UTILIZATION OF ALASKAN SALMON CANNERY WASTE

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UTILIZATION OF ALASKAN SALMON CANNERY WASTE
PARTS I AND II

by

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UTILIZATION OF ALASKAN SALMON CANNERY WASTE [1/]

PART I

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PART I

INTRODUCTION

This is the first of two parts of a report on utilization of Alaskan salmon cannery waste. The project was carried out under the contract sponsorship of the Industrial Research and Development Division, Office of Technical Services, Department of Commerce. The research grant was made to the Alaska Fisheries Experimental Commission and research was carried out at the Fishery Products Laboratory, Ketchikan, Alaska, and the Fishery Technological Laboratory, Seattle, Washington.

There has been a considerable interest shown by industry in obtaining reports of the results of this work even before the research is completed. In view of this situation and with several orders for reports and requests to borrow preliminary progress reports having been received, it was decided to publish that portion of the work completed during the calendar year 1947 as Part 1 of the report and to include additional material in a later part or parts to be published when more research has been completed. The next part of this report, in addition to giving results of research, will contain a detailed introduction giving comprehensive background of the status of the Alaska salmon cannery waste situation, recommendations for future work, and suggested applications of research completed at that time.

The present report is confined to a very brief introduction followed by a detailed report of the survey made at the start of this investigation

to determine what possibilities seemed most promising before selecting fields for laboratory work. Also included are reports on research completed on processing salmon waste for vitamin A oils, and results of preliminary tests on feeding of salmon cannery waste to fish in fish hatcheries.

In salmon canneries in Alaska the fish is dressed in a machine known as the "Iron Chink" which cuts off the head, fins, and tail and removes the viscera. These waste portions, amounting to about one-third of the weight of the fish, are in most cases discarded. In Ketchikan, the Alaskan city closest to Seattle, and where there is a heavy concentration of canneries close to the city, this waste is towed by barge to a reduction plant just outside the city where it is rendered into fish meal and oil. In practically all other Alaskan localities, the waste is dumped at sea. It is estimated that well in excess of 100,000,000 pounds of such waste is discarded annually.

Utilization of this waste is hampered by a number of conditions, many of which are peculiar to the salmon cannery industry in Alaska. These include:

1. Location of most canneries at isolated spots far from any city.

2. Operation of cannery by large crews of persons shipped in for the season from "outside".

3. Short canning season, usually three to six weeks in length.

4. Likelihood that fish will occur in gluts on peak days during fishing season.

5. Highly perishable nature of salmon waste.

6. High operating costs in Alaska.

7. Dependence for transportation upon shipping which, in the past, has often been interrupted for months by labor difficulties.

In order to successfully utilize the Alaska salmon cannery waste, it will be necessary to (1) find a product or, better, several products which can be prepared from the waste with a high enough selling price that the high Alaskan costs will not be prohibitive, and (2) develop some method of handling the huge gluts of waste, presumably by finding some suitable preservation technic whereby processing can be carried out over a longer period of time than the very short fishing season. These problems will be treated in greater detail in Part 2 of this report.

The work under this project was divided into the following fields:

1. Literature search and survey of industrial and economic possibilities.

2. Collection of samples of Alaskan salmon cannery waste for subsequent research.

3. Research on nutritive value of waste as a source of food for hatchery fish.

4. Use of waste as a source of vitamin A oils.

5. Research on processing waste to prepare an edible salmon oil for human consumption.

6. Research on development of new products from Alaska salmon cannery waste.

Item 1 above is treated completely in Part I, while Items 3 and 4 are discussed in a preliminary way. The latter two subjects will receive further treatment in Part II as will the other items after further research has been completed.

Preservation of the salmon cannery waste is a problem of such magnitude, it was decided not to undertake any work in that field until some of the other problems had been solved. It is recognized that no solution to the problem of Alaska salmon cannery waste utilization can be reached until adequate means of handling the huge gluts of material are devised. It is believed that this problem can be better attacked after some indication is obtained as to what portions of the waste are most apt to be utilized. At any rate, facilities and personnel available at the early stages of the project did not permit consideration of the preservation problem at this time.

POSSIBILITY OF DEVELOPMENT OF NEW PRODUCTS FROM SALMON CANNERY WASTE: LITERATURE SURVEY

By G. Ivor Jones and Edward J. Carrigan 2/

Introduction

One of the projects initiated during an investigation of the possibility of a better utilization of Alaskan salmon cannery waste was the development of new products. The term new products is used here to include isolated substances and special preparations not at present prepared from salmon cannery waste and which could be used in the pharmaceutical and chemical industries. When it appeared that there might be an economic advantage in using salmon cannery waste as a raw material source, other industrial applications were also considered.

Before starting the actual chemical studies and pilot plant operations, it was deemed essential that a survey of the chemical literature be made. A thorough review of Chemical Abstracts was made starting with volume 1. Abstracts were selected on the basis of any reference to the chemical composition of fish and fish organs. Also recorded were references

2/ Biochemists, Seattle Technological Laboratory, U. S. Fish and Wildlife Service.

to techniques and methods of production. During the survey, abstracts of over 600 articles were typed on punched cards. Microfilm copies of these cards are available for purchase. At the end of this section is a more detailed description of the chemical literature survey including the classification code used and a subject matter index.

It was decided that in conjunction with the literature review, an economic survey of the industrial possibilities of utilizing materials from salmon cannery waste would be extremely helpful in deciding the direction of the subsequent research activities. Accordingly, a trip was made so that personal interviews could be held with research staff members and executives of leading U. S. pharmaceutical manufacturing companies. Interviews were held with scientists in medical research centers and universities. Several chemical manufacturers were also interviewed with regard to a possible industrial utilization of Alaskan salmon cannery waste. In all, 18 cities were visited and interviews held with individuals in 31 different laboratories.

In the following report, the information obtained from both the literature review and the economic survey will be drawn upon in discussing the possibility of using salmon cannery waste for the manufacture of special products. A number of chemical substances will be considered and pertinent information will be discussed under each separate substance.

Proteins

Chemical nature

Proteins constitute one of the three important classes of foodstuffs. They are found in all animal and vegetable tissues and their major function is not primarily to furnish energy, but to act as building blocks in the formation of the organism itself. The proteins form a distinct class of biological substances because of their peculiar chemical and physico-chemical properities. In addition they are usually highly characteristic of the species of plant or animal in which they are found. Upon hydrolysis, proteins first yield a series of ill-defined intermediate fragments known as proteoses, peptones, and polypeptides which in turn are broken down to amino acids. Protein quality, as the term is usually applied, is judged on the basis of the amino acid analysis and refers to whether or not the ten "essential" amino acids are present in such a proportion as to promote optimum growth in test animals.

Uses

Proteins are very widely used. Their greatest use is, of course, as human and animal food. Other uses include the manufacture of pharmaceutical and industrial products such as: protein hydrolysates for medicinal use; amino acids; growth media for the production of antibiotic substances as penicillin and streptomycin; and many others.

Present sources of raw material

Each of the many industrial applications may require proteins of different composition and with specific characteristics. Protein hydrolysates have been successfully prepared from such widely divergent materials as yeast, blood fibrin, casein and lactalbumin from milk, and residue from tuna and mackerel cannery operations. Most infant and special dietary foods use milk proteins as the basic part of their formulae. Proteins from several sources have been used as the raw material for the preparation of amino acids. For example, glutamic acid, and its salt, mono-sodium glutamate, the form in which it is widely used as a food flavoring, can be prepared from the gluten of wheat and is also being produced on a large scale from "Steffens waste" which is recovered as a by-product during the refining of beet sugar.

Methods of manufacture

Some proteins must be highly purified before they can be used for certain industrial applications, while other processes do not require such purity or specificity and can utilize the protein in its natural form without separation from such impurities as carbohydrates, fats, lipids, and mineral salts. The two most important proteins in milk, casein and lactalbumin, are prepared in various degrees of purity depending upon the use for which they are intended. The protein is precipitated chemically and further purified by removal of fat and ash. Specifications for semi-purified proteins require a nitrogen content above 12.0 percent; fat, 4.0 percent maximum; and ash, 3.5 percent or less. Moisture should be held below 5.0 percent. Proteins intended for the subsequent manufacture of protein hydrolysates should be as free as possible from carbohydrate substances since these latter are converted to "humin", which in undue amounts is undesirable and also causes greater difficulty in preparing the finished product.

Economics of manufacture

Purified and semi-purified animal proteins are at present in great demand and command a good market price. For example, the milk proteins are being quoted at the price of 55 cents a pound for casein (purified--for food use) and 72 cents a pound for lactalbumin. Although production of these two proteins is very large, it could be increased by installation of additional processing equipment because large quantities of skim milk and whey are not being fully utilized. Egg albumin is also produced in large quantity and its market price has at times gone above $2.00 a pound.

When casein and lactalbumin are used directly in preparing infant and special dietary foods, the cost of the basic ingredients is a very important item in determining the price of the finished product. However, when extensive chemical testing, biological evaluation and clinical investigation are required before final marketing of a product such as a protein hydrolysate, then the cost of the basic ingredients do not represent a major item in the selling price. It can, therefore, be readily seen that in attempting to enter any given market with a competitive protein product, the cost of the raw or basic material is not the only factor to be condidered.

Presence of Proteins in Salmon Cannery Waste

The largest amount of protein in salmon cannery waste is present in the head, collar and tail sections. A fairly large amount of this proteinaceous material is composed of flesh similar to that present in canned salmon. Many reports present in the scientific literature attest to the high biological value of this protein. The parts constituting the next largest portion of the salmon waste are the gonads or roe and the milt. The proportion of roe and milt in the total salmon waste increases greatly as the fishing season progresses, and the fish prepare for spawning. Both of these organs contain characteristic proteins of a very special nature not found in other parts of the fish. The roe is reported to be a good source of the histone bases and the solids of milt contain a very high percentage of a protamine. Proteins with very specific properties are also found in the organs comprising the digestive tract, liver, heart and fins. These latter organs are present in salmon cannery waste in much smaller proportions than the roe and milt.

Percent Composition of Salmon Cannery Waste

Analytical data developed by the Fishery Products Laboratory of the Fish and Wildlife Service at Ketchikan, Alaska show the average percentage composition of the portions or organs present in the salmon cannery waste and also the average nitrogen content of each part or organ for the five species of salmon taken there in commercial quantities. By referring to Table 1 and Figure 1 it can be seen that the head and collar account for about half of the entire salmon cannery waste, for all five species. It must be remembered, however, that these are average values for a portion of the season only and that there is considerable variation as the season progresses due to increase in the proportion of roe and milt.

Table 1.--Percent Composition of Salmon Cannery Waste

Portion of fish	Percent of Total Salmon Cannery Waste				
	Species of Salmon				
	Pink	Red	Chum	King	Coho
	Percent	Percent	Percent	Percent	Percent
Head and Collar	57	61	54	50	60
Tail and Fins	16	14	11	11	11
Liver	5	5	5	3	4
Roe	8	9	16	15	8
Milt	5	5	6	4	6
Digestive Tract	9	6	8	18	11
Heart	0.8	0.8	0.7	0.7	0.7

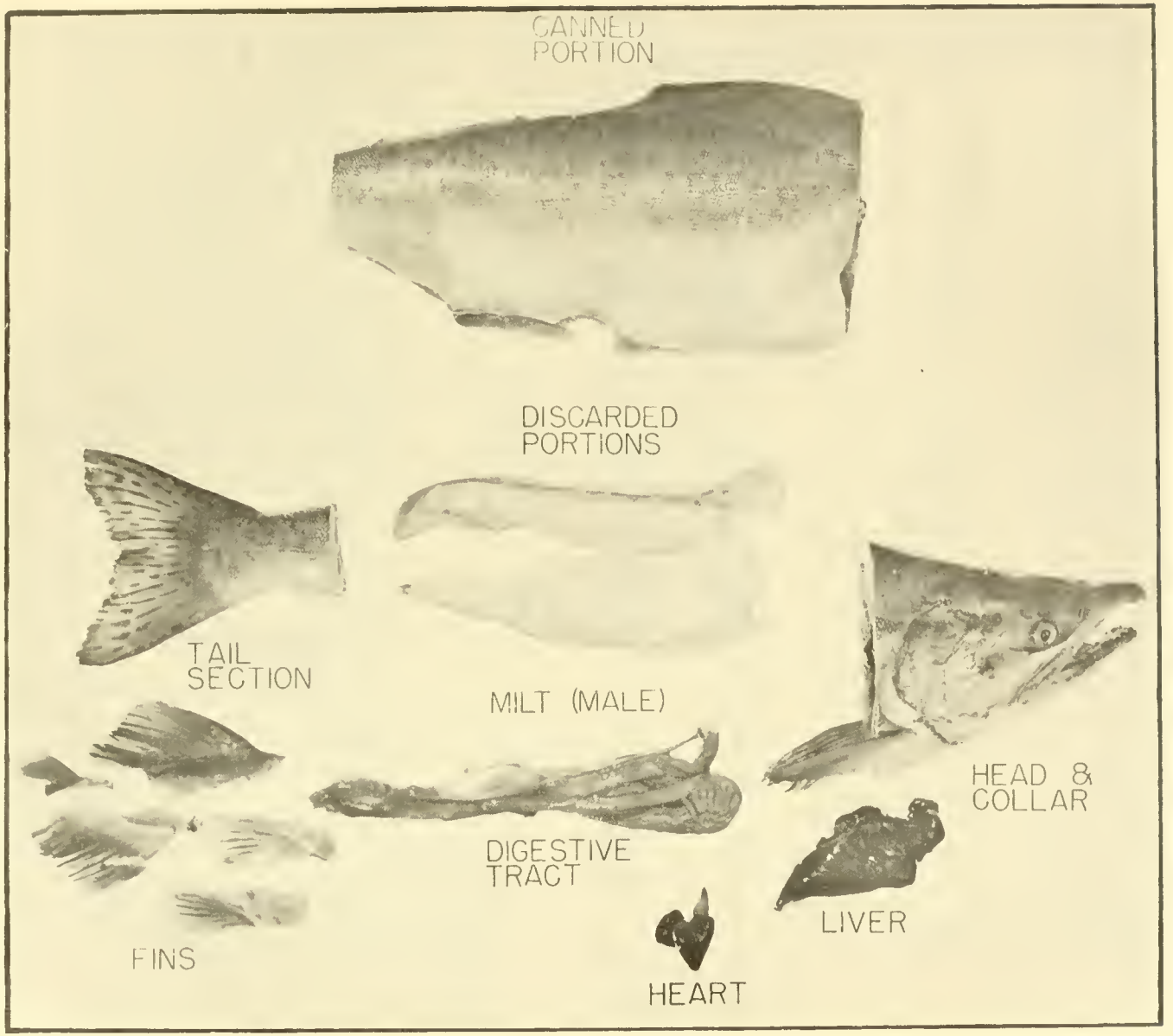

Figure 1.--Illustration of Canned and Discarded Portions of a Male Pink Salmon

Average values for the protein content of each part of the salmon cannery waste have been determined by assaying each part for total nitrogen content and multiplying this value by the factor 6.25. This calculated protein value obviously will be in error when the protein in question has an amino acid distribution which differs from the average so that the nitrogen-protein ratio is greater or smaller than 6.25. In Table 2 are listed the average protein values determined for various parts of salmon cannery waste by the Fishery Products Laboratory. These figures are not to be construed as representative for the entire season or all of Alaska since they were obtained by analyses of single samples collected during 1946 from one cannery located in Ketchikan, Alaska.

Table 2.--Protein Content of Salmon Cannery Waste

Portion of fish	Protein Content in Salmon Cannery Waste				
	Species of Salmon				
	Pink	Red	Chum	King	Coho
	Percent	Percent	Percent	Percent	Percent
Head	12.6	----	----	11.8	13.3
Collar	16.8	----	18.8	18.5	17.0
Tail (edible portion)	18.5	----	----	18.2	19.2
Liver	16.5	18.8	17.6	18.8	19.3
Roe	27.1	26.4	28.8	22.5	25.7
Milt	16.5	----	23.3	----	16.3
Digestive Tract	12.4	----	15.8	17.1	17.9

Sorting Problems

The problem of sorting salmon cannery waste in order to separate certain portions or organs for subsequent processing can probably be overcome to a large extent by mechanical means. It would be very desirable to limit the need for hand sorting to an absolute minimum due to the labor conditions existing in Alaska. By installing some additional equipment and making only minor changes in present cannery operations, it is believed that the major portion of the salmon cannery waste could be sorted mechanically. The type of utilization intended would determine whether or not and to what extent sorting would be necessary. Even with the butchering equipment now in use the head-collar section and the tail section could be segregated from the balance of the waste with only slight alterations to the existing disposal chutes. Segregation of the various intact parts or organs of the body cavity from the balance of the waste would pose a problem if such sorting were necessary.

Manufacturing Difficulties

Among the difficulties that would be immediately encountered in attempting to utilize the proteins present in Alaskan salmon cannery waste

for manufacturing purposes would be that of assembling and transporting the raw material. Many Alaskan salmon canneries are located at small isolated communities. In a few areas several canneries are located close together but this is not the rule. Therefore it would be necessary to collect and transport the raw material over a considerable distance unless it proved to be sufficiently economical or profitable to process the raw product at each individual source of waste. The salmon canning season in the various areas of Alaska are comparatively short, ranging from less than four weeks in the Bristol Bay area to approximately six weeks in Southeastern Alaska. To further complicate the picture, even though the season might be of four weeks' duration, most of the pack might be produced in a ten day interval. Thus it would be necessary to handle and at least start the initial processing of tremendous quantities of salmon cannery waste during a period of ten days. At many of the cannery locations, fresh water is quite limited and often is not adequate for sustained cannery operations at peak capacity. Because of the precipitous nature of the terrain at many cannery sites, very little area would be available for additional buildings except at prohibitive cost and any chemical operation that might increase the fire hazard adjacent to the cannery would probably not be considered in any case. The nature of the salmon waste itself might offer additional problems. For example, in considering protein recovery from salmon cannery waste, the presence of various tissues and organs would undoubtedly complicate the problem of preparing a semi-purified homogeneous, uniform product. Salmon cannery trimmings also contain a considerable amount of oil which oxidizes so easily that fat removal would probably be required in order to produce a high quality protein suitable for subsequent pharmaceutical or chemical application.

Possibility of Preparing Proteins from Salmon Cannery Waste

Many of the problems described in the preceding paragraph are peculiar to Alaska and perhaps can be re-solved or overcome by further study or by changing conditions in the future. The difficulties which might be encountered in processing the cannery trimmings with regard to protein recovery are more applicable to the head, collar and tail sections than to some of the other parts or organs. For example, salmon eggs or salmon milt could easily be separated from the remaining waste material. Since all of the nutritional elements are present in the salmon egg for reproduction of the fish, the egg protein should be of excellent quality. In preparing this protein, which amounts to about 25 percent of the total weight of the salmon roe, it would be advantageous also to recover at the same time the oil and lipide constituents. An operation designed to prepare a fat-free semi-purified protein and simultaneously to recover the valuable fat fractions of salmon eggs seems to offer good economic possibilities. Salmon milt, likewise, could be separated readily from the salmon cannery waste. Milt contains a simple protein, protamine, which is used to prepare protamine insulin, a valuable modified insulin for treatment of diabetes. The present market for protamine is comparatively small, but should additional uses be discovered for protamine, its production on a much larger scale would be relatively simple. This protein is exceedingly rich in the amino acid arginine and should its use be

enlarged as present trends appear to indicate, then the possibility of using salmon milt more extensively seems excellent. This use will be dealt with more fully under the section of this report devoted to a discussion of amino acid production from salmon cannery waste.

Protein Hydrolysates

Chemical nature

Protein hydrolysates, as the name implies, are a mixture of substances, mainly amino acids, resulting from the hydrolysis of proteins. This hydrolysis can be accomplished by several well known methods. Proteins can be hydrolyzed by digestion at a suitable temperature with acid, alkali, or enzymes. Each method possesses certain advantages and disadvantages. Digestion with acid causes destruction of tryptophane, an essential amino acid, which if lost during processing, must be added to the hydrolysate before the material would be considered nutritionally adequate. Hydrolysis of proteins with alakli causes a racemization of most of the amino acids resulting in a hydrolysate of much lower nutritional value. Enzymatic hydrolysis of proteins results in a product of excellent nutritive characteristics, but the process must be carried out under carefully controlled conditions. The complete hydrolysis of proteins by enzymes without the concomitant formation of pyrogens--substances that are dangerous in parenteral or intravenous solutions--is difficult if not impossible to accomplish without resorting to very expensive processing procedures.

Uses

Protein hydrolysates are used in medicine for feeding patients who cannot or should not take the whole protein. Hydrolysates are administered both parenterally and orally. Parenteral administration is used with patients unable to ingest protein because of gastro-intestinal disease or gastric surgery, and in burn and fracture cases where sufficient protein cannot be eaten. Hydrolysates for intravenous injection must be very carefully produced to be free from pyrogens and antigens while oral preparations need not meet such rigid standards of purity.

Protein hydrolysates are also used to some extent in the preparation of microbiological culture media and for the production of antibiotics and other substances.

In addition to the uses described above, protein hydrolysates also have been used to add a "meaty" flavor to various foods, such as soup mixes and soup stocks.

Present sources of raw material

At the present time the majority of the protein hydrolysate products are prepared from casein and lactalbumin. Some of these preparations are

being made from soy bean protein, others are from blood fibrin, and some are being made from fish proteins such as those of tuna or mackerel. Proteins to be used for the preparation of hydrolysates for human consumption must meet certain standards of purity. Specifications have been established for various proteins limiting the amount of moisture, fat and ash that may be present. A typical example of acceptable material was given in the section on methods of manufacture under proteins.

Methods of manufacture

Protein hydrolysates are at present being manufactured by the acid hydrolysis and the enzymatic digestion methods. The acid hydrolysis method yields a product which has a reduced tryptophane content and the customary practice is to supplement the preparation with the synthetic form of the amino acid, tryptophane. Since the synthetic tryptophane is only 50 percent active biologically, it is necessary to add double the amount required to bring the tryptophane content up to the level that was present in the original protein from which the hydrolysate was prepared. Since the amino acid tryptophane is comparatively expensive, this required supplementation adds considerably to the cost of the finished product.

Enzymatic hydrolysis is usually carried out by subjecting a solution of the protein (casein is most widely employed) to the action of the enzymes present in the ground tissue of mammal pancreas. The digestion is allowed to proceed until the proteins are reduced largely to amino acids and to a lesser extent to small polypeptides. If the final product is to be used intravenously, extreme care must be taken to avoid the formation of pyrogens during the processing. Pyrogens are substances, presumably carbohydrate in nature and formed by bacterial action, which when injected into the blood stream cause undesirable reactions and an increase in body temperature.

Economics of manufacture

Protein hydrolysates are being widely used in medicine. Both oral and parenteral preparations enjoy a large market. However, the supply of these products appears to be more than adequate for the demand with competition at present between more than two dozen different market preparations. The cost of the raw protein material, even though seemingly expensive when viewed by itself, actually represents only a fraction of the cost of the finished article. For example, the hydrolysate solutions when marketed usually contain only five percent of the hydrolyzed protein, but because of the expense involved in the extensive testing, both laboratory and clinical, in addition to high marketing and promotion expenses, the final cost bears little relation to the cost of the basic ingredients.

Possibility of using salmon cannery waste for preparation of hydrolysates

Suitability of Proteins in Salmon Cannery Waste

Proteins suitable for the preparation of hydrolysates are present in the fleshy portions of the head, collar, and tail sections and possibly in the roe. Fish flesh proteins have been reported by many investigators to be of high biological value, so that hydrolysates prepared from them should be well balanced nutritionally. Proteins present in the salmon liver and in the digestive tract might also be valuable for the preparation of hydrolysates but the necessity for careful sorting would present a difficult problem.

Percent Composition of Salmon Cannery Waste

The composition of salmon cannery waste in regard to proteins for the subsequent manufacture of hydrolysates has been discussed in detail under the section on proteins. Average values for the percent protein and for the percent of the part, organ or gland, in the salmon cannery waste can be found by referring to Tables 1 and 2.

Sorting Problems

In preparing protein hydrolysates for medicinal use from salmon cannery waste, it is believed that it would be necessary to sort out the tissue desired and to partially purify the protein. It would be necessary to use material which had not undergone decomposition and the fat content would need to be reduced to a minimum. As described in a previous paragraph, mechanical sorting could in all probability be arranged to separate the large parts and organs. Hydrolysates for uses other than medicinal such as for microbiological media or animal feeding could most probably be made from the waste without the need for much sorting.

Manufacturing Difficulties

In addition to the manufacturing difficulties due to location of the raw material discussed in the preceding section under proteins, some problems concerned with the nature of the material itself would be encountered.

In the preparation of oral hydrolysates, odor and flavor are important factors to be considered. Protein hydrolysates, in general, possess a disagreeable taste and it is possible that use of salmon cannery waste for manufacture of oral hydrolysates might be considered undesirable due to its fishy odor and taste. This same objection, of course, would not be manifested in a hydrolysate intended for parenteral administration.

Considerable oil and pigment are present in salmon cannery waste. It is believed that it would be necessary to at least remove the greater proportion of the fat content before suitable hydrolysates could be prepared. If color of the finished product were of any concern then it would also be necessary to destroy or remove the naturally occuring colored pigments present in the salmon cannery waste.

Possibility of Preparing Protein Hydrolysates from Salmon Cannery Waste

The utilization of salmon cannery waste for the preparation of protein hydrolysates appears to be entirely possible. As far as is known at present, the salmon proteins do not possess any unique advantages over other animal proteins such as casein or lactalbumin. It is possible that fish proteins are more easily hydrolyzed than some other proteins so that loss of tryptophane would be somewhat minimized but this advantage would not appear to be of great importance. That salmon cannery trimmings can be used to produce hydrolysates with excellent nutritional value has been demonstrated by Deas and Tarr (4) who reported in 1946 on the food value of protein hydrolysates prepared from waste materials from salmon cannery and fish liver plant operations. The hydrolysates were produced by using the pyloric ceca enzymes of salmon. There is little question that satisfactory hydrolysates can be prepared from various parts of salmon waste. The greatest difficulties are to be encountered in marketing, especially if the highly competitive market of human medicinal products is to be entered. In developing a protein hydrolysate for use in medicine, it is first necessary to carry out extensive and costly laboratory and clinical investigations. Marketing and promotional campaigns consume a large amount of time and money. Before any large pharmaceutical house would commit itself to the manufacture of a protein hydrolysate from salmon cannery waste, it would have to be assured of a constant and uniform supply of the raw material. Since the protein hydrolysate market appears to be already overcrowded with different preparations, it seems doubtful whether still another one prepared from salmon proteins could be successfully marketed unless further investigations demonstrate that it possesses some unique advantage over those now available.

Amino Acids

Chemical nature

Amino acids are commonly called the "building stones" of the protein molecule. Prctein molecules are composed of hundreds and, in some cases, thousands of amino acids combined with each other. There are 22 or more recognized amino acids. All the amino acids thus far isolated from natural proteins are alpha amino acids, that is they have an amino (NH_2) group attached to the same carbon atom that holds the acid (COOH) group. Amino acids are the end prcducts resulting from the hydrolysis of proteins. They can be prepared from natural proteins or they can be synthesized chemically. It is interesting and very important that the naturally occurring amino

acids are biologically active, that is, they are metabolized in the animal organism, while the synthetic amino acids are only 50 percent active due to the fact that they are made up of an equal mixture of the active and the inactive forms. This means that double the amount of synthetic amino acid is required to give the same biological response as the natural form of the amino acid. In addition, it has been reported by Albanese and Irby (1) that the introduction of the inactive form into the nutrition of the animal has an inhibiting or damaging effect.

The amino acids are classified further into what are termed "essential" or "indispensable" and "non-essential" or "dispensable" amino acids. The "essential" or "indispensable" amino acids have been determined by experimental animal feeding tests and have the special significance that when any one of the 10 indispensable acids are absent from the diet, growth of the animal ceases.

Uses

Amino acids and their salts are used in a number of different ways. Certain individual amino acids and mixtures of them are used in medicine, in the treatment of ulcer and burn cases, in the treatment of shock, and after major abdominal surgery. Tryptophane and methionine are used to fortify protein hydrolysates in order to enhance their nutritional value. The amino acid, lysine, is being promoted for the supplementation of various vegetable protein feeds which are deficient in this essential amino acid. Sodium glutamate is being widely used to add a meat flavor to soup mixes, sauces, and food concentrates. Experimental investigations designed to determine special functions of certain amino acids such as arginine in the treatment of some pathological conditions may lead to an enlarged use of amino acids.

Present sources of raw material

Both natural and synthetic amino acids are at present being used commercially and in medicine. The natural occurring amino acids are being prepared from tuna and mackerel fish residues, gluten of wheat, soybean protein, "Steffens waste" liquor, and other animal and vegetable materials.

The synthetic amino acids are produced chemically from simpler organic compounds such as hydrocarbons and cyanide.

Methods and economics of manufacture

The production of natural amino acids from various animal and vegetable sources can be accomplished by preparation of a hydrolysate of the protein followed by isolation of the amino acid by means of heavy metal precipitation and fractional crystallization. The isolation process is expensive and time-consuming, so that the cost of the natural amino acid is usually higher than the corresponding synthetic form, when both are available. However, since the natural form is twice as active as the synthetic, the natural is more desirable for some purposes. There are some amino acids

which have not been successfully synthesized on a commercial scale, so that where their use is indicated obviously the natural form is the only one available. Chemical synthesis has been successful in producing adequate quantities of the amino acids: methionine, isoleucine, valine, threonine, phenylalanine, tryptophane, and lysine. The resulting synthetic acids are equal mixtures of the natural (biologically active) and unnatural (biologically inactive) forms. Although it is theoretically possible to resolve these two forms by chemical methods, the procedures are laborious and impractical for quantity production.

Many different kinds of materials are used in the preparation of the natural-occurring amino acids. At the present time a number of competing raw materials are being exploited. For example, sodium glutamate, which has been mentioned before in this report is being successfully prepared from a gluten byproduct in the milling of wheat, and also from "Steffens waste", a byproduct occurring during the refining of beet sugar. Soybean hydrolysates have also been used. A number of important amino acids have been produced in sizeable quantities from fish residues of the tuna and mackerel canning operations.

The present market for amino acids is somewhat limited due to their comparatively high cost. When this cost is reduced, it is believed that certain of the amino acids will gain wider usage in medicine and in human and animal nutrition.

Possibility of utilizing salmon cannery waste for production of amino acids

Location in Salmon Cannery Waste

The proteins of salmon waste suggested as being suitable for the preparation of protein hydrolysates (head, collar, tail and roe portions) would, of course, serve as a potential source for the isolation of many of the amino acids. In addition a few of the visceral organs are rich sources of certain of the amino acids. For example, the protein, salmine, present in salmon milt, is reported to contain, on the dry basis, about 88 percent arginine. This, from all accounts, is one of the richest sources of arginine yet examined. The protein of salmon eggs, without doubt, could serve as a good source of all of the "essential" amino acids.

Percent Composition

The percent composition of salmon cannery waste in regard to proteins for the subsequent manufacture of amino acids has been presented in detail under the paragraph on proteins. Average values can be found by referring to Table 2.

Numerous articles are present in the scientific literature reporting on the amino acid content of fish flesh. Pottinger and Baldwin (10) reported the content of the five amino acids arginine, histidine, lysine, tryptophane, and cystine, found in the protein from the edible portions of a number of fishery products. The values for the five amino acids from the protein of various species of salmon are listed in Table 3.

Table 3.--Amino Acid Values of the Protein from the Edible Portions of Various Species of Salmon

Species	Composition of Protein				
	Arginine	Histidine	Lysine	Tryptophane	Cystine
	Percent	Percent	Percent	Percent	Percent
Chum	5.55	1.30	5.69	1.33	----
King	5.02	1.41	6.27	1.20	1.27
Pink	----	----	----	1.09	1.15
Silver	5.68	1.87	6.57	1.44	1.39
Sockeye	----	----	----	1.25	----

Pottinger and Baldwin concluded that the distribution of amino acids in fish flesh was similar to that in other high quality proteins such as beef, egg albumin, and casein.

Sorting Problems

It is possible that very little, if any, sorting would be required in order to isolate amino acids from salmon cannery waste. Since the protein must be hydrolyzed before the amino acids can be extracted, it might be possible to digest the entire salmon cannery waste at a suitable temperature so that the enzymatic activity of the salmon digestive tract could be utilized in solubilizing the proteins. However, in case it was desired to exploit a rich source of an amino acid such as the salmon roe or milt, then it might be more efficient to separate these organs from the remaining waste. In general, it should be emphasized that, although it might be considered uneconomical to sort the salmon cannery waste for the recovery of only one part or one substance, a combined operation designed to recover a number of materials for subsequent use would reduce the sorting charge against any one item to a relatively low figure.

Manufacturing Difficulties

The difficulties to be encountered in extracting and isolating amino acids from salmon cannery waste can only be speculated upon at this time. It is commonly known that a sizeable production of natural-occurring amino acids is at the present time being prepared from other fish wastes. It seems entirely possible that the commercial methods of extraction and isolation now being used with other protein sources could be readily adapted to the processing of salmon cannery waste proteins. Of course, considerable technical "know-how" must be available in order to set up

and operate these methods. Processing equipment must be adequate to handle the volume of material required for economic production.

Possibility of Preparing Amino Acids from Salmon Cannery Waste

Isolation of amino acids from natural proteins is a comparatively expensive procedure. The content of any particular amino acid ranges from less than 1 percent to 5 or 6 percent in most proteins. This means that a large quantity (20 to 100 times) of raw material must be processed to obtain a small amount of the pure amino acid. There are, of course, some notable exceptions. For example, while the protein of salmon flesh is reported to contain about 5 percent of the amino acid a arginine, salmon milt yields a simple protein "salmine" which is reported to contain about 88 percent arginine. In the case that the market for this amino acid increased greatly, it appears that production of arginine from Alaskan salmon milt would be economically possible. The raw Alaskan salmon milt could be obtained at very low cost. Markets for amino acids, while somewhat limited and competitive at present, seem likely to expand in the future. In considering the advantages of using certain portions or all of the salmon cannery waste because of its low procurement cost and the possibility of its being a rich source of the materials sought, it must be kept in mind that various disadvantages are to be encountered in attempting to operate recovery processes in Alaska. Further investigation into methods of isolation of amino acids from salmon cannery waste is indicated. Determination of yields of specific amino acids from various portions of the waste is necessary before any definite conclusions concerning this type of utilization can be considered for economic commercial development.

Fats and Lipids

Chemical nature

Fats and lipids constitute a group of naturally occurring organic compounds characterized by their insolubility in water and their solubility in "fat solvents" such as ether, chloroform, hot alcohol, and benzene. Simple lipids are esters of fatty acids and glycerol; those solid at room temperature are termed fats and those that are liquid are called oils. Compound lipids are esters of fatty acids combined with other substances, for example, phosphatides (lecithin and cephalin) and cholesterol. Fats are further classified into saturated and unsaturated depending upon whether or not the bonds between the carbon atoms are saturated with hydrogen or other chemical groups. The special properties of the various types of lipids make them especially valuable for a number of uses. The simple saturated fats are useful because of their freedom from rancidity and spoilage, while the unsaturated fats are highly valuable because of their ability to combine with oxygen to form hard films. The unsaturated fats are widely used as food, soap stock, etc., after having their unsaturated bonds hydrogenated. The phospholipids (lecithins) are particularly valuable because of their emulsifying power which is widely used in the chocolate, baking and cosmetic industries.

Cholesterol-fat-esters are useful as a source of cholesterol and fatty acids which are recovered by employment of a comparatively simple hydrolytic process.

Uses

Specific fats and mixtures of them are widely used for a great number of different purposes. Simple saturated fats are used in the following industries, food, baking, cosmetic, soap making, and chemical, to name a few. Unsaturated fats are used for the preparation of drying oils for use in paints, enamels, and lacquers. Unsaturated fats are often hydrogenated in order to harden them and to improve certain properties. These hydrogenated fats then serve in the cooking and baking processes, and as a source of the various fatty acids which are subsequently used in very large amounts in various manufacturing industries such as tire making, etc. Phospholipids, such as lecithin, are useful for a number of different purposes. Lecithin has wide usage in the chocolate, cosmetic, margarine, soap, baking and other industries. Lecithin also possesses properties which make it valuable as an antioxidant for oils and fats. Cholesterol finds many important uses in industry. It is used extensively in cosmetic articles and pharmaceuticals. Recent progress in scientific research has developed efficient methods for preparing biologically active vitamin D_3 from cholesterol. This process requires a cholesterol of high purity. Much of the cholesterol for vitamin D_3 manufacture is prepared from the spinal cords of meat animals.

Present sources of raw material

Fats, lipids, and cholesterol are being manufactured from a large number of animal and vegetable tissues. The possibility of utilizing salmon cannery waste as a source of fish oil for ordinary industrial use or as a source of vitamin A is considered elsewhere in this report. This section therefore will limit the discussion of fats and lipids to new or special products derived from oils and fats or to substances associated with oils and fats present in the salmon cannery waste. Discussion will be restricted mainly to cholesterol, lecithin, and unsaturated fatty acids.

Cholesterol is produced from a variety of materials. Spinal cords of meat animals are used to prepare a pure cholesterol for the subsequent manufacture of synthetic vitamin D_3. Cholesterol is also obtained from vegetable oils and from wool fat. A recent report in the technical literature states that a new method has been developed to produce pure cholesterol on a large scale from wool fat. Lecithin is produced largely from vegetable oils, soybean being the one most commonly used. Special unsaturated fatty acids are obtained from many vegetable oils although marine oils have recently been used to some extent for this purpose.

Methods of manufacture

Cholesterol is generally prepared by saponification of the oil in which it is contained followed by extraction and purification with solvents. When vegetable oils are used the resulting nonsaponifiable material is largely cholesterol and can be used directly in the semi-purified form.

Lecithin is prepared in a number of ways. One commercial method removes lecithin and other phospholipids by emulsifying the oil with hot water and then desludging the mixture by means of a high speed centrifuge, the lecithins being recovered from the aqueous phase.

Unsaturated fatty acids are usually prepared by fractional distillation although recently a process based on selective crystallization of the acids from organic solvent solutions has become of importance.

Economics of manufacture

Not a great deal of information is available on this phase of the problem. While it is true that the present market prices of cholesterol and other special substances are relatively high, it is believed that this is only a temporary situation and that reports of new processes being put into commercial production will definitely cause these seemingly high prices to fall. Competition will exist between many animal and vegetable byproducts from which these special substances can be isolated. For example, spinal cords and wool grease as sources of cholesterol will no doubt be widely used since they are rich sources of the material. Oils which are valuable as fat and which contain only a small percentage of cholesterol would obviously not be used for its manufacture. Lecithin is used in carload quantities; however, its production on a large scale from soybean oil is well founded and it is doubtful if any other material could compete unless it was found to be a very rich source.

As far as is known, the production of the higher unsaturated fatty acids has not yet been developed on a commercial scale.

Possibility of utilizing salmon cannery waste for fat, lipid, and cholesterol production

Location in Salmon Cannery Waste

Some parts of the salmon cannery waste are much richer in certain of the lipids than are others. The salmon head with adjoining collar section contains a relatively high percentage of oil. Salmon flesh yields a light colored oil in fair amount. Salmon viscera yields a lesser amount of oil which has some value for animal feeding because of its vitamin A content. Salmon eggs yield a light colored oil which is very highly unsaturated chemically and which, with possibly only slight

modification, would be well adapted for use as a rapid drying oil in the manufacture of enamels or lacquers. Salmon livers contain only a small amount of oil, but they do have a higher vitamin content than the oils prepared from other parts of the waste with the possible exception of the viscera already mentioned above. Samples of salmon liver oils have assayed as high as 40,000 units of vitamin A per gram of oil.

Percent Composition

The amount of fat, lipid, or cholesterol present in various parts of salmon cannery waste has not been determined accurately except for a few incomplete reports appearing in the scientific literature. The fat content of the various parts, glands, and organs varies from as low as 1 to 4 percent oil in salmon milt to as high as 22 percent fat in salmon roe. These oils also differ in composition as regards the amount of unsaturated acids present, the amount of lecithin and the percent of cholesterol. Koenig and Grossfeld (8) reported that the fat from fish roe contains as high as 49 percent lecithin and from 4 to 14 percent cholesterol. Schmidt-Nielsen and coworkers (11) reported in 1943 that cholesterol was present in fish sperm to the extent of 10-25 percent of the total fat. A report by Anno (2) in 1940 stated that the total unsaponifiable matter present in the lipids of salmon eggs was essentially cholesterol. Research by Harrison, et al (5) on Pacific salmon oils showed that the oil from salmon eggs has an iodine number as high as 220 indicating a large content of highly unsaturated fatty acids.

Sorting Problems

The recovery of a general purpose fish oil from total salmon cannery waste would require no sorting. The production of special oils or other lipids from various parts or organs would, of course, involve some sorting. The degree of sorting needed would depend on the nature and required degree of purity of the product or products to be recovered. Again, as mentioned in a previous section, mechanical separation might be sufficient to furnish the material for extraction. It is believed that salmon eggs at least might be handled separately, to an economic advantage, so as to recover a high quality protein material as well as to obtain the fat which should be highly valuable for its content of both lecithin and highly unsaturated fatty acids.

Manufacturing Difficulties

The extraction and isolation of special fractions of the fats and lipids present in salmon cannery waste would encounter some difficulties not met with in processing most vegetable tissues. The ordinary reduction of entire waste results in a dark colored oil which has undergone considerable decomposition. This comparatively low grade oil has not found wide usage except in possibly the leather and soap industries. Solvent extraction methods might be adaptable to certain portions of the waste such as salmon eggs. Naturally, this type of processing equipment is comparatively expensive and requires a considerable amount of technical

"know-how" to operate the equipment economically and safely. There are some disadvantages, of course, to this type of processing in Alaska but these might be outweighed by the advantages of a specialized product of superior quality. Equipment adequate to process large amounts of material by solvent extraction methods would present a definite engineering problem under the conditions outlined for Alaskan operations. Use of inflammable solvents would raise a serious objection to the increased fire hazard. Solvents would need to be transported to Alaska. Processing the material, or at least selected portions of it, would necessitate costly shipment of the preserved material in a raw or wet state over a considerable distance. However, it is believed that solvent extraction of salmon cannery waste would produce oils and fats of a much higher market value and, in addition, make possible, because of the less severe heat treatment during the drying process, the recovery of a more highly marketable protein material and possibly the subsequent recovery of lecithin and perhaps cholesterol. Pilot plant operations would need to be carefully studied before accurate predictions concerning the economic feasibility of this type of processing could be made. The isolation of cholesterol from fish oils might be expected to present some difficulties not encountered with the use of some other animal sources because the unsaponifiable matter from marine oils often contains substances other than cholesterol which would necessitate additional purification. Extraction of lecithin from salmon eggs would not be expected to present any unusual difficulties and it is believed that the present commercially used methods would be satisfactory.

Possibility of Preparing Special Fat and Lipid Materials from Salmon Cannery Waste

Exploitation of the fats and lipids present in salmon cannery waste for the separation of special products appears to have good economic possibilities. Utilizing salmon oils, especially the egg oil, for their property of having a large percentage of long chain highly unsaturated fatty acids might be accomplished by removing them from the saturated triglycerides by distillation. The separated unsaturated portion, with possibly slight modification, should possess excellent drying properties which would make it valuable for incorporation into lacquers and quick-drying enamels. Since the salmon oils as well as other fish oils are unique in their property of containing long chain highly unsaturated fatty acids, additional research into special application of these acids might uncover a specialized market where commercial development of salmon cannery waste was indicated in order to supply these materials. Lecithin, which is reported to be present in considerable amount in salmon egg oil, is widely used in many industries. Lecithin is bought and sold in carload lots and at present has a comparatively high market value. Separation of commercial lecithin from salmon egg fat seems to be possible. Recovery of cholesterol from salmon egg and other oils on an economical basis depends on whether or not the actual cholesterol content is as high as some of the reports in the literature indicate, and whether or not the present high market prices continue in the light of recently reported methods for the commercial extraction of cholesterol from "wool fat". It is believed, that although there is at present a strong market demand for

fats for the manufacture of soap, glycerine, etc., the utilization of oils from salmon cannery waste for these purposes ultimately will encounter strong competition from other fats. Economically successful utilization of salmon cannery waste for production of these less valuable oils will then depend upon how efficiently they can be produced in Alaska.

Enzymes

Chemical nature

Enzymes are organic catalysts produced by living organisms. The many chemical transformations that constantly take place in living tissues are largely the result of reactions guided and speeded by enzymes. A catalyst is defined as a substance capable of altering the speed of a chemical reaction without itself undergoing any permanent change. Generally it is believed the enzyme catalyzes or changes the speed of a reaction by momentarily attaching itself to the molecule undergoing the change, thereby increasing its instability and thus hastening the reaction rate. Thus an extremely small amount of enzyme may effect a comparatively tremendous amount of work by virtue of its rapid and repeated action.

Enzymes are usually named by using the suffix "ase" with the name of the substances being acted upon. Thus the enzyme catalyzing the breakdown of peroxides to oxygen and other products is termed peroxidase. However, specific names were given to enzymes in early investigations and, as a matter of convenience, are still used. In the following discussion the enzymes will be limited to and defined as proteolytic or protein splitting, lipolytic or fat splitting, and amylytic or starch splitting. Thus a proteolytic or protein splitting enzyme breaks down a protein to sub-products such as proteoses, peptones and sometimes completely to the basic constituents, i. e., amino acids. A lipolytic or fat splitting enzyme such as lipase, acts on fats to yield fatty acids and glycerine while an amylytic enzyme such as amylase splits starch to maltose, its basic sugar unit.

Uses

Enzymes find wide use in medicine and industry. Human digestive processes function almost entirely through enzymatic action. Thus disturbances in these processes respond very favorably to proteolytic enzyme medication. Various other enzymes have been found useful in treating high blood pressure, allergies, skin disorders, asthma, sloughing wounds, etc. In recent years, industrial application of enzymes has expanded greatly. The leather industry has long used enzymatic "bates" for removing hide glands, certain tissue fat and proteins, and reticular tissue before pickling and tanning, thus producing a much smoother, finer grained leather. Other industrial uses include the clarification of fruit juices and jellies, the chill proofing of beer, the tenderizing of meat, and the preparation of protein hydrolysates by enzymatic degradation of selected proteins. Enzymes are also used in de-sizing textiles, de-gumming silk, paper making,

and textile cleaning; in short, any process where the removal of minor amounts of proteins, fats, or carbohydrates present as impurities in a product or material is desired.

Present sources of raw material

It is readily seen that the above applications of enzymes would necessitate volume production and thus many sources have been investigated and utilized. Probably the largest single enzyme source is the mammal digestive tract, particularly the pig pancreas and gastric mucosa. Certain vegetable tissues, such as the Carica papaya, pineapple and Mexicain, yield extracts high in proteolytic enzymes. Enzymes are also successfully produced from certain molds and bacteria grown on appropriate media.

Methods of manufacture

The easiest method for producing enzymes commercially is an acid aqueous extraction of active tissues and precipitation of the enzymic protein by adding alcohol or acetone. In the case of vegetable enzymes such as papain from papaya, the process is simplified to the collection of latex and subsequent drying. However, the preparation of a single purified enzyme requires a considerable amount of repetitious reprecipitation and recrystallization since the aqueous solution extracts a mixture of enzymes which must then be segregated by selective precipitation. It must be remembered that enzymes are specific; that is, they catalyze the reaction of one and only one substance. Thus to achieve a definite reaction and product, the appropriate enzyme must be used in pure form; otherwise undesirable side reactions and non-uniform products would result.

Economics of manufacture

The numerous sources used for enzyme preparation are a result of an increasing demand conflicting with a limited supply. Thus, originally enzymes were extracted mainly from mammal organs, but as competing uses of these organs developed, such as the preparation of insulin from mammal pancreas, it became necessary to utilize papaya, molds, and bacteria to provide an adequate and yet economical supply of enzymes. In view of the increasing use of enzymes in industry and as new applications are found, it would appear that the market could readily absorb, and, in fact, will eventually require, an increased production of purified enzymes.

Possibility of utilizing salmon cannery waste for enzyme production

Location in Salmon Cannery Waste

The digestive tract of the fish is a potent source of the common hydrolytic or digestive enzymes. In general, peptic, tryptic, amylytic, and lipolytic enzymes are present in fish entrails, the type and amount varying considerably, however, with the species and the specific organ. Norris and Elam (9) isolated from the stomach mucosa of the king salmon a pepsin having unique properties and differing in specificity from mammal pepsins. Johnson (6,7) has done a considerable amount of investigating into the enzymatic constituents of fish and he concludes that only the pyloric ceca and the intestinal mucosa offer commercial possibilities, with the pyloric ceca yielding about four times as much enzyme as the intestinal mucosa. He notes that a dehydrated pyloric ceca powder is approximately seven times as active as the usual commercial leather bate prepared from animal pancreas. Numerous other workers have reported the presence of many specific enzymes in fish; however, the reports are mainly of scientific interest and these enzymes hold little commercial promise in the present economic picture.

Percent Composition

Experiments have shown that the digestive tract amounts to approximately 6-9 percent of the total salmon waste as it comes from the "Iron Chink" during cannery operations. Thus the pyloric ceca, constituting the major portion of the salmon digestive tract, would approximate 4-6 percent of the total waste. Thus, from 1,000 pounds of waste, 50 pounds of pyloric ceca could be separated yielding about 10 pounds of a dried enzymatic bate with a potency of 5 to 8 times that of the ordinary commercial bates. This concentrate, containing several enzymes, primarily exhibits proteolytic activity together with low peptic and lipolytic activity. The amount of purified enzymes that could be extracted from this concentrate is unknown at present, but in view of the high proteolytic activity, it may be safely assumed that an adequate yield could be recovered.

Sorting Problems

One of the primary factors in preparing an enzymatic powder from salmon waste is the cost of separating the pyloric ceca. The salmon ceca is a spongelike multi-lobed organ attached to the lower end of the stomach, partially surrounding the duodenum, and attached to each by elastic membranes. Ideally, the "Iron Chink" eviscerates the fish cleanly, leaving the digestive organs intact. In practice, however, the machine usually mutilates the organs, tearing off portions of the ceca and the intestines. The segregation of the ceca from the visceral mass would have to be done by hand, and would range from a simple stripping of the intact digestive tract to screening and sorting the voluminous mass of fragments. Obviously, the necessity for utilizing extensive hand labor will mean an increased cost of raw material; however, it is believed that with an efficient method of separation, pyloric ceca could be collected at a

comparatively nominal cost per pound. In view of the extremely high cost of pig and beef pancreas due to their competing uses, the raw material cost of pure pyloric ceca from salmon waste might not be too excessive.

Manufacturing Difficulties

As previously pointed out, the installation of any comprehensive chemical plant in Alaska would be very costly and its subsequent operation and maintenance expenses excessive compared to those of plants located in the States. It appears that the most feasible utilization of waste would include preparation of crude concentrates in Alaska with the refining processes being carried out in the United States. The production of a crude enzymatic powder from pyloric ceca is relatively simple and inexpensive. The fresh ceca need only be defatted and dehydrated at low temperature to yield a product that is five to eight times more active than the usual commercial leather bate. This product could be sold as concentrated bate or shipped to chemical plants in the States for the separation and purification of the enzyme constituents.

Possibility of Preparing Enzymes from Salmon Cannery Waste

The above discussion of the possibility of preparing enzymes from salmon cannery waste outlines the major aspects of the problem. The information available indicates that salmon waste provides a good source of proteolytic enzymes with commercial utilization of the ceca economically feasible. It should be noted that little information is available concerning the properties and characteristics of fish enzymes. From the preliminary investigations carried out, it appears that these enzymes exhibit unique features that enhance their utility. For example, the property of being active at low temperature, found in fish enzymes, might enjoy widespread use in the cold tenderizing of meat or in the preparation of protein hydrolysates where high temperature is an undesirable factor because of the degrading action on the hydrolysate. With enzymes finding increasing use and greater demand in medicine and industry, a new relatively cheap source of hydrolytic enzymes would be of great benefit.

It should be recognized that the proposals presented in the preceding paragraphs are based upon, and therefore limited to, scientific data already known. Many recent discoveries in biochemistry suggest promising avenues of research with reference to salmon waste. For example, an enzyme termed hyaluronidase, lately isolated from bull testes, exhibits the unique property of increasing a cell's permeability and thus may prove useful in animal husbandry and in the medical treatment of human malfunctions that result in sterility. The salmon testes offer a probable source of this enzyme, but until extensive investigations are carried out, the occurrence and concentration of hyaluronidase in these organs remains a matter of conjecture only. It is readily apparent that a great deal of study including not only applied research, but fundamental investigation also, must be undertaken before all the possibilities are exposed.

Hormones

Insulin

The myriad chemical processes taking place in fish, as in mammals, are subject to control and integration by means of specific chemical substances known as hormones. These substances are secreted by the endocrine glands into the blood stream and are distributed throughout the body, serving to maintain a proper balance of the various body functions.

With certain exceptions, most hormone therapy remains in the experimental stages. Insulin, a pancreatic hormone, controls carbohydrate metabolism in the body and has found very large scale use in the treatment of diabetes. In recent years, the incidence of this disease in older people has increased and the demand for insulin has increased accordingly. At present, beef pancreas is the main source of insulin, the hormone being extracted by dilute acid alcohol, after which it is precipitated, purified, and modified when so desired as a zinc or protamine complex for clinical use. However, the total available supply of beef pancreas is limited and competition exists between insulin and enzyme manufacturers for its procurement.

Possibility of Utilizing Salmon Cannery Waste as a Source of Insulin

The producers of insulin have made several attempts to extract fish insulin and have met with some degree of success. They have found that codfish pancreas is a good source, yielding several times as much insulin as an equivalent amount of beef pancreas. However, the raw material cost was too great due to the large amount of hand labor involved in obtaining sufficient quantities of the minute organs.

Unfortunately, the preparation of insulin from the waste salmon pancreas would present even greater difficulties. Instead of being a single small organ, the salmon pancreas is a diffuse tissue spread along the outside of the intestinal tract from the lower part of the stomach to the intestine. The pancreatic cells within this tissue are difficult to recognize and their separation in the volume necessary for commercial production of insulin would be uneconomical. It is possible, however, that a method of isolation could be worked out utilizing the entire section of the digestive tract stripped of the pyloric ceca. This section would either have to be used fresh or would have to be frozen and held in cold storage in order to attain the maximum yield when subsequently processed. Considering the fact that preliminary explorations in producing insulin from salmon are yet to be made, and in view of the many obvious difficulties that would be encountered due to the nature of the material, it becomes apparent that the possibility of utilization of salmon waste for insulin production at the present time is entirely unfeasible.

Other hormones

Again it should be noted that further investigations of the constituents of salmon waste might prove fruitful. The literature does not present any data on the estrogenic and androgenic hormone content of the salmon genital organs. However, it may be safely assumed that sexual hormones are present in these organs and, considering the large volume of reproductive tissue available, it is possible that salmon waste would provide another source for the commercial production of these increasingly important hormones.

Another promising opportunity for additional research is presented by the recent discovery of a growth factor present in certain proteins. The present knowledge of the growth factor, called strepogenin, is slight, but it appears that fish flesh is an excellent source. The hormone seems to contribute to more efficient protein metabolism and is sometimes termed the "protein utilization factor". Undoubtedly, after processes for the extraction and purification of the growth factor have been worked out, this material will find a ready market. As mentioned previously, it becomes increasingly obvious that full and efficient utilization of salmon wastes rests upon the instigation of a comprehensive and long term research program in order to fully ascertain the possibilities of the lesser known constituents.

Miscellaneous Organic Compounds

The preceding discussions have been confined to more or less specific substances capable of being derived from salmon waste in sufficient quantity for commercial production. In addition to these compounds, numerous miscellaneous compounds of lesser importance that are normally present in higher animals have been experimentally determined in fish. Those of minor interest include xanthophyl, carotene, astacin, xanthine, carnosine, taurine, betaine, choline, creatine, and creatinine. Others, such as bile acids, guanine, nucleic acid and glutathione, may prove significant in the future pending further development of current research.

The bile acids have become important in recent years, finding increased use in synthetic organic chemistry and medicine. These acids are secreted by the liver into the duodenum with the gall bladder serving as a reserve storage organ. The bile contents are intimately involved in body metabolism, serving to promote the digestion and absorption of fats in the intestines. The bile acids perform these functions by combining with fatty acids in the intestines to form compounds that are soluble and diffusible into the blood stream. The bile acids are also closely related in structure to cholesterol and are probably formed in the body from cholesterol. This basic configuration, common to cholesterol, bile acids, and sex hormones, enables chemists to synthesize the sex hormones from bile acids collected from mammalian gall bladders. Inasmuch as the cost of mammalian bile is rather high and the supply limited, investigations have been carried out to evaluate the economics of fish bile.

The salmon gall bladder is a very small organ and requires hand separation from the viscera. Cooke (3) presents an excellent perspective of the problems of collection and the commercial possibilities of salmon bile. He found an average bile content per fish of 0.73 grams. Thus the theoretical yield from Alaska is approximately 93,000 pounds of bile. However, Cooke notes that only 7-8 percent of the gall bladders came through the "Iron Chink" unbroken, thus reducing the potential yield to 7,000 pounds. Assuming Cooke's separating time of 2 hours per pound of bile, the cost of collecting would be close to $2.50 per pound. This price, of course, would be non-competitive with the present selling price of 75-90 cents per pound for ox bile. Thus, until mechanical means of separating the gall bladders are devised or until conditions require the simultaneous segregation of several visceral organs, the utilization of salmon bile as a source of bile acids is unwarranted.

The technologies of the nucleic acids and glutathione are other fields being currently investigated. Glutathione is found in every body tissue and appears to act as a coenzyme in the metabolic processes of carbohydrates. It is believed that glutathione also occupies an important role in intracellular oxidation-reduction processes. Should the need arise, fish waste would provide a cheap source as experiments have found the spleen, kidney, heart, and liver of salmon to possess an unusually high content of glutathione. A similar situation holds in the case of the nucleic acids. Recent work has shown that nucleosides (decomposition products of nucleic acids) are useful in treating anemia and blood pressure irregularities. Should the clinical use of nucleosides and other components of nucleic acids become widespread, the milt of salmon waste will offer one of the largest and most readily available natural sources. It has been shown that the solids of fish spermatozoa contain over 70 percent nucleic acid. The sperm could be obtained by mechanically stripping the mature salmon testes, which are extremely large organs in spawning salmon and contain from 5 to 10 percent sperm.

It is realized that large scale production of certain substances mentioned above may not be necessary for many years. However, these substances occupy an important place in the biochemistry of the human body and they will undoubtedly find their proper position in medical therapy.

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Bibliographies on Chemical Nature of Fish and Fish Nutrition

As previously mentioned, a literature survey of Chemical Abstracts was conducted. The recorded chemical studies of fish entailing pertinent information applicable to possible utilization of salmon waste was transferred to punched file cards3/. A cross-reference system was set up, dividing fish data into four general groups: (1) anatomical portions of fish, (2) chemical constituents, (3) uses, (4) operations. The detailed subheading breakdown within these four groups is as follows:

*1-4. AUTHOR	6. L	12. T
1. A,B	7. M	13. U,V,W
2. C,D	8. N,O	14. X,Y,Z
3. E,F	9. P,Q	
4. G,H	10. R	
5. I,J,K	11. S	

3/ Punched cards for indexing scientific data. C. F. Bailey, Robert S. Casey, and Gerald J. Cox. Science 104, 151 (1946).

* The numbers 1 to 4 in second margin are not coded but refer to the first block of 7,4,2,1 series. By using these numbers singularly or together, a total of 14 may be reached; these numbers have been coded to the first letter of the senior author's surname as shown.

WASTE PORTION
**5. Fish
6. Flesh, Muscles
7. Intestines, Stomach, Viscera
8. Roe and Milt
9. Glands
10. Bile
11. Heart, Kidneys, Gills
12. Liver
13. Pyloric caeca
14. Head, Eyes
15. Blood
16. Fins, Scales, Bones
17. Skin
18. Stickwater
19-23. Blank
24. Miscellaneous

CHEMICAL CONST.
25. Enzymes
26. Protein
27. Protamines
28. Amino Acids
29. Carbohydrates
30. Oil
31. Fats, Fatty Acids
32. Lecithin
33. Other Phospholipids
34. Sterols
35. Vitamin A
36. Vitamin D
37. Thiamine
38. Riboflavin
39. Other Vitamin B Complex
40. Vitamin C, E, etc.
41. Growth Factor
42. Hormones
43. Miscellaneous Organic Constituents
44. Inorganic Constituents
45. Protein Hydrolysates
46. Mechanics of Feeding
47-55. Blank

USES
56. Fish Food
57. Fur Animal Food
58. Other Animal Food
59. Human Food
60. Fertilizers
61. Paint, Lacquer, Etc.
62. Adhesives
63. Plastics
64. General
65. Vitamin Products
66. Other Pharmaceuticals
67. Minerals
68-72. Blank
73. Miscellaneous

OPERATIONS
74. Refrigeration
75. Preservation
76. Grinding
77. Hydrolysis
78. Dehydration
79. Filtering
80. Chemical Processing
81. Centrifuging
82. Purification Deodorizing
83. Analysis
84. Composition
85. Nutrition
86. Extraction
87. Fractionation
88-91. Blank

** Subsequent coding refers to numbers in second margin.

Thus it is possible to readily separate specific data as desired from a total of approximately 640 cards.

In addition to the chemical bibliography, another survey was made to assemble all possible information on the nutrition of fish. Some 240 cards were prepared and coded, primarily under fish food in the "Uses" group.

Copies of these bibliographies in microfilm will be available at nominal cost on application to the Office of Technical Services, Department of Commerce, Washington 25, D. C.

THE PREPARATION OF VITAMIN OILS FROM SALMON CANNERY OFFAL BY THE ALKALI DIGESTION PROCESS

By Charles Butler and David Miyauchi 4/

Introduction

The salmon offal available at Alaska Canneries is one of the potential sources from which we may be able to augment our diminishing supplies of

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vitamin A. A series of experiments are being conducted to test the adaptability of the alkali digestion process now employed commercially for the manufacture of vitamin A oils from fish livers to the preparation of vitamin A oils from the salmon offal. Anderson (1) has published a report of preliminary research utilizing the alkali digestion technique for the recovery of fish body oils from the head and collar section of the salmon cannery offal. During the past summer this method was tested on a larger scale at the cannery of the Alaska-Seldovia Packers, Incorporated at Seldovia, Alaska.

(1) Anderson, L. A preliminary report on an alkali process for the manufacture of commercial oil from salmon cannery trimmings. Fishery Market News, Vol. 7, pp. 4-7, 1945.

Purpose of the Investigation

Two phases of the investigation were carried out at Seldovia. The technique for the alkali digestion of cannery offal was developed to conform to the most efficient methods practicable under the conditions to be found in a small cannery and with the limited facilities available for a field operations laboratory. After the digestion technique proved to be satisfactory, representative samples of offal direct from the cannery's "Iron Chink" were processed into oils for subsequent vitamin A analyses.

Collection of Raw Materials

The salmon as received at the cannery, are sorted by species into separate bins holding approximately 10,000 fish. When the canning operation begins the fish are moved from the storage bins to the mechanical butchering machine--the "Iron Chink"--by a sluice and a slatted conveyor. The head is severed from the body by the butchering operation in equipment known as the header before the fish is sent to the "Chink" proper. The severed head (and the major part of the liver) falls through a hole in the floor into a flume where a stream of water carries it to the offal scow. A representative sample of the heads was thus obtainable by the removal of an occasional head as they passed to the offal scow.

The "Iron Chink" proper removes the fins, tail and viscera of the decapitated fish body. Since the machine performs these several removal operations at different points in the traverse of a circular path, the offal is reasonably well segregated as to type. Assuming that the fish's tail is engaged at a point corresponding to 3 o'clock and that the fish then travels in a counter-clockwise direction, the fins and tail (and occasionally the balance of the liver) drop to the sluice-way almost exclusively in the first 90-degree sector and the last 90-degree sector of the circular traverse. The viscera, consisting for the most part of the digestive tract (stomach, caeca, spleen and intestine), part of the liver, and the gonads is swept from the body cavity in the middle portion of the cycle. A chute to direct this portion of the offal over a sorting table facilitated the collection of a representative sample of the viscera. At first, only

materials in the viscera which were definitely identifiable were collected. As nearly as possible a correct proportion of the individual organs were included in the sample. For example, to each digestive tract, one liver and alternately the eggs and milt were selected. Sampling for each species was continued until 10,000 fish had been butchered provided that many fish were available at the time.

As the work progressed it was obvious, from preliminary analyses of oil samples, that the head portion of the waste contributed the major portion of the quantity of oil, but the viscera contributed the major portion of the vitamin A concentration. For this reason a series of samples of offal were collected to determine the relative contribution of oil and vitamin A for each of these portions of the offal. It was also of interest to ascertain the effect of the presence or absence of any one or more parts of the offal upon the alkali digestion technique. For these particular experiments, then, the additional materials were sorted from the waste. In several experiments the control, consisting of heads and total viscera, was compared to : (1) livers alone, (2) entire viscera alone, (3) viscera less gonads, (4) viscera less milt. All the offal in these particular tests was segregated from the same lot of fish to approximate uniformity of raw material.

Equipment Used for Alkali Digestion

The digester used was a wooden barrel approximately 30 inches in diameter and 42 inches in height. A length of iron pipe, ½-inch in diameter, was bent in a semi-circle to conform to the shape of the bottom of the barrel. This pipe was then placed in the bottom of the barrel and connected, by means of a second vertical pipe and a hose, to a source of high-pressure steam. A portable stirrer unit, powered with one-quarter horse-power 1750 RPM direct-drive motor, and equipped with two 3-inch boat-type propellors mounted on a 5/8-inch shaft 28 inches in length was clamped over the side of the barrel.

For the separation of the oil from the liquor upon completion of the digestion, a DeLaval oil purifier, Model No. 202, was employed.

Procedure for Alkali Digestion

The digestion procedure adopted as standard for the preparation of the samples of oil from the cannery offal was as follows:

(1) The salmon waste to be processed and an equal weight of potable water was placed in the digestion barrel.

(2) The stirrer motor was started and the steam turned on.

(3) A solution made by mixing one-half gallon of water with sodium hydroxide flakes equivalent to from 1.5 to 3.0 percent of the weight of the waste was added.

(4) Heating was continued for from 15 to 30 minutes at full steam pressure until contents of the barrel had reached 200° F.

(5) Heating at 200° F., with agitation, was continued until a sample of the liquor showed little if any solids remaining other than bone particles. The digestion time was 70 to 90 minutes depending on the size of the heads and the initial temperature of the offal.

(6) When the test sample of the liquor indicated complete digestion, the heating and agitation was discontinued and the liquor was allowed to stand for 10 minutes to facilitate settling out of solids (bony materials for the most part).

(7) Meanwhile the centrifuge had been started and thoroughly heated by the passage of 10 gallons of hot water (210° F.) through the machine.

(8) The contents of the barrel (other than the solids) were passed through the centrifuge.

(9) The oil recovered was weighed, the color was noted, and the samples for vitamin A analyses were hermetically sealed in tin cans.

Suitability of Alkali Digestion Method for Processing Salmon Offal

The alkali digestion technique advocated by Anderson (1) as being suitable for the recovery of oil from salmon heads and collars was used as the starting point for the project. These former experiments had shown that salmon heads and collars could be satisfactorily processed, using sodium hydroxide to the amount of 1.5 percent of the weight of the fish.

When this project was begun, it was assumed that the oil from the salmon heads would be needed to collect, by oil-solvent extraction, the vitamin A known to be present in the relatively less oily visceral material. After the first few digestions it became apparent that no particular difficulties existed with respect to the processing of total cannery waste by the technique outlined above. The protein portion of the waste was readily liquified, the bony portion settled out of the liquor, and upon centrifuging, an oil of excellent color and odor was obtained. Next a digestion was completed using only the soft portions of the waste--the gonads, liver, and digestive tract--and again there was no great difficulty encountered. The quantity of oil recovered was small--about 1.5 percent of the weight of the waste used--but the color and odor were as good as for the oil obtained from the heads. The vitamin A content tests of the oil so produced brought out the diluting effect of the oil contributed by the salmon heads when they were digested with the visceral portion of the waste.

Variations Made in Procedure for Alkali Digestion

In conjunction with the preparation of samples of oil from the waste for each of the species of salmon, variations were made in the technique of the digestions in an attempt to improve the method and at the same time to concentrate the vitamin A recovery into as small a volume of oil as possible.

Processing salmon livers alone

In one experiment the salmon livers were collected separately for an alkali digestion. As had been expected, there was no oil recovered upon centrifuging. The total liquor from the centrifuge was saved and salmon head oil of known vitamin A content was agitated with this hot liquor to oil-solvent extract any vitamin A that might be present.

Digestion of total viscera

As was described in the section on "Collection of Raw Materials", commercial operations on salmon cannery waste might have to be predicated on the use of the waste material as it comes from the "Iron Chink". Since the head-collar section could be separately conveyed from the header as mentioned earlier, the balance of the waste would then be available for processing into vitamin A bearing oil. Representative lots of this total viscera were alkali digested in exactly the condition they were discharged from the machine. Precautions previously taken to wash out blood, sea-water, coagulated egg materials, etc., with fresh water prior to the digestion were dispensed with.

Removal of both gonads

Earlier studies of the vitamin content of salmon waste by Harrison (2) have shown that although the salmon eggs do contain approximately 7 percent of oil, the vitamin A content is considerably less than 500 U. S. P. units per gram of oil. One would not expect the male gonads or testes to contain any more vitamin A that the eggs because of the specialized nature of the organs. Both these sexual products were, therefore, sorted from the viscera and discarded as diluents of the raw material. Digestions made on material thus sorted were said to be from "viscera less gonads".

(2) Harrison, R. W.; Anderson, A. W.; Holmes, A. D.; and Pigott, M. G. Vitamin content of oils from cannery trimmings of salmon from the Columbia River and Puget Sound regions. Investigational Report No. 36, U. S. Bureau of Fisheries, 1937.

Removal of the testes from the waste

The soft portion of the waste contains the male gonads or testes. The mature testes are composed, in large measure, of protein and water. Since

the alkali digestion process is based on the conversion of proteinaceous matter to a colloidal or semi-liquid state to facilitate separation of the oil in the tissue, this large proportion of protein increases the amount of material to be processed in a ratio disproportionate to any possible oil and/or vitamin A content that could be recovered therefrom. In some of the digestions the testes were, therefore, removed in order to observe the effect of the variation on the processing procedure.

Processing viscera less gonads with salmon head oil added

The diluting effect of the head oil has been mentioned before. If there is specific need for oil to act as a solvent for the vitamin A in less oily waste the amount of the dilution may be controlled within the limits deemed most advantageous by the addition of oil previously prepared by the alkali digestion of salmon heads. One such test was made.

Miscellaneous other variables considered

In Anderson's (1) report there is a list of variables which he found significant, in varying degree, to the proper functioning of the alkali digestion process. In a field study such as this paper describes, not all these suggestions could be checked because of the lack of adequate laboratory facilities at the cannery. At a later date the results of work now in progress at the Ketchikan Laboratory of the Fish and Wildlife Service on some of these factors will be published. At Seldovia the following variables were investigated:

(1) Particle size. Facilities were not available for grinding the salmon heads, but they were chopped to reduce the particle size to approximately 2 inches in diameter principally in the case of king salmon heads weighing 6 to 8 pounds each.

(2) Amount of alkali. The proportion of sodium hydroxide added was varied from 1.5 to 3.0 percent based on the amount of protein present in the waste used. The only criterion for judging the effect of this variation was the difference in the time of digestion, consistency of the material at various stages, and the extent of emulsification in the final liquor.

(3) Temperature of digestion. The effect of the temperature at which the digestions were carried out was noted for 180°, 190°, 200°, and 210° F.

(4) Effect of sea-water. Digestions were made on material containing sea-water and on similar material washed free of sea-water.

(5) Effect of blood. The blood was carefully washed from the waste in some digestions, but in others the waste was used exactly as it came from the butchering operation.

Discussion of Results

The experiments conducted were not sufficiently numerous on any one of the variations mentioned to warrant conclusive statements regarding the potential supply of vitamin A oils that could be obtained from Alaska salmon cannery waste. In Tables 1 through 5 there are presented the pertinent vitamin A data on the oils prepared and tested from pink, chum, coho, king, and red salmon waste respectively. Our aim in this preliminary project was primarily the evaluation of the alkali digestion process. In view of the satisfactory manner in which the alkali digestions progressed, it may be stated that the process can be utilized to recover the oils from the waste.

Salmon heads were digested separately to give a reference point for subsequent combinations of parts of the waste. There was no difficulty in the digestion of the heads whether they were processed whole or chopped. The time required for completion of processing was somewhat longer in the former instance. Emulsions were more frequently encountered in the preparation of the head oils than in the viscera oils, but in all such cases re-centrifuging of the emulsion resulted in a satisfactory oil. Probably further refinements in minor details of the procedure would minimize or eliminate this difficulty with emulsion formation. Since the vitamin A content of the head oils was uniformly very low, ranging from 175 U. S. P. units per gram of oil for chum salmon to 540 units for coho salmon, no further experiments seem to be indicated if vitamin A oils are the primary interest. The E value ratios at 300/328 millimicrons ranged from 0.891 to 1.577. In the vitamin A industry the customary maximum acceptable ratio at 300/328 millimicrons is 0.72. Higher ratios usually mean that there are substances present which increase the apparent vitamin A content so that the true biological vitamin A value of the head oils may be even less than the small potencies given.

Observations made during the digestions using the total visceral portion of the waste differed somewhat from those made when other portions were processed. When the viscera was added to the agitated mixture of water and sodium hydroxide (3.0 percent) a very viscous mass resulted. The material could be picked up on a paddle and stretched into a fine sheet resembling cellophane. This condition continued for the first 30 to 45 minutes of the digestion, then as stirring and heating continued, the liquor became gradually more fluid, and after a total period of 1½ to 2 hours the digestion went to completion. These tests were made using both cold water and hot (190°) water at the beginning of the process. No differences were apparent with respect to the viscous stage or to the digestion as a whole.

The oil yield from the viscera ranged from 1.2 percent for chum salmon to 4.2 percent from red salmon. Not too much significance should be attached to oil yields in small scale tests because the mechanical losses could be high and variable from one lot to another, but these results do give some conception of the approximate oil yield that could be expected from the material by alkali digestion. The vitamin A content, in U.S.P. units per gram of oil, varied from 2,844 units for pink salmon to 66,820

units for chum salmon. E value ratios at 300/328 millimicrons for the viscera oils were within the acceptable range: chum salmon viscera oil, 0.623, and pink salmon viscera oil, 0.698.

The concentrations of vitamin A reported here should not be considered necessarily typical or representative of those to be obtained in a commercial operation. It is impossible to generalize from the tests made on a few samples of the raw material taken at one cannery to the actual potential production from the several species over all of Alaska for the entire fishing season. These data do give some conception of the range that might be expected for the Seldovia district. Very probably if an average value were computed from these data, additional tests at other canneries or at a different time in the fishing season would result in oils even more divergent than those reported here. Material as variable as fish waste is very difficult to sample accurately even on a considerably larger scale of operations. The data reported is presented merely as the best criterion available for the evaluation of this potential source of vitamin A oils.

The removal of the testes from the waste eliminated the viscous stage in the digestion. Two percent of sodium hydroxide was sufficient to process the material in 1½ hours at 200° F. The oil recovered from the viscera less testes did not show any consistent difference in oil yield or in vitamin A concentration over that from the total viscera.

The combination of heads and viscera less gonads, was adequately processed within 2 hours at 200° F. using 1.5 percent of sodium hydroxide. No emulsion difficulties were encountered and the appearance, odor, color, and flavor of the oils was excellent. Oil yields ranged from 5.3 percent for king salmon to 9.5 percent for coho salmon. The vitamin A content varied from 14,690 units for the king salmon oil to 2,126 units for the coho salmon oil.

With raw material that varies from lot to lot and from day to day as much as fish does, it is difficult to account for the specific proportion of oil and of vitamin contributed by each of several component parts without elaborate control experiments. One method used to give an approximation of the relative contribution from the salmon heads was the substitution for the heads of a definite amount of salmon head oil previously prepared from material as nearly comparable as possible. The vitamin A concentration of this head oil was known and the volume of oil added was known. The total vitamin A from the head oil could, therefore, be calculated. The total vitamin A in the oil derived from the digestion of the viscera and the added head oil minus the total vitamin A supplied by the head oil would give the amount of vitamin A contributed exclusively by the viscera. In the experiments on red salmon waste, Lot 35 was on heads yielding an oil containing 154,000 U. S. P. units of vitamin A per pound of oil. Lot 36, prepared from heads and viscera less gonads, yielded an oil containing 2,369,000 units per pound of oil. The heads therefore supplied at least 6.5 percent of the total vitamin A recovered. Lot 42, for which 5 pounds of head oil

from Lot 35 was added to viscera less gonads for alkali digestion, resulted in an oil containing 8,060,000 units per pound of oil. In this instance the head oil contributed at least 1.9 percent of the vitamin A in the final oil. On the basis of the previous experiments, some oil would be expected from the viscera itself, but in this experiment there was no additional recovery of oil, probably due to saponification and/or mechanical losses in processing.

A similar series of experiments were conducted using pink salmon waste. The oil recovered from heads and viscera less gonads contained an average of 1,207,500 units per pound of oil. The oil from the digestion of the heads alone contained 116,700 U. S. P. units of vitamin A per pound of oil. When this head oil was added to the viscera less gonads the oil (Lot 12) recovered contained 985,180 units of vitamin A per pound, an apparent net loss compared to Lots 7 and 8. Here again the heads contributed only 1.2 percent of the total vitamin A obtained.

In Lots 5 and 6 pink salmon heads were combined with pink salmon livers for digestion. The results were at considerable variance. For Lot 5 the heads contributed 116,700 units per pound of oil, or 42 percent of the total 278,000 units per pound of oil recovered. The heads in Lot 6 supplied 13 percent of the total vitamin A recovered, assuming their vitamin A content was the same as that found for pink salmon heads in Lot 1.

The oils shown as Lots 13,23,28 were composites of all the oils prepared from the waste of pink, chum, and red salmon respectively. All oils from king and coho salmon waste were pooled to make up Lot 28. The E value ratios indicate that Lot 13, pink salmon oil, contained a disproportionately high amount of head oils although the vitamin A content is approximately representative of the combined lots. The chief purpose in saving these composites was to get an evaluation of the salmon oils in animal and poultry feeding tests. Thus any gross discrepancies in the vitamin A concentration as measured by physico-chemical methods compared to the actual biological vitamin A concentration may be brought out.

Summary

The alkali digestion process was found to be adaptable for the preparation of vitamin A bearing oils from total salmon cannery waste.

Several variations were made in the type of raw material selected from the total cannery waste to observe the effect of the presence or absence of specific parts of the waste on the digestion process and on the concentration of vitamin A in the oil produced therefrom. If the processor wishes to recover the vitamin A in an oil with the highest possible concentration, the best portion of the cannery waste to utilize is the viscera. Some increase in the facilitation of the digestion may be made by the removal of the gonads from the viscera.

The head-collar section may be satisfactorily processed by the alkali disgestion method to prepare a fish body oil of excellent quality, but of negligible vitamin A content. The protein portion of the fish waste is not usually recovered as fish meal when the alkali digestion process is employed. The liquified tissue could be re-precipitated, neutralized, and dried, but the denaturation of the protein might be sufficiently severe to limit the uses to which this meal could be put.

Examples, by species, of the yield of oil and of the vitamin A concentration include:

Species of Salmon	Heads		Heads and Viscera less Gonads		Total Viscera	
	Oil Content	Vitamin A Concentration in the oil	Oil Content	Vitamin A Concentration in the oil	Oil Content	Vitamin A Concentration in the oil
	Percent by weight	USP units per gram	Percent by weight	USP units per gram	Percent by weight	USP units per gram
Pink, *Oncorhynchus gorbuscha*	6.0	257	4.2	2,515	2.8	2,844
Chum, *Oncorhynchus keta*	4.75	175	6.0	7,319	1.2	66,820
Coho, *Oncorhynchus kisutch*	8.0	540	9.5	2,126	2.5	6,079
King, *Oncorhynchus tschawytscha*	10.6	270	5.3	14,960	2.2	20,182
Red, *Oncorhynchus nerka*	5.5	335	6.2	5,218	4.2	13,907

Table 1.--Vitamin A Oils from Pink Salmon Cannery Waste by Alkali Digestion

Lot No.	Preparation Date	Raw Material Used, in Pounds	Oil Yield by Weight, Percent	Vitamin A Concentration in the Oil, USP Units Per Gram	E Value 300/328 mmu., Ratio
1	7/19/47	Heads, 125	6.0	257	1.577
2	7/29/47	Heads, 65; Total viscera, 35	6.1	886	0.826
3	8/1/47	Heads, 40; Total viscera, 40	4.4	940	0.814
4	8/2/47	Heads, 40; Total viscera,40	6.25	908	0.844
5	7/23/47	Heads, 35; Livers, 25	5.4	613	0.909
6	8/1/47	Heads, 40; Livers, 45	4.75	2,036	0.727
7	7/9/47	Heads, 80; Total viscera less gonads, 45	5.5	3,248	-----
8	7/13/47	Heads, 80; Total viscera less gonads, 40	4.2	2,515	0.687
9	7/23/47	Heads, 60; Total viscera less gonads and liver, 40	6.8	1,175	0.780
10	8/2/47	Total viscera, 125	2.8	2,844	0.698
11	8/2/47	Total viscera less testes, 60	3.6	6,123	0.670
12	7/25/47	Head oil 1/, 5.75; total viscera less gonads, 60	(98) 2/	2,170	0.778
13	8/6/47	Composite oil sample from pooling all pink oils	----	2,075	0.778
14	7/18/47	Head oil 3/, 7.5; Livers, 60	(100)2/	3,344	0.697
15	7/27/47	Head oil 3/, 5; Total viscera,40	(100)2/	884	0.903

1/ Pink salmon head oil from Lot 1 was used in preparation of Lot 12.
2/ King salmon head oil from Lot 31 was used in preparation of Lots 14 and 15
3/ The oil yield (when head oil is added) is expressed as that percent of the added oil recovered, assuming no oil is obtained from the balance of the raw materials.

Table 2.--Vitamin A Oils from Chum Salmon Cannery Waste by Alkali Digestion

Lot No.	Preparation Date	Raw Material Used, in Pounds	Oil Yield by Weight Percent	Vitamin A Concentration in the Oil USP Units Per Gram	E Value 300/328 mmu. Ratio
16	7/3/47	Heads, 75	4.75	175	1.244
17	7/24/47	Heads, 150	4.0	198	1.514
18	7/30/47	Heads, 68; Total viscera, 32	3.0	2,640	0.722
19	7/12/47	Heads, 65; Total viscera less gonads, 35	----	4,099	0.688
20	7/24/47	Heads, 60; Total viscera less gonads, 40	6.0	7,319	0.696
21	7/30/47	Heads, 50; Total viscera less gonads, 25	2.0	4,692	0.664
22	7/6/47	Total viscera, 110	1.2	66,820	0.623
23	7/2/47	Total viscera less gonads, 40	3.3	7,134	0.670
24	7/10/47	Heads 1/ ,80; Total viscera less gonads, 60	3.6	5,236	0.680
25	8/6/47	Composite oil sample from pooling all chum oils	----	2,874	0.672

1/ Heads used in Lot 24 were from pink salmon.

Table 3.--Vitamin A Oils from Coho Salmon Cannery Waste by Alkali Digestion

Lot No.	Preparation Date	Raw Material Used, in Pounds	Oil Yield by Weight Percent	Vitamin A Concentration in the Oil USP Units per Gram	E Value 300/328 mmu. Ratio
26	7/24/47	Heads, 75	8.0	540	0.891
27	7/24/47	Heads, 70; Total viscera less gonads, 35	9.5	2,126	0.703
28	8/5/47	Total viscera, 50	2.5	6,079	0.676
29	8/5/47	Total viscera less testes, 70	2.8	6,112	0.655
30	8/6/47	Composite oil sample from pooling all king and coho salmon oils	---	6,542	0.654

Table 4.--Vitamin A Oils from King Salmon Cannery Waste by Alkali Digestion

Lot No.	Preparation Date	Raw Material Used, in Pounds	Oil Yield by Weight Percent	Vitamin A Concentration in the Oil USP Units per Gram	E Value 300/328 mmu. Ratio
31	7/11/47	Heads, 140	10.6	270	-----
32	7/31/47	Heads, 45; Total viscera less gonads, 40	5.3	14,690	0.631
33	7/11/47	Total viscera less gonads, 80	0.7	176,100	0.618
34	8/3/47	Total viscera, 45	2.2	20,182	0.643

Table 5.--Vitamin A Oils from Red[1] Salmon Cannery Waste by Alkali Digestion

Lot No.	Preparation Date	Raw Material Used, in Pounds	Oil Yield by Weight Percent	Vitamin A Concentration in the Oil USP Units Per Gram	E Value 300/328 mmu. Ratio
35	7/16/47	Heads, 150	5.5	335	1.135
36	7/13/47	Heads, 60; Total viscera less gonads, 40	6.2	5,218	0.678
37	7/17/47	Heads, 60; Total viscera less gonads, 40	5.0	9,155	0.643
38	7/27/47	Heads, 70; Total viscera, 35	5.25	4,930	0.651
39	7/31/47	Heads, 65; Total viscera less gonads, 35	5.25	5,414	0.656
40	8/3/47	Total viscera, 125	4.2	13,907	0.668
41	8/5/47	Total viscera less testes, 70	3.75	15,769	0.643
42	7/24/47	Total viscera less gonads, 40; head oil[2], 5	(100)[3]	17,759	0.647
43	7/20/47	Heads, 60; Total viscera less gonads, 40[4]	8.0	10,115	0.675
44	8/6/47	Composite oil sample from pooling all red salmon oils	---	8,480	0.641

1/ Red salmon waste for the most part was from Cook Inlet red salmon which are said to more nearly resemble the blueback salmon of the Columbia River area.

2/ Red salmon head oil from Lot 35 was used in the preparation of Lot 42.

3/ The oil yield (when oil is added) is expressed as that percent of the added oil recovered, assuming no oil is obtained from the balance of the raw materials.

4/ The waste for Lot 43 was from Kenai red salmon which are said to be larger than the Cook Inlet reds and to more nearly resemble the typical Alaska red salmon.

A BIOLOGICAL ASSAY OF THE NUTRITIONAL VALUE OF CERTAIN SALMON CANNERY WASTE PRODUCTS

By Roger E. Burrows and Neva L. Karrick5/

Introduction

There is a critical need for a large source of an inexpensive and nutritionally adequate supply of food for feeding fish. In the past few years the demand for hatchery-bred fish has increased tremendously. At the same time the cost of fresh meat and meat waste which has been a large part of diets in the past has increased and the supply of these foods has decreased. As a result, the amount of fish used in the diet of hatcheries has increased during this period. In the hatcheries in Washington State alone, the consumption of fish products has increased from 270,590 pounds in 1934 to approximately 4,000,000 pounds in 1946, and directors of hatcheries have stated that they could use a much larger amount if the waste could be properly processed. This means that a method of processing and preserving must be developed that is economical and retains in the finished product the essential nutritive factors known to be present in the raw fish waste.

The necessity for an adequate supply of food for fish will become even greater as the number of dams along the Columbia River increases and the demand for hatchery-bred fish becomes greater. Another possible market demand is due to the current trend in private and state sports fish hatcheries toward a longer holding period. Some hatcheries do not release fish until they are full size, with the obvious result that more food is needed than previously.

Not only must a cheap and plentiful source of food be found, but also a diet must be worked out that will supply the factors necessary for optimum fish growth and for the production of healthy fish. These factors are still an unknown quantity to those working on fish nutrition. A review of the work which has been done to determine the composition of an adequate diet for fish has been prepared by the U. S. Fish and Wildlife Service and will be published as a Fishery Leaflet. This leaflet includes a discussion of the mechanics of feeding, present knowledge of the necessary nutritional elements of foods which have been tested for their content of the anti-anemia factors, and of methods of preservation which have been tried. The abstracts of the references in the bibliography of "The Nutrition of Fish" are available on microfilm for purchase from the Office of Technical Services, Department of Commerce, Washington 25, D. C.

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Raw fresh meat contains a substance or combination of substances which are essential for sustaining life and growth. These have been called "Factor H" and the lack of them results in anemia and death. It has been shown that these anti-anemia factors are present in salmon viscera but not in the salmon flesh. A method of preservation must be worked out to include these anti-anemia factors in the preserved material.

Many workers think it is possible that the components of the vitamin B complex are important parts of the factors which prevent the anemic condition of fish that results from an inadequate diet. It has been proved that a deficiency of thiamine or riboflavin will cause characteristic diseases and high mortality. It has also been reported that lack of sufficient pantothenic acid resulted in a non-bacterial gill disease. For these reasons, analyses for the vitamin B complex content of the fish meals used in the diets are being made and will be reported later.

Many hatcheries have included waste from the salmon canneries of Washington and Oregon in their diets. Under present conditions the demand is greater than the supply from these canneries. Consequently, with millions of pounds of cannery waste available in Alaska, a huge potential source of food was waiting to be utilized. The problem of preservation must be solved if this source of supply is to be used advantageously. The salmon canneries in Alaska and most hatcheries in the United States do not have facilities for freezing or for storing the frozen product. Therefore, although it is important to test the frozen material to prove that the basic material contains the essential growth and anti-anemia factors, it is also necessary to find other methods of preservation so that the resulting product can be stored without refrigeration facilities yet at the same time will retain the essential growth factors. Since this project was begun at a time when it was impossible to obtain material from Alaska, it was decided to prepare meals from the frozen viscera of Columbia River salmon.

At the time the project was approved, only a small part remained of the normal growing season for the salmon at the Leavenworth Hatchery where feeding tests were to be carried out. Since time for research on preservation methods was not available, methods which has previously been worked out were used in the preparation of the materials for the feeding tests. The results of these abbreviated tests are, then, to be used only as an indication of the logical subsequent feeding tests to be made on waste from the Alaska canneries over the entire normal growing season next year.

In addition to the comparison of salmon viscera meals, confirmation of results of the previous years at the Leavenworth Hatchery was desired. It had been found that frozen salmon viscera permitted better growth than a diet of 100 percent beef liver. For this reason, as well as for comparison with the fish meals, one diet consisting of 90 percent salmon viscera bound by 10 percent apple pomace, and another diet of 100 percent raw frozen total salmon waste were included.

The investigation was conducted at the Leavenworth Laboratory of the Division of Fishery Biology, Fish and Wildlife Service, in cooperation with the Seattle Laboratory of the Division of Commercial Fisheries. Its primary purpose was to explore the possibilities of Alaska salmon cannery waste products

as a source of fish food for salmon and trout. The experimental work was limited in scope because of a lack of material available for test during the first season's operations. For this reason the work was confined to the testting of three meals prepared from salmon products and two types of frozen waste plus the necessary controls.

Selection of Products for Evaluation

The cost of transportation is a major item in the economical utilization of Alaskan cannery waste products. Since no artificial propagation of either salmon or trout is practiced on a large scale in the Territory at the present time these products must be processed in some manner to prevent spoilage during shipment if they are to be available for use where needed. Meals, which reduce the weight by about 80 percent and require no refrigeration, offer a practical answer to the problem of the cost of transportation. However, previous experimentation has proved that fish meals dried at the high temperatures encountered in the usual commercial fish meal plants are not a complete ration for the feeding of salmon or trout.

To further explore the possibilities of salmon meals and to determine if an alteration in the technique of preparation might preserve the anti-anemia factors, two meals were prepared in the Seattle Laboratory of the Division of Commercial Fisheries. Both meals were derived from salmon viscera secured from the Columbia River canneries. Salmon viscera, including the eggs and testes of the fish, was selected because previous work had indicated that this product in the raw fresh, or frozen form was nutritionally adequate for salmon fingerling when fed for an 18-week period. Therefore it obviously contained the anti-anemia factors in the raw state. If the meals prepared from this material would not support salmon fingerling when fed as the single diet component, the loss of these factors could be attributed to the methods of preparation.

The first meal, which has been designated as low-temperature-dried salmon viscera meal, was prepared by the tunnel drying process in which warm air, at a temperature of from 140° to 150° F., was circulated over thin layers of ground, pre-cooked viscera. This is a standard procedure and meals prepared in this manner are known to be deficient in anti-anemia factors. This meal would serve as a control in survival tests.

The second meal, designated as acetone-extracted salmon viscera meal, was prepared by the use of acetone to extract the oil and water from the viscera. The anti-anemia factors have been demonstrated to be heat labile and, as the acetone extraction was made at room temperature, it was possible that these factors might be retained if they were not contained in the water soluble or oil fractions of the salmon viscera.

These two meals plus a beef liver control formed the first phase of the experimental program. The purpose of this phase was merely to measure the presence of these factors as indicated by the survival of the stock with an absence of anemia.

The growth potential of cannery waste products, either processed or in the raw state, presented another problem for evaluation. Rations which

produce good growth are essential in the diet of salmon fingerling. Experimental data indicate that higher survival rates to the adult stage may be anticipated from large fingerling than from smaller fingerling of a comparable age when liberated. Analyses of protein content are not necessarily indicative of the growth potential of that particular product when used as fish food. Apparently salmon and trout are capable of utilizing certain proteins more readily than others. Heat is one factor responsible for an alteration in the structure of a protein and, reports indicate, those alterations due to intense heat are responsible for a reduction in growth rate when fish meals of this type are fed to salmonoid fishes. On this basis, an acetone-extracted meal might have a higher growth potential than a tunnel-dried meal if a portion of the protein were altered and made unavailable by the latter process.

Another fish meal, designated as flame-dried salmon offal meal, was available for experimental evaluation. This meal was a commercially prepared product derived from the whole cannery waste and dried by intense heat. Although derived from a different source and therefore not strictly comparable to the other meals tested, its value as a protein supplement in the ration of fingerling salmon had not been determined. Its inclusion in the diet studies would be a marked contribution to the evaluation of the potential utilization of Alaska salmon cannery waste for hatchery feeding purposes.

The freezing of the raw products offers another solution to the transportation of salmon waste without spoilage. The cost of transportation for the frozen material undoubtedly would be increased over that for fish meal. If, on the other hand, growth rates were increased and nutritionally adequate products found, these advantageous features might adequately compensate for the higher transportation cost. Because of the short term of the experimental period it was impossible to determine nutritional adequacy except in markedly deficient diets.

As stated previously, the number of products to be tested was limited at the time of initiation of the experiment. Unfortunately only two frozen products, salmon viscera and salmon trimmings, were procurable at the time. Both were secured from Columbia River canneries.

Salmon viscera had been tested previously and found to have a growth potential superior to any meat product customarily used in the diets of salmon fingerling. On short-term experiments (18 weeks) it contained adequate amounts of the anti-anemia factors to support fish life. The salmon viscera was included in these studies both to confirm these earlier results and to compare it with the fish meals being tested.

The salmon trimmings consisted of the scrap from hand-butchering operations. It did not include the viscera, testes or eggs, but was composed of heads, collars, fins, etc. It, together with salmon viscera, would represent the total waste material available from the salmon canneries. The salmon trimmings were included in these evaluations primarily to determine if this segment of the total cannery waste would be comparable to the visceral portion as a growth producing ration.

Procedure

Blueback salmon (Oncorhynchus nerka) were selected as the experimental animal. All fish used on the experiments were from a single age group with a comparable history. Distribution into troughs was made with the Leavenworth sampler to insure random sampling.

Survival experiment

This phase of the experiment consisted of 4 diets: 100 percent beef liver, 100 percent acetone-extracted salmon viscera meal, 100 percent low-temperature-dried salmon viscera meal, and 90 percent frozen salmon viscera-10 percent apple pomace. For the first 3 diets, quarter troughs were stocked at 250 grams or 179 fish each, with 2 troughs on each diet. The fish were fed all they would eat which was in excess of the amount usually required for this species. The 90 percent frozen salmon viscera-10 percent apple pomace diet was also included in the growth evaluation studies. Consequently, it was tested on a full trough containing 1,000 grams or 715 fish. This group was fed on the basis of the ratio of the amount of food to the total weight of fish as required by this species.

The experiment was initiated on July 9. On September 30 the group which was being fed the frozen salmon viscera diet showed no evidence of anemia and only 1 fish (0.1 percent) had died. This was a lower mortality rate than that of the beef liver control group. Thus it can be said that for the 12-week experimental period the salmon viscera diet was at least the equivalent of the beef liver diet.

On July 27, the fish fed the 100 percent acetone-extracted salmon viscera meal showed evidence of a nutritional deficiency. The symptoms observed indicated that there was a thiamine deficiency. On July 28, this diet was altered to include 0.8 grams of thiamine hydrochloride per 1,000 grams of fish weight--approximately 4 times the amount required to produce maximum storage in trout. To bind the vitamin into the diet, 10 percent gelatin plus water were added. The first week after the symptoms were noted and the diet changed, the mortality amounted to 70 percent of the stock, for the next 2-week period the loss was 65 percent of the remaining fish on hand. For the next 2-week period the loss was 31 percent of the stock. The total mortality of the lot for the entire experiment was 93 percent. The symptoms of a thiamine deficiency gradually disappeared but anemia was apparent when the experiment was concluded on September 3.

The fish fed 100 percent low-temperature-dried salmon viscera meal exhibited the same symptoms of a thiamine deficiency on August 8, after 31 days on the experimental diet as contrasted with 19 days for the fish fed the acetone-extracted meal. On August 8, the diet was altered as in the preceding instance to include thiamine and gelatin in the same proportions. In the next 10 days following the diet change the loss amounted to 54 percent of the fish on hand. For the next two weeks the mortality was 58 percent of the remaining fish. In all, 81 percent of the initial number

of fish were lost during the experimental period. On September 3 the experiment was discontinued due to the presence of anemia in this lot of fish.

Throughout the experimental period from July 9 to September 3 the loss of the control lot, fed 100 percent beef liver, amounted to 4 percent of the initial number of fish. There were no symptoms of anemia in this group when the experiment was concluded.

From this phase of the experimental work it was concluded that both the acetone-extracted salmon viscera meal and the low-temperature-dried salmon viscera meal were deficient in adequate amounts of thiamine to meet the requirements of blueback salmon. It was apparent also that the acetone extraction removed more of the available thiamine than did the drying process. If this were not true the symptoms of a thiamine deficiency should have appeared concurrently in both groups and not with a 12-day lag period between the two diets. The results of the thiamine assays of these meals will be included in a later report.

The addition of thiamine hydrochloride to the diet appeared to be successful in alleviating the deficiency although the treatment was hampered by the loss of appetite in the fish which is one of the symptoms of the deficiency. The survival of some of the stock of affected fish for 30 days after the symptoms first appeared is clear-cut evidence to support this conclusion.

Acetone-extracted salmon viscera meal does not appear to contain the anti-anemia factors although the picture is partially obscured by the thiamine deficiency. The presence of an anemia and this thiamine deficiency are not necessarily associated. Erythrocyte counts, which were made on moribund fish showing the typical retracted head, deflated flanks, and nervous spasms associated with a thiamine deficiency, disclosed an average normal count of 1,200,000 per cubic millimeter of blood. These counts were taken at the time the thiamine deficiency first made its appearance. However, as the experiment progressed, examination of the fish for gill coloration revealed a gradual diminution of the red coloration until, when the experiment was concluded on September 3, all of the remaining fish showed positive evidence of an anemia. A significant fact is that the course of this development was closely paralleled in the fish fed the low-temperature-dried salmon viscera meal which does not contain the anti-anemia factors. However, the loss of the desire to feed associated with the thiamine deficiency may have been responsible for the development of the anemia. This experiment should be repeated using a thiamine supplement throughout its course.

Growth evaluations

The growth evaluation experiments differed from the survival experiments in that each of the paired troughs on a single diet contained 1,000 grams of fish or 715 fish per diet. The fish were retained in the standard Leavenworth deep troughs and the full length of 16 feet was utilized as contrasted to the survival experiments in which but one-quarter of the length

was used. The amount of food fed per day was determined by reference to feeding charts for the species in which the size of fish and water temperture indicate the percentage of food required in terms of the total weight of the fish. Each trough of fish was weighed at bi-weekly intervals and the amount of food fed was then altered to conform to the increased weight of the fish. The design of this experiment was such as to allow the use of statistical methods for the determination of the significance of differences in final weights between diets. The initial population of each trough was retained intact, except for loss, throughout the course of the experimental period.

The diets were ground, mixed, and fed within an 8-hour period after the removal of the individual components from cold storage. Salt, at the rate of 2 grams per 100 grams of the mixed diet, was added where it would improve the diet consistency. After the diets were prepared they were held under refrigeration at a temperature approximating 29° F. until they were fed. The fish were fed twice daily by means of a hand ricer.

Seven diets were incorporated into the growth evaluation studies. Their composition and their place in the scheme of the experiment will be discussed individually.

Diet 1, consisting of 100 percent beef liver, is the standard control diet in fisheries nutritional studies. It was included not because it is considered the acme of perfection but because it supplied a point of reference by which the results of this experiment may be compared to those of other workers.

Diet 2 served as the actual control for the salmon meal evaluations. It consisted of 22.2 percent each, of beef liver, hog liver, and hog spleen and 33.4 percent of salmon viscera bound by the addition of salt. This mixture of meals and salmon viscera in conjunction with 10 percent fish meal has been tested both experimentally and in actual production diets and has given excellent results. It has been found to fulfill the nutritional requirements of blueback salmon as measured by good growth and the absence of discernable dietary deficiencies. In order to measure the contribution of the various fish meals to the growth rate it was necessary to feed the standard meat and viscera mixture alone.

Diet 3 consisted of the standard mixture of meats and viscera (20 percent each, of beef liver, hog liver, and hog spleen and 30 percent salmon viscera) plus 10 percent of low-temperature-dried salmon viscera meal bound by the addition of salt. The difference in weight between the fish fed this diet and those fed Diet 2 would serve as a measure of the contribution of this fish meal to the growth of blueback salmon.

Diet 4 varied from Diet 3 only in the composition of the fish meal. It consisted of the standard meat and viscera mixture and 10 percent flame-dried salmon offal meal bound by the addition of salt. It also was included to measure the contribution of variations in fish meal preparation and composition to the diet.

Diet 5 contained the standard meat and viscera mixture and 10 percent acetone-extracted salmon viscera meal bound with salt. This meal derived from the same source as the low-temperature-dried salmon viscera meal varied only in its method of preparation. Comparison with Diets 2 and 3 would measure the effect of this meal on the growth rate.

Diet 6 included 90 percent salmon viscera and 10 percent apple pomace. Salmon viscera, because of its semi-liquid nature, cannot be fed without the addition of some absorbing agent. Otherwise a large portion of the water-soluble components of the viscera dissolve into the water and are unavailable to the fish. Apple pomace was selected as the absorbing agent because previous experimental work indicated that its contribution to the growth rate when added to a diet at the 10 percent level was insignificant. The salmon viscera had been ground, mixed with the apple pomace, packaged in small containers, and stored at -10° F. until used. During the grinding and mixing operations the viscera was kept in a frozen condition. Only the amount sufficient to meet the daily requirements of the troughs on this diet was removed from cold storage on the day it was to be used. The diet was allowed to soften, then was cut into small chunks and placed in the mixer where the particle size was reduced to a point where it could be fed with the hand ricers. This procedure was necessary in order that its method of preparation be comparable to Diet 7. Comparisons with Diets 1 and 2 would measure the growth potential of salmon viscera.

Diet 7 consisted of 100 percent salmon trimmings. The method of preparation of this product differed from normal procedures. At the cannery the waste was run through a disintegrator, packaged in 50 pound boxes, and quick-frozen. Its method of preparation for feeding duplicated the procedure used for Diet 6. The effect of this diet on the growth rate of blueback salmon could be determined by comparison with Diets 1, 2, and 6.

The experiment was initiated on July 9, 1947, and concluded on September 30, 1947. The summarized data for the experimental period will be found in Table 1.

The mortalities in all diets, with the exception of Diet 7 (100 percent salmon trimmings), compared favorably with Diet 1--the beef liver control. Examinations of sample lots of fish from each diet for the presence of anemia as determined by gill coloration indicated that only Diet 7 was so affected. These fish were on the verge of an acute anemia when the experiment was discontined. Although the fish fed the salmon viscera diet (Diet 6) showed an intense red coloration of the gills comparable to control groups (Diets 1 and 2), it cannot be positively concluded that this diet contains adequate amounts of the anti-anemia factor because of the short term of the of the experimental period.

Analysis of variance was used to evaluate the significance of differences in gains in weight between diets and between troughs on a single diet. Using this procedure it was found that a highly significant difference, well below the 1 percent level, existed between diets and an insignificant difference between the troughs on single diets. The coefficient of variation due to experimental error and biological variation between troughs on the separate diets amounted to 2.6 percent. The fiducial limits of the diet means were used to determine the significance of difference between diets.

Table 1.--Summary of Experimental Diets

Diet No.	1	2	3	4	5	6	7
	Beef Liver 100%	Beef Liver 22.2% Hog Liver 22.2% Hog Spleen 22.2% Sal. Visc. 33.4% Salt	Beef Liver 20% Hog Liver 20% Hog Spleen 20% Sal. Visc. 30% Low Temp. Sal. Visc. Meal 10% Salt	Beef Liver 20% Hog Liver 20% Hog Spleen 20% Sal. Visc. 30% Flame Dried Sal. Offal Meal 10% Salt	Beef Liver 20% Hog Liver 20% Hog Spleen 20% Sal. Visc. 30% Acetone Ext. Sal. Visc Meal 10% Salt	Sal. Visc. 90% Apple Pomace10%	Salmon Trimmings 100%
No. of fish 7/9/47	715	715	715	715	715	715	715
No. of fish 9/30/47	702	712	708	706	704	714	661
Mortality	13	3	7	9	11	1	54
Percent Mortality	1.8	0.4	1.0	1.2	1.5	0.1	7.6
Weight in gms. 7/9/47	2,000	2,000	2,000	2,000	2,000	2,000	2,000
Weight in gms. 9/30/47	5,262	7,312	9,274	8,636	9,187	8,092	3,421
Gain in gms.	3,262	5,312	7,274	6,636	7,187	6,092	1,421
Percent Gain	163.1	265.6	363.7	331.8	359.4	304.6	71.0
Food Fed in gms.	16,154	19,078	19,491	18,786	19,435	19,123	14,134
Conversion	5.0	3.6	2.7	2.9	2.7	3.1	9.9

Using this criterion, it was found that there was no significant difference between the growth rates of the 3 salmon meals tested (Diets 3, 4, and 5) but that all these diets showed a highly significant difference from the control (Diet 2). From these results it was concluded that dry meals at the 10 percent level in the diet made a highly significant contribution to the growth rate. These analyses leave no alternative but the conclusion that the protein in fish meals prepared by these 3 processes is not altered in such a manner as to make it unavailable to the fish. The second conclusion, that intense heat has no effect on the growth potential of fish meals, is subject to further corroborative evidence. An additional variable, that of a different meal composition, was introduced into Diet 4 and this may have a direct bearing on the results. Another factor, the short term of the experimental period, may have obscured the significance of any differences that may exist between the low-temperature and flame-dried meals. However, there can be no question but that flame-dried salmon offal meal makes a significant contribution to the growth rate of blueback salmon.

Diet 6, salmon viscera and apple pomace, showed a significant difference in weight when compared with Diet 2 and a highly significant difference when compared with Diet 1. From these data it may be inferred that salmon viscera contains an excellent growth potential and may be responsible for the highly significant difference in growth rate which exists in favor of Diet 2 over Diet 1.

The salmon waste from which the viscera had been removed (Diet 7) showed no promise as a food for fingerling salmon when fed in the raw state. The fish fed this diet showed the lowest rate of growth of any of the diets tested. A highly significant difference existed between the mean weight of the troughs fed salmon waste and that of the next lowest diet—the beef liver control. When compared with salmon viscera the difference is startling.

The conversion shown in Table 1 is the number of grams of food required to produce a gram of fish weight. The conversion factor serves as a measure of the efficiency of a diet. If the conversion factor shows a specific diet to be very efficient, the relatively high cost of one or more of the ingredients of this diet would be perfectly acceptable by reason of the significantly better gain per unit of feed consumed. On this basis the use of salmon viscera and salmon meals are more than justified since these products cost from 5 to 7 cents per pound as compared to an average cost of 10 cents per pound for frozen meats and much more efficient conversions to fish flesh result from the use of the salmon products.

Summary

The experiment was divided into two parts, (1) a survival experiment to establish the presence or absence of the anti-anemia factors in salmon meals, and (2) growth evaluations of five salmon waste products. Both phases were conducted using blueback salmon fingerlings (Oncorhynchus nerka) as the test animals.

The results of the survival experiment indicated:

1. that the salmon viscera diet gave no evidence of being deficient in either the anti-anemia factors or thiamine. A lower mortality rate resulted from the salmon viscera diet than from any of the other diets.

2. that the acetone-extracted salmon viscera meal and low-temperature-dried salmon viscera meal were deficient in thiamine and that the acetone extraction procedure removed more of the thiamine than did the procedure employed for the preparation of the low-temperature-dried meals.

3. that the addition of thiamine hydrochloride to the diet was successful in alleviating the symptoms of the thiamine deficiency.

4. that the anti-anemia factors known to be present in the raw salmon viscera were not retained in adequate amounts in meals prepared by the acetone-extraction process.

The growth evaluation investigations indicated:

1. that salmon viscera, including the eggs and testes, contained an excellent growth potential when fed either as the single diet component or in conjunction with meat products. Observations previously made were substantiated that, for the 12-week experimental period, the salmon viscera diet permitted better growth and lower mortality than the 100 percent beef liver diet.

2. that low-temperature-dried salmon viscera meal, acetone-extracted salmon viscera meal, or flame-dried salmon offal meal when fed at the 10 percent level in the diet each made a highly significant contribution to the growth rate.

3. that temperatures up to 150° F., when used to dehydrate salmon viscera, do not alter the protein in such manner as to make it unavailable to fish.

4. that intense heat such as that used in the dehydration of flame-dried salmon offal meal has no effect on the growth potential of the meal. This conclusion, although indicated in the statistical analysis of the data, is subject to further corroborative evidence because of the introduction of an additional variable and the short term of the experimental period.

5. that salmon waste, minus the viscera, produced very poor growth rates and that the fish fed this diet were on the verge of an acute anemia at the end of the 12-week experimental period.

6. that frozen salmon cannery waste (visceral portions) is superior to beef liver as a food for growth. Methods attempted to make the salmon viscera non-perishable without refrigeration (i.e. dehydration without heat by means of acetone extraction and air drying at 150° F.) caused serious losses of nutritional factors and some other preservation methods will have to be found if freezing and cold storage is dispensed with.

PART II

INTRODUCTION

The first part of the report on the utilization of Alaskan salmon cannery waste was published in December 1947. At that time the results of the preliminary industrial and economic surveys were given and the studies on the use of the waste to prepare vitamin A oils and as a source of food for hatchery fish were discussed. The projects included in both the first and second reports were made possible by a research grant from the Industrial Research and Development Division, Office of Technical Services, U. S. Department of Commerce, to the Alaska Fisheries Experimental Commission. Originally a long term research program was planned, but, because of the liquidation of the Industrial Research and Development Division, the projects had to be terminated within one year.

The projects included in the second report were chosen from the various possibilities because it was felt that some indication concerning their value could be given in the limited period of time before the termination of the project. The phases of the program discussed in this report include the collection of the raw materials in Alaska; utilization of salmon eggs for the production of cholesterol, protein and industrial fat; the addition of salmon head oil to canned salmon; the vitamin content of the fish waste products which were used for hatchery foods; and the processing of the cannery waste to obtain vitamin A oils.

COLLECTION OF RAW MATERIAL IN ALASKA

By John A. Dassow[1]

Collection

In planning the collection of salmon cannery waste samples, the most important problem was that of obtaining a representative sample of the waste directly from the "Iron Chink." Of the six salmon canneries in the immediate vicinity of Ketchikan, two were arranged so that the flow of the waste material from the "Iron Chink" was easily accessible for the collection of material. One of these two canneries, that of the New England Fish Company, used a step conveyor to transfer the solid waste material from the chutes below the "chinks" to a bin located at the cannery floor level. During this operation, most of the liquid and much of the smaller parts of the salmon waste are washed out and lost. The larger parts of the waste such as the heads, tails, eggs, milt, and the digestive tract could be collected from the conveyor. Samples from this source were to be used only for chemical analysis and the preparation of products from the specific portions of the viscera. Waste

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PLATE 1.—SALMON WASTE COLLECTING SCOW AT THE CANNERY OF THE NEW ENGLAND FISH COMPANY, KETCHIKAN, ALASKA

material from this cannery could not be utilized for the samples to be used for feeding purposes since the waste from the conveyor was not representative of the total salmon waste coming directly from the "chinks." Approximately 100 pounds per hour of total materials consisting of salmon eggs, milt, and digestive tract could be collected from the conveyor by two workers.

The second cannery, that of Ketchikan Packing Company, was the more suitable place for the sampling. Here the waste from two "Iron Chinks" drops into two galvanized metal chutes below the floor and is flumed directly to the top of a bin located underneath the cannery and near the edge of the dock. This bin is emptied once or twice daily into a scow, and the waste is towed to the nearby reduction plant. In order to collect the waste in as fresh condition as possible and also to be able to sample that of the species being dressed at the time, a basket and a guide chute were designed to intercept and recover the waste discharging from the flume. After constructing a light wooden runway from the cannery floor down to the discharge end of the chute and by using a 1/4 inch wire mesh basket 18" by 16" by 9" with an attached rope for lowering, a representative sample of approximately 5 gallons of the total waste could be collected at a time. The contents were allowed to drain free of excess water and then dumped on a nearby table for sorting or into tubs for transportation to the laboratory where the waste was sorted or ground. In this manner approximately 1,000 pounds of the whole waste could be collected in an hour by two workers. Two additional workers were necessary for hauling, sorting or grinding the waste.

Preparation and Storage

When the whole waste or viscera was to be used for subsequent hatchery feeding tests, the material was taken to the laboratory for the grinding operation. In order to grind the whole waste effectively with a 5 hp. Rietz disintegrator, it was necessary to grind the waste first with a 7½ hp. Hercules meat and bone chopper. The Rietz grinder was equipped with 1/4-inch hole screens to produce the proper particle size for later preparation of the feed. Due to the somewhat limited capacity of the disintegrator, not over 400 pounds of the waste could be ground per hour. The ground whole waste and viscera were put into 5-gallon size friction top cans, frozen, and later transported to the Seattle laboratory where facilities were available for the preparation of the hatchery meal. To minimize the amount of decomposition of the waste during these processes, it was necessary to handle not over 300 to 500 pounds at a time. Using this quantity, it was possible to transport the waste to the laboratory, grind, fill into cans, and transport to the sharp freezer of the local cold storage in 4 hours or less.

Sorting of the whole salmon waste into the various parts was carried out on a table at the cannery and also on a specially built table at the

PLATE 2 - GRINDING SALMON WASTE FOR HATCHERY FEED AT FISHERY PRODUCTS LABORATORY KETCHIKAN, ALASKA

PLATE 3. - SORTING CANNERY WASTE PRIOR TO CHEMICAL ANALYSES.

laboratory. The various organs such as eggs, milt, liver, digestive tract and heart were separated rapidly and placed into 5-gallon cans or into large enameled pans. To determine the gross composition of the cannery waste, 200 or 300 pounds of the whole waste were carefully sorted and the various parts were weighed. In addition to the visceral parts, the heads, tails and miscellaneous fins were segregated and weighed. This was done at intervals throughout the cannery season on the various species of salmon in order to evaluate the percentage yield of each part of the total waste.

Two workers could sort 200 to 300 pounds of whole waste per hour. In most cases the sorted material was transferred to another can for inspection to insure against error in the sorting operation. The segregated parts were placed in 5-gallon size friction-top cans and placed in the sharp freezer as rapidly as possible to minimize changes in the material. With the temperature of the freezer varying from 0° to -20°F., a period of 20-25 hours was required to lower the temperature of the contents of a full can to that of the freezer. Each can was labeled on the outside with a code number referring to the species, part of offal, particle size (whole or ground), and cannery information. Also, a parchment label with this information was placed inside the can in case the code number was obliterated.

Most of the salmon cannery waste handled was from bright, slightly immature, trap-caught salmon. Seine fish are usually more mature salmon and often the quality of the whole fish is poorer due to the difference in location and method of catching. Samples of the waste from seine-caught fish were obtained during the season at the New England Fish Company cannery. The salmon run from which most of the samples were obtained during the cannery season were quite uniform. It would be expected that samples of the waste from fish caught in other areas or at a more mature stage would vary in percent composition. It is estimated that approximately 35,000 pounds of salmon waste were processed or sorted in order to obtain the desired samples.

UTILIZATION OF SALMON EGGS FOR PRODUCTION OF CHOLESTEROL, PROTEIN, AND INDUSTRIAL FAT

By G.Ivor Jones, Edward J Carrigan
and John A.Dassow 2/

Introduction

The findings of the preliminary survey by Jones and Carrigan(7) carried out during the initial stage of the research program on utiliza-

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tion of Alaskan salmon cannery waste directed attention to an investigation of the possible use of salmon eggs for the production of cholesterol, protein, and industrial fat. It was necessary to limit the proposed investigation to a period of not longer than six months, or the duration of the Industrial Research and Development Division contract with the Alaska Fisheries Experimental Commission. The study was planned so that the necessary factual information required to evaluate the possibilities of further development could be collected in the time allotted for experimental study. From analytical data reported in the literature, the use of salmon eggs as a source of cholesterol appeared promising. It was thought that commercial development might be practical if experimental tests showed the cholesterol content of the salmon eggs studied to be as high as had been previously 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 1/ fraction which would have a number of important industrial applications.

Cholesterol, a monatomic alcohol and a member of the group of compounds known as sterols, has the empirical formula $C_{27}H_{45}OH$. It is a primary cell constituent and is present in fairly large amounts in nerve tissue. As pointed out in the preliminary report by Jones and Carrigan (7), several investigators have reported on the presence of cholesterol in fish roe. Koenig and Grossfeld (8) reported that the fat from fish roe contains from 4 to 14 percent cholesterol. Anno (1) found that the unsaponifiable matter present in the lipides of salmon eggs was essentially cholesterol.

Cholesterol has been in much demand for the manufacture of synthetic vitamin D and for use in the preparation of various pharmaceutical and cosmetic articles. Pure cholesterol for subsequent manufacture of synthetic vitamin D commands an average market price of $12.00 a pound. Considerable quantity of pure cholesterol is prepared from the spinal cords of meat animals. Improved methods of cholesterol production from wool grease recently announced in the technical literature (2,10) may cause a drop in the present favorable market price. There is also a considerable demand for lecithin, which is an important constituent of the lipide fraction of salmon eggs. However, lecithin at present is being produced commercially on such a large scale from soy beans and other vegetable sources, that it is extremely doubtful if its production from salmon egg fat would be economically possible unless it was obtained incidental to the recovery of other substances. If salmon eggs were being processed for cholesterol and protein, it is possible that economic recovery of lecithin could be developed. Koenig and Grossfeld (8) in considering fish roe as food for man found the egg fat to contain as much as 49 percent lecithin. Halpern (5) reported in 1945 that the roe from sockeye salmon yielded 12.5 percent oil and 6.2 percent phospholipide. The phospholipides of salmon eggs are composed principally of

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1/ The term "lipide" used in this report includes both neutral fats and the phospholipides.

lecithin and cephalin, with the lecithin fraction predominating. 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 (4). In this process the lecithin is removed from the crude fat or oil by washing it with 2-5 percent 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 removed by repeated extractions with acetone.

Recovery of a semi-purified 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 1/ salmon to about 28 percent in the chum 2/. Although very little information regarding the amino acid content of salmon egg protein has appeared in the scientific literature, an investigation of the protein of the casings of salmon eggs was reported in 1938 by Young and Inman (11). They found that the protein in the egg casing was insoluble in the ordinary protein solvents and was slowly hydrolyzed by pepsin. On analysis the protein yielded the following values expressed in percentage of the moisture- and ash-free material: total nitrogen, 15.3; cystine, 1.84; tryptophane, 5.79; and glucosamine, 1.04. The casing constitutes 6.2 percent of the weight of the unfertilized egg. Hugouneng (6) in 1906 reported an analysis of an albumin extracted from the eggs of herring. Upon hydrolysis, the albumin, termed "clupeovine," yielded arginine, histidine, lysine, tyrosine, leucine, valine, alanine, serine, phenylalanine, and aspartic acid. Comparing this protein with vitellin from hens' eggs, Hugouneng found the products formed to be identical and he concluded that probably the two proteins are built upon the same plan.

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 4500 tons of salmon eggs are at present discarded each year.

Collection of the raw salmon eggs in Alaska should not interpose any difficult problems. The salmon are dressed prior to canning in a machine known as the "Iron Chink" which cuts off the head, fins and 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 only to a moderate degree. The large, and in most cases, intact skeins of salmon eggs

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1/ King Salmon - Oncorhynchus tschawytcha.

2/ Chum salmon - Oncorhynchus keta.

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 could be readily accomplished by salting, freezing, or possibly by the use of adding a small amount 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

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 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 in order to furnish a fairly representative approximation of the cholesterol content to be encountered. It was believed that if a fairly high concentration of cholesterol were found in certain samples, additional studies would be justified in order to promote commercial exploitation.

The egg samples for the analyses presented in the following report for all species of salmon except king 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 and maturity for the area. 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 four hours after collection. All samples were held at 0°F. storage until thawed and ground prior to chemical analysis. Each analysis reported in Table 1 (page 71) was made on a representative sample drawn from the entire 35-pound lot of thoroughly mixed ground eggs. This small rep-

resentative sample of about 250 to 300 grams was homogenized in a Waring blendor prior to removal of a sample for the determination of cholesterol and ether-soluble fat. The value reported for king salmon 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 found to be 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 official method for determination of cholesterol in eggs and egg products included in the A.O.A.C. Methods of Analysis 1/ was used in the initial experiments on salmon eggs. In this method the cholesterol is isolated from a saponified sample as the dibromide and determined by an iodine liberation-titration method using sodium thiosulphate. This method is considered precise and accurate but has the disadvantage of being somewhat 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. method and the colorimetric method of Cook and Mehlenbacher (3). The colorimetric method is based on the Lieberman-Burchard color reaction for cholesterol using the unsaponifiable fraction of the ether extract. Lampert (9) found that a Mojonnier modification of the Rose-Gottlieb method of extraction was useful in work on ice cream mixtures. Good agreement was observed by Lampert between the results obtained with this modified method and the digitonin precipitation method when applied to powdered egg samples. Cook and Mehlenbacher (3) suggested the use of a lower temperature during color development and reading and also suggested the use of a spectrophotometer to obtain the transmittance values. In this method the concentration-transmittance curve with pure cholesterol standards is determined at 640 millimicrons, the point of maximum absorption.

Initially the cholesterol content of dried (lyophilized 2/) chum salmon eggs (see sample chum (dehydrated) in Table 1) 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 of Cook and Mehlenbacher (3).

1/ Methods of analysis of the Association of Official Agricultural Chemists, VI Edition, page 349, 1945.

2/ The term "lyophilized" is used to designate the process of dehydration in the frozen state by vacuum sublimation.

Since the variation was considered small, subsequent values for cholesterol in salmon eggs were determined in duplicate by the colorimetric procedure. Due to the higher cholesterol content of salmon eggs as compared to the samples of egg white contaminated with small amounts of egg yolk for which the colorimetric method was originally adapted, it was not necessary to use more than two or three grams of ground salmon eggs for each analysis. With this small sample it was possible to saponify the eggs directly by the addition of 30 milliliters of 95 percent ethanol and 3 milliliters of 50 percent KOH followed by a period of 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 milliliters with ethyl ether. Five milliliter 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 five milliliters 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. However, the period of color development was not critical as it was found that a period of from 20 to 30 minutes gave reasonably good agreement on replicates. Transmittance values were determined at 640 millicrons with the Beckman spectrophotometer using 1 centimeter corex cells. Conversion values were obtained from a standard transmittance-concentration curve with concentrations of 0.04 to 0.12 milligrams cholesterol per milliliter chloroform in the final dilution. Blank determinations showed no absorption caused by impurities in the reagents used.

Fat Determination

The fat content represented by the total ether soluble fraction of the raw eggs was determined with a modified Mojonnier method using an initial acid hydrolysis. This treatment was necessary to break up lipid-protein complexes which are relatively insoluble in an ordinary ethyl ether extraction of raw eggs. The following procedure when applied to raw salmon eggs gave close agreement on duplicate samples.

A representative sample of 250-300 grams of ground salmon eggs was homogenized for 30 seconds in a Waring blendor. Two-to five-gram samples were removed immediately after mixing and weighed into 50-milliliter beakers. A small glass rod and 0.5 gram of purified sand were used to mix and distribute 5 milliliters of concentrated hydrochloric acid throughout the sample. A digestion period of 10 minutes on a warm hot plate was usually adequate to hydrolyze the eggs sufficiently so that a fluid mixture free of lumps was produced. This was transferred with the aid of 7-10 milliliters of 95 percent ethanol to a Mojonnier tube. Fifty milliliters of ethyl ether were added. The tube was

shaken for 30 seconds, swirled to facilitate separation, and then allowed to stand for 15 minutes. With salmon eggs a more satisfactory separation was obtained by using only ethyl ether, instead of the 25-25 mixture of ethyl ether and petroleum ether usually recommended. The ether layer was poured into a 250-milliliter separatory funnel. Two subsequent 35-milliliter washes with ethyl ether were made and added to the first extraction. Fifty milliliters of water were added to the combined ether extractions and the whole shaken and allowed to separate for 10 minutes. The water layer was drawn off and discarded. The ether layer was filtered through a medium porosity sintered glass filter provided with a 1/4-inch layer of anhydrous sodium sulfate. The filtrate was collected in a 150-milliliter tared beaker, and the ether evaporated in a current of warm air. The extractive was then placed in an air oven at 105°C. for one hour, cooled in air for 30 minutes, and weighed.

Unsaponifiable Matter

The unsaponifiable fraction of the fat was determined in duplicate on the ether extract from the above described fat determination using the official method of the A.O.A.C. for unsaponifiable residue in oils, fats and waxes. The final unsaponifiable residue as isolated by this procedure was dried to constant weight at 105°C. and allowed to cool in air for 30 minutes before weighing.

Results and Discussion

Although published reports indicated that the cholesterol content of salmon egg fat varies from 4 to 14 percent, the carefully controlled experiments reported here failed to disclose any values higher than 3.53 percent. As indicated in Table 1, the cholesterol content of the egg samples of the five species of salmon examined did not exceed 3.53 percent on the lipide fraction or 0.40 percent on the raw egg basis.

TABLE 1 .--Average Composition of Salmon Eggs

Species of Salmon	Moisture	Fat 1/	Cholesterol In Raw Eggs	Cholesterol In Fat
	Percent	Percent	Percent	Percent
Pink	59.5	11.1	0.29	2.61
Red	54.7	13.9	0.39	2.82
Chum	55.4	11.9	0.38	3.15
" (dehydrated)2/	0.5	28.0	0.86	3.06
King	62.2	12.8	0.34	2.64
Coho	60.3	11.4	0.40	3.53

1/ Total ether extract after acid hydrolysis of sample.
2/ This is a dehydrated sample prepared by lyophilization.

When compared with the average cholesterol content of hens' eggs of 1.32 percent in the yolk or 0.446 percent for whole edible egg, salmon eggs would not appear to be a rich source of this substance. Further, when considered on the lipide basis, the total fat of hens' eggs contains an average of 4.24 percent cholesterol as compared with 3.53 percent cholesterol in the egg fat of Coho salmon. The proximate analysis of hens' eggs is given in Table 2. Although the comparison of the cholesterol content of hen and salmon eggs is interesting, it is not economically significant inasmuch as hens' eggs are so valuable as a source of human food.

Table 2.--Average Composition of Hens' Eggs 1/

	Moisture Percent	Protein Percent	Fat Percent	Ash Percent	Yolk Percent	White Percent
Whole Egg	73.7	13.4	10.5	1.0	30.39	59.35
Yolk	49.5	15.7	33.3	1.1	-----	-----
White	86.2	12.3	0.2	0.6	-----	-----

1/ Allen's Commercial Organic Analysis, V Ed.,Vol.IX,p.537-43.

The average fat content of 12.2 percent for the eggs of all five species of salmon is slightly higher than the value of 10.5 percent fat for hens' eggs.

Anno (1) reported that the unsaponifiable matter extracted from the eggs of pink salmon was essentially cholesterol. In the present study, an attempt was made to verify this report. The unsaponifiable fraction was determined by the A.O.A.C. method mentioned previously. As indicated in Table 3, it was found that approximately one-half of the unsaponifiable matter did not respond to the reactions for cholesterol.

Table 3.--Cholesterol Content of Unsaponifiable Matter Of Salmon Eggs.

Species of Salmon	Unsaponifiable 1/ In Fat Percent	Cholesterol In Fat Percent	Cholesterol in Unsap. Percent
Pink	5.44	2.61	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. V Methods of Analysis.

When salmon eggs are compared with certain other materials as a potential source of cholesterol, they appear to be somewhat inferior. For instance, the spinal cords of meat animals are comparatively high in cholesterol content and are recovered for cholesterol production at some of the larger meat packing centers. The yield of pure cholesterol from spinal cords amounts to 3.0 to 3.5 percent of the fresh tissue. Upon the basis of our experimental findings, it can be seen that the cholesterol content of the spinal cords is about 8 to 10 times greater than that of salmon eggs. A compensating factor, however, is that the recovery of the spinal cords from the carcasses of meat animals might be somewhat more costly than the collection of salmon eggs.

To examine the problem further upon an economic basis, we can, by calculation, arrive at the probable value of salmon eggs as a source of cholesterol. From the experimental results using the cholesterol value of 0.40 percent, it can be readily calculated that, presuming the optimal recovery, about 250 pounds of raw salmon eggs would yield one pound of cholesterol. At the present price of $12.00 per pound, the cholesterol recoverable from one pound of salmon eggs would be valued at about five cents. To this, of course, must be added processing costs. Unless a profitable recovery of protein, fat and possibly lecithin could be accomplished from the same material, it seems unlikely that salmon eggs could be profitably processed for their cholesterol content.

Protein, Fat and Lecithin Recovery

In order to obtain the salmon egg protein in a fat-free form, as well as to recover the fat itself, 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 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.

Before commencing pilot plant studies of the solvent extraction process, it was decided to determine the proximate analysis of eggs from various species of salmon. Table 4 presents the analytical results obtained using the A.O.A.C. V Methods of Analysis on the raw salmon eggs. The samples of the various species used for analysis were from the same containers as those used for cholesterol determinations presented in Table 1, page 19.

Table 4. -- Proximate Analysis of Salmon Eggs

Species of Salmon	Moisture Percent	Protein 1/ Percent	Fat 2/ Percent	Ash Percent
Pink	59.5	27.1	11.1	2.3
Red	54.7	26.4	13.9	1.8
Chum	55.4	28.8	11.9	2.7
King	62.2	22.5	12.8	1.5
Coho	60.3	25.7	11.4	1.3

1/ Protein equals total kjeldahl nitrogen x 6.25
2/ Ethyl ether soluble lipide after acid hydrolysis of sample.

Solvent extraction of salmon eggs - preparation of protein meal.

After completion of the proximate analysis of the salmon eggs of various species, it was decided that fairly large quantities of egg protein and fat should be prepared by the acetone solvent extraction method described briefly above. Approximately 40 pounds of pink salmon eggs were thawed and ground in a meat chopper through a plate with 1/8-inch holes. Four parts by volume of acetone were added and the mixture stirred intermittently over a period of 4 hours. Continuous stirring of the mixture would doubtless reduce the time required for efficient extraction. The undissolved solid matter was allowed to settle out and the liquid phase was separated by decantation. The acetone extraction was repeated three times followed by a final treatment with ethanol at a temperature of 76-78°C. Final traces of solvent were removed from the dehydrated-defatted residue by evaporation in a vacuum desiccator using a water trap immersed in dry ice between the desiccator and the vacuum pump. The nearly colorless, odorless, fine, light powder was found to be not deliquescent. However, in order to maintain these samples without change for use in subsequent studies, they were vacuum packed (25 inches va.) in tin cans.

Analysis of the dehydrated-defatted salmon egg powder for protein, fat (ether extract after acid hydrolysis) moisture (vacuum oven--28 inches--5 hours at 80°C.), and ash are given in Table 5.

Table 5. --Composition of dehydrated-defatted salmon egg meal.

Species of Salmon	Moisture Percent	Protein 1/ Percent	Fat 2/ Percent	Ash Percent
King	9.76	84.1	2.45	3.13

1/ Total nitrogen x 6.25
2/ Fat determined as ether extract after acid hydrolysis.

Samples of the salmon egg protein meal were sent to various research laboratories for evaluation in the production of microbiological antibiotic substances and as a substitute for other proteins in the growth of poultry and small animals. Results of these investigations will be available for publication at a later date.

A study of the salmon egg protein meal to determine any residual toxicity toward rats was carried out because of a report the authors had received in a personal communication to the effect that rats did not thrive on salmon egg 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:

	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

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 6.

Table 6. - Evaluation of Salmon Egg Protein Meal for Growth.

Diet Designation	Sex	Initial Weight (Grams)	Length of Experiment (Weeks)	Gain in Live Weight (Grams)	Food Consumed (Grams)	Grams Food to Grams Gain
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	80	203	2.5
	M	69	4	110	225	2.0
	F	45	3	41	131	3.2

1/ Carried out at the College Park, Md., laboratory of the Fish and Wildlife Service.

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 meal and that its nutritional value is very nearly equivalent to that of casein.

Further investigations to delineate more precisely the special values of salmon egg protein are now being contemplated.

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, and the major part of the acetone 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. In pilot plant studies, it would probably be more expedient to remove the last traces of solvent by washing the oil with water and clearing by centrifugation. This treatment would also remove the lecithin fractions from the oil which might make it more desirable for certain purposes. In the laboratory studies being described here, the final ethanol extract was also concentrated and the extractives added to the previous acetone soluble lipides. It was later determined that the acetone not only extracts the water and essentially all the free oil, but also a considerable amount of the combined phospholipides which are not ordinarily considered soluble in acetone. The final extraction with hot (boiling) ethanol serves to break up the phospholipide and lipide protein complexes to make them more readily extractable. The resulting oil was a dark red color. In order to remove some of the dark color and to refine the product somewhat, the phospholipides present in the oil 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 fresh acetone, the solvent was again removed from the oil layer 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 purified further by repeated extraction with fresh acetone to remove any residual oil. Some darkening of the product was observed as the purification steps proceeded due undoubtedly to exposure to air during the processing steps. This darkening could probably be averted to some extent by processing the lecithin fraction in an inert atmosphere which would be more easily accomplished on a larger scale operation than on a laboratory experiment.

The phospholipide fraction after complete removal of solvent appeared as a dark brown greasy solid exhibiting many of the characteristics common to commercial lecithin.

The process of fat and phospholipide purification was applied to solvent extractives from the eggs of each species of salmon. Yields of the separate fractions are given in Table 7.

Table 7. --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 (5) selective extraction method.
4/ Phosphorous determination, A.O.A.C. V, page 21.
5/ Based on the phosphorous determination.

As indicated in Table 7, 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. Commercial recovery of the lecithin fraction would appear to be warranted in any process where preparation of a purified glyceride fraction of the fat was considered advantageous.

Discussion and Summary

The purpose of this investigation was to determine, if possible, by laboratory experimentation the feasibility of utilizing the salmon eggs from Alaskan salmon cannery waste for the production of cholesterol, protein and industrial fat. Some of the literature references used in the preliminary survey (7) held promise that the salmon roe would be an especially valuable source of cholesterol. The reputedly high values for cholesterol have not been confirmed by our findings. As mentioned previously, unless the salmon eggs can be economically processed for recovery of a high grade protein, along with a high yield of a good quality fat or oil, it appears to be unlikely that salmon eggs could be profitably processed for only their cholesterol content. The protein, fat, and lecithin fractions prepared by the solvent extraction process described in the present report appear to be of high quality. The protein material, judged on its appearance, odor, and preliminary nutritional evaluation, would appear to be worthy of further

study. Nutritional evaluation of protein meals prepared from salmon eggs by several different types of processes would provide interesting and valuable information. It is also possible that salmon egg protein may possess special properties found to be 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 200), 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 non-edible, 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 the 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 on a pilot plant scale in order to develop cost data upon which a profitable commercial operation might be built.

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VITAMIN CONTENT OF EXPERIMENTAL FISH HATCHERY FOODS

By Neva L.Karrick and Mabel A. Edwards 1/

Introduction

Members of the B vitamin complex are thought by some workers to be among the components of the growth and anti-anemia factors required by fish. It is known that if the diet lacks certain vitamins, characteristic deficiency diseases will develop. Examples of these are the non-bacterial gill disease caused by a low pantothenic acid content or the anoraxia, paralysis and eventual death caused by the lack of thiamine. For these reasons, it was decided to determine the correlation, if any, between the vitamin content of the diet and its nutritional effect on the fish. The preliminary results of feeding the hatchery diets were published in Part I, Section III of this report, and the results of the second year's work will be published later. The information to be determined about the components of the diet included the effect of the various methods of preparation of the meals on the vitamin content and the nutritional value of the material.

Preparation of the Salmon Meals

The materials used to prepare the air-dried salmon meals include Columbia River viscera, Alaska pink viscera, Alaska pink offal and spoiled Alaska pink offal. Offal is the designation for the total cannery

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waste. The frozen materials from Alaska were collected as described in Section I, "Collection of Raw Materials in Alaska." The Columbia River viscera used in the preparation of the meals was Chinook salmon waste and was obtained from the supply the Leavenworth Hatchery used for its standard production diets.

For the processing of the air-dried meals the frozen, ground viscera was thawed at room temperature. Two pounds of viscera were spread on each of nine hardware cloth trays. These loaded trays were stacked in a basket with a conical aluminum drain plate between each tray. The basket was then placed in the vertical pressure cooker and, after venting the entrapped air, the fish were processed at 15# steam pressure for 10 minutes. The object of the spaced trays and drain plates was, respectively: 1) to subject the material to moderate and uniform heat treatment as possible to facilitate coagulation of the protein, and 2) to minimize leaching of the water-soluble materials from the cooking fish by the condensed steam and freed liquor.

The cooked material was cooled and spread on hardware cloth screens. These screens, loaded with approximately one and one-half pounds of material per square foot of surface, were stacked in trucks and placed in a tunnel-type hot air drier. The meals were dried at 100°F. and 145°F. Drying was continued until the materials contained approximately 10 percent moisture, usually one and one-half to two hours. The dried material was ground in a Wiley mill using the 3/16" hole size screen. All meals were mixed, sampled for analysis, packed into tin cans, and stored at 0°F. until used for feeding tests.

In the preparation of the acetone-extracted meals, enough acetone was added to the thawed ground viscera to coagulate the protein. The solids were allowed to settle and the liquid, consisting of water, acetone and oil separated by decantation. The solid material was extracted twice with acetone. This entire procedure was carried out at room temperature.

The flame-dried salmon offal meal was commercially prepared from the whole cannery waste and is a standard by-product prepared from cannery waste. The history of the raw material used to prepare the meal is unknown. The meal is dried by intense heat with a direct flame.

The Puget Sound pink salmon viscera was the visceral waste from the "Iron Chink" and was conveyed to a storage tank where the excess water was siphoned off and discarded. The viscera was brought to a temperature of 210°F. by injection of live steam. The cooked mass was then pumped to a centrifugal separator (Sharples Super-decanter) where the solids were continuously separated from the liquid material. The solids, containing approximately 63.5 percent moisture, were packed into 5-gallon cans and 55-gallon drums. These filled containers were frozen and held in frozen storage until the feeding tests were to be made.

The above material was used for the following preparation. The frozen material was thawed, spread on hardware cloth screens, and air-dried at 100°F. until it contained approximately 10 percent moisture. The dried meal was ground, sampled for analysis, packed in 5-gallon tins, and placed in the cold storage at 0°F. until used for the feeding tests.

Frozen, ground Alaska pink salmon offal was thawed and dumped into a large wooden box and allowed to spoil. At intervals the mass was stirred thoroughly to keep the action uniform throughout. Since the experiment was conducted over a two-week period during the winter season, the decomposition by enzymatic and bacterial action was not as rapid or complete as had been desired. After two weeks the spoiled material was cooked and dried as described previously; drying was done at 100°F.

Fish solubles concentrate was prepared from the liquors drained from the retort during the cooking process for the Alaska pink salmon viscera. The liquors, consisting of the condensed steam, dissolved protein and water-soluble vitamins (B vitamin complex) and any oil or suspended protein solids, were placed in shallow stainless steel pans and acidified to pH 1.5 to 2 with concentrated hydrochloric acid. These pans were placed on shelves in a Stokes vacuum-drier oven. The liquors were concentrated by the simultaneous application of live steam to the jacketed oven and shelves and the vacuum evacuation of the oven. The maximum temperature attained by the concentrate was 135°F. when the gauge reading on the vacuum line was 29. Concentration was continued until the material contained approximately 50 percent solids. The hot concentrate was placed in 1/2-pound size flat cans, hermetically sealed and stored until ready for use. Prior to the feeding tests, the various lots of concentrate were warmed in the unopened cans, the cans were then opened, and a homogeneous mass made of the lots. Samples were taken for analysis. The balance of the concentrate was placed in small vials of 10 milliliters capacity to facilitate addition of small amounts to the diets as required without undue exposure of the unused portion to the action of air. The vials were stored at room temperature during the feeding test period.

Methods of Vitamin Assay

The general methods used for the riboflavin and niacin assays are those set up by the Association of Vitamin Chemists (1947) and Roberts and Snell (1946). The samples of raw material were composite samples taken when the meal was prepared. They were macerated in a Waring blendor and kept frozen. The ground meal samples were stored at 35°F. The hog liver and spleen and the beef liver are samples from material being used in the diets at the Leavenworth Hatchery. Approximately 10 pounds of each were ground in a meat grinder while still frozen, thoroughly mixed, sealed in cans and stored at 0°F.

Riboflavin

The samples were extracted by incubating overnight at 37°C. and pH 4.5 with 30 mg. each of papain and takadiastase and a few drops of toluene 1/. The enzymatic method was used for two reasons: It eliminated one pH adjustment and the one sample could be used for both the niacin and thiamine assays. The digested samples were filtered at pH 4.5 to remove the fat and then were diluted to 100 cc. Aliquots of the samples, except for the acetone extracted meals, the raw viscera and meat samples, were washed with ether to remove any remaining fat. The samples were then diluted to approximately 0.1 micrograms per cc. for the assay.

The medium used for the early riboflavin assays was that recommended in (1) and contained alkali-treated peptone, cystine, yeast supplement solution, mineral salts and dextrose. The medium suggested by Roberts and Snell (2) contained enzymatic casein digest, crystalline vitamins and amino acids, mineral salts and dextrose. This was used for both the riboflavin and niacin assays. The results of the riboflavin analyses checked and the recoveries were comparable using both media. Straight line standard curves were obtained in both cases. Acid production in the blanks was neutralized with less than 2 cc. of 0.1 normal sodium hydroxide.

Lactobacillus casei was the culture used for the riboflavin assays, and was carried as a stab culture on a medium recommended in (1). This consisted of 1.5 percent agar, 3 percent yeast extract, and 0.5 percent dextrose. After inoculation of the stab culture, it was incubated overnight at 37°C., and then stored in the refrigerator. The stock culture was transferred once a week. For use in an assay, the culture was inoculated into a broth tube containing 10 cc. of the complete medium used for the assay. The broth tubes were incubated overnight at 37°C. The cells were centrifuged down, the liquid decanted, and 10 cc. of sterile physiological saline solution were used to resuspend the cells. A sterile hypodermic syringe and needle were used to inoculate each assay tube with this suspension.

For the assay, 5 cc. of the basal medium of Roberts and Snell (2) minus the riboflavin were put in each tube. The standard tubes were set up in duplicate. Eight levels at 0.05 microgram intervals and ranging from 0.00 to 0.4 micrograms of standard riboflavin were used. The samples were run at five levels, but duplicate tubes were not run. An attempt was made to add samples of such a quantity that the tubes would contain from 0.05 to 0.25 micrograms of the vitamin. After the different amounts of sample were added the volume in each tube was adjusted to 10 cc. with water. The tubes were plugged, autoclaved at 15 pounds pressure for 15 minutes, cooled, and inoculated with the inoculum suspension.

1/ Extraction by autoclaving with 0.1 normal hydrochloric acid at 15 pounds pressure for 15 minutes was also satisfactory.

The medium and inoculum were thoroughly mixed and incubated for 72 hours at 37°C. The tubes were then cooled, and the acid production was measured by titrating with approximately 0.1 normal sodium hydroxide to pH 6.8 using a Beckman pH meter, model G. The same sodium hydroxide solution was always used for both the standard and the samples. The micrograms of riboflavin per tube were then determined for each level by reading the amount on the standard curve. The average value per cc. was calculated and any result which was not within ± 10 percent of the average was not used. If at least three of the levels were not within this range, the assay was not included in the final average for the material. The formula for the calculation was:

$$\text{Average microgram per cc.} \times \text{cc} \times \text{dilution} \times \frac{1}{\text{weight of sample}} = \text{Micrograms per gram}$$

In order to determine the accuracy of the assay, a recovery determination was run with each batch of samples by adding a definite amount of riboflavin to one of the unknown samples. The recovery sample and the unknown were assayed at the same time. The amount of riboflavin found in the recovery sample minus the amount found in the unknown was compared with the amount added. Ninety to 110 percent recovery was usually obtained.

Niacin

For the niacin assays Lactobacillus arabinosus was grown on the medium recommended by Roberts and Snell (2). Good growth, low blanks, a straight line standard curve, and excellent recoveries were obtained using this medium. The only modification was that 0.1 gram of additional cystine and 0.1 gram of tryptophane per liter of medium were added. This seemed to increase the growth of the organism. The culture was kept as a stab culture on agar medium as recommended in (1): 2.5 percent yeast extract, 0.5 percent dextrose, 0.5 percent anhydrous sodium acetate and 1.5 percent agar. The broth medium for the daily inoculum was the same as that used for the riboflavin assay and the inoculum suspension was prepared in the same manner.

The samples for the niacin assays were extracted by the same method as that used for the riboflavin assay so that both assays could be run on the same sample 1/. Niacin samples extracted by the enzymatic method were kept in the refrigerator for as long as five days to test the destruction of the vitamin during storage and there was no observable decrease in the niacin content during this time. Consequently, the niacin

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1/ Results on the unknown and recovery samples indicated that it was also possible to extract the niacin by autoclaving with 100 cc. of 1 normal sulfuric acid or with 100 cc.of water for 1/2 hour at 15# pressure.

extracts were often kept 24 hours after the extraction was completed because more samples could be run by assaying the two vitamins on separate days. It was not necessary to wash the samples used for the niacin assays with ether, but otherwise, they received the same treatment as that described for the riboflavin assays. A greater dilution of the samples was usually necessary for the niacin assay. The standard curve covered a slightly wider range - 0.00 to 0.5 micrograms. An attempt was made to run the levels of sample between 0.05 and 0.4mmicrograms per tube.

Thiamine

The samples for the thiamine assay were extracted as follows 1/: 75 cc. of 0.1 normal hydrochloric acid were added to 5 grams of sample and heated for 1/2 hour on the steam bath. This was cooled and 5 ml. of an enzyme solution containing 6 grams of takadiastase in 100 ml. of 2.5 molar sodium acetate were added. The final mixture was adjusted to pH 4.5 - 5.0. A few drops of toluene were added and the samples incubated overnight at 37°C. The samples were filtered and diluted to 100 cc.

The fish meal and raw viscera extracts had to be run through decalso tubes for purification and concentration. This step did not have to be included for the meat samples because the latter contained more thiamine and less fluorescing materials than the fish waste samples. The samples were prepared as described above. An amount of extract containing from 3 to 10 micrograms of thiamine was put through decalso. After the decalso was washed with three 10 cc. portions of hot water, approximately 20 cc. of hot acid potassium chloride solution (8.5 ml. of concentrated hydrochloric acid per liter of 25 percent potassium chloride) were added and the eluate was collected in a 25 cc. volumetric flask. This was made up to volume with acid potassium chloride. Twenty-five cc. of the standard solution containing 0.2 micrograms of thiamine per cc. was also run through the decalso each time and compared with the standard solution which was prepared directly from the stock solution. There was never any difficulty in recovering at least 92 percent of the standard from the decalso step.

The reaction tubes for the thiochrome conversion were test tubes to which standard tapers and stoppers had been fused so that there was no difficulty shaking the reaction mixtures. Five cc. of the standard or sample were added to each of two tubes. One tube was used as a blank; the other for the analysis. The two tubes were run at the same time and received the same treatment except that to one 3 cc. of alkaline potassium ferricyanide solution (3 cc. of 1.0 percent potassium ferricyanide per 100 cc. of 15 percent sodium hydroxide) were introduced and 15 ml. of redistilled isobutyl alcohol added. To the second tube, the blank,

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1/ It was also possible to use the samples prepared by enzyme extraction for the microbiological assays.

3 cc. of 15 percent sodium hydroxide and 15 cc. of the isobutyl alcohol were added. These were shaken vigorously for 1 1/2 minutes. The tubes were centrifuged for 1 minute and the aqueous layer siphoned off. Two to three grams of anhydrous sodium sulphate were added, thoroughly mixed, and the tubes were again centrifuged. The clear solution was decanted into cuvettes to be read immediately in the previously adjusted Coleman photofluorometer. For standardization, the instrument was set to read 70 with a 0.3 mg. per liter quinine sulphate solution. The instrument was checked with the quinine sulphate solution before each sample was read. The following formula was used to determine the thiamine content:

$$\frac{\text{Sample reading - Blank}}{\text{Standard reading - Blank}} \times \frac{1}{5} \times \frac{25}{\text{Vol. decalsoed}} \times \frac{100}{\text{Wt. of Sample}} = \text{Micrograms per Gram}$$

Recovery samples were run on the various types of products by adding a definite amount of thiamine to a sample and running the regular assay. Recovery assays with the raw materials and fish meals were high enough to indicate that there was no significant destruction of the thiamine during the extraction process. From 95 to 105 percent of the thiamine added was usually recovered.

Proximate Analyses

Proximate analyses were run to obtain the moisture, ash, protein and oil content of the raw materials and fish meals. These determinations were made by standard A.O.A.C. procedures.

Results and Discussion

The results of the vitamin and proximate analyses are given in Table I. Since the components of the diets prepared for the feeding tests are added to the diets on a wet basis, the vitamin analyses are also reported on a wet basis.

The stickwater concentrate was the liquor formed during the preparation of the air-dried Alaska pink viscera meal. Since the stickwater concentrate has a high vitamin content, the loss of vitamins during the processing of the meals seem to be due to the solution of the vitamins rather than to their destruction.

There was very little difference between the vitamin content of the fish meals dried at 145° F. and those dried at 100°F. However, the flame-dried meal prepared commercially had a significantly lower vitamin content than these air-dried meals.

The low vitamin content of the acetone extracted meals is due to the removal of the water soluble vitamins during the treatment with acetone. Since the acetone extraction process is expensive, the meal would have to be better than the meals prepared by other methods for the process to be commercially feasible. However, when the meals were compared, both in vitamin content and as to component of the hatchery diets, the acetone-extracted meals were of poorer quality.

The moisture determinations show that the air-dried meals, with one exception, had a lower moisture content than the meals extracted with acetone at room temperature. This is important from the standpoint of preservation, since the amount of moisture is an important factor in determining whether or not mold growth will occur during the storage of the meal.

It has been recommended that a diet fed to hatchery fish have a low fat content. The percentage of oil in the raw offal was 8.09 percent as compared with 4.56 percent in the raw viscera samples. As a result, the meals made from the total offal contained more oil than the meals made from the viscera.

A high protein content is said to be desirable in hatchery diets. The raw viscera samples have 20.0 and 18.05 percent protein as compared to 15.25 percent in the raw total offal. This differentiation again appears in the meals where there is an average of 15 percent more in the air-dried meals prepared from viscera than there is in those prepared from the total offal.

There has been much controversy as to whether hog liver is as effective in a fish hatchery diet as beef liver. Both the proximate and vitamin analyses of the hog liver and beef liver are similar. Beef liver has been considered a better material to feed the fish. From the analyses, it is indicated that, as some hatchery workers have suggested, this may be due to a difference in texture rather than to a superiority in the constituents of the beef liver over the hog liver. Hog spleen, which is said to be of poor quality for the fish diets, had a lower vitamin and protein content than beef and hog liver.

Since beef liver is considered the standard for fish hatchery diets, it is interesting to note the comparison of the analyses of the beef liver and the salmon viscera. The proximate analyses of the two materials are all within the same range, although the water content of the viscera is slightly higher and the protein content slightly lower than that of the beef liver. However, there is not enough difference to feel positive that the relationship would not change in different samples of the two materials. The vitamin content of the beef liver is significantly higher than that of the salmon viscera. Since the daily vitamin requirement for salmon is not known, possibly the vitamin content of the salmon viscera may still be high enough to furnish sufficient thiamine, riboflavin, and niacin for the salmon. The results of the feeding tests will give an indication of the answer to this question.

TABLE I. - ANALYSES OF COMPONENTS OF HATCHERY DIETS

Material Analyzed	Proximate Analyses in Percent				Vitamin Content in Micrograms per gram (Wet Basis)		
RAW CANNERY WASTE (FROZEN)	Water	Protein	Ash	Fat	Thiamine	Riboflavin	Niacin
Columbia River viscera	75.10	20.0	1.85	4.38	0.45	11	31
Alaska pink viscera	76.45	18.05	1.49	4.56	0.6	5	25
Alaska pink total offal	73.75	15.25	2.90	8.09	0.55	3	24
Alaska pink total offal (spoiled)	74.4	16.24	3.44	8.94	0.5	2.5	25
Puget Sound pink viscera	64.0	28.5	2.20	6.72	0.35	4	11
Stickwater concentrate	49.40	42.60	6.07	0.36	5.0	19	143
SLAUGHTER HOUSE WASTE (FROZEN)							
Hog liver	72.05	20.4	1.35	3.33	2.4	20	112
Hog spleen	75.3	16.6	1.28	6.72	1.8	3	39
Beef liver	68.85	21.6	1.43	4.75	2.6	27	107

Material Analyzed	Drying Temp. ° F.	Proximate Analyses in Percent				Vitamin Content in Micrograms per Gram		
FISH MEALS		Water	Protein	Ash	Oil	Thiamine	Riboflavin	Niacin
Columbia River viscera 1/	145					0.8	19	40
Columbia River viscera 1/ (acetone extracted)	70					0.7	14	15
Columbia River viscera	145	8.18	65.25	5.56	21.40	1.0	28	71
Alaska pink viscera	100	9.38	66.42	5.10	23.74	1.0	16	67
Alaska pink viscera	145	3.87	64.4	7.40	24.00	1.5	15	64
Alaska pink viscera (acetone extracted)	70	9.51	71.7	10.16	10.00	0.4	10	13
Alaska pink offal	100	6.93	49.1	10.43	33.06	0.7	8.0	63
Alaska pink offal	145	5.45	51.75	10.93	31.88	0.7	7.0	55
Alaska pink offal (acetone extracted)	70	10.95	69.3	13.67	6.57	0.2	5.0	14
Puget Sound pink viscera	100	7.98	74.4	5.12	12.04	0.5	9.0	23
Spoiled pink offal	100	8.73	47.0	11.41	35.83	0.3	6.0	58
Flame-dried commercial meal		6.94	61.56	15.90	15.99	0.3	4.0	31

1/ prepared in June 1947 and used in hatchery diets July to September, 1947. Results of feeding tests reported in IRDD - Part I.

Summary

Fish meals to be used in hatchery feeding tests were prepared from cannery waste materials. Those used were Columbia River chinook salmon viscera, Alaska pink offal, and partially dried Puget Sound pink viscera. The meals were prepared by cooking to coagulate the protein and then drying in air at 100° F. and 145°F., and by acetone extraction at room temperature. Some of the Alaska pink waste was allowed to spoil and was then cooked and air dried at 100°F. Vitamin B and proximate analyses were run on the raw materials and meals as well as the stick water concentrate and samples of the hog and beef liver and hog spleen being used at the Leavenworth Hatchery. Microbiological assays were used to determine niacin and riboflavin and the fluorometric method in the determination of thiamine.

There was no great difference between the analyses of the meals dried at 100°F. and 145°F., but the vitamin content of the flame-dried meal was lower. Many of the vitamins lost during the processing of the meals were dissolved in the stickwater concentrate. The acetone extraction of the fish waste resulted in a lower vitamin content of the meal than that of the air-dried meals. Hog spleen had a lower protein and vitamin content than either the hog liver or beef liver. Salmon viscera had approximately the same amount of protein, but a lower vitamin content than the beef liver.

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EVALUATION OF SALMON HEAD OIL FOR ADDITION TO CANNED SALMON

By Charles E. Butler 1/

Introduction

For many years salmon oil, prepared from fresh salmon heads and collar bones, has been added to canned salmon. This practice began on

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the Columbia River and has since spread to Puget Sound and Alaska. The process used for the preparation of the oil has consisted of the following steps:

1. Pressure cooking of the raw material.
2. Segregation of liquors from solids by settling and/or screening.
3. Separation of the oil from the liquor by centrifugation.
4. Storage of finished oil in tin containers.

In connection with the utilization of Alaska salmon cannery waste, one possible use for a portion of the offal would be the preparation of an edible or canning grade oil similar to that now being used. Since the visceral portions of the waste lend themselves to alkali digestion in the preparation of a vitamin A oil, the head and collarbone section of the offal was utilized to prepare canning oil by the same method. A series of packs of the five species of salmon were prepared to evaluate and compare any changes in the odor and flavor attributable to the added salmon oil. The effect of salmon oil prepared by the alkali digestion process was also compared with the salmon oil prepared by boiling the heads.

Selection of Raw Material

The salmon heads were collected at the header machine just before the decapitated fish is sent to the "Iron Chink" for the balance of the butchering operation. The severed head usually contains a small part of the liver and possibly the heart. The heads were washed thoroughly by spraying them with high pressure salt water from a hose, after which they were re-washed in potable water. Slime and blood were removed in this washing operation.

Material was collected in lots of approximately 200 pounds for each of the five species of salmon. Each of these lots was divided into two equal parts. One hundred pounds of heads from each lot were processed into oil by the alkali digestion method. The other hundred pounds were processed by boiling the heads at atmospheric pressure.

Equipment Used for Alkali Digestion

The digester used was a wooden barrel approximately 30 inches in diameter and 42 inches in height. A piece of iron pipe, 1/2-inch in diameter, was bent in a semi-circle to conform to the shape of the bottom of the barrel. This pipe was then placed in the bottom of the barrel and connected, by means of a second vertical pipe and a hose, to a source of high-pressure steam. A portable stirrer unit, powered with one-quarter horsepower 1750 RPM direct-drive motor and equipped with two

3-inch boat-type propellers mounted on a 5/8 inch shaft 28 inches in length, was clamped over the side of the barrel.

For the separation of the oil from the liquor upon completion of the digestion, a DeLaval oil purifier, Model No.202, was employed.

Procedure for Alkali Digestion

The digestion procedure adopted as standard for the preparation of the samples of oil from the cannery offal was as follows:

1. The salmon waste to be processed and an equal weight of potable water were placed in the digestion barrel.

2. The stirrer motor was started and the steam turned on.

3. A NaOH solution was added. This was made by mixing one-half gallon of water with sodium hydroxide flakes equivalent to 1.5 percent of the weight of the waste.

4. Heating was continued for 15 to 30 minutes at full steam pressure until contents of the barrel had reached 200° F.

5. Heating at 200°F. with agitation was continued until a sample of the liquor showed few, if any, solids other than bone particles remaining. The digestion time was 70 to 90 minutes depending on the size of the heads and the initial temperature of the offal.

6. When the test sample of the liquor indicated complete digestion, the heating and agitation was discontinued and the liquor was allowed to stand for 10 minutes to facilitate settling out of solids (bony materials for the most part).

7. Meanwhile the centrifuge had been started and thoroughly heated by the passage of 10 gallons of hot water (210°F) through the machine.

8. The contents of the barrel (other than the solids) were passed through the centrifuge.

9. The oil recovered was weighed, the color was noted, and the samples for addition to the pack of canned salmon were hermetically sealed in tin cans.

Procedure for Boiling Method

The same equipment was used as in the alkali digestion method. The procedure differed only in that no sodium hydroxide solution was added.

Preparation of the Canned Salmon Packs

During the course of a day's canning of each of the five species of salmon, a case of 48 cans was selected from the flow of filled cans passing the patching table. The criterion for selection was that all cans chosen were as nearly uniform in appearance and color of the exposed portion of salmon as possible and that adequate headspace and voids in the packed flesh were available to insure retention of the added oil during the can evacuation and seaming operation.

The 48 cans selected were separated at random into three lots of 16 cans each. Lot 1 was kept as the control. To each can of Lot 2, 15 ml. of the salmon oil prepared by the alkali digestion was added per pound of fish. The cans of Lot 3 were similarly fortified using the salmon oil prepared by the boiling method. Lots 2 and 3 were each suitably coded. The case of salmon was then evacuated, sealed and retorted in the standard manner along with the cannery pack.

These five cases of salmon were shipped from Seldovia to the Seattle laboratory of the Fish and Wildlife Service by water transport.

Storage Conditions for Canned Salmon

Upon arrival at Seattle, the canned salmon samples were placed in storage in an unheated warehouse for the duration of the testing period. Twenty-four hours before samples were to be opened for the monthly examinations, they were brought into the laboratory where they could come to room temperature prior to the inspection and taste testing.

Examination of Samples

At approximately 30-day intervals over a nine-month period, the pack of each of the five species of salmon were examined. One can was selected at random from each lot: The control, the lot fortified with salmon oil prepared by the alkali digestion method, and the lot to which salmon oil made by boiling the heads had been added. The vacuum readings were measured and recorded. All cans examined exhibited a vacuum averaging between 5 and 10 inches. Any peculiarities of color, appearance, or odor of the fish, or of the headspace of the opened can were noted. The liquor was poured from each can into beakers coded to correspond with the sample code. The degree of discoloration, if any, and the location of the discoloration on the inside of the can, body and ends was observed upon removal of the fish.

The first examination was made using the flesh portion without the liquors. The solid content of the three cans were placed on three plates

designated by the code letters A, B and C. The panel of testers was told the species of fish and requested to rate each sample on a flavor basis only. The ratings used were as follows:

Excellent	10	Minus	5
Very good	9	Fair	4
Good	8	Poor	3
Plus	7	Very poor	2
Medium	6	Inedible	1

Whenever possible, at least ten tasters were used. Over the period of the storage tests, the specific individuals present each month varied so that it was impossible to have the benefit of the same panel of tasters throughout. The number of samplings at which the several individual tasters were present varied from three to ten. The results from those tasters who had shown the most aptitude for and interest in the evaluation of the organoleptic properties of fishery products were used as the basis for formation of the opinions and conclusions reported here.

After the first examination the procedure for preparation of samples for the tasting panel were changed to accentuate any possible differences and to render the portion tasted by each of the persons as nearly comparable as possible. The contents of each can were broken up with a spoon and then blended together in a Waring blender. The homogenous mass was used as the sample. Considerable sacrifice in the enthusiasm of tasters was the result of using a uniform sample since the blended fish flesh presented a less appealing appearance and the texture was less desirable. The conditions were, therefore, severe in comparison to the normal practice of a housewife, for example, who frequently discards liquors and adds seasoning and other ingredients before the salmon is eaten.

Discussion of Results

Upon the completion of the taste tests at each examination date, the ratings as indicated by the several individual judges were totaled and the average score for each sample was calculated. The flavor ratings of each of the seven persons who participated most frequently on the judging panel were tabulated individually and by species of salmon to determine whether their taste preferences were consistently in favor of any one of the three lots of canned fish. There were seven such panel members evaluating five species of salmon or a total of 35 average scores for comparison.

The control samples were preferred by the judges in 19 out of 35 tests; the samples with alkali process oil added were preferred in 8 instances; the samples to which the boiling process oil was added were

preferred in 5 instances. On two occasions the judges rated the control and the alkali process sample equally superior to that containing the boiling process oil and on one occasion the control and the sample with boiling process oil added were preferred equally over the sample to which the alkali process oil had been added.

One judge gave samples containing the boiling process oil the lowest average flavor rating and a second judge rated this pack lowest for four out of the five species over the entire period of the examinations. This was one of the few consistent trends in the observations of the tasting panel. Other judges thought they could distinguish particular samples on the basis of oil preparation method, but their average flavor ratings did not consistently agree with their preconceived opinions.

In order to check on the number of points required to indicate significant differences in flavor scores for the several individuals, a comparison test was conducted. A single can of red salmon with alkali process oil added was opened and the entire contents were blended thoroughly. When the blended material was as nearly homogeneous in appearance and composition as possible, it was divided into three equal portions. The judging panel members were asked to rate the samples, marked A, B and C, just as in all other instances. They were told only that the samples were red salmon. Of the nine judges, three found no difference, two had a flavor rating spread of one point between the replicate samples, two judges had a two point spread, and two found as much as three points difference.

If the flavor ratings for the eight monthly examinations are evaluated in the light of this diversity of organoleptic sensitivity in the judging panel, it becomes evident that a difference in average preference score of approximately 1.3 points should be found between samples in any given examination to indicate perceptible flavor differences.

In organoleptic tests it is difficult to judge flavor without a conscious or unconscious bias from texture and color when flavor is the variable being evaluated. The texture of the salmon was materially different in the lots to which the oil was added. From comments of the tasting panel, it was evident that these differences in fat content were apparent to them. Each judge was asked whether he preferred lean, medium fat, or fat fish. The stated preference was then used in studying the flavor ratings for the fish samples. Two of the judges who had a decided preference for lean fish chose the control samples as best for 60 percent of the samples tried. On the other hand, the persons signifying a preference for medium fat and fat fish rated the control samples best in approximately 60 percent of the tests. This would seem to minimize any bias in flavor scores attributed solely to differences in fat content.

No effort was made to counteract the color factor since tasters had to know the species of salmon to make an intelligent evaluation of the flavor. Any prejudice for the more highly colored salmon, as red or king salmon, would still influence the flavor score, even though the color variable had been controlled if the taster knew the species of salmon being tested.

The average flavor ratings obtained at the eight examination periods may represent some degree of bias. The factor of color and species bias seems to be ruled out by the fact that the second and seventh examination of king salmon and the seventh examination of red salmon brought out as great spreads in flavor scores as were reported for chum salmon, which is generally classed as the least popular salmon on the basis of color and species.

The flavor scores for the king salmon samples show a more consistent trend in favor of the lot to which alkali process oil was added. Lower scores were given to the samples containing the boiling process oil. There was a general drop in the total average scores from 9.1 at the first to 7.0 at the last examination.

The pink salmon samples were judged equally often in favor of the control lot. There was very little to choose between the ratings of the two lots containing added oil. Again the total average scores per examination declined from an initial 7.9 to 6.8 at the eighth examination.

For Coho salmon, the lot with added oil prepared by the boiling process made the best showing with the taste panel. The pack containing the alkali process oil was the least favored. The same decline in total average scores was noted, with a change from 8.9 to 6.8 in the flavor rating.

The control samples were scored highest in six of the eight examinations of the red salmon packs. The lot containing the boiling process oil was almost as consistently rated least acceptable. The decline in flavor score over the entire period of the tests was considerably less than in the previous three species; initial average score was 7.7 and final score 7.2.

The chum salmon series resulted in top flavor rating for the control samples. There was little to choose between the packs having added oil. The total average flavor score of 7.2 at the first examination declined to 6.3 at the eighth examination.

Summary

Experimental packs of each of the five species of salmon were prepared with added salmon head oil made by two procedures: 1. by alkali

digestion, and 2. by boiling at atmospheric pressure. During a storage period of eight months, samples from these packs have been compared at monthly intervals with samples from a control pack to which no oil was added. A tasting panel was used to determine whether the added salmon oil imparted any undesirable flavors to the canned salmon. Flavor ratings have been expressed in terms of numbers with the best possible rating scored as 10 and the lowest possible rating scored as zero.

The flavor scores for each species of salmon indicate several general trends. The pack of king salmon with added alkali process oil and of coho salmon with added boiling process oil were rated highest in these respective species. The control samples were rated highest in the packs of red, pink and chum salmon. In those instances where there was a degree of difference between second and third choice, the lots containing the alkali process oil were slightly favored over those containing the boiling process oil.

It is very possible that had equipment been available to prepare the oil by pressure cooking as originally planned, instead of by the process of boiling at atmospheric pressure, an oil more comparable to that now used commercially would have resulted. For this reason any inferences drawn from these tests should be rechecked by the storage and examination of salmon packs in which the vacuum cooked salmon head oil is compared with that prepared by the alkali digestion process.

There was a consistent trend in that the combined average flavor scores for the three lots at each examination period declined as the tests proceeded. There was some difference in the rate of flavor score decline with species. King and coho salmon lead with the highest initial scores of 9.1 and 8.9 but declined 2.1 points each to approximately the final average of 6.8 for the five species. Pink salmon ratings dropped only 1.1 points from the initial 7.9. Chum salmon was rated lowest at the first examination and at the last, but changed only 0.9 points. Red salmon samples decreased by only 0.5 points from the first rating of 7.7.

On the basis of the flavor preference of the seven tasters who rated the three lots for the five species of salmon, the control samples were best in 19 of the 35 tests. The samples with alkali process oil added were best in 8 tests, the samples with oil by boiling added were best in 5 instances. The balance of three tests were inconclusive with respect to a definite first choice.

The difficulties in obtaining an unbiased judgment based entirely on flavor differences by the use of a tasting panel are recognized. The apparent preference scores were, therefore, not considered to be significant unless there was a difference of 1.3 points between the ratings

assigned the three lots at an examination. There was considerable correlation between the lot most frequently rated highest by the panel and the fact that a flavor score spread of approximately 1.5 points was reported at that examination. For example, in the king salmon examinations, the alkali process added oil lots were rated best in the two instances where scores as diverse as 1.5 points were involved. For the two examinations in which the range in scores most nearly approached 1.5 points, the best score was in favor of the alkali process added oil sample. Chum salmon showed the best correlation with four instances of 1.5 points range in scores. In each case the control was given the highest rating. The two other examinations in which therange of scores approached 1.5 points likewise showed the control receiving the best score.

Conclusions

The flavor score rating of the tasting panel indicate that there was a slight but significant preference for the salmon samples without added oil. From the opinions of individual tasters at each examination, the reason for the preference was the presence of a slight but persistent after-taste, especially in the lots containing the oil prepared by boiling the salmon heads. Occasional comments of a similar nature were made regarding samples with alkali processed oil added, but confirmation by the flavor ratings was not as definite in the latter case.

The sales appeal of the cans of the salmon, as measured by the opinion of the appearance of the can when opened, was invariably in favor of the added oil packs. This was true even for the supposedly oily species, such as king salmon.

If the added oil was removed from the canned salmon before the taste panel tested the flavor, rating scores for any of the five species were not significantly in favor of any of the three lots.

At the first few examinations the cans of salmon to which the alkali process salmon head oil was added showed more discoloration of the head space portion of the cans than was evident for either the samples with the oil by boiling added or for the control samples. As the tests progressed, there was a gradual increase in the degree of discoloration of the tinplate in the control series and in the series to which the oil made by boiling the heads had been added. By the sixth to eighth examinations there was no consistent difference in the appearance of the tin plate either on the main body of the several cans or in the head space area. The discoloration was in no instance sufficiently pronounced to indicate an abnormal degree of action on the cans by reason of the added oil by either method of preparation.

Further tests, especially those employing a salmon canning oil prepared by pressure cooking the heads, should be carried out. These tests would offer additional evidence on the question of imparting flavor to the canned salmon. On the basis of the organoleptic tests herein reported, there is not sufficient conclusive evidence that the reported preference for the unoiled salmon is due entirely to flavors attributable to the added oil. From the standpoint of accelerated undesirable changes in storage over a period of eight months, there is some slight evidence that the samples containing added oils may be more susceptible. This trend will have to be followed for a longer storage period before definite evidence can be obtained on this point.

PROCESSING SALMON CANNERY WASTE FOR RECOVERY OF VITAMIN A OILS

By Clarence J.Carlson and Harris W.Magnusson 1/

Introduction

During the summer of 1947 an investigation of the alkali digestion of salmon cannery waste on a pilot plant scale was started. This work was conducted in a cannery in Seldovia, Alaska. The results of this research were reported by Butler and Miyauchi (1). The procedure used was a modification of the method recommended by Anderson (2) on the basis of studies of very small lots of salmon head and collar sections. The investigation at Seldovia studied the application of this method to various portions of salmon cannery waste. Waste from each of the five species of salmon were digested experimentally, and numerous studies were made to test the effectiveness of processing only certain parts. Especially considered were the parts in which the oil or the vitamin A were most concentrated, such as head, liver and viscera. Tests were also performed using whole waste less the eggs and milt, which two parts are most likely to cause emulsions.

At the Fishery Products Laboratory in Ketchikan, beginning in the summer of 1947 and continuing through the following winter, a study was made to determine the most effective procedure for recovery of maximum amounts of oil and vitamin A from whole waste. In this study none of the parts were removed. The particle size of the raw material, the concentration of alkali used, and the time and temperature of the digestion were varied. For each variation of the digestion procedure the yields of oil and vitamin A were determined.

Collection of Raw Materials

The raw materials used in this series of experiments consisted of the whole waste of only one species of fish, the pink salmon (Oncorhynchus gorbuscha). All of the waste was collected from the cannery of the Ketchikan Packing Company, which handles only trap-caught salmon.

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PLATE 4. - GRINDING SALMON WASTE IN HERCULES MEAT-BONE CHOPPER FOR PREPARATION OF HATCHERY FEED.

A two-rail slide was built extending from the main floor of the cannery down into the gurry bin situated beneath the floor. The slide was placed so that the whole waste from the "Iron Chinks" would drop into a movable basket. The basket, of about 5-gallon capacity, was 18-1/2 inches long, 16-1/2 inches wide, 9 inches deep, and was constructed of 1/4-inch mesh wire screen. Screen of this mesh was sufficient to permit adequate drainage and yet to retain the solid materials.

In order to determine the representativeness of the material collected, several basket loads of the whole waste were separated into their unit parts, e.g. head, tail, eggs or milt, liver, etc. The ratio of these parts was found to be very nearly 1:1:2:1, etc., for each basket load. This indicated that a fairly representative sample was being collected.

The waste was removed from the basket and poured into 5-gallon liver cans which were frozen and stored at 0° to -20°F. When needed the cans of waste were removed from cold storage and allowed to thaw in the open air at room temperature.

Equipment Used

Three different pieces of equipment were used to vary the particle size of the raw waste: A California Press Grinder, No.B-12067, with 7/8-inch hole plate, powered with a 15 hp. electric motor; a Hercules Meat-Bone Chopper, powered with a 7-1/2 hp.electric motor; and a Rietz Disintegrator, Model RD-8, using a 1/8-inch hole screen and powered with a 5 hp.electric motor.

An upright iron retort 25 inches in diameter and 30 inches in height was used as the digester. Steam under a pressure of 40 pounds was introduced into the digester through two 3/4-inch pipes located on opposite sides of and at the bottom of the digester. The stirring apparatus was built into the digester about 4 inches above the bottom in a horizontal position and consisted of two blades, one 7-1/2 inches in length and the other 5-1/2 inches in length, mounted on a 3/4-inch steel rod. The stirring apparatus was powered by a 1 hp.1750 rpm motor with a driving ratio of 2 to 1, rotating the stirring propellers at about 875 rpm.

A DeLaval Centrifugal Oil Purifier, Model No.302, was used to separate the oil from the liquor upon completion of the digestion.

General Procedure for Alkali Digestion

The general procedure used in all of the experiments except for certain variations which will be described later was as follows:

1) To approximately 100 pounds of salmon cannery waste in the digester an equal weight of cold fresh water was added.

2) The desired percentage of alkali (NaOH) by weight of salmon waste used was dissolved in 1 to 2 gallons of fresh water and added to the mixture in the digester. 1/

3) The stirring apparatus was turned on and steam added so that the desired temperature was reached within 10 to 30 minutes.

4) Heating was continued at the desired temperature or for the prescribed length of time until a sample of the liquor showed little if any solids remaining except bone particles.

5) When the digestion was complete the stirring apparatus and steam were turned off. The mixture was allowed to stand for 15 minutes to permit most of the bones and the remaining few other solids to settle.

6) While the mixture in the digester was settling, the centrifuge was started and preheated by passing about 10 gallons of hot water (200°F. to 210°F.) through the machine.

7) After settling for 15 minutes the bottom half of the liquor was allowed to drain off as waste. The remaining liquors in the digester were passed through the centrifuge.

8) The oil recovered was weighed and the samples for vitamin A analyses hermetically sealed in 1/2-pound flat cans.

Variations in Procedure for Alkali Digestion

Anderson (2) reported several variables he used in his laboratory studies of the alkali digestion of salmon heads and collars. Butler and Miyauchi (1) mentioned a few of the variables but were unable to study them thoroughly. The present studies carried out at the Fishery Products Laboratory, Ketchikan, Alaska, considered the four principal variables in the processing procedure: particle size, amount of alkali, digestion temperatures, and digestion time.

Amount of Alkali

The first variation considered was the percentage of alkali used. In all of the tests of this series the waste was ground through the California Press Grinder and the temperature was kept constant at 200° F. The

1/ The 1 to 2 gallons of water used for dissolving the alkali was part of the equal weight of water used in part 1)

percentage of alkali, (sodium hydroxide) by weight of the salmon waste, was varied from 1 to 5 percent. The digestion was continued until test samples of the liquor indicated complete digestion.

Particle Size

Using 1-1/2 percent sodium hydroxide, by weight of waste, in each test, four variations in the particle size of the raw material were tried.

1) Some tests were run with the whole waste as it comes from the "Iron Chink" of the cannery.

2) In other tests the raw material was passed through a California Press Grinder with 7/8-inch hole plate; this 15 hp.grinder handled material far more rapidly than it could be fed by hand, and therefore it was impossible to determine its capacity.

3) When the whole waste was passed through the Hercules Meat-Bone Chopper the soft parts were well mascerated, the head and other bony parts were sliced thin, but many tail pieces came through whole; this hogger had little difficulty handling the salmon cannery waste.

4) In order to determine the effect of very fine grinding, tests were run on material that had been passed through a Rietz Disintegrator with 1/8-inch hole screen. Before passing it through this disintegrator, it was necessary to hog the material through the meat-bone grinder.

Temperature of Digestion

With each of the variations in particle size of raw material, digestions were run at 190°, 200° and 210°F. In each test the digestion was continued until test samples indicated completion of the process.

Time of Digestion

In 28 of the experiments the proper digestion time was determined by removing test samples of the liquor. In two pairs of the experiments the effect of the digestion time was specifically considered. Using 1-1/2 percent alkali by weight of waste, which had been disintegrated, digestions at 200° F. were continued for 30 minutes and for 60 minutes.

Determination of Oil Content and Oil Yield

In those tests in which the raw material had been disintegrated or passed through the California Press Grinder, it was possible to secure a representative sample for an oil determination. About five pounds were removed from the ground or disintegrated mass and this was reground

and quartered to give a representative sample of a few grams. The latter samples were analyzed by the standard Stansby-Lemon acetone-ether extraction method, using a Bailey-Walker extraction apparatus. At the end of the centrifuging process as much as possible of the oil was flushed over with hot water. The liquor remaining in the bowl of the DeLaval Separator were centrifuged in bottles at 2000 rpm. The oil layer thus obtained was added to the clear oil obtained from the DeLaval Separator. The total weight of oil was taken as the oil yield.

Determination of Vitamin A in Oils

In addition to determining the weight of oil recovered in each test, the vitamin A content of the recovered oil was determined by chemical and spectrophotometric methods.

All oil samples were analyzed by the Carr-Price Blue Color method as detailed in "Methods of Vitamin Assay" (3). This procedure includes the "increment technique" for comparing an unknown with a known standard. This technique tends to correct for the presence of substances in the salmon oil which modify the intensity of the blue color. A Coleman Universal Spectrophotometer, Model No.11, was used to determine the transmittance at 620 mmu. of the oil solution treated with antimony trichloride. A vitamin A concentrate prepared by the Distillation Products,Inc., laboratories was used as the known standard.

Twenty-five of the oil samples were also tested by the usual spectrophotometric method, determining the transmittance at 328 mmu. with a Beckman Spectrophotometer. A conversion factor of 1894 was employed to convert E values to USP units.

In order to give some indication of the validity of the spectrophotometric results at 328 mmu., the E values at 300 mmu. and 350 mmu. were also determined on three samples. The ratios of optical densities at 300 and 328 mmu. and the ratios at 350 and 328 mmu. were calculated:

Sample No.	E 300/328	E 350/328
12	0.79	0.61
18	0.93	0.60
28	0.79	0.63

The vitamin oil industry customarily accepts E value ratios 300/328 of less than 0.72 and E value ratios 350/328 of 0.65 to indicate vitamin A potency. Only one of these two ratios is satisfactory in the case of the salmon oil under investigation. This indicates the presence of moderate amounts of interfering materials. It is believed the Carr-Price blue color results are perhaps more accurate than the spectrophotometric since they are somewhat lower.

TABLE 1. - RECOVERY OF OIL AND VITAMIN A FROM PINK SALMON CANNERY WASTE.

Particle Size of Raw Material	Amount of Alkali(NaOH) Used	Temperature of Digestion	Time to Reach Temperature of Digestion	Total Time of Digestion from Cold Water Start
Grinder	% by weight of waste	Degrees F.	Minutes	Minutes
2	1.0	200	16	90
2	1.5	200	18	60
2	2.0	200	10	50
2	2.5	200	14	20
2	3.0	200	17	25
2	4.0	200	15	18
2	5.0	200	25	25
2	5.0	190	12	12
1	1.5	210	20	60
1	1.5	210	20	65
1	1.5	200	20	60
1	1.5	200	20	60
1	1.5	200	20	75
1	1.5	190	25	70
1	1.5	190	12	75
3	1.5	210	20	50
3	1.5	210	30	50
3	1.5	200	15	50
3	1.5	200	20	60
3	1.5	190	13	65
3	1.5	190	20	65
4	1.5	210	20	50
4	1.5	210	25	50
4	1.5	200	18	60
4	1.5	200	15	50
4	1.5	200	17	50
4	1.5	200	10	30
4	1.5	200	10	30
4	1.5	200	10	60
4	1.5	200	10	60
4	1.5	190	15	60
4	1.5	190	20	60

(1) Not ground.
(2) Ground in California Press Grinder.
(3) Shredded in Hercules Meat-Bone Chopper.
(4) Disintegrated in Rietz Disintegrator.

Oil Content of Raw Material	Oil Yield by Alkali Digestion	Efficiency of Oil Recovery	Vitamin A Content of Oil	
			U.S.P.units per gram	
% by weight	% by weight	Percent	Carr-Price	Ultra Violet
9.08	3.30	36.3	660	---
8.09	5.43	67.1	890	---
8.92	3.89	43.6	830	---
8.50	4.74	55.8	810	---
9.28	5.88	63.4	820	---
8.45	5.15	60.9	950	---
7.86	4.41	56.1	810	1190
8.16	3.06	37.5	820	---
---	5.24	---	1060	1230
---	4.73	---	1060	1150
---	5.36	---	910	1090
---	4.95	---	950	1110
---	5.09	---	1150	1270
---	5.17	---	1370	1530
---	5.23	---	1040	1200
---	5.21	---	1040	1220
---	5.00	---	930	1140
---	5.14	---	1020	1220
---	5.24	---	1090	1240
---	4.95	---	1070	1200
---	5.05	---	1130	1340
---	5.14	---	1040	1240
8.19	4.75	58.0	880	1140
8.46	4.42	52.2	1010	1230
---	5.10	---	840	1140
---	5.05	---	1010	1220
7.41	4.95	66.8	990	1230
7.83	5.10	65.1	970	1180
8.37	5.58	66.7	990	1170
7.73	5.35	69.2	1190	1320
---	5.03	---	1070	1360
---	4.89	---	800	1130

Discussion of Results

The numerical data obtained in the thirty-two trial alkali digestions of whole pink salmon waste are summarized in Table 1. The data exhibit no clear-cut advantage for any one of the digestion procedures tried. Therefore it is necessary to rely considerably on the visual observations which are not easily recorded in a table. Notes were kept on the following pertinent factors: rate of digestion of the fleshy parts, the formation of soap and foam, the occurrence of difficult emulsions, and the appearance of the separated oil and of the discarded mixture.

Effect of Particle Size

The lack of or the degree of grinding of the salmon cannery waste prior to digestion has a noticeable effect on the time required for completing the digestion. However, the data shows no correlation between the particle size of the raw material and the oil or vitamin A recovery. Since variation between duplicate experiments was quite high, any small apparent differences in oil yield or vitamin content are probably of no significance. Coarse grinding of the cannery waste reduces the digestion time sufficiently so as to warrant inclusion of this step in any recommended procedure.

Amount of Alkali

When only 1 percent sodium hydroxide by weight of the waste was used, the total digestion time was excessive; even after 90 minutes a large share of the oil had not yet been released. Using between 1-1/2 and 3 percent alkali the digestions proceeded satisfactorily. In the tests where 4 to 5 percent alkali were employed excessive amounts of soap were formed and emulsions hindered efficient separation of the oil. As 1-1/2 percent alkali gave satisfactory digestions and good oil yield, increasing the quantity of alkali, and thus the operating costs, seems to be without advantage.

Temperature of Digestion

In the range between 190° and 210° F. the temperatures of the digestion seem to have little effect on the oil or vitamin yields. At 210° F. the incoming steam caused violent agitation of the mixture; there was a tendency toward foaming and some of the mixture was lost from the retort. Digestion at 200° F. proceeded nearly as rapidly as at 210° F. and the temperature was easier to control. Because there is no saving in time the lower digestion temperature would use steam more economically. Digestion at 190° F. was equally easy to control, probably just as economical, but required slightly more time for completion.

Time of Digestion

The time necessary for completing the digestion would of course depend on factors such as, particle size, amount of alkali, and temperature of digestion. Shortly after all of the fleshy parts have dissolved the digestion is considered complete, and the oil readily separates. There is no noticeable advantage in further digestion. Comparison of the present set of experiments with those reported by Butler and Miyauchi (2) indicate that increasing the degree of agitation shortens the digestion time and does not necessarily cause the formation of emulsions.

Summary

It is recognized that additional experiments must be performed before the most efficient procedure can be recommended. However, the present data are sufficient to indicate a completely satisfactory basic procedure. Studies to improve this basic procedure could best be made using the exact equipment to be operated commercially.

The present investigation indicates that the whole pink salmon waste should be ground or shredded to break up the head and collar section. The digestion retort should be equipped with efficient agitators. The ground material, together with an equal quantity of water containing 1-1/2 percent sodium hydroxide by weight, should be heated as rapidly as possible to 190°-200°F. and held at that temperature, with agitation, until the fleshy parts are completely digested, and then a few minutes longer, the total digestion period being approximately 50 minutes. The digested mixture must be allowed to stand for approximately 15 minutes to allow bones to settle and oil to rise. The top layer can then be drained off and passed through a centrifuge. The liquor should not be drained through the bottom of the retort because the oil layer tends to absorb on the solids which have settled to the bottom of the vessel.

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