

DEVELOPMENT OF AN ENZYMATIC FISH HYDROLYSATE AND ITS USE IN INSTANT SOUP BASES¹

*Amanda Gálvez², Josefina Morales de León³, and
Héctor Bourges Rodríguez³*

Departamento de Ciencia y Tecnología de Alimentos
Instituto Nacional de la Nutrición "Salvador Zubirán",
México, D. F., México

SUMMARY

The successful conservation of fish products, at low costs, is a subject of special interest in the developing countries. Conscious of this fact, our group has been studying several fish conservation methods, such as autolysis with high salt concentrations, and has obtained a sauce of high nutritive value and long shelf life. Nevertheless, the reaction process takes from four to six months.

In the study herein reported, the hydrolysis was accelerated and controlled by using the following enzymes: papain, HT proteolytic, and Brew (N) zyme. The hydrolysate was then mixed with cereals to prepare instant soups. As results indicated, the best hydrolysate was obtained with Brew (N) zyme at 50°C and 8.30 hours. This hydrolysate contains 93.0 g/100 g crude protein with a protein efficiency ratio (PER) and a net protein utilization (NPU) of 60% that of casein's NPU as well as a content of 0.8% ether extract. The lowest-cost mixtures with the highest nutritive value were: hydrolysate-wheat-soymeal, and hydrolysate-rice-soymeal, with 38.3 and 29.7 protein per 100 g of mixture, respectively, and a NPU of 79.0 and 79.8% in relation to casein, respectively.

The soups prepared had a satisfactory acceptance rating. There were no significant differences in flavor and aroma at a confidence level of 95%. The cost per gram of protein is about US\$ 0.22 per kg.

INTRODUCTION

Even though in Mexico the fish catch potential is approximately two million tons/year, the Mexican population consumes very little sea food

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- 2 Present address: Universidad Nacional Autónoma de México, Departamento de Alimentos, Ciudad Universitaria, México D. F., México.
- 3 Instituto Nacional de la Nutrición "Salvador Zubirán", Vasco de Quiroga No. 15, Col. Tlalpan, C. P. 14000-México D. F., México.

with a *per capita* intake of only 4 kg per year. The main reason for this low consumption seems to be the high fish cost at the consumer level, determined mainly by the expensive transportation and conservation systems used for these products.

At the present time, basically only high income families of urban areas consume fish; for the rest of the population, fish is virtually unknown. Our group has been working in the development of inexpensive technologies for conservation and distribution of fish; one of these technologies is now in its last stages of development prior to marketing (1, 2). Aside from these techniques in which the origin of the products is apparent to the consumer, we considered of interest to explore the possibility of obtaining fish protein concentrates and hydrolysates of neutral characteristics which could be used as ingredients in high-protein products (beverages, cereals, soups, baby foods, and so forth) for nutritional programs (3).

Previous projects allowed us to develop a fish autolysate and concentrates. The concentrates were easy to preserve, but their functional characteristics were poor. On the other hand, the procedure for obtention of the autolysate involved from four to six months (4). The objectives of the present study were: 1) to develop a shorter procedure to obtain a fish protein hydrolysate (hours instead of months), with a solubility index of at least 80^o/o, an emulsification capacity of at least 30 ml of oil/100 mg of enzymatically-hydrolyzed-fish-protein remaining stable for one hour, and with the least possible color and odor when incorporated into a high moisture food system; and 2) to develop cereal-hydrolysate mixtures with no less than 25 g of protein/100 g, at least 80^o/o of the NPU of casein and with a cost below the equivalent to US\$ 0.65/16 g of nitrogen.

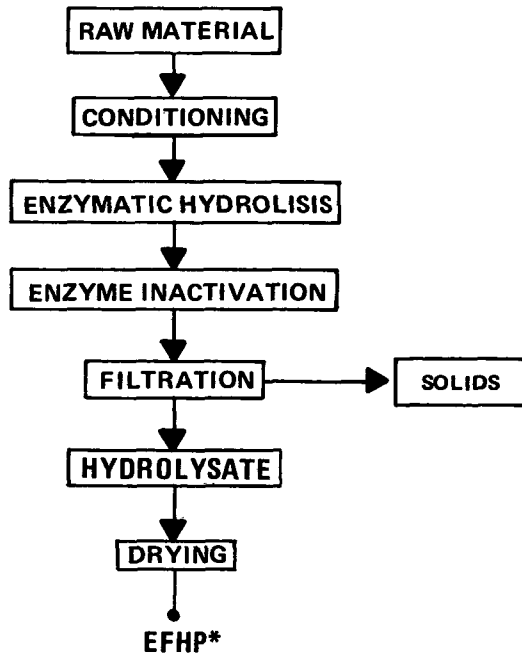
MATERIAL AND METHODS

Raw Material. Mullet (*Mugil cephalus* L.), wheat, rice and defatted soybean flour were selected considering their amino acid composition, cost and availability.

Procedure. The general procedure for the preparation of the hydrolysate is shown in Figure 1. Hydrolysis time and enzyme type and concentration were the variables tested as detailed in Table 1.

Formulation. The mixtures were calculated according to the FAO/WHO 1973 provisional pattern, using a specific computer program wherein composition, protein quality and cost are taken in consideration.

Analysis. Proximal and microbiological analyses were performed using the AOAC methods (5, 6). Physical determinations as solubility (7, 8), emulsification capacity (7, 9) and viscosity, measured with a Brookfield Viscosimeter, were also undertaken. The protein quality was evaluated by the chemical score (10, 11), protein efficiency ratio (PER) (12), and net protein utilization (NPU) (13), following the traditional techniques. For the sensory analyses, a consumer panel (non-experts) tested the products using a questionnaire of attribute classifications (14);



* Enzymatic fish protein hydrolysate.

FIGURE 1

Enzymatic fish protein hydrolysate

TABLE 1

ENZYME SELECTION FOR FISH HYDROLYSIS

Enzyme	pH	Temperature (°C)	Enzymatic concentration (g/100 g fish-meat)	Time (hours)
Vegetal*	5.5	60	0.1	1, 2, 3, 4, 8, 8:30, 9, 20 & 36
			0.2	
			0.3	
			0.4	
Microbial*	7.5	50	0.2	1, 2, 3, 8, 8:30, 9, 20 & 36
			0.3	
			0.4	

* Obtained from Mixim Laboratories, S. A.

** Obtained from ENMEX, S. A.

the statistical analyses was carried out according to referred techniques (15).

RESULTS AND DISCUSSION

The proximal analysis of *Mugil cephalus* showed a content of 69.80/o humidity, 19.50/o protein, 18.50/o ether extract, 1.40/o ashes and 0.850/o carbohydrates.

The optimum enzyme concentration was 0.3 g/100 g of fish meat. Figure 2 shows the behavior of two of the enzymes tested. Although after 36 hr, hydrolysis was near 900/o, the curves revealed a point of

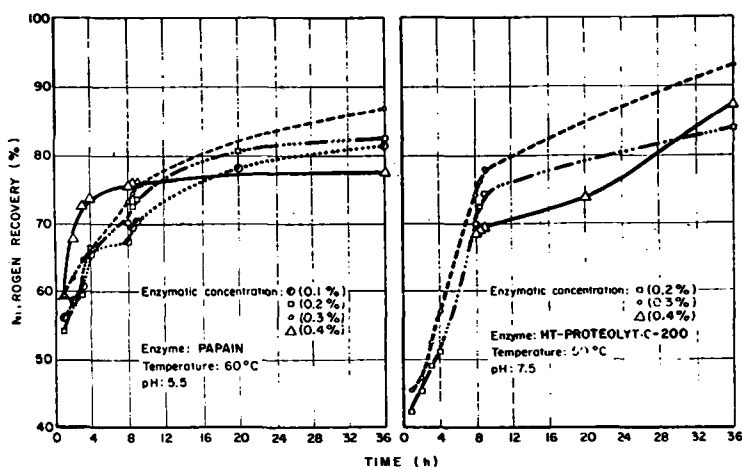


FIGURE 2

Nitrogen recovery (o/o) during hydrolysis of fish with papain and HT-proteolytic-200 at different enzyme concentrations

inflection about the 10th hour, after which little increase in hydrolysis was obtained. Furthermore, bacterial contamination appeared after hour 12. The activity of the three enzymes at a concentration of 0.30/o, is compared in Figure 3. It is clear that the most adequate reaction time was eight hours, reaching 800/o of hydrolysis. Table 2 presents the cost per kilogram of the three enzymes; it shows that HT-proteolytic-200 is the most efficient from the economic point of view. The hydrolytic conditions selected were: HT-proteolytic-200, fish meat concentration, 0.3 g/100 g, reacting at 50°C, at a pH of 7.5 for eight-and-a-half hours. Using these conditions, a 200 kg batch was prepared following the procedure shown in Figure 4. The yield of the procedure was 80/o in relation to ground fish, value which is similar to the 110/o reported by Pigott (16). The composition of the protein hydrolysate appears in Table 3. As the data demonstrate, it contains 930/o protein, only 0.820/o fat and 3.380/o minerals. The microbiological analysis (Table 4), indicates a total count

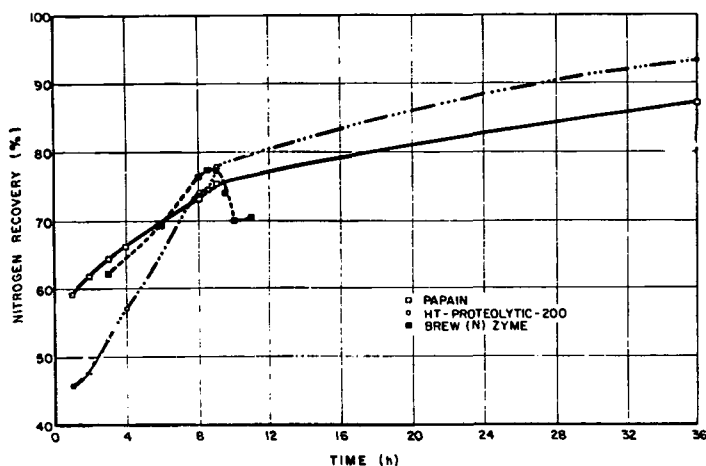


FIGURE 3

Nitrogen recovery (o/o) during hydrolysis of fish with papain HT-proteolytic-200 and Brew (N) zyme
(Enzyme concentration 0.3)

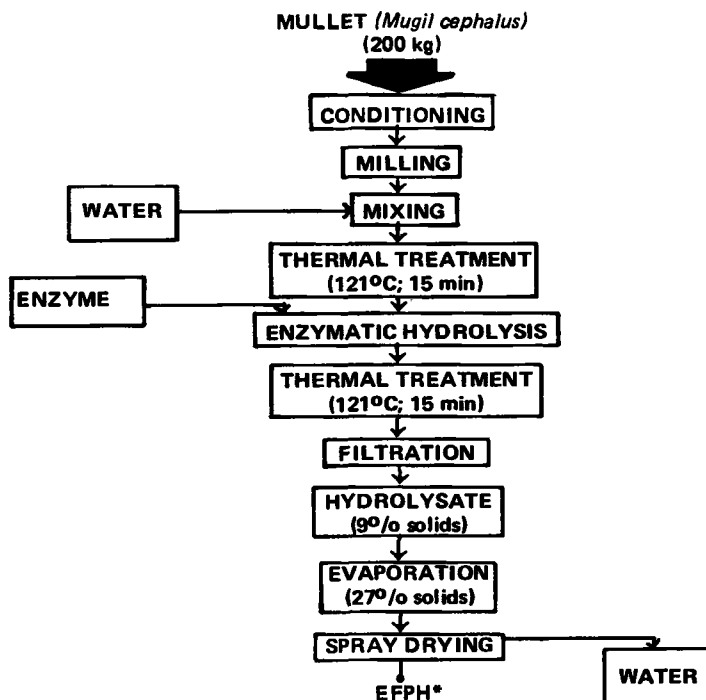
TABLE 2

COST OF ENZYMES USED IN THE HYDROLYSIS

Enzyme	Cost US\$/kg	Enzyme activity
Papain	87.00	0.32 M. C. U.
HT-proteolytic-200	18.00	200 Northrop units
Brew-N-zyme	39.00	700 Northrop units

of 390 colonies/g, 10 molds and 20 yeasts per gram, and no coliform organisms. The amino acid analysis is shown in Table 5, and as shown, tryptophan was the only limiting essential amino acid (700/o as compared to the FAO/WHO 1973 provisional pattern). The cost of the enzymatic hydrolysate calculated from raw material and process yield was equivalent to US\$ 11.30 per kg.

With the product obtained, two mixtures with cereals, shown in Table 6, were prepared. In both cases, the hydrolysate provided 350/o of the total protein. According to their proximal analyses (Table 7), Mixture 2 provided more protein. Although the PER and NPU of the fish hydrolysate were only 540/o in comparison to casein's, its mixture with cereals



* Enzymatic fish protein hydrolysate.

FIGURE 4

Experimental diagram for the preparation of enzymatic fish protein hydrolysate

TABLE 3

PROXIMAL ANALYSIS OF ENZYMATICAL FISH PROTEIN HYDROLYSATE

Determination	g/100 g of product
Moisture	2.8
Crude protein	93.0
Ether extract	0.8
Ash	3.4

raised these values to 80% those of casein (Table 8). Both mixtures were used to prepare two bases for instant soups with the addition of species (Table 9). The emulsification capacity and viscosity of these bases were adequate. Mixture 1 was better accepted by the panel than

TABLE 4

**MICROBIOLOGICAL ANALYSIS OF THE ENZYMATIC
FISH PROTEIN HYDROLYSATE**

Determination	CFU/g
Total count	390
Molds	10
Yeasts	20
MPN coliforms	—

TABLE 5

**AMINOGRAM OF ENZYMATIC FISH PROTEIN HYDROLYSATE IN
COMPARISON WITH THE FAO/WHO 1973 PROVISIONAL PATTERN
(mg aa/16 mg N)**

Amino acids	Enzymatic fish protein hydrolysate	FAO/WHO 73
Phenylalanine + tyrosine	6.61	6.0
Isoleucine	3.64	4.0
Leucine	7.25	7.0
Lysine	8.20	5.5
Methionine ± cystine	4.76	3.5
Threonine	4.79	4.0
Tryptophan	0.71	1.0
Valine	3.99	5.0
Aspartic acid	10.33	
Glutamic acid	19.49	
Alanine	6.50	
Arginine	7.54	
Glycine	6.43	
Histidine	3.22	
Proline	2.36	
Serine	4.18	

Mixture 2 (70% against 47%) for flavor and palatability. Color and aroma were practically similar for both.

The soup bases developed in this study have a higher protein content than any similar product available in the Mexican market (34% vs 10%); aside from this fact, they also render a lower cost per nitrogen content (0.52 vs 2.30 US\$/16 g N).

TABLE 6
SELECTED PROTEIN MIXTURES
(percentage of protein)

Mixture 1:	EFPH* 35%o	Soybean flour 50%o	Rice flour 15%o
Mixture 2:	EFPH* 35%o	Soybean flour 50%o	Wheat flour 15%o

* Enzymatic fish protein hydrolysate.

TABLE 7
PROXIMAL ANALYSIS OF PROTEIN MIXTURES

Determination (g/100 g sample)	Mixture 1 EFPH*-soybean-rice	Mixture 2 EFPH*-soybean-wheat
Crude protein	29.7	38.3
Ether extract	1.4	2.8
Ash	3.1	4.0
Crude fiber	1.4	2.2
Carbohydrates**	57.7	47.2
Moisture	6.7	5.5

* Enzymatic fish protein hydrolysate.

** By difference.

TABLE 8
DETERMINATION OF PER AND NPU OF ENZYMATIC FISH PROTEIN
HYDROLYSATE (EFPH) AND MIXTURES 1 AND 2

Product	PER	PER as %o of casein's PER	NPU	NPU as %o of casein
Casein	2.5	100.0	60.0	100.0
EFPH	1.5	60.0	32.3	53.8
Mixture 1	2.2	88.0	47.4	79.0
Mixture 2	2.4	96.0	47.9	79.8

TABLE 9

**FORMULATION OF SOUPS WITH ENZYMATIC FISH PROTEIN
HYDROLYSATE AND CEREALS**

Ingredients	Mixture 1 (g)	Mixture 2 (g)
Proteinic mixture	65.8	76.7
Salt	7.5	6.5
Pepper	1.0	1.0
Garlic	1.5	1.5
Onion	0.5	0.5
Monosodium glutamate	0.5	0.125
Water	1 liter	1 liter

RESUMEN

**DESARROLLO DE UN HIDROLIZADO ENZIMATICO DE PESCADO, Y SU
USO EN BASES PARA SOPAS INSTANTANEAS**

El desarrollo satisfactorio de productos a base de pescado, a bajo costo, constituye un tema de especial interés en los países en vías de desarrollo. Consciente de ello, nuestro grupo ha venido estudiando varios métodos de conservación de pescado, tales como autólisis con altas concentraciones de sal, y ha logrado obtener una salsa de alto valor nutritivo y larga vida de anaquel. No obstante, el proceso de reacción toma de cuatro a seis meses.

En el estudio de que aquí se informa, se logró acelerar y controlar la hidrólisis mediante el uso de las enzimas siguientes: papaína, HT proteolítica, y Brew (N) zima. El hidrolizado se mezcló entonces con cereales para preparar sopas instantáneas. Como lo indican los resultados, el mejor hidrolizado se obtuvo con Brew (N) zima a 50°C y un término de 8.30 horas. El hidrolizado contiene 93.0 g/100 g de proteína cruda, un índice de eficiencia proteínica (PER) y una utilización proteínica neta (NPU) de 60% de la NPU de caseína, así como un contenido de 0.80% de extracto etéreo. Las mezclas de menor costo y mayor valor nutritivo fueron: hidrolizado-trigo-harina de soya, e hidrolizado-arroz-harina de soya, con 38.3 y 29.7 g de proteína por 100 g de mezcla, respectivamente, y una NPU de 79.0 y 79.8% en relación con la caseína, respectivamente.

Las sopas preparadas tuvieron una tasa de aceptación satisfactoria. No se constataron diferencias significativas en cuanto a sabor ni aroma a un nivel de 95% de confiabilidad. El costo por gramo de proteína es de US\$0.22 por kg, aproximadamente.

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