A CHEMICAL EVALUATION OF TUNA-LIVER AND BEEF-LIVER MEALS PREPARED BY DIFFERENT METHODS

By G. Ivor Jones* and William H. Hoyer**

ABSTRACT

MEALS PREPARED FROM BEEF LIVER, ALBACORE TUNA LIVER, AND YELLOWFIN TUNA LIVER BY THE PROCESS OF LYOPHILIZATION RETAINED THE GREATEST AMOUNT OF THE THIAMINE, NIACIN, AND RIBOFLAVIN PRESENT IN THE RAW LIVER.

DEHYDRATION OF THE RAW LIVER BY MEANS OF REPEATED EXTRACTION WITH ACETONE CAUSED CONSIDERABLE LOSS OF THE THREE VITAMINS STUDIED. ACETONE, HOWEVER, REMOVED MOST OF THE FAT WHICH IS EASILY OXIDIZABLE AND, THEREFORE, OBJECTIONABLE WHEN PRESENT IN THE FINISHED MEAL.

VACUUM DRYING OF THE RAW LIVER AT 100° F. PRODUCED DARK-COLORED MEALS WHICH RETAINED APPRECIABLE AMOUNTS OF THIAMINE, NIACIN, AND RIBOFLAVIN. THIAMINE WAS THE MOST EASILY DESTROYED OF THE THREE VITAMINS INVESTIGATED. MEAL COULD NOT BE SUCCESSFULLY PREPARED FROM ALBACORE TUNA LIVER BY THIS PROCESS BECAUSE OF THE PRESENCE OF A LARGE AMOUNT OF FAT.

- - - - -

INTRODUCTION

As part of a continuing project on the utilization of wastes of the fishing and fish-canning industry, a survey of tuna canning plants in the latter part of 1948 revealed a large quantity of yellowfin and albacore tuna livers available in storage with little market value for production of vitamin A oil. Since reduction of the tuna livers to commercial fish meal would yield only the minimum potential value of the raw material, it was decided to investigate the possibility of preparing high-grade meals by several different methods. The meals could then be evaluated as to possible use as supplemental feeding materials for fish hatcheries, animal and poultry nutrition, or for isolation of valuable chemical substances. In the present report, the preparation of the various meals by different methods are discussed in detail, followed by the results of proximate chemical analysis and data on three members of the vitamin B complex, namely: thiamine, niacin, and riboflavin. Biological evaluation of the raw tuna liver in the feeding of hatchery fish will be presented in another report considering the nutritional value of many raw natural foods for the propagation of hatchery-reared red or sockeye salmon. Comparison of the raw tuna livers and meals with that of beef liver was decided upon since the latter is a standard article of commerce whose value in nutrition of hatchery fish has been established over the years by numerous investigations.

EXPERIMENTAL PROCEDURES

The frozen raw beef liver was obtained from the regular supply of "fluky" beef liver used in the production hatchery of the Fish and Wildlife Service at

* CHEMIST, FISHERY TECHNOLOGICAL LABORATORY, BRANCH OF COMMERCIAL FISHERIES, U. S. FISH AND WILDLIFE SERVICE, SEATTLE, WASHINGTON.
** FISHERY TECHNOLOGIST, PACIFIC OCEANIC FISHERY INVESTIGATIONS, U. S. FISH AND WILDLIFE SERVICE, HONOLULU, T. H.

Leavenworth, Washington. The frozen raw tuna livers were furnished through the courtesy of the Columbia River Packers Association of Astoria, Oregon. Neither the size of the fish nor the geographical location of capture are known, as the livers were received in five-gallon tin cans. Upon its receipt in December 1948, the frozen liver material was chopped into smaller chunks while still semi-frozen and then passed through a meat grinder having a plate with 1/8-inch holes. The ground material was thoroughly mixed before drawing samples for analyses of the raw liver and for the preparation of meal. Samples were packaged in one-gallon fibreboard cartons, frozen, and held at 0° F. until used.

Three types of meals were prepared from the raw liver. The methods used included "lyophilization" or vacuum-freeze drying, vacuum drying at 100° F., and dehydration by repeated extraction with acetone. It was believed that the preparation of a meal by vacuum drying of the ground livers while frozen would be least destructive of the essential vitamins and other nutritional elements. Vacuum drying at 100° F. in a commercial-type steam-jacketed dryer would undoubtedly cause some nutritional loss but would be expected to produce a meal superior to those prepared in conventional direct-flame fish-meal dryers. Dehydration by repeated extraction with acetone would have the advantage of preparing a meal without the application of heat, and in addition reduce the fat content to a lower level, which would be especially advantageous with livers having a high fat content. However, it is quite possible that this method would remove a portion of the water-soluble B complex vitamins which would, of course, be a distinct disadvantage.

PREPARATION OF LYOPHILIZED MEAL: In preparing the lyophilized meal, the frozen ground liver samples were thawed at room temperature and then further blended and mixed with one-half their volume of distilled water in a Waring Blendor. Approximately 600 ml. of this mixture was placed in a 3-liter round-bottom distilling flask equipped with a standard-taper glass connector. The material was frozen in a thin layer on the inside surface of the flask by alternately rotating it and then immersing it in a mixture of dry ice and alcohol. After the samples were frozen, the flasks were connected to a vacuum system containing a condenser immersed in a bath consisting of dry ice and alcohol. The contents of the flask were dry in approximately 12 hours. The meals prepared by this method were vacuum-packed in 1/2-pound flat, hermetically-sealed cans and held at 0° F. for subsequent analysis.

PREPARATION OF 100° F. VACUUM-DRIED MEAL: Preparation of a liver meal vacuum dried at 100° F. was accomplished by charging 40 to 50 pounds of the thawed-ground liver sample into a small Stokes horizontal, rotary-paddle, steam-jacketed, vacuum dryer. The charge was maintained at a temperature of 100° F. by admitting steam at a pressure of 5 to 10 pounds into the external jacket of the dryer and by maintaining a vacuum of 27 inches in the drying chamber. Some difficulty was encountered with all three types of livers. Beef liver and yellowfin tuna liver had a tendency for forming into round balls. Midway during the drying process it was found expedient to break up these balls of semidried liver in order to hasten drying. The difficulties might have been overcome by changing the technique but, since our interest was in the meals and not primarily in the processing methods, no attempt was made to improve the procedure.

Albacore livers did not yield a satisfactory product by this method. The fat content of these livers is relatively high (about 18 percent fat in raw liver) and, under the conditions existing in the dryer, produced a sticky mixture with the appearance of grease. Since it did not appear that a satisfactory albacore

meal could be readily prepared by this method, this part of the experiment was abandoned.

After drying the beef and yellowfin tuna liver in the Stokes vacuum dryer, the residue was finely ground and packed into 1/2-pound flat cans, vacuum-sealed, and held at 0° F. storage until analyzed.

PREPARATION OF ACETONE DEHYDRATED MEAL: Dehydration of the thawed, ground, raw liver material with acetone was carried out by covering 3 kg. of the liver with 12 liters of C. P. acetone in a large glass jar fitted with a plywood cover. The mixture was stirred intermittently during the extraction process, which was allowed to proceed for 8 hours. At this time, the mixture was allowed to settle and the supernatant liquid was siphoned off without disturbing the solid residue. The residue was then covered with a second portion of acetone. A total of 3 extractions were made. The third acetone extraction appeared to remove only a trace of color from the liver solids and it was surmised that the moisture content of the residue had by then been reduced to less than 10 percent, which later proved to be correct upon analysis. The last traces of acetone were removed from the solids by air drying before a fan in a well-ventilated room. When dry, the powdered meal was vacuum-packed in tin containers and held at 0° F. during storage.

ANALYTICAL DETERMINATIONS: Determination of moisture, total nitrogen, fat, and ash on both the raw livers and the prepared meals were made using modifications of the A.O.A.C., VI (1945) methods of analysis. These modifications have been developed at the Seattle laboratory after many years of study on analytical methods applicable to fish products and by-products. The modified procedures are as follows:

Moisture: Weigh 5 to 10 g. of sample into tared aluminum dishes provided with covers. For wet material, mix sample with about 20 g. of purified sand. Place dishes in an air oven at 115° C. for 3½ hrs., or in a vacuum oven (1 mm. mercury or less) at 80° C. for 5 hrs. Cool samples in a desiccator for 1 hr. and weigh. Return samples to oven for 30 min., cool, and weigh to determine if weight is constant.

Total Nitrogen: Weigh 0.5 to 2.5 g. of sample (percent of moisture governs amount) into clean, glass cells. Transfer cells to Kjeldahl flasks. Add 6 to 8 glass beads, 10 g. of anhydrous Na_2SO_4 and a granule of selenium catalyst. Finally, add 25 ml. of concentrated H_2SO_4 and digest the mixture until it becomes clear, plus an additional 30 to 60 min. to assure complete digestion. Cool the digested mixture and add 180 to 200 ml. of distilled water. Place 25 ml. of 0.5 N HCl plus 100 ml. distilled water, plus 4 drops of indicator composed of 0.2-percent methyl-red and 0.1-percent methylene-blue in the receiving flask. Add a few drops of phenolphthalein indicator to the digest. Pour carefully into the tilted flask about 100 ml. of 45-percent NaOH solution. Immediately connect flask to the distillation assembly. Distill 150 ml. volume or until "bumping" begins. Add an additional 4 drops of the indicator described above to the receiving flask to enhance the end point during titration. Total nitrogen multiplied by factor 6.25 was used to calculate the value for protein.

Fat: Weigh 4 to 6 g. of sample into tared alundum thimbles, cover sample with a thin layer of cotton and extract for 16 hrs. with 35 ml. of ethyl ether in a Bailey-Walker extractor.

Wet materials, such as raw liver, are weighed on several grams of dry pumice powder placed in a tared thimble. After weighing, the sample and pumice are mixed. The pumice prevents seepage of water through the pores of the thimble.

The surplus solvent in the extract is distilled off to a low volume, and the last traces are removed on a hot plate at low heat. Place flasks in a vacuum oven at 80° C. with a vacuum of 24 to 25 in. for 1 hr. Cool in a desiccator for 45 min. and weigh.

Ash: Weigh 3 to 4 g. of sample into tared crucibles. Dry samples in an air oven at 80° C. for 2 hrs. Carbonize samples carefully over an open flame, then place in an electrically-heated muffle at a temperature of 550° C. for 4 hrs. Cool in a desiccator and weigh.

Thiamine: Determination of this vitamin in the raw and processed samples was carried out using the thiochrome method described in Methods of Vitamin Assay of The Association of Vitamin Chemists (1947).

Niacin and Riboflavin: These two vitamins were determined using the microbiological procedures of Roberts and Snell (1946).

ANALYTICAL RESULTS: The results of the proximate analyses of livers and liver meals of beef, albacore tuna, and yellowfin tuna are presented in Table 1. The values are averages of 3 or more replicates in all cases except where an individual replicate value was at large variance from the others, in which event it was discarded. No special difficulty was experienced in making any of the determinations. It was noted, however, that in titrating the ammonia distillate in the determination of total nitrogen, a sharper end point was obtained when an indicator mixture composed of equal parts of 0.2-percent methyl-red and 0.1-percent methylene-blue in ethanol was used in place of the straight methyl-red indicator. The color change of this mixture began as a red-violet at pH 5.2 with a change to a grey-blue at pH 5.4 and finally to a green at pH 5.6, which was taken as the correct end point.

Table 1 - Proximate Analyses[1/] of Livers and Liver Meals of Beef, Albacore Tuna, and Yellowfin Tuna

Type of Liver	Condition	Moisture	Solids	Protein	Fat	Ash
		%	%	%	%	%
Beef	Raw	68.4	31.6	18.9	9.3	1.3
Beef	"Lyophilized"	4.8	95.2	61.2	18.8	4.2
Beef	Vacuum-dried at 100° F.	3.5	96.5	61.6	20.5	4.2
Beef	Acetone dehydrated	6.7	93.3	75.4	4.6	3.7
Albacore tuna	Raw	60.3	39.7	16.6	18.5	1.1
Albacore tuna	"Lyophilized"	3.9	96.1	39.4	50.8	2.5
Albacore tuna	Acetone dehydrated	9.2	90.8	73.0	4.8	4.4
Yellowfin tuna	Raw	69.8	30.2	23.2	3.1	1.5
Yellowfin tuna	"Lyophilized"	4.6	95.4	72.0	11.6	4.6
Yellowfin tuna	Vacuum-dried at 100° F.	2.9	97.1	72.3	10.7	4.9
Yellowfin tuna	Acetone dehydrated	9.0	91.0	79.8	2.1	5.0

1/ Values reported represent an average of 3 to 6 replicates.

The results of the vitamin analyses are presented in Table 2. The thiamine values represent an average of duplicate samples. Several confirmatory assays

were made in order to check some questionable values. Recovery experiments indicated that 95 percent recovery could be expected. A standard thiamine sample was carried through all the steps of the determination each time that samples were assayed.

DISCUSSION OF RESULTS: Meals prepared by drying in a vacuum at 100° F. had the lowest moisture content (Table 1). Drying by lyophilization produced a meal nearly as low in moisture content. Undoubtedly, the moisture content of the lyophilized meals could have been reduced still further by allowing the process to continue for a longer period of time. The acetone-dehydrated and partially-defatted meals had a higher moisture level than those prepared by the other two methods, but was still under 10 percent moisture. All of these meals had apparently good storage qualities, except that the fat present, especially in the lyophilized albacore tuna-liver meal, oxidized rapidly resulting in a rancid-smelling product. This meal darkened rapidly when exposed to air.

The meals appeared to have no tendency to pick up moisture on exposure to air at room temperature nor was there any tendency of the material to mold or to spoil.

The high fat content of lyophilized albacore tuna-liver meal is objectionable in that it would reduce the storage life of the finished product and may be undesirable in the diet of young hatchery fish. However, the recovery of this fat, either from the raw liver during meal preparation or by extraction from the dried meal, would seem to pose no insurmountable problems, and the oil so obtained might even be of considerable economic value.

In order to compare the effect of the various drying methods on the vitamin content of the finished meals, these values were calculated on the moisture-free basis and are presented in Table 2. It will be noted that dehydration by means of lyophilization caused no significant loss of thiamine in the beef-liver meal. The thiamine content of the lyophilized albacore and yellowfin tuna-liver meals is approximately twice that present in the raw material when compared on the moisture-free basis. These seemingly irreconcilable data can best be explained by the fact that the thiamine and other vitamin assays were not performed on either the raw material or the several meals until all of the meals had been prepared. The lyophilized meals were prepared first, early in the 6-month period. Thus, the raw-ground liver had been held in frozen storage at 0° F. for about 6 months before the vitamin determinations were made. The reason for the values of thiamine in the raw material being lower than in the lyophilized meal can best be explained by destruction of the vitamin due to liberation or excitation of enzyme systems in the raw liver caused by grinding prior to storage. The data in Table 2 indicate the lyophilized yellowfin tuna-liver meal to be the best source of the 3 vitamins tested. The liver meals prepared by vacuum drying at 100° F. appear to retain niacin and riboflavin without any appreciable destruction, but they do sustain a considerable loss of thiamine amounting to approximately 50 percent loss in the beef-liver meal and about 66 percent in the yellowfin tuna-liver meal.

Dehydration of the raw liver by means of acetone causes nearly total loss of thiamine in the tuna-liver meals. Acetone extraction also removed one-half to two-thirds of the niacin and riboflavin content. This method of meal preparation produces a light-colored meal, low in fat, but undoubtedly removes, to some degree, members of the vitamin B complex present which, of course, disturbs the nutritional balance of the final product.

Table 2 - Thiamine, Niacin, and Riboflavin Content of Livers and Liver Meals of Beef, Albacore Tuna, and Yellowfin Tuna

Type of Liver	Condition	Moist Basis			Moisture-free Basis		
		Thiamine	Niacin	Riboflavin	Thiamine	Niacin	Riboflavin
		..... (Micrograms per gram)					
Beef	Raw	2.8	99	20	8.9	313	63
Beef	"Lyophilized"	8.6	365	87	9.0	383	91
Beef	Vacuum-dried at 100°F.	4.6	423	94	4.8	438	97
Beef	Acetone dehydrated	3.5	77	65	3.8	83	70
Albacore tuna	Raw	0.3	48	16	0.8	121	40
Albacore tuna	"Lyophilized"	1.4	110	25	1.5	115	26
Albacore tuna	Acetone dehydrated	0.2	39	32	0.2	43	35
Yellowfin tuna	Raw	0.9	83	19	3.0	275	63
Yellowfin tuna	"Lyophilized"	5.7	262	78	6.0	275	82
Yellowfin tuna	Vacuum-dried at 100°F.	2.0	269	67	2.1	277	69
Yellowfin tuna	Acetone dehydrated	0.2	28	43	0.2	31	47

SUMMARY

Meals of good appearance and apparently good keeping qualities can be prepared by lyophilization, vacuum drying at 100° F., and by dehydration by repeated extraction with acetone. Lyophilization caused the least destruction of nutritive components. Dehydration by means of acetone resulted in very appreciable loss of thiamine, niacin, and riboflavin in the dried meal, but this method possessed the advantage of removing most of the fat which in tuna livers is highly susceptible to oxidation. Great difficulty was encountered in attempting to prepare a meal from albacore livers by vacuum drying at 100° F. due to the large amount of fat present. Although the tuna-liver meals do not contain as much thiamine, niacin, or riboflavin as does beef-liver meal, it appears possible that by furnishing a higher proportion of them in the diet than is customary with beef liver, they might supplant the latter in the feeding of hatchery fish. It also seems possible that a good-quality tuna-liver meal would possess nutritive factors valuable as a vitamin supplement in animal and poultry feeds.

CONCLUSIONS

(1) Meals prepared from beef liver, albacore tuna liver, and yellowfin tuna liver by the process of lyophilization retain the greatest amount of the thiamine, niacin, and riboflavin present in the raw liver.

(2) Dehydration of the raw liver by means of repeated extraction with acetone causes considerable loss of the three vitamins studied. Acetone, however, removes most of the fat which was easily oxidizable and, therefore, objectionable when present in the finished meal.

(3) Vacuum drying of the raw liver at 100° F. produces dark-colored meals which retain appreciable amounts of thiamine, niacin, and riboflavin. Thiamine is the most easily destroyed of the three vitamins investigated. Meal could not be successfully prepared from albacore tuna liver by this process because of the presence of a large amount of fat.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of Miss Neva Karrick and Mrs. Mabel Edwards of the Seattle Technological Laboratory in carrying out the determinations of niacin and riboflavin.

LITERATURE CITED

ROBERTS, ELIZABETH CUNNINGHAM AND SNELL, ESMOND E.
1946. AN IMPROVED MEDIUM FOR MICROBIOLOGICAL ASSAYS WITH LACTOBACILLUS CASEI. JOUR. BIOL. CHEM. VOL. 163, NO. 2, PP. 499-509.

THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS
1945. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. WASHINGTON, D. C., SIXTH EDITION.

THE ASSOCIATION OF VITAMIN CHEMISTS, INC.
1947. METHODS OF VITAMIN ASSAY. INTERSCIENCE PUBLISHERS, INC., NEW YORK, N. Y.

UTILIZATION OF FISHERY BYPRODUCTS IN WASHINGTON AND OREGON

Very little fish scrap is being discarded in the states of Washington and Oregon. The small amount not utilized is either in an area where the supply is inadequate to support a commercial operation, or else the material is of such a nature that it does not command a market. Companies have failed because the supply of waste has been insufficient. Others have lost money on the production of materials not in demand. Anyone who intends to enter the field of byproducts should, therefore, make a thorough survey of the source of supply and the market for the finished product.

The byproducts industry is not static. Changes are taking place, and the field is becoming increasingly competitive. Fish waste, in earlier years, was thrown away. Later, it was utilized only by reduction plants. Now it is in demand for reduction purposes and for mink feed and other uses. With few exceptions, the operations have not produced appreciable revenue, and many firms have operated largely on a marginal basis. For this reason, there is a continuing and increasing pressure to find more remunerative uses for the waste. The problems to be solved are not easy; but with rapid acceleration in technological knowledge and the demands of a growing population, further changes are inevitable.

By F. Bruce Sanford

--Fishery Leaflet 370 (March 1950)