

# Biochemical, nutritional and microbiological quality of fresh and smoked mud eel fish *Monopterus albus*—a comparative study

W. Vishwanath, H. Lilabati & M. Bijen

Department of Life Sciences, Manipur University, Canchipur - 795003, Manipur, India

(Received 7 January 1997; accepted 13 May 1997)

A comparative study of the biochemical, nutritional and microbiological quality of the fresh (FF) and smoked (SF) symbranchoid mud eel fish, *Monopterus albus* was carried out. SF had lower percentages of total protein (79.0 vs 76.0), pure protein (66.7 vs 57.1), lipid (10.74 vs 9.82) and ash (7.00 vs 6.00) contents than FF. The pH values of SF were more acidic than those of FF (6.90 vs 7.25). Digestibility and protein efficiency ratio (PER) were significantly less ( $P < 0.05$ ) in SF. Total plate counts of bacteria (TPC) and fungi (TFC) were  $10^6$ – $10^7$  g<sup>-1</sup> and  $10^2$  g<sup>-1</sup>, respectively in FF and  $10^9$ – $10^{10}$  g<sup>-1</sup> and  $10^5$  g<sup>-1</sup>, respectively, in SF. *Salmonella* and *E. coli* were not present in any samples examined. However, coliform bacteria, *Staphylococcus aureus* and faecal *Streptococci* were detected in both. Seven genera of fungi were present in FF, the dominant one being *Fusarium* sp. Five genera of fungi were detected in SF, out of which *Penicillium* was dominant. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Smoking is an important technique for the preservation of fish in the north eastern part of India. Among smoked fish, the symbranchoid mud eel fish, *Monopterus albus*, is widely consumed in the state of Manipur. The technique of smoking of fish in the state has been detailed by Singh *et al.* (1990). The fish are consumed after frying or roasting or as an ingredient in vegetable curry preparations to add flavour and taste (Singh *et al.*, 1990).

Nutritional quality and microbiological quality are important considerations of all forms of food processing. Commercial hot-smoking of eels caught in contaminated water caused an outbreak of *Salmonella* poisoning in Holland (Van der Brock, 1948). Pathogenic bacteria (*Salmonella*, *E. coli*, faecal *Streptococci*, coliform, *Staphylococcus aureus* and pathogenic fungi, namely, *Aspergillus* sp. and *Penicillium* sp. etc.) which contaminate food are a health hazard and pose a significant public health problem. Detection of these organisms in raw and processed foods is important. Although smoking or dehydration at temperatures no higher than 42°C does not significantly affect the amino acid composition of protein, severe heat treatment may result in the non-availability of lysine (Carpenter, 1960).

There is no detailed information available on the quality of fish of Manipur, fresh or smoked in the traditional

style. The present paper reports a comparative study of the biochemical, nutritional and microbiological quality of fresh (FF) and smoked (SF) eel fish, *M. albus*, available in the state of Manipur, India.

## MATERIALS AND METHODS

Fresh and smoked fish were purchased randomly from the fish sellers of the Imphal market. For biochemical and nutritional analyses, fish muscle was used and for microbiological analysis, the whole body was used. Such sampling was done weekly for three consecutive months, i.e. from January to March 1996.

### Biochemical analysis

Total N (TN), pure protein N (PPN), non protein N (NPN), moisture, lipid and ash were determined according to AOAC (1975). Total protein (TP) and pure protein (PP) were obtained from their respective N values as described by Singh *et al.* (1990). The pH value was measured using a pH meter (Valsan, 1975).

### Nutritional evaluation

Feeding trials for nutritional evaluation were carried out using  $21 \pm 1$  day-old male albino rats. Six rats

weighing 30–40 g from the same colony were used for a particular diet. Four sets of experiments were performed using the following diets: (1) casein diet as control diet (CD); (2) fresh fish diet as test diet A; (3) smoked fish diet as test diet B; and (4) a protein-free diet (PF) for determination of metabolic N. The protein contents of the diets were calculated taking into account the proximate composition of wheat flour, fresh and smoked *M. albus*, as in Table 1. The compositions of the diets are given in Table 2. Feeding was carried out for 28 days at ambient temperature, with food and water provided *ad libitum*. At the end of every fourth day, the amount of food consumed and weight gain by the rats were noted. N contents in food, excreta and faecal matter were estimated. Digestibility and protein efficiency ratios (PER) were determined according to Pomeranz and Meloan (1971).

### Microbiological analysis

Enumeration of the total plate count of bacteria (TPC), and total plate count of fungi (TFC), most probable number (MPN) of coliforms and detection of pathogenic bacteria (*Salmonella*, *E. coli*, *Staphylococcus aureus* and faecal *Streptococci*) were done by the procedure of APHA (1976) and Bergan and Norris (1978). The suspected pathogenic bacterial colonies were further tested using the methods of APHA (1976) and Kiss (1984). Fungi were identified up to generic level base according to the procedures of Gilman (1957) and Ellis (1971, 1976). Doubtful cultures were sent to the Central Research Institute, Kasauli, and the International Mycological Institute, Kew, UK, for confirmation of genera.

## RESULTS AND DISCUSSION

Table 1 shows the biochemical composition of the FF and SF. TP and PP contents of SF were lower than those of FF. Loss of protein during smoking has also been reported in the case of Atlantic mackerel and catfish by Bhuiyan *et al.* (1986a) and Lilabati *et al.* (1993).

Lipid content was also low in smoked *M. albus*. Lipid may be lost along with the body fluid leached in the process of hot smoking. Drying of lipids during technological

processing causes a substantial loss of the more volatile fractions, such as short chain fatty acids and methyl esters (Colowick and Kalpan, 1969). Thus, length of time of processing may have a bearing on the lipid content of the samples.

The lower value of ash in SF might be due to the loss of water-soluble minerals as drip on heating. SF was more acidic than FF. It is presumed that the phenolic/acidic constituents deposited on the fish muscle during smoking might be the reason for this (Joseph *et al.*, 1987).

PER and digestibility values are shown in Table 3. PER of SF was significantly lower ( $P < 0.05$ ) than those of FF and of CD. The lower value of PER may be related to the loss of essential amino acids on processing. Bhuiyan *et al.* (1986b) reported a significant decrease of PER value of mackerel in the process of hot smoking. This change in protein quality was related to a loss of essential amino acids such as lysine and tryptophan and also to the availability of lysine on processing. However, the PER value of SF was more than 2.0 which is graded as a protein-rich food. Digestibility values of CD, FF and SF were 86.6%, 91.5% and 85.4%, respectively. Heat changes the structure of the protein which results in the binding of amino acids such as lysine, aspartic acid and glutamic acid by a linkage which is resistant to enzymatic action (Bhuiyan *et al.*, 1986b).

Bacterial and fungal counts of FF and SF are shown in Table 4. The values of TPC, TFC, coliform, *Staphylococcus aureus* and faecal *Streptococci* in SF were higher than those of the fresh fish. Contamination during processing, storage, handling and selling in the market may be the reason for the higher count of bacteria. The moisture content of SF was high. Microfloral count is related to the moisture content of the sample. It plays an important role in bacterial spoilage. Lowering of moisture retards the spoilage of fish (Stansby, 1963).

Stansby (1963) reported TPC counts of  $10^7$  and  $10^{10} \text{ g}^{-1}$  in smoked fish. In the present study the value for TPC was up to  $10^{10} \text{ g}^{-1}$ . Fungi are important organisms that grow rapidly during preservation/storage. However, the smoking process is effective in controlling the onset of fungal attack (Joseph *et al.*, 1987). The occurrence of moulds to a magnitude of  $10^5$ – $10^6 \text{ g}^{-1}$  fish is a matter of great concern (Graikoski, 1973). The

Table 1. Compositions of wheat flour and fresh and smoked *M. albus*

Composition	Wheat flour	Fresh <i>M. albus</i>	Smoked <i>M. albus</i>
Moisture	14.00 ± 0.22	77.00 ± 0.08	45.70 ± 0.00
Crude protein (% DWB)	10.3 ± 0.21	79.0 ± 0.20	76.0 ± 0.45
Non-protein N (% DWB)	—	1.9 ± 0.64	3.03 ± 0.29
Pure protein N (% DWB)	—	66.7 ± 0.71	57.1 ± 0.67
Lipid (% DWB)	0.80 ± 0.04	10.74 ± 0.48	9.82 ± 0.53
Ash (% DWB)	0.70 ± 0.02	7.00 ± 0.57	6.00 ± 1.00
pH	—	6.90 ± 0.07	7.25 ± 0.03

DWB, dry weight basis. Results are mean ± S.D. of 12 samplings.

**Table 2. Composition (g per 100 g diet) of diets containing fresh and smoked *M. albus***

Ingredients	Control diet	Fresh <i>M. albus</i>	Smoked <i>M. albus</i>	Protein-free diet
Casein vitamin-free (Hi-Media, India)	10	—	—	—
Fish powder (lipid-free)	—	11.30	11.86	—
Refined groundnut oil (Dalda, India)	9.0 ml	9.0 ml	9.0 ml	5.0 ml
Vitamin mixture (AOAC, 1960)	1.0	1.0	1.0	1.0
Salt mixture (AOAC, 1960)	4.0	4.0	4.0	4.0
Sucrose	—	—	—	20.0
Cellulose	—	—	—	5.0
Starch	—	—	—	65.0
Wheat flour	76.0	74.70	74.14	—
Final protein content	17.83	17.69	17.64	—

**Table 3. N intake, N output, digestibility (%) and protein efficiency ratio of diets of casein, fresh and smoked *M. albus* (mean value for 28 days of feeding experiments)**

Sample	Total N in diet	Total N in excreta	Apparent digestibility (%)	True digestibility (%)	PER
Casein	9.04	1.85	79.5	86.6 <sup>a</sup>	2.44 <sup>a</sup>
Fresh	8.32	1.25	85.0	91.5 <sup>b</sup>	2.56 <sup>b</sup>
Smoked	9.31	2.01	78.4	85.4 <sup>a</sup>	2.31 <sup>c</sup>

TD and PER values with different superscripts are significantly different. Values are the average of six replicates.

**Table 4. Microflora of fresh and smoked *M. albus* (*E. coli* and *Salmonella* were not detected in either sample)**

Sample	TPC g <sup>-1</sup> (bacteria)	TFC g <sup>-1</sup> (fungi)	Coliforms (MPN g <sup>-1</sup> )	<i>Staphylococcus aureus</i> g <sup>-1</sup>	Faecal <i>Streptococci</i> g <sup>-1</sup>
Fresh <i>M. albus</i>	1.2×10 <sup>6</sup> –1.0×10 <sup>7</sup>	1.1×10 <sup>2</sup> –1.3×10 <sup>2</sup>	0–3.6×10	1.6×10–7.8×10 <sup>2</sup>	0–7.1×10
Smoked <i>M. albus</i>	2.36×10 <sup>9</sup> –1.52×10 <sup>10</sup>	3.0×10 <sup>5</sup> –7.5×10 <sup>5</sup>	2.3×10–1.2×10 <sup>2</sup>	2.7×10 <sup>2</sup> –5.3×10 <sup>3</sup>	4.6×10 <sup>2</sup> –1.7×10 <sup>3</sup>

Results for 12 samplings

noticeable feature of smoked fish during storage is the presence of moulds. Table 4 shows the fungal flora of FF and SF. Flora isolated from FF were *Penicillium*, *Cladosporium*, *Fusarium*, *Rhizopus* and some sterile mycelia and those of the SF were *Penicillium*, *Cladosporium*, *Colletotrichum*, *Gleosporium* and some sterile mycelia. The mode of handling of fish in the market also contributes to the incidence of fungi on the fishes. The possibility of the incidence of toxic fungi or fungal metabolites leading to food poisoning cannot be ruled out unless proper care is taken.

*Staphylococci* can also grow best in foods in which the competing organisms are present in low numbers. The food might be contaminated during processing and handling. Sanjeev and Iyer (1988) also isolated the organisms from palms and throats of workers from fish-processing factories of Cochin. Small numbers of this organism in fishery products is not a serious problem but food poisoning may occur if the product is handled carelessly during processing resulting in multiplication of the organism (Iyer, 1979).

Faecal contamination is evidenced by the presence of faecal *Streptococci*, coliforms and *E. coli*. Their presence in foods possibly indicates the presence of enteric pathogens (Frazier and Westhoff, 1983). *E. coli* was

totally absent in both FF and SF. The presence of even a single cell of *Salmonella* makes the fish unfit for human consumption as it poisons the food (Iyer, 1979). These harmful bacteria were not detected in either of the samples.

From the results of the present experiment, the nutritive value of SF is considered high. However, it is lower than that of FF and CD. High fungal and bacterial count in SF is a matter of concern for the health and hygiene of consumers. Attempts may be made to reduce the moisture content of SF by extending the smoking time or by exposing to sunshine. Thus, the microbial

**Table 5. Fungal flora present in fresh and smoked *M. albus* (in % of total flora)**

Fungi	Fresh <i>M. albus</i>	Smoked <i>M. albus</i>
<i>Penicillium</i> sp.	20.5–50.0	50.0–60.0
<i>Cladosporium</i> sp.	12.3–25.0	10.0–40.0
<i>Fusarium</i> sp.	33.8–50.0	ND
<i>Rhizopus</i> sp.	0.00–12.5	ND
<i>Colletotrichum</i> sp.	ND	0.00–16.7
<i>Gleosporium</i> sp.	ND	0.00–16.7
Sterile mycelia	0.00–12.50	10.0–20.0

ND, not detected. Results for 12 samplings.

quality of fish may be improved. As microorganisms readily propagate in the fish, proper sanitary care should be taken during and after processing the fish.

#### ACKNOWLEDGEMENT

One of the authors, H. Lilabati Devi, is grateful to the council of Scientific and Industrial Research (CSIR), New Delhi for financial support (Research Associate award No 9/476(13)97/EMR-I dt 7.3.1997)

#### REFERENCES

- AOAC (1960) *Official Methods of Analysis*, 9th edn. Association of Official Analytical Chemists, Washington DC.
- AOAC (1975) *Official Methods of Analysis*, 12th edn. Association of Official Analytical Chemists, Washington DC.
- APHA (1976) *Compendium of Methods for Microbiological Examination of Foods*, ed. M. L. Speak. American Public Health Association, Washington.
- Bergan, T. and Norris, J. R. (1978) *Methods in Microbiology*. Academic Press, New York, 268 pp.
- Bhuiyan, A. K. M. A., Ratnayake, W. M. N. and Ackman, R. G. (1986a) Effect of smoking on the proximate composition of Atlantic mackerel (*Scomber scomber*). *Journal F Sci* **51**, 327–329.
- Bhuiyan, A. K. M. A., Ackman, R. G. and Lall, S. P. (1986b) Effect of smoking on protein quality of Atlantic mackerel (*Scomber scomber*). *Journal F Proc Preserv* **10**, 115–126.
- Carpenter, K. J. (1960) The estimation of the available lysine in animal protein foods. *Biochem Journal* **77**, 604.
- Colowick, S. P. and Kalpan, N. O. (1969) *Methods of Enzymology* Vol. 14. Academic Press, New York and London.
- Ellis, M. B. (1971) *Determaticeous Hypomyces*. CMI, Kew, Surrey, UK, 608 pp.
- Ellis, M. B. (1976) *More Determaticeous Hypomyces*. CMI, Kew, Surrey, UK, 507 pp.
- Frazier, W. C. and Westhoff, D. C. (1983) *Fundamentals of Food Microbiology*. Tata Mc Graw Hill, New York.
- Gilman, J. C. (1957) *A Manual of Soil Fungi*. The Iowa State University Press, Iowa, USA, 450 pp.
- Graikoski, J. T. (1973) Microbiology of cured and fermented fish. In *Microbial Safety of Fishery Products*, ed. C. O. Chichester and H. D. Graham, Academic Press, New York, 334 pp.
- Iyer, T. S. G. (1979) Pathogens and faecal organisms in fish and fishery product. In *Quality Control in Fish Processing*. Central Institute of Fisheries Technol., Cochin (ICAR), pp. 45–59.
- Joseph, A. C., Phrabhu, P. V. and Balachandra, K. (1987) Studies on dried/smoked Barracuda. *Fish Technol* **24**, 96–98.
- Kiss, I. (1984) *Testing Methods in Food Microbiology*, ed. I. Kiss. Elsevier, Amsterdam, Oxford, New York, 437 pp.
- Lilabati, H., Bijen, M. and Vishwanath, W. (1993) Comparative study on the nutritive value of fresh and smoked catfish *Clarias batrachus*. *Journal Freshwater Biology* **5**, 325–330.
- Pomeranz, Y. and Meloan, C. E. (1971) *Food Analysis: Theory and Practice*. AVI, Westport, CN.
- Sanjeev, S. and Iyer, K. M. (1988) Antibiotic resistance of *Staphylococcus aureus* strains isolated from fish processing factory workers. *Fish Technol* **25**, 139–141.
- Singh, M. B., Sarojnalini, C. and Vishwanath, W. (1990) Nutritive value of sundried *Esomus danricus* and smoked *Lepidocephalus guntea*. *Food Chemistry* **36**, 89–96.
- Stansby, M. E. (1963) Cured fishery products. In *Industrial Fishery Technology*, ed. M. E. Stansby and E. Robert. Krieger Publ. Co., Hunlington, New York, 415 pp.
- Van der Brock, C. J. H. (1948). Onderzoek naar de bactericide werking van het palingroken. *Central Inst. Voedingson derzock*. T. N. O. Utrecht Mededel. Publ., No. 72.
- Valsan, A. P. (1975) A comparative yield and biochemical evaluation of the existing fish curing methods in India. *Proc. Symp. Fish. Processing Industry in India*. Assn. of Food Scientists and Technologists (India) and Central Food Technological Research Institute, Mysore, pp. 77–79.