PREPARATION OF FISH PROTEIN ISOLATE AND HYDROLYZATE *(Mugil cephalus)* AND THEIR INCORPORATION INTO MEXICAN FOODS[1]

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SUMMARY

Fish protein isolates (FPI) and hydrolyzates (FPH) were obtained from mullet *(Mugil cephalus)* through alkali solubilization and HCl precipitation for FPI, as well as enzymatic hydrolysis for FPH. The powdered products showed solubilities of 50 and 89%, and emulsifying capacities of 36 and 39 ml oil/100 mg for FPI and FPH, respectively, with protein contents of 90% and oil contents lower than 1.6%. Both products were used to enrich cereals and legumes in order to increase their protein content and quality. The resulting mixtures were used to prepare common Mexican dishes. When up to 20 and 35% of the total protein was provided by FPI and FPH, respectively, the dishes were well acepted by 70% of the panel.

INTRODUCTION

Despite a potential catch of two million tons of fish per year in Mexico, fish consumption by the population barely reaches 9 kg *per capita*; furthermore, this average figure does not reveal that consumption is concentrated in some urban areas, while for rural population these products are virtually unknown. This may be partly

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explained on the grounds of eating habits, as well as on the very high prices fish reaches at the consumption point. Most of the people in Mexico are settled in the central plateau of the country and sea products have to be transported, usually frozen or refrigerated, very long distances. On the other hand, the income of most Mexicans does not allow them to pay the high resulting prices.

Among the approaches that have been proposed to increase the consumption of fish protein in Mexico, some non-traditional techniques have been particularly attractive since they provide easy preservation and transportation, and permit preparing products that can be incorporated into traditional dishes. Spinelli (1, 2) and Rasekh and Metz (3) proposed isolation techniques; Sen *et al.*(4), Hale (5), Cheftel *et al.* (6) and Rutman and Heimlich (7) have reported hydrolysis procedures. This study was conducted in order to adapt protein isolation and enzymatic hydrolysis procedures to mullet *(Mugil cephalus)*. A second part deals with the incorporation of the isolate prepared into commom Mexican dishes such as tortillas and refried beans, as well as the preparation of instant soups containing the hydrolyzate, provided its high solubility.

MATERIAL AND METHODS

Raw Material for Hydrolysis and Isolation

Mullet *(Mugil cephalus)* was purchased from Productos Pesqueros Mexicanos, S.A., and conditioned by washing and grinding the fish meat through a Hobart Dayton meat grinder, provided with plate with 1/8 in diameter holes.

Enzymes

Papain showing a pH optimum of 5.5 and optimum temperature of 60°C, with a standarized activity of 0.32 M.C.U. (Milk Clotting Units), was obtained from Laboratorios Mixim, S.A. HT Proteolytic-200 and brew (N) zyme, both from Enmex, S.A., are food-grade bacterial proteases obtained by controlled fermentation of *Bacillus subtilis var*; they show a pH optimum of 7.5 and optimum temperature of 50°C, a standardized activity of 200 and 700 NU/g (Northrop Units per gram) respectively, and a broad substrate specificity.

Hydrolysis Procedure

Based on data reported by Sen *et al.*(4), Cheftel *et al.*(6) and Rutman and Heimlich (7), the enzymatic hydrolysis was performed in batch experiments using 10g fish meat suspended in 10 ml water plus the enzyme, and adjusting the pH and temperature to reach the manufacturers recommendations for each protease. In order to avoid microbial contamination, thermal treatments (121°C, 15 min) were performed before and after hydrolysis for inactivating the enzymes, and

to ensure the quality of the final product. The following variables were tested: time (1 to 36 hours) and enzyme concentration (0.1 to 0.4g of enzyme/100g of fish meat). All hydrolyzates were filtered in order to obtain a liquid fraction assayed for total nitrogen solubilized, using the total nitrogen determination from the AOAC (1975) methods.

Isolation Procedure

Mullet protein isolation essentially consisted of the following operations: a protein solubilization, with 0.12 NaOH at 70°C, H_2O_2 decoloration protein precipitation at the proper pH, filtration, oil extraction, solvent elimination and drying, based on the methods of Tannenbaum, Ahern and Bates (8), Meinke, Rahman and Mattil (9), and Rasekh and Metz (3). Six different experiments were performed for testing the variables for each operation. The most adequate conditions were selected after the sensory evaluation results of each isolate prepared. This evaluation considered the two physical characteristics most likely to be affected by the procedure of each batch: color and aroma, which were graded using an arbitrary scale for an acceptability test. These tests were sequentially performed, so that the results of each experiment could be applied to the following: Protein solubilization was carried out for periods of time of 50 and 120 min. Decoloration was performed with 2 and 5% v/w H_2O_2, during 20 to 45 min at temperatures from 40 to 60°C. The protein was precipitated at pH 4.5, at 25 and 50°C; a double precipitation was also performed. The oil was extracted using isopropanol; isolate ratios (v:w) of 0.8:1, 1.6:1 and 2.5:1, performing it 1 to 3 times at temperatures of 22, 60 and 75°C during 15 and 30 min. The excess solvent was eliminated with hot water (80°C) using three washings at a water:isolate ratio of 1.2:1 (v:w), and a further experiment was done with no washings. Drying was performed in a vacuum oven at 30 and 37°C.

Analysis of Products

Fish protein hydrolyzate (FPH) and isolate (FPI) were analyzed according to the AOAC (10) procedures for proximate analysis, and to the method recommeded by Fernández, Costarrica and Parrilla (11), for microbial analysis. Solubility index was determined for both products according to the technique reported by Rasekh and Metz (3) and López (12), and emulsifying capacity according to Carpenter and Saffle (13) adapted by Rasekh and Metz (3) and Téllez-Sill (14). In order to select the best FPI, color and aroma were evaluated by a panel of non-experts, for grading them using an arbitrary scale.

Formulation and Evaluation of the Mixtures

Mixtures of either FPH or FPI with cereals and legumes meals were formulated, calculating the proportions needed for 1) the high-

est chemical score; 2) protein contents higher than 15g per 100g of mixtures; 3) a protein quality of at least 80% of the NPU casein value, and 4) an amino acid content no less than 75% with respect to the FAO/WHO 1973 provisional pattern for lysine, tryptophan and sulfur-amino acids. The FPI mixtures fulfilling such characteristics were utilized to prepare tortillas and refried beans, and their attributes (color, aroma, flavor, texture and palatability) were evaluated in comparison with tortillas and refried beans not containing the isolate. Salt and spices were added to FPH mixtures for the preparation of instant soups, since a highly-soluble hydrolyzate is expected (solubility higher than 80% of the total solids); these soups were evaluated through a test of attributes, and for acceptability. Both evaluations were performed with non-experts panels, following the techniques recommended by Hirsch (15), and statistically evaluated, using an unpaired "t" Student test (16).

Evaluation of Protein Quality

Protein quality was evaluated for both FPH, FPI and their mixtures. Amino acid analysis was done by ion exchange chromatography using the techniques of Moore and Stein (17) and tryptophan by the method by Spies and Chambers (18). PER and NPU were measured according to the techniques of Campbell (19) and Miller (20), using casein as a control.

Equipment

For testing the final hydrolysis conditions in larger batches, the following pilot plant equipment was utilized: meat grinder (Hobart Dayton Mexicana); steam-jacketed reactor with agitation (Poli-Ingenieros, S.A. de México); evaporator (LUWA S.G. Zurich), and Niro Atomizer dryer (Soeborg Denmark).

RESULTS AND DISCUSSION

Fish Hydrolysis

The total nitrogen recovery for the experiments with papain and HT-proteolytic-200 at different enzyme concentrations are shown in Figure 1. For all the protease concentrations employed, an inflection point was shown around nine hours of reaction time with nitrogen recoveries close to 80%; a longer period of time increased the recovery only by 10%.

The experiments with an enzyme concentration of 0.3% showed the highest nitrogen recovery. An enzyme concentration increase to values higher than 0.3% showed no further increase in soluble nitrogen recovery. Therefore, hydrolysis using 0.3% enzyme must be examined closer during the period of time before the plateau is reached (Figure 2), for the three proteases mentioned above, and

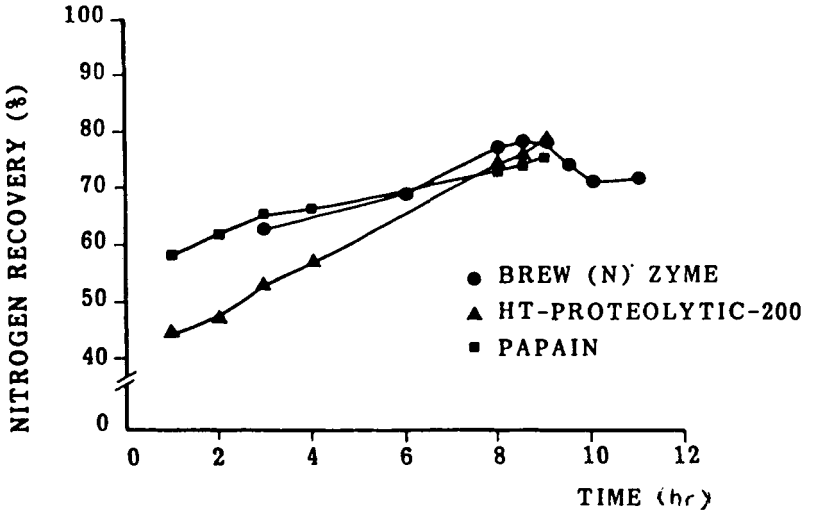

FIGURE 1

Percentage of nitrogen recovery during fish meat hydrolysis with papain and HT-proteolytic-200

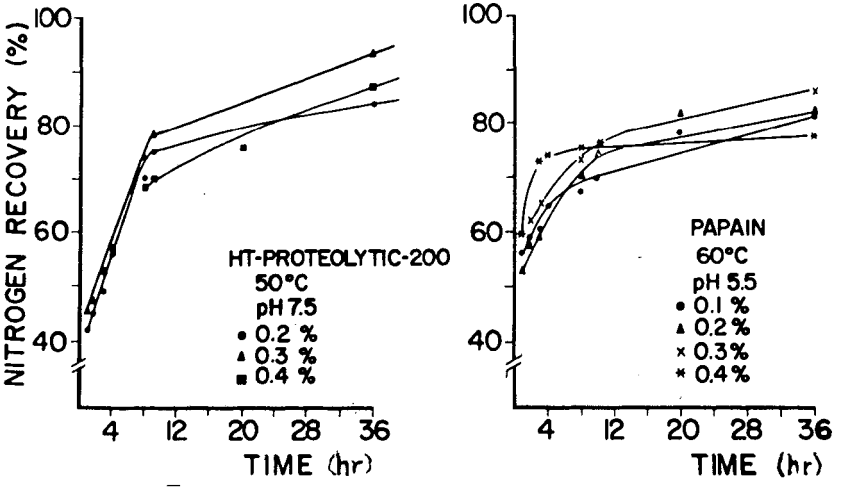

FIGURE 2

Percentage of nitrogen recovery during fish meat hydrolysis with papain, HT-proteolytic-200 and brew (N) zyme at 0.3% concentration

using the optimum pH and temperature for each enzyme as recommeded by the manufacturer. Given that the activities were very similar, obtaining a nitrogen recovery of 78%, 76 and 75% for brew (N) zyme, HT-proteolytic-200 and papain, respectively, it was necessary to take in account the price and availability of these enzymes. HT-proteolytic-200 showed the lowest price ($15.00/kg) and being a microbial enzyme, it has a good availability, just as brew (N) zyme which has the same source but is more concentrated ($17.00/kg). Papain is the most expensive of them all ($45.00/kg) (prices from the Mexican market for October, 1987). Therefore, HT-proteolytic-200 was selected as the most convenient enzyme, and the final hydrolysis conditions were: 0.3%, as enzyme concentration, 50°C, pH 7.5 and a reaction time of 8.5 to 9 hours.

A 16 kg batch of minced meat was further processed using the final conditions already established (Figure 3). Thirty-two liters of hydrolyzate were obtained, which were then filtered to yield a liquid fraction of 9% solid content; this was subsequently vacumm-evaporated at 60°C, to increase the solid content up to 30%. This concentrate was spray-dried at an inlet temperature of 143°C and outlet temperature of 90°C. The process showed a weight yield of 8% based on minced meat, a comparable value to that reported by Pigott (21). The protein recovery in the final powder with respect to initial raw material was only 38.5%. The difference with the results obtained at the laboratory may be due to inefficient filtration when working with this amount of material. The final product, yellowish-white in color, has a faint bitter taste, and a very slight fish odor.

Fish Protein Isolation

Protein solubilization at 70°C with 0.12 NaOH did not demonstrate a higher yield by increasing the period of time from 50 to 120 min; therefore, 50 min were selected as the appropriate time for solubilizing more than 90% of the protein. H_2O_2 was added to the protein suspension, and the mixture was held for different times. By increasing decoloration time up to 45 min and holding the temperature at 50°C and 0.3% H_2O_2 concentration, no improvement was shown. A higher temperature (60°C) did not yield a light-colored isolate. Only by increasing the H_2O_2 concentration up to 5%, the decoloration step showed a color improvement. A double precipitation step decreased the yield of the process, since the resolubilization of the first precipitate was not complete, and using temperatures higher than 25°C render a colloidal suspension too difficult to handle; therefore, a single precipitation step at 25°C was chosen. For the oil extraction, higher temperatures and isopropylalcohol (IPA) proportions gave better results; however, the results of Spinelli, Koury and Miller (1) show that IPA extraction at temperatures over 70°C destroy some functional characteristics such as the emulsifying capacity; a solvent; isolate ratio of 0.8:1 was chosen for performing three isopropanol extractions by holding it for 15 min during each extraction at 60°C in order to accomplish a minimal effect to the functional

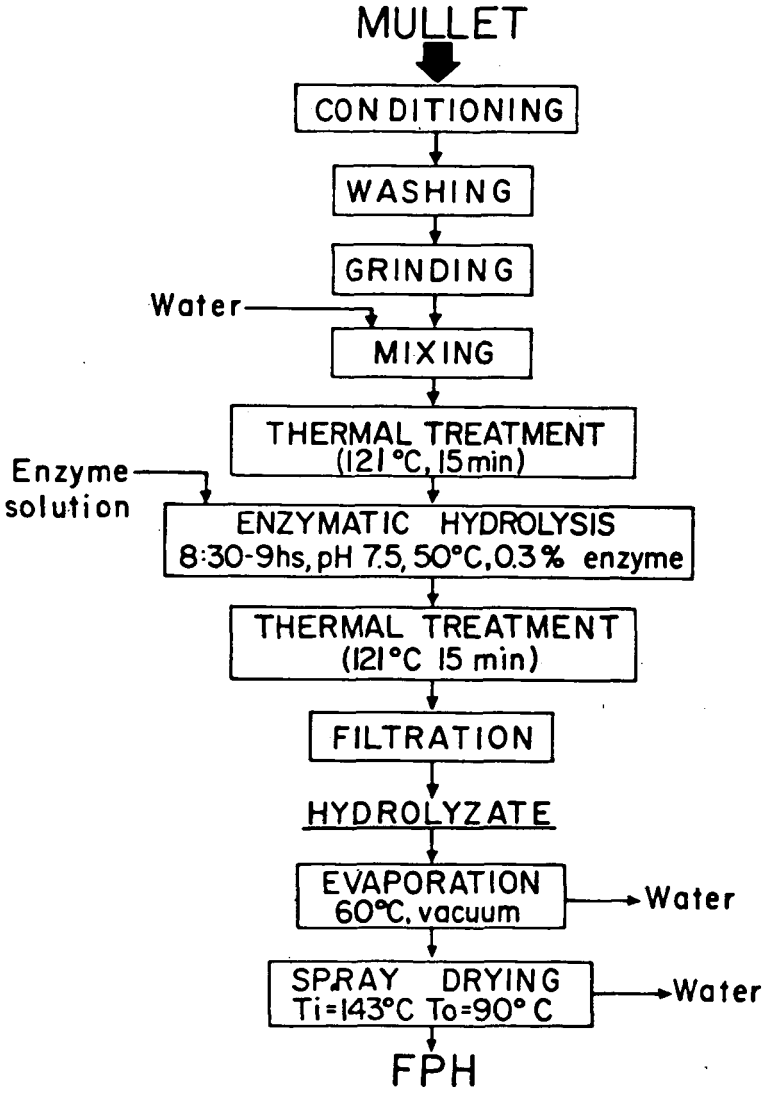

FIGURE 3

FPH final procedure

properties of the isolate. The excess solvent was removed with water washings of the wet isolate as recommended by Spinelli, Koury and Miller (2), but using hot water (80°C) to improve elimination. The experiment performed with no washings was discarded, given the fact that a 60% IPA would remain in the isolate. Vacuum drying at 30°C was selected in order to avoid secondary reactions due to the presence of oxygen and higher temperatures. The final product was ground and sieved through a No. 150 mesh, packaged and stored. The final FPI, prepared by following the parameters reported in Figure 4, revealed a very faint beige color and a slight and acceptable fish aroma, according to the panel test results.

The protein recovery for this process was around 51%, with a weight yield of 10.2% with respect to whole raw fish, and 12% with respect to mullet muscle. These values are comparable to those informed by Power (22) and Lawler (23), which run from 12 to 15% for deboned fish.

Characteristics of the Final Product

FPH and FPI proximate analysis appear in Table 1. The low fat content is an advantage, since it renders better flavor and odor characteristics as well as a greater stability against rancidity. The microbial analysis showed that these products are suitable for human comsumption (Table 2).

TABLE 1

PROXIMAL ANALYSIS OF FPH AND FPI

Determination (g/100g product)	FPH	FPI
Moisture	2.8	5.8
Protein	92.7	88.7
Ether extract	0.8	1.5
Ash	3.7	4.0

TABLE 2

MICROBIAL ANALYSIS OF FPH and FPI

Determination (CFU*/g)	FPH	FPI
Standard plate count	390	100
Plate count for molds	10	50
Plate count for yeasts	20	Negative
MPN coliforms	Negative	Negative

* CFU colony forming units.

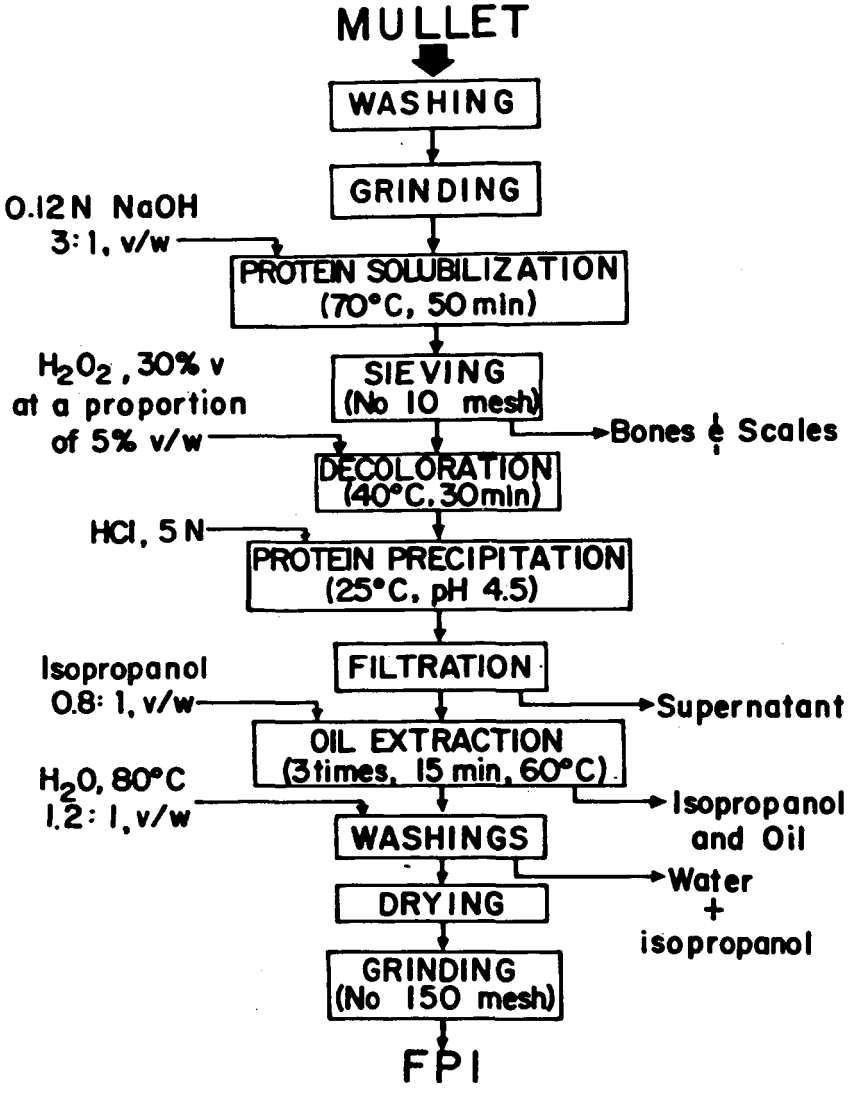

FIGURE 4

FPI final procedure

Both products were able to form dense and smooth emulsions with corn oil: FPH and FPI showed an emulsifying capacity of 327 and 326g of oil per gram of protein and an oil phase volume of 39 and 37.5% respectively, values higher than those of Spinelli, Koury and Miller (2); emulsions were very stable, not showing a phase separation at least within the 360 min of observation. With respect to solubility, FPH showed a very good index: 86 to 89% in the pH interval from 1 to 7; on the other hand, FPI was much less soluble: 5 to 10% for pH values between 3 and 7, and 15 to 40% in the pH interval from 1 to 2.5.

Protein Quality of FPH, FPI and their Mixtures

Table 3 presents the essential amino acid composition of FPH and FPI. Tryptophan was limiting for FPH (71%); valine reached 80% with respect to the FAO/WHO 1973 provisional pattern. The lysine content attained 150% of the pattern value for both products. The chemical score of FPI was excellent, since all of the essential amino acids were found at levels higher than those required by the FAO/WHO 1973 pattern.

TABLE 3

AMINO ACID ANALYSIS OF FPI, FPH AND FAO/WHO 1973 PATTERN
(g/100 g of protein)

Essential amino acids	FPI	FPH	FAO/WHO 1973 pattern
Phenylalanine + tyrosine	7.6	6.6	6.0
Isoleucine	5.7	3.6	4.0
Leucine	8.5	7.3	7.0
Lysine	8.0	8.2	5.5
Methionine + cystine	4.5	3.7	3.5
Threonine	4.6	4.8	4.0
Tryptophan	1.3	0.7	1.0
Valine	6.2	3.9	5.0

The designed mixtures of FPH or FPI with cereals and legumes that fulfilled the requirements established above of the formulation of mixtures showed protein contents from 13 to 39% (Table 4). The amino acid content of FPI mixtures was found to be over 70% the FAO/WHO 1973 pattern, and biological experiments demonstrated its suitability for supplementing cereals and legumes deficiencies, since mixtures showed PER values of 80% of casein's (casein control of 2.5), and NPU values were superior to the 80% of the casein NPU (50 to 58 versus 60 for casein). The six mixtures of FPI were used to prepare tortillas and refried beans.

TABLE 4

COMPOSITION OF THE SELECTED MIXTURES

Mixture	Component	% of total protein supplied by the component	% protein of mixture	% of FAO/WHO provisional pattern		
				Lys	Met + Cys	Tryp.
1	FPH-Soy-Rice	30-50-15	30.3	141	84	105
2	FPH-Soy-Wheat	35-50-15	38.9	138	87	106
3	FPI-Wheat	80-20	13.1	60	99	113
4	FPI-Soy-Rice	15-50-35	14.5	105	100	123
5	FPI-Soy-Wheat	10-50-35	15.9	79	87	111
6	FPI-Nixtamal	47-53	18.4	99	110	99
7	FPI-Black bean	58-42	31.6	126	109	132
8	FPI-Kidney bean	58-42	28.3	126	109	132

On the other hand, FPH gave a PER value of 60% that of casein, and a NPU value of 54% that of casein. These values, however, were overcome in the mixtures —in the case of FPH, the best mixtures were those prepared with soy, rice and wheat and were formulated as instant soups— in which PER and NPU values were higher than 78% in relation to casein's, and the mixtures attaining 80% of the limiting essential amino acids in comparison to the FAO/WHO 1973 pattern.

For the sensory tests, the panel evaluated series of three samples for grading flavor, aroma, color, texture and palatability, using a 5-points scale from "dislike very much" to "like very much"; the samples were compared to controls with no isolate or hydrolyzate in them. No significant difference was found in the "t" Student test at all levels of FPI addition, with a contribution which was lower than 20% of the total protein. The samples containing FPH were accepted by more than 70% of the panelists for mixture 1, and 47% of the panel for mixture 2 (Table 4) for levels of FPH addition up to 30% of the total protein.

CONCLUSIONS

No extractions were necessary for obtaining a fish protein hydrolyzate (FPH) with low fat content (0.8%). FPH is deficient in tryptophan and valine, but its high lysine content allows it to supplement cereal foods; its high solubility is an advantage for its use in liquid foods. Thermal treatments could be eliminated by using lower hydrolysis times, which provide less microbial contamination probabilities; such a milder procedure might increase FPH nutritive value.

The overall preparation procedure, and specifically the fish protein isolate (FPI) solvent extraction at a relatively high temperature, such as 60°C, did not affect the emulsifying capacities: 327 and 326g oil per g protein for FPH and FPI, respectively, while reports from Spinelli, Koury and Miller (2) indicate much lower values for fish isolates and hydrolyzates: 145 and 225 g oil per g protein.

Storage at low temperatures, in light protective packages, is recommended for both FPI and FPH in order to avoid deleterious effects on the functional properties of the protein, due to free radical reactions from a possible lipid oxidation.

A protein isolate and an enzymatic hydrolyzate of mullet *(Mugil cephalus)* were succesfully incorporated in common Mexican foods. The resulting formulations have a protein content higher than any similar products in the Mexican market.

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RESUMEN

OBTENCION DE UN AISLADO Y UN HIDROLIZADO DE PROTEINA DE PESCADO *(Mugil cephalus)* Y SU INCORPORACION A LOS PLATILLOS DE LA COCINA MEXICANA

Se obtuvieron aislados proteínicos de pescado (APP) mediante solubilización con álcali y precipitación con HCl, así como hidrolizados de pescado (HP) por vía enzimática. Los productos en polvo mostraron solubilidades de 50 y 89%, un índice de emulsificación de 36 y 39 ml de aceite/100 ml de APP y HP, respectivamente, con contenidos proteínicos de 90% y un extracto etéreo menor de 1.6%. Ambos productos se adicionaron a diversos cereales y leguminosas para incrementar la calidad y cantidad de sus proteínas. Las mezclas resultantes se utilizaron para preparar platillos comunes de la cocina mexicana, los cuales fueron aceptados como "buenos" por el 70% de los jueces que evaluaron los platillos.

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