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Fermented Fish (Ngari) of Manipur –Preparation Technique and its Potential as a Functional Food Ingredient

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ABSTRACT

Ngari is a non-salted fermented fish product of Manipur. Sundried *Puntius sophore (Ham.)* are fermented anaerobically with no intentional addition of starter culture for 4 to 12 months. LAB, Bacillus and yeast were involved in its fermentation. They are prepared at household level and marketed locally. Ngari are good source of protein, amino acids and minerals. The product has potential probiotic properties, antioxidant and antihypertensive characteristics. However, the fermented product if not processed hygienically can pose threat to food borne illness due to cross contamination. This review study is to know the processing technique and potential health benefits of Ngari.

Introduction

Fermentation is one of the oldest and most valued technologies used for food preservation (Sarkar and Nout, 2014). It is one of the oldest and most economical methods for producing and preserving foods (Tamang, 1998). It is a household art handed down through generation by generation in north-east, India (Jeyaram *et al.*, 2009).

Traditional processing of fish, such as fermentation, salting, drying and smoking are the principle method of fish preservation in Southeast Asia (Cooke *et al*., 1993). Fish and other seafood are also widespread as fermentation substrates for the production of sauce and paste condiments across a range of traditional Asian cultures (Van Veen and Steinkraus, 1970). The people of the Himalayas—mostly the eastern Himalayan regions of Nepal, Darjeeling hills, Sikkim, Assam, Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Mizoram, and Tripura, and some regions in Bhutan—consume different types of traditionally processed smoked, sun- or air-dried, fermented, or salted ethnic fish products (Tamang, 2010). The north east region of India comprised of a cluster of eight states namely Assam, Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Mizoram, Sikkim and Tripura (Chatterjee et al., 2006).

Among the indigenous fermented products, fermented fishery products have been widely consumed in Southeast Asia (Muzaddadi and Basu, 2012). Fish and fish products have been associated with the economic life of the people of northeast, India from time immemorial (Karthikeyan et al., 2007).

Many tribes of northeast, India prepare fermented fish generally from locally available small species of freshwater fish (Das and Deka, 2012). In Manipur, one of the states in Northeast, India, fermented foods and beverages are consumed as a regular food item. Ngari is a non salted fermented fish product of Manipur. Ngari is gifted to the people of the Manipur since decades for its enormous values. The people of Manipur consumed it almost everyday; hardly a day passes without Ngari being cooked (Asem et al., 2012). It forms an intrinsic part of the diet of the people of Manipur and has become an important commodity amongst the people (Sarojnalini and Suchitra, 2009; Das and Deka, 2012). It is one of the essential ingredients of every household in the area accounting to its taste, therapeutic properties and strong appetizing nature. Due to its ever-growing popularities, its value as a food ingredient has grown into other states of the North-eastern region (Singh et al., 2010). Ngari preparation and consumption reflects the typical food culture of Manipur (Tamang, 2010). Similar, fermented fish has several local names in Northeast, India which are commonly called as shidal, seepa and hidal in Assam, Tripura, Mizoram, Arunachal Pradesh and Nagaland (Ahmed et al., 2013). Puntius sophore is used to make the fermented fish product called tungtap in Meghalaya. This product is made at the village level and is sold throughout the district at the weekly markets (Agrahar and Subbulakshmi, 2006). Production of Ngari is confined only in the valley region of Imphal, the capital of Manipur and in the surrounding area. Ngari is known for its characteristics flavour and taste.

Processing of Ngari

Fermented fish processing is an artisanal activity and the process differs from one country to another (Sarojnalini and Suchitra, 2009). The methods of preservation are traditionally used with cultural identity and these household arts are handed down through generation (Das and Deka, 2012).

Ngari is fermented from small and less priced sundried fish, Puntius sophore (Ham.) locally known as Phoubu nga (Devi and Kumar, 2012). Ethnic people of northeast, India catch fishes from rivers, lakes and wetland and these are traditionally fermented, but for commercial production, fish is procured both from local market and from the Brahmaputra river of Assam or Bangladesh or from distant states like West Bengal and Andhra Pradesh (Tamang, 2001).

The processing involve a brief washing of sundried fishes (whole) with water using porous bamboo baskets followed by draining and drying the water for 24-48 hours. The washed-drained fishes were pressed hard by using legs covered with gunny bags or stone rollers to remove excess water and also

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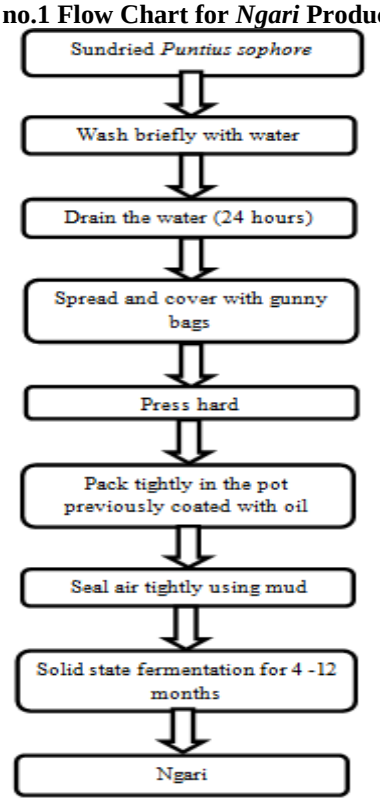
to breakdown heads and bones. Oil released from the head during pressing is believed to initiate fermentation. Earthern pot / vat of capacity 45-50 kg locally known as Kharung were used for the processing. The pot is a round bottomed, narrow necked, although breakable, it can be used for many years. The older the pot, the better the product is supposed to be. The inner surface of the pot is coated with mustard oil or any other vegetable oil to check porosity (Asem et al., 2012; Devi and Kumar, 2012; Jeyaram et al., 2009; Sarojnalini and Viswanathan, 1998; Sarojnalini and Suchitra, 2009; and Thapa, 2002).

The oils used in the smearing process may be of any vegetable oils like sunflower, mustard or palm oil. As the oils are getting absorbed and finally fully dried, another step of oil layering is done and it is dried once again (Singh et al., 2010). For new pots, 8-10 times oil coating are required with interval of 7 to 10 days. In old pots, only one coat is sufficient for retaining fermentation. Oil coating are believed to create anaerobic environment inside the chamber during the fermentation process (Jeyaram et al., 2009). This also avoids the fish from sticking on the inner surface of the jar (Singh et al., 2010).

The pressed or rolled fish were packed tight mechanically inside the oil coated pot using wooden stick or pressed by using legs. The pots were sealed airtight with a dough like paste prepared from fish powder or trash fish discarded during washing- drying process which has been sundried, grounded and moistened with water. Then it is covered with polythene or cover leaves (such as broad bottle gourd or banana leaves) or even newspapers. Finally the earthern pot is covered with thick mud/ clay prepared from humus rich fine soil as permanent seal till maturation and the earthen pot are tightly bound by wires to facilitate easy handling and to avoid leakage and breakage. The sealed pot is subjected to fermentation for 4 to 12 months at room temperature (Asem et al., 2012; Jeyaram et al., 2009; Sarojnalini and Suchitra, 2009; Singh et al., 2010). Figure 1 depicts the procedure for production of Ngari.

Figure no.1 Flow Chart for *Ngari* Production



To accelerate the fermentation process, the starter culture consisting of three species of Bacillus and three species of Micrococcus isolated from ngari was used. Proper fermentation was noticed only after 40 days in starter culture-inoculated fish, whereas in naturally fermented fish, the fermentation was complete after 5–6 months or longer (Sarojnalini and Suchitra, 2009).

Microorganisms Involved

The use of microorganism in the preparation of fermented foods dates back many centuries (Sarojnalini and Suchitra, 2009). Like hawaijar (fermented soya of Manipur), no starter culture are intentionally added during its preparation; it is a naturally fermented product (Keishing and Banu, 2013).

Study has shown the involvement of various microorganisms during its fermentation. The microorganisms isolated from Ngari are LABs (lactic acid bacteria), Bacillius and Yeast strain confirming its microbial diversity. The LABs isolated were identified as Lactobacillus fructosus of heterofermentative; Lactobacillus amylophilus, Lactobacillus coryniformis subsp. torquens and lactobacillus plantarum of homofermentative. Coccal LABs identified were Lactococcus plantarum, Lactococcus lactis subsp. cremoris and Enterococcus faecium. Endospore forming rods identified were Bacillus subtilis and Bacillus pumilus. Aerobic cocci were identified as Micrococus. Yeast strains present were identified as Candida and Saccharomycopsis (Thapa et al., 2004).

Significant increase in total bacterial counts indicates the bacterial role of fermentation during the fermentation of Ngari, which finally resulted in the production of flavours. It was also observed that bacteria are responsible in the ripenning process of Ngari (Sarojnalini and Suchitra, 2009).

Nutrient Composition

Microorganism converts the chemical constituent of raw substrates of plant or animal origin during food fermentation and enhances the nutritional value of the products, fortifying the product with health promoting bioactive compounds, vitamins and minerals degrade undesirable compounds and antinutritive factors (Tamang, 1998; Farhad et al., 2010). Fermented fish products are a good source of protein, peptides and amino acids. Protein and peptides derived from fermention have been found to be physiologically active or bioactive which have regulatory functions in the human body, apart from serving as important nutrients (Vignesh et al., 2012; Ngo et al., 2012). Traditionally processed fish products are unique in these regions, and they are important because they supplement the nutritive value of the local diet (Tamang, 2010).

The major bacterial enzymes produced during fermentation result in the hydrolysis of macronutrient molecules (proteins, complex carbohydrates, and triglycerides), enhancing digestibility and nutrient bioavailability of the fermented product compared with the raw food substrate (Wang and Fung, 1996). There is a range of fermented fish and other seafood products consumed throughout traditional food cultures in Asia. These foods generally have high levels of protein with high biological value, but are often consumed more as condiments than staples (Van Veen and Steinkraus, 1970).

The nutritional properties of Ngari, have been analysed, examined and reported by many. Table no.1 and 2 shows the proximate nutrient and mineral composition of Ngari respectively. Fermented fish products are generally high in protein and amino compounds (Puwastien et al. 1999). The total amino acids, free amino acids and fatty acid contents are increased during fermentation of fish (Rabei et al., 2009; Chang et al., 1994). During fermentation, the quantity of most of the free amino acids and peptides increases markedly, which contribute significantly to the characteristics of fermented fish. Total volatile-based nitrogen (TVBN) value and amino nitrogen

increase, probably due to the microbiological and biochemical changes in the fish muscle, which impart flavor during fermentation (Sarkar and Nout, 2014).

The utilisation of whole fish including bones and scales in the fermentation increases the ash content and higher minerals. Ngari shows an increase in protein content which can be attributed to microbial synthesis of proteins. Further protein in Ngari might have been degraded to amino acids by the activities of the fermenting microbes and used in the metabolic activities (Taorem and Sarojnalini, 2012). This degrative process also bringsout certain characteristics flavour that is essential for the quality of the final product (Amano, 1962; Ito and Salo, 1963).

The pH value of Ngari increase due to non-involvement of LABs (Taorem and Sarojnalini, 2012) that allows bacteria to become more dominant mainly the Bacillus sp. which may be due to the formation of basic nitrogeneous compounds (Soyiri, 2003). FFA (free fatty acid) liberation was more at high temperature (40 °C) in ngari due to protein denaturation and lipid hydrolysis but no rancid odour occurs (Taorem and Sarojnalini, 2012).

The traditional process of drying and further fermentation during fish fermentation weakens the bones considerably, almost dissolving them in the flesh portion, which may be the reason for the increase in calcium (Tamang, 2010). Similar fermented fish, nuoc-nam of Vietnam had high calcium (350mg/L), magnesium (1.3/L), phosphorus (25mg/L) (Van Veen, 1965).

In relation to water-soluble vitamins, fermentation can result in a 3-fold increase in thiamine and riboflavin contents and a 5-fold increase in vitamin B12 content, compared with the raw substrate (Reddy et al., 1982) however, theres has been no report on the vitamin contents of nagri.

Therapeutic value of *Ngari*

During the process of fermentation fermented foods can produce anti oxidant and anti microbial compound that stimulate the probiotic function (Farhad et al., 2010). Fermented fish products apart from serving as a good source of protein might possess bioactives, especially ACE inhibitory properties and anti oxidants activity (Phadka et al., 2014). The inhibition of ACE (angiotensin converting enzyme) activity is a major target in the prevention of hypertension (Ondetii et al.,1977). Fermentation is an effective way to generate ACE inhibitory peptides, which are released from long-chain protein of the food matrix in the role of protease secreted by microorganisms (Sarkar and Nout, 2014).

With increase in fermentation period and higher protein concentration the ACE inhibitory activity of Ngari showed higher ACE inhibition proving a good antihypertensive property (Phadke et al., 2014). Alkaline Fermented Foods-derived ACE inhibitory peptides show a great promise in the development of a novel physiologically functional food for preventing hypertension (Sarkar and Nout, 2014). Furthermore, they perhaps can be considered as starting compounds for development of antihypertensive drugs against ACE (Li et al., 2004).

Fermented food products are good source of peptides which possess antioxidant properties (Shimada et al., 1992). Nagri has hydrogen donating ability and could react with free radicals to convert them to more stable product, thus terminating the radical chain reaction (Phadke et al., 2014).

Abdhul et al., (2014) has also reported the scavenging activity of exopolysaccharides (EPS) extracted from Enterococcus faecium BDU7, isolated from Ngari increased with increasing concentration by determing hydroxyl, DPPH and superoxide radical scavenging activity. The extracted EPS exhibited equal scavenging activity compared to the known standard ascorbic acid showing strong anti-oxidant activity of Ngari.

Probiotic potential

Probiotics are live microbes which, when administrated in adequate amounts, confer a health benefits to the host. It provides numerous health benefits beyond providing basics nutritional values (FAO/WHO, 2001). The uses of probiotics have been shown to turn many health benefits to the human and plays a key role in normal digestive process (Song et al.,2012). To provide health benefits, probiotic must be capable of surviving and colorizing the intestinal tract (Lee and Salminen, 1995), expressing high tolerance to acid and bile (Kirjavainen et al., 1998).

Enterococcus faecium BDU7 isolated from Ngari showed acid tolerant and survival at pH value as low as 2.0 at least for 1hour but showed tolerance at pH values as low as 2.0 at least for 1 hour but showed tolerance at pH of 3-5. The gastric pH in healthy human is about 2-2.5 which causes the destruction of most microorganisms ingested. The same strains showed high resistances to bile showing 57.6 and 8.4% survival of E. faecium in 1 and 5% bile respectively. The physiological concentration of human bile is between 0.3% and 0.5% (Abdhul et al., 2014).

Functional effects of probiotic bacteria include adherence to the intestinal cell wall for colonization in the gastrointestinal tract with the capacity to prevent pathogenic adherence of pathogen activation (Salminen et al., 1996).

Auto segregation ability is related to the cell adherence properties which play an important role in the adhesion of bacterial cells to intestinal epithelium and it increases the chance of bacterial maintenance in the gastrointestinal tract (Del et al., 2000).

Hydrophobicity is an important attribute which helps the probiotics to colorize, modulate immune system and contribute adhesion of bacterial cells to host tissue (Ram and Chander, 2003). A high degree of hydrophobicity by some strains of LAB from the lesser-known Himalayan fish products probably indicates a probiotic character, since hydrophobicity is one of the important factors in determining the probiotic property of the microorganisms (Tamang and Holzapfel 2004). LAB isolated from Ngari showed high degrees of hydrophobicity indicating their potential advocating probiotic character (Thapa et al., 2004). Similar study reported strong autoaggregation and hydrophobicity of E.faecium BDU7 from Ngari validating its potential probiotic characters (Abdhul et al., 2014). Accordingly, these products could be marketed as health foods (Phadke et al., 2014).

The lactobacillus species associated wuth the traditionally fermented fish product- tungtap- an important component of the diets of the ethnic Khasi and Jaintia tribes of Meghalaya were found to possess many health promoting probiotic properties which included tolerance to acid and bile, cell surface hydrophobicity, and cholesterol lowering (Rapsang and Joshi, 2013).

Safety aspects of Ngari

The quality of a fish product is judged by its microbiological characteristics. 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). During fish processing change of microbial contamination are plenty.

Table No.1 Proximate Nutrient Composition of *Ngari* Product

Parameters	Amount %
Moisture	33.5
Carbohydrate	31.62
Protein	34.1
Fat	13.0
Ash	21.1
pH	6.2

Table No.2 Mineral Composition of *Ngari* Product

Minerals	Amount (mg/100 g)
Calcium	41.7
Iron	0.9
Magnesium	0.8
Manganese	0.6
Zinc	1.7

Presence of S. aureus in fermented fish product could be attributed to contamination during processing. The growth of Staphylococcus in food presents a potential health hazard since many strains produce enterotoxins, which cause food poisoning if ingested (Sarojnalini and Suchitra, 2009). Lien (2002) reported an outbreak of Staphylococcus aureus food poisoing in Nesby in 12 to 22 people who consumed 'rakeorret' (a fermented fish product).

Investigation has reported the presence of S. aureus in Ngari however the counts were for below the above and occurred within acceptable limit (Thapa et al., 2004 and Taorem and Sarojnalini, 2012). Staphylococcus count exceeding 6.0 log cfu g^{-1} is considered hazardous (Abraham et al., 1993; Rao, 2001; Nychas and Arkoudelos, 1990; Bergdoll, 1979).

Taorem and Sarojnalini, (2012) reported that coliform and salmonella were not detected in Ngari conforming it safe for consumption. Similar study on Shidal a fermented fish of Assam also detected the non presence of E. coli and Salmonella sp. in the product (Ahmed et al., 2013).

Though there has been no reported case of toxicity or illness due to consumption of fermented fish products, presence of pathogenic microbes due to gross contamination can be overcome which wouldsubsequently increase its quality by improving the hygienic conditions of the processing. There should be emphasison the proper vending and packaging of product (Ahmed et al., 2013), as it is directly related to the health and hygiene of consumer (Taorem and Sarojnalini, 2012).

Conclusion

Ngari produced traditionally by the people of Manipur are prepared rudimentarily at household level without proper hygienic and processing technique. The product is known for its flavour, enhancement of nutritional quality and its probiotic properties. Studies have reported potency as a potential probiotics, anti oxidant and anti hypertensive characters and can find wide application in functional food and pharmaceutical formulation. It is also known that Ngari is an intricate part of the diet of the people Manipur. On the other hand, it is also indicated that there are presence of pathogenic microbes, though no cases of illness due to its consumption are reported. Keeping the above facts in mind need arises to improve the quality of Ngari processing and utilized the best out of it for its health benefits. Formulation of new technique (s) to ferment the product hygienically by avoiding cross contamination; proper packaging and marketing strategies would help get the potential health benefits for consumers and also increase economy. Therefore, the fermented fish product Ngari has bioactive molecules with potential biological and therapeutic activities provided the microbial contaminants are removed and not to pose any threat to food borne illness to human health.

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