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Comparative Nutritive Value of Fish Protein Concentrate (FPC) from Different Species of Fishes

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Fish protein concentrate (FPC) prepared from three different species of fishes [catfish, family Arridae (*Tachysurus arius*, *T. dussumieri*, *T. thalassinus*), picked ribbonfish, family Trichuiridae (*Trichiurus savala*, *Trichiurus lepturus*), milkfish, family (*Chanos chanos*)], available in plenty on the western coast of India, have been analyzed for their nutritive value, i.e., PER (protein efficiency ratio), NPR (net protein retention), amino acids, proximate composition, and organoleptic evaluation. The protein quality index based on PER and NPR at the 10% protein level was found to be highest in FPC made from picked ribbonfish (*T. savala*), followed by FPC from catfish (*T. arius*) and milkfish (*Chanos chanos*). PER value of *T. savala* was significantly higher than casein. Chemical score based on the essential amino acid content of FAO/WHO pattern (1973) and egg indicated the level of the first limiting amino acid methionine + cystine. The essential amino acid index and biological value were also calculated.

The nutritional needs of a significant part of the world population of developing countries are yet to be satisfied. In most cases the nutritional insufficiency is attributed to the unavailability of protein. Although vegetable protein is being used to increase the intake of protein quantity, the lack of an economic source of animal protein prevents the immediate improvement of protein quality of diet of these people.

An underutilized resource of animal protein is present in the world's oceans, but the main problem is to provide this resource of protein economically to the people of these developing countries. The perishability and lack of storage facilities in preventing spoilage of this valuable source add to the cost of marine products. Solvent extraction methods have been developed in defatting and dehydrating whole fish to fish protein concentrate (FPC) containing at least 70–90% protein (Snyder, 1967; Moorjani and Lahiry, 1970) in different countries. Taking into consideration these factors and potential for indigenous (FPC) production, the nutritional quality of (FPC) prepared from some of the important species of fishes available in plenty on the western coast of India and other parts of the world was taken in hand to find out which of the species give better product of higher nutritional value.

MATERIAL AND METHODS

The total yearly catch of fish in the Indian Ocean is about 2.8 million tons (FAO, 1970), out of which the Indian contribution is about 1.4 million tons yearly. This catch could be increased many fold. Some of the catch consists of certain varieties that are cheap and fairly abundant. Table I shows some of the cheap and abundant varieties of fish and the quantities likely to be available for FPC manufacture. Production of FPC in India seems both feasible and economical. FPC properly prepared can be stored for years without deterioration. The method developed for manufacture of FPC in India is described briefly.

Table I. Some of the Cheap and Abundant Varieties of Fish That Could Be Used for FPC Production

name of fish	family	quantity, Tons
catfish (marine)	Arridae[a] (*T. dussumieri*, *T. thalassinus*)[a]	57 000
picked ribbonfish	Trichuiridae[a,b]	26 000 to 45 000
	(*Trichiurus lepturus*,[a] *Lepturacanthus savala*)[a]	
milkfish	*Chanos chanos*[a–c]	20 000

[a] Commercially important species. [b] Plate XIII, Figure 5, p 30, Jhingran (1970). [c] Plate III, Figure 3, p 20, Jhingran (1970).

Alcohol Extraction. The three species, catfish, picked ribbonfish, and milkfish, were chosen as the raw material because of their cheapness and availability on India's West Coast in abundance during a large part of the year.

Ethanol was chosen as solvent because of its availability, use in food processing, low toxicity, low boiling point, antibacterial properties, and low price. The method for

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Table II. Mean Values of Food Intake, Protein Intake, Gain in Weight by Albino Rats, and PER of FPC Made from Different Species of Fishes at 10% Protein Level (Results Expressed at PER Adjusted to 3.0 for Standard Casein)

protein source	food intake, g/28 days per animal	protein intake, g/28 days per animal	mean wt gain, g/28 days per animal	PER	F value obsd
catfish (FPC)	283[b]	28.3 ± 0.18[a]	78.2 ± 2.4[a]	2.99 ± 0.06[a]	5.9[c]
picked ribbonfish (FPC)	280	28.0 ± 0.57	87.3 ± 3.3	3.38 ± 0.06	13.5[d]
milkfish (FPC)	233	23.3 ± 0.68	55.5 ± 3.5	2.56 ± 0.09	31.6[d]
casein	239	23.9 ± 0.76	66.2 ± 7.8	3.0 ± 0.03	

[a] Standard error. [b] Average value of one animal. [c] F value, significant at $P = 0.05$. [d] F value, significant at $P = 0.01$.

preparing FPC was essentially that of Lahiry et al. (1962) with slight modification.

The three species of fishes were dressed to remove head, tail, visera, and scales. The dressed fish was covered with water at the rate of 1 L for 1 kg, cooked in steam for 5 min, and pressed in a hand-driven screw press. The pressed material was dried in hot air at 60 °C until a moisture level of 5–10% is reached. The dried material was ground coarsely and extracted with boiling 95% alcohol at a solid to liquid ratio 1:4 for 1 h. After three extractions the material was dried in hot air for 5 h until the solvent vapor was completely driven off. The sample was then heated up to 100 °C for 1 h to drive off any traces of alcohol (AOAC, 1970) and it was then powdered in a micropulverizer to 100-mesh size.

Samples were dried in a hot-air oven at 105 °C for 6 h for the determination of moisture. Ash, nitrogen, and calcium were determined according to methods of the Association of Official Agricultural Chemists (AOAC, 1970). Oil was determined as in the AOAC method for oil in cotton seed except that the samples were extracted for 8 h with petroleum ether, bp, 40–60 °C. Phosphorus was determined by King's method (1932), a modification of the method of (Fiske and Subbarow, 1925). The protein content of the samples was calculated by multiplying the Kjeldahl N by 6.25. The amino acid composition was studied using a Technicon sequential multisample amino acid analyzer (TSM). Defatted samples containing 5 mg of protein were hydrolyzed by refluxing with 6 N HCl for 22 h. After removal of acid by evaporation under reduced pressure, the residue was dissolved in 2 mL of citrate buffer (pH 2.875). An aliquot (0.4 mL) was used for the determination of amino acids according to the method of Moore and Stein (1954). Tryptophan was determined by the method of (Spies and Chambers, 1949).

Diets. The powdered samples of three species of fishes were used for making diets. The diets for all the biological experiments were prepared at a 10% protein level. The composition of 100 g of diet was as follows: test sample flour (weight calculated to give 10% protein), 10 g groundnut oil (containing 1 mg or 100 IU of vitamin E), 4% mineral mixture U.S.P. XVII 4 (Sikka et al., 1975), 5 g of glucose and 5 g of complete vitamin mixture (Manna and Hauge, 1953), and 2 drops of Adoxline containing vitamin A (12000 IU/g) and vitamin D_2 (I.P. 2000 IU/g) was fed orally twice a week. The N content was adjusted by adding the required quantity of N free potato starch.

Protein Efficiency Ratio (PER). PER was determined by the method of (AOAC, 1975) except that in place of ten male animals, six animals (three female and three male) were used. Weanling albino rats about 22 days old and weighing 30–40 g were divided into six groups. All groups within each experiment had the same average initial weight. Each group consisted of three males and three females.

The rats were placed in individual all-wire cages with a raised platform. Water was available to them at all times. Food intake was measured every day, and spilled food was collected daily and used to correct the amount of food intake. The animals were weighed twice a week for 28 days.

Net Protein Retention (NPR). NPR was determined by the method of (Bender and Doell, 1957). One-month-old albino rats, three males and three females in each group (five groups in all), having the same initial weight, were used. All FPC flours in the diet were at the 10% protein level. A nonprotein diet was prepared by replacing FPC flour with protein-free starch. Three groups were fed with three different FPC flours, one group with nonproteinous diet and one group with standard casein diet. The experiment was continued for 10 days. The weight of each rat was recorded every third day. NPR was calculated as follows:

$$\text{NPR} = \frac{\text{wt gain of TPG + wt loss of NPG}}{\text{wt of protein consumed}}$$

where TPG = test protein group (FPC flours) and NPG = nonprotein group.

Biological Value (BV). BV was determined by calculation according to regression equation given by (Duggal and Eggum, 1977) for proteins with methionine + cystine as limiting amino acids.

RESULTS AND DISCUSSION

Table II shows food intake, protein intake, weight gain, and PER. The PER values reveal that FPC made from picked ribbonfish had better protein quality than FPC made from catfish and milkfish. Table II shows that FPC made from picked ribbonfish was better than casein followed by FPC made from catfish which was equal to the casein value, while FPC from milkfish had a lower value than casein. The results agree very well with those obtained by Sidwell et al. (1970) in the case of FPC prepared from Atlantic menhaden, ocean paut, Moroccan sardine, and northern anchovy and with FPC prepared from oil sardine (Moorjani and Lahiry, 1970). The analysis of variance shows that PER values were significant for FPC prepared from all three species. Lower PER values obtained in the case of FPC made from milkfish may be due to a comparably lesser amount of essential amino acids present than those in FPC from picked ribbonfish and catfish (Table IV).

The NPR values of FPC made from three species are presented in Table III. The values listed for NPR in Table III also confirm the pattern obtained in the case of PER for three different species. Bender and Doell (1957) reported that NPR is a far more accurate measure of protein value since it measures protein efficiency based on both growth and maintenance. The NPR values for FPC made from three species are slightly lower than casein, which indicates that the growth with FPC is slightly lower in initial stages but in the longer run it is better than casein.

Amino Acid, Chemical Score, BV, and EAAI. The amino acid composition of FPC made from three different species of fishes was determined and presented in Table

Table III. Mean Values of Food Intake, Protein Intake, Gain in Weight by Albino Rats, and NPR of (FPC) Made from Different Species of Fishes at 10% Protein Level

food source	food intake, g/10 days per animal	protein intake, g/10 days per animal	mean wt gain, g/10 days per animal	loss in wt, g/10 days per animal	NPR
catfish (FPC)	79[b]	7.9 ± 0.24[a]	24 ± 1.69[a]	5.9	3.76 ± 0.15[a]
picked ribbonfish (FPC)	70	7.0 ± 0.04	21 ± 1.05	5.9	3.96 ± 0.19
milkfish (FPC)	65.9	6.59 ± 0.17	17.3 ± 0.6	5.9	3.53 ± 0.12
casein	71.8	7.18 ± 0.24	27.0 ± 1.05	5.9	4.60 ± 0.08

[a] Standard error. [b] Average value of one animal.

Table IV. Amino Acid Composition of FPC from Different Species of Fishes, Egg, FAO/WHO Provisional Pattern (1973) (g/16 of N)

amino acid	picked ribbonfish (FPC)	FPC catfish	FPC milkfish	egg	FAO/WHO (1973)*
essential aa					
isoleucine	5.53	4.78	4.56	5.8	4.0
leucine	9.08	8.76	7.76	8.9	7.0
lysine	10.71	9.10	8.37	6.7	5.4
total aromatic aa	8.49	8.26	7.39	10.3	6.1
phenylalanine	4.44	4.46	3.84	6.7	3.05
tyrosine	4.05	3.80	3.55	3.6	3.05
total sulfur aa	4.03	3.52	3.77	5.3	3.5
cystine	0.94	0.84	0.75	3.0	
methionine	3.09	2.68	3.02	2.3	
threonine	5.14	4.78	4.93	5.1	4.0
tryptophan	1.31	1.32	1.34	1.5	1.0
valine	5.76	5.66	5.41	7.5	5.0
nonessential[a] aa					
arginine	6.91	6.88	6.86	6.7	5.2
glycine	6.88	6.56	7.40	3.6	2.2
aspartic acid	11.26	10.58	10.26	10.40	7.7
serine	4.37	4.22	4.28	6.0	7.7
histidine	2.48	2.69	2.13	3.5	2.5
alanine	6.36	6.42	6.84	3.5	6.1
glutamic acid	18.86	16.32	15.87	25.2	14.7
proline	3.40	3.78	5.10		10.7
ammonia	1.02	1.10	0.92		
nitrogen %	14.60	11.73	10.13		

[a] A mixture of nonessential amino acids in FAO/WHO provisional pattern based on the proportion of these amino acids as found in skim milk protein (Eggum, 1968).

Table V. E:N, E:P, E:T Ratios, Chemical Score, EAAI, and BV of FPC Made from Different Species of Fishes[a]

food source	E:N, %	E:P, %	E:T, %	EAAI, %	chemical score, %	BV, %	chemical score, %
catfish (FPC)	1.01	0.52	0.504	89.2	65.7	59.7	100.0
picked ribbonfish (FPC)	1.03	0.560	0.506	95.2	75.2	66.3	114.5
milkfish (FPC)	0.943	0.496	0.485	84.3	70.3	62.9	107.1
egg				100.0	100.0	100.0	
FAO/WHO (1973)							100.0

[a] EAAI (essential amino acid index) is based on the ratios of the amounts of essential amino acids in a protein relative to their amount in whole egg protein (Oser, 1951). Chemical score is the percentage of the most deficient essential amino acid in the protein as compared to the requirement pattern (Mitchel and Block, 1946). E:N, ratio of essential amino acids to nonessential amino acids. E:P, ratio of essential amino acids to protein, 100 g. E:T, ratio of essential amino acids to total amino acids.

Table VI. Distribution of Chemical Constituents in FPC from Different Species of Fishes

FPC source	moisture, %	ash, %	protein N × 6.25	crude fat, %	calcium, g	phosphorus, g
catfish (FPC)	2.5	12.23	73.13	0.13	3.8	2.4
picked ribbonfish (FPC)	2.4	8.9	91.25	0.12	3.6	2.1
milkfish (FPC)	2.6	12.5	63.3	0.12	3.9	2.3

IV. It was observed that the level of amino acid in FPC made from three different species approaches closely to the amino acid in egg and is significantly higher than FAO/WHO pattern (1973). The first limiting amino acids were found to be sulfur amino acids (methionine and cystine), and their level was significantly lower than that of egg. The results agree very well with those of Duggal and Eggum (1977). The amino acid composition of FPC made from three different species by ethanol extraction agrees very well with the amino acid composition of FPC made from different species of fishes by isopropyl alcohol extraction method (Sidwell et al., 1970).

E:N (ratio of essential amino acids to nonessential amino acids), E:P (ratio of essential amino acids to protein, 100 g), E:T (ratio of essential amino acids to total amino acids), chemical score, EAAI, and BV in FPC made from three different species are presented in Table V. The results show that E:N, E:P, and E:T ratios are highest in the case of FPC made from picked ribbonfish, followed closely by catfish and milkfish. This may be the reason for higher PER and NPR values for FPC from picked ribbonfish and catfish.

Picked ribbonfish FPC has also given the highest value for EAAI, chemical score, and BV, which further confirms the higher PER and NPR values obtained in its case. The EAAI for the specie picked ribbonfish approaches very closely the EAAI for egg taken as 100.

Chemical Composition. Results on the distribution of chemical constituents in FPC from different species (Table VI) showed a minimum amount of moisture and a neglegible amount of crude fat present in the samples. The ash content is lower in FPC prepared from the picked ribbonfish specie.

Acceptability of the Product. Sensory evaluation showed that bread containing 5% FPC was as acceptable with respect to texture and flavor as bread with no FPC to a panel of judges. The results agree very well with those obtained by Sidwell et al. (1970), Revanker et al. (1965), Lahiry et al. (1962). The product was quite stable and there was no reversion of flavor when stored in glass jars with screw caps at 20–30 °C for more than a year. The product was free of *Escherichia coli*, *Salmonella*, and pathogenic anaerobes (total plate count below 2000/g).

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Some Chemical and Nutritional Properties of Acylated Fish Protein

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Data are presented on some of the chemical and nutritional properties of acylated fish protein. These include the extent of sulfhydryl group reaction, amino acid composition, protein efficiency ratio (PER) values, the rates of enzyme digestion, and the utilization of ^{14}C-labeled derivatives by rats. The PER of highly acetylated (about 75%) myofibrillar protein was 85% that of reference casein and the PER of medium succinylated (about 50%) myofibrillar protein was 73% that of reference casein. The feeding of ^{14}C-labeled acetyllysine and ^{14}C-labeled acetylated protein to rats showed that both free and protein-bound acetyllysine were partially utilized. ^{14}C-labeled succinyllysine and the succinyllysine in an enzyme hydrolysate of ^{14}C-labeled lysine protein, both administered intravenously were not utilized. Some utilization of orally administered free and protein-bound succinyllysine may have occurred; however, these utilization values may have been distorted by gut microbial activity.

The preparation and functional properties of acylated fish proteins have been described (Groninger, 1973; Groninger and Miller, 1975; Chen et al., 1975; Miller and Groninger, 1976). Kruckenberg (1956) was granted a patent on the production of acyl lysines for animal feed supplements. Also, White and Britton (1959) were granted a patent on the use of ε-acyl lysine derivatives in baked products. Continued interest in these free and protein-bound lysine derivatives as food materials makes information on the chemical and nutritional properties of these derivatives increasingly important.

The chemical properties of acylated proteins depend on the kinds of derivatives that are formed when a protein is reacted with an acylating agent. Acylating agents can react with the amino, thiol, hydroxyl, phenolic, and imidazole groups of protein. The main reaction is with the amino groups of protein (Grant-Green and Friedberg, 1970). Reaction with protein thiol groups has been reported by Habeeb et al. (1958), Hass (1964), Mühlrad et al. (1968), and Meighen and Schachman (1970). Reaction with protein hydroxy groups has been reported by Gounaris and Perlmann (1967), Brattin and Smith (1971),

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