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Microbiological and Nutritional Evaluation of Fermented *Setipinna* Species

Ch. Sarojnalini and T. Suchitra
Department of Life Sciences
Manipur University, Canchipur - 795 003

Biochemical composition, digestibility and microbiological quality of fermented *Setipinna* sp. were evaluated. The average percentage of moisture, total protein, lipid, ash content and pH values were 36.44, 36.25, 6.66, 10.22 and 7.68 respectively. Thiobarbituric acid number was within the permitted level, but total volatile basic nitrogen values were above the acceptable limits. *In vitro* digestibility of fermented *Setipinna* was 54.11 percent in pepsin phase and 82.78 percent in pepsin + trypsin phase in comparison with the value for casein of 98.03 percent in pepsin + trypsin phase. Apparent digestibility value of fermented fish was 83.72 percent as against the value of 87.20 percent for reference casein. Colony forming unit of bacteria and fungus reached up to 5.74 log cfu/g and 3.28 log cfu/g respectively. The predominant bacteria include *Bacillus* and *Micrococcus* species. Pathogenic bacteria *viz. Staphylococus aureus*, faecal *Streptococci* were detected above the permitted level but coliform, *E coli* and *Salmonella* were not detected. *Aspergillus, Penicillium, Rhizopus Cladosporium* and *Fusarium* were the fungi isolated from fermented fish.

Key words: *Setipinna* sp., Fermentation, Microbiological quality, Nutritional quality.

Fermentation is one of the oldest and low cost methods of producing and preserving food. For centuries, fermented foods in varied forms are widely being used by different races of the world especially from Southeast Asia and Africa. It has wide scope on nutritional possibilities in future and considered as a superior food in terms of protein value, minerals and vitamins (Sorasuchart, 1971). Fermented fish in general have favourable amino acid composition which can be compared well with other meat products.

In Manipur, a fermented fish product 'Ngari' or 'Utongna', prepared from small sized low valued fish (*Puntius* spp) is widely used by a large number of people. Recently sun dried *Setipinna* species is introduced as raw material for the preparation of Ngari. It is used as a compulsory condiment and flavouring agent in curry preparations by the people of Manipur (Sarojnalini and Vishwanath, 1988). The importance of this food to the people of Manipur was noticed by Chaudhuri and Banerjee (1965) and recommended dry fish for the preparation of Ngari to meet the growing demand.

In Manipur, reports on the fermented fish products were scanty except the work done by Sarojnalini and Vishwanath (1987, 1988, 1995). There was no report so far on Ngari prepared from *Setipinna* species. This paper reports the results of the nutritional and microbial studies of *Setipinna* from the Imphal market of Manipur.

Materials and Methods

Sun dried and fermented *Setipinna* sp. having length 10.80-13.60cm and weight 3.80–9.14g were collected randomly from different fish sellers of Imphal market for analysis. Sampling was done seasonally during 2003 – 2004. Colour and odour of the samples were recorded. Reconstitution property was evaluated as percentage of water imbibed by 100g of the sample soaked in 400 ml of water for a period of 3½ hours (Valsan, 1975). Sensory evaluation was carried out by the method described by Lilabati and Vishwanath (1996).

Moisture lipid, ash, protein, non-protein nitrogen (NPN) and soluble protein nitrogen (SPN) were determined by AOAC (1975). Total volatile base nitrogen (TVBN) was estimated by the Conway Micro-diffusion Method and free fatty acid (FFA) by the method of Lilabati and Vishwanath (1996). Thiobarbituric acid (TBA) number was determined as per the methods of Sinhuber and Yu (1958). pH value of the sample was measured using a pH meter.

Digestibility was estimated as per the procedure of Singh *et al.* (1990). For determination of digestibility *in vivo*, a feeding experiment with 6 rats for each 4 groups were carried out using 21 ± 1 day old albino rats. Rats from the same colony having a weight of 30-35g were used. Control diet was prepared using 10 percent of the standard protein casein (Hi media). In test diets 10 percent of defatted and dehydrated fish proteins were added as the animal protein. The rats were divided into 4 groups viz. those fed with 1. Casein diet, 2. Protein free diet for the determination of metabolic N and 3. Sundried *Setipinna* 4. Test diet (fermented *Setipinna*). The composition of the diets is given in Table 1. The amount of food consumed and weight gain by the rats were noted at the end of every 4th day. N content in the food, excreta and faecal matter was estimated. Apparent digestibility was obtained by the differences between N intake and N in the faecal excreta and given as percentage of N intake. True digestibility was obtained by correction with metabolic N. Protein efficiency ratio (PER) was calculated as weight (g) gain per dietary protein intake (g). Chemicals and reagents used were analytical grade supplied by E-Merck (Danstardt, Germany) and Sigma (St. Louis, U.S.A.).

Total colony forming units of bacteria and fungi were determined by dilution plate method (APHA, 1976) using Trypton glucose agar and Potato dextrose agar medium respectively. The fungi isolated were stained with lacto phenol cotton blue and identified based on Gilman (1957) and Eliss (1971, 1976). The bacterial colonies (Cocci and Bacilli) were identified by the Institute of Microbial Technology (IMTECH), Chandigarh for confirmation. Most probable number (MPN) of coliform and detection of pathogenic bacteria viz; *Escherichia coli, Staphylococcus aureus, Salmonella,* Faecal *Streptococci* and *Bacillus cereus* were done as per the method of APHA (1976).

Results and Discussion

Table 2 shows the pH, water reconstitution and sensory characteristics of sun dried and fermented *Setipinna.* The pH of the dried *Setipinna* increased from 6.40 to 7.65 on fermentation for 6 months. The increase in pH shows that no lactic acid fermentation occurred. Higher pH allows bacteria to become dominant and helps in the anaerobic breakdown of fish proteins during fermentation. Soyiri *et al.*, (2003) suggested that the

Table 1. Composition of the diets in the feeding experiments

Ingredients	A	B	C	D
Casein vitamin free, g	10.00	-	-	-
Fish powdered (lipid free), g	-	22.03	27.58	-
Refined groundnut oil, ml	9.00	9.00	9.00	4.00
Vitamin and salt mixture, mg	0.50	0.50	0.50	0.50
Sucrose, g	-	-	-	20.00
Cellulose, g	-	-	-	5.00
Starch, g	-	-	-	70.50
Wheat flour, g	80.50	68.47	62.92	-

A = Casein, B = Sun dried *Setipinna,* C = Fermented *Setipinna,* D = Protein free diet

increase in pH was due to the formation of basic nitrogenous compounds. The percentage of water absorbed by the fish was reduced by fermentation. The sensory qualities of both the samples were good.

Table 2. pH, reconstitution of water and sensory evaluation of the sun dried and fermented *Setipinna* sp.

	Sun dried	Fermented
pH	6.40 ± 0.02	7.68 ± 0.05
Reconstitution,	157.60 ± 2.11	56.53 ± 1.61
Sensory qualities		
Colour	Yellowish	Yellowish
Odour	No rancid odour	Characteristics sweet odour
Texture	Hard	Moderately soft

Fermented fish showed higher values for moisture, SPN, NPN, TVBN and TBA and lower values for lipid, ash and crude protein (Table 3). During fermentation, proteins might have been hydrolyzed to peptides and amino acids by microorganisms. Higher values for TVBN, SPN and NPN might be due to the action of microorganisms during fermentation. Although the values of TBA increased, it was within the permitted limit. According to Sinhuber and Yu (1958), TBA number less than 3.0 mg/1000g of cured fish is considered to be in good condition. The high values of free fatty acids (FFA) in fermented *Setipinna* might be due to the lipid hydrolysis during storage.

Table 3. Chemical characteristics of sun dried and fermented *Setipinna* sp.

Composition	Sun dried	Fermented
Moisture,%	18.70±0.66	36.44±0.89
Total lipid, % (DWB)	10.76±0.95	10.48±0.57
Ash content, % (DWB)	23.04±0.60	16.08±0.44
Crude protein, % (DWB)	62.52±1.44	57.03±1.93
Soluble protein nitrogen, %	1.05±0.13	2.13±0.11
Non protein nitrogen, %	1.59±0.11	1.86±0.23
FFA, % as oleic acid, (DWB)	66.00±2.61	97.76±0.97
TVBN, mg %	117.30±0.30	206±1.40
TBA, mg malonaldehyde/kg	0.45±0.15	0.62±0.16

Results are Mean ± SD of 6 Replicates
DDB- Dry Weight Basis, FFA – Free Fatty Acids.

The *in vitro* digestibilities were found low in sun dried and fermented fish as compared to reference casein (Table 4). The lower values might be due to the presence of bones, scales and utilization of the whole body for the estimation (Singh *et al.*, 1990). The digestibility (pepsin and pepsin +trypsin digestion) of the dried fish increased on fermentation. The present value obtained for Ngari was higher than that for the fermented fish paste "Hentak" which had a value of 46.82 percent in pepsin digestion and 71.89 percent in pepsin + trypsin digestion (Sarojnalini and Vishwanath, 1988).

Table 4. Digestibility of protein *in vitro*.

Sample	Digestibility	
	Pepsin phase	Pepsin + Trypsin phase
Casein	88.00 ± 0.50	98.03 ± 0.45
Sun dried fishes	48.40 ± 1.42	79.95 ± 0.70
Fermented fishes	54.11 ± 0.60	82.78 ± 0.58

Results are mean ± SD of 6 replicates.

In vivo digestibilities of casein and fish diets are given in Table 5. The group of rats fed with fermented fish showed increased food intake over the group fed with unfermented fish and casein, indicating the acceptability of the former. The nitrogen absorbed was higher in Ngari (11.25g) than casein (7.84g). According to Barnes *et al.*, (1946), the relative proportion of dietary nitrogen entering into growth and maintenance depend upon the nutritive quality of dietary proteins. Casein diet was the most digestible of the three, followed by fermented fish. The lower *in vivo* digestibility of the fish might be due to the fact that the digestive enzymes in growing rats are not able to digest food protein efficiently due to the presences of scales and bones in the fish

Table 5. Nutritional characteristics of dry and fermented *Setipinna* in comparisons with casein. Mean values for 28 days of feeding trials.

Digestibility data	Casein	Unfermented	Fermented
Total N in diet, g	8.28± 0.12	11.87 ± 1.54	13.13 ± 1.18
N in excreta, g	1.05 ± 0.05	1.93 ± 0.20	2.13 ± 0.26
Nitrogen absorbed, g	7.84 ± 0.39	10.94 ± 1.48	11.25 ± 1.20
Apparent digestibility, %	87.20 ± 0.90	83.57 ± 2.25	83.72 ± 1.34
True digestibility, %	89.78 ± 0.61	85.35 ± 2.12	85.57 ± 0.99
Biological value, %	97.15 ± 0.37	96.91 ± 0.75	97.90 ± 0.29
PER	2.40 ± 0.39	1.52 ± 0.41	2.33 ± 0.17

Table 6. Microbial profile of sundried and fermented *Setipinna* as colony forming unit per gram of samples.

Microflora	Sundried		Fermented
Total plate count (Bacteria)	Range Mean	4.60-5.95 log cfu/g 4.77	3.77-5.60 log cfu/g 5.74
Total plate count (Fungus)	Range Mean	2.77-3.47 log cfu/g 3.25	2.44- 2.53 log cfu/g 2.49
Coliform (MPN)	Range Mean	1.14 -1.33 log cfu/g 1.25	ND
Faecal *Streptococci*	Range Mean	4.30-4.47 log cfu/g 4.36	2.95-3.47 log cfu/g 3.25
Staphylococcus aureus	Range Mean	4.20- 4.25 log cfu/g 4.24	3.49-4.74 log cfu/g 3.63
Bacillus cereus	Range Mean	0.00-0.20 0.04	0.00-0.60 0.21
Salmonella	ND		ND
E. coli	ND		ND

ND-Not detected.

consumed (Ammu *et al.*, 1986; Sarojnalini and Vishwanath, 1995). Biological value and PER of Ngari (97.90%) was higher than casein (97.15%) indicating higher quality of the proteins.

The fermentation process increased the PER values from 1.52 to 2.33, and hence it was comparable to casein (Steinkraus, 1983). Thus, Ngari has a value appropriate for a protein rich food, as the value was more than 2.00 (ISI, 1982).

Table 6 shows the microbiological qualities of sundried and fermented fish. The fermentation increased the bacterial load

Table 7. Fungal flora of sun dried and fermented *Setipinna* as percentage of total fungal flora.

Fungal flora	Sun dried	Fermented
Rhizopus oryzae	8.69	ND
Fusarium dimerium	34.78	6.25
Penicillium regulosum	17.39	ND
P. citrinum	8.69	25.00
P. rubrum	21.73	ND
Aspergillus fumigatus	4.34	12.50
A. versicolar	ND	31.25
Cladosporium species	ND	18.75
White sterile mycelium	ND	6.25
Unidentified species	4.38	-

ND – Not detected

moderately. Gram +ve rod, *Bacillus* and gram +ve cocci, *Micrococcus* predominate in the indigenous fermentation. The presence of *Bacillus* and *Micrococcus* species during fermentation suggested that spore forming bacilli as well as nonsporing cocci might play an active role during fermentation.

Significant amount of *Staphylococcus aureus* (4.20-4.74 log cfu/g) and faecal *Streptococci* (2.95-4.47 log cfu/g) were noticed in dried and fermented fish. *Staphylococcus* count exceeding 6 log cfu/g is considered to be hazardous (Bergdoll, 1979). However, in the present investigation, the *Staphylococcal* count was below the above value. According to Almas (1981) fish flesh containing below 10 million (10^8) bacteria per gram is considered suitable for food. The increase in *Bacillus* and *Micrococcus* produce flavour due to their proteolytic and lipolytic enzymes (Sand and Crisan, 1974) and assist in the breakdown of fish tissues (Beddows, 1985). Achinewhu *et al.* (2004) and Rose (1982) also reported the occurrence of *Staphylococcus, Bacillus* and *Micrococcus* species as the predominant organisms from the naturally fermenting *Sardinella* sp. and in patis. Coliform was detected only in sun dried but not recovered from the fermented samples. Pathogenic bacteria *viz., Salmonella* and *E. coli* were also absent. Total plate count of fungus was up to 3 log cfu/g in both the sun dried and fermented product. *Aspergillus, Penicillium* and *Fusarium* species were dominant among the fungus identified (Table 7).

The result of the study showed that the fermented fish product, Ngari, prepared from *Septipinna* species is nutritionally rich except for the higher counts of *Staphylococcus* in which food poisoning may occur.

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