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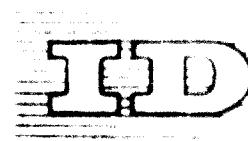
ANALYSIS, TESTING AND USES OF FISH PROTEIN CONCENTRATE 1/

by

V.D. Sidwell, B.R. Stillings, and G.M. Knobl, Jr.
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SUMMARY

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ANALYSIS, TESTING AND USES OF FISH PROTEIN CONCENTRATE

During the past decade a considerable amount of research has been conducted on the processing and utilization of FPC (fish protein concentrate). In 1961 the U.S. Bureau of Commercial Fisheries initiated a program to investigate chemical, biological and physical processes for the manufacture of FPC. Major emphasis was given to a chemical process, which involved the extraction of water and lipids from whole fish with isopropyl alcohol. Multiple stage co-current or countercurrent extraction processes were developed, and they have been used to prepare FPC from red hake and other species of fish. The FPC has been extensively evaluated to determine its chemical composition, nutritional quality and use in foods.

The proximate composition of FPC made from various species of fish—alewife, northern anchovy, Atlantic herring, Atlantic menhaden, Gulf menhaden, red hake, ocean pout and Moroccan sardine—was as follows, expressed as percent: protein, 71.4 to 77.5; lipids, 0.01 to 0.54; ash, 10.8 to 21.6; and volatiles, 1.5 to 6.1.

The nutritive quality of the FPC made from the various species of fish was equal to or better than casein. The essential amino acid pattern of the protein in FPC compared favorably to that of egg protein. For FPC made from red hake, the first limiting amino acid was methionine. Histidine, tryptophan and threonine were nearly equally second limiting.

Extensive work has been conducted on the use of FPC in food products, including bread, pasta, tortillas, cookies and crackers.

Bread. In rheology studies on doughs made from mixtures of high-protein wheat flour and varying amounts of hake-FPC (0, 5, 10, 15, 20, and 25 percent), we found that more water was needed with each increment of FPC, except with 25 percent FPC, to bring the dough to the same degree of development. FPC increased the stability of the dough, but it decreased the extensibility. The best results were obtained when 5 or 10 percent FPC was used. In general, the texture of the bread, made with 5 percent FPC improved the texture of the crumb. "Gigliotti and Tipton found that 5 or 10 percent FPC had an effect similar to that with 10 FPC. Breads with higher levels of FPC were less acceptable.

FPC made from six other species of fish was incorporated into the basic bread formulation at the expense of 10 percent of the flour. Each FPC decreased the volume of the bread as follows: 3 percent for Atlantic menhaden, 7 percent for anchovy, 11 percent for ocean pout, 12 percent for herring, 15 percent for whitefish, and 23 percent for drift menhaden. The panelists found no significant difference in flavor or texture between the six experimental breads and the control made with hake-FPC. The color of the bread made with the various FPC's differed markedly.

Bread made with varying amounts of hake-FPC was evaluated nutritionally. The nutritive quality of the bread enriched with FPC increased from a PER value of 1.01 for bread with no FPC, to a PER value of 2.62 for bread with 10 percent FPC.

Pasta. Mixtures of semolina and varying amounts of FPC (0, 3, 6, 9, 12 percent) were used to prepare pasta. The product became darker with each increment of FPC. Upon cooking, the pasta lightened markedly. Only small amounts of protein leached into the cooking water. Panelists liked the texture, odor, and flavor of the pasta made with from 3 to 9 percent FPC equally as well as that made without FPC.

Crackers. FPC was used at levels of 4, 8, 12 and 16 percent in saltine crackers. The flavor of crackers with 4, 8 and 12 percent FPC was as acceptable as that with no FPC. Crackers with 16 percent FPC were less acceptable. At the 8 percent level of supplementation, the nutritive quality of the protein in the cracker was approximately four times better than the plain non-enriched cracker.

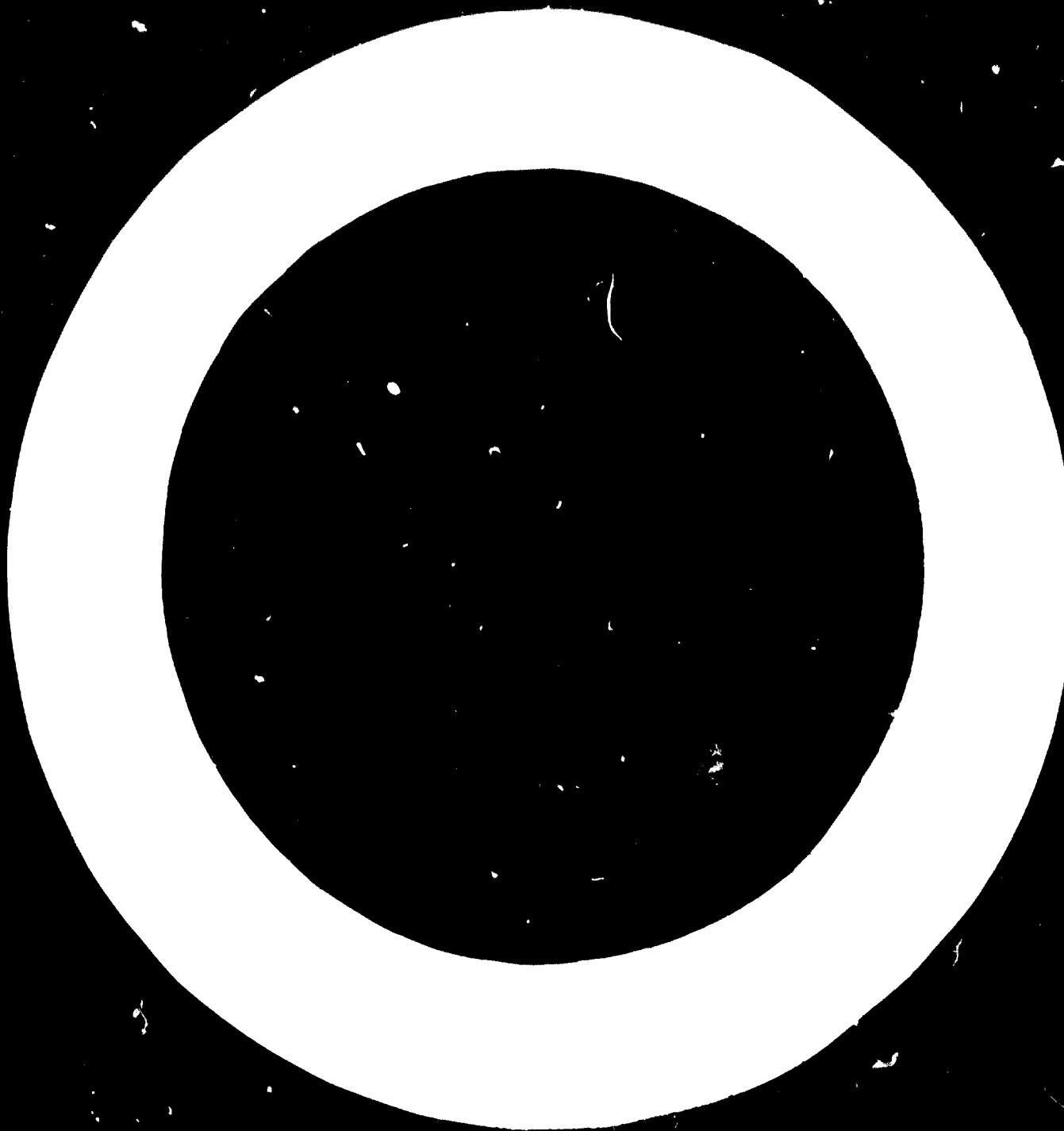
Cookies. FPC was added to a plain cookie to evaluate its effect on flavor, texture and appearance. The FPC affected the appearance primarily; for example, anchovy-FPC darkened the cookie. There was a variation in flavor with the different FPCs; but in most cases, when up to 10 percent FPC was used, the flavor was not adversely affected. When the flavor was affected by the FPC, it could be masked by adding other common flavors to the cookies.

The nutritive quality of a butter cookie was improved nearly three times by using a mixture of 10 percent baked-FPC and 90 percent soft wheat flour in place of the wheat flour alone.

Beverage. With the use of emulsifiers and stabilizers, a beverage was developed that contained FPC as the sole source of protein. The percentage composition of the beverage was as follows: Protein, 3.5; fat, 3.0; and carbohydrates, 10.5.

Soup. FPC has been used at levels of 10 percent of the total solids in soups. The addition of 10 percent FPC to soup reduced the intensity of the flavor, but the enriched soup was as acceptable as that with no FPC.

These studies have demonstrated that FPC can be used in a variety of food products at levels which significantly increase their protein content and value. In some cases, slight changes occur in the physical and organoleptic characteristics, but these do not adversely affect the acceptance of the fortified products.



Introduction

In 1951, and 1952, the Canadian Research Institute made a broadly based survey of the properties of the most abundant naturally occurring organic acids found in the soil. The results of the research will be published in a series of papers in the Journal of the Canadian Soil Science Society. The present paper is concerned with the properties of citric acid, which is one of the most abundant organic acids found in the soil. It is also a major product of the microbial metabolism of organic materials.

While useful in the financial aspect of this research program, however, it became apparent that the United States Soil Conservation Service (USCS) would not without justified apprehension be compelled, by virtue of the financial support given to the project, to accept the findings of the research. Consequently, the results of the research were limited to 60 concentrations of citric acid, leaving, for each different data to provide the soil scientist with a minimum of information. This is reflected in the 10 additional tables and figures which were to be selected and developed, and on the condition was to be exhaustively examined for completeness, accuracy, and sufficiency.

On the basis of knowledge... obtained to date, particularly that of the Canadian researchers, we decided to adopt a chemical approach (solvent extraction) using isopropyl alcohol as the solvent. Isopropyl

alcohol was chosen because it was known to be highly effective in the removal of water and fat from raw fish, and since it was prepared by a continuous process, the variability could be carefully controlled. In addition, isopropyl alcohol was known to be reasonably priced, to be an effective bacteriostat, and to be safe for use in food processing.

The fish we chose for the manufacture of FPC was red hake (Urophycis chrysosoma). Red hake was already being eaten in small quantities, and was an underutilized schooling species of fish, and is also a lean fish (as it contains a relatively low amount of fat). Thus it appeared to us that we could quickly develop a satisfactory method by working with this species of fish.

Our accelerated FPC program eventually resulted in the development of one process which can be defined as a three-stage countercurrent batch extraction (1). In short, the work at the Bureau of Commercial Fisheries showed that FPC can be produced from whole fish at a relatively low cost by a solvent extraction method. It also showed that the FPC produced was highly nutritious, safe and wholesome and that it therefore should be entirely satisfactory as a dietary supplement for human consumption. A petition incorporating these findings was submitted to the FDA and on February 2, 1967, the FDA of the United States approved the process using fish of the hake family for FPC manufacture.

Recently, activities at the National Center for FPC have been directed to extend the FDA approval of FPC from hake and hake-like fish to other fish which are available in large quantities and are edible but which are not widely used for human consumption. We have made FPC from the

following species of fish: alewife (Alosa pseudoharengus), Atlantic mackerel (Scomber scombrus), Atlantic herring (Clupea harengus imberbis), Northern anchovy (Engraulis mordax), and ocean pout (Myrophis macrurus). The uses of hake and three additional species have been evaluated and will shortly be presented to the FDI with the expectation that they will permit FDC to be made from these and related species of fish in addition to hake and mako-like species.

The purpose of this paper therefore is to review and summarize the chemical and nutritive evaluation of FPCs prepared from these species of fish and to present information showing how these FPCs can be used in a variety of foods including bread, pasta, crackers, cookies, beverages and soups. Data is presented indicating the effects of the different FPCs on the sensory characteristics and nutritive value of many of the food products.

CHEMICAL COMPOSITION

The chemical composition of FPC has been studied extensively by our analytical group. The work has not been limited to the analysis of the usual macrocomponents, but it also includes analysis of the microcomponents, including flavor compounds.

Table 1 shows the proximate composition of ten representative samples of FPC, which were processed from ten different batches of red hake. The crude protein averaged 30.66 percent, volatiles 7.71 percent, ash 23.50 percent and lipids 0.16 percent. In general, the composition of the samples was quite uniform. In Table 2 you will notice that the

proximate composition of six batches of Atlantic salmon-IPC was also uniform. The percent volatilized was about one-half of that present in raw salmon-IPC. The average residual protein content of this more bony fish was lower, and the ash and moisture content 5 percent higher. The percent volatilized was less in the salmon-IPC in this case because slightly different deproteinizing conditions were used.

Table 3 shows the proximate composition of IPCs made from seven other species of fish. As would be expected, there was a greater variation in composition of fish meal than different species of fish than of that made from different batches of the same species. Ocean pout-IPC had the highest percentage of crude protein. The amount of residual lipids in all samples was less than 0.50 percent. Moroccan sardine-IPC was comparable to hake in composition.

The amino acid composition has often been used as an indicator of the nutritive quality of IPC. Table 4 shows the amino acid composition of the IPCs made from various species of fish, along with that for whole egg. The protein in whole egg is considered as being a natural protein of high nutritive quality. The different IPCs compared favorably with whole egg. The most obvious differences were the lower values for tryptophane and cystine.

NUTRITIVE QUALITY OF IPC

The data from chemical analyses of the various IPCs are only indications of nutritive quality. Animal feeding studies are needed to evaluate the utilization of the protein in the IPC, both as a sole source and as a supplementary protein.

Nutritive Value of FPC as a Sole Source of Protein

In our studies, we incorporated FPC into diets at a 10 percent protein level, and the diets were fed to normal weanling rats for 28 days. A diet containing 10 percent protein from casein was used as a control. Weight gain and feed intake were recorded and the PER (protein efficiency ratio) was calculated. Table 5 summarizes the PER values obtained from a number of studies in which hake-FPC and Atlantic menhaden-FPC were evaluated. In general, the PER values were equal to or better than casein. The average value for the 22 samples of hake-FPC was significantly better than casein ($P = 0.01$). Atlantic menhaden-FPC was comparable to casein.

In another study we compared the nutritive value of single samples of FPC made from seven different species of fish. Table 6 shows that FPC made from the various species of fish was also equal to or better than casein. Northern anchovy-FPC was the best. FPCs made from Atlantic menhaden, ocean trout, and Moroccan sardines were comparable to casein. From the results of our many investigations we may conclude that FPC prepared by the isopropyl alcohol method will probably have a nutritive value equal to or better than casein.

Stillings *et al.* (2) conducted a series of experiments to determine the sequence of limitation of the essential amino acids in FPC produced by isopropyl extraction of whole red hake. Diets were prepared containing 1.28 percent nitrogen from FPC and 0.32 percent nitrogen from various combinations of amino acids. The diets were fed to weanling rats for four weeks; weight gain, feed intake and protein efficiency ratio (PER)

were determined. From the results he obtained with two different samples of hake-PPC prepared by the same process, the amino acids were grouped according to their limitation from greatest to least, as follows: (1) methionine; (2) histidine, tryptophane and ureidone; (3) valine, isoleucine and phenylalanine; (4) leucine, lysine and arginine.

Nutritive Value of Fish meal as a Protein Supplement

Fish protein concentrate is intended for use only as a **protein supplement**, and not as a sole source of protein. This cannot be emphasized strongly enough. In this respect, numerous nutritional studies have been conducted on the effect of supplementing various vegetable protein sources with fish protein concentrate. Substantial increases in nutritive value have been obtained in all cases. Only the results of one study will be presented to illustrate the point. In this study (3) wheat flour was replaced with 5 to 25 percent PPC. The mixtures were then incorporated into diets at a 10 percent protein level and fed to weanling rats for four weeks. Table 7 shows the results of this feeding trial. Supplementing wheat flour with 1% percent hake-PPC markedly increased the weight gain, food intake and PNI value. The higher levels of supplementation had little additional effect on any of the variables investigated.

USES OF FPC IN FOOD PRODUCTS

Bread

There is very little published information on the changes that take place in the rheology of doughs and in the characteristics of bread made from flour that contains varying amounts of FPC. We, therefore, conducted a study to examine the changes. Studies were conducted using FPC made from hake and from other species of fish.

Bread Supplemented With Hake-FPC.--For this study (4), mixtures of high-protein wheat flour and hake-FPC were prepared that contained 0, 5, 10, 15, 20 and 25 percent FPC. Table 6 shows that more water was required to bring the dough to the same degree of development with each increment of FPC, *i.e.*, from 59 percent for 0 percent FPC to 70.2 percent for 20 percent FPC. Less water--66 percent--was needed for the 25 percent FPC mixture. The replacement of 5 percent of the flour with FPC increased the stability of the dough markedly. From 5 to 20 percent FPC, it remained about constant. It increased further, however, when 25 percent FPC was used in the mixture.

Tolerance index and 20-minute-drop were indices that marked the rate of the dough breakdown. Tolerance index was measured 5 minutes after the Farinogram curve reached its peak. The 20-minute-drop is measured 20 minutes after the water was first added to the flour mixtures. Both of these indices showed that the addition of FPC improved the stability of dough.

Table 9 shows the values obtained for the consistency of the doughs containing FPC. The extensibility of the doughswas less at the end of

the 180-minute relaxation period for the 0 and 5 percent FPC doughs than at the end of the 45-minute relaxation period. It increased for the 10 and 15 percent FPC doughs, but remained constant for the 20 and 25 percent FPC doughs.

The addition of FPC increased the resistance against deformation, i.e., the stiffness and shortness of the dough as measured by the height of the curve. At the end of the 45-minute rest period, the resistance increased with the addition of 5 percent FPC to the dough, then remained about constant for the 10 to 20 percent FPC doughs. With 25 percent FPC, the resistance to deformation increased further. At the end of the 180-minute rest period, the maximum resistance increased with each increment of FPC except for the dough with 20 percent FPC.

The energy needed to bring about a break in the dough along a predetermined path is proportional to the area under the curve. At the end of the 45-minute rest period, the amount of energy needed to rupture the dough was about the same for the 0 and 5 percent FPC doughs. The energy requirement decreased when 10 and 15 percent FPC were used in the dough. The amount of energy needed for the 20 percent FPC dough increased slightly.

After 180 minutes of relaxation, more energy was needed to rupture the doughs with 5 to 15 percent FPC than was needed to rupture the doughs with no FPC. The energy needed for the doughs with 20 and 25 percent FPC was a little less than that for the dough with no FPC.

The loaf volume of the bread containing varying amounts of FPC decreased markedly with each increment of FPC—from 12 percent at the 5 percent FPC level to 36 percent at the 25 percent FPC level. The

loss of volume in our studies was somewhat greater than the loss reported by the South African investigators (5) who used a 90 percent extracted flour and calcium carbonate in a formulation similar to that which we used. They obtained a decrease in loaf size of 3-5 percent at the 5 percent level of enrichment and 10 percent at the 10 percent FPC level. The variability in the percent loss was not due entirely to the addition of the FPC but to the baking quality of the original wheat flour that they used in the tests.

Table 10 shows the results of the sensory evaluation. With each increment of FPC in the formulation, the bread crust became a darker brown. The judges liked the appearance of the bread crust containing 5 percent or 10 percent FPC nearly as well as they liked the standard with no FPC. The appearance of the bread containing 15, 20 or 25 percent FPC was less acceptable to the judges.

In this investigation, texture referred primarily to mouth-feel and chewiness, since the judges were blindfolded. We did not want the color of the bread to influence the judges' evaluation of texture and flavor. They found very small differences between the bread with no FPC and the ones with 5 or 10 percent FPC. The bread containing 10 percent FPC was somewhat crumbly in texture. This textural characteristic became more pronounced as the amount of FPC in the bread increased.

As the level of FPC increased, the typical flavor associated with bread decreased. The judges preferred the flavor of the bread with 5 or 10 percent FPC as well as they did that of the bread with no FPC. Bread with higher levels of FPC, however, was less acceptable.

Donoso et al. (6) in Chile found that panelists accepted bread enriched with 3 percent fish flour just as well as bread with no fish flour. At the 6 percent fish flour level, color influenced the acceptability of the bread more than flavor. Bread with 9 or 12 percent fish flour was good, but differed from regular bread in color, flavor, and texture.

Scillings et al. (7) conducted an animal feeding study to determine the nutritive quality of the enriched bread. The bread was incorporated into the diets in two different ways. First, the diets were formulated to contain 1.6 percent nitrogen from the bread samples. Second, the diets were formulated to contain 1.6 percent by weight of the bread samples. Both diets had the same caloric value. Table 11 shows the results from this study. When diets contained 1.6 percent nitrogen from the bread, there was a steady increase in nutritive value with each increment of FPC. When diets contained 1.6 percent bread, a level of 10 percent FPC produced near maximal weight gains. Table 12 points out that very little lysine was lost during the processing of the FPC-wheat flour mixtures into bread.

Morrison and Campbell (8) reported that the addition of 10 percent FPC to white bread increased the PDI value by 103 percent. Yanez et al. (9) found that 6 percent FPC and 12 percent dried milk solids produced similar increases in the protein values of the bread. With both supplements--FPC or dried milk solids--however, there was some loss in the quality of the protein during baking.

Bread Supplemented with FPC made from Other Species of Fish.-- FPC made from other species of fish was also incorporated into bread at the 10 percent FPC level and evaluated by a taste panel. The experimental bread was compared to the bread containing 10 percent hake-FPC and the results are shown in Table 13. The flavor and texture of the bread with these other types of FPC were as acceptable as that with hake-FPC. There was a significant objection to the appearance of the bread containing anchovy-FPC and alewife-FPC, which were quite gray in color.

Pasta

Pasta Supplemented with FPC.--Semolina and varying amounts of hake-FPC (0, 3, 6, 9, and 12 percent) were used to make the macaroni products (10). Enough water was incorporated into the semolina-FPC mixture so that it was free flowing, yet cohesive under pressure. The mixture was extruded and resulting pasta air-dried overnight.

The pasta became darker with each increment of hake-FPC. The color went from a bright yellow for the plain semolina pasta to a darkish gray yellow for the pasta with 12 percent hake-FPC. During cooking, much of the dark color leached into the cooking water.

Table 14 shows the percent solids and the protein in the cooking water. The percent solids in the cooking water remained about the same within each cooking time (8, 16, and 26 minutes) regardless of the amount of hake-FPC in the pasta. There was an increase of solids in the cooking water, however, as the time of cooking increased. The protein in the cooking water increased, however, as the amount of FPC in the pasta was increased. The maximum amount of protein in the water was found at the 16-minute cooking time.

Table 15 shows the effect of adding FPC on the swelling and the water absorption of the pasta during cooking. The addition of 3, 6, and 9 percent FPC appeared to retard the swelling of the pasta at the end of the 8-minute cooking time, yet about the same amount of water was absorbed as with the control. At the end of the 10-minute cooking time, the 9 percent FPC pasta still did not increase in volume like the others. At the end of 16 minutes, the 9 and 12 percent FPC pasta had become quite soft and no longer retained its shape.

A sensory evaluation was conducted of the pasta containing the various amounts of hake-FPC. When FPC is one of the ingredients of a food product, any odors are more likely to be detected when the food is hot. The pasta, therefore, was served in warm, mildly salted distilled water. No differences were detected between the 0 and 3 percent FPC. A few of the judges were able to detect a slight odor difference in the 6 and 9 percent FPC pastas. There was a distinct odor difference with the 12 percent FPC pasta. The judges liked the flavor of the 0, 3, and 6 percent FPC-semolina pasta, but had a definite dislike for the 12 percent. The addition of 6 percent FPC to semolina did not change the texture of the cooked pasta. Levels of 6 and 9 percent FPC tended to harden the pasta, but with 12 percent FPC the pasta cooked to the same degree of hardness as the 0 percent FPC.

Table 16 shows the results of a nutritional evaluation of mixtures of semolina and hake-FPC before and after processing into pasta. The nutritive quality was increased when FPC was added to semolina. When the various mixtures of semolina and hake-FPC were processed into pasta, there was a slight decrease in nutritive quality (11).

Kwee et al. (12) found that nutritious and acceptable pasta could be made from mixtures of varying amounts of corn, soya, rice, and tapioca flours, along with 10 or 20 percent alewife-FPC and 15 to 25 percent semolina. The pasta that contained a high proportion of rice flour was particularly acceptable. The other samples, containing corn, soya, and tapioca as basic ingredients, were also acceptable except for the ones that contained 60 percent corn and 10 percent rice; 35 percent soya and 25 percent tapioca; and 60 percent tapioca and 10 percent soya. The pasta with a higher percentage of tapioca tended to be too soft. The cooking losses were high for the pasta that contained sweet potato powder. The greatest protein losses in the cooking water were observed in the pasta that contained high percentages of soya. In most cases, the nutritional quality of the cooked pasta was equal to or better than casein.

Pasta Supplemented with FPC Made from Other Species of Fish.--Pasta made with 10 percent FPC from other species of fish was also evaluated, and Table 17 shows the results. Due to the variation in color of the FPCs used in preparation of the pasta, the final product varied in color. Pasta made with alewife-FPC, anchovy-FPC, and herring-FPC was especially dark in color. Upon cooking, the colors bleached markedly, still they remained grayish in tone. The pasta with ocean pout-FPC and Atlantic menhaden-FPC were equally as acceptable as the pasta with hake-FPC.

Texture in this case refers to degree of hardness when the pasta was cooked in boiling water for 10 minutes. The pasta was evaluated against a 9-point scale--1, hard; 5, al dento; and 9, soft. The results

showed that the pasta made from hake-FPC was a little softer than that of the other enriched pasta. The difference, however, was not significant at the 5 percent level of significance.

To eliminate the effect of color on the evaluation of flavor and odor, this portion of the sensory evaluation was done in a darkened room. No significant differences were found by the judges in the flavor or odor of the pastas that contained the various FFCs.

Crackers

A study was conducted to evaluate the effect of adding hake-FPC to saltine crackers on their acceptability and nutritional quality. The crackers were made in the pilot plant of the National Biscuit Company according to their formulation. FPC was added to the formulation at the expense of wheat flour at levels of 0, 4, 8, 12 and 16 percent. A little more water was needed to bring the dough to the proper consistency; otherwise the ingredients and the processing procedures were the same. Table 18 shows the proximate composition of the enriched crackers. The protein content of the cracker nearly doubled when 12 percent of the flour was replaced with FPC.

The nutritional quality of the crackers was evaluated in a rat-feeding study in which diets contained 8 percent protein from either the crackers, casein or hake-FPC. Table 19 shows that substantial increases were obtained in weight gain, feed intake and PER-values when crackers were enriched with 4, 8, and 12 percent FPC. There was no significant improvement between the 12 percent and 16 percent enrichment. In all cases the results were lower than those for casein or hake-FPC alone.

Table 20 shows the results of the sensory evaluation. The appearance of the fortified crackers was less acceptable than the unfortified crackers. The texture and flavor, however, of crackers enriched with 4, 8, and 12 percent FPC was nearly as acceptable as that of the unenriched crackers. The addition of FPC to the saltine type cracker, seemed to make it more crispy and crumbly. The addition of FPC at the 4 and 8 percent level of enrichment gave the crackers an interesting flavor which was described as "shrimpy."

Cookies

Cookies are not notably high in protein content, but they are eaten in large quantities as a light dessert or a snack; therefore they can contribute significantly to the dietary intake of individuals, especially children.

Cookies Supplemented with Milk-FPC.--The formulation used to evaluate the nutritional quality of a FPC-enriched cookie appears in Table 21. For this particular cookie the addition of 10 percent FPC at the expense of the flour not only increased the crude protein content from 5.4 percent to 8.1 percent, but also did not result in any undesirable or unacceptable changes in flavor, odor or appearance. The two obvious changes were: (a) as the amount of FPC increased, the degree of sweetness in the cookies decreased, and (b) the color went from a bright yellow to a dull gray-yellow. The nutritive quality of the cookies containing 0 percent and 10 percent FPC was evaluated in a feeding trial. The animals were fed a mixture of cookies, vitamins and minerals. We found that the cookies with no FPC had a PER value of 0.9 while those with 10 percent FPC had

a PER value of 2.3. In this feeding study the control diet, casein, had a value of 3.1 (13).

Cookies supplemented with FPC made from Other Species of Fish.--

We have used a standard sugar cookie to evaluate the effects of adding FPC prepared from various species of fish on the sensory characteristics. The formulation used was the same as given in Section 10-50 of the American Association of Cereal Chemists Methods (14). FPC was added at levels of either 5 or 10 percent--at the expense of flour. At the 5 percent level, the FPCs that we have evaluated have not significantly affected the sensory characteristics.

Table 22 shows the results of the sensory evaluation when 10 percent FPC was used. The appearance of cookies made from anchovy- and alewife-FPC was less acceptable than that of the control. No significant differences were found, however, in the flavor and texture of the cookies containing the various FPCs.

The characteristics of cookies were sometimes changed when FPC was used at a level of 10 percent. The products, however, were still usually very acceptable to judges. We have found that any slight objectionable differences in flavor can be overcome by the addition of flavor components.

FPC-beverage

Recently we have worked on a formulation for make-FPC beverage. Our aim is to make a product that can be spray-dried and reconstituted at the time it is to be used.

Fish protein concentrate can be utilized to prepare an attractive, tasty, and nutritious beverage. The composition of the formulation tested in our laboratory is comparable to cow's milk in protein and fat content,

but with twice the amount of carbohydrate. In order to make a stable suspension, a stabilizer-emulsifier was used in the formula. The beverage, as prepared, was then dried and the resulting material examined. The powder dissolved on the tongue and left no gritty residue. The basic powder can be used as the basis of infant formula. Or, it can be flavored and colored to appeal to older age groups. The chocolate flavored powder has a very acceptable taste; in fact, with minor changes, it has the possibility of further application in candy bars or frozen desserts.

Forty-four grams of the dried powder dispersed in 200 grams of water will make a drink comparable to an 8-ounce glass of milk (Table 23). The caloric value of the baked-FPC beverage is higher than cow's milk, due to the higher carbohydrate content. The essential amino acids, lysine and methionine, are higher in the FPC drink; the others are equal to or a little lower than in cow's milk.

In a preliminary study on the nutritive value of the FPC-beverage we found that it was bitter than casein and just a little lower than the FPC used to make the beverage.

Soups

Soups offer the possibility of a large variety of flavors and combinations. They can be prepared from FPC alone with the addition of spices, flavoring, etc., or from a mixture of FPC, legumes, and/or vegetables. Since legumes play an important role in the diets of many population groups as a protein source, it may be prudent to devise formulations that utilize combinations of FPC and legumes.

Our laboratory has done only a small amount of work on this group of foodstuffs. The investigations we have conducted on split pea and

tomato soups, for instance, indicate that with the proper flavorings and formulations, it is possible to prepare very palatable products.

In several experiments, attempts were made to freeze-dry the soups. The resulting powdered material had a good color, texture, and flavor. The proximate compositions shown in Table 24 are the values for the dried products. The FPC soup was prepared using FPC as the sole source of protein. The pea soup was a combination of legume protein and FPC.

In a sensory evaluation of pea soup containing varying amounts of hake-FPC, the panelists liked the soup that contained 5 or 10 percent FPC equally as well as the soup with no hake-FPC.

Thirty-four grams of the FPC soup powder dispersed in 170 grams of water will make a brown soup base containing 8.9 grams of protein. A similar serving of pea soup will supply 6.3 grams of protein.

SUMMARY

We have shown that FPC can be prepared from several species of fish by an isopropyl alcohol extraction process. The composition of the FPCs made from different species of fish was slightly more variable than when made from the same species. Usually, however, the protein content ranged from 80 to 85 percent and the fat content was less than 0.3 percent. The nutritive quality of the FPCs was equal or slightly better than that of casein.

Several studies have been conducted on the effects of incorporating FPC into a variety of food products, such as bread, pasta, crackers, cookies, and soups. These studies have shown that, with only minor changes in formulation, FPC can easily be added to food products at

levels of 5 and 10 percent without markedly altering the characteristics of the products. At the same time, the addition of FPC to food products significantly improves their nutritional quality.

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Table 1. Proximate analyses of fish protein concentrate (FPC) prepared by $\text{CH}_3\text{CO}_2\text{H}$ propyl alcohol extraction of ten 150-pound batches of red hake (*Urophycis chrysosoma*)

Sample	Crude protein (N x 6.25)	Percent of sample			Lipids
		Moisture	Crude fat	Nitrogen-free extractives	
1	81.73	7.55	14.35	0.17	
2	81.26	7.36	13.72	0.15	
3	78.04	10.78	13.06	0.17	
4	80.74	7.58	13.80	0.13	
5	80.63	7.53	13.22	0.19	
6	79.30	8.91	13.42	0.15	
7	81.85	6.25	13.48	0.21	
8	82.28	7.67	12.92	0.19	
9	81.17	6.74	13.47	0.19	
10	81.53	6.72	13.56	0.22	
Average	80.86	7.71	13.50	0.18	
Standard deviation	1.2876	1.2953	0.4055	0.0283	
Standard error of mean	0.4072	0.4096	0.1282	0.0090	

Table 2. Proximate analyses of fish protein concentrate (FPC) prepared by isopropyl alcohol extraction of six batches of Atlantic menhaden (see Fig. 1 for sample)

Sample	Canned weight g./100 g. of sample	Protein content percent of sample	NH ₃ nitrogen percent of sample	Nitrogen percent of sample
1	78.44	3.80	19.56	0.16
2	78.01	3.60	20.06	0.29
3	78.14	3.55	19.51	0.26
4	78.75	3.66	19.44	0.13
5	77.77	4.48	19.56	0.15
6	80.01	3.68	18.39	0.10
Average	78.52	3.80	19.42	0.18
Standard deviation	0.81	0.34	0.55	0.08
Standard error of mean	0.33	0.14	0.22	0.03

Table 3. Proximate analyses of fish protein concentrate (FPC) prepared by isopropyl alcohol extraction of various species of fish

Species of fish used	Crude protein (N x 6.25)	Volatile			Lipids
		Percent of sample	Ash	Lipids	
Red hake-FPC	80.9	7.7	13.5	0.18	
Atlantic menhaden-FPC	78.5	3.8	19.4	0.18	
Atlantic herring-FPC	87.5	5.9	10.8	0.19	
Northern anchovy-FPC	80.0	5.1	15.8	0.07	
Ocean pout-FPC	86.0	1.5	15.0	0.24	
Alewife-FPC	86.0	2.3	15.7	0.09	
Moroccan sardines-FPC	79.7	4.4	-	0.21	

Table 4. Essential amino acid composition of fish protein concentrate (FPC) prepared by isopropanol extraction of various species of fish and of dried whole eggs

	Whole egg	Atlantic hake	Mediterranean mackerel	Alewife	Northern anchovy	Atlantic herring	Ocean pout	Moroccan sardine
				Percent of protein				
Lysine	6.40	8.26	7.89	8.19	8.06	8.53	7.93	8.58
Histidine	2.40	2.05	2.29	2.08	2.31	2.13	1.99	2.82
Arginine	6.56	6.47	6.41	6.31	6.25	6.12	6.89	6.26
Threonine	4.98	4.15	3.96	4.05	4.23	4.23	4.10	4.14
Valine	7.42	4.88	5.13	4.96	5.06	5.15	4.52	5.20
Methionine	3.14	2.93	2.96	3.03	3.05	3.19	2.91	3.05
Cystine	2.31	0.86	0.58	0.65	0.72	0.71	0.70	-
Isoleucine	6.64	4.33	4.12	4.25	4.34	4.37	4.00	4.40
Leucine	8.80	7.54	6.95	7.25	7.38	7.62	6.64	7.18
Phenylalanine	5.78	4.49	3.84	4.06	3.98	4.05	3.69	4.14
Tryptophane	1.65	0.97	1.11	1.27	1.31	1.20	1.12	0.97

Table 5. Nutritive quality of fish protein concentrate (FPC) prepared by isopropyl alcohol extraction of various batches of red hake and Atlantic menhaden compared to casein

	No. of analyses	Average protein efficiency ratio ¹	Range
Hake-FPC	22	3.29**	2.93-3.63
Atlantic menhaden-FPC	6	3.05	2.97-3.11
Casein	8	3.00	-

¹/ Values adjusted to a casein value of 3.00.

**Significantly different than casein at the 0.01 percent level.

Table 6. Nutritive quality of fish protein concentrate (FPC) prepared by isopropyl alcohol extraction of various species of fish

Species of fish	Average protein content (%)	Average protein digestibility (%)	Protein efficiency ratio ^{1/}
Sea bass-FPC	4.21	14.6	3.19 ^{2/}
Atlantic mackerel-FPC	4.60	13.9	3.05
Atlantic herring-FPC	5.32	15.0	3.15
Northern anchovy-FPC	5.18	14.6	3.25
Ocean trout-FPC	4.68	13.8	3.06
Herring-FPC	5.26	15.2	3.17
Moroccan sardine-FPC	4.98	15.7	2.96
Casein	4.35	13.0	3.00

^{1/} Protein efficiency ratio = $\frac{\text{Weight gained}}{\text{protein consumed}}$

^{2/} Values adjusted to a casein value of 3.00.

Table 7. Nutritive value of wheat flour supplemented with fish protein concentrate (FPC) when fed to rats in 10 percent protein diets

Protein source	Average daily weight gain g	Average daily feed intake g	Protein efficiency ratio
100% wheat	0.90	8.45	.92 ^{2/}
95% wheat, 5% FPC	3.17	13.34	2.07
90% wheat, 10% FPC	4.70	15.55	2.65
85% wheat, 15% FPC	5.79	16.64	3.04
80% wheat, 20% FPC	5.77	16.86	2.99
75% wheat, 25% FPC	5.43	15.53	3.06
0% wheat, 100% FPC	5.75	16.10	3.13
Casein	4.85	14.24	3.00

^{1/} Protein efficiency ratio = $\frac{\text{weight gained}}{\text{protein consumed}}$

^{2/} Values adjusted to a casein value of 3.00.

Table 8. Farinograph characteristics of doughs made from mixtures of wheat flour with varying percentages of fish protein concentrate (FPC)

Characteristics	Units	0	1	2	3	4	5	6	7
Absorption	%	59.0	61.0	63.6	67.0	70.2	70.0	68.0	
Arrival time	Min.	1.2	2.0	2.2	4.2	4.0	4.0	4.0	
Stability	Min.	10.8	17.2	18.2	26.0	18.0	34.0		
Peak time	Min.	5.5	8.5	10.2	10.0	10.0	7.0		
Tolerance index	B.U. ^{1/}	30.0	20.0	20.0	20.0	0.0	20.0		
20-minute drop	B.U. ^{1/}	70.0	50.0	30.0	30.0	0.0	0.0		

^{1/}Brabender units.

Table 9. Consistency of doughs made from mixtures of wheat flour containing varying percentages of fish protein concentrate (FPC) after a 45-minute and 180-minute rest period

FPC %	Total extension		Maximum resistance		Area under curve	
	45 min. mm.	180 min. mm.	45 min. Brab. units.	180 min. Brab. units.	45 min. sq. cm.	180 min. sq. cm.
0	185	150	460	560	116	104
5	178	148	480	660	114	125
10	102	130	530	700	103	116
15	105	118	520	740	73	113
20	98	95	515	680	72	91
25	68	68	850	880	82	78

^{1/}Brabender units.

Table 10. Sensory evaluation of crumb from bread supplemented with varying percentages of fish protein concentrate (FPC)

FPC %	Appearance	Texture	Flavor
0	3.3 ± 0.12 ^{1/}	3.0 ± 0.08 ^{1/}	3.1 ± 0.06 ^{1/}
5	3.0 ± 0.16	2.8 ± 0.13	2.8 ± 0.19
10	2.8 ± 0.14	2.6 ± 0.16	2.6 ± 0.14
15	2.6 ± 0.16*	2.0 ± 0.16*	2.1 ± 0.25*
20	2.3 ± 0.17*	2.4 ± 0.16*	2.4 ± 0.12*
25	1.8 ± 0.13*	1.5 ± 0.15*	1.4 ± 0.12*

*Tukey's W 0.4
P .05

^{1/}Standard error of mean

Table 11. Nutritive value of bread supplemented with varying amounts of FPC, which was incorporated into the diet either at the 10 percent protein level, or at 80 percent by weight of the total diet

Mixtures used to make bread	Diets containing 10% protein		Diets containing 80% bread	
	Average daily weight gain g	Protein efficiency ratio	Average daily weight gain g	Weight gain /100 g bread intake
			g	
100% wheat, 0% FPC	1.13	1.13	1.07	16.4
95% wheat, 5% FPC	2.89	2.04	4.20	37.6
90% wheat, 10% FPC	4.31	2.53	5.93	51.4
85% wheat, 15% FPC	4.98	2.86	6.22	56.3
80% wheat, 20% FPC	5.24	3.01	6.39	58.4
75% wheat, 25% FPC	5.99	3.35	6.34	58.1
Casein	5.34	3.28		

^{1/} Protein efficiency ratio = $\frac{\text{weight gained}}{\text{protein consumed}}$

^{2/} Calculated by dividing the grams of weight gained by the grams of protein consumed.

Table 12. Crude protein and lysine content of bread supplemented with FPC

Supplement, ^{1/} <u>FPC</u>	Crude protein % of dry matter	Lysine % of crude protein	Lysine % of theoretical
0	16.0	1.97	97
5	19.6	3.32	97
10	23.2	4.35	100
15	27.4	5.09	101
20	31.7	5.36	97
25	34.5	6.06	102

^{1/}Values shown are percentages of the mixtures of wheat flour and supplements used to make bread.

Table 13. Sensory evaluation of bread supplemented with 10 percent FPC made from various species of fish

	Appearance	Flavor	Texture
Hake-FPC (control)	2.9 ± .13 ^{1/}	3.0 ± .21 ^{1/}	3.0 ± .26 ^{1/}
Ocean pout-FPC	2.5 ± .22	2.5 ± .17	2.4 ± .48
Anchovy-FPC	1.5 ± .17*	2.4 ± .20	2.5 ± .27
Herring-FPC	2.3 ± .30	2.8 ± .33	3.0 ± .21
Atlantic mackerel-FPC	2.1 ± .18	2.5 ± .22	1.9 ± .23
Alevilfe-FPC	1.9 ± .07*	2.1 ± .23	2.3 ± .33

*Tukey's W
(P < .05)

^{1/}Standard error of mean

Table 14. Percent solids and protein in the cooking water of pasta made from semolina and varying amounts of fish protein concentrate (FPC) after cooking for 8, 16 and 28 minutes.

Concentration of FPC Percent	Cooking time (minutes)			Solids/ Protein ² %
	8	16	28	
100	0	.6	.57	.9
97	3	.7	.65	.6
94	6	.7	.60	.80
91	9	.6	.65	1.0
88	12	.8	.91	1.1

¹/ Percent by weight of the cooking water.

²/ Percent by volume of the cooking water.

Table 15. Percent increase in volume and weight of pasta made from semolina and varying amounts of fish protein concentrate (FPC) after cooking for 8, 18 and 26 minutes

Composition of pasta	Semolina	FPC	Cooking time (minutes)								
			8	18	26	Volume	Weight	Volume	Weight	Volume	Weight
%	%	%	%	%	%	%	%	%	%	%	%
0	0	0	111	165	166	271	271	179	342		
97	3	3	101	165	159	271	271	183	346		
94	6	6	105	167	153	271	271	162	354		
91	9	9	100	162	133	269	269	143	334		
88	12	12	136	164	164	275	275	179	338		

Table 16. Nutritive quality of mixtures of semolina and varying amounts of hake-FPC before and after processed into pasta

Protein source	Before processing		After processing	
	Average daily weight gain g	Protein efficiency ratio	Average daily weight gain g	Protein efficiency ratio
100% semolina 0% FPC	0.85	.98 ^{2/}	0.81	.90 ^{2/}
97% semolina 3% FPC	2.47	1.90	2.31	1.83
94% semolina 6% FPC	4.20	2.52	3.80	2.38
91% semolina 9% FPC	5.12	2.91	4.74	2.69
88% semolina 12% FPC	5.56	3.12	4.98	2.94
Casein	5.02	3.00	4.78	3.00

1/ Protein efficiency ratio (PER) = $\frac{\text{weight gained}}{\text{protein consumed}}$

2/ All values were adjusted to a PER of 3.00 for casein.

Table 17. Sensory evaluation of cooked pasta with 10 percent FPC made from various species of fish

Source of FPC	Appearance	Texture	Flavor	Odor
Hake-FPC (control)	3.0 ± .00 ^{1/}	6.1 ± .61 ^{1/}	3.0 ± .11 ^{1/}	2.6 ± .29 ^{1/}
Ocean pout-FPC	3.1 ± .26	4.2 ± .49	2.8 ± .20	2.8 ± .30
Anchovy-FPC	1.0 ± .00*	5.6 ± .69	2.5 ± .25	2.5 ± .22
Herring-FPC	1.8 ± .13*	5.2 ± .59	2.9 ± .31	2.9 ± .23
Atlantic menhaden-FPC	2.6 ± .27	4.4 ± .62	3.0 ± .15	2.8 ± .32
Alewife-FPC	1.5 ± .22*	5.1 ± .48	2.7 ± .26	2.8 ± .24
*Tukey's <i>t</i> (P = <.05)	0.8	2.6	1.1	1.2

^{1/}Standard error of mean

Table 13. Protein composition of saltine crackers containing varying amounts of fish protein concentrate (FPC)

Amount of FPC	Protein (6.25 x 1)	Percent of sample		Lipid
		Crust	Interior	
0% FPC	9.4	3.6	10.0	3.4
4% FPC	12.0	3.2	9.4	3.6
8% FPC	15.3	2.7	9.6	4.0
12% FPC	17.2	3.1	9.0	4.5
16% FPC	20.2	2.3	10.1	4.9

^{1/}Determined by ether extraction

Table 19. Nutritive quality of crackers supplemented with varying levels of fish protein concentrate (FPC) and incorporated into diets at an 8 percent protein level

DIETARY PROTEIN SOURCE	AVERAGE DAILY WEIGHT GAIN g	AVERAGE DAILY FEED INTAKE g	PROTEIN EFFICIENCY RATIO
Crackers with:			
0% FPC	0.32 ± 0.02 ^{2/}	6.57 ± 0.20 ^{2/}	0.61 ± 0.04 ^{2/}
4% FPC	1.34 ± 0.04	10.26 ± 0.46	1.75 ± 0.05
8% FPC	1.95 ± 0.07	10.69 ± 0.26	2.31 ± 0.05
12% FPC	2.75 ± 0.13	12.60 ± 0.43	2.75 ± 0.05
16% FPC	2.87 ± 0.08	12.83 ± 0.22	2.77 ± 0.04
Casein	2.93 ± 0.11	12.22 ± 0.39	3.01 ± 0.10
FPC	3.46 ± 0.06	12.92 ± 0.22	3.34 ± 0.07
Tukey's W (P < 0.05)	0.35	1.42	0.26

^{1/} Protein efficiency ratio = $\frac{\text{weight gained}}{\text{protein consumed}}$

^{2/} Standard error of the mean

Table 20. Mean values for 50 sensory evaluations on crackers containing varying percentages of fish protein concentrate (FPC)

FPC % of flour	Appearance	Texture	Flavor
0	3.9 ± .15 ^{1/}	3.1 ± .11 ^{1/}	3.0 ± .10 ^{1/}
4	3.4 ± .11*	2.9 ± .08	2.9 ± .09
8	3.1 ± .10*	2.9 ± .08	2.7 ± .08
12	2.8 ± .06*	2.7 ± .10	2.8 ± .11
16	2.1 ± .12*	2.3 ± .12*	2.4 ± .13*
* Tukey's w (P = <.05)	0.4	0.4	0.4

^{1/} Standard error of the mean.

Table 21. Formulation for a butter cookie

	g ^m
Butter or oleomargarine	110
Sugar	200
Egg	50
Water	60
Vanilla	4
Cake flour	¹ 222 ₂
Salt	1
Baking powder	7

1/The percentages 5, 10, and 15 of FPC were added to the flour at the expense of the cake flour used in the formula.

Table 22. Sensory evaluation of cookies containing 10 percent FPC made from various species of fish

Source of FPC	Appearance	Flavor	Texture
Hake-FPC (control)	2.9 ± .23 ^{1/}	2.8 ± .13 ^{1/}	2.9 ± .18 ^{1/}
Ocean pout-FPC	2.9 ± .23	3.1 ± .23	2.3 ± .39
Anchovy-FPC	1.6 ± .22*	2.6 ± .26	2.6 ± .36
Herring-FPC	2.2 ± .13	2.4 ± .26	2.8 ± .25
Atlantic menhaden-FPC	3.1 ± .38	2.7 ± .33	2.8 ± .36
Alewife-FPC	1.4 ± .16*	2.1 ± .23	2.7 ± .36
Tukey's w (P< .05)	1.0	1.1	1.4

^{1/}Standard error of mean

Table 23. Comparison of the composition of an 8-ounce serving of the plain FPC beverage and whole milk

	FPC beverage ^{1/}	Milk ^{2/}
Weight (gm)	244	244
Weight (gm)	8	8
Calories	221	158
Protein (gm)	9.2	8.5
Fat (gm)	12.0	11.9
Lysine (gm)	.762	.664
Methionine (gm)	.298	.210
Threonine (gm)	.398	.393
Valine (gm)	.458	.586
Phenylalanine (gm)	.369	.415
Histidine (gm)	.180	.224
Arginine (gm)	.628	.312
Tryptophan (gm)	.089	.120
Leucine (gm)	.674	.839
Isoleucine (gm)	.402	.544

^{1/} Amino Acid values shown are averages of 25 analyses except 11 analyses for tryptophane of the FPC used in beverage

^{2/} Values for composition were from "Amino Acid Content in Foods, U.S.D.A. Home Economics Research Report No. 4, 1957.

Table 24. Proximate composition of freeze-dried soup containing fish protein concentrate (PPC)

<u>Product</u>	<u>Crude protein ($\text{N} \times 6.25$)</u> <u>%</u>	<u>Fat (ether)</u> <u>%</u>	<u>Ash</u> <u>%</u>	<u>Moisture</u> <u>%</u>
PPC soup	26.2	29.5	7.9	20.9
Pea soup	24.0	31.0	8.5	4.0

25 · 1 · 72