Food Chemistry **12** (1983) 275–286

Proximate, Fatty Acid and Amino Acid Composition of the Brazilian Freshwater Fish *Prochilodus scrofa*

Everardo L. Maia,* Delia B. Rodriguez-Amaya

&

Jaime Amaya-Farfan

Faculdade de Engenharia de Alimentos e Agrícola,
CP 6121, Universidade Estadual de Campinas,
13100 Campinas, São Paulo, Brazil

(Received: 25 January, 1983)

ABSTRACT

The proximate, fatty acid and amino acid compositions of the skinned fillets of the Brazilian freshwater fish Prochilodus scrofa *were determined. The water, protein, lipid and ash contents (%) were: 76·5 ± 2·2, 20·4 ± 1·0, 2·7 ± 1·5 and 1·3 ± 0·0, respectively. The marked variation in total lipid content was reflected in the fatty acid composition where qualitative and quantitative differences could be discerned between samples. The principal fatty acids were palmitic, oleic, palmitoleic, stearic, linolenic and linoleic acids. The amino acid composition showed that the* P. scrofa *proteins were high in lysine and methionine but low in cysteine/cystine.*

INTRODUCTION

Fish consumption in Brazil has been based principally on marine species. The potential of freshwater fishes has been largely underrated. Recently, however, the SUDEPE, a government agency entrusted with the task of developing fish resources, has given impetus to increased utilisation of the

* Present address: Departamento de Química, Universidade de Amazonas, 69000 Manaus, AM., Brazil.

Food Chemistry 0308-8146/83/$03·00

freshwater species, encouraging research on their production, commercialisation and preservation. Along with this incentive comes the concomitant need for information about the chemical composition and nutritive value of these species.

Of the various Brazilian freshwater species, those belonging to the genus *Prochilodus* are especially promising because of their wide geographical distribution, adaptability to different conditions, facility for artificial fertilisation and fast reproduction and development (Ihering & Azevedo, 1934). Of these species, *Prochilodus scrofa*, Steindachner 1881, popularly known as 'curimbatá' or 'corimbatá', is the most extensively studied in terms of its biological characteristics while very little information about its chemical composition has been published. In this study, therefore, the proximate, fatty acid and amino acid compositions of *Prochilodus scrofa* collected from various places in the state of São Paulo (Central Southern Brazil) were determined.

MATERIALS AND METHODS

Materials

For the determination of the proximate composition, six samples, consisting of between one and eight fish, were analysed. The collection date, origin and average weight of the fish are presented in Table 1. Except for the samples taken from a local fish wholesale distributor, the fish were collected live, stored in ice and transported immediately to our laboratory. All proximate analyses, carried out in duplicate, were completed within 24 h.

Six samples, consisting of between one and six fish (two samples from Promissão, SP and four from the local fish distributor), obtained between October, 1979 and August, 1981, were analysed for fatty acid composition. Two composite samples of four and six fish, respectively, were used for the amino acid determination.

The fish comprising each sample were washed, filleted and skinned. The fillets were homogenised in a Waring blender and the homogeneous mass was used for the various determinations.

Determination of the proximate composition

The moisture and ash contents were determined according to the method

TABLE 1
Description of Samples of *Prochilodus scrofa* Used for Proximate Composition Determination

Sample	*Collection date*	*Number of fish*	*Average weight (g)*	*Source*
A	4 July, 1979	8	750	Mogiguaçu River, Pirassununga, SP
B	7 July, 1979	3	890	Aquaculture Station, Pirassununga, SP
C	7 July, 1979	1	280	Aquaculture Station, Pirassununga, SP
D	19 July, 1979	2	1 100	Frigorífico Comercial (Wholesale market), Campinas, SP
E	23 January, 1980	6	760	Aquaculture Station, Promissão, SP
F	5 May, 1980	6	1 000	Aquaculture Station, Promissão, SP

of Umemoto (1972), the total protein by the semi-micro Kjeldahl method described by Pearson (1973) and the total lipids by the method of Bligh & Dyer (1959).

Determination of the fatty acid composition

The lipids were extracted according to the method of Bligh & Dyer (1959) and the methyl esters of the fatty acids were prepared according to the technique described by Metcalfe *et al.* (1966). Gas chromatography was performed with a Perkin-Elmer gas chromatograph, Model 990, fitted with FID. Stainless steel columns, 1·8 m × 3·5 mm inside diameter, packed with 10% DEGS in Chromosorb W (80–100 mesh) and with 10% EGSSX in Chromosorb W, were used. The flow rates were: N_2, 30 ml/min; air, 500 ml/min and H_2, 35 ml/min. The operating temperatures were: detector, 250 °C; injector, 250 °C; DEGS column, 180 °C and EGSSX column, 190 °C.

The identification of the fatty acids was based on: (1) comparison of retention time and spiking with standard methyl esters of fatty acids from Polyscience Corp. (USA); (2) semi-logarithmic plots of relative retention

times against carbon chain lengths of the standards and fitting in these plots the log of the relative retention times of the fatty acids under investigation; (3) calculation of separation factors (Kates, 1972; Kinsella *et al.*, 1977).

Quantitative results are expressed as area percentages. The peak areas were calculated by multiplying peak height by the width at half-height.

Determination of the amino acid composition

The residue left after extraction of the lipids was dried in an oven at 50–60 °C to constant weight and finely ground in an abrasion mill. A sample containing approximately 20 mg of protein was hydrolysed in a screw-cap tube filled with 6 N HCl at 110 °C for 22 h. After fast removal of the excess acid in a rotary evaporator, the amino acids were chromatographed in a Beckman 119-CL automatic analyser according to standard ion-exchange procedures (Beckman Instruments, 1977). Individual amino acids were quantitated by comparison of the peak areas with those of a commercial standard mixture of amino acids (Beckman Instruments, Palo Alto, CA, USA). The molecular weights of the free amino acids were used for the calculation of the amino acid per cent composition. Tryptophan determination was undertaken according to the procedure of Spies (1967).

TABLE 2
Proximate Composition of Skinned Fillets of *Prochilodus scrofa**

Sample	*Amount (%)*			
	Water	*Protein*	*Lipids*	*Ash*
A	76·1	20·7	2·5	1·3
B	76·5	21·5	1·3	1·3
C	80·0	19·0	0·7	1·3
D	77·4	19·9	2·2	1·3
E	75·6	20·1	3·2	1·3
F	73·6	21·5	4·0	1·4
Range	73·6–80·0	19·0–21·5	0·7–4·0	1·3–1·4
Mean and standard deviation	76·5 ± 2·2	20·4 ± 1·0	2·3 ± 1·2	1·3 ± 0·0
Coefficient of variation (%)	2·7	4·9	52·1	0·0

* Values corresponding to samples specified in Table 1.

TABLE 3
Fatty Acid Composition of the Muscle Lipids of *Prochilodus scrofa*

Fatty acids	*Percentages Samples*						*Mean and standard deviation*
	A	*B*	*C*	*D*	*E*	*F*	
12:0	—	—	—	—	Trace	—	
13:0	Trace	—	—	—	Trace	—	
13:1	Trace	Trace	—	—	—	Trace	
14:0	3·2	3·8	3·2	3·3	4·0	3·0	3·4 ± 0·4
14:1	0·4	0·6	0·4	Trace	2·2	Trace	
NI	Trace	—	—	—	Trace	Trace	
15:0	0·7	Trace	Trace	Trace	0·3	Trace	
NI	Trace	Trace	—	—	Trace	Trace	
15:1	Trace	Trace	—	—	Trace	Trace	
16:0	33·1	36·8	29·6	32·0	31·3	31·4	32·4 ± 2·4
16:1	13·9	15·2	14·9	14·6	11·9	12·7	13·9 ± 1·3
17:0	0·2	Trace	Trace	Trace	0·2	Trace	
NI	Trace	—	—	—	—	Trace	
16:2	1·3	—	—	0·6	1·2	Trace	
17:1	0·3	0·7	0·8	0·6	Trace	Trace	
18:0	8·9	7·2	7·5	8·3	6·8	9·8	8·1 ± 1·1
18:1	24·1	20·3	26·4	22·9	21·6	23·9	23·2 ± 2·1
16:4 or 19:0	Trace	—	Trace	Trace	—	Trace	
18:2	5·0	8·0	6·1	3·7	4·4	5·5	5·4 ± 1·5
19:1	Trace	—	—	—	—	Trace	
20:0	1·1	—	—	Trace	—	Trace	
17:4 or 19:2	—	—	—	—	Trace	—	
18:3	8·8	7·4	6·8	5·6	9·6	8·1	7·7 ± 1·4
NI	—	—	—	—	Trace	—	

(*continued*)

TABLE 3—*contd.*

Fatty acids	*Percentages Samples*						*Mean and standard deviation*
	A	*B*	*C*	*D*	*E*	*F*	
18:4	0·7	—	—	—	Trace	Trace	
20:2	—	—	—	—	Trace	Trace	
21:1	—	—	—	—	Trace	—	
22:0	Trace	Trace	Trace	0·7	1·6	Trace	
NI	—	—	—	—	Trace	—	
20:3	Trace	Trace	1·8	5·0	3·8	5·7	
22:1	—	—	—	—	Trace	—	
20:4 or C_{23}	—	—	—	—	1·0	Trace	
20:5 or $C_{22:2}$	—	—	2·4	2·6	Trace	Trace	
22:3	—	—	—	—	Trace	Trace	
22:4	—	—	—	—	Trace	Trace	
Total saturated	47·2	47·8	40·3	43·6	45·2	44·2	44·7 ± 2·7
Total monoenoic	38·7	36·8	42·5	38·1	35·7	36·6	38·1 ± 2·4
Total polyenoic	15·8	15·4	17·1	18·2	19·0	19·3	17·5 ± 1·6
Total lipids (%)	2·8	3·2	4·0	5·6	4·7	1·7	
Source of fish	Wholesale market	Aquaculture station	Aquaculture station	Wholesale market	Wholesale market	Wholesale market	
Collection date	24.10.79	23.1.80	14.5.80	6.6.80	23.6.80	13.8.81	
Number of fish	1	6	3	4	2	3	

NI—not identified.

RESULTS

The proximate compositions of the samples described in Table 1 are presented in Table 2. Of the various constituents determined, only total lipids showed pronounced variation (coefficient of variation = 52·1).

The ranges and average percentages of the fatty acids of six composite samples, varying from 2·5 to 5·6 % in total lipids, are presented in Table 3.

TABLE 4
Amino Acid Composition of Homogenised Flesh and Intramuscular Bones of Defatted Samples of *Prochilodus scrofa*[a]

Amino acid	*g/16 g N*	*g/16 g N (Condensed value)*[b]
Aspartic acid	12·9 ± 1·63	11·2
Threonine	5·44 ± 0·57	4·62
Serine	4·89 ± 0·76	4·05
Glutamic acid	18·6 ± 0·62	16·3
Proline	4·11 ± 0·21	3·47
Glycine	5·86 ± 0·49	4·46
Alanine	7·84 ± 0·47	6·26
Cystine[c]	0·51 ± 0·01	0·43
Valine	6·78 ± 0·82	5·74
Methionine	2·69 ± 0·38	2·37
Iso-leucine	5·74 ± 0·69	4·95
Leucine	9·67 ± 0·57	8·34
Tyrosine	3·78 ± 0·17	3·40
Phenylalanine	5·19 ± 0·30	4·62
Histidine	2·97 ± 0·02	2·63
Lysine	11·4 ± 0·10	9·98
Arginine	7·78 ± 0·31	6·98
Tryptophan[d]	1·32 ± 0·04	1·20
Total	117·72	100·96

[a] Means and standard deviations from separate analyses of two composite samples of four and six fish.
[b] Mean value using the molecular weight of condensed amino acids.
[c] Value not corrected for losses.
[d] Determined separately with *p*-dimethylamino-benzaldehyde.

Thirty-five fatty acids were detected, of which palmitic, oleic, palmitoleic, stearic, linolenic and linoleic acids were the major components.

The amino acid composition, expressed in terms of grams of amino acid per 16 g of total nitrogen, is shown in Table 4.

DISCUSSION

Proximate composition

The well documented variability of total lipid content is immediately apparent from Table 2. Samples A, B, D and E demonstrate the variability of fish collected from different locations. Samples E and F show seasonal effects.

The inverse relationship between moisture and lipid content of the fish, observed by many authors, was also exhibited by *P. scrofa*. The sum of these constituents was 78–80 %.

The inverse relationship between water content and the size of the fish, observed by Thurston *et al.* (1959) in fish of different species, was also shown by samples B and C, which came from the same batch of fish and were separated according to size. This relationship was not followed by the other samples, but, since these samples were collected at different times from different places, other factors might have exerted some influence.

P. scrofa from the Mogi Guaçu River (Pirassununga, SP) had already been investigated by Lessi (1968) who obtained the following composition of the fish muscle: water, 72·50 %; protein, 20·50 %; ether extract, 6·68 % and minerals, 1·38 %. For a composite sample of six fish collected from the same river but at a different locality, Camargo *et al.* (1973) obtained the following data: water, 78·07 %; ether extract, 2·79 %. The results of the latter study fall within the ranges reported in the present paper. However, of ten samples analysed for total lipids (Tables 2 and 3), the highest amount obtained in the present study (5·6 %) is lower than the 6·68 % reported by Lessi. Since the number of samples and time of collection were not specified in Lessi's work, it is difficult to explain this discrepancy. In any case, for the most part, *P. scrofa* falls into the low fat, high protein fish category (Stansby, 1972).

Fatty acid composition

Table 3 shows the qualitative and quantitative variability of the fatty acid

composition. These results agree with the often cited assertion that dietary fatty acids are the principal determining factors of the tissue fatty acid composition of fish. The number of fatty acids in samples B and C, consisting of fish kept at an aquaculture station fed with commercial fish diet, was much less than in samples of fish captured from the natural habitat where the dietary fatty acid composition could be more variable.

The fatty acid composition of *P. scrofa* agrees with the general finding that freshwater species have higher levels of C_{16} and C_{18} acids and lower levels of C_{20} and C_{22} acids compared with marine fishes.

Although much discussion has appeared in the literature about the difference in the fatty acid composition of freshwater and marine species, a comparison between freshwater species has not been encountered. Although such a comparison would be subject to the numerous factors that affect lipid content and fatty acid composition, from the data already presented in the literature, a characteristic pattern can be discerned, differentiating freshwater species taken from different regions.

In five Canadian species studied by Ackman (1967), the principal fatty acids, in decreasing quantitative order, were: oleic, palmitoleic, palmitic and eicosapentaenoic acid ($C_{20:5}$), with the exception of one species in which the positions of palmitoleic and palmitic acids were interchanged.

Docosahexaenoic acid ($C_{22:6}$) was found to be a major constituent of freshwater fishes of the USA. In three species from the state of Washington the order was: oleic, palmitic, $C_{22:6}$, palmitoleic and $C_{20:5}$, except in one species where $C_{22:6}$ and palmitic acid exchanged positions (Gruger *et al.*, 1964). These same five fatty acids predominated in eighteen species from the state of New York. However, the first three fatty acids competed for the first position, with $C_{22:6}$ surpassing oleic and palmitic acids in six species (Kinsella *et al.*, 1977).

Oleic and palmitic acids were also the predominant constituents in six Indian species (Viswanathan Nair & Gopakumar, 1978). In contrast to the species already cited, stearic acid assumed third place, surpassing palmitoleic acid. Oleic, palmitic and stearic acids were also the most abundant fatty acids in four species of mussels, also from India (Sen *et al.*, 1976). Arachidonic acid ($C_{20:4}$) was, however, comparatively higher; in fact, occupying the first place in one species.

Based on the above discussion, it came as no surprise that the fatty acid composition of *P. scrofa* resembled that of *Pimelodus clarias*, another Brazilian freshwater fish from São Paulo, rather than that of freshwater species of other countries. According to Andrade (1975), the principal

fatty acids of the latter fish were: oleic, palmitic, palmitoleic, stearic, linoleic and linolenic acids.

Of the total calculable fatty acids, the saturated acids comprised 44·7 % and the unsaturated acids 55·6 %. The ratio of unsaturated to saturated fatty acids was 1·2 for *P. scrofa*, about 1·6 for *P. clarias* and an average of 1·3, 3·0 and 3·2 for the species investigated by Viswanathan Nair & Gopakumar (1978), Kinsella *et al.* (1977) and Ackman (1967), respectively. The freshwater species of Canada and the USA (temperate water) were thus richer in unsaturated fatty acids than the freshwater fishes of India and Brazil (tropical water). Even the Indian species contained appreciable amounts of the polyunsaturated C_{20} and C_{22} fatty acids which were detected only in traces in *P. scrofa*. While these findings limit the utilisation of *P. scrofa* in special low fat, highly polyunsaturated diets, from the technological standpoint, they could indicate greater resistance to oxidative deterioration. In fact, fish patties formulated from *P. scrofa* maintained their good quality throughout a storage experiment (92 days at −20 °C), without the addition of antioxidants or preservatives (Maia *et al.*, 1982).

Amino acid composition

Amino acid determinations demonstrated the presence of lysine- and methionine-rich proteins in *P. scrofa*. In relation to other freshwater fishes such as the white sucker, burbot, black crappie, walleye pike and yellow perch (Mai *et al.*, 1980), the proteins of *P. scrofa* have an unusually high lysine content, comparable only with that of bolti or tilapia (10·6 g/100 g protein) (Khalil *et al.*, 1980). *P. scrofa* is also rich in methionine, an amino acid often limiting in diets where leguminous protein sources are staples. Our values for cysteine/cystine do not account for losses (about 30 %) during preparation of the sample but do suggest that this sulphur amino acid is present in amounts lower than those found in the fishes mentioned above or even much lower than that found in the rainbow trout roe (3·8 g/100 g protein) (Kaitaranta *et al.*, 1980).

Microbiological estimation of some essential amino acids in an earlier study on *P. scrofa* proteins (Lessi, 1968) showed reasonable agreement with our results for threonine, cysteine, valine, leucine, phenylalanine and lysine. The concentration reported therein for methionine (5·49 g/16 g N), however, seems to overestimate this amino acid.

REFERENCES

Ackman, R. G. (1967). Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. *Comp. Biochem. Physiol.*, **22**, 907–22.

Andrade, M. O. (1975). *Preparo, seleção, armazenamento e estudos químicos e sensoriais de conservas de mandi* Pimelodus clarias *Block*. MSc thesis, Universidade de São Paulo, São Paulo, Brazil.

Beckman Instruments, Spinco Division (1977). *Amino acid analyzers' instruction manual.* Palo Alto, CA, USA.

Bligh, E. G. & Dyer, W. L. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–17.

Camargo, L. A. A., Yasaka, W. J. & Oga, S. (1973). Constantes físicas e químicas dos extratos estereos de alguns peixes brasileiros. *Arch. Latinoamer. Nutr.*, **23**, 135–44.

Ihering, V. R. & Azevedo, P. A. (1934). A curimbata dos açudes Nordestinos (*Prochilodus argenteus*). *Arch. Inst. Biol.*, **5**, 143–84.

Gruger, Jr., E. H., Nelson, R. W. & Stansby, M. E. (1964). Fatty acid composition of oils from 21 species of marine fish, freshwater fish and shellfish. *J. Am. Oil Chem. Soc.*, **41**, 662–7.

Kaitaranta, J. K., Lamppu, R. & Linko, R. R. (1980). Amino acid content of baltic herring and rainbow trout roe. *J. Agric. Food Chem.*, **28**, 908–1111.

Kates, M. (1972). *Techniques of lipology.* North Holland Publishing Co., Amsterdam.

Khalil, M. E., Moustafa, E. K. & Osman, H. O. A. (1980). Composition of bolti (*Tilapia nilotica*) muscle proteins. *Food Chem.*, **5**, 175–84.

Kinsella, J. E., Shimp, J. L., Mai, J. & Weihranch, J. (1977). Fatty acid content and composition of freshwater finfish. *J. Am. Oil Chem. Soc.*, **54**, 424–9.

Lessi, E. (1968). Aspectos químico-bromatológicos do corimbata (*Prochilodus scrofa* Steindachner 1881). Estudo da fração proteica. *Rev. Fac. Fram. Odont. Araraquara* **2**, 121–32.

Mai, J., Shetty, J. K., Kan, T. M. & Kinsella, J. E. (1980). Protein and amino acid composition of select freshwater fish. *J. Agric. Food Chem.*, **28**, 884–5.

Maia, E. L., Rodriguez-Amaya, D. B. & Moraes, M. A. C. (1982). Formulation, acceptability and stability of fish patties made with the freshwater fish *Prochilodus scrofa* Steindachner. *Ciênc. Tecnol. Aliment*, **2**, 33–46.

Metcalfe, L. D., Schmitz, A. A. & Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.*, **38**, 514–15.

Pearson, D. (1973). *Laboratory techniques in food analysis.* John Wiley & Sons, London.

Sen, P. C., Ghosh, A. & Dutta, J. (1976). Fatty acids of the lipids of murrels. *J. Sci. Food Agric.*, **27**, 811–18.

Spies, J. R. (1967). Determination of tryptophan in proteins. *Anal. Chem.*, **39**, 1412–16.

Stansby, M. E. (1972). Proximate composition of fish. In: *Fish in nutrition* (Heen, E. & Kreuzer, R. (Eds)). Fishing News, London, p. 55.

Thurston, C. E., Stansby, M. E., Karick, N. L., Miyauchi, D. T. & Clegg, W. C. (1959). Composition of certain species of freshwater fish. II. Comparative data for 21 species of lake and river fish. *Food Res.*, **24**, 493–502.

Umemoto, S. (1972). Fundamentals of chemical experiments. In: *Utilization of marine products* (Okada, M., Hirao, S., Noguchi, E., Sozuki, T. & Yokosuki, M. (Eds)). Overseas Technical Cooperation Agency, Tokyo, pp. 147–58.

Viswanathan Nair, P. G. & Gopakumar, K. (1978). Fatty acid composition of 15 species of fish from tropical waters. *J. Food Sci.*, **43**, 1162–4.