IMPROVED UTILIZATION OF MARINE SPECIES OF LOW COMMERCIAL VALUE THROUGH THE ELABORATION OF HYDROLYSATES[1]

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SUMMARY

Although Mexico is a country with a great fishing potential, fish consumption remains very low. An important reason for this situation is the difficulty faced in regard to its preservation and distribution, a factor which notably increases the final price of the product. As is known, in some countries fish preservation is carried out through autolysis, using high concentrations of sodium chloride. This was the type of work carried out by us, in an effort to adapt the procedure to the species and conditions prevalent in Mexico.

The raw material was selected according to its availability and cost. The selected species were mojarra *(Archosargus unimaculatus)* and sardine *(Sardinops caerula).* Three different fish-to-salt ratios were tested (1.5:1, 4:1 and 6:1), with incubation periods ranging from 4 to 24 weeks, at both 20 to 23°C, and 37 to 39°C.

Results indicated that a fish-to-salt ratio of 4:1, at a temperature of 37°C and an incubation period of 12 weeks, represent the optimun conditions for obtaining a fish sauce which is acceptable in flavor, with a protein content of 12% per 100 ml, and a storage life of at least 90 days. The recovery of the final product was 22%, reaching 35% in a second extraction.

Sensory evaluation tests were undertaken by adding the sauce to cooked unsalted rice. According to the results, there was a favorable acceptance of the final product. The price calculated for the elaboration of the sauce at the household or rural level was lower, as compared with the price of protein from meat or egg which is 3-to-4-fold higher.

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INTRODUCTION

In spite of the fact that Mexico has more than 10,000 km of shoreline, and over 6,500 km^2 of inland waters with an estimated immediate fishing potential of 2.5 million metric tons, fish consumption in rural Mexico is scarce.

The reasons for the above situation are complex. Most of the country's population lives inland, separated from the coast by wide mountain ranges, so that fish has to be preserved and transported long distances. Freezing, cooling and canning are commonly used for preservation, resulting in high customer prices which often are 100% or more those at the coastline. Therefore, the market is very limited, restricted only to the higher economic strata of a few big urban centers. Even at the coastlines, fish consumption is rather low because of the lack of preservation facilities, and it is not uncommon that part of the fish, especially those species of low commercial value, get spoiled, thus becoming useless.

The development of simple and less expensive preservation techniques could increase utilization and consumption of this important food resource. In several European and Asian Countries (1-4), advantage has been taken of the autolytic capacity of fish, to obtain a sauce-like product named by the Romans "Garum". Fish is mixed with salt in alternate layers, allowing the proteolitic enzymes present in the fish to digest the protein and make it soluble in water without undesirable bacterial contamination (5, 6).

The objective of the present study was to determine the best conditions (fish-salt ratio, temperature and time) required to obtain the fish sauce, and to adapt the procedure to some of the most abundant species in Mexican waters.

MATERIALS AND METHODS

A. *Materials*

Because of its high availability and low cost in Mexico, the raw materials utilized in this work were: mojarra *(Archosargus unimaculatus)*, with an average length of 20 to 25 cm, and sardine *(Sardinops caerula)* with an average length of 10 to 15 cm. The first one was bought fresh and the sardine was obtained frozen at a local market in Mexico City. In both cases, the raw material was stored frozen until it was used. Ground salt (mesh 30) was also utilized.

B. *Methods of Analysis*

1. *Chemical analyses* (7, 8). The following determinations were done: moisture using the thermobalance method (7); ashes, by the calcination procedure (8); ether extract; protein, by the Kjeldahl method; carbohydrates, by difference; ammoniacal nitrogen, by the magnesium oxide technique; aminic nitrogen, by the Sörensen method (9), and sodium chloride, by the Mohr methodology. All determinations were done in duplicate.

2. *Microbiological analyses.* These included the standard plate count of viable microorganisms; most probable number of coliforms (MPN); mold and yeast count, and confirmative test for *E. Coli* (10).

3. *Amino acids.* These were determined by the Moore and Stein (11) technique in an automatic Beckman analyzer, Model 116. Tryptophan was measured according to Spies and Chambers (12).

4. *Sensory tests.* Flavor, odor and preference, by means of a hedonic scale, were evaluated in all the sauces, and compared with a commercial sauce using a panel of untrained judges. For these tests, the sauces were added to boiled rice prepared without salt (13).

C. *Fish and Sauce Elaboration*

In order to establish the conditions of temperature, fish: salt ratio, and incubation time for the preparation of autolysates from mojarra and sardine, 26 different tests were carried out. These conditions are described in Table 1.

The general procedure followed to prepare the sauce is presented in Figure 1. Once the frozen fish was thawed and washed, it was placed in a

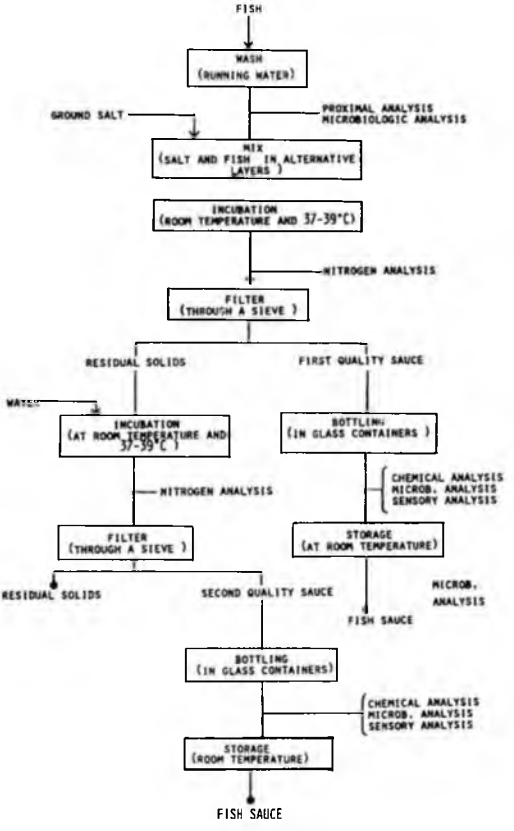

FIGURE 1

Diagram of the process for elaboration of the fish sauce.

TABLE 1

EXPERIMENTAL TESTS FOR THE FISH SAUCE ELABORATION

Sample No.	Raw material	Added water (lt)	Fish-salt ratio	Incubation temperature (°C)	Incubation time (weeks)
1	Mojarra	—	1.5:1	37 - 39	4
2	Mojarra	—	4:1	37 - 39	4
3	Mojarra	—	6:1	37 - 39	4
4	Mojarra	—	1.5:1	20 - 23	4
5	Mojarra	—	4:1	20 - 23	4
6	Mojarra	—	6:1	20 - 23	4
7	Sardine	—	1.5:1	37 - 39	12
8	Sardine	—	4:1	37 - 39	12
9	Mojarra	—	1.5:1	37 - 39	12
10	Mojarra	—	4:1	37 - 39	12
11	Sardine	—	1.5:1	20 - 23	12
12	Sardine	—	4:1	20 - 23	12
13	Mojarra	—	1.5:1	20 - 23	12
14	Mojarra	—	4:1	20 - 23	12
15	Sardine	—	1.5:1	37 - 39	24
16	Sardine	—	4:1	37 - 39	24
17	Mojarra	—	1.5:1	37 - 39	24
18	Mojarra	—	4:1	37 - 39	24
19	Sardine	—	1.5:1	20 - 23	24
20	Sardine	—	4:1	20 - 23	24
21	Mojarra	—	1.5:1	20 - 23	24
22	Mojarra	—	4:1	20 - 23	24
23	Residue 7	2.5	—	37 - 39	12
24	Residue 8	2.5	—	37 - 39	12
25	Residue 11	2.5	—	20 - 23	12
26	Residue 12	1.5	—	20 - 23	12

clay container of 20 lt capacity. Fish was placed at the bottom, followed by alternate layers of salt and fish (four layers of each); the top layer of salt was twice as thick as the previous one. The containers were incubated either at 37-39°C, or at room temperature, 20-23°C. The fish-to-salt ratios (w/w) tested were 1.5:1, 4:1 and 6:1. Samples were drawn from each container at zero time, every 24 hours during the first week, and then at the completion of 2, 3, 6, 8, 10, 12, 16, 20 and 24 weeks. Every sample was analyzed in duplicate for total nitrogen, ammonium nitrogen and formaldehyde.

A "second extraction" was performed using the residual solids obtained from four of the experiments involving sardine which had given the best yield in soluble nitrogen per minute of time. Water was added to samples 23 to 26 (Table 1), and reincubated at 37-39°C or at 20-23°C for 12 weeks, in order to obtain a "second extraction sauce". Moisture, sodium chloride, standard plate count and yeast and mold counts were evaluated

both in the products obtained from the first and from the second extraction. Amino contents were also determined, and the sensory tests compared with those of a commercial sauce.

RESULTS AND DISCUSSION

The chemical analyses of the raw material are detailed in Table 2. As stated therein, the protein content was similar for both species, although sardines had a higher fat content. Microbiological analyses are shown in Table 3.

TABLE 2

PROXIMAL ANALYSIS OF THE RAW MATERIAL
(AVERAGES)
(g/100 g)

Determination	Mojarra	Sardine
Moisture	72.5	71.3
Protein (Nx6.25)	17.5	18.5
Ether extract	0.47	6.4
Ashes	5.9	3.7
Carbohydrates (by difference)	3.5	0.0

TABLE 3

MICROBIOLOGICAL ANALYSIS OF THE RAW MATERIALS

Determination	Mojarra (ice preserved)	Sardine (frozen)
Standard plate count	101,000	25,000
Yeast and fungus	830,000	670,000
Most probable number of coliforms	2,400	78
Escherichia coli	+	+

Figure 2 exhibits the organic nitrogen content of mojarras during the incubation period. As the Figure depicts, the organic nitrogen in the aqueous portion increased from the beginning in the three cases, reaching its highest levels after 12 weeks. The organic nitrogen content for sardines is shown in Figure 3; this was similar to that of mojarras.

It is important to observe that the organic nitrogen concentration is more directly related to the salt-fish proportion than to the temperature.

Optimum conditions (37 to 39°C, incubation period of 12 weeks and fish-salt ratio 4:1) were favorable for both species since the environmental temperature, common in most Mexican coastal areas, varies from

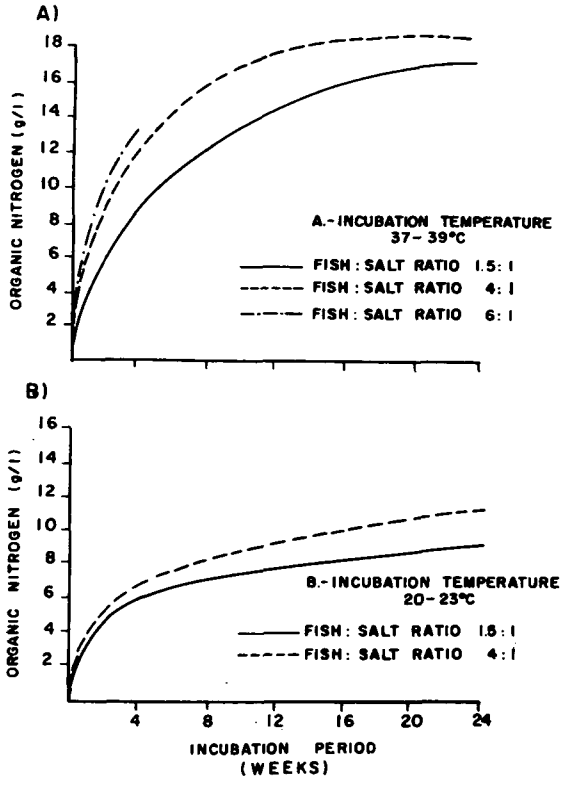

FIGURE 2

Organic nitrogen concentration during the incubation period in the sauce made with mojarra

37°C to 39°C. In addition, the 4:1 fish-salt ratio produces a more acceptable sauce than a 1.5:1 ratio. In the case of the 6:1 fish salt ratio, the total amount of microorganisms and ammonia nitrogen increased, thus giving undesirable characteristics to the product. Therefore, in further experiments this ratio was no longer employed.

Autolysis was higher with a 4:1 ratio. The efficiency of the process became very low in the three cases after a 12-week period.

The procedure yield is presented in Table 4. As the figures indicate, under previously selected optimum conditions, this yield was almost 32.6o/o for sardine, but only 11.4o/o for mojarra.

Once the conditions for the procedure were set, analysis of the different types of nitrogen was obtained for both species during a 12-week incubation period. The results are shown in Figure 4. In the case of

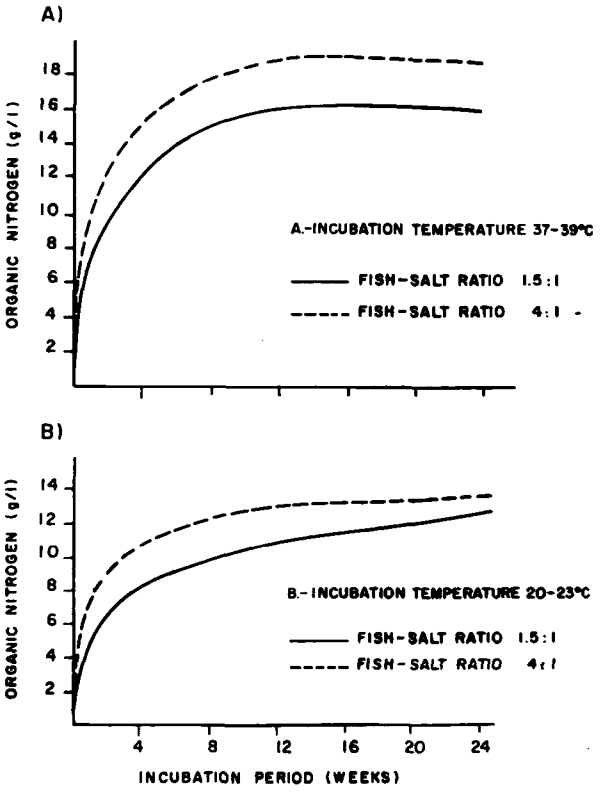

FIGURE 3

Organic nitrogen concentration during the incubation period in the sauce made with sardine

mojarra, total nitrogen increased steadily to more than 20 g/lt at 12 weeks; most of it was organic nitrogen, which showed the same tendency. Ammonia nitrogen increased during the first six weeks to 8 g/lt, thereafter reaching a plateau. The amino acid nitrogen increased to almost 6 g/lt, representing 30% of the total nitrogen. The results for sardines were similar, but total nitrogen increased steeply, reaching 16 g/lt by the 4th week; in this case, amino nitrogen was 8 g/lt at 12 weeks representing, therefore, 40% of the total.

Ammonia nitrogen was lower for sardines –no more than 1 g/liter at the end of the period– while for mojarras this content was higher (2.5 g/lt). The mojarras did not affect the odor of the product but this did show up when flavor was evaluated.

Since the efficiency of extraction levels at 12 weeks was low, the possibility of making a "second extraction" was considered. Mackie and

TABLE 4

WEIGHT YIELD OF THE FISH SAUCE OBTAINED FROM MOJARRA AND SARDINE UNDER EXPERIMENTAL CONDITIONS

(Incubation temperature 37-39° C, incubation period 12 weeks, fish-salt ratio 4:1)

Ingredients/yield	Mojarra	Sardine
Fish (kg)	7.0	6.0
Salt (kg)	1.75	1.5
Sauce obtained (ml)	820.00	2,000.00
Residue (kg)	4.66	4.07
Density of the sauce at 15oC (g/cm^3)	1.217	1.227
Yield (ml/100 g fish)	11.4	32.6

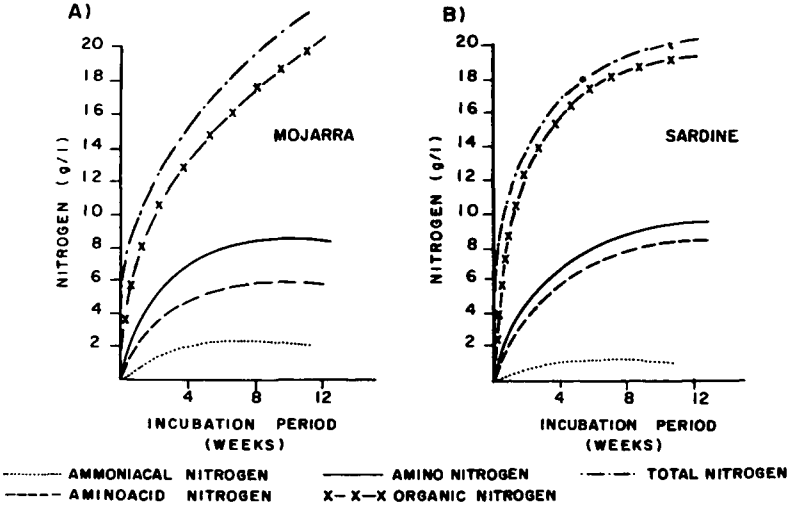

FIGURE 4

Variation of the nitrogen content in the fish sauce elaborated under the most adequate experimental conditions during the incubation period (Incubation temperature, 37-39°C, fish-salt ratio, 4:1)

Hardy (5) and Throung Tan Quan (3) have indicated that draining of the sauce and addition of water, allow the extraction of considerably more material. This possibility was tested with sardines under the conditions presented in Table 1. Variation of the organic nitrogen concentration, in this case, is shown in Figure 5. As may be seen, four weeks after the addition of water, the organic nitrogen was 12 g/lt and slowly increased to 15 g/lt at the end of 12 weeks.

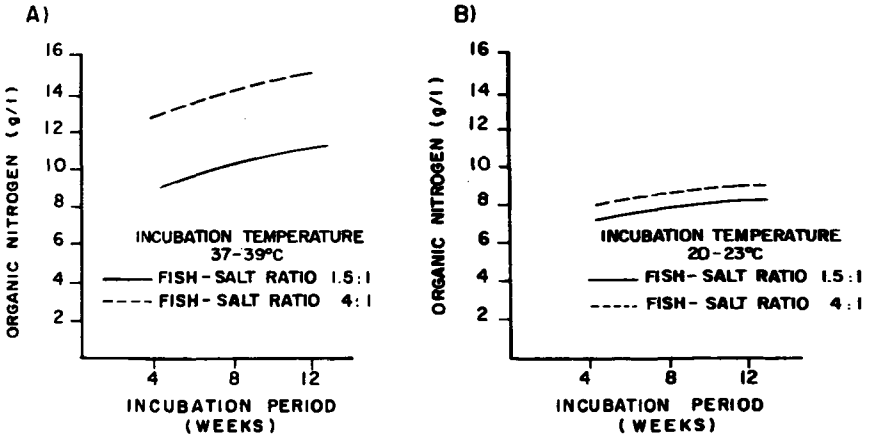

FIGURE 5

Variation of the organic nitrogen concentration during the incubation period in the sauces from the second extraction
(Sardine)

Concentration of the different nitrogen compounds was similar to that of the extraction done at four weeks of incubation; once again, better results were obtained with the 4:1 fish-salt ratio. After four weeks of incubation, little gain in extraction occurred.

Taking as example the extraction of sardines at 37°C during 12 weeks with a 4:1 fish-salt ratio, we observed (Table 4) that starting with 5 kg of fish containing a total of 177.6 g of nitrogen, 2,000 ml of sauce, with a total N content of 40.4 g was obtained in the first extraction, which represents 22.7°/o of the original nitrogen. On the second extraction, 1,475 ml of sauce were obtained with a total nitrogen content of 23.3 g, which represents 13°/o of the original nitrogen in the fish. Therefore, considering both extractions, 36°/o of the total nitrogen was extracted.

In some countries where this kind of product is of usual consumption, the sauce from the second extraction is mixed with that of the first extraction. Nevertheless, in other countries the two products are commercialized separately, but that of the first extraction is more concentrated and more expensive than the other. Although the second extraction produced a more diluted product, 15.8 g N/lt compared to 20.2 g

N/lt in the first extraction, a higher proportion of the nitrogen consisted of free amino acids (49.8o/o compared to 43o/o), indicating that a greater hydrolysis was obtained in the second extraction.

The chemical analysis of the sardine sauces rendered by the first and second extractions is compared to that of two commercial sauces in Table 5. As the data show, the composition of the sardine sauce from the first extraction is quite similar to that of commercial sauces. In all the cases, the total nitrogen content of the product was higher than that of the "Sing chuen" sauce.

TABLE 5

CHEMICAL ANALYSIS OF THE SAUCES FROM THE FIRST AND SECOND EXTRACTION, COMPARED WITH THE COMMERCIAL SAUCES (g/liter)

Determination	Sardine extraction		Pattis Bayaniham (Philippines)	Sing Chuen (Hong Kong)
	1	2		
Moisture	651	702	645	656
NaCl	280.5	228.0	286.1	285.5
Nitrogen total	20.2	15.8	19.3	16.94
organic	19.1	14.98	17.15	15.39
ammoniacal	1.01	0.90	2.12	1.55
amino	9.50	8.78	9.65	10.40
amino acid	8.58	7.87	7.53	8.85

The amino acid content of the sauces obtained from the first extraction at 37oC - 39oC, with a 4:1 fish-salt ratio, from both mojarra and sardines, is shown in Table 6. As stated therein, the FAO/WHO 1973 provisional pattern is also included for comparison purposes.

It is apparent that most amino acids are present in good quantities, especially lysine, which is about twice the required level. Tryptophan is very low in both cases, thus lowering the nutritional quality of the product. In the case of sardines, methionine is also low. From these results it may be concluded that the sauce is an excellent source of lysine but, if consumed alone, its protein quality is inadequate. Therefore, the idea is that sauces be added to foodstuffs such as rice, low in lysine, but sufficient or high in tryptophan content.

Since fish in general is not tryptophan deficient, this amino acid might have been destroyed by the process. This point, however, needs further research.

The differences found in the amino acid content of the sauces obtained from different fish species, demonstrate the influence that composition of the raw material has on the final product.

A microbiological analysis of the final product was undertaken. The mojarra sauce obtained under optimum conditions at four weeks,

TABLE 6

ESSENTIAL AMINO ACIDS CONTENT IN THE SAUCES COMPARED WITH THE 1973 FAO/WHO PROVISIONAL PATTERN (g/100 g of protein)

Essential amino acids	Sauces of		FAO/WHO 73
	Mojarra	Sardine	
Valine	5.14	5.38	5.0
Isoleucine	4.32	3.96	4.0
Threonine	3.68	4.25	4.0
Tryptophan	0.26	0.29	1.0
Phenylalanine + tyrosine	2.64	2.70	6.0
Leucine	6.71	7.03	7.0
Lysine	13.33	10.73	5.5
Methionine + cistein	2.67	1.31	3.5

had a bacterial count of 94,000 col/g, decreasing to 1,600 col/g and 200 col/g at 12 and 24 weeks. This reduction continued during the storage period. The bacterial estimate of the sardine sauce was much lower, and also showed a decreasing trend as time went on. The bacterial estimate of the commercial sauce was in the same range.

The product sauce resulting from the first extraction, by incubation at 37°C for 12 weeks and stored for three months at room temperature, was evaluated by sensory analysis, against a fish sauce from the Philippines. The panel gave "moderate" grades to all the products, particularly regarding smell characteristics (Table 7). This result was to be expected since, as stated before, this kind of product is not commonly used in our country. The products obtained in the present study, however, were given the same grades as the commercial products, and in some cases they even obtained higher marks.

There were no cases of rejection of any of the products in the different sensory tests.

In order to estimate the production prices at both household and community level, the procedure considered, shown in Figures 1 and 6, was followed. For example, it was considered that the volume of raw material on each occassion would be of 6 kg and a two yearly sauce production was assumed, derived from the first and second extraction, using sardine as raw material and a fish-salt ratio of 4:1 at a temperature of 26°C during the incubation period.

The variables considered for the quotation of production prices at community or household levels were: equipment, raw and other materials. These variables gave an estimate of US$0.068 for the production of a liter of sauce. If the final product is assumed to contain an average of 112.5 g of hydrolyzed protein per liter, the price per gram of protein would be approximately US$0.251.

From these results the following conclusions may be derived:

In order to obtain a sauce from mojarras and sardines, the most

TABLE 7

FISH SAUCE SENSORIAL EVALUATION, AFTER A STORAGE PERIOD OF 3 MONTHS AT ROOM TEMPERATURE AND INCUBATED 12 WEEKS AT 37-39°C

Sensory characteristics	Fish-salt ratio 1.5:1	4:1	Control[1]
		Mojarra sauce	
Flavor	8.6	8.0	8.2
Odor	8.4	7.4	7.8
Preference	1.6	2.2	2.2
		Sardine sauce	
Flavor	9.6	9.0	8.3
Odor	8.5	8.1	6.8
Preference	1.3	1.8	3.1

1 Commercial fish sauce (Pattis Bayaniham, Philippines).

adequate conditions are: a 4:1 fish-salt ratio, and a 37-39°C incubation temperature during a 12 week period. Special equipment is not necessary to obtain the product, even under rural conditions.

To recover more nitrogen, it is convenient to make two extractions. The product resulting from the second extraction can then be mixed with that obtained from the first, or else, be employed separately.

– After two extractions a 36°/o nitrogen yield is obtained, most of it organic nitrogen, and about 45°/o amino acid nitrogen.

– The sauce turned out to be very rich in lysine but poor in tryptophan and, in the case of sardines, low in methionine. All the other amino acids are present in sufficient quantities. Investigation of the nature of the tryptophan loss, as well as the effect of the kind or raw material used, on the final composition of the product, is recommended.

– The sauces are bacteriologically safe due to their high salt content; in the sensory tests, they obtained similar grades to those given to commercial products of the same kind.

– A simple, inexpensive procedure for the utilization of fish excedents under rural conditions was developed. A combination of this product with other foods rich in tryptophan such as rice, is ideal and inexpensive.

– This kind of product is exotic in the Mexican diet; educational programs would therefore be needed to promote its production and consumption. An alternative would be to establish small communal units for the production of the sauce which could be sold to the Oriental restaurant market. In this last case no direct nutritional advantage would be obtained; however, the income of the fishermen and their families would increase. Another advantage would be the greater usage of an already available raw material.

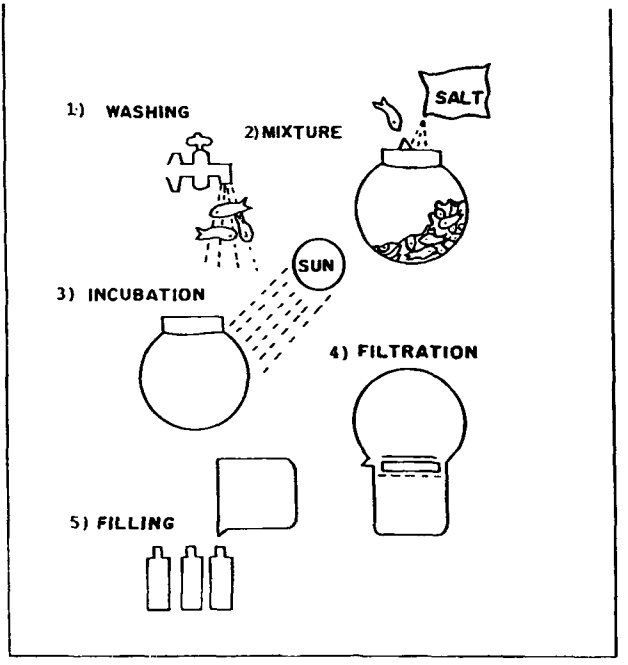

FIGURE 6

Diagram for the elaboration of fish sauce at household level

RESUMEN

UTILIZACION MEJORADA DE ESPECIES MARINAS DE BAJO VALOR COMERCIAL A TRAVES DE LA ELABORACION DE HIDROLIZADOS

A pesar de la riqueza pesquera potencial de México, el consumo de pescado sigue siendo escaso, debido fundamentalmente a las dificultades actuales que se enfrenta para su conservación y distribución a bajo costo. Se sabe que en algunos países se conserva el pescado mediante su autólisis en presencia de altas concentraciones del cloruro de sodio. El presente estudio se realizó con el fin de adaptar este procedimiento a las especies y condiciones ambientales más comunes en México.

Las materias primas seleccionadas como las más adecuadas, con base en su disponibilidad y costo, fueron la mojarra *(Archosargus unimaculatus)* y la sardina *(Sardinops caerula)*. Se sometieron a prueba tres relaciones en peso de pescado-sal (1.5:1, 4:1 y 6:1), y los períodos de incubación variaron de cuatro a 24 semanas a dos temperaturas de incubación, 20 a 23°C, y 37 a 39°C.

De acuerdo con los resultados, con la relación pescado-sal de 4:1, a una temperatura de 37oC y con un período de incubación de 12 semanas, se obtiene una "salsa" organolépticamente aceptable, con 12 g de equivalente proteínico por 100 ml y una vida de anaquel de 90 días. El rendimiento es de 22o/o, y se alcanza el 35o/o en una segunda extracción.

Se efectuaron pruebas sensoriales adicionando la salsa a arroz cocido sin sal, con lo cual se obtuvo una aceptabilidad satisfactoria. El costo estimado para la elaboración de la salsa a nivel doméstico o rural se compara favorablemente con el costo de la proteína de carne y huevo, que es tres o cuatro veces mayor.

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