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continuing in this issue:

the Fish Protein Concentrate story

6. Quintero Fish Protein Concentrate: Protein Quality and Use in Foods

ENRIQUE YANEZ, ITA BARJA, FERNANDO MONCKEBERG, ALEJANDRO MACCIONI and GONZALO DONOSO

ABOUT THIS SERIES

☐ *Continuing a series—previous installments appeared in 1967 issues of* Food Technology *as follows: Parts 1–3, July, p. 70; Part 4, Aug., p. 56; Part 5, Sept., p. 70.* ☐ *The series will continue in a future issue.*

■ THE MANUFACTURE of Fish Protein Concentrate is particularly suited to conditions existing in Chile. An abundance of raw material available there is one decided advantage, and lysine-rich FPC is especially suited for enrichment of lysine-deficient products from wheat (a staple in Chile).

In 1956 the government of Chile, with the financial and technical assistance of UNICEF, established a pilot plant at Quintero for production of defatted, deodorized FPC suitable for children. This plant is now able to manufacture annually 300 tons of FPC from the local hake, *Merluccius gayi.*

Manufacturing Process

THE PLANT AT Quintero consists of a horizontal steam-jacketed air-swept raw fish dehydrator; a horizontal steam-jacketed rotary extractor with integral cloth filters and the necessary connections for vacuum, solvent flow and steam stripping; a solvent recovery and storage system; a hammer mill for the dehydrated meal; and a hammer mill, flour sieve and bagging arrangement for the deodorized product (see Fig. 1).

Raw fish is chopped and then dried in a steam-jacketed horizontal vessel. Drying temperature ranges from 70–100°C and is controlled by adjusting the rate of air circulation. It takes about six hours to dry a two-ton batch, which yields about 400 kg of dried meal containing 5% moisture. The meal is then ground to pass through 1/16" mesh screen.

Defatting and deodorizing of the ground meal is accomplished by solvent extraction in a jacketed rotary extractor. Six to eight successive washings are made; initially hexane and ethanol was used, later only 95% ethanol. The unit has a total volumetric capacity of 4500 L and is equipped for use with either hexane or ethanol, or both.

The solvent is removed and recovered first by draining the cake, and then by agitation and heating under vacuum. Residual solvent is removed completely by vacuum steam

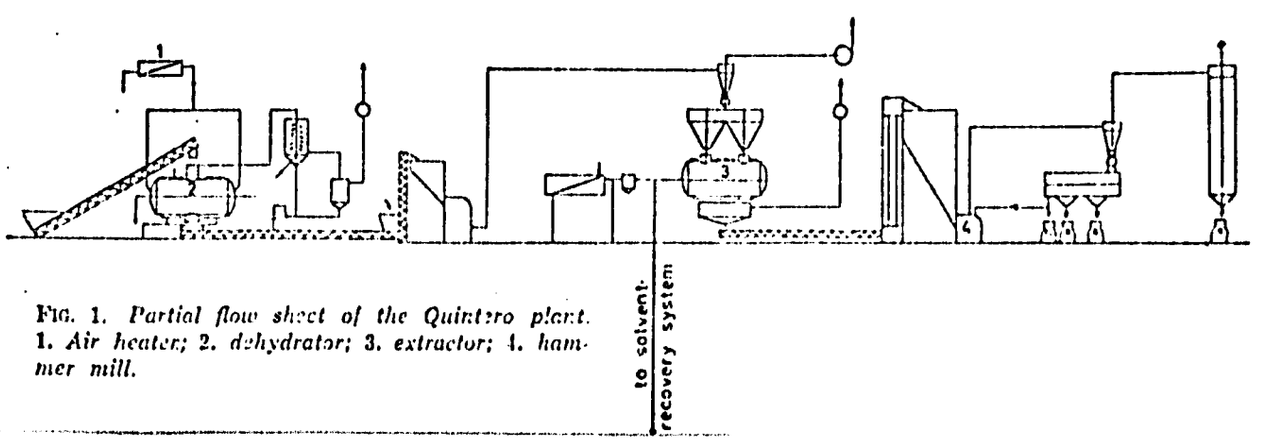

FIG. 1. *Partial flow sheet of the Quintero plant. 1. Air heater; 2. dehydrator; 3. extractor; 4. hammer mill.*

distillation. The temperature during this operation does not exceed 80°C.

The dried extracted product is finished by grinding and sieving to give a powder passing 1/16" mesh screen.

This powder is of very light tan color, practically odorless and tasteless. The yield is about one-seventh the weight of the fresh starting material. Hexane in the miscella is recovered by distillation and condensation, followed by gravity separation of entrained water. Ethanol is recovered from the miscella by simple distillation, followed by standardization to pH 5 with hydrochloric acid and rectification to 95% by volume (Allen, 1963).

Chemical Composition and Protein Quality

THE COMPOSITION of the hake used in the manufacture of FPC is quite variable, especially in its fat content (Table 1). This might be important in the intensity of the treatment necessary to reduce the fat content below the maximum amount permitted.

The chemical composition of different batches of Quintero FPC is given in Table 2. Shown for comparison is the composition of a similar product manufactured in the United States (Viobin Co.). The protein content of the samples is uniformly high (over 73%), the fat content is very low, and water is present in amounts compatible with good storage properties.

The protein quality of different batches of FPC was determined in rat feeding studies as net protein utilization (NPU) according to the method of Miller and Bender (1955) in diets containing FPC at the 10% protein level. The protein quality was good (Table 3), comparing well with values obtained in our laboratory for casein at a similar concentration (number of assays 10, average 71.3, standard deviation 2.6, mean error 0.8). NPU values for FPC after months of storage under somewhat unfavorable conditions (paper bags, room temperature, warehouse adjacent to seashore) corresponded to those of a protein of good quality. Such was the case with batch FF-A 23 (Table 3) which was assayed more than two years after the date of manufacture.

The nutritional properties of proteins can be damaged through heat treatment or solvent extraction procedures (Morrison *et al.*, 1962), especially in regard to the biological availability of lysine and sulfur-bearing amino acids (Morrison *et al.*, 1963). Miller (1956) has reported that methionine has been shown to be the limiting acid in fish meals of different origins. The possibility that lysine or methionine becomes the limiting amino acid in FPC was investigated by adding these amino acids to two different batches of the product.

Table 4 shows that NPU was increased by adding methionine, but not by adding lysine. The results can be interpreted in the sense that, for the rat, sulfur-containing amino acids are the ones that limit the biological value of the Quintero FPC.

The conservation of a high content of biologically available lysine is important even when it is not the limiting amino acid. Such is the case for fish protein, where the excess lysine -if biologically available- can be of value when fish products are added to lysine-deficient diets.

Lysine availability is also a measure of the intensity of heat treatment experienced by a protein concentrate during its manufacture (Carpenter, 1958). Table 3 (last column) shows the "available lysine" content of different batches of FPC, determined by the chemical method of Carpenter (1960). Recent reports, however, have shown that this amino acid may not be the only one that is altered (Donoso *et al.*, 1962).

Table 1. Chemical composition[a] of dried hake (*Merluccius gayi*).

Sample	Ash	Fat	Protein[b]
Quintero 1	15.6	7.7	73.4
Quintero 2	18.4	8.5	73.2
Quintero 3	13.1	14.8	70.1
Santiago 1[c]	18.3	6.3	74.8
Santiago 2[c]	16.7	9.1	75.0
San Antonio 1	12.2	26.6	61.2
San Antonio 2	19.1	9.2	73.3
San Antonio 3	13.7	15.7	69.1

[a] g/100 g dry matter.
[b] N × 6.25.
[c] Eviscerated.

Table 2. Chemical composition of Chilean (Quintero) FPC (g/100 g).[a]

Batch[b]	H_2O	Ash	Fat	Protein
FF-HA-7	5.9	16.5	0.2	76.4
FF-HA-9	8.2	14.9	0.2	76.2
FF-HA-10	8.3	16.3	0.2	74.7
FF-HA-19	7.1	17.2	0.2	73.8
FF-HA-20	8.4	17.3	0.2	73.9
FF-A-8	6.7	14.9	0.3	78.1
FF-A-12	9.4	14.1	0.2	76.1
FF-A-13	7.7	18.5	0.2	72.8
FF-A-14	6.3	16.6	0.2	76.8
FF-A-15	6.4	13.9	0.1	74.2
FF-A-16	6.4	19.0	0.1	74.1
FF-A-22	6.4	15.9	0.2	76.6
FF-A-23	7.1	11.0	0.2	81.6
Viobin	5.6	20.9	0.1	71.9

[a] Determined by AOAC methods.
[b] HA = hexane- and ethanol-extracted; A = ethanol-extracted.

Table 3. Net protein utilization[a] and "available" lysine (A.L.)[b] of Quintero FPC.

Batch	NPU[a]	A.L.[b]
FF-HA-9	66.9	8.4
FF-HA-10	67.9	—
FF-HA-19	—	9.5
FF-HA-20	—	8.4
FF-A-8	64.3	8.3
FF-A-11	70.7	8.5
FF-A-16	—	8.8
FF-A-23	63.5	8.4
Viobin	69.2	8.7

[a] Net protein utilization operative at 10% protein calories.
[b] Available lysine (g/100 g crude protein).

Table 4. Effect of amino acid supplementation on NPU[a] values of Quintero FPC.

	NPU		
Batch	Control	+ 0.5% L-lysine	+ 0.3% DL-Methionine
FF-HA-9	66.9	66.2	70.7
FF-HA-10	67.9	61.3	70.2

[a] Net protein utilization operative at 10%

. . . the FPC story . . .

To judge the importance of heat treatment on the protein quality of hake, two batches of fish (whole, 500 g per batch) were subjected in the laboratory to three different methods of drying: freeze-drying; oven drying for 6 hr at 105°C, and at 200°C.

Table 5 shows the values for available lysine and NPU (10% level) of these products. It is surprising to see that the NPU of the freeze-dried materials is quite similar to that of the 105°C oven-dried products. Available lysine was lost in these preparations, which is not reflected in their NPU values. This could be expected to occur in a protein that is limited by its sulfur-bearing amino acids rather than by lysine. More in line with expectations, the intensely heated preparations (200°C) lost considerable protein quality and available lysine.

Available lysine is of the same order in the different batches of Quintero FPC as in the freeze-dried laboratory preparations (Tables 3, 5). This could be interpreted to mean that the protein damage in the industrial product is slight.

Growth and Toxicity Studies

Animal tests. The toxicity of Quintero FPC was investigated by Allison *et al.* in 1957. They found no evidence of toxicity of FPC for rats fed diets containing 5% to 25% of the product. However, the material used in those tests was of an experimental nature: the fish had been dried by a process no longer in use, then sent to Berlin for deodorization by extraction with heptane and ethanol according to the procedure of Dabsch (Yanez *et al.*, 1961).

In our studies, a group of 35 (18 male, 17 female) weanling albino rats were fed a diet containing 21% FPC (batch FF-A-23) for 14 weeks. Their growth performance and general aspect proved similar to those of a group of animals that received a diet containing 24% casein. Histology of liver and kidney was normal.

A small number of rats were fed a diet containing 30% FPC (batch FF-HA-19) for three generations. Each generation consisted of three male and three female rats (18 total) that received the FPC diet from weaning. The first generation was sacrificed at 20 weeks of age, the second generation at 42 weeks, and the third generation at 25 weeks.

Growth of the rats was comparable to that shown by a similar group of animals that received a commercial rat food. Both groups looked lively and behaved normally, and their coat was always in good condition. No special pathological signs appeared in any of the animals, and no abnormalities were recorded in the macroscopic postmortem examination. The histology of liver and kidney

Table 5. Net protein utilization and "available" lysine (A.L.) of hake (*Merluccius gayi*) dried at different temperatures (laboratory preparation).

	NPU	A.L.*
Sample 1		
Freeze-dried	63.0	7.9
Dried at 105°C	66.4	6.7
Dried at 200°C	17.2	0.8
Sample 2		
Freeze-dried	65.1	8.4
Dried at 105°C	65.2	8.2
Dried at 200°C	31.9	5.1

* g/100 g crude protein.

in the 18 rats examined was normal and comparable to that of the animals on the commercial rat food.

Tests with infants. The lack of toxicity in tests with rats permitted us to try FPC as the only source of protein in feeding a group of five human infants three to five months old. The babies received a formula containing 22.5% in water of the following preparation (g per 100 g): FPC 15, butter 12, maize starch 17, sucrose 18, lactose 35, mineral and vitamin supplement 3. The formula was autoclaved and fed to the infants in the quantity of 150 ml per kg of weight.

The increase in weight over the two months of observation was 25 g per day, which can be considered satisfactory for children of this age. A comparable group of children was treated in the same way—but with Viobin FPC as the source of protein—with results entirely similar to those related above. These trials are definite proof of the good quality of the protein in FPC.

FPC as Food Additive

IN THE ADDITION of FPC to foodstuffs, two questions have primarily concerned us:

a) how does added FPC affect food acceptability? and
b) what is the protein value of the product, once enriched?

Chile is a wheat-eating country. Surveys show (Ballester *et al.*, 1962) that at least 36% of the total calories and 29% to 38% of the protein of Chilean diets are derived from cereal products. The most important of these are: bread flour (80% extraction); parboiled wheat (*trigo mote*); flour and semolina pastes (noodles, spaghettis, soups, pastas, etc.); roasted whole-wheat meal (*harina tostada*); lime treated cooked wheat (*mote de trigo*); and so-called baby foods (up to 20% wheat flour).

Acceptance—A problem. The odor and taste of fish are generally disliked by Chileans. Although Chile has the largest fish consumption per capita (10 kg per year) in Latin America, this figure is small when compared with that of Japan or the Scandinavian countries.

This means that products with the smell and/or taste of fish can be said, *a priori*, to be not readily acceptable to the national palate. Consequently the food products to be enriched should have a good masking capacity for the slight but definite fish odor and taste of FPC.

A promising area. The most promising of the wheat products to be enriched with FPC seemed to be: 1) bread; 2) pastas; 3) roasted whole wheat meal.

Bread

Acceptability studies. The bread consumed in Chile is a small loaf, 80-150 g, called "marraqueta." Bread was prepared containing 0, 3, 6, 9, and 12% by weight of FPC. This addition considerably and progressively deteriorated the properties of the dough and the quality of the baked bread. By altering the standard fermentation and baking times and by the addition of sugar, a suitable though darker bread was produced (Donoso *et al.*, 1963).

Adults and school children were given the enriched bread. Three groups of 30 adults each were used; university students of both sexes, nursing mothers, and male manual workers. The test consisted in asking each to give two judgments:

1) on the normality (normal-different) of color, odor, and taste; and

2) on the taste (good, indifferent, bad)
of the samples of bread (0, 3, 6, 9, and 12% FPC in random order).

Table 6 shows the results of the acceptability test in

Table 6. Acceptability ratings* of bread enriched with FPC at different levels.

Level of enrichment (%)	GOOD			INDIFFERENT			BAD		
	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	16	12	5	14	4	1	0
12	18	22	16	5	8	6	7	0	8

* Total number of tasters = 30. W = workmen, M = mothers, U = university students.

adults. At the 3% level there is little difference in acceptability between the enriched sample and the control, but at the 6% level poor quality judgments increase especially among university students, a more sophisticated group. Reasons are not available for the reversal of the trend for this group as shown by ratings of the 9% FPC bread.

The acceptability of bread enriched at the 9% level was also tested on 175 school children of both sexes, seven to fourteen years old, from working class families.

At lunchtime their normal bread was exchanged for a "marraqueta" (80 g) of enriched product. No bread was left over, every child said he liked it, and 140 of 175 expressed personal preference for the FPC-enriched bread.

While these results are very encouraging, there are limitations involved in the method employed. Long-term feeding trials must be carried out before the enriched product is used in mass feeding programs.

Protein quality and protein value. The net protein utilization operative (NPUop) of bread, bread enriched with FPC at the 6% level, bread enriched with 12% skim milk powder, and bread plus FPC and 0.5% L-lysine hydrochloride, was determined according to the method of Miller *et al.* (1955). The bread and the bread enriched with FPC was of the same batch as the one used in the acceptability tests.

Table 7. Properties of bread enriched with FPC, with FPC plus lysine, and with skim milk powder, including net protein utilization operative (NPU op) and standardized (NPU st) on protein concentration (P)* and protein value (NDp Cals%)*.

Material	NPU op	P	NDp Cals%	Score	NPU st
Bread	35.4	10.4	3.7	45	35.4
Bread with 6% FPC	42.8	14.3	6.1	72	48 9
Bread with 6% FPC + 0.5% L-lysine HCl	58.8	14.6	8.6	70	71.4
Bread with 12% skim milk powder	44.5	13.5	6.0	71	50.4

* Protein calories percent total calories.
* Net dietary protein calories percent.

Table 7 shows the experimental values obtained, the protein concentration of the different materials, and the protein value as net dietary protein calories percent (NDp Cals%), calculated according to Miller *et al.* (1963). It also shows the calculated net protein utilization standardized (Miller *et al.*, 1961), which can be considered as a biological equivalent to the protein score (also given).

The addition of the FPC to bread increases the protein value (as NDp Cals%) of bread from 3.7 to 6.1, an increase similar to that obtained by the addition of 12% skim milk powder. The increase is obtained through the higher content of protein in the enriched bread (14.3 vs 10.4) and also through an increase in its protein quality (48.9 vs 35.4).

The protein score of the FPC enriched bread shows that the increase in quality is much smaller than it should have been (72 vs 48.9). This is brought out clearly by the increases in protein value and protein quality to the predicted level through the addition of lysine to the FPC enriched bread. Possibly the protein of the enriched bread may have been damaged by heat. This suggestion is strengthened by the considerable loss in protein quality also suffered in milk enriched bread. It should be remembered that we use only 80 g per unit and that therefore the proportion of crust to interior is much higher than in the type of loaf commonly produced in countries well to the north of the equator.

Pastas

Acceptability studies. Spaghettis and other pastas are of considerable importance in Chilean diets, especially in lower income groups. Dry pastas offer, from the all-important food supply point of view, a very definite advantage over bread, i.e., their good keeping quality (3-6 months). Use of FPC in pastas would considerably simplify mass feeding programs through centralized manufacture of the enriched product, which permits proper control.

An important technical consideration is the use of only low temperatures in the industrial preparation of spaghettis and other pastas. Such products are therefore less likely to suffer protein damage than is bread subjected to the high temperatures involved in baking.

For our experiments, a commercial firm prepared spaghettis enriched with FPC at the 10% level. The usual methods involve preparation of a dough (using semolina as a base), lamination, cutting, and drying at 37°C for 18 hr. Because of the higher absorption of water by the dough containing FPC, the drying had to be extended to a total of 36 hr.

The acceptability studies were conducted with adults and children. Adults were hospital personnel (150, male and female) and hospital patients (300, male and female). They were given a ration of 300 g of cooked FPC enriched spaghetti, without knowledge of the actual composition.

In no case was there a refusal or suspicion of the real nature of the spaghetti. Many said spontaneously that the dish was better than what they usually have.

In total, 150 school girls six to fifteen years of age were subjects for testing. The children regularly attended a school of a benevolent society. They were given a ration of 300-400 g of cooked spaghetti three times a week for three months. The spaghetti was cooked in plain water, and salt and a little shortening (3 g per ration) were added before serving. Acceptability was excellent. No case of refusal, initial or acquired, was reported. Also, no abnormal gastrointestinal complaints were reported by the girls in their medical check-up.

Protein quality and protein value. The NPU of spaghetti, FPC-enriched spaghetti, and of the mixture of semolina plus fish used in their manufacture was determined by the method of Miller *et al.* (1955). The spaghetti

was of the same batch as that used for the acceptability tests in school children.

Table 8 shows the experimental values obtained, the protein concentration, the calculated NPU, the protein scores from tables (and the protein values as Net Dietary protein Calories percent) of the different products tested.

Table 8. Properties of industrially prepared enriched spaghetti and of the mixture used in preparation of the enriched spaghetti, including net protein utilization operative (NPU op) and standardized (NPU st), protein concentration (P)[a] and protein value (NDp Cals%)[b].

Material	NPU op	P	NDp Cals%	Score	NPU st
Spaghettis	38.4	11.9	4.6	47	40.2
Enriched spaghetti	47.0	19.1	9.0	73	64.4
Mixture used in manufacture of enriched spaghetti	51.6	19.2	9.9	73	71.8

[a] Protein calories % total calories.
[b] Net dietary protein calories %.

The results show that there is a relatively small loss in protein quality (NPUop values of 47.0 vs 51.6) of the enriched spaghettis through the industrial processes. They also show that sufficient biologically available lysine is present in FPC (batch FF-A-23) to supplement the deficiency of this amino acid in wheat protein. The results also confirm that through the preservation of the protein quality of FPC, the enrichment of spaghetti is superior to that of bread. Finally, the protein value of the enriched material is excellent and more than sufficiently good for the age group tested, judging from the recommendations of Platt *et al.* (1961).

Roasted Whole Wheat Meal

Acceptability studies. Roasted whole wheat meal (*harina tostada*) is consumed by lower income groups in Chile, especially in rural areas. It is either homemade (by roasting the whole grain over an open fire on a large earthenware dish called "callana") or industrially manufactured by direct fire roasting for 10 min at about 250°C. The roasted grain is ground in a stone or hammer mill, and the final product is slightly tan with a pleasant odor and taste. It is consumed in the form of a gruel, prepared by the addition of either cold or hot water (no cooking); milk is sometimes used. This gruel is called "ulpo" (*oolpuch*) and is much liked by toddlers and school children. Wide acceptability and the considerable advantage in needing no culinary preparation seem to make it very suitable for enrichment with FPC.

A mixture (MHTP5) was prepared which contained, in g per 100 g, roasted whole wheat meal 70, FPC (batch FF-A-23) 10, refined sugar 20.

The acceptability of this mixture was tested at a health center in a poor community by giving 250 g of a 33%

Table 9. Properties of roasted whole wheat meal, MHTP5[a], including net protein utilization operative (NPU op) and standardized (NPU st), protein concentration (P)[b] and protein value (NDp Cals%)[c].

Material	NPU op	P	NDp Cals%	Score	NPU st
Roasted whole-wheat meal	41.2	8.8	3.6	47	41.2
MHTP5[a]	70.5	11.6	8.2	72	81.6

[a] Roasted whole-wheat meal plus FPC.
[b] Protein calories % total calories.
[c] Net dietary protein calories %.

gruel in cold water to 300 toddlers and 50 adult women. Everyone accepted it readily, and many asked for a second helping. The women were not aware that the product contained fish.

Protein quality and protein value. The protein quality of the mixture was determined according to the method of Miller *et al.* (1955). Table 9 shows its NPUop, NPUst, protein concentration, and protein value. The heat treatment suffered by the wheat grain in roasting can reduce the quality of the protein. In fact, Ballester *et al.* (1962) found that the value of 40.3 (NPU units) for wheat protein falls to 34.1 for roasted whole wheat meal.

An increase in the protein quality of wheat meal should be expected with the addition of FPC, because the excess lysine in fish protein should supplement the deficiency of this amino acid in wheat protein. Table 9 shows that this indeed occurs at the 10% level of addition in mixture MHTP5 (one part of protein from FPC and one from wheat). In fact, the mixture showed an NPU even higher than that of the FPC itself (Table 3; batch FF-A-23).

This means that FPC contains enough lysine to supplement the deficiency (made greater by roasting) in this amino acid of wheat protein. This conclusion is in good agreement with that obtained in the biological assay of protein quality of bread and spaghetti enriched with Quintero FPC.

An important point to consider is that 10% added FPC increases the protein value of roasted whole wheat meal from 3.6 to 8.2. This last figure would make the product highly recommendable for toddlers and school children, and even suitable for infants.

Outlook for FPC

THE FUTURE of FPC depends on many factors, the most important of which is its price. It is difficult to calculate the real cost of the Quintero product because the plant is such a mixed enterprise: it was built with the technical and financial aid of an international agency, it is owned by the Chilean government, and it is operated by private industry. The product has been sold at 35 to 50 cents per kg. This means that per gram of protein it is half the price of skim milk powder. However, milk is more readily acceptable than FPC, its protein is of better quality, and it also carries energy value (calculated as sugar) equivalent to 5 cents per kg. All these points would make half the price not low enough for economic competition.

But price alone is not the only deciding factor. FPC has the advantage over milk in that it is 75% protein. The addition of a small quantity (5-10%) to staple foods low in protein can increase their protein value to adequate levels, without considerable alteration of their normal organoleptic properties. Also, if a local product can partly replace milk now imported, the balance of payments will come into the picture. The government—which in Chile is the principal purchaser of milk because of free milk and other mass-feeding programs—can be expected to favor the local product.

The cost of FPC is strongly influenced by two factors.

1) *Near-complete fat extraction.* If 1% of fat were acceptable (such as in FAO specifications for grade B FPC), the extraction procedures would be considerably less costly and perhaps the product would be just as useful when added to staple foods.

2) *Price of fish used.* Hake (lean, medium sized fish) will never be the cheapest, because it has a large market as fresh, filleted, frozen, and canned fish. Millions of tons of anchovy (*Engraulis ringens*) are caught yearly in Peru and Chile and made into meal used in animal feeding. The price of anchovy meal is one-fifth to one-sixth of that of FPC. Perhaps the final competing product should be FPC made from anchovy or other fish meal. No fully accepted technology for the manufacture of such a product exists.

• EARLIER articles in this series were published in *Food Technology* July (p. 70), August (p. 56) and September (p. 70), 1967.

• Planned future articles will describe development of laboratory and pilot plant scale equipment, a new approach to FPC production, and a government view.

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