Microbial profile of starter culture fermented fish product 'Ngari' of Manipur

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ABSTRACT
Bacteria isolated from Ngari (a fermented fish product of Manipur), prepared in large-scale industrial products were identified. The starter culture isolated from 'Ngari', consisting of 3 species of \textit{Bacillus} and 3 species of \textit{Micrococcus} served as inoculum for the initiation of fermentation at 30 °C in laboratory conditions. Proper fermentation was noticed in 40 days in starter culture inoculated fish whereas in naturally fermented fish, fermentation was noticed after 5 to 6 months. The results confirmed that bacteria are responsible in the ripening process of 'Ngari'. Total plate count of bacteria reached upto 10^8 cfu g^{-1}. Coliforms, \textit{Escherichia coli} and \textit{Salmonella} were not detected during the fermentation period. Sensory quality of the products so obtained were comparable with that of commercial 'Ngari'. The spoilage indices such as thiobarbituric acid (TBA) number and total volatile base nitrogen (TVBN) were within the acceptable limit.

Keywords: Fermented fish products, Microbial profile, Ngari, Starter culture

Introduction
Fermented fish products are consumed almost everywhere in south-east Asia, generally as a condiment for rice dishes (Borgstrom, 1962). It has become very popular in the developed countries also due to their high nutritive value and organoleptic characteristics (Sanjeev et al., 1990). The use of microorganisms in the preparation of fermented foods dates back many centuries. Fermented foods in varied forms are widely consumed by different races of the world and have their own models of fermentation. Fermented fish processing is an artisanal activity and the process differ from one country to another.

'Ngari' is an indigenous fermented fish product of Manipur, India. It is prepared from small and less-priced sun dried fishes such as \textit{Puntius sophore} (Ham) and \textit{Puntius ticto} (Ham) subjecting to fermentation in the absence of salt for 5 to 6 months or more at room temperature. The process of 'Ngari' preparation involves a brief washing of the sun dried fishes, followed by draining and drying for 24-48 h. The fishes were then pressed hard using stone roller to breakdown head and bones. Before filling the fishes, a thin layer of mustard oil was applied to the inner wall of the earthen pot 'Kharung' to check porosity. Fishes were pressed hard mechanically inside the pots using wooden stick. The pots were then sealed airtight and incubated at room temperature for 6 months. Because of its special flavour, it is used as a compulsory item in daily curry preparation (Sarojnalini and Vishwanath, 1988). It has become an important commodity amongst the people of north-east India, hence its development and production in shorter period is necessary to meet the demand of the growing population. To fulfil the above objective, effects of microbes, their role in fermentation and finally elimination of unwanted strains are necessary. The use of starter culture to accelerate the fish fermentation process was reported by many workers (Apilado and Mabisa, 1991; Aryanta et al., 1991; Joshi and Rudra Setty, 1994; Asiedu and Sanni, 2002; Paludan et al., 2002).

The quality of fermented fish products is judged by their microbiological characteristics. Determination of standard plate count, the faecal coliform count and \textit{Staphylococcus} count are the widely accepted parameters in inspection of fish food (Anon, 1964). Microbial contamination in processed foods is not only a cause of concern to the health of the consumers but also an economic loss to the canner (Rao, 1980). The growth of \textit{Staphylococcus} in food presents a potential health hazard since many strains produce enterotoxins, which cause food poisoning if ingested. Barber and Deibel (1973) reported the incidence of \textit{Staphylococcus} food poisoning associated with fermented food causing gastroenteritis in human. Lien (2002) reported an outbreak of \textit{Staphylococcus aureus} food poisoning in Nesby in 12 to 22 people who consumed 'rakeorret' (a fermented fish product). Rieman and Bryan (1979) reported that certain strains of \textit{E. coli} cause enteric disease in man, 10^8-10^9 cells of this organism in human...
system causes symptoms of food poisoning as reported in infantile diarrhoea.

Reports on the fermented food in our state are scanty except some work done by Sarojnalini and Vishwanath (1987; 1988; 1995). But there are no reports on the full identity of the fermenting microorganisms. Attention should be paid on the microbiological aspects of such products from quality point of view and also for safeguarding the consumers’ health. This paper is a preliminary investigation on the acceleration process of fermentation using bacterial inoculum.

Materials and methods

Collection of samples, isolation and identification of microorganisms

Good quality 'Ngari' was brought from the Imphal market of Manipur asceptically to the laboratory. Microorganisms were isolated from the 'Ngari' by conventional dilution technique using sterile physiological saline solution and pour plate technique making use of appropriate media. The isolated bacterial strains were maintained on Trypton Glucose Agar slopes at 37 °C for 48 h and finally at 5 °C. The isolated strains include 3 species of Bacillus and 3 species of Micrococcus. All these strains were further checked for purity and identified at the Institute for Microbial Technology (IMTECH), Chandigarh, India and CABI Biosciences, UK before inoculation. The isolated bacterial strains were maintained and grown on respective slants and propagated at 37 °C after which a suspension was made to a concentration of 10^9 cfu ml^-1. Pots containing sun dried P. sophore were inoculated at the rate of 0.25 %, 0.5 % and 1.0 % of mixed strains and subjected to fermentation at 30 °C in BOD incubator. Samples without inoculum was allowed to ferment naturally as a control. Panels of examiners monitored the rate of fermentation organoleptically. All the process were carried out in aseptic condition in a laminar flow cabinet and incubated at 30±1 °C.

Microbiological and biochemical analysis

Total colony forming units of fungi and bacteria were determined by dilution plate method (APHA, 1976) using Potato Dextrose Agar and Tryptone Glucose Agar Medium respectively. Coliform and E. Coli, were detected by Most Probable Number (MPN) method and Staphylococcus aureus and faecal Streptococci were enumerated as per the method of APHA (1976). Samples were tested for the presence of Salmonella (APHA, 1976). Coliform was determined by using brilliant green lactose broth, E. coli using Eosine methylene blue agar, Staphylococcus on Bairded Parker agar, Salmonella on brilliant green agar and faecal Streptococci on K. F. Streptococcal agar (Hi media).

Moisture content, total lipid and crude protein were determined by AOAC (1975). Total amino acids was determined as per Moore and Stein (1948). Total volatile base nitrogen (TVBN) and free fatty acid (FFA) were estimated using TCA extract in Conway unit following the method of Morris (1959). Thiobarbituric acid (TBA) number was estimated by the method of Sinhuber and Yu (1958). α-amino nitrogen was estimated by formol titration as per Sorensen's method (AOAC, 1984). pH was measured using a pH meter (Valsan, 1975).

Results and discussion

The total bacterial counts reached upto 10^6 and 10^8 cfu g^-1 in natural fermentation and inoculated samples respectively (Table 1). Increase in the viable count of microflora from 10^6 to 10^7 in 72 h of fermentation using starter culture was reported in African fermented fish (Aseidu and Sanni, 2002). Aryanta et al., (1991) also observed a population of 10^7-10^8 cfu g^-1 by 48 h in fermented fish sausage using Pediococcus acidilactici as starter culture. Nagao (1951) observed an increase in the bacterial load from 10^4 to 10^7 g^-1 in 'Shiokara' during 41 days of fermentation and indicate the possibility of bacterial role in the ripening of the product. Joshi and Rudresetty (1994), observed a total plate count value of 10^6 cfu g^-1. In the present study, the total plate count reached upto 10^6 cfu g^-1, which was similar to ensure finding of Durairaj et al. (1975), in which a total plate count of 10^6 g^-1 was found to ensure proper course of fermentation. In the present investigation, significant

<table>
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<tr>
<th>Parameters</th>
<th>Starter culture fermented at</th>
<th>Naturally fermented</th>
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<tbody>
<tr>
<td></td>
<td>0.25 %</td>
<td>0.50 %</td>
</tr>
<tr>
<td>Total plate count of bacteria</td>
<td>3.26 X 10^7</td>
<td>6.43 X 10^7</td>
</tr>
<tr>
<td>Total Fungal count</td>
<td>1.05 X 10^2</td>
<td>1.66 X 10^2</td>
</tr>
<tr>
<td>Staphylococci count</td>
<td>2.03 X 10^3</td>
<td>2.28 X 10^3</td>
</tr>
<tr>
<td>Faeal Streptococci count</td>
<td>2.33 X 10^4</td>
<td>2.60 X 10^3</td>
</tr>
<tr>
<td>Coliform count (MPN)</td>
<td>ND</td>
<td>ND</td>
</tr>
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MPN = Most Probable Number
ND = Not Detected
increase in total bacterial counts indicates the bacterial role in fermentation, which finally resulted in the production of flavours.

Coagulase +ve Staphylococci count reached 10^5-10^6 cfu g^-1 in matured condition. High count of Staphylococci (10^5-10^7) was also noticed in fermented fish sausage (Barber and Deibel, 1972; Aryanta et al., 1991). Staphylococci count exceeding 10^6 cfu g^-1 is considered to be hazardous (Bergdoll, 1979) and a count of 100 million (10^8) per gram is considered unfit for food (Almas, 1981). In the present study, Staphylococcal count did not exceed 10^6 cfu g^-1 and occurred within the acceptable limit. The occurrence of small number of Staphylococcus in the fishery product is not a serious problem but food poisoning may occur if the products are handled carelessly during processing (Iyer, 1979). Amongst the fungus identified, Aspergillus, Penicillium and Cladosporium species were dominant. Gram-positive rod, Bacillus and Gram-positive cocci, Micrococcus were predominant among the bacterial flora of both the naturally fermented and starter culture fermented fish. Bacillus species identified were B. coagulans, B. pumilis, B. subtilis and B. pantothenticus. The presence of Bacillus suggested that spore forming bacilli might play an active role during fermentation. The occurrence of Micrococcus species also indicates the possible involvement of non-sporing microorganisms (Rose, 1982). These bacteria may contribute to the development of flavour and odours in fermented fish products due to their proteolytic and lipolytic activities (Sand and Crisan, 1974). They also assist in the breakdown of fish tissue and the development of flavour and aroma (Beaumont, 2002), which are essential for the quality of the final product (Amano, 1962).

Results revealed that the application of starter cultures accelerate the rate of fermentation. It does not bring about adverse changes in flavour and texture (Apilado and Mabisa, 1991). The products with 0.5 % and 1.0 % mixed strains showed the most desirable organoleptic properties than 0.25 % and reached maturity at 40 days. The organoleptic analysis revealed that all the products with starter culture possess characteristics comparable to those of indigenous one and showed consumer acceptability. Panelists did not report any off flavours in ngari with inocula where the total plate count raised to 10^6 cfu g^-1. Its flavour, texture and odour were comparable with the products prepared traditionally which took around 5-6 months. Higher inoculum concentration always resulted in rapid hydrolysis of protein (Aseidu and Sanni, 2002). More softening of fish muscle was observed with the samples of higher inocula and comparable to naturally fermented fish for 5-6 months period.

The mean value for moisture, protein and lipid are shown in Table 2. Changes in pH were minimal. A pH level of 6.74 was observed for starter fermented 'Ngari' while a pH of 6.49 was recorded for the naturally fermented sample after a period of 40 days. This finding was similar to that of 'Hentak' (a fermented fish product of Manipur) with a pH value 6.90 (Sarojnalini and Vishwanath, 1987). Higher pH allows bacteria to become dominant and also favors the anaerobic breakdown of proteins that releases amine compounds. Total volatile basic nitrogen, total amino acids, free fatty acid and alphaamino nitrogen increased rapidly as compared to that of uninoculated products. Variation in the total amino acid was noticed between the naturally and starter culture fermented fish. During fermentation, most free amino acids and peptides increased markedly which contribute significantly to the characteristics of fermented fish. TVBN value reached up to 245 mg % in 1.0 % inoculum. Increase in TVBN and amino nitrogen were also reported by many workers (Yang and Chung, 1995; Cho

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<tr>
<td></td>
<td>0.25%</td>
<td>0.50%</td>
</tr>
<tr>
<td>Moisture (DWB %)</td>
<td>24.73 ± 0.28</td>
<td>24.53 ± 0.65</td>
</tr>
<tr>
<td>Total lipid (WWB %)</td>
<td>15.33 ± 0.57</td>
<td>14.33 ± 0.65</td>
</tr>
<tr>
<td>pH</td>
<td>6.80 ± 0.03</td>
<td>6.66 ± 0.02</td>
</tr>
<tr>
<td>TVBN (mg %)</td>
<td>2.10 ± 0.57</td>
<td>246.93 ± 0.98</td>
</tr>
<tr>
<td>TBA No. (mg melonaldehyde kg^-1)</td>
<td>0.42 ± 0.07</td>
<td>0.43 ± 0.19</td>
</tr>
<tr>
<td>FFA (%) as oleic acid</td>
<td>56.08 ± 0.37</td>
<td>61.76 ± 0.98</td>
</tr>
<tr>
<td>-amino nitrogen (mg g^-1)</td>
<td>10.96 ± 0.30</td>
<td>12.04 ± 0.33</td>
</tr>
<tr>
<td>Total amino acid (mg g^-1)</td>
<td>4.85 ± 0.23</td>
<td>5.00 ± 0.17</td>
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Results are mean values of six replicates ± standard deviation
DWB = Dry Weight Basis
WWB = Wet Weight Basis
et al., 2000; Kuda et al., 2001) in fermented fish. Increase in these values were probably due to the subsequent microbiological and biochemical changes in the fish muscle, which impart flavour during fermentation. TBA value increased slightly during fermentation but remained almost unchanged as it attained maturity. Although the value of TVBN and TBA increased, the values were within the acceptable limits as according to Morris (1995) and Sinhuber and Yu (1958) respectively. According to Sinhuber and Yu (1958), TBA number less than 3.0 mg per 1000 g sample of cured fish is considered to be in good condition. Increase in free fatty acid could be attributed to mainly hydrolysis of lipid by endogeneous or exogeneous lipolytic enzyme systems (Change et al., 1994).

The results show that the occurrence of faecal Streptococci and Staphylococcus aureus in ‘Ngari’ is of concern as it is directly related to the health and hygiene of consumers. The use of starter cultures are recommended in order to prevent the growth of undesirable microbes, to improve the product quality and to avoid contamination.

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References


Kuda, T., Miyamoto, H., Sakajiri, M., Ando, K. and Yano, T. 2001. Microflora of fish nukazuke made in
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