ENZYMES AS AN AID IN SEPARATING OIL FROM PROTEIN IN SALMON EGGS

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ABSTRACT

THE DIGESTION OF SALMON EGGS BY ENZYMES OFFERS ADVANTAGES IN SEPARATING OIL FROM PROTEIN IN SALMON EGGS, PARTICULARLY IN ALASKA WHERE THE SOURCE OF EGGS IS WIDELY SCATTERED AND OFTEN IN REMOTE LOCATIONS.

INTRODUCTION

Stansby and Associates (1953A) stated that the oil from salmon eggs was highly unsaturated (iodine value over 200). These authors also found that salmon-egg oil contained significant quantities of cholesterol, although less than reported by earlier workers (Koenig and Grossfeld 1913: Anno 1940), and phospholipids (25-39 percent of the total oil) that might have commercial interest. Research is now being carried out in U. S. Fish and Wildlife Service laboratories and in industrial laboratories on new chemical products (Anonymous 1955) that can be made from fish oil. Kyte (1956) determined the distribution of the fatty acids of which salmon-egg oil is composed and indicated the amount of unsaturation in the fatty acids. The double bonds in the unsaturated salmon egg oil make this oil particularly attractive in the synthesis of polymers and highly substituted compounds and as a reactive intermediate for the synthesis of other organic compounds.

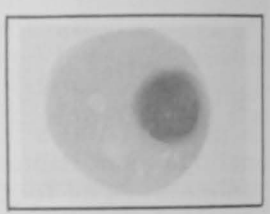

FIG. 1 - PHOTOGRAPH OF SALMON EGG SHOWING OIL DROPLET.

Seagran, Morey, and Dassow (1954) determined the amount of the ten essential amino acids in the protein of salmon eggs. The amount and distribution of these acids indicated salmon-egg protein would be a good animal feed and, being relatively rich in methionine, isoleucine, and lysine, would balance the vegetable protein concentrates which are often deficient in these amino acids. Robinson, Palmer, and Burrows (1951) reported salmon eggs to be an excellent feed for hatchery fish, particularly when water temperatures are over 50° F. and the fish are eating relatively large amounts of food.

Many of the present or potential uses of salmon eggs depend on either the protein fraction or the oil fraction, not the two together. In dried protein concentrates such as fish meals, or in this case egg meal, oil may be an undesirable diluent. Oil for chemical uses or industrial processing is subject to even more rigid purity requirements than is the protein concentrate. Traces of protein matter are undesirable. The purpose of this paper is to discuss methods for separating oil from protein in salmon eggs and, in particular, to stress the role enzymes can play in this process.

METHODS FOR SEPARATING OIL FROM PROTEIN

Sinnhuber (1943) developed a simple method for recovering the highly pigmented oil from the free-oil droplet in salmon eggs (fig. 1). He broke the egg shell or membrane by grinding, then added warm dilute brine (4 percent NaCl) and allowed the oil to separate. The oil was further clarified by centrifuging to remove traces of suspended protein and moisture. This clear brightly-colored oil and the body meat oil derived from the cannery trimmings other than the viscera were used by canners as additives to canned salmon. The Sinnhuber method, however, recovered only one-third of the total oil in the egg and left the protein much diluted. Recovery of the protein was not considered in this method of separating the oil and the process would be expensive.

The dry-rendering process--the drying of fish in a steam-jacketed vessel--is ordinarily used for reducing fish and fish wastes of low oil content to meal. The pressing of the dry-rendered product for removal of oil is usually not carried out because of the difficulty with which the oil is expressed. Salmon eggs processed by this method give a low yield of oil and the protein meal has a very high and undesirable oil content.

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The wet-reduction process is used extensively on a commercial scale for the reduction of fish with high oil content such as menhaden and herring. In this process the fish is cooked, then pressed to remove most of the oil and a large portion of the moisture. Laboratory experiments indicated, however, that cooking ground salmon eggs for periods as long as three hours freed only about 65 percent of the available oil (fig.2). The remaining oil was retained with the protein probably bound as a lipo-protein complex. The particle size of the cooked egg protein is very small and pressing this material by itself in the standard fish meal press would be extremely difficult because the cooked protein does not form the necessary mat or shoulder. Thus, neither of the processes most commonly used for the reduction of fish is satisfactory for the handling of salmon eggs.

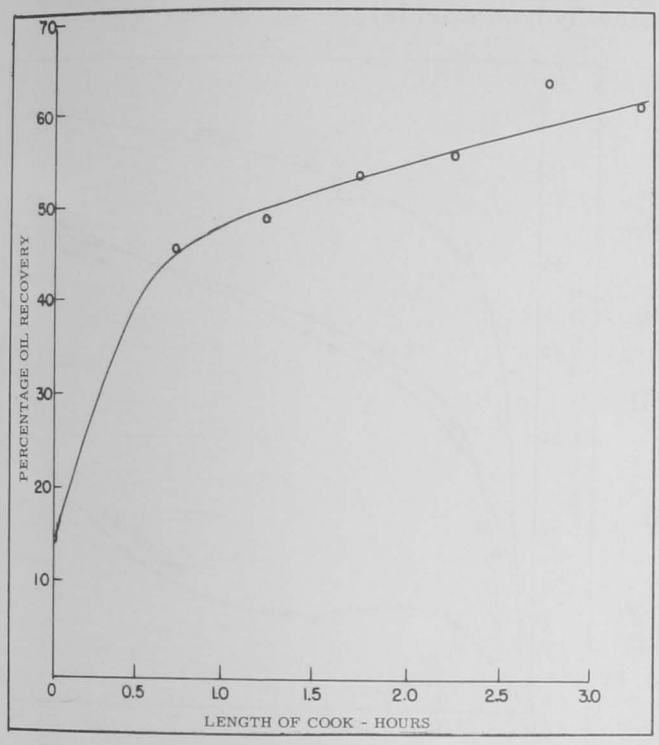

FIG. - 2 THE EFFECT OF COOKING TIME ON OIL RECOVERY FROM PINK SALMON EGGS.

Levin and Lerman (1951) reported an azeotropic distillation process for the dehydration and oil extraction of vegetable and animal products. Tests in this laboratory using an azeotropic distillation with benezene, n-butyl alcohol, or di-chlorethane as solvents for the dehydration and oil extraction or raw fresh salmon eggs, removed from 75 to 80 percent of the oil from the eggs. It was only by the use of several solvents (acetone, ethanol, and a methanol:chloroform mixture) that essentially all of the lipid was removed from the raw salmon eggs.

Chargaff and Cohen (1939) reported the enzymes in rattlesnake venom to be effective in separating certain of the oils in egg yolk. Lovern (1955), however, reported proteolytic enzymes to be ineffective in freeing bound oil from the protein with which it was associated. He reported that the oil was bound quite as strongly to the peptides and other products of enzyme hydrolysis as to the original protein. Nevertheless, proteolytic enzymes might play a desirable role in the separation of oil from protein in salmon eggs.

Thomson, MacLeod, and Idler (1954) report that proteolytic enzymes are used extensively to reduce the viscosity of stickwater in the production of fish solubles. Salmon canneries--the source of supply for salmon eggs--are scattered over a large area and in rather remote locations in Alaska. Preliminary processing at the canneries with minimum equipment and manpower requirements would permit a gross separation of oil and protein and allow the oil and protein concentrates to be collected and transported to centralized processing facilities where efficient utilization might be possible.

EXPERIMENTAL ENZYME DIGESTION

The action of a number of proteolytic enzymes on salmon eggs was investigated. Conditions of pH and temperature reported to be within the active range of the enzymes were maintained and the rates of solubilizing the protein and liberating the oil were observed. The general pattern of action was the formation of a clear protein solution, a free oil, an oil emulsion layer, and, in some cases, a small amount of

sediment. Figure 3 shows the rate of formation of clear solution from salmon eggs using pepsin (N. F. grade), Rhozyme B-6 and Rhozyme A-4 (commercial proteolytic enzymes) and Bromelain (a mixture of several proteolytic enzymes obtained from the stem of the pineapple plant). Bacterial action was inhibited by the addition of 20 p.p.m. of aureomycin (chlortetracycline hydrochloride).

Even though the protein solution of the enzyme digest was clear after centrifuging, 10 to 20 percent of the dry weight of the protein solution was oil (as determined by acid hydrolysis). A benzene or dichloroethane azeotropic distillation of the enzyme digest removed one-half to two-thirds of the oil but the dried residue still contained 6 to 8 percent oil. The enzyme digestion did, however, solubilize or put into solution the egg protein and did permit a gross separation of an oil emulsion and protein solution by simple settling or centrifuging.

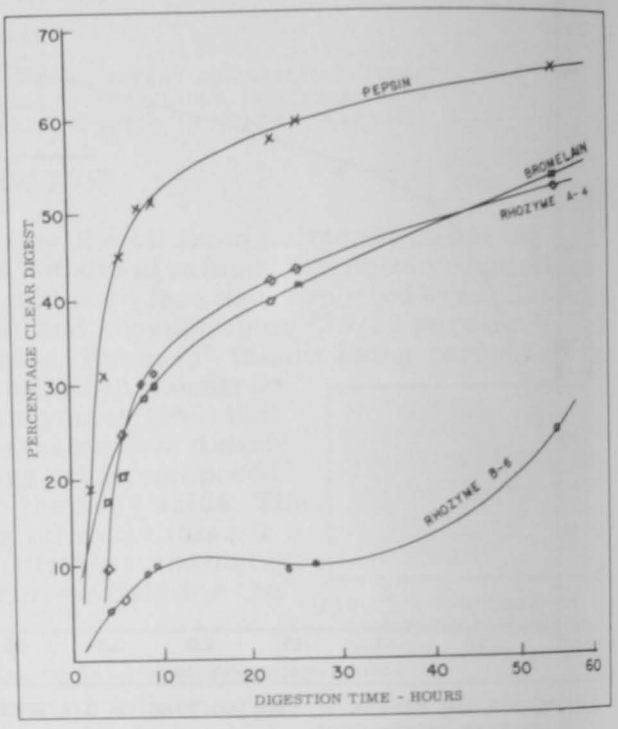

FIG. 3 - THE EFFECT OF DIGESTION TIME ON THE AMOUNT OF CLEAR DIGEST FORMED IN THE DIGESTION OF SALMON EGGS USING ONE PERCENT BY WEIGHT OF FOUR DIFFERENT ENZYMES.

The oil emulsion phase contained 40 to 60 percent oil and 5 percent protein. The dried protein residue from the enzyme digestion had a bright orange glassy to dull semiplastic appearance depending on the enzyme and length of digestion.

The oil of the salmon egg was recovered as free oil and oil emulsion on centrifuging or allowing the digest to settle. The emulsion was very stable. The oil could be recovered from the emulsion, however, by drying or solvent extraction. The oil recovered from the emulsion was bright red, and was similar in appearance to brine-extracted oil. However, its chemical characteristics were not determined.

The digestive tract of fish contains a variety of proteolytic enzymes (Stansby and Associates 1953B: Kenyon 1925) which probably attack peptides and peptones (protein fragments) as well as whole proteins. The enzyme system in the digestive tract of pink salmon was effective in solubilizing the salmon egg protein (a direct comparison of rate with the other enzymes was not obtained) and permitted an azeotropic distillation extraction to remove essentially all of the oil from the protein residue. It is suggested that the enzyme system of salmon digestive tracts either broke the salmon egg protein molecule to very short fragments from which the lipid could be readily extracted or contained an enzyme which split the protein lipid bond.

NUTRITIVE QUALITY OF ENZYME DIGEST

The effect of enzyme digestion on the nutritive value of the salmon egg protein had not been established. A single evaluation of three samples of dried salmon egg protein was carried out by Dr. Grau of the University of California by a method found effective in evaluating fish protein in poultry rations (Grau 1955). The protein was prepared from (a) salmon eggs with fat removed by exhaustive solvent extraction, (b) salmon eggs digested with the enzymes of the digestive tract of salmon and solvent extracted to remove the fat, and (c) salmon eggs digested with the commercial enzyme Rhozyme B-6 and not solvent extracted. The results are shown in table 1 and indicate that, in chick feeding, enzyme digestion did not affect the protein quality.

Table 1 - Quality of the Protein of Salmon-Egg Meals Produced With or Without Enzyme Digestion as Determined by Chick Feeding Tests

	Sample	Gain/Day	Gain/Feed
		. . . .(Percent). . . .	
Salmon-Egg Meal	Defatted by exhaustive solvent extraction	4.8	0.42
	Enzyme digested[1], solvent defatted	4.9	0.38
	Enzyme digested[1], high fat content	4.8	0.42

1/ENZYME USED WAS THE DIGESTIVE TRACT OF A PINK SALMON, ONE PERCENT OF THE WEIGHT OF THE EGGS.
2/ENZYME USED WAS RHOZYME B-6 AT 0.5 PERCENT WEIGHT OF THE EGGS.

With the salmon egg-enzyme systems investigated, bacterial decomposition became a factor of major importance in the pH range of 4.5 to 8.5. Aureomycin (chlortetracycline hydrochloride) at 20 p.p.m. was found effective in controlling bacterial action for over one week. Twelve p.p.m. aureomycin did not adequately inhibit bacterial action as judged by the formation of offensive odors.

Preliminary experiments with the complete offal from the canning of salmon indicated that enzymes were effective in dissolving most of the protein and separating a large part of the oil from the proteins.

SUMMARY

The oil and protein contained in salmon eggs are valuable when separated from each other. Common methods of reduction are not entirely satisfactory for separating oil from protein in salmon eggs.

The action of certain enzymes on salmon eggs partially separates the oil from protein as it solubilizes the protein.

An enzyme digestion might find application to salmon cannery offal as well as to salmon eggs as a processing step in producing concentrates of oil and protein at scattered and remote canneries in Alaska.

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LITERATURE CITED

ANNO, K.
1940. INVESTIGATIONS OF THE LIPIDES OF SALMON EGGS. I. THE ACETONE SOLUBLE FRACTION. JOURNAL AGR. CHEM. SOC. JAPAN, VOL. 16, P. 181.

ANONYMOUS
1955. SALTONSTALL-KENNEDY ACT FISHERIES PROJECTS. COMMERCIAL FISHERIES REVIEW, VOL. 17, NO. 6, JUNE, P. 55.

CHARGAFF, E. AND COHEN, S. S.
1939. LYSOPHOSPHATIDES. JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. 129, P. 619.

GRAU, C. R. AND WILLIAMS, M. A.
1955. FISH MEALS AS AMINO ACID SOURCES IN CHICK RATIONS. POULTRY SCIENCE, VOL. 34, NO. 4, JULY, PP. 810-816.

KENYON, W. A.
1925. DIGESTIVE ENZYMES IN POIKILOTHERMAL VERTEBRATES; AN INVESTIGATION OF ENZYMES IN FISHES WITH COMPARATIVE STUDIES IN THOSE OF AMPHIBIANS, REPTILES AND MAMMALS. U. S. DEPT. OF COMM. BUREAU OF FISHERIES, DOCUMENT 977.

KOENIG, J. AND GROSSFELD, J.
1913. FISH ROE AS FOOD FOR MAN. BIOCHEMICAL JOURNAL, VOL. 54, PP. 351-394.

KYTE, R. M.
1956. POTENTIAL BY-PRODUCTS FROM ALASKA'S FISHERY. PART I. UTILIZATION OF SALMON EGGS. U. S. FISH AND WILDLIFE SERVICE, WASHINGTON, D. C. (IN PREPARATION).

LEVIN, E. AND LERMAN, F.
1951. AN AZEOTROPIC EXTRACTION PROCESS FOR COMPLETE SOLVENT RENDERING OF RAW TISSUE. J. AMER. OIL CHEMISTS' SOC., VOL. 28, PP. 441-444.

LOVERN, J. A.
1955. THE CHEMISTRY OF LIPIDS OF BIOCHEMICAL SIGNIFICANCE. JOHN WILEY AND SONS, INC., NEW YORK, P. 71.

ROBINSON, L. A., PALMER, D. D., AND BURROWS, R. E.
1951. TESTS OF HATCHERY FOODS FOR BLUEBACK SALMON 1949. U. S. DEPT. OF INTERIOR, FISH AND WILDLIFE SERVICE, SPECIAL SCIENTIFIC REPORT: FISHERIES NO. 60, P. 18.

SEAGRAN, H. L., MOREY, D. E., AND DASSOW, J. A.
1954. THE AMINO ACID CONTENT OF ROE AT DIFFERENT STAGES OF MATURITY FROM THE FIVE SPECIES OF PACIFIC SALMON. JOURNAL OF NUTRITION, VOL. 53, NO. 1, MAY, PP. 139-150.

SINNHUBER, R. O.
1943. PRODUCTION OF SALMON EGG OIL. INFORMATION CIRCULAR 302, AGRI. EXP. STATION, OREGON STATE COLLEGE.

STANSBY, M. E. AND ASSOCIATES

1953A. UTILIZATION OF ALASKA SALMON CANNERY WASTES. U. S. DEPT. OF INTERIOR, FISH AND WILDLIFE SERVICE, SPECIAL SCIENTIFIC REPORT: FISHERIES NO. 109, P. 78.

1953B. IBID., P. 28.

THOMSON, W. A. B., IDLER, D. R., MACLEOD, R. A.
1954. THE CONVERSION OF HERRING STICKWATER SOLUBLES. II. PREVENTION OF BACTERIAL DECOMPOSITION OF HERRING STICKWATER AT HIGH TEMPERATURES. FISHERIES RESEARCH BOARD OF CANADA, PROGRESS REPORTS OF PACIFIC STATIONS, NO. 101, P. 6.

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DOMESTIC SOAP FROM SEAL OIL

In localities where seal oil is easily available it would probably be advantageous if it could be used in the form of domestic soap. But raw seal oil contains unsaturated molecules which are responsible for the softness of the soap and its tendency to turn rancid rapidly. In order to obtain a hard, stable soap, it would therefore be necessary to change the unsaturated condition of the oil. This could be accomplished by hydrogenation, a process which is used mostly for edible oils; but because of the special apparatus required, it is not adaptable to home use. It was thought that polymerization, which can be carried out at home, might furnish a solution to the problem.

Polymerization makes the unsaturated molecules react among themselves; the extent of which depends upon the temperature used and the length of time the reaction is allowed to proceed.

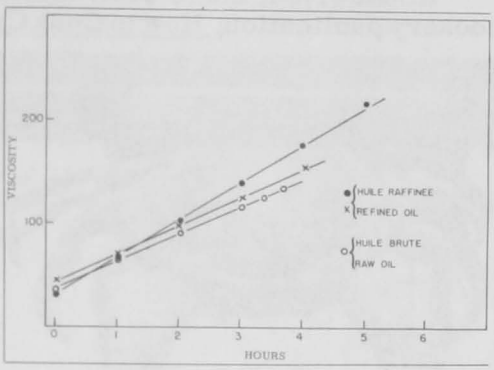

Fig. 1 - Variation in iodine value with time of polymerization.

In a previous study, it was established that polymerization of seal oil proceeds readily at a temperature of 525° F. However, the speed of the reaction in an open air vessel was unknown. Therefore, several experiments were carried out to follow the course of the reaction over a period of hours. Since polymerization produces a lowering in iodine value, and an increase in viscosity, the iodine value and viscosity of seal oil heated at 525° F. were followed on samples withdrawn at hourly intervals. Figure 1 shows the rate at which the iodine value decreases during the polymerization of raw and of refined seal oil. It also shows that the reaction is at first rapid and that it then slows down gradually, the rate being the same for refined and unrefined oil.

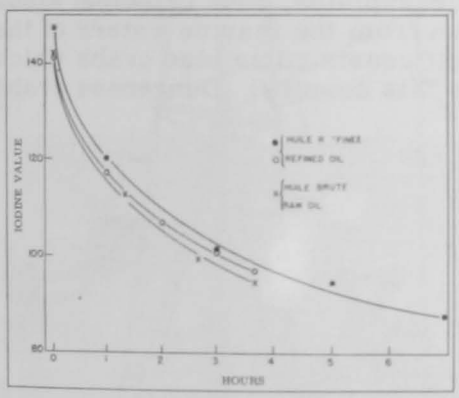

Fig. 2 - Viscosity change with time of polymerization.

The iodine value of seal oil is about 145. It must be lowered to at least 90 before the oil shows resistance to oxidation. Thus, it is apparent that the oil must be heated for about four hours to obtain the desired iodine value.

Figure 2 shows the change in viscosity which accompanies polymerization. It shows that the viscosity of the oil increases regularly with time. In practice, the viscosity index could be used to determine the extent of polymerization and thus to indicate whether uniform products were obtained.

Since the object of this investigation was the preparation of soap, some oil was first heated at 525° F. for four hours, then subjected to the usual procedure for the preparation of domestic soap. A product of suitable firmness was obtained. With the addition of rosin, it showed good lathering properties.

Thus the polymerization process offers a cheap means of preparing domestic soap from seal oil. No special apparatus is required since polymerization and saponification can be done in the same kettle. The technique is also very simple: it consists in heating the oil at 525° F. for about four hours. After polymerization the oil is transformed into soap by means of the standard procedure used for the preparation of domestic soap.

At 525° F. the oil gives off smoke and care must be taken not to heat it much higher because it might catch fire.

The process described above could, in all probability, be applicable to other oils, such as fish oils, to yield a domestic soap of fair quality. Depending on the kind of oil used, certain modifications of the temperature and heating time would no doubt be required.

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