Role of Microorganisms in Traditional Fermented Foods

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Introduction

Use of microorganisms in preparing foods from locally available plant and animal materials is a traditional practice since pre-historic times. Development of "Spontaneous" food fermentation was primarily governed by climatic conditions, the availability of typical raw materials, socio-cultural ethos and ethnical preferences. Fermented foods are defined as foods that have been subjected to the action of selected microorganisms by which a biochemically and organoleptically modified substrate is produced, resulting in an acceptable product for human consumption. Growth and activity of microorganisms play an essential role in biochemical changes in the substrates during fermentation. Traditional fermented foods are generally nutritious and form the basic components of the diet as staple, adjunct, condiment and beverage, providing calories, proteins, vitamins and minerals to the people. With respect to the substrate, traditional fermented foods are generally categorized into products of plant, dairy, meat and fish origin.

Microorganisms associated with traditional fermented foods are present in or on the ingredients and utensils, in the environment, and are selected through adaptation to the substrate and by adjusting the fermentation condition. Three major types of microorganisms are associated with traditional fermented foods and beverages and these are :

- ***Filamentous fungi***

Species of *Aspergillus, Amylomyces, Actinomucor, Monascus, Mucor, Neurospora, Penicillium* and *Rhizopus*.

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- ***Yeasts***

Species of *Candida, Debaryomyces, Geotrichum, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Saccharomycopsis, Torulopsis* and *Zygosaccharomyces.*

- ***Bacteria***

Species of lactic acid bacteria (LAB)- *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Pediococcus, Tetragenococcus* and *Streptococcus*, Species of *Acetobacter, Citrobacter, Klebsiella, Bacillus, Brevibacterium and Propionibacterium.*

Most of the traditional fermented foods are prepared by processes of solid substrate fermentation in which the substrate is allowed to ferment either naturally or by adding starter cultures. The majority of fermented foods and beverages involving filamentous fungi are produced in East and South-East Asia. In Africa, Europe and America, fermented products are prepared exclusively using bacteria or bacteria-yeasts-mixed cultures. Moulds seem to be little or never used (Hesseltine, 1979; Geisen, 1993). In India, mostly due to wide variation in agro-climatic conditions and diverse form of dietary culture of the various ethnical groups, in most cases, three major groups of microorganism are associated with traditional fermented foods and beverages. (Table 1) shows some popular traditional fermented products of India (Ramakrishnan, 1979; Batra, 1986; Soni and Sandhu, 1990; Tamang, 1996). Microorganisms play essential roles and bring about some transformation of the substrates during fermentation.

Biopreservation

Foods may be preserved without refrigeration and without expensive or energy intensive operation by lactic fermentation, which produces lactic acid and sometimes also acetic acid, which lowers the pH of the food and inhibits the growth of other pathogenic organisms (Steinkraus, 1983; Holzapfel *et al.*, 1995). During fermentation of *Gundruk* and *Sinki*, common traditional non-salted fermented vegetable products of Himalaya, spe-

Table 1. Common Traditional Fermented Foods of India

Food	Substrate	Microorganism	Nature and use
Traditional Fermented Products of South India			
Idli	Rice-blackgram	LAB, yeasts	Steamed, spongy cake; breakfast food
Dosa	Rice-blackgram	LAB,. yeasts *B.amyloliquefaciens*	Spongy pan cake, shallow-fried staple food
Ambali	Millet, rice	LAB	Steamed sour cake; staple food
Kanji	Carrot/beet roots	Starter culture used is *TORANI* which contains LAB, yeasts	Strong-flavoured alcoholic beverage
Traditional Fermented Products of North India			
Ballae	Blackgram	LAB, yeasts, *B. subtilis*	Deep-fried patties; snack
Vadai	Blackgram	*LAB*, yeasts B. subtilis	Deep-fried patties; snack
Papad	Blackgram	*LAB*, yeasts	Circular wafers; snack
Wari	Blackgram	*LAB*, yeasts	Ball-like hollow, brittle; condiment
Bhatura	Wheat	LAB	Flat deep-fried, leavened bread; snack
Nan	Wheat	Yeasts, LAB	Leavened flat baked bread; Staple food
Jalebi	Wheat	LAB, yeasts	Crispy, deep-fried, pretzel sweet confectionery
Paneer	Milk	LAB	Soft mild-flavoured cheese; fried, curry
Traditional Fermented Products of Western Regions of India			
Dkokla	Bengalgram	LAB, yeasts	Steamed, spongy cake; snack
Khamam	Bengalgram	LAB	Spongy cake; breakfast food
Rabadi	Wheat/pear-millet/ maize/barley- buttermilk mixture	LAB, *Bacillus* spp.	Cooked paste; staple food
Shrikhand	Milk	LAB	Concentrated sweetened, savoury
Traditional Fermented Products of Eastern Regions of India			
Misti dahi	Milk	LAB,	Thick-gel; sweet savoury
Tari	Date palm	Yeasts, LAB	Sweet cloudy white alcoholic beverage
Traditional Fermented Products of the Himalaya			
Kinema	Soybeans	*Bacillus subtilis, Enterococcus, faecium,* yeasts	Sticky with typical flavour; side-dish curry
Hawaijar	Soybeans	*Bacillus* spp.	-do-; fish substitute
Gundruk	Leafy vegetables	LAB	Sun-dried, sour-acidic taste; soup/pickle
Sinki	Radish tap root	LAB	-do-
Mesu	Bamboo shoot	LAB	Sour-acidic pickle
Jaanr	Finger-millet/rice maize/barley	Starter culture used is *marcha* which contains Filamentous moulds, Yeasts, LAB	Mild alcoholic, slightly sweet-acidic beverage

LAB, lactic acid bacteria mostly spp. of *Lactobacillus, Leuconostoc, Lactococcus, Enterococcus and Pediococcus.*

cies of *Lactobacilllus* and *Pediococcus* produce lactic acid and acetic acid, which lower the pH of the substrates making the products more acidic in nature (Tamang and Sarkar, 1993; Karki, 1994). Due to low pH (3.3.-3.8) and high acid content (1.0-1.3%), *Gundruk* and *Sinki*, after sundrying, can be preserved without refrigeration and addition of any synthetic preservative for more than two years. This can be cited as an example of biopreservation and physical preservation of perishable vegetables, which are plenty in the winter season in the Himalayan regions. Common fermented vegetable products preserved by lactic acid fermentation are *Kimchi* in Korea, *Sauerkraut* in Germany and Switzerland, *Sunki* in Japan etc. Pickled vegetables, cucumbers, radishes, carrots, even some green fruits such as olives, papaya and mango are acid-fermented in presence of salt.

Bioenrichment

Bioenrichment of food substrates by traditional fermentation with proteins, essential amino acids and vitamins enhances nutritive value of the raw material. This has high significance for developing countries, where the majority of the people cannot afford commercially available and expensive fortified nutritive foods. In *Tempe*, a traditional fermented soybean food of Indonesia, the levels of vitamins such as niacin, nicotinamide, riboflavin and pyridoxine are increased by *Rhizopus oligosporus*, whereas cyanocobalamine is synthesized by non-pathogenic strains of *Klebsiella pneumoniae* and *Citrobacter freundii* during fermentation (Leim *et al.* 1977; Keuth and Bisping, 1994). Thiamine and riboflavin contents in *idli*, a traditional fermented black gram-rice breakfast food in South India, has been found increased during fermentation (Rajalakshmi and Vanaja, 1967). Increase in methionine from 10.6 to 60% during *idli* fermentation has been observed (Rao, 1961; Steinkraus *et al.*, 1967). *Pulque*, produced by lactic acid fermentation of juices of the cactus (*Agave* sp.) plant, is one of the oldest traditional fermented beverages of Mexico, which is rich in vitamins such as thiamine, riboflavin, niacin, pantothenic acid, pyridoxine and biotin and serves as important diet of low-income children in Mexico (Steinkraus, 1985). Total amino acids, free amino acids and mineral contents are increased during *Kinema* fermentation. This is a traditional soybean food of the Himalaya, fermented by *Bacillus subtilis* by which the nutritive value of the product is enriched (Tamang, 1995; Nikkuni *et al.*, 1995).

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Dawadawa, *Bacillus*-fermented locust bean food condiment is an important source of riboflavin in the local diet of West Africa (Campbell-Platt, 1980).

Microorganisms That Produce Enzymes

Microorganisms associated with the fermentation of foods produce desirable amounts of enzymes, which may degrade unsatisfactory or antinutritive compounds and thereby convert the substrates into edible products with enhanced flavour and aroma. *Bacillus subtilis (natto)* produces several enzymes such as proteinase, amylase, mannase, cellulase and catalase during Natto fermentation, a sticky fermented soybean food of Japan. *Bacillus subtilis* KK2:B10 (MTCC 2756) and GK2:B10 (MTCC 2757), isolated from *Kinema*, produce desirable amounts of protease and α-amylase (Tamang and Nikkuni, 1996).

Microorganisms That Destroy Undesirable Components

During *Tempe* fermentation, trypsin inhibitor is inactivated by *Rhizopus oligosporus*. It also eliminates the flatulence causing indigestible oligosaccharides, such as stachyose and verbascose into the absorbable di- and mono-saccharides (Hesseltine, 1983). Microorganisms associated with *Idli* fermentation reduce the phytic acid and content of the substrate (Reddy and Salunkhe, 1980). Bitter varieties of cassava *(Manihot esulenta)* tubers, the main staple crop in West Africa, contain the cyanogenic, glycoside linamarin, which can be detoxified by spp. of *Leuconostoc*, *Lactobacillus* and *Streptococcus* in I *Gari*, a fermented cassava product. Cassava tubers are thereby rendered safe to eat (Westby and Twiddy, 1991).

Enrichment of the Diet

In fermented milk products, lactic acid bacteria produce diacetyl and other desirable flavour (Kosikowski, 1977). Biotransformation of bland vegetable proteins into meat-flavoured amino acids sauces and pastes by mould fermentation is common in Japanese *Miso* and *Shoyu*, Chinese soy-sauce and Indonesian *Tauco* (Steinkraus, 1989). Halopthilic microorganisms contribute flavour and quality to fermented fish products, common in South-East Asia (Itoh *et. al.*, 1993).

During *Tempe* fermentation, mycelium of *R. oligosporus* knits the soybean cotyledons into a compact cake resembling bacon slices. Similarly, in *Ontjom*, an Indonesian fermented peanut presscake product, *Neurospora intermedia* knits the particles into firm cakes, imparting meat-like texture (Steinkraus, 1994).

In *Ang-kak*, a traditional fermented rice food of South-East Asia, *Monascus purpureus* produces a purple-red water-soluble colour in the product, which is used in colouring meats and rice wine (Beuchat, 1978).

Mixed Starter Culture

In many Asian countries, mixed-culture dough inocula in the form of dry powder or hard balls are widely used as starter cultures to prepare various fermented alcoholic beverages from starchy substrates. These starter cultures are known under different names such as Marcha/Bakhar/Phab in India, Nepal, Bhutan and Tibet in China, Ragi in Indonesia, *Nuruk* in Korea, *Bubod* in the Philippines, *Chiu-yueh* in China and *Loogpang* in Thailand. These starter cultures contain mixed microflora of filamentous moulds such as species of *Amylomyces, Mucor, Rhizopus, Actinomucor,* yeasts such as *Saccharomyces fibuligera, Saccharomyces* sp. *Pichia* spp; *Homsemula* spp *and lactic acid bacteria, mostly* spp *of Lactobacillus* and *Pedicoccus* (Hesseltine *et al.*, 1988; Tamang and Sarkar, 1995). These mixed-culture innocula degrade starch into reducing sugars and ethanol with enhanced aroma and flavour to the product (Yokotsuka, 1991). The sweet and sour alcoholic beverages prepared by these starters are *Tape ketan* in Indonesia, *Jaanr/Chiang* in the Himalayan regions of India, Nepal and Bhutan, *Krachae* in Thailand, *Lao-Chao* in China and Taiwan. *Koji,* an amylolytic mycotoxin-free *Aspergillus oryzae* culture on steamed rice is used along with a culture of *Saccharomyces cerevisiae* to prepare the common alcoholic drink *Sake* in Japan (Inoue *et al.*, 1992).

Time has come that we have to commercialize our culturally acceptable traditional fermented foods.

Traditional Fermented Foods of Medicinal Value

Koumiss, a fixy gray acidic-alocoholic beverage is prepared from horse or donkey milk in Russia. It contains *Lactococcus lactis, Lactobacillus bulgaricus* along with some yeasts *Candida kefyr* and *Torulopsis* spp. and has been used in treating pulmonary tuberculosis (Kosiko-wski, 1977). *Kvass*, a rye/wheat-based sour-alcoholic beverage of the Ukraine, is fermented by *Saccharomyces cerevisiae* and *Lactobacillus* spp., and is suggested to provide protection to the digestive tract against cancer (Wood and Hodge, 1985). Consumption of *Natto* presents hemorrhage caused by vitamin K deficiency in infants in Japan. Consumption of *TEMPE* reduces the cholesterol level which is due to inhibition of hydroxymethylglutaryl co-enzyme A reductase, a key enzyme in cholesterol biosynthesis, by oleic acid and linoleic acid during fermentation (Hermosilla *et al.*, 1993).

Conclusion

Most of the traditional fermented foods and beverages of other countries have been well investigated and documented. Statistical data on production, consumption, socio-economy, microbiology, biochemistry, nutritional profile, laboratory scale as well as large scale optimized production methods, etc., of traditional fermented foods of some countries are available. In India, most of the traditional fermented foods and beverages are yet to be investigated. Only a few common fermented foods such as *idli, dosa, dahi* etc., have been studied so far. Isolation, purification and identification of dominant microorganisms involved in traditional fermented foods and beverages are important aspects of such studies. Genotypic identification using molecular methods such as DNA base composition, DNA hybridization and ribosomal RNA sequences and chemotaxonomic tools such as cell wall studies, cellular fatty acids and isoprenoid quinones are helpful, when the conventional approach of identification is not reliable. Due to diverse dietary cultures of the various ethnical groups and wide-variations in agro-climatic conditions of India, microbial diversity associated in lesser-known fermented foods may contribute a significant gene pool, which must not be lost in this generation. The diversity of microorganisms in food ecosystems has not been sufficiently assessed and they may contain undescribed strains, which could be of scientific interest or may have potential industrial application. Time has come that we have to commercialize our culturally acceptable traditional fermented foods, some of which may present unidentified potent gene pools of microorganisms as part of well-known food eco-systems.

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References

Batra, L.R.(1986) Microbiology of some fermented cereals and grain legumes in India and vicinity. In: Indigenous Fermented Food of Non-Western Origin) (Hesseltine C.W. and Wang H.L., eds.) *Cramer J.*, Berlin, pp 85-104.

Beuchat, L.R.(1978) Traditional fermented food products. In: Food and Beverage Mycology (Beuchat L.R., ed.). AVI Publishing co., Westport, CT, pp 224-253.

Campbell-Platt, G. (1980) African locust bean *(Parkia* species) and its West African fermented food product, *dawadawa*. *Ecol. Food Nutr.* **9**: 123-132.

Geisen, R. (1993) Fungal starter cultures for fermented foods: Molecular aspects. *Trends Food Sci. Technol.* **4**: 251-256.

Hermosilla, J.A.G., Jha, H.C. Egge, H. and Mahumud, M. (1993) Isolation and characterization of hydroxymethylglutaryl coenzyme A reductase inhibitors from fermented soyabean extracts. *J. Clin. Biochem. Nutr.* **15**: 163-174.

Hesseltine, C.W. (1983) Microbiology of oriental fermented foods. *Ann. Rev. Microbiol.* **37**: 575-601.

Hesseltine, C.W. Rogers, R. and Winarno, F.G. (1988) Microbiological studies on amylolytic oriental fermentation starters. *Mycopathol.* **101**: 141-155.

Holzapfel, W.H. Geisen, R. and Schillinger, U. (1995) Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int. J. Food Microbiol.* **24**: 343-362.

Inoue, T., Tanka, J. and Mitsui, S. (1992) Recent Advances in Japanese Brewing Technology. Gordon and Breach Science Publishers, Tokyo.

Itoh, H., Tachi, H. and Nikkuni, S. (1993) Halophilic mechanism of isolated bacteria from fish sauce. In: Fish Fermentation Technology (Lee C.H., Steinkraus K.H. and Reilly P.J.A., eds.). The UNU Press, Tokyo, pp 249-258.

Karki, T. (1994) Food processing Industries in Nepal. In: Processing of the International Workshop on Application and Control of Microorga-nisims in Asia (Komagata K., ZYoshida T., Nakase T. and Osada H. , eds.). STA, Tokyo, pp 71-87.

Kosikowski, F.(1977) Cheese and Fermented Milk Food, 2nd Edn. Edwards Brothers, Ann Arbor, MI.

Keuth, S. and Bisping, B. (1994) Vitamin B_{12} production by *Citrobacter freundii or Klebsiella pneumoniae* during *tenpeh* fermentation a proof of enterotoxin absence by PCR. *Appl. Environ. Microbiol.,* **60**: 1495-1499.

Liem, I.T.H., Steinkraus, K. H. and Cronk, T.C. (1977) Production of vitamin 12B in *tempeh*, a fermented soyabean food. *Appl. Environ. Microbiol.,* **34**: 773-776.

Nikkuni, S., Karki, T.B., Vilku, K.S., Suzuki, T., Shindoh, K., Suzuki, C. and Okada, N. (1995) Mineral and amino acid contents of *Kinema*, a fermented soyabean food prepared in Nepal. *Food Sci. Tehnol., Int.* **1**: 107-111.

Rajalakshmi, R. and Vanaja, K. (1967) Chemical and biological evaluation of the effects of fermentation on the nutritive value of foods prepared from rice and grams. *Brit. J. Nutr.* **21**: 467-473.

Ramakrishnan, C.V. (1979) Studies Indian fermented foods. *Baroda J. Nutr.* **6**: 1-57.

Rao, M.V.R. (1961) Some observations on fermented foods. In: Progress in Meeting Protein Needs of Infants and Preschool Children. National Academy of Sciences, Washington, DC, pp 291-293.

Reddy, N.R. and Salunkhe, D.K. (1980) Effect of fermentation on phytate phosphorus, and mineral contents in blackgram, rice, and blackgram and rice blends *J. Food Sci.* **45**: 1708-1712.

Soni, S.K. and Sandhu, D.K. (1990) Indian fermented foods: Microbiological and biochemical aspects. *Indian J. Microbiol.* **30(2)** : 135-157.

Steinkraus, K.H. (1983) Lactic acid fermentation in the production of foods from vegetables, cereals and legumes. *Antonie van Leewen.* **49**: 337-348.

Steinkraus, K.H. (1985) Bio-enrichment: Production of vitamins in fermented foods In: Microbiology of Fermented Foods (B.J.B. Wood, ed.). Elsevier Applied Science Publication, London, pp 323-344.