UTILIZATION OF SALMON EGGS FOR PRODUCTION OF CHOLESTEROL, LIPIDE, AND PROTEIN

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ABSTRACT

QUANTITATIVE DETERMINATION OF CHOLESTEROL, FAT, PHOSPHOLIPIDE, AND PROTEIN WERE CARRIED OUT ON THE ROE (EGGS) OF FIVE SPECIES OF SALMON. THE CHOLESTEROL CONTENT WAS FOUND TO VARY FROM 0.29 TO 0.40 PERCENT, ON THE RAW-EGG BASIS. WHEN CALCULATED ON THE LIPIDE FRACTION (ETHER-SOLUBLE FAT = 11.1 TO 13.9 PERCENT OF THE WHOLE EGG), THE CHOLESTEROL VARIED FROM 2.21 TO 3.53 PERCENT.

PRELIMINARY ANIMAL-FEEDING STUDIES SHOWED THAT THE DEFATTED SALMON EGG PROTEIN COMPARED FAVORABLY WITH CASEIN IN NUTRITIONAL QUALITY. PROTEIN CONTENT OF THE ROE VARIED FROM 22.5 TO 28.8 PERCENT AND THE ASH CONTENT FROM 1.3 TO 2.7 PERCENT.

PHOSPHOLIPIDE, BASED ON THE PHOSPHOROUS CONTENT OF THE LIPIDE FRACTION, RANGED FROM 10.4 TO 12.4 PERCENT OF THE EGG (MOISTURE-FREE BASIS) OR 25.8 TO 39.2 PERCENT OF THE ETHER-SOLUBLE FAT.

UTILIZATION OF SALMON EGGS FOR THE EXTRACTION OF THE RELATIVELY SMALL AMOUNT OF CHOLESTEROL PRESENT IS UNWARRANTED AT THIS TIME. HOWEVER, COMMERCIAL EXPLOITATION OF THE LIPIDE AND PHOSPHOLIPIDE FRACTIONS AND PERHAPS THE PROTEIN OF SALMON ROE APPEARS TO BE PRACTICAL.

INTRODUCTION

Attention was directed to an investigation of salmon eggs as a possible commercial source of cholesterol, lipide, and protein by the findings of a preliminary survey by Jones and Carrigan (1947) carried out during the initial stage of the research program on utilization of Alaskan salmon-cannery waste. The period of study was necessarily limited to the six months' contract of the Industrial Research and Development Division of the Office of Technical Services with the Alaska Fisheries Experimental Commission, under which the investigation was possible. Accordingly, the experimental work was arranged so that the information required to evaluate the possibilities of further development could be collected in the allotted time. From analytical data reported in the literature, the use of salmon eggs as a source of cholesterol appeared promising. It was hoped that commercial development might be practical if experimental tests showed the salmon eggs under study to be as high in cholesterol content as had previously been reported.

It was believed very likely that in addition to cholesterol extraction, processes could be developed which would also permit recovery of a high quality protein meal from salmon eggs as well as a fat or lipide fraction which might have a number of important industrial applications.

The presence of cholesterol in the roe of fish has been reported on by several investigators. Koenig and Grossfeld (1913) reported that the fat from fish roe contains from 4 to 14 percent cholesterol. Anno (1940) found that the unsaponifiable matter present in the lipides of salmon eggs was essentially cholesterol. In addition to cholesterol, the lipide fraction of fish roe has a

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high content of lecithin. Koenig and Grossfeld (1913) in considering fish roe as food for man found the egg fat to contain as much as 49 percent lecithin. Halpern (1945) reported that the roe from sockeye salmon (Oncorphynchus nerka) yielded 12.5 percent oil and 6.2 percent phospholipide.

The separation of lecithin from the extracted egg fat would appear to be easily accomplished by a process developed by the German oil industry and described by Goss (1947). In this process, the lecithin is removed from the crude fat or oil by washing it with 2 to 5 percent by volume of hot water and removing the resulting sludge in a centrifuge. Two successive washings are required to insure maximum recovery of lecithin. The lecithin is recovered from the sludge by removal of water at 60° C. with the aid of vacuum, followed by a bleaching of the residue with hydrogen peroxide. Residual oil remaining in the lecithin is then recovered by repeated extractions with acetone.

Recovery of a semipurified protein of high nutritional quality may have considerable importance in the economic utilization of salmon eggs. The protein content of salmon eggs varies from about 22 percent in king or chinook salmon to about 28 percent in the chum.1/

The quantities of salmon eggs available in Alaska for processing are enormous. Since the eggs constitute about 8 to 10 percent of the entire salmon-cannery waste, which amounts to more than 100,000,000 pounds annually, it can be readily calculated that about 9,000,000 pounds or 4,500 tons of salmon eggs are at present discarded each year.

Collection of the raw salmon eggs in Alaska should not interpose any very difficult problems. The salmon are dressed prior to canning in a machine known as the "Iron Chink" which in a single cycle cuts off the head, fins, tail and removes the viscera. The eggs as a part of the viscera, are swept out of the body cavity in the middle cycle of the rotating wheel of the "Iron Chink." Separation of the eggs from the rest of the abdominal contents would necessitate hand sorting to only a moderate degree. The large and, in most cases, intact skeins of salmon eggs could be readily separated from the other waste parts while they are traveling along a belt, chute, or trough.

Problems of handling and storing salmon eggs for subsequent processing are expected to be somewhat easier to overcome than those of other fractions of salmon-cannery waste, because the eggs are individually encased in a tough semi-permeable membrane and the entire egg mass is held together in a skein structure which offers ease of handling and some protection from contamination. If it were found necessary to hold or store the salmon eggs for a considerable period of time before processing, this no doubt could be accomplished by salting, freezing, or by addition of a chemical preservative. Salmon eggs appear to offer a unique material for chemical processing due to their special constituents and because of the size of the roe in salmon waste and the enormous quantity that is available in Alaska.

EXPERIMENTAL PROCEDURES

Before accurate assessment of the possibility of recovering cholesterol from salmon eggs could be made, it was necessary to determine the quantity of cholesterol present in this portion of the cannery waste as it occurs in Alaska. Since a complete survey of the variation in cholesterol content due to size of fish, maturity, and location of capture, would require an expenditure of a large

1/ UNPUBLISHED DATA OF THE AUTHORS.

amount of time and money, it was decided to limit the preliminary analysis to a sample of eggs from 25 to 100 fish of each species. It was believed this sample would furnish a fairly representative approximation of the cholesterol content to be encountered.

The egg samples for the analyses presented in the following report for all species of salmon, except king or chinook, were collected during the 1947 fishing season at two salmon canneries located at Ketchikan, Alaska. The samples of king eggs were collected from Columbia River chinook salmon at a cannery located at Astoria, Oregon. Each sample of approximately 35 pounds of raw eggs represented the roe from 25 to 100 salmon of the individual species. Samples of each species other than king were obtained directly from the "Iron Chink" butchering operation with no attempt to segregate the material according to size or maturity. The salmon were trap-caught, in most instances, and represented fish of average size which were semi-mature, as evidenced by development of the gonads. The eggs were inspected for the presence of other waste parts before being sealed in five-gallon tin containers and frozen in a sharp freezer at -20° F., within 4 hours after collection and about 24-36 hours after the salmon were caught. All samples were held at 0° F. storage until thawed and ground prior to chemical analysis.

TABLE 1 - AVERAGE CHOLESTEROL AND FAT CONTENT OF SALMON EGGS

SPECIES OF SALMON	CHOLESTEROL		FAT 1/
	IN RAW EGGS	IN FAT	
	PERCENT	PERCENT	PERCENT
PINK	0.29	2.61	11.1
RED	0.39	2.82	13.9
CHUM	0.38	3.15	11.9
CHUM, DEHYDRATED	0.86	3.06	28.0
KING	0.34	2.64	12.8
COHO	0.40	3.53	11.4

1/ TOTAL ETHER EXTRACT AFTER ACID HYDROLYSIS OF SAMPLE.

Each analysis in Table 1 was made on a representative sample drawn from the entire 35-pound lot of thoroughly mixed ground eggs. This small representative sample of about 250 to 300 grams was blended in a Waring Blendor prior to removal of a sample for the determination of cholesterol and ether-soluble fat. The value reported for king or chinook eggs was determined on a representative sample drawn from a 35-pound lot collected at Astoria, Oregon, during August 1947. Due to the large size of this species, a 35-pound sample of eggs represents only 15 to 20 fish.

CHOLESTEROL DETERMINATION

Numerous methods for the quantitative determination of cholesterol are found in the chemical literature. However, many of these methods are modifications of the Lieberman-Burchard reaction, and are designed primarily for the determination of small quantities of cholesterol present in blood. Methods commonly used for the determination of cholesterol in hen egg yolks or in other food products containing egg yolk appeared to be the most logical to use for the analysis of salmon eggs. Accordingly, the method described in Methods of Analysis of the Association of Official Agricultural Chemists, VI Edition, 1945, page 349, for determination of cholesterol in eggs and egg products was used in the initial experiments on salmon eggs. In this method the cholesterol is isolated from a saponified sample as the dibromide and subsequently determined by an iodine liberation-titration method using sodium thiosulphate. This method is considered precise and accurate, but has the disadvantage of being laborious and time consuming. In order to examine a larger number of samples, a simpler method was resorted to after a preliminary check analysis had been made using an aliquot of the same sample in the determination of cholesterol by both the A.O.A.C. VI method and the colorimetric method of Cook and Mehlenbacher (1946). The Cook and Mehlenbacher method is based on the Lieberman-Burchard color reaction for cholesterol using the unsaponifiable fraction of the ether extract. Cook and Mehlenbacher suggested the use of a lower temperature during color development

and subsequent reading and also the use of a spectrophotometer to obtain the transmittance values.

Initially the cholesterol content of dehydrated (lyophilized) chum salmon eggs was determined by the A.O.A.C. method. The amount of cholesterol calculated on the basis of the total oil fraction was found to agree within the limits of experimental error with that found for the oil from raw eggs when analyzed by the colorimetric method. Subsequent values for cholesterol in salmon eggs were determined in duplicate by the colorimetric procedure. It was not necessary to use more than two to three grams of ground salmon eggs for each analysis. With this small sample, it was possible to saponify the eggs directly by addition of 30 ml. of 95 percent ethanol and 3 ml. of 50 percent KOH followed by refluxing on a steam bath for 30 minutes. The combined ether extractions of the unsaponifiable fraction were washed with distilled water until the washings were neutral to phenolphthalein. The extract was then made up to a volume of 100 ml. with ethyl ether. Five ml. aliquots were placed in dry test tubes for color development; the ether was removed by immersion in a water bath maintained at 60° C., and 5 ml. of C. P. chloroform were added when the ether had evaporated. The color was developed at 18° C. for 25 minutes in accordance with the Cook-Mehlenbacher technique using acetic anhydride-sulfuric acid mix. The period of color development was not critical as it was found that a period from 20 to 30 minutes gave reasonable good agreement on replicates. Transmittance values were determined at 640 mmu. with a Beckman spectrophotometer using 1 cm. corex cells. Values were obtained from the transmittance-concentration curve with a range of 0.2 to 0.60 mg. cholesterol per 5 ml. chloroform. Blank determinations showed no absorption caused by impurities in the reagents used.

Transmittance values for known amounts of cholesterol subjected to color development are shown in Figure 1.

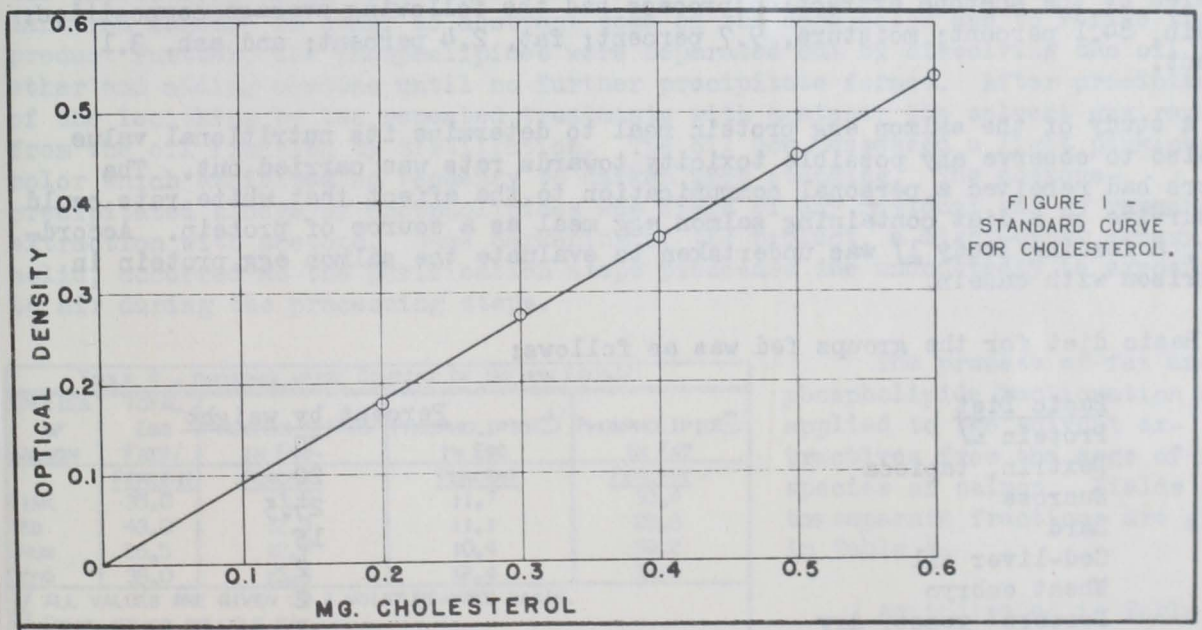

FIGURE 1 - STANDARD CURVE FOR CHOLESTEROL.

The values for cholesterol present in salmon eggs of five different species of salmon are given in Table 1.

These data indicate that the cholesterol content of salmon eggs lies near the lower part of the range of 4 to 14 percent cholesterol in fish-roe fat as reported in the literature. For comparison purposes, it is pointed out that the fat fraction of hens' eggs contains an average of 4.24 percent cholesterol as compared with 3.53 percent cholesterol in the fat of coho salmon eggs.

Anno (1) reported that the unsaponifiable matter extracted from the eggs of pink salmon was essentially cholesterol. Observations made during the present study failed to confirm this report. As indicated in Table 2, approximately one-half of the unsaponifiable matter did not respond to the reactions for cholesterol.

TABLE 2 - CHOLESTEROL CONTENT OF THE UNSAPONIFIABLE MATTER OF SALMON EGGS

SPECIES OF SALMON	UNSAPONIFIABLE RESIDUE 1/ IN FAT	CHOLESTEROL IN FAT	CHOLESTEROL IN THE UNSAPONIFIABLE RESIDUE
	PERCENT	PERCENT	PERCENT
PINK	5.44	2.16	48
RED	4.44	2.82	64
CHUM	6.46	3.15	49
KING	5.16	2.69	52
COHO	7.10	3.53	50

1/ UNSAPONIFIABLE RESIDUE DETERMINED BY A.O.A.C. VI METHODS OF ANALYSIS.

PROTEIN, FAT, AND LECITHIN RECOVERY

In order to obtain the lipide fraction and protein fraction of salmon eggs for evaluation, an extraction process was developed using acetone directly on the raw salmon eggs. In this process, the acetone removes the water content of the egg and a major portion of the fat. Final extraction of some of the remaining lipoidal material is accomplished with hot ethanol. By distillation of the acetone-water-fat solution at atmospheric pressure, the solvent is recovered and the oil or fat separates as a layer on top of the water in the still pot. The oil is then separated from the water by decantation.

The pilot plant studies carried out on the acetone extraction of salmon eggs will not be included in this report. A sufficiently large quantity of protein meal and salmon egg fat were prepared for evaluation studies. The protein meals prepared by the acetone extraction process had the following average composition: protein, 84.1 percent; moisture, 9.7 percent; fat, 2.4 percent; and ash, 3.1 percent.

A study of the salmon egg protein meal to determine its nutritional value and also to observe any possible toxicity towards rats was carried out. The authors had received a personal communication to the effect that white rats could not survive on a diet containing salmon egg meal as a source of protein. Accordingly, a feeding study 1/ was undertaken to evaluate the salmon egg protein in comparison with casein.

Basic diet for the groups fed was as follows:

Basic Diet	Percent by weight
Protein 2/	30
Dextrin, tapioca	20
Sucrose	25.5
Lard	15
Cod-liver oil	2
Wheat embryo	2
Brewers' yeast, dry	2
Liver extract, Lilly	0.5
Mineral mixture, USP XIII, No. 2	3

1/ CARRIED OUT AT THE SERVICE'S COLLEGE PARK FISHERY TECHNOLOGICAL LABORATORY.

2/ FOR THE CONTROL GROUP, 25-PERCENT TECHNICAL CASEIN AND 5-PERCENT DEXTRIN WERE SUBSTITUTED FOR THE 30-PERCENT SALMON EGG PROTEIN.

White rats were allotted to the two groups at random and kept in individual cages. Food and water were allowed ad libitum. Data, including rat weight and food consumption, were recorded weekly. Gain in body weight to food consumption was calculated and is presented in Table 3.

TABLE 3 - EVALUATION OF SALMON EGG PROTEIN MEAL FOR GROWTH OF RATS

DIET DESIGNATION	SEX	INITIAL WEIGHT	LENGTH OF EXPERIMENT	GAIN IN LIVEWEIGHT	FOOD CONSUMED	RATIO OF GRAMS OF FOOD TO GRAMS OF GAIN IN WEIGHT OF RAT
		GRAMS	WEEKS	GRAMS	GRAMS	
CASEIN	M	75	4	88	216	2.5
	M	82	4	80	219	2.7
	M	42	4	79	215	2.7
KING SALMON EGG PROTEIN	F	88	3	30	203	2.5
	M	69	4	110	225	2.0
	F	45	3	41	131	3.2

The data show that the rats on the diet containing salmon egg protein grew about as well as those fed casein. No gross symptoms of toxicity were manifested at the termination of the experiment. While it is realized that these experiments are not extensive, they do indicate that no acute toxicity resides in the defatted salmon egg meal and that the nutritional value appears to be very nearly equivalent to that of casein.

RECOVERY OF SALMON EGG FAT

The solvent in the acetone-water solution of salmon egg extractives was removed by distillation in a simple pot still at atmospheric pressure. The major part of the acetone was recovered by heating the mixture to 60° C. From all appearances this temperature was not measurably destructive to the lipide fraction which separated out as an oily layer. This oil layer was removed by decantation and subjected to further solvent removal at reduced pressure. The water phase was discarded after decantation. The final ethanol extract was also concentrated and the extractives added to the acetone soluble lipides. The resulting oil was a dark red color. In order to abstract some of the dark color and to refine the product further, the phospholipides were separated out by dissolving the oil in ether and adding acetone until no further precipitate formed. After precipitation of the lecithins by two repeated treatments with acetone, the solvent was removed from the oil fraction by distillation. The oil now possessed a light pinkish-red color which exhibited no tendency to darken upon standing. The acetone-precipitated sludge of phospholipides was freed of any residual oil by repeated extraction with acetone. Some darkening of the product, a light-brown greasy solid, occurred as the purification steps proceeded due undoubtedly to exposure to air during the processing steps.

TABLE 4 - PHOSPHOLIPIDE CONTENT OF SALMON EGGS[1]

SPECIES OF SALMON	TOTAL EGG FAT[2]	PHOSPHOLIPIDE[3] IN EGG	PHOSPHOLIPIDE[4] IN EGG	PHOSPHOLIPIDE[5] IN FAT
	PERCENT	PERCENT	PERCENT	PERCENT
PINK	35.0	13.6	11.7	33.4
RED	43.0	12.3	11.1	25.8
CHUM	26.5	12.9	10.4	39.2
COHO	38.0	15.3	12.4	32.6

1/ ALL VALUES ARE GIVEN ON A MOISTURE-FREE BASIS.
2/ ETHYL-ETHER SOLUBLE FAT.
3/ HALPERN (1945) SELECTIVE EXTRACTION METHOD.
4/ PHOSPHOROUS DETERMINATION, A.O.A.C. V, P. 21.
5/ BASED ON THE PHOSPHOROUS DETERMINATION.

The process of fat and phospholipide fractionation was applied to the solvent extractives from the eggs of four species of salmon. Yields of the separate fractions are given in Table 4.

As indicated in Table 4, the phospholipide fraction constitutes about one-third of the total fat. It appears that recovery and partial purification of the lecithin fraction of the extracted fat would be relatively simple.

DISCUSSION AND SUMMARY

One of the purposes of this investigation was to determine the possibility of utilizing salmon eggs from Alaskan salmon cannery waste for the production of cholesterol, lipide, and protein. Some of the references in the chemical literature held promise that salmon roe would prove to be an especially valuable source of cholesterol. Our observations have shown salmon eggs to be only average in cholesterol content, for example, approximately the same as hens' eggs. In view of these findings it appears unlikely that salmon eggs could be profitably processed for their cholesterol content alone. However, the protein, fat, and lecithin fractions prepared by solvent extraction of the raw eggs appear to be of high quality and to offer promise of economic recovery. The egg protein, judged on its appearance, odor, and preliminary nutritional evaluation appears worthy of further study. It is also possible that salmon egg protein may possess special properties desirable in certain industrial applications, such as the sizing of paper, manufacture of plastics, etc.

The salmon egg fat fractions, either combined or separated into glyceride and phospholipide portions, seem to be worthy of commercial exploitation. For example, because of the highly unsaturated nature of salmon egg oil (iodine number of about 220), it is believed that either directly or after slight modification, it would be suitable for incorporation into quick-drying paints and varnishes. The existing prices for oil, both edible and nonedible, and for commercial lecithin makes the recovery of these two materials from salmon eggs a promising possibility. With the fat content of salmon eggs ranging from 11 to 14 percent on the raw material basis, and with lecithin comprising about one-third of the total fat, the possibility of recovering these materials along with a high quality protein, seems to warrant further investigation.

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LITERATURE CITED

ANNO, K.

1940. J. AGR. CHEM. SOC., JAPAN, VOL. 16, NO. 181.

COOK, J. H., AND MEHLENBACHER, V. C.

1946. IND. ENG. CHEM., ANAL. ED., VOL. 18, NO. 785.

GOSS, W. H.

1947. FOOD INDUSTRIES, VOL. 19, NO. 108.

HALPERN, G. R.

1945. NATURE, VOL. 155, NO. 110.

JONES, G. I. AND CARRIGAN, E. J.

1947. DEPT. OF COMMERCE, OTS REPORT, "UTILIZATION OF SALMON CANNERY WASTE--PART I" CAC-47-17.

KOENIG, J., AND GROSSFELD, J.

1913. BIOCHEM. Z., VOL. 54, NO. 351.