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Introduction

The selection of foods best suited for promoting good health has been found by continuous trial and error by ancestral human beings (Swaminathan 1992). In the long way of journey of human civilization, cereals and legumes have become the two most important raw materials for preparation of plant food.

Edible leguminous seeds (pulses) are used in Asia to prepare human food much more than in any other continent (Padmaja and George 1999). These are an important source of proteins, B-vitamins and minerals in the dietaries of millions of people in the Asian countries. Legume proteins are, in general, a good source of lysine and threonine in which cereal proteins are deficient. They are, however, poor sources of sulphur-containing amino acids and tryptophan of which cereals are good sources. Hence, legume proteins effectively supplement cereal proteins. In the developing countries, like India, where problem of poverty, malnutrition and protein deficiency prevails, legumes offer a low-cost alternative source of protein. However, this property is underestimated due to some negative factors,

including unavailable carbohydrates, phytates, trypsin inhibitors, haemagglutinins, goitrogenic factors, cyanogenic glucosides and saponins present in legumes. Beneficial micro-organisms may play an important role in detoxifying these chemicals as well as fortifying foods with vitamins and minerals through fermentation (Khader 2001). However, pathogenic micro-organisms present in the foods may cause diseases due to infection, intoxication and toxicoinfection, causing severe gastrointestinal problem. Foodborne illnesses are a health problem in developing countries, particularly among infants and children. It is estimated that about 70% of the episodes of diarrhoeal diseases are foodborne, resulting from food contaminated by dirty utensils, poor processing, handling and storage conditions (Motarjemi and Nout 1996). Therefore, it is essential to check and improve the microbiological quality of foods through regular inspection.

A food is considered fermented when one or more of its constituents have been acted upon by micro-organisms to produce a considerably altered final product acceptable for human use (van Veen 1957). The use of micro-organisms to ferment foods goes back to prehistoric time. It is well approved that the science of fermentation is not an invention, rather a discovery. The credit goes solely to those ancient wise men who observed that when a few grains of barley are left in the rain, opportunistic micro-organisms ferment the starch-derived sugars into alcohols. Their patience of observation and capacity to harness and encourage these fermentations are worth-mentioning. According to some anthropologists, the knowledge of preservation of the otherwise perishable fruits and grains by fermentation changed the nomadic wanderers into settled farmers.

Descriptions of fermented foods go back as far in time as inscriptions are available. The art of food fermentation, used as a means of improving the keeping quality of food, probably originated around 7000 to 8000 BC in the tropical areas of Mesopotamia and the Indus Valley (Adams and Moss 1995). The Egyptians, Sumarians, Babylonians and Assyrians knew about the use of barley to produce alcoholic beverages; a cuneiform inscription on a Babylonian brick from 2800 BC gave a recipe for the production of barley wine (Borgstrom 1968). Records of soya sauce and also miso production in China go back to around 1000 BC with the transfer of knowledge of these production processes to Japan occurring around 600 AD (Yokotsuka 1985). Early Europeans were known to be making flat sourdough bread from rye in 800 BC. Around 100 BC, there were 250 bread bakeries operating in ancient Rome (Pederson 1979). Accounts of using dahi (curd) throughout India, dadhanvat (milk product) and kali (fermented rice) in southern part of India and panir (milk product) in Punjab are as old as 2000 BC (Om Prakash 1961). Records of dosa and idli go back to 1100 AD (Gode 1955). The history of using bhat bajraka (bajra flour product) in southern India, rabdi (maize flour product) in Rajasthan and rasgulla (milk product) in Bengal is found in 1500 AD (Pathak 1970). As soon as man started collecting milk from animals, sour milk became an item in his diet. Accounts of production of fermented dairy products can be found in early Sanskrit and Christian works, while recipes of sour fermented milks were given in Roman times around 200 AD (Oberman 1985). The origins of most fermentation technologies have been lost in the mists of history. Many fermented foods are now receiving world attention for their health-promoting or disease-preventing effects. Fermented foods are palatable and wholesome, prepared from raw or heated raw materials by microbial activity (Holzapfel 1997). The micro-organisms produce the proper enzymes, which bring about specific transformations of the substrate: improved flavour and appearance, destruction of undesirable components, improved keeping quality, enhanced nutritional value, improved digestibility, changing physical state of the product from raw material, imparting colour to the products, and providing dietary variety to otherwise monotonous meal. Moreover some fermented foods have medicinal importance, and the products require less cooking than the original substrate (Hesseltine 1965, 1979, 1983; Hesseltine and Wang 1980; Ramakrishnan 1979a; Steinkraus 1996) .

Traditional or indigenous fermented foods are those popular products that since early history have formed an integral part of the diet and that can be prepared in the household or in cottage industry using relatively simple techniques and equipment (Aidoo *et al.* 2006; Hesseltine and Wang 1980). Several plant foods are preferred in a fermented state, e.g. tempe to unfermented soya beans; and gari to unprocessed cassava tubers (Holzapfel 1997). Traditional fermented foods are essential for the well being of many people of the world, especially people of the Near East, southeast Asia, India, Far East, and Africa - south of the Sahara desert (Hesseltine and Wang 1980).

India is a treasure of different fermented foods. The plant-based fermented foods of India are mostly acid products prepared by bacterial and yeast fermentation of cereals such as rice supplemented with a protein source, like locally-grown legumes. Indeed, Indians are credited for developing the methods of souring and leavening cereal-legume batters (Padmaja and George 1999). Micro-organisms used in the production of legume-based traditional fermented foods of India are typically those present in or on the ingredients and are selected by adjusting the fermentation conditions. Fermentation processes of these foods have been developed mainly to add nutrition and flavour to a bland cereal-legume batter making organoleptically acceptable and easily digestible in addition to pertaining shelf-life enhancement. Today, consumer awareness for minimally processed, nutritionally rich and chemical preservative-free foods has paved the path for popularization of these fermented foods.

Fermented foods have generally been considered as less likely to be vehicles for foodborne infection or intoxication than fresh foods due to the competitive activity and metabolites of the functional microflora (Nout 1994). But due to unhygienic handling, external contamination, contaminated water and inferior quality of raw material, many of these foods may get contaminated by bacteria, such as *Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Escherichia coli, Salmonella, Shigella* and many other pathogens. In most of the fermented foods, especially in lactic acid bacterial fermented ones, the inhibition of growth of bacterial pathogens is common and can often ensure safety where levels of contamination are low (Adams and Nicolaides 1997). But with infectious pathogens, particularly those with a small low infectious dose, some degree of inactivation may be necessary to provide an acceptable level of safety (Beumer 2001).

Human illness from the consumption of foods contaminated with factors other than poisons or chemical toxic agents was recognized long before the understanding of the role of pathogens in foodborne diseases (Ray 2001). For healthy and well-nourished people, most food poisoning is an unpleasant episode from which recovery is normally complete after a few days (Adams and Moss 1995). But for the old and immunocompromised persons, foodborne illnesses can be fatal when caused by pathogens with low infectious doses. Moreover foodborne illnesses cause suffering, discomfort, and debilitation among the survivors. The economic losses from various factors could be high. The factors include medical treatment, lawsuits, lost wages and productivity, loss of business, recall and destruction of products, and investigation of the outbreaks (Garvani 1987). Statistics covering foodborne illnesses are notoriously unreliable. Many countries have no system for collecting and reporting data on gastrointestinal infections and even where these exist the reported data is acknowledged to represent only a fraction of the true number of cases. It is widely accepted that the real figure for foodborne illness may be 25-100 times higher than the recorded data due to factors such as under-reporting not seeking medical treatment. The World Health Organization (WHO) has estimated that only 10% of incidents occurring in most European countries are reported. Reported cases are most likely those who have sought medical attention and probably represent those who are most seriously ill or those who are most at risk such as the very young, the elderly and those with pre-existing illness or compromised immune systems. Unreported cases are usually those with mild illness or those with milder illness who treat themselves or who consult a doctor but for some reasons do not get reported (Adams and Moss 1995).

The increased incidence of foodborne diseases due to microbiological hazards is the result of a multiplicity of factors, all associated with our fast changing world. Demographic profiles are being altered, with increasing proportions of people who are most susceptible to micro-organisms in food. Changes in farm practices, more extensive food distribution systems and the increasing preference for meat and poultry in developing countries all have the potential to increase the incidence of foodborne illnesses. Extensive food distribution system raises potential for rapid, widespread distribution of contaminated food products. Changes in food products result in new types of food that may harbour less common pathogens. Changes in eating patterns, such as a preference for fresh and minimally processed food, the increasingly longer interval between processing and consumption of foods and the increasing prevalence of eating food prepared outside the home all contribute to the increased incidences of foodborne illness ascribed to micro-organisms. The emergence of new pathogens and pathogens not previously associated with food is a major public health concern. The results of these risk assessments will provide the scientific basis for undertaking measures to reduce illness from microbiological hazards in foods. Effective management of microbiological hazards is enhanced through the use of tools such as microbiological risk assessment (MRA) and hazard analysis and critical control point (HACCP) systems. Sound microbiological risk assessment provides an understanding of the nature of the hazard, and is a tool to set priorities for interventions. HACCP is a tool for process control through the identification of critical control points (CCPs). The ultimate goal is improvement of public health, and both MRA and HACCP are meant to that end.

Microbiological safety of fermented foods is an important issue in developing countries, including India. Processing technologies that ensure food safety are required at both the rural and urban levels, particularly in view of the frequently poor sanitary conditions and high ambient temperatures. As the population is increasing tremendously, lots of mobile shops, restaurants, hotels and cafeterias are mushrooming to cater the hungry mouth as well as to earn money to sustain in the ocean of unemployment. Most of the people working in these catering establishments have no basic knowledge of self-hygiene. Irregular disposal of sewage, scarcity of potable water, increasing pollution, warm and humid climatic condition also may contribute to the development of foodborne diseases. The production of safe foods is the responsibility of the producers. But the authorities need to regularly verify and validate those through inspection and product testing both at the site of production and at the point of sale. Originally the legume-based fermented foods in India were prepared traditionally in household level from a long time. Works on microbiology and biochemistry have established these foods as wholesome, nutritionally rich, easily digestible and organoleptically well-accepted. These attributes have made them popular as breakfast, tiffin and light midday meals resulting in their commercialization. However, incomplete fermentation, contaminated water, low-grade raw materials and unhygienic environmental condition may allow profuse growth of pathogenic contaminants in commercial products. Many traditional legume-based fermented foods including idli, dosa, dhokla and wadi contain considerable level of lactic acid lowering the pH to 4-5 that could prevent the survival or growth of pathogens in the cereal-legume batter or dough. However, bacteria like *Bacillus* and clostridia produce endospores, which can sustain unfavourable environmental conditions. Moreover, post-preparative contamination after cooking of batter or dough to make final product may occur from unhygienic storage condition and food handlers. The fact that several bacteria have a remarkable ability to survive different environmental stress conditions makes it very difficult for the food industry to exclude them from their products (Andersson *et al.* 1995).

In the State of West Bengal in India, fermented foods like amriti, papad and wadi are popular from time immemorial. In the passage of time, southern and western Indian fermented foods, like idli, dosa and dhokla have also become the choice for restaurant hunters as nutritious and delicious light

midday meal. The traditional method of preparation of amriti and appearance of the product are similar to those of jalebi, excepting that in pretzel-looked jalebi, blackgram dal (dehulled split beans) is replaced by refined wheat flour (*maida*) and the fermentation time is longer (Campbell-Platt 1987; Steinkraus 1996). The preparation of dhokla, dosa and idli are similar, excepting that in dosa and idli Bengalgram is substituted with blackgram, and while dosa is a highly seasoned griddled pancake, idli is a steamed pancake resembling dhokla. While all the aforementioned ones are ready-to-eat (RTE) foods, marketed papad and wadi are cooked before consumption. Though these foods are consumed throughout the country, no published data regarding the microbiological quality of these fermented foods except a few for idli and wadi have been found. In this context, necessity for checking the microbiological quality of the legume-based traditional fermented foods sold in markets and studies to better defined conditions affecting the behaviour of the isolates are of public health importance.

Hence, the protocol adopted to attain these objectives was as follows:

(i) observing hygienic status of the retailed outlets of these foods in West Bengal, and sampling a variety of foods through a cross-section of the retailers;

(ii) isolating major foodborne bacterial pathogens from the samples using their respective selective media;

(iii) characterizing the isolates in order to determine their taxonomic status;

(iv) enumerating the identified isolates to reveal the microbiological quality of the samples;

(v) generating antibiograms of the representative isolates against antimicrobials to find out their resistance patterns;

(vi) studying thermal inactivation of the sporeformers in order to minimize potential health hazards;

(vii) evaluating the production of extracellular enzymes to predict shelf-life evaluation;

(viii) examining the influence of different hurdles such as pH, sodium chloride, benzoic acid, sorbic acid and nisin on the growth of the isolates with a view to control a possible menace that may be caused by them;

(ix) protein-typing of the isolates to find out the diversity among them;

(x) studying antagonistic activity of lactic acid bacteria from legume-based traditional fermented foods against some isolated pathogens; and

(xi) determining survivability and growth following spiking of foods with isolated pathogens.