Effect of Temperature on Biochemical and Microbiological qualities of Ngari

Suchitra Taorem and Ch. Sarojnalini

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

ABSTRACT: Ngari, a traditional fermented fish product prepared from sundried *Puntius* sp were fermented in different temperature and its biochemical and microbiological qualities were analyzed. Changes in the different parameters were observed. During fermentation, the value of moisture changes at different temperature. Ash content was low, at 180 days of 20° C followed by 40°C and room temperature 30°C. The values of Total protein, amino acid, non protein nitrogen soluble protein nitrogen, free fatty acid, were found to vary during the course of fermentation indicating of fish fermentation. The values of Cholesterol were 3.90, 3.67 and 3.22 at 20°C, room temperature and 40°C respectively. Changes in pH were minimal. Variations in the microbial count during the fermentation of *Puntius* were analyzed at various intervals. The colony forming unit of bacteria was observed in the range of 10^4-10^6. *Bacillus* and *Micrococcus* spp. were dominant amongst the bacteria identified. *Staphylococcus aureus* and faecal *Streptococci* count reaches 10^5 and 10^6 cfu/g respectively. *Vibrio parahaemolyticus* and Pathogenic bacteria such as *Salmonella* and *E,coli* were not detected during the course of study. *Aspergillus* spp and *Penicillium* spp were the dominant fungal species during the period of fermentation. The results shows that several microflora with varying percentage were found to be associated with the fermentation of sundried *P sophore* and they might play an important role during fermentation.

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INTRODUCTION

Fermentation is an easy and safe means for preservation of fish which involves various techniques. Ngari (a fermented fish product) have a high consumer acceptance and have contributed in many ways to the diet of northeastern part of India. This indigenous product is prepared from sundried *P. sophore* (Ham), *P. ticto* (Ham) and *Setipinna s*p (Swainson) subjecting to fermentation for five to six months in an eartherned pot at room temperature. Because of its special flavor, it is used as a compulsory item in daily curry preparation (Sarojnalini and Vishwanath., 1988). Nutritive value of such processed fish has been reported to be high and also serves as a preservative making the food tastier and easier to digest. Sometime, processing tends to affect the nutritional value of food products. The important factor which determines the value of a fish product is its biological composition. The desirable changes produced by the action of microbes enhance the flavour improve the nutritional value and extend the shelf life (Amano, 1962. Essuman, 1992). On the other hand, the deterioration caused by microorganisms, proteolytic, enzymes, oxidative particularly lipid and various non enzymatic reactions can be controlled. Examination of fermented fish may therefore provide clues as to how its production and preservation can be expanded and thereby contributed to improve nutrition in future. Steinkraus (1983) determined the optimum condition for fermentation, the essential microorganisms, the biochemical, nutritional flavour texture and toxicological problem that could arise during fermentation. Jafri (1968) suggested that evaluation of biochemical composition was a primary factor relating to its nutritive value, acceptability quality and sensitivity of various processing methods.

The quality of a fish product is judged by its microbiological characteristics. Uncontrolled growth of microbes, in such fishery products may lead to serious implications on the storage quality and safety of the product (Abrahim *et.al.,* 1993. During fish processing, chances of microbial contamination are plenty. Fungal organisms are known to produce a wide variety of chemical substances. On the other hand, a constant check must be maintained on the quality of the inoculums since, unwanted microorganisms or alteration by mutation results in reducing quality and yields (Rao, 2001).

In Manipur, the traditional fermented fish has been an important commodity and thus has important market value. With increasing consumption of Ngari, the present work on biochemical and microbiological quality has been undertaken. The work has relevance to human nutrition and health of the consumers and aims at improvement of local processing techniques to a more scientific based technique to make the product nutritionally rich at the time retaining the acceptability and special flavour of choice. Information on the scientific study of fermented fishes of Manipur is scanty except the work done by Sarojnalini and Vishwanath, (1987, 1988, 1994, 1995). This paper deals with the composition variation and microbial

changes during the fermentation of Ngari at different temperature. It also aimed at finding out the different types of microorganisms in fermented products and its changes in the count, prepared in the laboratory to study the role of various predominant microbial types during the course of fermentation.

Materials and method

Sundried *P sophore* was fermented under laboratory condition following the traditional method. The fish pots were then stored at different temperature (20°C, 30°C and 40°C) using BOD incubator at room temperature as control. It was analyzed for every 30 days of fermentation for six months.

Biochemical Process Moisture content, total lipid, ash, crude protein, non protein nitrogen was determined by AOAC (1975). Total volatile basic nitrogen (TVBN) and free fatty acid were estimated using TCA extract in Conway unit following the method of Moris (1959). Thiobarbituric acid (TBA) number was estimated by the method of Sinhuber and Yu (1958) .PH values of the samples were measured using a PH meter (Valsan, 1975). Cholesterol was determined following the method described by Plummer (1979). Estimation of total amino acid was done by Ninhydrin method introduced by Moore and Stein (1948) and total soluble sugar by Dubois *et.al,* (1951).

Microbiological Analysis Enumeration of total viable counts of bacteria were determined by the dilution plate method of APHA (1976) using trypton agar medium (PH=7.0). Dilution and inoculation were carried out in aseptic conditions in a Laminar flow (Klenzaids, India) and the plates were incubated at 37 ± 2^{c} in BOD incubator for 48 hours. The colonies present were counted using colony counter and expressed as colony forming unit per gram of samples. Total plate counts of fungi were also determined by the dilution plate method of APHA (1976), using PDA (potato Dextrose Agar) medium at pH 3.5. Inoculated Plates were incubated at $28 \pm 2^{°C}$ for 72 hours. Most probable number count for Coliform and detection of pathogenic bacteria viz. *Escherichia coli, Staphylococcus, Salmonella,* Faecal *Streptococci and Bacillus cereus* were tested as per the method of APHA(1976).Coliform count was determined by using diluted sample in brilliant green lactose broth using Durham's tubes. *.E coli* were determined by using brilliant green lactose broth Eosine methylene blue and further tested using the methods of APHA (1976) and Kiss (1984).

For determination of *Staphylococcus aureus*, 1 ml of the diluted samples was inoculated in Baired Parker Agar medium containing egg yolk solution and 1.0% potassium tellurite and incubated at 35-37oC for 48 hours. *Salmonella* was determined by using Bismuth sulphite agar medium (Sulphadizine agar) BGA medium Faecal *Streptococci* were inoculated in KF agar enriched with trichorotetrazoliumchloride (TTC). *Bacillus cereus* was determined by using Polymixin Pyruvate. Egg yolk Mannitol Bromothymol Blue agar (PEMBA) medium. *Vibrio parahaemolyticus* was estimated by using thiosulphate citrate bile salt sucrose medium (TCBS).

Identification of microflora Different strains of bacterial colonies were identified upto generic level based on Buchanan and Gibbons (1974) Kiss (1984), APHA (1976) and finally confirmed by the Institute of Microbial Technology (IMTECH) Chandigarh and CABI Bioscience UK Centre, Egham. Fungal flora was identified upto the species level based on Gilman (1957), Elis (1971, 1976).

RESULTS AND DISCUSSION

The fermented fishes in the present study exhibited after complete fermentation had moderately soft texture. On the organoleptic point of view, these fermented products showed, good quality with a typical characteristics odour and desirable flavour. Ngari stored at room temperature achieved the best quality as compared to others. Ngari at 40°C had slightly dark colour and possessed pungent smell due to prolong storage at high temperature. However, there is not so much variation in the chemical composition of all the products.

The moisture content of the products varied from 24.1 percent to 24.55 percent over 180 days at different temperature as shown in table 1. This fluctuation could be attributed to their fats content. Sebaiy and Metwalli (1989) noticed a reduction in moisture content during fermenting of Bowri fish muscles prepared from *Mugil cephalus*. The percentage of ash content was increased during the initial stage but a tendency towards stabilization occurred at 90 days of fermentation. The ash fraction observed at $20^{°C}$ was lower than the value at control and $40^{°C}$. The utilization of the whole fish, including bones and scale in the fermentation also increased the ash content in all the samples. Scales were reported in contributing higher ash (Quadrate *et.al.,* 1964) and also rich in mineral. The lipid content also increases and obtained the maximum value at 60 to 90 days at various temperatures but remain almost unchanged at the later stage. This finding was similar with the finding of Ijong and Ohta (1995) in bakasang in which the crude lipid of 14.17 percent was obtained.

In the present analysis, cholesterol content has a value of 3.90 mg/g at 20° C, 3.22mg/g at 40° C and 3.67mg/g at room temperature. It is lower at higher temperature. The food borne bacteria capable cholesterol, degradation especially in fermented foods. *B subtilis* isolated from Korean traditional fermented flate fish, could produced a high level of cholesterol oxides, which degrade cholesterol (Kim *et.al.,* 2002). Sugar was greatly reduced after fermentation. Carbohydrate in the form of soluble sugar might be absorbed by fungi present in the fishes for supply of energy required for their general metabolism It might also related to the declined of fungi, especially the *Aspergillus* sp. Ngari stored at 40° C, reduces the value from 0.62 to 0.47mg/g.

The percentages of total proteins at the initial stage of fermentation (30days) were 38.7, 38.60 and 38.75percent at 20°C, at room temperature and 40°C respectively. At the end these values increased slightly to 38.90 percent for 20°Cand 38.87 percent for 30°C.However at 40°C, it was reduced to 35.25 percent. The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles. Our present analysis shows the microbes are temp dependent. Prolong storage at high temperature the colour becomes dark with strong odour indicating that higher temperature was not applicable for fermentation. Protein might have been degraded to amino acids by the activities of fermenting micro organism and used in the metabolic activities. Sanni *et.al.,* (2002) reported protein value of 16.8-21.9 percent in 'Mamoni', a Ghanian fermented fish condiment, which was lower than the value of the present study. Amano (1962) observed a loss of 30% nitrogen in the fermenting fish products of fermented fish, Shiokario. The fraction of the decomposed proteins remains in the fish muscles as free amino acids content increased steadily indicating that some polypeptide is being formed in addition to amino acids. This degradative process however brings out certain characteristics flavour that is essential for the quality of the final product (Amano, 1962, Ito and Sato 1963). Ijong and Ohta (1995) noticed a lower liberation of amino acids under variable temperature than when fermented at constant temperature. At 20° C the value was low. This is due to lower hydrolysis or degradation of protein releasing less amino acid due to low temperature.

Unlike other fermentation, pH values increase due to non – involvement of lactic acid bacteria. It might also be related to the decrease in the population of fungi, since they prefer to grow satisfactorily in slightly acidic pH. The increased in pH allows bacteria to become dominant mainly the *Bacillus* sp. Soyiri (2003) suggested that the increased in pH was due to the formation of basic nitrogenous compounds. At the same time it also favours the anaerobic breakdown of proteins. Yatsunami and Takenaka, (1996) reported that the increase in pH during the latter part of fermentation has been attributed to the formation of volatile basic compounds. Increase in PH was observed from the initial stage of fermentation. Non protein nitrogen increased due to the utilization of the whole body in the fermentation process. This was supported by Oetterer (2003) in which the treatment of enzymes present in the digestive system increased the NPN during fermentation. The value was also attributed to the proteolytic activities of the fermenting microorganisms (Babu *et.al.,* 2005). The soluble protein nitrogen might be synthesized from some organic substances by the fermenting microbes as a result, soluble protein increased during fermentation.

TBA value were high reaching upto 1.11 at 20°C, 0.73 at room temperature and 1.20 at 40°C which all lie within the acceptable limit according to Sinhuber and Yu (1958). The levels of TVBN increase gradually as storage period increased and obtained a value of 218.0 for 20°C, 246.0 for 40°C and 206.0 at room temperature. It is due to the elevation of temperature and subsequent microbiology and biochemical changes in the fish muscles. It also indicates the continuous production of volatile bases due to the breakdown of proteins by the action of microbes Babu *et.al.,* (2005) and responsible for the generation of typical flavor and aroma of the final product (Majumdar *et.al.,* 2005) and also shows that higher liberation of TVBN were correlated with bacterial activity (Vanderzant *et.al.,* 1973))

It has no significant effect on the organoleptic qualities. Connell (1975), Oetterer *et.al.,* (2003) reported that processed fish presented a higher level of TVBN. The gradual increase of TVBN at the later stage of maturation is probably due to the enzymatic and bacterial action of the fish. Maximum FFA was liberated at high temperature of 40°C due to higher protein denaturation and lipid hydrolysis but no rancid odour was noticed.

When sundried *Puntius* were subjected to different temperatures of 20°C, 30°C and 40°C in laboratory condition, the total population of microbes increased during the early stage of fermentation and then decreased. Normally food that is produced, ripened or fermented by the actions of bacteria will yield high total counts and a known flora of non pathogenic organisms should be present (Hall *et.al.,* 1967).

Increased in TPC was reported in various kinds of fermented fish. This observation was in general, in agreement with those reported by Yang and Chung (1995), Ijong and Ohta (1995), Syaefudin *et.al.,* (1992), and Aijedah *et.al.,*.(1999). This shows that bacteria play a very important role in fish fermentation.

They degrade fish protein leading to the production of volatile compounds from amino acids and small peptides (Lopetcharat and Park, 2002).At 20$^{\circ C}$ and 30$^{\circ C}$, maximum count was observed in 60 days and in 30 days at 40$^{\circ C}$, At 40$^{\circ C}$, proliferation of microbes was high, however longer storage at 40°C, for six months, induced products to become dark with pungent ammmoniacal odour and finally loss of flavour. In this case the count decreased drastically upto 10^4 cfu/g. However, it was in contrast to that reported by Hamm and Clague (1950), who observed that at high temperature fermented fish can be accelerated by storing at 45$^{\circ C}$. In our present study high temperature cannot be applied to fermentation. According to Lonsane *et.al.,* (1985), a temperature of 25^{o}C - 35^{o}C is generally employed in solid substrate fermentation Fluctuation in the temperature prevents proper growth of fermenting bacteria. Ngari subjected to 20^{o}C required longer period of time than the normal period. It results in autolysis and possible spoilage by microorganism. Voskresenky (1965) suggested the rate of ripening also depended on the quality, type of fish and temperature.

The fungal count decreased after fermentation. According to Abraham *et.al.,* (1993), fungal count of cured fishes ranged from 10^3 to 10^4 cfu/g. Microbial proliferation is inhibited is inhibited at moisture of 20% and below. During 6 month of fermentation at different temperature, fungal count declined slowly as the total population of microbes die. Anaerobic condition caused the declined of mould during the aging process (Chou *et.al.,* 1988). At higher temperature (40^{o}C), fungal reduces to 10^2 cfu/g ,since they are less resistant to heat than the endospore of bacteria (Lacey, 1989).On the other hand ,temperature affects spore germination, growth and sporulation of fungi (Lonsane, 1985), which is also one of the factor of reducing fungal count. Coliform which is an indicator of faecal pollution was not detected from all the fermented samples. It is a measure of the sanitary quality and presence of pathogenic bacteria. Bacteria belonging to the genus *Salmonella* were not detected in all the samples analysed or in the fish processing environment. The absence of *Salmonella* sp from the fermented fish was due to low water activity of the products (Essuman, 1992). These conditions do not favour the growth of *Salmonella*. As the Ngari was prepared from sun dried fishes, they might be destroyed during sun drying, as *Salmonella* is heat labile. This shows that the products were free from Salmonellosis. Coagulase positive *Staphylococcus aureus* was recovered from all the sample analysed .Variation in *Staphylococcus* count was observed at different temperatures. At 40$^{\circ C}$, upto 90days the count was slightly reduced and then increased upto 150 days. At 30°*C*, count increased during the early stages i.e., 90 days and declined slowly till 150 days At 20 ^{o}C, maximum count was noticed at 60 days (10^6) and gradually reduced to 10^4 cfu/g. Tanasupawat *et.al.,* (1991) reported the occurrence of gram positive and catalase positive *Staphylococci* was reported from fermented fish. He also isolated several strain from fermented fish of Thailand. Fish flesh containing 100million (10^8) bacteria per gram is considered as unsuitable for food (Almas, 1981). *Staphylococcus* count exceeding 106/g is considered to be hazardous (Bergdoll, 1979) .In the present study, *Staphylococcal* count did not exceed 106cfu/g and occurred within the acceptable limit. The presence of small numbers of *Staphylococcus* 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).

Processing caused considerable changes in the composition of the microflora in counts as well as in species found (Pivovarou *et.al.,* 1988). The result of the present investigation indicated that gram positive rod, *Bacillus* and gram –ve cocci, *Micrococci* species predominates the bacterial flora of the fermented fish studied. The bacterial flora comprised strain of *Bacillus cereus*, *B. coagulans, B. pumilis, B. subtilis, B. Panthothenticus, Staphylococcus* and *Micrococcus species*. The occurrence of this spore – forming *Bacillus* sp. in the completely fermented product may reflect the resistant nature of these microorganisms (Crisan and Sand, 1975). The presence of large percentage of *Bacillus* species during initial and end of fermentation process suggested that spore-forming *bacilli* might play an active role during fermentation. The presence of 3 species of *Micrococcus* throughout the fermentation also indicates the possible involvement of non sporing microorganisms in every stage of fermentation (Rose, 1982). At the same time, these bacteria are considered to produce metabolites that contribute to flavour and odour in the fermented fish products due to the proteolytic and lipolytic activities (Sands and Crisan, 1974; Mackie *et.al.,* 1971). Their degradation process brings out certain characteristic flavour, which are essential for the quality of the final product. They assist in the breakdown of fish tissue as well as the development of aroma and flavour (Beddows, 1985). Amongst the *Bacillus* strains isolated, *B. subtilus*, which grow at pH 7.0 and 1% of salt content were the best proteolytic bacteria (Cha and Lee, 1989). Table 7, shows the distribution pattern of the isolates of fermented *Puntius* under different temperatures. Amongst the *Bacillus* species identified, *B. subtilis* was the dominant bacterial flora till the end of fermentation period with13.60-30% and 26.66% - 27.27% at 60 days and 180 days respectively at 20^{o}C. The value was higher when stored at room temperature. At higher

temperature of 40°C, *Bacillus coagulans* which was isolated at the initial stage was not detected latter on. Percentage of *B.coagulans* (0.00-9.09) was low. *Micrococcus* sp was dominant with 25.00% - 54.54% while some bacteria also remain unidentified.

The occurrence of natural fungal flora coming from the staple and surroundings is very common in fish fermentation. In all the samples, *Aspergillus* species were observed and shows variations in their percentage during different storage of fermentation and at different temperatures. At 20°C, *Penicillium* followed by *Aspergillus* constituted the dominated genus as shown in Table 8. These two genera were very common in various fermented including fish sauces and fish paste. Crisan and Sands (1975) observed the presence of *P. notatum*, *Cladosporium herbarum* and *A. fumigates* from fermented fish sauces. Sarojnalini and Vishwanath, 1987 observed many varieties of mould, from fish paste "hentak" of Manipur. The authors also reported that enzymes released by the ferments on the microorganisms caused the fermentation. The use of fungi as amylolytic agent by the oriental people for preparation of fermented foods is an old art. The *Aspergillus* produce acid, neutral and alkaline proteases that were used for complete hydrolysis of proteins (Aunstrup, 1983) and also accelerated the ripening of the fish paste (Amano, 1962).

Table 1: Changes in various Biochemical constituents during Ngari fermentation at 20°C temperature.

Parameter	Fermentation period in days					
	30	60	90	120	150	180
Moisture (%)	24.65±0.78	24.57±0.50	24.64±0.35	24.56±0.01	24.58±0.27	24.55±0.73
Total lipid (WWB %)	14.50±0.57	14.79±0.78	14.75±1.09	14.67±0.60	14.66±1.00	14.68±0.44
Ash content (DWB %)	12.66±0.95	12.50±0.54	12.33±0.57	12.30±0.60	12.36±0.79	12.32±0.54
Total protein (DWB %)	38.73±0.95	38.74±1.08	38.97±1.10	38.72±2.34	38.75±1.44	38.90±1.28
pH	6.36±0.01	6.39±0.04	6.41±0.04	6.41±0.01	6.43±0.19	6.45±0.01
NPN (DWB %)	2.53±0.50	2.62±0.32	2.70±0.33	2.59±0.26	2.48±0.35	2.55±0.60
SPN (DWB %)	2.19±0.83	2.53±0.24	2.20±0.35	2.23±0.19	2.33±0.20	2.38±0.22
Total soluble sugar (mg/g)	0.82±0.16	0.78±0.04	0.73±0.12	0.70±0.17	0.74±0.20	0.72±0.18
Total amino acid (mg/g)	4.45±0.32	4.74±0.46	5.20±0.60	5.33±0.72	5.62±0.35	6.90±0.10
TBA No. (melonaldehyde/kg)	0.60±0.07	0.63±0.04	0.73±0.12	0.70±0.11	0.94±0.03	0.97±0.05
TVBN (mg %)	164.83±0.70	162.0±0.26	171.00±0.55	2.02±0.820	212.0±0.52	218.0±0.29
FFA (% as oleic acid)	84.57±0.06	84.74±1.56	85.18±1.74	88.11±3.01	89.03±2.00	96.17±1.70
Cholesterol (mg/g)	3.85±0.08	3.57±0.83	3.46±0.05	3.29±0.33	3.96±0.05	3.90±0.22

Table 2. Changes in various Biochemical constituents during Ngari fermentation at room temperature (30-32°C)

Parameter	Fermentation period in days					
	30	60	90	120	150	180
Moisture (%)	24.18±1.17	24.14±0.66	24.10±2.66	24.05±2.20	24.06±1.68	24.10±1.73
Total lipid (WWB %)	14.43±2.00	14.47±2.08	14.75±1.17	14.60±0.63	14.66±0.46	14.67±0.81
Ash (DWB %)	13.72±0.68	13.87±0.74	13.88±0.21	13.83±2.31	13.80±1.30	13.71±0.47
Total protein(DWB %)	38.60±0.89	38.66±1.02	38.75±0.76	38.91±0.19	38.85±1.90	38.87±0.15
pH	6.34±0.06	6.40±0.02	6.44±0.06	6.47±0.01	6.54±0.05	6.55±0.03
NPN(DWB %)	2.14±0.34	2.16±0.30	2.20±0.42	2.40±0.20	2.37±0.50	2.52±0.17
SPN (DWB %)	1.86±0.46	1.70±0.14	1.73±0.11	2.06±0.30	2.13±0.14	2.22±0.30
Total soluble sugar (mg/g)	0.83±0.13	0.83±0.60	0.78±0.28	0.76±0.15	0.75±0.25	0.74±0.11
Total amino acid (mg/g)	5.24±0.28	5.90±0.35	6.30±0.34	7.36±0.64	8.76±1.30	8.83±0.70
TBA No. (melonaldehyde/kg)	0.59±0.10	0.60±0.02	0.70±0.05	0.72±0.21	0.82±0.04	0.93±0.04
TVBN (mg%)	117.30±0.30	133.30±0.11	149.0±1.06	160.0±0.12	170.0±0.61	286.0±0.27
FFA (%as oleic acid)	82.51±1.81	83.49±096	84.60±1.24	85.12±0.90	87.08±1.17	89.36±0.86
Cholesterol (mg/g)	3.46±0.11	3.55±0.19	3.77±0.25	3.80±0.05	3.70±0.51	3.67±0.49

Table3. Changes in various biochemical constituents during Ngari fermentation at 40^0C temperature

Parameter	Fermentation period in days					
	30	60	90	120	150	180
Moisture (%)	24.45±0.37	24.43±0.52	24.42±0.31	24.48±0.68	24.47±0.28	24.46±0.32
Total lipid (WWB%)	14.66±1.21	14.66±0.56	14.61±0.98	14.66±0.56	14.53±1.96	14.50±0.60
Ash (DWB %)	13.33±1.86	13.25±0.15	13.26±0.54	13.33±1.36	13.35±0.46	13.31±1.11
Total protein(DWB %)	38.75±1.03	38.10±0.10	36.84±1.0	35.48±1.01	35.15±1.02	35.25±0.90
pH	6.35±0.03	6.30±0.01	6.43±0.10	6.44±0.10	6.55±0.06	6.58±0.50
NPN (DWB %)	2.26±0.30	2.91±1.72	2.31±0.17	2.30±0.32	2.25±0.10	2.36±0.11
SPN (DWB %)	2.48±0.86	3.08±0.50	2.58±0.59	2.78±0.50	2.85±0.26	2.91±1.11
Total soluble sugar (mg/g)	0.62±0.14	0.77±0.27	0.58±0.12	0.49±0.11	0.45±0.20	0.47±0.05
Total amino acid (mg/g)	5.00±0.04	5.04±0.74	5.15±0.74	5.82±0.70	7.34±0.56	8.06±0.15
TBA No. (melonaldehyde/kg)	0.66±0.06	0.70±0.11	0.90±0.15	0.80±0.15	0.94±0.09	1.17±0.20
TVBN (mg %)	163.33±0.10	169.33±0.25	190.0±0.25	218.0±0.89	226.0±0.11	246.0±0.49
FFA (%as oleic acid)	85.28±2.56	88.75±0.42	89.46±0.45	89.96±1.24	92.31±2.80	99.45±3.00
Cholesterol (mg/g)	2.86±0.19	2.84±0.18	2.90±0.54	3.49±0.39	3.37±0.38	3.22±0.88

Note: Results are mean ± SD of 6 samples.
DWD=Dry weight Basis, NPN=Non protein nitrogen, TVBN=Total volatile base Nitrogen, TBA=Thiobarbituric acid, FFA= Free fatty Acid.

Table 4: Microbiological analysis of Ngari fermented at 20^0C in cfu/g.

No. of days	30 days	60 days	90 days	120 days	150 days	180 days
TPC (Bacteria)	1.87 10^5-1.27 10^6 6.52 10^5	2.10 10^6-1.27 10^6 3.27 10^6	1.50 10^6-2.5 10^6 1.81 10^6	3.45 10^5-1.0 10^5 6.25 10^5	2.2 10^5-5.4 10^5 3.9 10^6	3.0 10^5- 9.0 10^5 3.1 10^5
TPC (Fungi)	6.0 10^2-3.0 10^3 1.86 10^3	2.8 10^3-3.4 10^3 3.1 10^3	3.0 10^2-4.1 10^3 3.23 10^3	8.0 10^2-1.0 10^3 5.7 10^2	7.0 10^2-4.0 10^3 1.93 10^3	5.0 10^2- 4.0 10^3 1.66 10^3
Staphylococcus aureus	3.15 10^5-5.5 10^5 4.32 10^5	1.63 10^6-1.85 10^6 1.74 10^6	1.0 10^6-1.96 10^6 1.48 10^6	1.7 10^5-2.6 10^5 2.13 10^5	2.6 10^4-5.0 10^4 3.3 10^4	2.5 10^4- 3.1 10^4 2.76 10^4
Faecal *streptoccocci*	1.66 10^5-3.33 10^5 2.50 10^5	2.10 10^5-3.5 10^5 2.93 10^5	2.8 10^5-3.2 10^5 3.0 10^5	2.3 10^5-4.5 10^5 3.23 10^5	5.0 10^5-6.5 10^5 5.90 10^5	1.31 10^5- 3.0 10^5 1.72 10^5

E. coli, Salmonella and Coliforms were not detected.

Table 5: Microbiological analysis of Ngari fermented at room temperature (30-32°C).

No. of days	30 days	60 days	90 days	120 days	150 days	180 days
TPC (Bacteria)	7.0 10^5- 8.6 10^6 8.2 10^5	1.9 10^6- 8.0 10^6 4.3 10^6	7.0 10^6- 8.0 10^6 3.3 10^6	1.8 10^6- 2.9.0 10^5 2.3 10^5	4,0 10^5- 9.2 10^5 7.6 10^6	8.5 10^5- 9.4 10^5 8.9 10^5
TPC (Fungi)	3.0 10^4- 9.0 10^4 1.9 10^3	3.5 10^4- 6.64 10^4 5.6 10^4	2.0 10^4- 3.6 10^4 2.6 10^4	1.0 10^3- 6.0 10^3 3.6 10^3	1.3 10^2- 5.1 10^3 1.1 10^3	5.0 10^2- 1.7 10^3 1.1 10^3
Staphylococcus aureus	2.6 10^4-4.7 X10^5 2.6 10^4	1.0 10^6- 1.4 10^6 1.1 10^6	1.4 10^6- 1.8 10^6 1.5 10^6	1.4 10^5- 2.1 10^5 1,4 10^5	2.0 10^4- 3.7 10^5 2.1 10^5	2.0 10^5- 2.7 10^5 2.5 10^5
Faecal *streptoccocci*	1.9 10^6- 4.2 10^6 2.6 10^5	1.0 10^6- 1.5 10^6 1.2 10^6	2.0 10^5- 7.3 10^5 5.2 10^5	10^5- 4.2 10^5 4.0 10^5	2.1 10^5- 3.3 10^5 2.3 10^5	1.21 10^5- 2.3 10^5 1.6 10^5

E. coli, Salmonella and Coliforms were not detected.

Table 6: Microbiological analysis of Ngari fermented at 40^0C in cfu/g.

No. of days	30 days	60 days	90 days	120 days	150 days	180 days
TPC (Bacteria)	4.0 10^6- 9.0 10^6 6.0 10^6	7.0 10^4- 1.25 10^5 1.7 10^5	1.9 10^4- 1.5 10^6 1.15 10^6	1.1 10^6- 1.7 10^6 1.5 10^6	5.0 10^4- 1.2 10^5 7.2 10^4	5.9 10^3- 4.0 10^4 2.6 10^4
TPC (Fungi)	1.5 10^2- 2.0 10^3 1.0 10^3	2.0 10^3- 4.1 10^3 2.8 10^3	4.0 10^2- 6.1 10^3 4.6 10^3	3.0 10^2- 3.5 10^3 3.1 10^3	2.0 10^2- 4.0 10^3 2.66 10^3	2.2 10^3- 3.4 10^3 2.6 10^3
Staphylococcus aureus	2.1 10^5- 3.0 10^5 2.4 10^5	3.5 10^4- 3.0 10^5 7.1 10^4	3.4 10^4- 4.0 10^4 3.4 10^4	2.2 10^5- 5.8 10^5 4.0 10^5	2.1 10^5- 2.3 10^4 2.2 10^5	9.0 10^2- 3.0 10^3 1.4 10^4
Faecal *streptoccocci*	2.0 10^4- 3.0 10^4 2.3 10^4	1.0 10^3- 3.0 10^7 2.2 10^3	1.5 10^4- 4.0 10^4 2.6 10^4	1.0 10^6- 1.1 10^6 1.1 10^6	1./2 10^4- 2.3 10^4 1.7 10^5	9.0 10^2- 3.0 10^3 1.8 10^3

E. coli, *Salmonella* and Coliforms were not detect

Table 7: Percentage of Bacterial flora isolated from fermented *Puntius sophore* stored at different temperature

Bacteria	20^{0}C			30^{0}C			40^{0}C		
	60 days	120 days	180 days	60 days	120 days	180 days	60 days	120 days	180 days
Bacillus subtilis	13.60	8.45	26.66	25.00	14.28	15.49	17.80	18.18	9.07
	-30.00	-16.48	-27.27	-33.33	-26.78	-28.08	-23.07	-28.57	-25.00
Bacillus pumilis	20.00	14.0	13.63	25.00	19.04	12.76	7.69	9.09	0.00
	-21.73	-16.66	-14.68	-37.50	-35.7	-19.71	-15.06	-14.28	-9.09
Bacillus panthothenticus	15.00	14.28	12.50	8.12	7.14	15.95	15.38	25.00	20.00
	-27.27	-15.38	-22.72	-18.33	-9.52	-18.30	-19.17	-28.57	-27.27
Bacillus coagulans	4.54	9.76	13.12	8.33	8.92	11.26	8.22		
	-10.00	-17.28	-14.54	-18.75	-14.28	-17.02	-16.43	ND	ND
Staphylococcus	0.00	2.81	12.50	0.00	9.52	8.45	0.00	9.09	0.00
	-15.00	-17.58	-18.18	-12.50	-12.5	-11.70	-8.27	-12.5	-20.00
Micrococcus	25.00	42.85	18.18	16.66	8.92	13.82	15.06	28.57	25.00
	-40.00	-50.54	-35.50	-25.00	-34.61	-28.16	-46.15	-37.5	-54.54
Unidentified	0.00	0.00	3.12	0.00	2.81	10.63	0.00	14.28	20.00
	-10.00	-7.31	-6.66	-9.52	-11.53	-12.67	-8.21	-25.00	-25.00

ND = Not detected.

Table 8: Fungal flora isolated from the fermented *P. sophore* maintained at 20^{0}C (%).

Microflora	Fermentation period (days)		
	60 days	120 days	180 days
Aspergillus fumigatus	4.16-50.00	2.22-52.94	14.25-18.18
A. sydowi	0.00-25.00	3.22-38.23	0.00-18.18
Cladosporium sp.	4.16-13.85	0.00-33.33	9.09-25.00
Penicillium citrinum	0.00-16.60	0.00-22.22	25.00-28.57
P. fellutanum	0.00-20.83	8.33-16.66	ND
P. regulosum	28.57-50.00	11.10-12.90	ND
Gliocladium penicilloides	0.00-22.22	7.14-32.25	12.5-25.00
White sterile mycelium	0.0027.77	20.00-50.00	14.28-25.00
Rhizopus sp.	13.85-16.6	11.11-19.35	0.00-9.09
Humicola sp.	0.00-50.50	0.00-7.142	0.00-28.57
Helminthosporium nodulosum	25.00-33.33	ND	ND

Table 9: Fungal flora isolated from the fermented *P. sophore* maintained at room temperature (control)

Microflora	Fermentation period (days)		
	60 days	120 days	180 days
Aspergillus fumigatus	0.00-33.33	7.69-42.85	0.00-27.27
A. candidus	5.26-22.22	ND	ND
A. sydowi	50.00-66.66	0.00-20.00	0.00-14.28
Cladosporium sp.	0.00-40.00	20.00-42.85	12.5-18.18
Penicillium citrinum	0.00-33.33	14.28-50.00	0.00-29.41
P. fellutanum	14.28-20.00	33.33-62.50	11.76-45.45
P. regulosum	0.00-20.00	4.28-50.00	0.00-50.00
Cunninghamella sp	0.00-11.11	ND	ND
Curvularia pallescens	0.00-20.00	ND	ND
Gliocladium penicilloides	13.33-20.00	14.28-50.00	0.00-12.5
White sterile mycelium	5.26-11.11	0.00-20.00	9.09-12.5
Brown sterile mycelium	ND	0.00-33.33	ND
Humicola sp.	0.00-10.00	ND	ND

Table 10: Fungal flora isolated from the fermented *P. sophore* maintained at 40^{0}C (%).

Microflora	Fermentation period (days)		
	60 days	120 days	180 days
Aspergillus fumigatus	0.00-33.33	9.09-66.66	8.82-22.72
A. sydowi	16.66-40.00	25.00-50.00	18.18-38.23
Cladosporium sp.	14.28-25.00	14.28-25.00	29.41-63.63
Penicillium citrinum	20.00-66.66	18.18-50.00	ND
P. fellutanum	27.27-50.00	27.27-50.00	ND
P. regulosum	0.00-8.33	ND	ND
Curvularia pallescens	0.00-12.50	ND	ND
Gliocladium penicilloides	20.00-33.33	25.00-50.00	20.58-22.72
White sterile mycelium	0.00-16.66	0.00-14.28	0.00-4.54
Rhizopus sp.	16.66-20.00	0.00-9.09	ND

At higher temperature (40°C) most of the fungal species, which were observed during early stages, diminished at the latter stage. *Cladosporium* sp*, P.fellutanum, P.regulosum, Curvularia pallescans and Rhizopus* were completely absent. *Penicillium* sp was recorded as the highest dominant genus. These fungal floras were not able to adapt due to rancidity of oils in which the products were of dark colour with pungent odour. *A. fumigatus* and *P.citrinum* considered as toxigenic fungi was very common in our present study and hence proper care should taken up while consuming fermented fishes. The study reveals that several micro floras with varying percentage were found associated with the fermentation of sundried *Puntius* and they played an important role during fermentation. As indigenous fermented fish have been and will be continue to be important fish food in bringing vital protein to the consumers and as a part of the diet, quality of the fermented fish should be improved.

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