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Joint FAO/UNIDO/[illegible] Expert Group Meeting on the
Production of Fish Protein Concentrate

Rabat, Morocco, 8 - 12 December 1969

THE DETERMINATION OF NUTRITIONAL EFFECTIVENESS AND ACCEPTABILITY OF FISH PROTEIN CONCENTRATES 1/

by

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Expert Group Meeting on Fish Protein
Concentrate Production

Rabat/Agadir, Morocco, 15 - 19 December 1969

SUMMARY

THE DETERMINATION OF NUTRITIONAL EFFECTIVENESS AND ACCEPTABILITY OF FISH PROTEIN CONCENTRATES [1]

by

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The amino acid composition of fish protein concentrates is indicative of its excellent nutritional pattern. The utilization of mild processing techniques yields a product which retains high nutritional values, whereas high-temperature extraction, manipulation of solvents or high-temperature drying may yield products which have a lowered nutritional value, due to the loss of lysine and other amino acids. The acceptable removal of flavours and odours from whole fish protein concentrate lies somewhere between the application of drastic techniques and extremely mild ones.

In numerous experiments utilizing weanling rats to measure the protein efficiency ratio or the net protein utilization, it has been shown that fish protein concentrate may have values higher than casein. These high values are retained when the fish protein concentrate is incorporated in products which do not undergo substantial Maillard reactions during preparation.

In normally nourished children and in adults, fish protein concentrate prepared from either low-fat fish or fatty fish exhibits protein efficiency ratios equivalent to that of casein when fed as a portion of a fortified food. In normally-nourished human infants, fish protein concentrate from

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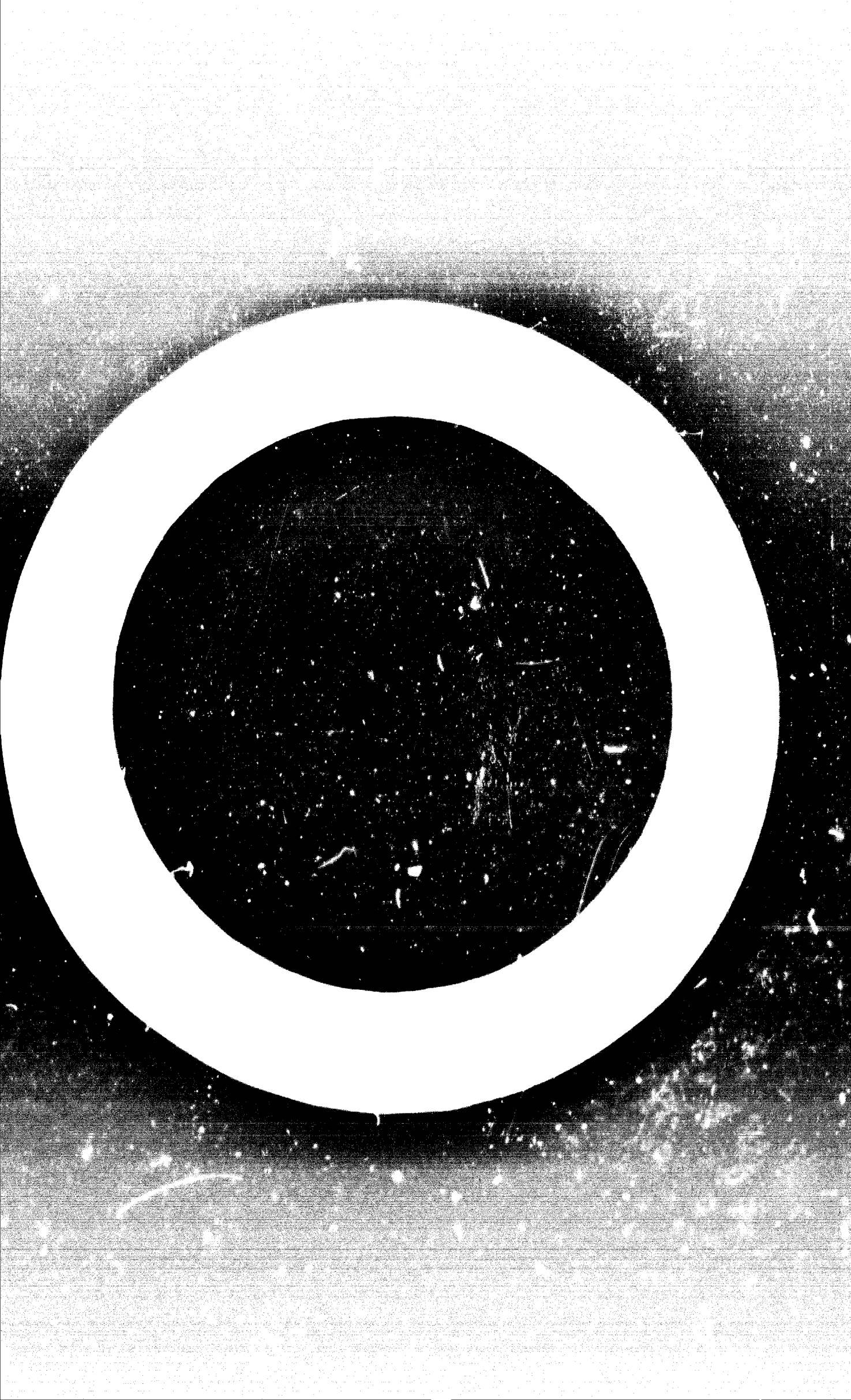

low-fat fish, when fed as the sole source of protein in the diet, displays a protein efficiency ratio or nutritional value equivalent to that of skim milk. In children suffering from severe malnutrition, the nutritional effectiveness of F.P.C. appears to be inferior to milk, as judged by its ability to correct hypoalbuminemia or maintain serum albumin levels. These observations, however, do not militate against the utilization of fish protein concentrate as a protein supplement in the prevention of protein malnutrition.

F.P.C. made from fatty fish by alcohol extraction techniques has protein efficiency ratios equivalent to those of F.P.C. made from low-fat fish, when tested in rats. The acceptability, as judged by taste panels which compared fish protein concentrate derived from high-fat and low-fat fish, is equivalent. In bread, cookies and macaroni there is essentially no difference in acceptability between fish protein concentrate made from fatty fish and that made from low-fat fish. Therefore, based upon these results, there is no reason to suspect that there would be any difference between fish protein concentrate derived from low-fat fish or high-fat fish when used as a dietary supplement to prevent protein deficiencies in human diets.

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Joint UNIDO/FAO Expert Group Meeting on the Production of Fish Protein Concentrate

Rabat, Morocco, 8 - 12 December 1969

THE DETERMINATION OF NUTRITIONAL EFFECTIVENESS AND ACCEPTABILITY OF FISH PROTEIN CONCENTRATES

by

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Corrigendum 1

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Table II. Amino Acid Composition of Fish Protein Concentrates.

(Per cent. of protein)

SOLVENT EXTRACTED

Amino Acid	Herring Sweden [1]	Hake U.S.A. [2]	Sardine Morocco [3]
Alanine	7.31	6.81	6.49
Aspartic Acid	11.20	10.35	10.37
Arginine	7.59	7.13	8.09
Cystine	--	0.77	0.36
Glutamic Acid	15.30	15.39	16.47
Glycine	6.83	8.09	5.12
Histidine	2.38	2.08	2.04
Isoleucine	4.47	4.56	4.26
Leucine	8.70	7.78	7.75
Lysine	9.14	8.41	9.02
Methionine	2.94	3.30	2.91
Phenylalanine	4.48	4.24	4.42
Proline	5.21	5.21	4.84
Serine	5.30	4.65	4.45
Threonine	5.24	4.47	4.68
Tyrosine	3.17	3.35	3.05
Valine	5.18	5.26	5.09
Tryptophan	1.40	1.03	*

[1] Astra Nutrition, Molndul, Sweden.

[2] Bureau of Commercial Fisheries, College Park, Maryland.

[3] K. Wetherell and C. Chichester, Department of Food Science and Technology, University of California, Davis (unpublished data).

* Undetermined

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Joint [illegible] Expert Group Meeting on the
Production of Fish Protein Concentrate

Rabat, Morocco, 8 - 12 December 1969

THE DETERMINATION OF NUTRITIONAL EFFECTIVENESS AND ACCEPTABILITY OF FISH PROTEIN CONCENTRATES

by

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The production of protein-rich foods from the sea has centered about the development of products which are not only low in cost, but stable without the utilization of normal preservation techniques. In order to maximize the use of marine proteins, it is obviously of importance to use marine products which are in large supply world-wide and are easily harvestable. The tremendous yields of fish meal from the anchovetta in Peru illustrates the tonnage of protein that may be taken from the sea at low cost. By avoiding the interconversion problem, i.e., the utilization of marine proteins in an intermediary animal such as chickens, hogs, etc., the efficiency of supplying a good quality protein in the human diet is increased. Most marine fishes that are heavily utilized for the production of fish meal are high in fat, and the products produced therefrom are moderately unstable, due to the ready oxidation of the unsaturated fats in the meal. The presence of the fats and phospholipids contributes to a characteristic fish odor and flavor, and in populations whose food habits do not include the consumption of fish flavoring materials, such products are unacceptable from a gustatory sense. Reduction techniques in the manufacture of fish meal also leave considerable to be desired in the way of sanitary conditions, and the direct utilization of commercially-produced fish meal in human consumption presents a significant problem, particularly if an infant population is to be the recipient.

There is mounting evidence that the group with whose nutrition we should be most concerned is children between birth and two years.[1] This group is also very susceptible to infection and other diseased states. The introduction of a supplementary protein product to this group is particularly fraught with danger. The inclusion of fat in infant nutrition has significant benefits, since a food material of high caloric density is desired in the growing infant. Nonetheless, in most instances the consumption of normally produced fish meal by this group is not the method of choice.

Initial attempts to produce an adequate grade of fish meal for human consumption involved the production of a fish meal under sanitary conditions which was subsequently deordorized and had the fat removed. In order to remove the fat materials it was necessary to employ solvent extraction techniques which removed the fat, and at least partially the flavoring components. By using semi-polar solvents it was also possible to remove the phospholipids, which apparently contribute significantly to the flavor. As solvent extraction must be utilized in order to produce a relatively odorless and tasteless product from fish, the marine resource of choice, at least initially, dictated the use of fish which contrined relatively little fat. This allowed the utilization of a minimum quantity of solvent.

The solvents must of necessity be recovered and purified in order to make any extraction system economically feasible. Using high solvent-to-product ratios is not desirable, because of the mechanics of

manipulating the solvent, and its subsequent recovery. The use of comparatively low-fat fish in the process minimizes the use of the solvent, and allows a simpler technique for the separation of the extracted fat and the recovery of the solvent. With this in mind, many of the major attempts to produce a fish protein concentrate suitable for human consumption from whole fish utilized fish of low fat content.[2]

Typical of a product produced from a low-fat fish is that produced by the isopropanol extraction process, such as that developed by the Bureau of Commercial Fisheries, College Park, Maryland. The composition of the final product made from red hake is shown in Table I.[3] Even at this level of fats, there is the possibility of some flavor revision.

A typical amino acid profile of fish protein concentrate is shown in Table II. Although the figures may vary one to another between amino acids, the general patterns of most fish protein concentrates are essentially similar. The comparatively high concentration of lysine compared to plant protein obviously presents a more balanced protein for human consumption than do many other sources of protein. The amino acid composition would indicate that lysine is not the limiting amino acid, but would suggest that other amino acids would assume this role in the utilization of protein. This can be shown by experiments on the supplementation of FPC produced from hake (Merluccius gayi) in Chile. Table III shows the effect of supplementing the FPC

Table I. Composition of Fish Protein Concentrate Produced by Isopropanol Extraction from Red Hake. (Bureau of Commercial Fisheries, College Park, Maryland.)

Protein	81.4%
Lipids	0.2%
Ash	13.5%
Moisture	6.7%

Table II. Amino Acid Composition of Fish Protein Concentrates.

(Per cent. of protein)

Amino Acid	SOLVENT EXTRACTED		
	Herring Sweden[1]	Hake U.S.A.[2]	Sardine, Morocco[3]
Alanine	7.31	6.81	5.49
Aspartic Acid	11.20	10.35	10.91
Arginine	7.59	7.13	10.17
Cystine	--	0.77	0.35
Glutamic Acid	15.30	15.39	18.42
Glycine	6.83	8.09	3.11
Histidine	2.38	2.08	2.54
Isoleucine	4.47	4.56	4.07
Leucine	8.70	7.78	6.75
Lysine	9.14	8.41	11.32
Methionine	2.94	3.30	3.26
Phenylalanine	4.48	4.24	3.41
Proline	5.21	5.21	2.84
Serine	5.30	4.65	4.43
Threonine	5.24	4.47	4.59
Tyrosine	3.17	3.35	3.41
Valine	5.18	5.26	4.63
Tryptophan	1.40	1.03	*

[1] Astra Nutrition, Molndul, Sweden.

[2] Bureau of Commercial Fisheries, College Park, Maryland.

[3] K. Wetherell and C. Chichester, Department of Food Science and Technology, University of California, Davis (unpublished data).

* Undetermined

Table III. Effect of Amino Acid Supplementation on NPU[a] Values of Quintero PPC (Chile).

Batch	NPU		
	Control	+ 0.5% L-lysine	+ 0.3% DL-Methionine
I	66.9	66.2	70.7
II	67.9	61.3	70.2

[a] Net protein utilization operative at 10% protein calories.

with lysine or methionine on the NPU determined in rats.[4]

A major advantage of fish protein concentrate is its stability in storage. As the material is low in moisture, carbohydrates and fats, the typical interactions of these constituents to reduce its biological value is not encountered to the extent that it may be in other products. An example of the stability of FPC made from monthly fish is shown in Table IV, in which the quality of the protein does not seem to vary significantly with age. Batch 5 in the table represents material which has been stored over two years in paper bags at room temperature in a warehouse. Batches 1, 2, 3, and 4 were stored between three months and one year under similar conditions.[4] Recent experiments with batches of FPC produced and stored under similar conditions for over five years show approximately the same NPU values.[5] Thus the product from a nutritional standpoint is stable over long periods of time. Similar results have been reported by Rakjat.[6]

Although the storage stability of a fish protein concentrate made from low-fat fish appears to be quite good, its use in food materials which undergo heating may present some difficulties. The effect of heating proteins or amino acids in the presence of carbohydrates decreases the availability of many amino acids. This loss is particularly noted in available lysine or methionine. Losses in protein quality in bread have been noted when the bread is enriched with milk or other proteins high in lysine. In the preparation of cereal

Table IV. Net Protein Utilization After Storage.

Batch	NPU[a]
1	66.9
2	67.9
3	64.3
4	70.7
5	63.5

[a] Net protein utilization operative at 10% protein calories.

products fortified with milk solids utilizing high temperature, significant losses in measured protein efficiency ratio were shown.[7] Heating amino acids with casein, or heating mixtures of various plant proteins in the presence of lysine and carbohydrates also causes significant losses in nutritional quality.[8] In experiments in which FPC was utilized to enrich bread, the increase in the nutritional quality of the bread was smaller than had been predicted and it was shown that the addition of lysine to the FPC-enriched bread increased its protein quality to that which would have been predicted, further indicating that high temperature treatment in the presence of carbohydrate materials may damage the protein of FPC. This is also noted in milk-enriched bread, which also shows a similar effect.[4,9] When lime-treated corn was enriched with FPC and used in the preparation of tortillas, it was shown that significant decreases of the protein efficiency ratio resulted from cooking. Steaming of the dough reduced the protein efficiency ratio by approximately 10%, while heating at 350°F, such as might be expected in deep-fat frying, caused a further reduction in P.E.R. It appeared in these experiments, however, that the FPC retained its biological value better than did mixtures enriched with soybean.[10]

There have been few proposals in the literature that fish protein concentrate be utilized directly. Almost all suggestions for its use propose that it be used as a nutritious ingredient of existing food materials, or perhaps as a physically inert but nutritionally

enriching component of new food materials. Its nature, that of a fairly tasteless and odorless powder, suggests its usefulness in products which will tolerate the addition of a filler. Physically, the extracted materials possess a slightly gritty character, and since the protein therein does not respond well in water without the addition of emulsifiers and stabilizers. These characteristics mitigate against its use in many products. The availability of a non-denatured or modified furcelleran protein could go far in extending the usefulness of FPC. The possibility of producing a non-denatured protein from fish by hexametaphosphate extraction or water extraction with the addition of fat is particularly attractive in this regard.

Based upon its properties as primarily a nutritional supplement with high available lysine, the obvious use of solvent-produced FPC would seem to be in plant protein mixes which are consumed as food products. Bread, pasta, and tortillas are obvious vehicles for the utilization of FPC. It can be incorporated, however, into many other products in which its physical characteristics do not interfere with the organoleptic properties of the product. In yeast-leavened bread it tends to degrade the baking qualities somewhat, resulting in a smaller loaf volume, a different texture and a different color.

In a series of tests on the enrichment of bread with FPC at different levels, individuals employed in a university hospital were

asked to rate breads containing FPC as to whether they were as good as conventional bread, worse, or acceptable but not equivalent. The results of this experiment are shown in Table V.[4] It is obvious from this that at the level of 3% there is no significant difference between the non-enriched and the enriched bread. At 6%, differentiation begins to become clear, and at 9% and 12% the color differentiation is sufficient to cause considerable detection. In a series of school lunches utilizing a population of 800 school children, in which color was of no importance, bread enriched with 9% FPC did not increase the rejection rate of materials.[11] The acceptability of enriched spaghetti was measured by utilizing 150 adults in a hospital staff and 150 patients. The spaghetti was enriched to a 10% level using FPC produced in Chile from hexane-alcohol extracted cake. The analysis of plate wastes showed that the enriched spaghetti had no greater rate of rejection than the conventional pasta. A similar experiment was carried out in a children's day school where spaghetti was a principal dish (served three times a week). The rejection in this case was identical to that of the non-enriched product. In a very recent test in Brazil, FPC produced by the isopropanol extraction method was incorporated into macaroni which was served as a portion of the diet in a school lunch program. Similar analysis of acceptance or rejection showed that from the pupils' standpoint the enriched product was not differentiated from the non-enriched product.[12] It thus appears that in most populations

Table V. **Acceptability Test of Bread Enriched with FPC at Different Levels. Taste Scored as Good (G), Indifferent (I), Bad (B).**

Level of Enrichment (%)	G			I			B		
	W	M	U	W	M	U	W	M	U
0	27	27	20	3	3	8	0	0	2
3	22	25	16	7	5	13	1	0	1
6	20	26	15	8	3	11	2	1	4
9	14	24	14	12	5	14	4	1	0
12	18	22	16	5	8	6	7	0	8

Number of tasters = 30.

W = workmen; M = mothers; U = university students.

bread can be enriched with FPC at moderate levels and pasta up to 10% without a significant rejection rate by consumers. It must be noted, however, that most of the testing of FPC in bread and macaroni has utilized that produced from non-oily fish.

In India, puree or chapatti, native Indian cereal-based foods, appear to be acceptable when fortified with 5% fish protein concentrate. The FPC in these trials was made from a lean animal, Bombay Duck (Harpodon nehereus). It appears in this case that the method of preparing the FPC influenced the acceptability. In bread fortified at 10% level, some of the subjects noted a fish odor or flavor.[13]

In the study of the addition of FPC to different types of foods such as soup, meat, beans and cornmeal tortillas to a level of 15 grams total intake protein there was no noticeable rejection of the food materials over the period of study (60 days), the conclusion being that at this level and with children there appeared to be no significant rejection of the FPC.[14]

The obvious conclusion one could make from these studies, which were concerned almost entirely with low-fat fish, is that FPC produced by the conventional methods which have so far been proposed produced a material which when mixed as an ingredient with other foods is acceptable to large proportions of the population, and particularly those which may have very limited food intake.

There have been relatively few studies of the acceptability of FPC produced from high-fat fish. In recent evaluation of FPC produced from the Moroccan sardine by the isopropanol process, several formulations using this FPC were evaluated as to the acceptability by an untrained test panel in the United States. Brown-sugar cookies were enriched at the level of 0, 3, 5, and 10% and served to 25 staff and students from the Department of Food Science and Technology, University of California, Davis, at 10:00 a.m. and 3:00 p.m. Cookies representing each of these four formulations were placed in separate dishes and located at a central table. Panelists were not informed about the nature of the cooky. Participants were observed in order to determine whether they would comment to each other or to themselves about the quality of the various cooky samples, or whether some of the formulations would be left uneaten or partially eaten, and other samples would be consumed more rapidly.

In a series of tests it was apparent that all of the samples were consumed with no adverse comments, however, the 10% enrichment appeared to have been slightly less well utilized than the other formulations. In a more formal presentation, 25 different staff members of the Department of Food Science and Technology were asked to sample the four formulations of cookies. They were not told that they had been formulated with FPC, but were instructed simple to sample the cookies and comment on their impression. All the judges found the 10% formulation to be less palatable than the lower formu-

lations. However, most of their comments were directed to the heavier, chewier texture of these cookies, rather than to the off-flavor. There were no substantial differences between the 0, 3, and 5% formulations.[15]

Using the Moroccan sardine fish protein concentrate, bread was prepared using 3, 5 and 10% levels and presented to two panels of 25 people. The panels were asked to sample the formulations and comment upon their flavor. A non-enriched bread formulation was available to the panels for comparison purposes. They were not told that the bread contained FPC. As in the case of the cookies, the panels found the 10% formulation less palatable in both terms of flavor and texture than the other three samples. In particular the comments were largely confined to the color and the low loaf volume. In four instances the judges did identify the off-flavor at the 10% formulation as "fishy". Surprisingly enough, however, in the bread formulation the 3% FPC was preferred by 50% of the people over the non-fortified material on the basis that it seemed to be "richer" and "more bakery-like" than the non-enriched bread. The 50% of the people who preferred the enriched formula were queried as to their bread-buying habits, and it was found that they normally purchased the specialty breads - either whole wheat, French-type, sour dough, or breads that differ markedly from the very white breads that are produced commercially in the United States.[15]

With respect to breads, in comparison with the FPC produced from non-oily fish, the sardine FPC seemed to be slightly lower in preference, but not sufficiently lower to mitigate against its use in the formulations.

The nutritional efficiency of fish protein concentrates, including that made from oily fish or non-oily fish, has been measured extensively in animals. In a large number of studies the fish protein concentrate was utilized to enrich a diet representative of the staple food of the country. In almost all of these cases the fish protein concentrate, whether made from oily or non-oily fish, demonstrated that it was equivalent to casein as a dietary source of protein. In some cases the fish protein concentrate appears to result in slightly better growth than skim milk powder when added to the mixed diet.[16-21]

When FPC is fed as the sole source of protein in a rat diet, it has in most cases as high a protein efficiency ratio as casein, and on occasion exceeds the casein. In a study by Schendel, four generations of rats were fed on a diet which supplied 19% of the total protein from FPC, and it was noted that the females on the casein diet appeared to have more nutritional difficulty than did those on the fish protein concentrate diet. Histological exmination of the organs showed no abnormality and no difference between the groups.[22]

In a similar experiment, three different groups of weanling rats were fed for six months on FPC at 20% protein calories. At the end of the six months' period, weights were determined for a large number of organs and histological examinations were made on an even larger number. A significant difference in weight was noted in the organs of some rats fed FPC but since histological studies revealed no alteration in the organs, it appears that the weight changes are of no particular significance.[11]

In a series of studies in rats of the protein efficiency ratio of different fish protein concentrates, Morrison showed considerable variation in the P.E.R. of different fish protein concentrates. In one that had been severely heat-damaged the difference appeared to lie in the destruction of histidine or methionine, since when these were added to the [illegible] which had been damaged, weight gains were increased significantly.[23,24] From these studies it can be concluded that in the process of manufacturing, the limiting amino acid (which was shown in other studies to be methionine) is the amino acid whose loss must be guarded against.

A study of the isopropanol-extracted flavor-free sardine FPC in rats showed that the product has protein efficiency ratios of 2.98 and 3.04 versus a casein control of 2.50. These experiments were performed using a 10% protein in the diet. The fish protein concentrate that was fed had a concentration of 82.0% protein. Weight gain and acceptability in the animal feeding studies were excellent.

No rejection of the feed was observed, and the animals showed normal growth patterns.[25]

In experiments in children and adults, fish protein concentrate was used to determine the effect of supplementation on the nutriture of the mixtures. In a study of children 9-10 years of age, fish protein concentrate derived from the oil-sardine was used to supplement a rice diet. Better nitrogen retention was achieved on the the rice-protein fed than on the control diet. All subjects remained in nitrogen balance and the diet was apparently accepted.[26] In a similar manner Korean diets supplemented with 10% fish protein concentrate were found to be better digested and gave a better nitrogen retention than an identical diet in which the fish protein concentrate was substituted for by other protein sources.[27] In a study involving a large number of premature infants the FPC appeared to give results equivalent to that of casein or amino acid mixtures.[28]

In a study of convalescing malnournished infants, Graham reported that a mixture of 10% fish protein concentrate with 90% wheat flour mixture gave weight gains and nitrogen retention indistinguishable from milk. Fish protein concentrate given to such convalescent subjects as the sole source of protein also elicited responses that were similar to milk.[29] In another study, fish protein concentrate was fed as the sole source of protein to normal infants in Chile. The formula consisted of fish protein concentrate, sugars, carbohy-

drates, water, salts and vitamins. The preparation was suspended at a level of 22.5% in water, and supplied 90 kcal and 2.4 grams of protein per 100 ml. Twelve normal infants two-and-one-half to five months old received this formula for a period of from 30-90 days, with an average protein intake of 3.6 kilograms per day. Figure 1 shows the weight gain of such infants receiving FPC as the sole source of protein. Hematological tests of the same infants showed normal values. Lowering the total intake of protein stepwise showed that 2.5 grams of FPC/kg body weight can support normal growth, but at 2.0 gm/kg reductions were noted. This is the same order of magnitude as might be expected in a milk formula.[11]

In a study of the nutritive qualities of fish protein concentrate in the convalescence of kwashiorkor patients, it was shown that cornmeal diets supplemented with dried skim milk showed no statistical differences in weight gain, nor in the protein and amino acid levels in the serum. It was concluded that the results indicate that fish protein concentrate may be of considerable value in the prevention of protein malnutrition.[30]

In contrast to this are the experiments which appear to show that in children suffering from kwashiorkor, and in which the major source of protein is fish protein concentrate, there is a problem in acceptability in children. The clinical and biochemical responses were similar to those receiving the diet containing skim milk, except that the weight gain was decreased on the FPC diet. It ap-

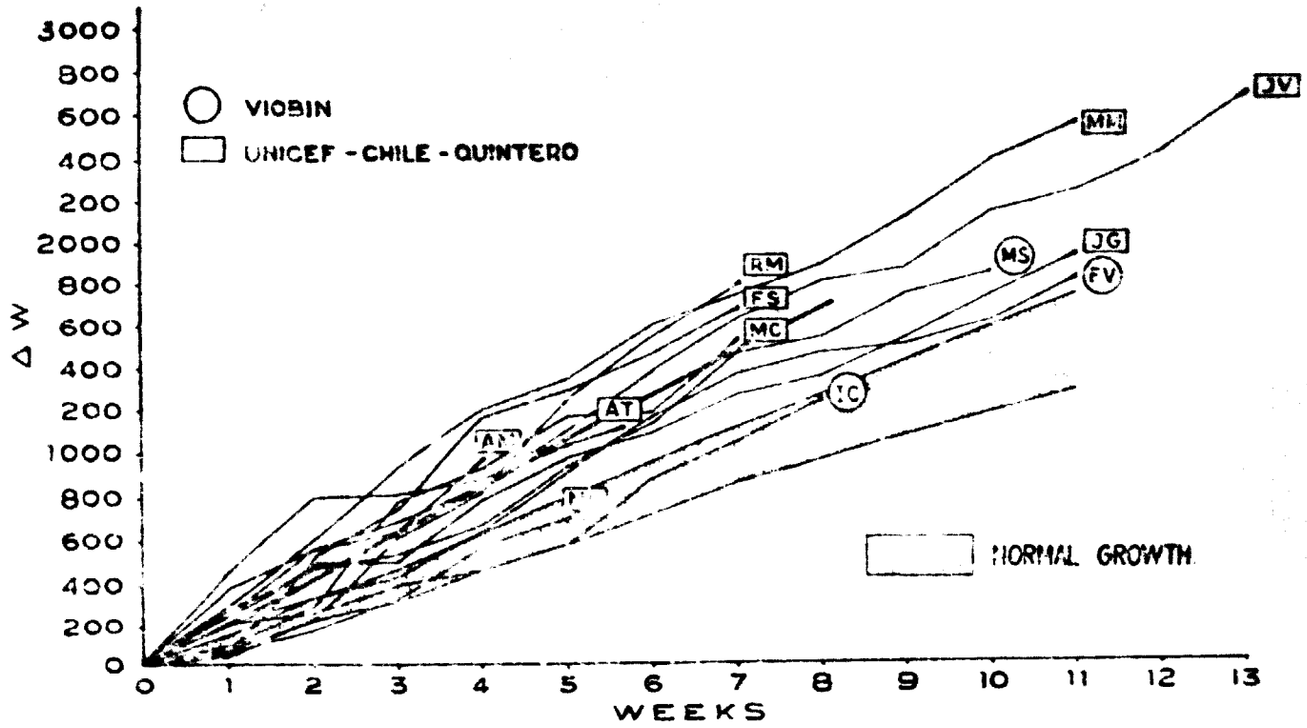

Figure 1. Weight Increment in Infants Fed Fish Protein Concentrate as Sole Source of Protein.

peared that there might be a shortage of available lysine in these diets. The addition of lysine to the diet, however, did not cause any significant increase in weight gains.[31] It has been noted earlier that the limiting amino acid in most FPC is methionine, and that this is decreased significantly upon heating. It is to be noted that the diets reported by Dr. Gopalin were cooked in the presence of sugar before being fed, and secondly it was assumed that lysine was the limiting amino acid. It is thus possible under the conditions of the experiment that the limiting amino acid methionine was further reduced by the cooking and thus led to the lower weight gains experienced in the experiment. In a similar set of experiments Graham found that in marasmic infants aged 5 to 54 months fed wheat enriched with fish protein concentrate as the only source of protein the weight gains observed were closely similar to milk. A significant difference, however, was noted in the ability of the fish protein concentrate diets to correct hypoalbuminemia.[29] The exact reasons for this difference have not been determined. In the convalescing subjects the fish protein concentrate did support nitrogen retentions and growth equivalent to that of milk-based diets.

The data thus far reviewed indicates that there is no question as to the nutriture of fish protein concentrate in normal experimental animals and humans from infancy onward. From a nutritional standpoint, however, there are some data which at the present time are

incompletely explained.

As a supplemental food in wheat, corn or other cereal diets, FPC offers an excellent protein for fortification. In products in which the FPC can be incorporated with cereal mixtures, where it does not have to contribute a functional property (for example, in bread, pastas, cookies, etc.), the product is acceptable to most populations at values approximating 10% of the total mix. There seems to be no large difference between FPC made from oily or non-oily fish, although acceptability appears to be better for non-oily fish.

From the standpoint of supplying protein to the world's population, FPC has significant attributes. The cost of the material appears to be lower than most other food protein sources. The product either in its present form or in alternate forms can be made acceptable to large populations. The nutritional qualities of the protein are excellent, and in addition, its stability is excellent in that it is able to retain its nutritional qualities without special attention being paid to its storage conditions.

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