13.5	1.0	83.0
	C	3.0	13.8	1.0	84.2

*THIS PORTION INCLUDED THE VENTRAL FINS WITH THE ADJACENT SKIN AND FLESH AS CUT BY THE IRON CHINK.
NOTE: - INDICATES NO DATA.

Table 1 presents the results of the proximate analyses of the pink salmon trimmings samples for each of three seasons. Each value in the table is the average of triplicate determinations on a composite sample of the samples taken at intervals during the season at the one cannery. The three results to be acceptable were required to have a difference range of less than the following: oil, 0.25 percent; protein, 0.2 percent; moisture, 0.2 percent; ash, 0.15 percent. Most of the triplicates exhibited ranges of less than half these limits.

The analytical procedures employed gave satisfactorily reproducible results. However, the proximate analysis totals (oil, protein, ash and moisture) frequently exceeded 100 percent. The excess was often much more than could be attributed to the limits of the analytical procedures. The "oil" as reported here probably included substances not strictly oil, yet extractable by acetone and soluble in ether. For example, cholesterol was probably a minor constituent of all the "oils" and the egg "oil" was probably about one-third lecithin (Jones, Carrigan, and Dassow 1948). The Kjeldahl procedure fairly accurately determines the total nitrogen content of a sample. However, the "protein content" data obtained by multiplying the nitrogen content figures by a constant factor (6.25) are subject to criticism.

As the proximate totals for milt were especially high, over 103 percent, a sample of milt was subjected to a more detailed analysis. The milt was twice extracted -- 3 hours and 21 hours -- with acetone. The two extracts were combined and the acetone removed by evaporation on a steam bath. Anhydrous ethyl ether was added to dissolve the oil residue. The ether insoluble matter was treated with, and appeared to dissolve completely in, water. The three separate fractions (1) acetone insoluble residue, (2) ether-insoluble residue of the acetone-soluble

material, and (3) ether-soluble portion of the acetone-soluble material, were dried in a vacuum oven. The nitrogen and ash contents of each were then determined. The proximate composition data, expressed as percentages of the whole raw milt sample, are summarized in table 2.

Table 2 - Proximate Composition of Solvent-Separated Fractions of Pink-Salmon Milt

Fraction of Milt	Proximate Composition in Percent of the Whole Raw Milt				
	Dry Matter	Oil	Ash	Protein (Nx6.25)	Total of Oil Ash Protein
Acetone-Insoluble Residue	16.7	0.0	1.9	17.0	18.9
Ether-Insoluble Residue of the Acetone-Soluble Material	1.0	0.0	0.2	0.7	0.9
Ether-Soluble Portion of the Acetone-Soluble Material	1.8	1.8	0.0	0.3	2.1

The data demonstrate that the factor used for converting nitrogen to protein, 6.25, is far too large for use with milt. This would be expected since fish milt is unusually rich in arginine, an amino acid with a high percentage of nitrogen. Judging from the data in table 2, the difference between the dry matter and ash, or 14.8 percent, is the maximum which the protein content could be. Therefore, the assay figure of 17.0 percent for protein is too high, and the conversion factor for this sample of milt cannot be higher than $\frac{14.8}{17.0} \times 6.25$ or about 5.5. The nitrogen content of the ether-soluble portion indicates that, if the nitrogen were present only as lecithin, then this phospholipid made up about half of the total "oil" in the milt.

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