Partial Characterization and Nutritive Value of Proteins from Pacu (*Colossoma mitrei*, Berg 1895)

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The edible portion (fillet) of pacu (*Colossoma mitrei*), a freshwater fish from the south of Brazil, was composed of 67.7% water, 13% protein, 17.2% total lipids, and 1.3% minerals. The muscle proteins were composed of 64.3% myofibrillar, 25% sarcoplasmic, and 8% stroma proteins. Simple polyacrylamide gel electrophoresis revealed six protein bands in the sarcoplasmic fraction; SDS–polyacrylamide gel electrophoresis revealed eight protein structural units with molecular weights ranging from 4000 to 149 000. Total protein nutritive value was similar to that of casein in terms of PER and growth-promoting capacity. Full-fat fillet protein did not differ from casein ($p = 0.05$) with respect to apparent digestibility and net protein utilization but was inferior to casein in the apparent biological value. On the other hand, defatted fillet protein was inferior to casein ($p = 0.05$) in all of the three indices calculated.

Pacu (*Colossoma mitrei*, Berg 1895) is a freshwater fish of the order Cypriniform, suborder Characoidei, and family Myleinae. It was first classified by Holmberg in 1887, as cited by Géry (1896), as *Piaractus mesopotamicus* and later by Berg (1895) as *C. mitrei*. This species has for its habitat the Paraguai–Uruguai rivers, which is extended also into the swamp of Mato Grosso, Brazil. Its large size, eating habits, and high prolificity make it adequate for the practice of fish culture (Silva, 1987). Reproduction in captivity requires hormonal induction. Most of the available literature on this species deals with reproductive cycle induced reproduction and conditions for intensive cultivation (Castagnolli and Donaldson, 1981; Romagosa et al., 1985; Almeida–Toledo et al., 1987; Chabalin et al., 1988a,b).

In this paper the general (percent) composition, amino acid composition, and some physicochemical and nutritional properties of the proteins from the fillet prepared from *C. mitrei* are described.

MATERIALS AND METHODS

Raw Material. Some of the fishes analyzed in this work were from the Aguaculture Research and Training Center at Pirassununga, São Paulo, Brazil, and were about 1.5 years old. These were collected alive, sacrificed in the laboratory, and analyzed within 24 h. Some were obtained frozen after capture in their natural environment at about full size and maintained in their best state of freshness for commercialization. Prior to analysis the whole fishes were thoroughly washed and eviscerated; the muscles were removed in the form of fillets, which included muscles of the dorsal and ventral regions. The muscles were then comminuted and homogenized in a blender. The homogenate was utilized for the various analytical procedures.

Chemical and Biochemical Determinations. Proximate percent composition was determined as follows: water content, determined in an oven (100–105 °C) to constant weight (AOAC, 1975); crude protein by the semimicro-Kjeldahl procedure (AACC, 1976) using the factor 6.25 to convert total percent nitrogen into percent protein; total lipids according to the method of Bligh and Dyer (1959); ashes by incineration (550–600 °C) according to the AOAC (1975) procedure. For amino acid determination the pulp was defatted and then freeze-dried and ground to a fine powder. A sample (20 mg of protein) was submitted to acid hydrolysis (6 N HCl, 110 °C, 22 h). After complete removal of the acid, the hydrolysate was analyzed in an automatic Beckman amino acid analyzer Model 119CL (Beckman Instruments, 1977). The proteins were extracted and fractionated according to the method of Dyer et al. (1950) using extraction with a 5% NaCl solution and centrifugation. The supernatant after centrifugation contained the sarcoplasmic and myofibrillar protein fractions, whereas the stroma proteins remained in the insoluble residue. After the supernatant was diluted 10-fold and left to stand at 0–4 °C for 24 h, the myofibrillar proteins precipitated out of solution, leaving the sarcoplasmic proteins in solution. The two fractions were separated by centrifugation (10000*g*, 15 min, 4 °C). The stroma proteins were determined in the insoluble residue. Simple polyacrylamide gel electrophoresis was performed on the sarcoplasmic protein fraction according to the method of Davis (1964), and SDS–polyacrylamide gel electrophoresis was done on the same fraction according to the method of Weber and Osborn (1969). Pepsin (36 000), ovalbumin (45 000), bovine serum albumin (66 000), and lima bean trypsin inhibitor (10 4000), all from Sigma Chemical Co., were used as molecular weight standards.

In vitro proteolysis of total protein in the muscle was determined according to the pepsin/pancreatin method of Akeson and Stahmman (1964); the reaction was stopped with 10% trichloracetic acid (TCA) solution, final concentration in the reaction mixture. Enzymatically liberated nitrogen was determined in the TCA-soluble fraction after TCA-soluble nitrogen originally present in the sample was discounted. The enzyme systems used were pepsin (Sigma; 1:10 000, 716 units/mg of protein, $E^{1\%}_{1cm(280)}$ in 0.1 N HCl) and pancreatin (Merck; 350 FIP/g of protease, 7500 FIP/g of lipase, 7500 FIP/g of amylase) in 0.1 M sodium phosphate buffer, pH 8.

Biological Assays. Nutritional evaluation was performed on weanling rats of the Wistar strain (males and females) weighing 40–50 g at the beginning of the experiment. During the assays the animals were maintained in individual bottom-screened stainless steel cages at a temperature of 22 ± 1 °C with alternating dark/light periods of 12 h. Diet and water were offered ab libitum, and special care was taken in recovering and considering food spillage in the calculation of food intake. The general composition of the diets was as follows: protein 10%; lipid 11% (corn oil or natural lipid in the fish flour); salt mixture 5% (Hegsted et al., 1941); vitamin mixture 2% (NBC, 1977/1978); fiber 1% (cellulose powder); carbohydrate mixture (25% sucrose/75% starch) to complete 100. In one diet the protein (10%) was provided by casein (reference protein). Two other diets were prepared in which the protein was furnished by the fish muscles. One contained whole fillet that had been lyophilized and ground; the other contained defatted ground fillet. Two types of assays were performed. A PER (protein efficiency ratio) using six rats per diet (four males and two females) with a duration of 28 days was calculated. The animals were weighed once a week, and the PER for each diet was calculated at the end of 28 days by the ratio of total weight gain to total protein consumed. Nitrogen balance

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Table I. Body Weight and Fillet Yield of Pacu (*C. mitrei*) Samples

sample	no. of fish/sample	mean body wt, g	fillet yield, %
A[a]	5	1050	48.6
B[b]	4	2200	50.2
C[b]	4	3200	55.1
D[b]	6	3840	52.0
E[b]	4	3600	54.6
F[b]	3	3800	55.6

[a] Obtained at the aquaculture station at Pirassununga, São Paulo, Brazil. [b] Frozen, from commercial distribution and originated from wild habitat.

Table II. Proximate Percent Composition for the Fillets of Pacu (*C. mitrei*)[a]

sample	water, %	total protein, %	total lipid, %	ash, %
A	68.6	12.7	16.6	1.3
B	67.7	12.8	17.0	1.4
C	68.5	12.9	16.9	1.4
D	66.4	13.5	18.2	1.3
E	68.3	13.1	17.3	1.3
F	66.9	13.3	17.6	1.3
range	66.4–68.6	12.2–13.5	16.6–18.2	1.3–1.4
mean ± SD	67.7 ± 0.9	13.0 ± 0.3	17.2 ± 0.6	1.3 ± 0.1

[a] Mean value of three analytical replications for each sample.

was performed in another test using six weanling rats (four males and two females). The test had a duration of 10 days divided into two periods: the first 5 days was considered an adaptation to the diets and to the laboratory environment, and no materials were collected for analysis; in the last 5 days feces and urine were collected and subsequently analyzed. Food intake and nitrogen ingested were also recorded in this period. Protein digestibility (apparent) was calculated by the ratio of nitrogen absorbed to nitrogen ingested, protein biological value (apparent) was calculated by the ratio of nitrogen retained in the animal body to nitrogen absorbed, and net protein utilization (apparent) was determined by the ratio of nitrogen retained to nitrogen ingested. The results are all expressed as percentage of nitrogen ingested. Analysis of variance and the Duncan test (Puri and Muller, 1980) were applied for the interpretation of the biological assays.

RESULTS AND DISCUSSION

The body weight and fillet yield for six samples (A–F) of fish are shown in Table I, and the proximate percent composition is shown in Table II. Yield of fillet ranged from 48.6 to 55.6% by weight in our samples. Suzuki (1981) presented data on the fillet yield of fish in the range 45–50% with a few species reaching 65%. Therefore, our values are well within the reported range. According to Zaitsev et al. (1969) water and particularly lipid content are the constituents showing the greatest variability in fish muscle, in contrast with low protein variability.

Our data (Table II) indicate relatively low variability in body water and lipid content in spite of differences in origin, age, and season of capture of the fishes used in the various samples. Variations in the same species are normally attributed to factors such as age, physiological state, diet composition, and season of the year when the capture occurred. For another freshwater fish common in Brazil, the Mandi (*Pimelodus claria*), Henderson and Tocher (1987) reported a range of variation for total lipids from 8.3 to 20.5% depending on the season. The inverse relationship between water and lipid contents reported by various authors (Stansby, 1962; Thurston et al., 1959) was also observed in this work as well as an inverse relationship between water content and size of the fish (Suzuki, 1981).

On the basis of our analytical data the pacu should be classified as a fish of high fat (>15%) and low protein (<15%) content, according to Stansby (1962). The high fat content could be explained by the inclusion in the fillet of the ventral muscles where the cavity fat concentrates. Variations in the fat content of various portions of the fillet were also reported by Henderson and Tocher (1987).

Table III. Amino Acid Composition of Fillets from Pacu (*C. mitrei*) and a Comparison with NRC (1980) Reference Protein

amino acid (g/16 g of N)	pacu protein[a]	ref std (NRC, 1980)
lysine	10.0	5.1
histidine	2.2	1.7
aromatic amino acids (Tyr + Phe)	8.1	7.3
leucine	8.8	7.0
isoleucine	4.5	4.2
total sulfur amino acids ($^1/_2$-Cys + Met)	3.6	2.6
valine	3.9	4.8
threonine	3.8	3.5
arginine	6.6	
phenylalanine	4.3	
tyrosine	3.8	
methionine	2.8	
$^1/_2$-cystine	0.8	
alanine	5.1	
glycine	4.8	
proline	4.7	
glutamic acid	19.8	
serine	4.8	
aspartic acid	10.5	

[a] Mean values from three samples, each composed of three fishes.

Amino acid composition of total protein in the fillet is given in Table III. The results are similar to those reported by Mai et al. (1980) for six freshwater species from the United States, except for lysine content which was higher in the pacu. High lysine, 11.4 g/16 g of N, was also found for curimbatá (*Prochilodus scrofa*) by Maia et al. (1983) and for tilapia (*Tilapia nilotica*) (10.6 g/16 g of N) by Khalil et al. (1980). By comparing the essential amino acids of pacu with the National Research Council (NCR, 1980) reference protein (Table III), one concludes that all essential amino acids in pacu, except valine, are in excess of the recommendation.

In vitro proteolysis of total pacu protein was studied in four different samples. Percent proteolysis from 83.5 to 85.6% (mean value 84.3 ± 1.2%) was inferior to 91.5% for casein. Jhaveri et al. (1984) encountered higher in vitro proteolysis in four of five saltwater species, i.e., Atlantic cod fish (*Gadus morhua*) 92.2%, monkfish (*Lophius piscatorius*) 91.3%, whiting (*Merluccius bilinearis*) 89.8%, saup (*Stenotomus chrysops*) 89.2%, and squid (*Loligo pealei*) 83.1%. Direct comparison of these data is not possible not only because we are dealing with different species but also because Jhaveri et al. (1984) used a different method for measuring proteolytic susceptibility.

Results of the nitrogen balance and PER determined on casein, full-fat, and defatted fillet from pacu are given in Table IV. The PER values calculated for the diets containing full-fat or defatted pacu were similar to those of casein, and no statistical differences were found among the three diets ($p = 0.05$). Also, food intake and body weight gain at the end of the experimental period (28 days) showed no statistical differences among the three protein sources (Table IV). Afolabi et al. (1984) reported PER values in the range 2.2–2.9 for five freshwater Nigerian species. On the other hand, Jhaveri et al. (1984) encountered PER values ranging from 3.2 to 4.0 for five New England marine species which were compared with a PER of 3.0 for casein. The values 2.9–3.0 for pacu seem to compare with the highest values reported for freshwater fishes. The results obtained in the nitrogen balance showed that, except for protein digestibility in the full-fat

Table IV. Results of Nitrogen Balance and PER Determination on Casein and Full-Fat and Defatted Fillets as a Source of Dietary Protein

determination	source of protein in the diet[a]		
	casein	full-fat fillet	defatted fillet
nitrogen intake[b]	976.1 ± 85.5	772.8 ± 48.1	636.1 ± 16.3
fecal nitrogen[b]	100.0 ± 18.6	76.3 ± 4.3	100.0 ± 9.0
urinary nitrogen[b]	74.6 ± 10.5	118.1 ± 20.2	93.6 ± 46.6
nitrogen balance[b]	801.4 ± 79.3[a]	578.2 ± 70.9[b]	442.4 ± 52.3[c]
app protein digestibility[c]	89.8 ± 1.5[a]	90.0 ± 1.4[a]	84.3 ± 1.2[b]
app protein biol value[c]	91.4 ± 1.6[a]	82.8 ± 4.4[b]	82.5 ± 9.0[b]
NPU[c] (app)	82.0 ± 1.8[a]	74.6 ± 4.8[ab]	69.6 ± 8.0[b]
diet intake[d]	228.0 ± 25.3[a]	284.2 ± 18.0[a]	272.0 ± 7.0[a]
body wt gain[d]	81.8 ± 4.5[a]	82.8 ± 9.2[a]	79.0 ± 4.6[a]
PER[e]	2.8 ± 0.1[a]	2.9 ± 0.3[a]	3.0 ± 0.1[a]

[a] Different superscript letters in the horizontal lines indicate significant statistical difference ($p = 0.05$). [b] Mean value (mg)/rat, 5 days. [c] Mean value (%) of six rats (four males and two females). [d] Mean value (g)/rat, 5 days. [e] Body weight gain/protein intake, 28 days.

pacu fillet, the indices of nitrogen absorption and retention are superior for casein compared with those for pacu proteins ($p = 0.05$). Nitrogen retention and protein digestibility were lower ($p = 0.05$) in defatted than in full-fat fillet. The reason for this is not apparent. It seemed that the ethyl ether used for fat extraction decreased protein digestibility and absorption. However, retention of nitrogen absorbed was not affected as shown by the protein biological value of full-fat and defatted fillet (Table IV). Burnette and Rusof (1978) and Bender (1978) stated that treating a proteinaceous food with fat solvents tended to decrease the protein nutritive value.

Extraction of the muscle proteins with a 5% NaCl solution at pH 7.2 resulted in 92% extraction of the total nitrogen. From the extracted nitrogen 64.3% was determined to be myofibrillar proteins, 25.0% sarcoplasmic proteins, and 2.7% nonprotein nitrogen. The 8% protein that remained in the insoluble residue was considered stroma and denatured or complexed proteins. Similar extraction (93%) was obtained by Awad et al. (1969) from the freshwater fish *Coregonus clupeiformis*. Khalil et al. (1980) found 66.7% for the myofibrillar proteins and 24.8% for the sarcoplasmic proteins in *T. nilotica*. Moorjani et al. (1962) found different values for extractable proteins in four species of freshwater fish in India, i.e., total extractable protein of 90.7, 77.8, 90.0, and 86% with sarcoplasmic protein contents corresponding to 29.0, 22.0, 35.0, and 35.7%, respectively.

Polyacrylamide gel electrophoresis of the sarcoplasmic proteins according to the method of Davis (1964) revealed six protein bands numbered I–VI in Figure 1B. Band V with mobility of 0.33 with respect to the dye was the strongest one. When the sarcoplasmic proteins were treated with SDS and 2-mercaptoethanol and run on SDS–PAGE according to the method of Weber and Osborn (1969), a total of eight protein bands were seen with the following structural units molecular weights (MW): I, 149 200; II, 127 000; III, 66 900; IV, 52 700; V, 38 300; VI, 21 900; VII, 6100; and VIII, 4100. Polyacrylamide gel electrophoresis has been largely used as a technique for characterization and identification of species (Cowie, 1968; Mackie and Jones, 1978; Keenan and Shaklee, 1985). It has also been used for detection of protein deterioration or adulteration during storage and processing of fish (Cowie, 1968; Draetta et al., 1985).

We have demonstrated that pacu (*C. mitrei*) is a freshwater fish having high fat and relatively low protein content. The proteins are of good quality with respect to growth promotion and PER. Digestibility and NPU values of pacu proteins are inferior to those of casein. By

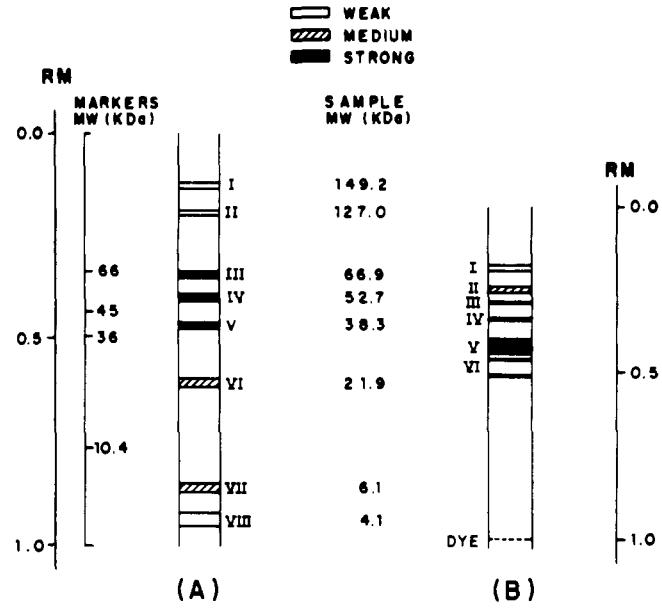

Figure 1. Simple PAGE (B) and SDS–PAGE (A) electrophoresis of the sarcoplasmatic proteins extracted from the fillet of pacu (*C. mitrei*). MW markers for the SDS–PAGE: bovine serum albumin, 66 000; ovalbumin, 45 000; pepsin, 36 000; lima bean trypsin inhibitor, 10 400.

extraction and fractionation of the muscle proteins we obtained 64.3% myofibrillar proteins, 25% sarcoplasmic proteins, and 8% stroma protein. Sarcoplasmic proteins submitted to simple PAGE revealed six protein bands and on SDS–PAGE gave eight structural units with molecular weights ranging from 4000 to 149 000.

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