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THE STUDIES ON FISH HYDROLYZATES AND FISH EXTRACTS FROM TELEOSTEAN FISHES OF THE ARABIAN SEA

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Abstracts. Fish hydrolyzates and extracts were prepared from some selected varieties of fishes as well as from junk or 'kachra' fish; in order to assess the industrial feasibility of the technique and quality of the product. Higher yield of product has been found between 17–23% in proteolytic hydrolysis.

By alkaline extraction the yield was between 2–9%. Similarly the range of protein in the alkaline extraction was also on the lower side (48–82%) as compared to hydrolysis by proteolytic enzyme (78–91%). The fat content of alkaline extract was on the higher side (up to 49%). Not much variations were noticed in the ash content of these products.

In the aqueous extract the yield of the product was better (4–13%) than the alkaline extract, but there was not much variation in their protein percentage. In the light of above observations it is concluded, that for the production of fish hydrolyzate in the laboratory, the enzymatic digestion by crude papain or raw papaya is more effective. Moreover, the procedure of enzymatic hydrolysis is less complicated and more economical.

Pakistan has fairly large fisheries resources, but the extent of these resources has not been fully assessed and utilised so far. According to Qureshi[1] there are over 200 varieties or species of marine fish available in our waters in which 80 varieties are of commercial importance. A statistical data collected by the Marine Fisheries Department, Government of Pakistan, Karachi, for the year 1970 indicates that the annual landing of fish in West Pakistan is approximately 173 thousand metric tons (including 53 thousand tons of other marine teleosteans or junk fish). Out of which only 50 thousand tons are consumed by the local population and the rest is sent to curing yards for curing and salting.

Fish is a well-known source of good quality protein. The superiority of fish protein over that of vegetable protein has already been established. It was observed[2] that chickens fed on a diet consisting 2–5% fish protein grow better than those on a diet composed wholly of vegetable food. Thus the addition of about 5% fish protein to their feed increased the growth rate of chickens, in the first year by about 7% and decreased the feeding requirements by about 20%. According to Slovtzov[3] fish protein is equal to that of beef. Moreover, when fish is substituted for beef the nitrogen is utilised better, resulting in a decreased excretion of uric acid in the urine. Further, fish meat produces good influence on the assimilation of magnesium and phosphorus. It has also been found that fish protein is superior to casein. The protein of mackerel, sardine and tuna was compared nutritionally with standard casein, and found superior to it.[4]

It is a paradoxical that in a country where malnutrition, and in particular, protein deficiencies are prevelent, huge fish catches are processed into fish meal and exported to developed countries for animal feeding.

Equally discouraging is the fact that ample supplies of fresh fish from the surrounding sea cannot find their way to the diet of the people even a few miles away from the sea shore. A good proportion of fish production goes waste due to factors like careless collection, faulty storage, defective means of transportation. Large supplies of trash fish also remain uncollected or are thrown away as waste. Lack of knowledge of fish preservation, food habits, taboos and low purchasing power also play their role. Perhaps due to these reasons, the consumption of fish in Pakistan is very low being only about 5 lb capita/year as compared to Japan (92 lb), Norway (42 lb), and U.K. (35 lb).

In view of the above-mentioned facts and keeping in mind the importance of fish protein, due consideration was given to investigate the possibilities of preparing fish hydrolyzate and fish extracts from surplus fish. Fish extracts is best recommended for special diet therapy as an important supplement to diets of convalescent patients and as medications to specific types of ulcer. Hydrolyzed fish protein will make possible the utilization of cheap species of fish particularly those with low commercial value into superior quality protein foods.

In the present study, fish hydrolyzates and fish extracts have been prepared by different methods, by using selected varieties of fish, the landing of which is constant round the year and after meeting the local demand they are available in abundance for fresh consumption. The industrial aspect of this study will be dealt with separately.

Experimental

With regard to palatability the edible fishes found in Arabian Sea have been grouped into: A, (surmai, rawas, white pomfret and black pomfret); B, (sua, aal, boi, palla, dhoter and dawan); and C, (mushka, khagga and kund).

Investigation on some of the important marine edible fishes of these groups have been undertaken for the study, for the preparation of fish hydrolyzate

and fish extracts by proteolytic enzyme, alkali and by water digestion in the following manner:

Fresh fish was procured from the Karachi Fish Harbour. After necessary identification, the fish was cleaned, washed under running tap-water, and eviscerated. The body flesh was minced in a mincing machine and thoroughly mixed. The minced meat was divided into four parts and each part was subjected to: (1) proteolytic extractions;[3] (2) alkaline extraction; aqueous extraction;[4] and drying.

Proteolytic Extraction. Fresh minced meat (600 g) obtained by thorough mixing, was mixed with double the amount of water in a 2-litre. Erlenmeyer flask The enzyme preparation obtained by saturating a suspension of 12 g crude papain in 50 ml water with H_2S gas and kept at 55°C for 2 hr. It was then added to the meat and incubated over night at 55°C. The pH of the medium was adjusted to 5.5 and a small amount of toluene was also added to avoid any contamination during the process of proteolysis.

After 12 hr of proteolysis, the extract was filtered off by suction. The residue was washed with water and the washings were added to the filtrate. The pH of the extract was adjusted to 4.5 the flask was placed in a steam sterilizer and heated at 100°C for 30 min. The whole volume of the extract was reduced to a minimum in a cyclone evaporator under reduced pressure and finally dried in a vacuum oven. The dried material was then ground to 60 mesh. After noting down the yield, the material was finally submitted to chemical analysis, according to standard techniques of A.O.A.C.[7] The results are given in Table 1.

Alkali Digestion (2% *Alkali*). Fresh meat (600 g) first cooked for about one hr in dilute acetic acid at 80°C, according to Mohanty and Roy[8] technique, until the muscle became thread-like, when pressed and the soluble nitrogenous constituents were removed and the connective tissues were broken down. The material was then washed in running water and then hydrolyzed by adding 600 ml water and 18 ml saturated aqueous solution of sodium hydroxide (equivalent to 12 g NaOH) with constant stirring. The mass was digested at 75–80°C for about 2 hr. This treatment resulted in a fairly good solution of the muscle in dilute alkali. The solution was then filtered through a cotton plug or a coarsed cloth. The filtrate was cooled to 50°C in order to facilitate precipitation in the subsequent stage. The precipitation was carried out by the addition of 1.0% acetic acid solution in water. The solution was continuously stirred during precipitation until completion when a curd like precipitate of protein appeared. The supernatant liquid was removed in a Buchner funnel. The protein was washed twice with distilled water and dried at about 65°C and finally ground to 60 mesh. This material was finally submitted to chemical analysis according to A.O.A.C.[7] standard techniques (Table 2).

Aqueous Digestion. Fresh minced (600 g) fish meat was transferred into 2-litre conical flask and 1200 ml water added to the meat. After plugging with cotton the flask was placed in a pressure cooker at 15 lb pressure for 30 min. The extract was filtered off. Two more extracts were taken in the identical way and filtrates were combined. The combined extracts was concentrated under reduced pressure in a cyclone evaporator. The concentrated extract was dried in a vacuum oven at 60°C. The dried mass was then ground to 60 mesh and after noting down the yield the dried material was finally submitted to chemical analysis according to A.O.A.C.[7] standard techniques (Table 3).

Dried Fish Meat. Fresh minced fish meat (600 g) was dried in a laboratory oven at 65°C, overnight,

TABLE 1. CHEMICAL COMPOSITION OF HYDROLYZATE BY PROTEOLYTIC ENZYME.

Local name	English name	Scientific name	Group	Yield (%)	Moisture (%)	Protein (%) (N×6.25)	Fat (%)	Ash (%)
Surmai	Mackerel	*Cybium*	A	20.0	7.07	86.56	0.15	4.71
Achchopitho or paplet	White pomfret	*Chondroplites chinesis*	A	18.5	5.49	87.12	0.49	5.50
Kalapitho or kala-chanda	Black pomfret	*Parastromateus niger*	A	20.0	2.98	90.62	0.28	4.04
Dhoter	Pomadasid grunt	*Pomadasys olioaceus*	B	17.0	2.54	91.10	0.03	6.18
Sua	Drums or croakers	*Sciaena diacanthus*	B	20.8	5.11	87.25	0.59	6.61
Aal	Leather jacket	*Chroineus tolocparah*	B	19.0	6.97	85.75	0.51	6.00
Hira	Snapper	*Lutjanus rivulatus*	B	17.4	3.60	90.56	0.07	5.50
Dawan	Tuna	*Thynnus*	B	23.3	5.64	85.87	2.33	6.01
Palla	Hilsa or shad	*Hilsa illisha*	B	16.0	9.25	80.50	1.05	6.66
Boi	Mullet	*Mugil speigleri*	B	12.6	4.96	86.56	0.22	7.31
Khagga	Cat fish	*Arius seratus*	C	18.1	7.30	78.65	3.45	6.81
Mushka	Drums or croakers	*Otolithas ruber*	C	22.2	8.14	83.81	0.08	5.18
Kachra	Trash or junk	—	C	18.1	7.30	78.65	3.45	6.81

TABLE 2. CHEMICAL COMPOSITION OF ALKALINE EXTRACT OF FISH.

Local name	Yield (%)	Moisture (%)	Protein (%) (N×6.25)	Fat (%)	Ash (%)
Surmai	9.60	5.60	79.56	8.98	4.68
Achchopitho or paplet	4.87	4.09	73.06	15.29	8.46
Kalapitho or kala-chanda	3.89	8.00	71.56	12.23	8.20
Dhoter	4.00	5.34	79.25	8.32	4.80
Sua	6.14	5.20	77.56	12.55	3.49
Aal	4.10	4.01	73.65	11.22	1.08
Hira	7.71	4.05	76.18	6.51	0.39
Dawan	5.50	2.48	48.93	49.14	1.00
Palla	2.00	6.68	48.34	39.73	0.54
Boi	9.37	6.82	73.81	12.83	6.60
Khagga	9.02	4.83	70.81	29.33	6.27
Mushka	5.00	5.93	82.08	4.67	9.14
Kachra	1.36	4.99	79.81	6.71	5.36

TABLE 3. CHEMICAL COMPOSITION OF AQUEOUS FISH EXTRACT BY PRESSURE COOKING.

Local name	Yield (%)	Moisture (%)	Protein (%) (N×6.25)	Fat (%)	Ash (%)
Surmai	8.02	5.78	73.25	21.44	2.44
Achchopitho or paplet	10.00	5.45	53.18	30.10	9.44
Kalapitho or kala-chanda	4.70	10.53	56.81	15.99	26.97
Dhoter	7.50	3.09	76.66	6.51	13.97
Sua	8.07	2.50	76.00	9.99	12.39
Aal	8.50	2.66	60.87	16.92	1.66
Hira	8.00	2.22	79.87	5.46	12.81
Dawan	7.56	5.10	84.00	2.85	12.70
Palla	13.30	2.07	60.43	34.04	7.34
Boi	12.85	4.42	65.75	19.93	8.03
Khagga	7.38	7.29	80.50	2.80	9.52
Mushka	9.37	3.82	80.25	3.61	12.47
Kachra	9.20	5.02	84.43	0.42	10.25

TABLE 4. CHEMICAL COMPOSITION OF WHOLE DRIED FISH (Dried at 65°C).

Local name	Yield (%)	Moisture (%)	Protein (%) (N×6.25)	Fat (%)	Ash (%)
Surmai	26.3	3.48	77.93	14.88	7.09
Achchopitho or paplet	27.0	3.66	63.30	26.37	6.95
Kalapitho or kala-chanda	27.2	5.86	67.62	17.68	7.10
Dhoter	21.2	2.96	72.93	10.10	16.17
Sua	22.7	3.01	81.40	12.61	7.13
Aal	31.2	1.32	63.98	24.07	10.83
Hira	23.1	5.48	71.18	6.70	17.93
Dawan	27.5	7.21	75.56	12.86	4.95
Palla	36.0	1.32	61.31	30.38	9.63
Boi	25.4	4.75	62.81	10.62	15.39
Khagga	30.0	4.96	62.42	15.64	15.29
Mushka	25.0	7.30	77.50	5.92	11.51
Kachra	22.2	1.80	72.10	6.74	20.03

the dried material was ground to 60 mesh and finally submitted to chemical analysis according to A.O.A.C.[7] techniques (Table 4).

Chemical Analysis

Weighed amounts of each dried material obtained by papain, alkali and water digestion techniques, together with the dried fish flesh evenly powdered were submitted to chemical analysis. Moisture, fat, ash and crude protein were estimated according to standard methods of analysis.[7] The results are given in the Tables 1–4. Total nitrogen contents done by the microkjeldahl method. The results are expressed as per cent crude protein by multiplying total nitrogen by 6.25. No attempt was made to separate protein from non-protein nitrogen, hence the term 'crude protein' used'.

Discussion

According to limited data available, Bertullo *et al.*, who prepared the fish hydrolyzates for human consumption by using proteolytic yeast, have found that the amount of protein in their product was much less (54–60%). In 1969 Perdomo found that hydrolyzed fish protein increases the blood sugar more than glucose but its effect on ketones was found less than that of either glucose or glycerol.

During the last two decades an exhaustive study on marine fishes of the Arabian Sea has been undertaken in these Laboratories.[9–17]

Due to these findings the studies were later on extended to teleostean marine fishes, specially because these fishes constitute a major item in the diet of the population around the coasts of Karachi and Mekran. The data obtained from this study can be used in regulating the composition of our daily diets with respects to vitamin A, proteins and fats. In an earlier communication[17] studies were conducted on the distribution of vitamin A in the skin flesh and liver of some of the important edible fishes. It has been reported that the flesh of group A fishes is poor in vitamin A while their livers are quite rich in it. On the other hand B and C group of fishes have most of their vitamin A in their flesh, the liver contain very little of it.

Following highly encouraging laboratory findings it was found necessary to extend these studies further to the utilisation of non-consumable fish. Fish hydrolyzate and extracts were prepared from some selected varieties of fishes from each of the above stated groups as well as, from junk or kachra fish, in order to asses the industrial feasibility of the technique and the quality of the product. It would be evident from the Tables that the higher yield has been found between 17–23% in proteolytic hydrolysis in all the three groups with an exception of boi or phal fish (12%). Perhaps it is due to the fact that boi or phal is a small fish which attains maximum length of about 6 in and the whole fish was used in this study.[18] By alkaline extraction the yield is between 2–9%. Similarly the range of protein in the alkaline extract is also on the lower side (48–82) as compared to hydrolysis by proteolytic enzymes (78–91%). But the fat content of alkaline extract specially of palla and dawan is on the higher side (39 and 49% respectively). The high percentage of fat in the alkaline extract might be due to the fact that during the process, some of the fat got

emulsified which hindered the separation. Moreover, the process of alkaline extraction is more complicated than the enzymatic digestion. However, ash content of the two products show not much variation.

In the case of aqueous extract the yield of the product is slightly better (4–13%) than the alkaline extract, but the amount of protein shows not much variation, with the exception of dawan in which protein has been estimated about 94% which is higher than the alkaline extract of the same fish (48%).

Perhaps it is due to the fact that alkaline extract contained more fat (49%) which could not be separated due to the above stated reason as compared to 2.85% fat in the aqueous extract. This process of aqueous extract is more simple.

The composition of the whole dried fish has also been determined for comparison.

In the light of above observations, it is concluded that for the production of fish hydrolyzate in the laboratory the enzymatic digestion by crude papain or raw papaya is more effective. Moreover, the procedure of enzymatic hydrolysis is less complicated and more economical. The industrial aspect of this study is being investigated separately.

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