

Biochemical, Nutritional and Microbiological Quality of Six Species of Smoked Fishes from Manipur

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Biochemical, nutritional and microbiological quality of six smoked fishes viz. *Esomus danricus*, *Puntius sophore*, *Mystus bleekeri*, *Amblypharyngodon mola*, *Notopterus notopterus* and *Glossogobius giuris* of Manipur were analysed. Non protein nitrogen (NPN) and total volatile base nitrogen (TVBN) were 0.50 to 1.86% and 40.00 to 80.00 mg% respectively. In vitro digestibility of one sample (*P. sophore*) was higher than that of casein. Alpha amino nitrogen liberated in pepsin phase were 28.44 to 49.75% of total N and in trypsin phase, 53.25 to 70.37% of total N. Available lysine content was 2.17 to 4.68 g/16g of N. Total plate count of bacteria (TPC), total plate count of fungi (TFC), most probable number (MPN) of coliform, *Staphylococcus aureus* and faecal *Streptococci* counts were 10^3 - 10^8 .g⁻¹, 10^2 - 10^4 .g⁻¹, 0- 10^2 .g⁻¹, and 10^2 - 10^5 .g⁻¹ respectively. But *Escherichia coli* and *Salmonella* were not detected in any of the smoked fishes studied. *Aspergillus niger* and *Cladosporium cladosporoides* were the dominant fungal flora.

Key words: biochemical composition, nutritive value, microbiological quality, smoked fish

Smoking of fish is practised in many parts of the world. The process not only imparts colour and flavour, but also has a preservative effect, since formaldehyde, phenol and other substances evolved from wood smoke are deposited on the fish during smoking (Stansby, 1963). Antioxidant effects of wood smoke were reported by Ohtani (1938). Fretheim *et al.* (1980) also reported that smoke condensate has antioxidant effect, even at low concentration. In Manipur, fishes of all types are smoked and they are consumed after frying or roasting or as an ingredient to add flavour and taste. Though there are reports on smoke curing of fish from India as well as from abroad (Chandrasekhar *et al.*, 1979; Muraleedharan *et al.*, 1986; Papov *et al.*, 1979; Motohiro, 1988), the age old technique adopted in Manipur is different, as salting is not involved in the processing. Lilabati *et al.* (1993, 1997) and Vishwanath *et al.* (1998) have reported the biochemical and microbiological quality of some smoked fishes of the state.

Smoked *Esomus danricus*, *Puntius sophore*, *Mystus bleekeri*, *Amblypharyngodon mola*, *Notopterus notopterus* and *Glossogobius giuris* are some of the important smoked fishes which are popular in Manipur. This paper reports the biochemical composition, nutritive value and microbiological quality of these six species of fish, smoked in the traditional Manipuri way.

Materials and Methods

In Manipur, smoking of medium size fish is done by spreading the fish on a wire tray and then exposing to flame briefly to burn the skin. The process is repeated after turning the fishes upside down. In case of small size fish they are simply spread on a wire tray. Then they are exposed to smoke from burning paddy husk from a distance of about 30 cm below for about 2-3 h at 70-80°C.

Smoked *E. danaricus*, *P. sophore*, *M. bleekeri*, *A. mola*, *N. notopterus* and *G. giuris* were collected from the fish vendors and then brought to the laboratory aseptically.

Total nitrogen (TN), pure protein nitrogen (PPN), non protein nitrogen (NPN), soluble nitrogen (SN), moisture, lipid and ash were determined according to AOAC (1975) methods. Total volatile base nitrogen (TVBN) was determined as per the method of Morris (1959). For determination of pH, 1 g of smoked fish was homogenized with 10 ml of distilled water. pH values were measured using a pH meter.

In *vitro* digestibility was estimated as per the procedure of AOAC (1960). For determination of in *vitro* digestibility, defatted and moisture free samples were taken. 1 g of powdered sample and 15 ml of 0.2% pepsin (activity 1:1) were taken in flasks and incubated at $40\pm1^{\circ}\text{C}$ for 2 h with constant shaking in a metabolic shaker. At the end of 2 h all the flasks were removed. Three flasks out of the six from each sample were taken. The contents were adjusted to pH 8.2 with 1 N NaOH and then 0.3 ml of trypsin (activity 1:250) was added to each. Incubation was continued for another 22 h. The suspensions, after digestion, were centrifuged at 3000 rpm for 20 min. The value of digestible nitrogen was obtained by subtracting the value of undigested nitrogen from total nitrogen of the sample. Digestible protein in pepsin phase, and combined pepsin and trypsin digestions, were expressed as percentage of total proteins of the samples. The results were compared with that of casein. Alpha amino nitrogen of smoked fishes was estimated by Sorensen's method of formol titration using barium

hydroxide (AOAC, 1984). Available lysine was also estimated using the method of Carpenter (1960).

Enumeration of total viable bacteria (TPC), total fungi (TFC), most probable number (MPN) of coliform and detection of pathogenic bacteria, viz. *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and faecal *Streptococci* was done as per the procedure of APHA (1976). The suspected pathogenic bacterial colonies were further tested using the methods of APHA (1976) and Kiss (1984). Fungal colonies on PDA were picked, stained with cotton blue in lactophenol and identified following the methods of Gilman (1957) and Ellis (1971, 1976).

Results and Discussion

Biochemical composition of the smoked fishes is listed in Table 1. Crude protein and pure protein contents of the smoked fishes were moderately high when compared with those of smoked fishes from outside Manipur reported by Lilabati & Vishwanath (1996). Protein content of these samples was slightly lower when compared with the fresh samples reported by Sarojnalini & Vishwanath (1988).

Total lipid content was in the range of 3.15 to 14.35% (DWB), highest being in *A. mola*. TVBN values were in the range 38.00-80.00 mg%. Muraleedharan & Valsan (1976) also observed the value of 80.42 mg% in smoked sardine fillets. Lu *et al.* (1988) did not consider TVBN value of about 10.5 mg%

Table 1. Biochemical composition of six species of smoked fish

Name of fishes	Moisture (%)	Crude protein (% DWB)	Pure protein (% DWB)	Total lipid (% DWB)	Ash (% DWB)	NPN (% DWB)	TVBN (mg %)	PH
<i>E. danricus</i>	7.34 $\pm$ 2.62	67.75 $\pm$ 0.8	60.23 $\pm$ 1.96	3.15 $\pm$ 0.01	18.76 $\pm$ 0.1	1.20 $\pm$ 0.1	40.00=5.00	6.8 $\pm$ 0.02
<i>P. sophore</i>	9.36 $\pm$ 1.51	57.50 $\pm$ 1.3	54.38 $\pm$ 2.01	12.14 $\pm$ 0.21	17.50 $\pm$ 0.10	0.50 $\pm$ 0.05	38.00=4.00	6.5 $\pm$ 0.05
<i>M. bleekeri</i>	36.72 $\pm$ 2.40	66.28 $\pm$ 2.3	52.00 $\pm$ 2.20	12.65 $\pm$ 0.25	18.10 $\pm$ 0.21	2.28 $\pm$ 0.1	45.00=5.00	6.6 $\pm$ 0.09
<i>A. mola</i>	11.33 $\pm$ 1.62	66.88 $\pm$ 3.1	62.63 $\pm$ 2.84	14.35 $\pm$ 0.17	14.54 $\pm$ 0.16	0.68 $\pm$ 0.1	40.00=5.00	6.7 $\pm$ 0.1
<i>N. notopterus</i>	25.53 $\pm$ 2.00	67.50 $\pm$ 2.6	60.20 $\pm$ 1.74	4.91 $\pm$ 0.98	21.31 $\pm$ 0.11	1.17 $\pm$ 0.12	80.00=6.00	6.6 $\pm$ 0.1
<i>G. giuris</i>	36.96 $\pm$ 2.10	69.17 $\pm$ 2.3	57.57 $\pm$ 1.56	3.93 $\pm$ 0.05	19.78 $\pm$ 0.15	1.86 $\pm$ 0.10	45.00=5.00	6.4 $\pm$ 0.1

NPN - Non protein nitrogen; TBVN - Total volatile base nitrogen

DWB - Dry weight basis, Values are Mean $\pm$ m SD of 6 sampling

Table 2. In vitro digestibility of six species of smoked fish

	Pepsin (%)	Pepsin + Trysin (%)
Casein	80.54±0.70	82.37±0.33
<i>E. danricus</i>	50.84±0.70	57.34±0.10
<i>P. sophore</i>	84.57±0.47	86.45±0.21
<i>M. bleekeri</i>	74.00±0.55	82.21±0.52
<i>A. mola</i>	75.09±0.61	78.20±0.25
<i>N. notopterus</i>	63.99±0.21	72.59±0.30
<i>G. giuris</i>	58.63±0.52	62.39±0.25

Values are Mean ± SD of 6 sampling

(DWB) to be high in smoked herrings. Joseph *et al.* (1987) reported the value of 38.12 and 39.86 mg% in barracuda smoked for 0 and 5 days, respectively. According to them, TVBN does not affect the organoleptic qualities of smoked samples. In the present study there were wide variations in the NPN values. Variation in TVBN and NPN levels may be due to the quality of the starting material, differences in smoking methods etc., on which the experiment had no control. pH values of the smoked fishes were acidic (6.4-6.9) which may be due to phenolic/acidic constituents deposited on the fish muscle during smoking (Joseph *et al.*, 1987).

Except in the case of *P. sophore*, the digestibility values were lower than that of casein (Table 2). *P. sophore* had the highest digestibility values both in pepsin (84.57%) and trypsin (86.45%) phases. Umoh *et al*

(1980) found digestibility in commercial smoked freshwater fish *Tilapia metanopleura* to be 75.3% compared to 96.9% for whole hen's egg.

Alpha amino nitrogen released during hydrolysis of these smoked fish samples are shown in Table 3. Highest level was observed in *M. bleekeri* (70.37%) which was comparable with that of casein (77.00%). Valanju & Sohoni (1957) reported that out of the total nitrogen present in fish protein, about 63 to 75% is made up of alpha amino nitrogen.

Available lysine concentrations in the smoked fishes was in the range of 2.17 to 4.68g/16 g of N (Table 4). The highest level was in *A. mola*. Carpenter (1960) reported the available lysine content for 24 commercial human foods. The values varied from 1.5g/16g to 8.6g/16g of N. The value for pork meat, after 24 h at 105°C was 5.3g/16g of N and for herring meal, which had been heated spontaneously, 5.2g/16g of N. He also observed that food products that had been heated had shown decreased nutritional value in feeding tests and showed lower available lysine values in the chemical analysis. Smoke contains carbonyl which react with lysine and reduce protein quality; and with more smoke the greater is the effect

Table 3. Alpha amino nitrogen released during hydrolysis of six species of smoked fish

Protein/fish powder	Mg of N in 5 g of defatted fish powder	Pepsin phase		Pepsin + Trysin phase	
		Mg of alpha Amino N in 5 g of defatted fish powder	Alpha amino N as % of total N	Mg of alpha amino N in 5 g of defatted fish powder	Alpha amino N ₂ as % of total N
Casein	80.00	46.20±1.60	57.75±0.12	61.60±0.80	77.00±1.15
<i>E. danricus</i>	47.60	20.44±2.00	42.85±0.52	29.27±1.65	61.36±1.00
<i>P. sophore</i>	43.35	14.31±1.52	33.08±0.52	32.69±1.20	75.58±1.44
<i>M. bleekeri</i>	60.70	30.20±1.71	49.75±0.23	42.72±1.35	70.37±0.46
<i>A. mola</i>	62.47	27.85±1.24	44.58±0.10	39.45±1.78	63.15±0.75
<i>N. notopterus</i>	56.79	20.20±2.41	35.57±1.50	30.24±1.64	46.21±1.0
<i>G. giuris</i>	57.60	16.38±2.50	28.44±1.84	38.62±1.10	67.05±0.66

Note: Value are mean ± SD of 6 sampling

Table 4. Available lysine in six species of smoked fish

Fishes	Available lysine (g/16 g of N)
<i>E. danricus</i>	3.80
<i>P. sophore</i>	2.17
<i>M. bleekeri</i>	3.13
<i>A. mola</i>	4.68
<i>N. notopterus</i>	4.32
<i>G. giuris</i>	2.63

Note: Mean value of 6 sampling

(Opstvedt, 1988). Whenever smoking is used, the duration of smoking and the concentration of smoke should be kept to the lowest possible level to preserve the nutritional quality of the product to the maximum.

Table 5 shows the bacterial and fungal count of smoked fishes. *M. bleekeri* had the highest TPC (10^8 .g⁻¹), followed by *N. notopterus* (10^7 .g⁻¹); the lowest being in *P. sophore* (10^3 .g⁻¹). Coliform was absent in *P. sophore* and *M. bleekeri*. However TPC and TFC were quite high in these samples reaching 10^8 and 10^4 cfu.g⁻¹ respectively. Abraham *et al.* (1993) found lower TPC and TFC in smoked tuna and sardines. These lower values may be due to the incorporation of salt in the processing of these fishes. Smoking in Manipur does not involve salting and this may be the reason for higher microbial counts. In general, TPC and TFC are high in smoked fishes of high moisture level. *S. aureus* and faecal *Streptococci* counts

Table 5. Bacterial and fungal count in six species of smoked fishes (mean value of 6 Observations of each sample)

	TPC/g	TFC/g	Coliform (MPN.g ⁻¹)	Staphylococcus/g	Faecal Streptococci/g
<i>E. danricus</i>	2.00×10^4	4.0×10^2	1.1×10^2	2.3×10^2	1.01×10^2
<i>P. sophore</i>	3.4×10^3	2.07×10^4	Nil	4.7×10^4	2.00×10^3
<i>M. bleekeri</i>	1.44×10^3	1.5×10^4	Nil	7.1×10^4	3.6×10^3
<i>A. mola</i>	4.5×10^5	3.00×10^2	3.6	3.0×10^4	2.0×10^2
<i>N. notopterus</i>	3.06×10^7	3.2×10^3	9.3×10	1.82×10^5	1.63×10^5
<i>G. giuris</i>	4.7×10^6	2.7×10^2	1.5×10	2.5×10^3	1.1×10^3

Table 6. Fungi isolates from six species of smoked fish

Fungi	<i>E. danricus</i>	<i>P. sophore</i>	<i>M. bleekeri</i>	<i>A. mola</i>	<i>N. notopterus</i>	<i>G. giuris</i>
<i>Aspergillus niger</i>	+	+	+	+	+	+
<i>Aspergillus candidus</i>	+	-	-	+	-	+
<i>Aspergillus flavipes</i>	+	-	-	-	-	-
<i>Aspergillus terreus</i>	-	-	-	+	+	-
<i>Aspergillus nidulans</i>	-	-	-	+	-	-
<i>Aspergillus repens</i>	-	-	-	+	-	-
<i>Curvularia senguensis</i>	+	-	-	-	-	-
<i>Phomopsis vexans</i>	+	-	-	-	-	-
<i>Penicillium chrysogenum</i>	+	-	-	-	+	-
<i>Penicillium rubrum</i>	+	+	+	+	+	+
<i>Penicillium funiculosum</i>	-	+	+	-	-	-
<i>Rizopus niger</i>	-	+	-	-	-	-
<i>Cladosporium cladosporioides</i>	-	-	+	+	+	-
<i>Trichoderma longibrachiatum</i>	-	+	-	+	-	-
<i>Collectrichum</i> sp.	-	-	+	-	-	-
<i>Mucor</i> sp.	-	-	-	-	-	+
Sterile mycelia	-	+	-	-	-	+

* Dominant fungi, + present, - absent

were also high, ($10^3 - 10^5 \text{ g}^{-1}$). *E. coli* and *Salmonella* were not detected in any of the samples analysed. Smoking of fish is reported to impart a degree of microbiological stability to the product which is a function of reduced water activity, heating and smoking (Eklund *et al.*, 1988).

Sixteen species of fungi were isolated from these samples (Table 6). Some were sterile mycelia. *Aspergillus niger* and *Penicillium rubrum* were present in all the six samples. They are the common air borne fungi. *A. niger* was the dominant species in five samples (*E. danricus*, *P. sophore*, *M. bleekeri*, *A. mola* and *G. giuris*). *Cladosporium cladosporoides* was the dominant species in *N. notopterus*. In the present analysis, four xerophilic fungi were observed. They were *A. niger*, *A. candidus*, *A. terrus* and *P. chrysogenum*. Pitt (1975) pointed out that all the xerophiles so far reported were mycotoxic and were *Aspergillii* (including *Eurotium* and *Emericella*) and *Penicillia*.

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