5

Discussion

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). Although several legume-based fermented foods are being prepared in different parts of India by employing indigenous techniques, the origins of most of these fermentation technologies are lost in the mist of history.

Indians are credited for discovering the methods of souring and leavening cereal-legume batters. Due to her diverse agroclimatic locations, social behaviour, cultural and religious beliefs, and dietary habits among the multi-ethnic population, India harbours an excellent source of legume-based indigenous fermented foods (Table 5). The traditional fermented foods, both alkaline and acidic, derived either from edible legume alone or in combination with different kinds of cereals, have made a significant contribution to human diet across the country. They not only add variety to the human diet, but also serve as an economic source of supplementary proteins for the large human population of the country like India, where bulk of the population is vegetarian. Many fermented foods are now receiving world attention for their health-promoting or disease-preventing effects.

Although some of these legume-based traditional products, native to different parts of the country have been well-studied (Aidoo *et al.*, 2006; Batra, 1981, 1986; Batra and Millner, 1976; Campbell-Platt, 1987; Desai and Salunkhe, 1986; Nout and Sarkar, 1999; Nout *et al.*, 2007; Ramakrishnan, 1979; Reddy *et al.*, 1982, 1986; Soni and Sandhu, 1989a, 1989b, 1990, 1991; Steinkraus, 1996; Tamang *et al.*, 1988; Venkatasubbaiah, 1985), there is dearth of information on other lesser-known, similar legume-based fermented foods indigenous to India. Due to changes in the life style of people, their rapid migration to cities, decreased time at home, lack of awareness of fermentation techniques and numerous other factors, and most importantly the ongoing modernization processes, there is a gradual inclination of the newer generation towards the modern fast foods, under-estimating their own traditional foods. Consequently, these factors have diminished greatly the tradition of fermenting foods at home. Since the production of indigenous fermented foods has remained a traditional village art practiced in homes following crude and technically ill manner, it is obvious that many of them are being replaced by industrially processed and convenience foods. But the unfortunate outcome of this replacement is the inevitable loss of traditional know-how and much valued resources before it is fully understood and harnessed for the future generations. In addition, the food industry's success with truly massive production, distribution, storage and merchandising of processed foods and designer foods has helped to relegate many earlier commonly consumed fermented foods to the dusty region of dim memory.

Detailed information on the types, traditional methods of preparation, modes of consumption, shelf-lives and ethnic values of a variety of legume-based fermented foods used traditionally by the local people was obtained by conducting a moderate survey through a well-structured pre-tested proforma and by personal interview method. Most of the fermented products are delicious and easily digestible. While some are consumed by all the communities irrespective of caste and creed, others are confined to the particular ethnic groups. The products were also suitable for ailing persons, pre- or post-natal women and children.

5.1. Dhokla

Dhokla is one of the fermented foods with appealing sour taste, colour, flavour and spongy texture. The recipes for dhokla preparation varied in details from home to home, which resulted in the inconsistent quality of product (Desai and Salunkhe, 1986; Mahajan and Chattoapdhayay, 2000; Ramakrishnan, 1979). In the present study, dhokla with organoleptically acceptable and desirable quality was obtained when the mixed batter of Bengalgram dal and white polished rice in the proportion of 4:1 was fermented at 32°C for 15 h under semicontrolled conditions. The product had a characteristic sour aroma and was soft, spongy and light yellow colour with a honeycomb structure inside. Desai and Salunkhe (1986) were of the opinion that the prime ingredients used in the preparation of dhokla are white polished rice and Bengalgram dal which can be substituted by suji (coarsely ground meal of wheat, maize or kodri) and soybean, split pea, redgram or moth beans, respectively. In the State of Maharashtra, dhokla is being prepared by fermenting the mixture of Bengalgram flour and curd for 16-18 h and steaming for 20 min (Joshi *et al.*, 1989). Although Mahajan and Chattoapdhayay (2000) reported that the batter mixture containing rice, Bengalgram dal and blackgram dal (1:6:3) was found to be the best in respect of minimum bulk density and hardness as well as acceptable taste of the products, the 4:1 ratio of the Bengalgram dal and rice employed in the present study imparted an optimum stickiness to the fermenting batter which retained adequate gas during fermentation resulting in about 2-fold increase in the batter volume. Most of the organoleptic scores and desirable biochemical changes during dhokla batter fermentation may be ascribed to the nature of substrates, fermentation environment and composition and activity of functional as well as accompanying microbiota.

Due to prolonged soaking and addition of water during grinding, acidic dhokla batter had a higher moisture content than the substrates (Table 7). A significant increase in total acidity as indicated by fall in pH probably helps in to prevent the growth and transmission of various foodborne pathogenic organisms. The total protein content of fermented batter was that of Bengalgram dal. The fermented batter was superior to raw substrates with regard to soluble nitrogen content. The highest carbohydrate content of fermented batter was less than that of rice, indicating the balanced dietary status of the product.

Dhokla batter ripening is essentially an autofermentation process, depending entirely on the environmental inoculum. The microbial study of the substrates as well as the fermented batter showed the involvement of both lactic acid bacteria and yeasts (Table 12). The lactic acid bacteria included *Leuconostoc mesenteroides*, *Pediococcus pentosaceus* and *Lactobacillus fermentum* (Table 10), and the yeasts included *Saccharomyces cerevisiae*, *Pichia membranifaciens* and *Pichia silvicola* (Table 11). *L. mesenteroides*, *P. pentosaceus* and *S. cerevisiae* were recovered from both the two types of substrates and the fermented batters. This suggests that substrates, Bengalgram dal in particular, might act as the primary source of functional microbiota responsible for the fermentation. *P. membranifaciens*, which occurred in one of the positive sample of marketed batter only, could be considered as a mere opportunistic organism. A number of interesting changes occurred during the fermentation under semicontrolled conditions. The occurrence of *L. mesenteroides*, *P. pentosaceus*, *L. fermentum*, *S. cerevisiae* and *P. silvicola* at the onset of fermentation was due to their presence in the substrates and subsequent passage through soaking. Joshi *et al.* (1989) reported that besides *L. mesenteroides* and *L. fermentum* the other dominant lactic acid bacteria involved in dhokla batter fermentation was *Lactobacillus lactis* and *Lactobacillus delbrueckii*, both of which eventually became extinct after about 12 h of fermentation. Although recovered from one of the substrates (Bengalgram dal) only, *L. fermentum* and *P. silvicola* occurred throughout the fermentation as a dominant candidate (Table 14).

Yeast growth can be essential for the development of typical texture and aroma profiles of certain fermented products, the outcome of their strong proteolytic and lipolytic activity (Jakobsen and Narvhus, 1996; Spinnler *et al.*, 2001). To have any effect on fermented products, yeasts need to reach high cell densities and must compete and interact with other microorganisms present, especially with the dominant lactic acid bacteria (Beresford *et al.*, 2001; Wouters *et al.*, 2002). While Joshi *et al.* (1989) reported participation of *P. silvicola* only; the present study shows that *S. cerevisiae* along with *P. silvicola* appeared as the dominant yeasts throughout the fermentation. Such anomaly in the composition of microbiota, particularly yeasts, involved in the fermentation of a particular food may depend on the quality of raw substrates, locality of production and deviations in the production method including the use of different types and varieties of substrates (Owuama, 1999). Moreover, it has been reported that *S. cerevisiae* provides essential metabolites such as pyruvate, amino acids and vitamins to the other microorganisms, including lactic acid bacteria, and stimulates their growth and easy proliferation. They, in turn, utilize certain bacterial metabolites as carbon sources (Gadaga *et al.*, 2001). However, in cocultures, yeast strains might use as an alternative source of energy the galactose moiety of lactose secreted by some lactic acid bacteria in the presence of an excess of lactose (Marshall and Tamine, 1997).

While the interactions between yeasts and lactic acid bacteria species have been reported (Alvarez-Martin *et al.*, 2008), the functional role of *L. mesenteroides* and *L. fermentum* along with *P. silvicola* in dhokla batter fermentation under controlled conditions was strongly advocated by Kanekar and Joshi (1993). Positive interactions include the stimulation of lactic acid bacteria through the production of CO_2, pyruvate, propionate and succinate (Leroi and Pidoux, 1993). Lactic acid bacteria

have nutritional requirement for many compounds, and can also be stimulated by the synthesis of vitamins or by the production of amino acids by yeasts (Roostita and Fleet, 1996). In addition, some lactic acid bacteria release galactose, which may favour the growth of lactose-negative yeasts (Marshall and Tamine, 1997). The combination of low pH produced by the bacterial starter plus the alcohol and CO_2 produced by the yeasts is inhibitory to many undesirable microorganisms (Ferreira and Viljoen, 2003).

The remarkable physicochemical changes occurred during the fermentation was acidification and leavening of the batter (Table 15). These have been used as criteria for judging the progress of fermentation. The changes in pH and total titratable acidity due to fermentation are well agreed with those of Joshi *et al.* (1989). Lactic acid is undoubtedly the main end product of sugar metabolism by lactic acid bacteria as growth of lactic acid bacteria and lactic acid production are coupled processes. They also produce small quantities of other organic acids such as acetic, butyric and propionic acids (Dellaglio *et al.*, 1994). Orotic acid is not only an intermediary in the synthesis of nucleotides, but also acts as a growth promoter for some lactic acid bacteria (Østile *et al.*, 2003). Succinic acid is consumed by lactic acid bacteria since in this bacterial group carbohydrates are metabolized following the citric acid route (Gadaga *et al.*, 2001). It is also well known that *Leuconostoc* species use citrate from which they produce acetoin and diacetyl (Schmitt *et al.*, 1997), but other lactic acid bacteria and yeasts also produce these compounds (Bartowsky and Henschke, 2004).

The batter increased 1.8 times its original volume. The increase was significant ($P < 0.05$) after every 3 h-interval till 12 h. The evolution of CO_2 by the heterofermentative members of lactic acid bacteria could be attributed for the significant rise in batter volume. Free fatty acidity content of the batter registered a 3.5-fold increase by the end of 15 h-fermentation. According to Joshi *et al.* (1989) acetoin and volatile fatty acids are the major compounds imparting characteristic flavour to dhokla at their optimum concentration. They further reported that the recovery of total titratable acidity, diacetyl, acetoin and total volatile fatty acids in unfermented batter could be due to their presence in curd, one of the ingredients. However, subsequent rise in these compounds during fermentation may be due to the corresponding rise in microbial population particularly of *L. fermentum*, *L. mesenteroides* and *P. silvicola*, and their increased metabolic activity. Kanekar and Joshi (1993) established that the development of characteristic flavour and desired sour taste of dhokla can be attributed to *L. fermentum* and *L. mesenteroides*, while the yeast contributes to the production of soft spongy and fluffy texture of the steamed product. The contents of total protein, total nitrogen and protein nitrogen increased marginally, however soluble protein content showed a 3-fold increase after fermentation.

As could be seen from the protein fingerprinting, most of the resolved major protein subunits of the mixed batter revealed the combination of both the substrates (Fig. 39). A significant proteolysis of the rice oligopeptides corresponding to molecular weight of 29 kDa and those ranging from 32 to 35 and 85 to 88 kDa was observed during soaking. Three major bands (with molecular weight ranging from 35 to 40 kDa) in the mixed-batter, which seem to be derived exclusively from the Bengalgram dal, resolved significantly as fermentation progressed. While protein subunits of the mixed batter corresponding to the molecular weight of approximately 43 and 75 kDa appeared after the first 3 h of fermentation and persisted till the end, the resolution of all the protein subunits of the mixed batter beyond 6 h having molecular weight of d"27 kDa, was significantly prominent than the corresponding bands of the substrates. The influence of fermentation on the electrophoretic pattern of protein subunits during fermentation of mixed batter was not significant. However, a different scenario was figured by Hatzikamari *et al.* (2007) during the submerged fermentation of chickpea for 8-10 h. They found that both high molecular weight and low molecular weight protein subunits were broken down into

smaller peptides during fermentation. They attributed the proteolytic activities of *Bacillus* spp. and clostridia for the change in protein fingerprint during the fermentation of chickpea.

The antioxidant activities of different polar and nonpolar solvent extracts from plant-derived foods were reported (Esaki *et al.*, 1996; Gûlçin *et al.*, 2003; Mokbel and Hashinaga, 2006; Negi *et al.*, 2005; Ordonez *et al.*, 2006). However, differences in the polarity of the extracting solvents could result in a wide variation in the polyphenolic contents of the extract. The methanolic extract was reported to possess higher antioxidant activity than that of other extracts in various model systems (Chyau *et al.*, 2002). So, in the present investigation, the antioxidant activities of the free and soluble esterified polyphenolics from the methanolic extracts, but not of the ones bound in cell walls, were evaluated. The lyophilized crude extract of fermented batter gave the highest yield in methanol, than that of nonfermented batter and steam-cooked dhokla (Table 16). The enhancement in the yield of the methanolic extracts after fermentation, observed in the present study, is consistent with the findings of Lin *et al.* (2006) in mould-fermented soybean-koji. However, the enhanced effect on the yield of crude extracts in soybean-koji varied with the starter organisms (Lin *et al.*, 2006).

In complex systems, such as foods, various mechanisms may contribute to oxidative processes, such as in Fenton reactions, where transition metal ions play a vital role. During such oxidative processes different reactive oxygen species might be generated and various target structures such as lipids, proteins and carbohydrates, can be affected. Therefore, it is important to characterize the crude extracts by a variety of antioxidant assays (Halliwell, 1997). In the Fe^{3+}-reduction assay, the general ability of the extracts to donate electrons is tested, whereas in the DPPH·-scavenging assay hydrogen atoms are involved as well. One of the important mechanisms of antioxidant action may be the chelating ability of Fe^{3+} which serves as a catalyst in Fenton reactions. Linoleic acid, a polyunsaturated fatty acid, acts as a model lipid for the assay of lipid peroxidation inhibitory activity.

The Folin-Ciocalteu phenol reagent, used to obtain a crude estimate of the amount of phenolic compounds present in the extract, undergoes a complex reaction with phosphotungstic acid and phosphomolybdic acid present in the reagent. However, the assay has been shown not specific to just polyphenols but to any other substances that could be oxidized by the Folin-Ciocalteu reagent. The phenolic compounds, depending on the number of phenolic groups they have, respond differently to this reagent, and various workers reported the poor specificity on this assay (Escarpa and Gonzalez, 2001; Singleton *et al.*, 1999). In spite of all these, the total phenol content assay is a convenient was to quantify it.

Phenolics are secondary metabolites, and in part, are produced as a result of the plant's interaction with the environment (Snyder and Nicholson, 1990). Phenolic compounds have been demonstrated to exhibit scavenging effect for free radicals and metal-chelating ability (McCue and Shetty, 2003; Shahidi *et al.*, 1992). The mean total phenol content of the crude extracts of batter significantly increased by 2.5-fold after fermentation (Table 16). The enhancement in the total phenol content batter after fermentation is consistent with the findings reported by other investigators (Lin *et al.*, 2006; Randhir *et al.*, 2004; Vattem and Shetty, 2002). However, the total phenolic content of fermented batter declined significantly after steam-cooking for 10-15 min, eventually leaving only 11% of total phenol content higher than that of nonfermented batter.

The reducing property of the compound indicates that these are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, and therefore, can act as both primary as well as secondary antioxidants. The crude lyophilized extract of fermented batter shows 187% higher reducing activity than the unfermented one. However, steam-cooking for 10-15 min resulted in

about two-fold decrease in the reducing activity of the batter (Table 16). The effect of fermentation on the enhancement of reducing power of fermented bean and bean products have been previously reported by several workers (Bergofer *et al.*, 1998; Chung *et al.*, 2002; Lin *et al.*, 2006; Wang *et al.*, 2004; Yang *et al.*, 2000). The increased reducing power observed may be due to the formation of reductants that could react with free radicals to stabilize and terminate radical chain reactions during fermentation. In addition, the intracellular antioxidant peptides of microbiota involved and their hydrogen-donating ability, may also contribute to this increased reducing ability (Yang *et al.*, 2000).

DPPH is a stable nitrogen-centered, lipophilic free radical that is widely used in evaluating the antioxidant activities in a relatively short time period. At different stages of dhokla preparation, the crude extracts at the same dose level, incubated for 50 min, exhibited various degrees of scavenging potential for $DPPH^{\cdot}$ (Table 17). Interestingly, Lin *et al.* (2006) reported that the *Aspergillus*-soy koji exhibited a 9-fold higher scavenging effect compared to unfermented soybean at 20 mg ml^{-1}. The scavenging activity of soy-koji varied from 55 to 100% depending on the starter organism (Lin *et al.*, 2006), while in furu, 30-50% scavenging was observed (Ren *et al.*, 2006). In rice-koji, however, a higher scavenging activity (25-79%) was observed at 200-800 μg ml^{-1} (Yen *et al.*, 2003). Though the antiradical activity of all the tested concentrations (10-50 mg ml^{-1}) of the extracts was the function of the tested concentrations (10-50 mg ml^{-1}), they increased significantly up to a certain extent (20 min) and then gradually leveled off despite further extension of incubation period (Table 17). With respect to the scavenging activity of $DPPH^{\cdot}$, IC_{50} and relative scavenging activity fermented batter were superior to those of its nonfermented counterpart and the steamed product.

Transition metal ions have a great importance in the generation of oxygen-free radicals in both living organisms and food systems. Iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. Fe^{2+} is the most powerful pro-oxidant among various species of metal ions encountered in the food system (Halliwell and Gutteridge, 1984; Lin *et al.*, 2006), while Fe^{3+} is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on the conditions, particularly pH (Strlic *et al.*, 2002), and oxidized back through Fenton type reactions, with the production of hydroxyl radicals or Haber-Weiss reactions with superoxide anions (Kehrer, 2000; Wong and Kitts, 2001). A metal chelating agent may inactivate metal ions and potentially inhibits the metal-dependent processes (Finefrock *et al.*, 2003). They possess dramatic effect in blocking the formation of chain initiators by preventing metal-assisted homolysis of hydroperoxides. Free iron is known to have low solubility, and a chelated iron (i.e., iron-ligand) complex, such as EDTA-Fe, has greater solubility in solution, which can be contributed solely from the ligand. Furthermore, chelated iron, such as EDTA-Fe, is also known to be active, since it can participate in iron-catalyzed reactions (Wong and Kitts, 2001).

Ferrozine can quantitatively form complexes with Fe^{2+}, which fades in the presence of chelating agents. By measuring the colour reduction, therefore, it is possible to estimate the chelating activity of the co-existing chelator (Yamaguchi *et al.*, 2000). In this assay, the natural antioxidants present in the extract interfered with the formation of the Fe^{2+}-ferrozine complex, suggesting that it has chelating ability. Hence, the chelating activities of Fe^{2+} by crude extracts of dhokla were estimated by the ferrozine assay.

The Fe^{2+}-chelating activity of all the extracts, regardless of their source, significantly increased at the lower concentrations. In fact, they disrupted the Fe^{2+}-ferrozine complex at the dose levels of 10-30 mg ml^{-1} methanol (Table 18). At 10 mg ml^{-1}, the extract of dhokla exhibited 51% of metal-chelating activity which was about 49% higher than that of fermented batter. However, beyond this dose level

the Fe^{2+}-chelating activity of the extracts of fermented batter was superior to that of unfermented batter and steamed product. The chelating ability of the extracts significantly increased up to a certain extent with the increase in dosage level, and then leveled off despite further increase in concentration. The chelating ability exhibited by the extracts revealed that dhokla had better IC_{50} (9.8 mg ml^{-1}) than those of batters, while higher relative Fe^{2+}-chelating ability was observed in the extracts of fermented batter. The overall increase in relative Fe^{2+}-chelating ability of fermented batter, therefore, revealed that the fermentation enhanced the Fe^{2+}-chelating ability significantly. Steam-cooking of the fermented batter showed a marginal decline in the relative Fe^{2+}-chelating activity. Metal chelating capacity was significant, since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion.

One of the major concerns in food technology is the auto-oxidation of lipids which occurs autocatalytically through free-radical intermediates and is generally initiated by trace metals and peroxides present as ubiquitous impurities in food systems. Lipid peroxidation results in the rapid development of rancid and stale flavour and is considered as a primary mechanism of quality deterioration in food lipids and oils (Guntensperger *et al.*, 1998).

In vitro assay of LPIA of the extracts on the peroxidation of linoleic acid emulsion system was performed. The extracts showed significant LPIA which increased significantly with increase in concentration (Table 19), however negatively correlated with the incubation time. The ability of the extract to retard lipid peroxidation could be attributable to the ability of its phenolic constituents to quench reactive oxygen species. The fact that fermentation enhanced the total antioxidant activity of the dhokla batter could be supported by the findings of Tseng *et al.* (2006). According to their observations, the methanolic extract of *Monascus*-fermented dehulled adlay showed higher antioxidant activity than the nonfermented counterpart. With the increase in concentrations (10-20 mg ml^{-1}), the antioxidant activities of the extracts of adlay increased to about 94%; indicating that the fermented adlay was superior to unfermented one (Tseng *et al.*, 2006). A different scenario was pictured by Esaki *et al.* (1997), while evaluating antioxidant activities of natto. The antioxidant activity of methanolic extract of natto, made by using a natto stain (*B. subtilis*) was almost equal to that of nonfermented soybean. However, they found that the methanolic extracts of *Aspergillus*-fermented soybean had a better antioxidant activity than the nonfermented soybean, and tempe was more effective than miso. The relationship between rates of lipid oxidation and water is complex. The amount of water, the water activity and the state of water in a food system along with other factors are vital (Frankel, 1998; Nelson and Labuza, 1992). Since nonlipid components such as proteins, sugars and minerals present in foods may also have a strong influence on the rate and mechanism of lipid oxidation mostly in the presence of water (Pokorny *et al.*, 1985), antioxidant activity of the crude extracts should be evaluated in a variety of model systems using several different indices because the effectiveness of such antioxidant materials is largely dependent upon the chemical and physical properties of the system of which they are added and a single analytical protocol adopted to monitor lipid oxidation may not be sufficient to make a valid judgment.

The change in the activities of the natural antioxidants during the processing of commodity is of utmost concern. The factors that influence the activity of natural antioxidants include temperature, pH, fermentation, presence of metal, as well as numerous microcomponents acting as prooxidants or synergists (Kamal-Eldin and Appelqvist, 1996). Moreover, the usefulness of natural antioxidant will be dependent on the fractions of components used in the foods or whether a crude extract can provide sufficient antioxidant activity (Hall, 2001).

As suggested by Yen and Dhu (1993), the pH of the system also dictates antioxidant activity. In the present study, the pH of the mixed batter of dhokla declined from 6.3 to 4.7 after the fermentation. The fermented batter exhibited superior antioxidant activities than the nonfermented one. The result was in consistent with the findings of Yen and Dhu (1993) who reported that the effectiveness of the methanolic extracts of peanut hull decreased as the pH increased from 3.0 to 9.0. At pH 7.0 the extract retained about 80% of the antioxidant activity which was completely lost when the pH was increased to 9.0.

Steam-cooking of fermented dhokla batter for 15-20 min dramatically changed the antioxidant activities of the crude extracts. The variation in temperature may change the mechanism of action as well as the order of effectiveness of the antioxidants (Marinova and Yanishlieva, 1992). In the earlier findings the effect of temperature on the antioxidative activity of different foods has been reported. A 5% decrease in the oxidative inhibition was found when methanolic extracts of peanut hull was heated at 185°C for 20 min prior to the addition to linoleic acid (Yen and Dhu, 1993). Similarly, Berghofer *et al.* (1998) reported that the steaming of the faba bean flour caused a reduction in the antioxidant activity. Because of its accelerating effect on lipid oxidation, the increase in temperature is undesirable as alternation in the structure of the phenolics, due to the application of heat, may be responsible for the loss of the hydrogen-donating activity. Frankel (1993) summarized several problems with using elevated temperatures to predict antioxidant activity. Although an increase in temperature accelerates oxidation by a large factor, the temperature may affect the mechanism of auto-oxidation, the stability and volatility of the antioxidant and oxidation products, and the partition of the antioxidant between different phases present in the food. At high temperatures where reaction rates are fast, transport of oxygen may become rate-limiting. Ragnarsson and Labuza (1977) claimed that antioxidants were normally less effective at elevated temperatures than that at ambient temperature. Lölinger (1991), therefore, suggested that the optimum oxidative stability can be achieved by minimizing exposure of lipids and lipid-containing food products to air, light and higher temperature during processing and storage.

Furthermore, amino acids and polypeptides are typical metal-chelating agents (Pokorny, 1987; Fujimoto *et al.*, 1984). The higher level of antioxidant activity in fermented batter compared to nonfermented batter could be attributed to the hydrolysis of protein during soaking and fermentation. The antioxidant activity of histidine-containing peptides is thought to be related to their metal chelating ability as well as to lipid radical trapping potential of the imidazole ring (Uchida and Kawakishi, 1992; Murase *et al.*, 1993).

Analysis of correlation coefficients between every two antioxidative parameters tested for the extracts of fermented and nonfermented batters and the steamed product (dhokla) reflects significant correlation (Table 20). In nonfermented batter the highest positive correlation was observed between metal-chelating activity and reducing power; while in fermented batter a strong correlation was found between reducing power and LPIA. Metal-chelating is an example of a secondary antioxidant mechanism by which many natural antioxidants can influence the oxidation process. Metal chelators can stabilize the oxide forms of metals, i.e. reduce redox potentials, thus preventing metals from promoting oxidation. In addition, they also form complexes with the metals making them unavailable to promote oxidation (Hall, 2001). The positive correlation between the reducing power and metal-chelating activity further indicates that the extracts of nonfermented batter capable of reducing ferric ions were also able to chelate Fe^{2+}. However, no clear relationship has been reported between Fe^{3+}-reducing ability with metal-chelating activities (Wong *et al.*, 2006). Similarly, dhokla registered the highest correlation between total phenol content and LPIA. The satisfactory correlation between every

two parameters in extracts of batters and dhokla in the present study was in consistent with the findings of Arnos *et al.* (2002), Mathew and Abraham (2006) and Dhu and Yen (1999).

Using regression analysis it is possible to ascertain the relation itself beyond developing a measure of relatedness of two variables with the assumption of unilateral causality (Kapsalis *et al.*, 1973). The regression equations and coefficients of correlations between different antioxidant parameters and total phenol content of the methanolic extracts of dhokla batters and the steamed product revealed that all the tested samples exhibited that the total phenol contents were positively correlated ($P < 0.001$) with DPPH$^{\cdot}$-scavenging, reducing power, metal-chelating and lipid peroxidation inhibitory activities (Table 21). The phenolic compounds and other chemical components present in the extract may inhibit lipid peroxidation through different chemical mechanisms, including free radical-quenching, electron transfer, radical addition or recombination (Mathew and Abraham, 2006). Relevant regression equations reveal that they possess curvilinear relationships which demonstrate that although correlation exists between total phenol contents and other antioxidant parameters, their relationships are not always linear. Kapsalis *et al.* (1973) studied the linearity curve fitting of higher order and transformation of data obtain linearity of relationship with possible predictions and found that correlations between the two variables may be the result of third variable which may be unspecified. Relevant regression equations exhibited that the total phenol content accounted for significantly higher percentage of DPPH$^{\cdot}$-scavenging activity in the extracts. Since antiradical activity depends on the structural conformation of phenolic compounds (Bors *et al.*, 1990), it was greatly influenced by the phenolic contents which accounted for 96%, 90% and 89% DPPH$^{\cdot}$-scavenging activities in nonfermented and fermented batters and dhokla, respectively. A similar correlation between total phenolic contents and antiradical activities was also reported by other workers (Miliauskas *et al.*, 2004; Ordonez *et al.*, 2006). The present study also revealed that polyphenols in the extracts may play a significant role of electron and hydrogen donors. The observation was in consistent with the findings in grams, herbs and spices (Choi *et al.*, 2007; Hinneburg *et al.*, 2006).

The metal-chelating ability of natural phenolics is also an important factor regarding their antioxidant activity (Afanasev *et al.*, 1989; Chen and Ahn, 1998; Nardini *et al.*, 1995). Total phenol content in nonfermented batter accounted highest Fe^{2+}-chelating activity closely followed by fermented batter. However, lesser correlation between total phenol content and Fe^{2+}-chelating activity of steamed product could suggest that the phenolic compounds alone might not be the main chelators of Fe^{2+}. Moreover, in a complex system, especially in food, organic acids, amino acids and sugars can be the main sequesters of transition metal ions. During dhokla batter fermentation, probably the organic acid contents of the batter increased (as revealed by significant fall in pH) along with release of amino acids and free sugars. The accumulation of the organic acids and amino peptides could act as the primary chelators of the Fe^{2+} in the fermented batter. Though phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals and metal-chelating activity (McCue and Shetty, 2003; Shahidi *et al.*, 1992), their ability to chelate metal ions depends on the availability of properly oriented functional groups (Van Acker *et al.*, 1996). A sample high in polyphenols might not chelate metal if the polyphenols present did not have suitable groups that could chelate the cations. Bidentate ligands are more powerful scavengers of metal cations than monodentate ligands, for example, catechol binds ferric ions tightly whereas phenol does not (Hider *et al.*, 2001). When a phenolic group is conjugated with a carbohydrate group, as in naturally occurring phenolic glycosides, it can no longer bind metals (Hider *et al.*, 2001).

The ability of the extract to retard lipid peroxidation is attributable to the ability of its phenolic constituents to quench reactive oxygen species. There are, however, reports of phytophenolics exhibiting

antioxidant/prooxidant activities, which depend on several factors such as metal reducing potential, chelating behaviour, pH, solubility characteristics etc. (Decker, 1997). Total phenol content also accounted 83%, 88% and 96% of LPIA in extracts of fermented and nonfermented batters and steamed products, respectively. Positive correlation were found between total phenol contents and antioxidant activities of red wines (Vinson and Hontz, 1995), vegetables (Kaur and Kapoor, 2002), and grape, pomace must, wine and juice (Yildirim *et al.*, 2005). Yen *et al.* (1993) reported that the antioxidant activity of the methonolic extract from peanut hull correlated with its content of total phenols. The high content of total phenol in the extracts might explain high antioxidant properties in the extracts. On the whole, total content of extracts exhibited a significantly high positive correlation ($P<0.01$) with all the antioxidant parameters tested. The good correlation between the results from total phenol analysis and the antioxidative assays has been previously reported (Zheng and Wang, 2001).

The importance of the antioxidants present in foods is well appreciated for both preserving the foods themselves and supplying essential antioxidants *in vivo*. With increasing experimental, clinical and epidemiological data which show the beneficial effects of antioxidants against oxidative stress-induced degenerative and age related diseases, cancer and ageing, the importance and role of antioxidants have received renewed attention (Shi, 2001). The present study on the antioxidative assay of dhokla samples during different stages of its preparations reveals that all the tested samples exhibited fair DPPH·-scavenging activity, metal-chelating ability, reducing power and LPIA in linoleic acid emulsion system. The antioxidant activities of fermented batter of dhokla was significantly higher ($P<0.05$) than those of nonfermented batter suggests the role of fermentation in enhancing these attributes. Statistically, all the antioxidative parameters used were positively correlated ($P<0.01$) with their respective total phenol contents. However, the possible mechanism and the essential biofactors contributing to the enhancement of antioxidative activity remained to be further investigated.

5.2. Dosa

Dosa is mostly preferred by restaurant hunters to other snacks relished as a breakfast food along with chutney and sambar, vegetables, tamarind juice, salt and herbs (Aidoo *et al.*, 2006; Nout and Sarkar, 1999; Soni and Sandhu, 1999; Soni *et al.*, 1985, 1986).

While thin consistency of the dosa batter (slurry) was due to high level of moisture (Table 22), the higher count of lactic acid bacteria (Table 27) could be attributed for the acidic nature (pH 4.5) of fermented batter. The overall protein and carbohydrate contents and energy value of fermented batter indicate dosa as a nutritionally rich fermented product. Hence, consumption of cereal-legume mixture is advantageous from the nutritional point of view, as these results in an improved balance of carbohydrates and proteins, particularly dietary essential amino acids (Aidoo *et al.*, 2006; Nout and Sarkar, 1999).

The microbial analysis of marketed batter of dosa showed the prevalence of both lactic acid bacteria and yeasts along with TAMB and their spores (aMBS) (Table 27). Lactic acid bacteria isolated from all the positive samples were identified as *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* (Table 25). While *L. mesenterioids* with $>10^9$ cfu g^{-1} load was the dominant lactic acid bacterium, *P. pentosaceus* with an average load of $>10^7$ cfu g^{-1} was recovered from only 50% of the samples. They possibly contribute the enhancement of the product flavour. The presence of *L. mesenterioids* along with *Enterococcus faecalis, Lactobacillus fermentum, Lactobacillus delbrueckii, Pediococcus cerevisiae, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus polymyxa* and *Enterobacter* sp. in fermented batter of dosa has been reported by Soni *et al.* (1985, 1986).

Likewise, three distinct groups of yeasts isolated from the fermented batter were identified as *Saccharomyces cerevisiae, Issatchenkia orientalis* and *Rhodotorula minuta* (Table 26). The occurrence of 67% each of *S. cerevisiae* and *I. orientalis* in fermented batter indicates that these two are the dominant yeast flora involved. In earlier reports, Soni *et al.* (1985, 1986) advocated *S. cerevisiae* as the most predominant yeast involved in the fermentation, followed by other members such as *Debaryomyces hansenii, Pichia anomala, Trichosporon beigelii, Oosporidium margaritiferum, Trichosporon pullulans, Kluyveromyces marxianus, Candida kefyr* and *Candida krusei*. However, the prevalence of *I. orientalis* and *R. minuta* in fermented batter of dosa was hitherto unreported. They further attributed the raw substrates of dosa as the primary source of inoculum of the functional microbiota responsible for the fermentation. However, *R. minuta* recovered from only 8% of the sample could be assumed as opportunistic.

5.3. Idli

Idli comprises the interesting group of fermented cereal-legume based steam-cooked product which is basically indigenous to the southern part of India but widely consumed throughout the country and many places of Sri Lanka. It imparts an appealing sour flavour, spongy texture and nutritionally rich with easy digestibility and relished mainly as a breakfast snack along with chutney and sambar (Nout and Sarkar, 1999).

The prolonged soaking and addition of water during grinding of substrates caused higher moisture content in batter which remained relatively constant throughout the fermentation (Tables 28 and 41). The fermented batter was acidic (pH, 4.5) in contrast to that of relatively neutral substrates (pH, 6.8-7.0). This supports the involvement of acid-producing microbiota, predominantly lactic acid bacteria and yeasts, during fermentation. The increase in titratable acidity after fermentation was in contrast to pH which probably helped to inhibit the proliferation and cross contamination of various foodborne pathogens. Total protein content was in descending order of blackgram dal - fermented batter - white polished rice. Blackgram dal had a significantly higher content of protein; although incorporation of rice lowered the protein level of the dal slurry, the mixed batter produced a balanced dietary status of carbohydrate and protein.

The microbial study of the substrates as well as fermented idli batter showed the involvement of both lactic acid bacteria and yeasts (Table 35). While the two morphologically distinct groups of lactic acid bacteria isolated from all the positive samples were assigned as *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* (Table 32), the four morphologically distinct groups of yeasts were identified as *Saccharomyces cerevisiae, Issatchenkia orientalis, Pichia membranifaciens* and *Rhodotorula minuta* (Table 33). While *S. cerevisiae* was the only yeast isolated from the raw blackgram dal, parboiled rice was devoid of any yeast flora. *Mucor racemosus*, which was recovered from 100% of the samples of nonfermented and 3 h-fermented batters, disappeared thereafter.

The traditional method of idli preparation varies in details from home to home resulting in inconsistent quality of the product. In order to prepare a reproducible quality product with acceptable texture and aroma attractive to large groups, and to scale up, the process parameters employed in the traditional fermentation conditions were optimized by sensory evaluation (Tables 37-39). Most of the organoleptic scores and desirable biochemical changes occurring during idli fermentation may be ascribed to the nature of substrates, fermentation environment, and composition and activity of functional as well as accompanying microbiota. Although blackgram dal is a rich fermenting substrate, leavening and souring of the mixed batter are more pronounced when the two major ingredients are fermented together rather than the sum total of their individual contribution. Parboiled rice, either

singly or in combination with blackgram dal, plays a significant secondary role supporting the growth of lactic acid bacteria and yeasts (Radhakrishnamurthy *et al.*, 1961). Though the proportion of rice and dal ranges from 1:4 to 4:1, the appropriate ratio of the ingredients is a matter of concern in idli preparation (Reddy *et al.*, 1986; Steinkraus *et al.*, 1967).

Idli with organoleptically acceptable and desirable quality was obtained when a mixed batter of parboiled rice and blackgram dal in the proportion of 2:1 was fermented at 30°C for 18 h. That product had a characteristic sour aroma and was white and spongy with a honeycomb structure inside, scoring 'good' grade. The batter did not develop the characteristic flavour until a period of 12 h had elapsed, before which the aromas of raw rice and raw dal were apparent. The 2:1 ratio of the ingredients imparted an optimum stickiness to the fermenting batter which caused adequate leavening. When the proportion was changed to 4:1, the products were hard and unacceptable. Idli prepared from 1:1 proportion tended to be sticky even though the batter registered a maximum increase in volume. Radhakrishnamurthy *et al.* (1961) also preferred the 2:1 ratio to others. However, in a study of Desikachar *et al.* (1960) idli made from batter fermented at 31°C for 16 h was found organoleptically better than that prepared at 41°C for 10 h. Steinkraus (1996) indicated a probable temperature of 30-32°C as optimum for fermentation of idli batter.

Idli batter ripening is essentially an autofermentative process, depending entirely on the environmental inoculum. A number of interesting changes occurred during the fermentation. The high initial load of *L. mesenteroides* and *S. cerevisiae*, even at the onset of fermentation, was due to their presence on raw blackgram dal and passage through soaking which did not change ($P < 0.05$) their load (Table 40). Although Venkatasubbaiah *et al.* (1985) found yeasts in parboiled rice, they were not recovered from any of the raw parboiled rice samples studied. Among the dominant microbiota isolated, *L. mesenteroides* was the only organism found in 100% of raw blackgram dal and fermented batter. More interestingly, this is the only bacterium which occurred in 100% samples of fermenting batter analysed at every 3 h-interval (Table 40). This suggests that *L. mesenteroides*, supplied by the ingredient, is an essential functional component of microbiota responsible for the fermentation of idli batter, followed by the only other bacterium *Pediococcus pentosaceus*, which first appeared after 3 h, and remained predominant till the end. The exclusive role of *L. mesenteroides* in idli batter fermentation was strongly advocated by Mukherjee *et al.* (1965). Ramakrishnan (1979) isolated *L. mesenteroides* along with *Lactobacillus fermentum*, *Lactobacillus delbrueckii* and *Enterococcus faecalis* from fermented batter of idli. All these organisms, excepting *L. fermentum* which occurred in rice only, were found to occur in both the major ingredients. Blackgram dal harbours *L. mesenteroides* and other lactic acid bacteria which play a major role in its fermentation (Mukherjee *et al.*, 1965; Ramakrishnan, 1979; Soni and Sandhu, 1990a). Steinkraus *et al.* (1967) were of opinion that the fermentation of idli batter was entirely due to heterofermentative *L. mesenteroides*.

However, the functional microbiota in idli batter fermentation has been a controversial point. Although Mukherjee *et al.* (1965) were unable to isolate yeasts from any stage of the fermentation, Lewis and Johar (1953) reported, for the first time, the role of *Galactomyces geotrichum* and *Candida holmii* in the production of gas during idli batter fermentation. Subsequently, Batra and Millner (1974) reported participation of *Candida saitoana*, *Candida holmii* and *Guehomyces pullulans*. Venkatasubbaiah *et al.* (1985) reported the isolation of *G. pullulans*, *Pichia anomala*, *C. holmii*, *C. saitoana*, *Candida glabrata*, *Candida tropicalis* and *Candida sake* as the dominant yeasts from fermenting batter. Soni and Sandhu (1991) reported that during fermentation, along with *L. mesenteroides*, several yeasts, namely *S. cerevisiae*, *Debaryomyces hansenii*, *P. anomala* and *G. pullulans* were predominant appearing first, and *Trichosporon cutaneum* developed subsequently; however, only *S. cerevisiae* persisted till the end. In the present

study the dominant yeast flora, next to *S. cerevisiae*, included *I. orientalis* and *P. membranifecians*, both hitherto unreported from idli batter (Table 35) Such a variation in the composition of yeast flora is not unusual, because composition of yeast population involved in the fermentation of a particular food may vary depending on locality and deviations in the production method including the use of different types and varieties of ingredients (Owuama, 1999). *S. cerevisiae* stimulates the growth of other microorganisms, including lactic acid bacteria, by providing essential metabolites such as pyruvate, amino acids and vitamins; the yeast utilizes certain bacterial metabolites as carbon sources (Gadaga *et al.*, 2001).

M. racemosus, the only mould species recovered from the initial stage of fermentation was present neither in raw ingredients nor in fermented batters. Since the source of the mould is not apparent, it is likely that the surfaces of utensils and grinder used, workers handling the preparation and the aerial environment are contributing to the initial inoculation. Their sudden disappearance after early phase of fermentation might be due to the inhibitory effects of the yeasts, particularly substrate competition. However, inhibition of the spore germination might occur due to the production of high concentration of organic acids (Halm and Olsen, 1996), or some possible synergism between diverse groups of predominant microbiota. Since among the isolates, *M. racemosus* is the only amylolytic organism, the mould certainly helps in the degradation of starch into maltose and glucose, essentially required for the growth and metabolism of the lactic acid bacteria and yeasts.

According to the Mukherjee *et al.* (1965), the souring and leavening attributes of idli batter are entirely due to the activity of heterofermentative *L. mesenteroides*. However, the role of yeasts in the production of gas during fermentation cannot be overlooked. They were responsible for the production of more than 50% of the CO_2 and 2-fold increase in the batter volume imparting acceptable texture and organoleptic qualities of idli, while lactic acid bacteria were confined to reduce the pH of batter and make them optimum for yeast activity (Venkatasubbaiah *et al.*, 1985).

During the fermentation, two significant changes are acidification and leavening of the batter. These have been used as criteria for judging the progress of fermentation. The pH decreased ($P < 0.05$) from 5.9 to 4.3 with three-fold increase in titratable acidity. The batter increased two times its original volume. The results agree well with those of Steinkraus *et al.* (1967) and Venkatasubbaiah *et al.* (1985). There was no influence of fermentation on the total protein content. An increase ($P < 0.05$) in nonprotein nitrogen content of the fermenting batter, as was found in this study, was also observed by Soni and Sandhu (1990a).

The occurrence of TAMB cells in 100% samples of the substrates (rice and dal) and also the fermenting batters indicates that the substrates as well as the batter provide a suitable environment to support their growth. *Bacillus* spp. are important as food-spoilage organisms (Johnson, 1984). Hence, the presence of aerobic mesophilic bacterial spores in 100% samples of ingredients and fermenting batters poses a threat to the shelf-life of idli. Although the batter is steamed at the final stage of idli preparation, the heating step could not eliminate them but only lower the count of these undesirable bacteria. This is because these bacterial cells escape the steaming process through certain 'cool pockets'. Indeed, total aerobic mesophilic bacteria and their spores were found in 100% and 85% of the samples, respectively, of freshly-prepared idli (Roy *et al.*, 2007).

As could be seen from the electrophoretic pattern of whole-cell proteins of the substrates and mixed-batter of idli at different stages of fermentation (Fig. 40), the rice protein resolved in as much as 10 major bands with molecular weights ranging between 14.3 to 97.4 kDa. Blackgram dal had one major band corresponding to molecular weight of 45 kDa and 6 minor but significant protein fractions

corresponding to molecular weight ranging between 45 to 55 kDa. However, as expected, the resolved protein fingerprint of the mixed batter revealed the combination of both rice and blackgram dal protein subunits. The electrophoretic patterns of mixed batters, which remained constant throughout the fermentation period, indicate the nonproteolytic nature of fermentation which was in contrast to the similar findings of Hatzikamari *et al.* (2007) in submerged fermentation of chickpea.

As was obtained in dhokla, the lyophilized crude extract of fermented idli batter in methanol gave the highest yield followed by nonfermented batter and steam-cooked product (Table 42). Fermentation caused 1.6-fold increase in total phenol content of the idli batter. This result obtained is in consistent with the findings of Lin *et al.* (2006), Randhir *et al.* (2004) and Vattem and Shetty (2002). Steam-cooking of fermented batter for 10-20 min significantly reduced the total phenolic content.

The reducing activity of the extracts of nonfermented and fermented batters and steamed product was estimated. Fe^{3+}-ferricyanide complex was reduced to the Fe^{2+} form by the formation of reductants (antioxidants) present in the extracts, which was then monitored by measuring the formation of Perl's blue at 700 nm (Oyaizu, 1986) to evaluate the reducing activity. Significant variation in the reducing activity of the crude extracts was observed during different stages of preparation (Table 42). While the extract of fermentation enhanced 125% of reducing activity, steam-cooking of the fermented batter for 10-15 min showed 2.3-fold decrease in the reducing activity. *In vitro* assay of antiradical activity of the extracts of idli during different stages of its preparation exhibited various degrees of DPPH·-scavenging activity (Table 43). Fermented batter inhibited DPPH· absorption, incubated for 50 min, at all the tested concentrations and was superior to that of nonfermented batter and steamed product. Enhancement of 2.8-fold scavenging activity of the nonfermented batter was observed when the concentration of the extract was increased from 10 to 50 mg ml^{-1} while the respective scavenging activity of the crude extracts of fermented batter and steamed idli was increased significantly by 3.4 and 4.5-fold. Thus the data reflect that DPPH·-scavenging activity of all the tested extracts was the function of their concentrations. However, the dose-time-response of extracts at all the tested concentrations revealed that the scavenging effect increased up to a certain extent with increase in concentration and leveled off despite further increase in time. The chelating activity of Fe^{2+} by crude extracts of the fermented and nonfermented batters and steamed product was estimated by the ferrozine assay (Table 44). Regardless of their source, the Fe^{2+}-chelating activity of all the tested concentrations of the extracts increased up to 20 mg ml^{-1}. In fact, the extracts disrupted the Fe^{2+}-ferrozine complex at 20 mg ml^{-1} concentration. At this dosage level, the extract of steamed product exhibited better chelating effect (44%). However, it was found that the chelating ability of the extracts of steamed product and batters increased up to the dosage level of 20 mg ml^{-1} and 30 mg ml^{-1}), respectively, and then leveled off despite further increase in concentration. Extract of fermented batter showed better IC_{50} and relative Fe^{2+}-chelating activity. In the earlier investigations Dhu and Yen (1999) reported that the methanolic extracts of mung beans were found to be prominent metal chelators. The LPIA of the methanolic extracts of idli batters and steamed product was estimated on linoleic acid emulsion system. The extracts showed a significant LPIA at all the tested concentrations (10-50 mg ml^{-1}) (Table 45). While the LPIA of the extracts increased significantly with the increase in concentration, the efficiency gradually decreased as the incubation period progressed. In all the cases, extract of fermented batter exhibited a better LPIA than others.

The analysis of correlation coefficients between every two antioxidative parameters tested for the extracts of nonfermented and fermented batters and steam-cooked product exhibited positive correlation (Table 46). While in nonfermented batter a strong correlation was observed between metal-chelating activity and LPIA, in fermented batter it was between metal-chelating activity and reducing power. However, in steamed idli a strong correlation was found between the antiradical and lipid

peroxidation inhibitory activities, closely followed by the correlation between total phenol content and metal-chelating activity. In earlier findings the free-radical scavenging capacity and ability to inhibit lipid peroxidation was found to be of considerable interest in *Cinnamomum verm* leaf extract (Mathew and Abraham, 2006). However, Dhu and Yen (1999) reported that the methanolic extracts of mung bean were found to be both metal chelators and radical scavenger. The data also reflect that all the 5 antioxidant parameters exhibited significant positive correlation among the every two parameters

The regression equations and coefficients of correlations between different antioxidant parameters and total phenol content of the extracts were analysed (Table 47). All the tested samples showed that the total phenol contents were positively correlated ($P < 0.001$) with DPPH-scavenging activity, reducing power, metal-chelating ability and lipid peroxidation inhibitory activity. The equations suggest that the total phenol content accounted for 62%, 92% and 90% DPPH-scavenging activity in respective extracts of nonfermented and fermented batters and steamed product. The structural conformation of phenolic compounds determines the antiradical activity of the extract (Bors *et al.*, 1990).

As revealed from the relevant equations, total phenol also accounted for 50%, 81% and 90% of reducing power in extracts nonfermented and fermented batters and steamed product, respectively. This indicates that polyphenols in extracts of fermented batter and the steamed idli, may generate reducing power. The observation was in consistent with the findings in grams, herbs and spices (Choi *et al.*, 2007; Hinneburg *et al.*, 2006). The total phenol content in fermented batter could account higher metal-chelating ability (82%) than the nonfermented batter and steamed product. This clearly indicates the effect of fermentation on enhancement of metal-chelating ability of the idli batter. On the other hand, lesser correlation between total phenol content and Fe^{2+}-chelating activity in unfermented batter and the steamed product might explain that the phenolic compounds alone might not be the primary chelator of ferrous ions. Total phenol content also accounted for 91% of LPIA in extracts of fermented batter, followed by nonfermented batter and steamed product. The ability of the extract to retard lipid peroxidation is attributable to the ability of its phenolic constituents to quench reactive oxygen species.

5.4. Kinema

Kinema constituents a significant dietary component for the people living in Nepal, and Darjeeling hills of West Bengal and Sikkim, India (Sarkar *et al.*, 2007). The respective yields of lyophilized methanolic extracts of cooked nonfermented (CNF) soybean and kinema were 9.1 g and 15.4 g (100 g)$^{-1}$ (dry weight) (Table 48). The corresponding values in CNF soybean and soybean kojis (fermented by different molds) were 5.3 and 6.7-16.8, respectively (Lin *et al.*, 2006). Recently, the antioxidant activities of different polar and nonpolar solvent extracts from plant-derived foods have been reported.

The total phenol content of kinema was 144% higher than that of CNF soybean (3.3 mg g^{-1} dry weight) (Table 48), the value which is consistent with the data (3.1 mg g^{-1} soybean, dry weight basis) provided by Bajpai *et al.* (2005). The reducing power in kinema was higher ($P < 0.05$) than that in CNF soybean (Table 48). The dose and time-response curve for the antiradical activities of the crude methanolic extracts of kinema and CNF soybean revealed that the scavenging effect increased ($P < 0.05$) with the increasing concentration of the extracts up to 50 mg ml^{-1} and up to 40 min of reaction, however leveled off with further increase in time. Kinema extract inhibited DPPH· absorption at all the tested concentrations and was superior to the CNF soybean (Table 49). Estimation of the IC_{50} of the methanolic extracts revealed that on average about 50.03 and 40.83 mg ml^{-1} of the methanolic extract(dry weight basis) of CNF soybean and kinema, respectively, could decrease the initial concentration of

DPPH· by 50% when the reaction mixture was incubated for 40 min. Since the yield of methanolic extracts of CNF soybean and kinema varies, the relative scavenging effect exerted by the different samples, taking into account the extract yield, were estimated. In this regard, kinema exhibited a relative DPPH·-scavenging effect of 2.1 compared with that of CNF soybean, which was assigned as 1.0 (Table 49). The chelating ability of methanolic extract of CNF soybean and kinema was examined against Fe^{2+}, since it is the most effective pro-oxidant that is found in the food system (Lin *et al.*, 2006). Metals abstract hydrogen atom from the fatty acids and affect both the speed of auto-oxidation and the direction of hydroperoxide breakdown to volatile compounds. At all the tested concentrations, the crude methanolic extract of kinema had a significant metal-chelating capacity, which was demonstrated by the decrease in purple colour formed due ferrozine- Fe^{2+} complex formation, as compared to that of the CNF soybean (Table 50). At 10 mg ml^{-1} concentrations the methanolic extract of kinema exhibited 64% metal chelation which was much higher than the activity shown by CNF soybean (22%). The chelating ability increased with the increase in concentration. Kinema exhibited better IC_{50} for Fe^{2+}-chelating activity than CNF soybean. Likewise, a 3.3-times higher Fe^{2+}-chelating ability was observed with the methanolic extract of kinema (Table 50). The significant increase in the relative Fe^{2+}-chelating ability of kinema, therefore, revealed that the fermentation enhanced the Fe^{2+}-chelating ability. The antioxidation effects of the methanolic extracts of CNF soybean and kinema, at different concentrations on the peroxidation of linoleic acid were investigated (Table 51). At 50 mg g^{-1}, after 24 h, while kinema exhibited antioxidant activity 44% inhibition of linoleic acid peroxidation, CNF soybean showed 36% inhibition. However, the peroxidation inhibition of both CNF soybean and kinema extracts was found to decline with time and reached merely 26% after 72 h. At lower concentrations, the methanolic extracts of CNF soybean and kinema showed almost similar level of peroxidation inhibition. Thus, the kinema strain (*B. subtilis*) is an effective producer of antioxidant activities. The sharp decline of the peroxidation inhibition in methanolic extract of kinema beyond 24 h can be attributed to the shelf life of kinema. After about 12 h of traditional fermentation, the surfaces of the beans are covered with a rough, white viscous mass when kinema is ready for cooking (Sarkar *et al.*, 1993). The shelf-life of kinema is 2 days, after which a rapid softening along with a strong off-flavour develops in the product, rendering it unacceptable for consumption. CNF soybean also followed a similar patter; however, it inhibited lipid peroxidation to a much lesser extent.

All the five antioxidant parameters from CNF soybean and kinema exhibited significant positive correlations among the every two parameters (Table 52). In CNF soybean the highest correlation was observed between radical-scavenging activity and metal-chelating activity, while in kinema it was radical-scavenging activity with lipid peroxidation inhibitory activity, being closely followed by metal-chelating ability.

The regression equations and coefficients of correlations between different antioxidant parameters and total phenol content of CNF soybean and kinema showed that the total phenol content was positively correlated ($P < 0.01$) with DPPH·-scavenging activity, reducing power, metal-chelating and lipid peroxidation inhibitory activity (Table 53). Antiradical activity is greatly influenced by the phenolic content which accounted for 68% and 92% DPPH·-scavenging activities in CNF soybean and kinema, respectively. Again, 68% and 88% correlations between total phenol content and reducing power in CNF soybean and kinema, respectively, were consistent with the findings in grains, herbs and spices (Choi *et al.*, 2007; Hinneburg *et al.*, 2006). This indicates that polyphenolics in methanol extracts of soybean and soybean products may play a role as electron and hydrogen donors. Total phenol content could also explain 58% and 91% metal-chelating ability, and 66% and 83% lipid peroxidation inhibitory activity of the CNF soybean and kinema extracts, respectively. On the whole,

it can be concluded that total phenol content of the extracts had a high positive correlation ($P < 0.01$) with all the antioxidant parameters tested.

The trend towards eating a healthier diet has led to an increase in consumption level of grains products. Soybean health claims on food labels have recently been approved by the food administrative authorities in several countries. The fact that kinema exhibited a better antioxidant activity than CNF soybean could be due to the fact that individual phenolic compounds; with high antioxidant activities might have been produced through the fermentation step. A high level of correlation between phenolics and antioxidant activities was shown earlier on the studies of fruits and vegetables (Kaur and Kapoor, 2002; Yildirim *et al.*, 2005). The higher level of antioxidant activity in kinema compared to CNF soybean could be attributed to the extensive hydrolysis of proteins and an increase of 58% in the overall content of phytosetrols (campesterol, stigmasterol and β-sitosterol) that happens during the *Bacillus*-fermentation of soybeans (Sarkar *et al.*, 1996, 1997). Since, the sequence and composition of amino acids in the peptides are critical, several peptides in soy protein hydrolysate were found to have good antioxidant activity while the others had marginal activity (Quinn and Tang, 1996).

5.5. Papad

Papad, a thin, usually circular, wafer-like product, constitutes an important legume-based traditional food adjunct, being manufactured and extensively consumed in India.

Though, extensive work on the proximate composition, packaging, storage and quality control of papads in India has been reported (Kulkarni *et al.*, 1996; Manan *et al.*, 1988; Pruthi *et al.*, 1984; Shurpalekar, 1986), there are no comprehensive reports available on the interplay of papad microflora and their consequent biochemical changes. The traditional methods of preparation, however, vary in details from place to place resulting in the inconsistent quality of the product. The variations in methods and ingredients used in the preparation of papads encounter difficulties in obtaining papad dough with consistent rolling properties and papads of uniform quality attributes. In order to produce papad of reproducible quality different parameters employed in the traditional methods were optimized under semicontrolled conditions by sensory evaluation. Since it was necessary to understand the functional attributes of the ingredients, attributes such as dough consistency, rolling property, texture, taste and post-frying expansion of papads, were considered as the main criteria for producing papad with acceptable quality.

The microbial analysis of the substrates and product exhibited the presence of both lactic acid bacteria and yeasts (Table 59). The representative strains were identified as members of *Pediococcus pentosaceus* and *Saccharomyces cerevisiae*. While TAMB were encountered in all the samples studied, the respective occurrence of their spore (aMBS) count in blackgram dal flour and marketed papads were 67% and 94% (Table 60). However, the load of *P. pentosaceus* and S. *cerevisiae* in papad were below the detection limits. The occurrence of TAMB indicates that these samples provide the suitable environment to support their growth. Their presence in the products possesses a threat to the shelf-life of papad. Indeed, TAMB and their spores (aMBS) were found in 100% of the raw papads collected from different retail sources (Roy *et al.*, 2007). Since *Bacillus* spp. are considered as important food-spoilage organisms (Johnson, 1984), the presence of this organism at high levels suggests potential risk to the consumers, because of the subsequent production of toxin associated with food poisoning (Banerjee and Sarkar, 2004).

The desirable papad dough with homogeneous and consistent rolling properties was obtained when 1 kg-blend of blackgram dal and mung dal flours (1 : 2) was mixed with 500 ml lukewarm water,

15 g papad khar and 70 g common salt (Table 61-64). Papad with acceptable, uniform quality attributes and satisfactory shelf-life was obtained when the corresponding dough, after fermenting at room temperature (28 ± 2°C) for 3 h, was rolled into thin circular discs and dried under controlled conditions (70 ± 5% relative humidity and 30 ± 1°C) for 8 h. The papad, dried under controlled conditions, was superior with respect to colour and texture and had a 'good' grade scoring.

Though blackgram dal flour is the indispensable constituent in papad dough because of the mucilaginous substances it contains, most of the commercially available papads are prepared from blends of blackgram dal with other legume or cereals flours, unless mentioned otherwise. Blackgram and mung bean dal flours in the proportion of 1:2 ratio imparted a desirable consistency and rolling property to papad dough (Table 61). The blackgram or mung bean dough, when used alone, was difficult to roll, and had a tendency to crack at the edges, resulting in papads with bean-like taste and unacceptable colour. This was in accordance with the findings of Shurpalekar (1986) who reported similar observations when papads were prepared from mung bean dal flour alone. However, the possibility of using other legumes, such as cowpea (Bharati *et al.*, 1995), Bengalgram, greengram, lentil and redgram (Saxena *et al.*, 1989), soy flour (Deepa *et al.*, 1992), with or without blackgram has been reported. Bhattacharya and Narasimha (1999) characterized the papads from different blends of blackgram dal flour with cereals and observed that the papads made by using 25% rice flour and 75% blackgram dal flour resembles the product made from blackgram dal flour alone. The use of cereal flours to partially replace blackgram dal flour is yet another option, which not only reduces the cost of production but also offers a balanced amino acid composition and improved the nutritional quality of the product (Almeida-Dominguez *et al.*, 1990; Juneja *et al.*, 1980). Bhattacharya *et al.* (1999) showed that the blackgram dal flour with 20-40% of wheat flour or up to 22% rice flour was suitable for making papad. Deepa *et al.* (1992) found that incorporation of soy-flour up to 40% with the blackgram dal flour was acceptable for making papads. Even the papads prepared from a blend of 1:1 ratio of blackgram dal flour and soy flour compared well with those from blackgram dal flour alone. Shurpalekar and Venkatesh (1975) reported that at least 20% of blackgram dal flour is essential in making greengram papad having desired quality attributes. However, replacing the legume flour with cereal flours at the level of 30% and above adversely affects the taste and acceptability of papad (Bhattacharya and Narasimha, 1999; Singh *et al.*, 1996)

'Papad khar' or 'saji khar' is traditionally used as an additive in the papad formulations. Papad khar, ashy in nature, is presumably used to improve the rolling properties of the dough and the frying quality of papad. They are obtained from saltworts by burning a variety of plant species, or from very alkaline deposits in the soil, e.g., dhobi's earth. The chemical composition of papad khar revealed mainly carbonates, chlorides and sulphates along with a trace of phosphorus, iron and sulphur (Shurpalekar, 1986). However, chemical analysis on different samples of papad khar offers an alternative choice of sodium carbonate for producing papads of desired taste and crispness.

Amount of water is another variable for obtaining papad dough of desired consistency and rolling characteristics, and papads of acceptable quality attributes and satisfactory shelf-life (Table 63). Optimization of the level of edible salt is equally important for obtaining dough with the desired characteristics and papad having a balanced and acceptable taste when roasted or fried (Table 64). Common salt not only imparts desirable taste to the product but also softens the papad dough and helps in rolling it out into thin sheets. Salt also facilitates uniform distribution of flour components such as protein, mucilages, and starch, and contributes to the blooming or expansion of papads on frying. While excessive addition of salt caused salt bloom on raw papads during storage (Shurpalekar, 1986) inadequate salt yields an insipid and unacceptable product.

The moisture level of the papads ranges between 12-17.5% above which they become susceptible to fungal spoilage (Shurpalekar, 1986). The pliability of papads was affected and tends to wrap when the moisture content was below the minimum level. In the traditional method of preparations, papads are dried in shade, under widely fluctuating conditions of atmospheric humidity and temperature. The nonuniform thickness of papads also influences their drying characteristics. Optimization of drying period of papad sheets under controlled temperature and humidity for desired moisture content avoids the fear of over or underdrying. However, moisture content in the market samples of papad ranged between 9-17%.

The initial load of *Pediococcus pentosaceus* and *Saccharomyces cerevisiae* in papad dough was probably inherited from the substrates (Table 65). However, after a significant ($P < 0.05$) increase at onset of fermentation, their count gradually declined to below the detection limit after 4 h-drying of the papad sheets. The decrease in the count of *P. pentosaceus* was significant after every 2 h-interval. The incorporation of common salt (70 g kg^{-1} flour blend) and papad khar (15 g kg^{-1} flour blend) could affect the luxuriant growth of and *P. pentosaceus* and *S. cerevisiae*. The high pH of the dough, which probably did not allow the proliferation of acidiphilic microbiota of papad, further justified the disappearance of *P. pentosaceus* and *S. cerevisiae* after 4 h-drying.

No significant influence of fermentation on the physicochemical properties of papad dough was observed except the increase ($P < 0.05$) in soluble nitrogen content and decrease in free fatty acidity, protein nitrogen and crude fat content. However, two significant changes occurred in papad sheets during drying were decrease ($P < 0.05$) in moisture content and corresponding increase ($P < 0.05$) in % diametric expansion of papad sheets after deep-frying in oil for 10-15 s (Table 66). These parameters have been used as criteria for judging the progress of drying the papad sheets. Besides, the other parameters were relatively constant, and changes in proximate compositions, if any, were insignificant during the fermentation and subsequent drying of papad sheets. Moreover, the period for which the papad dough fermented was not sufficient for its microbiota to cause any significant changes. The findings, therefore, revealed that fermentation of the papad dough and the subsequent drying of papad sheets have lesser effect on its proximate composition.

5.6. Wadi

Traditionally consumed in the northern and north eastern states of India, wadis are now popular in many places in India, Pakistan, Bangladesh and Nepal. A similar product, with slightly different in preparation techniques and raw substrate used, has been called by different synonyms in different regions of India (Punjabi wadi in states of Northern India (Batra, 1981; Soni and Sandhu, 1989b), bori in West Bengal and Orissa; adhauri or wadi in Bihar and Jharkhand, and masyuara in Darjeeling hills of West Bengal and Sikkim (Yonzone and Tamang, 1998).

Because of continuous drying for 60 h the mean moisture content of wadi declined significantly to 14.6% (Table 67). Though the raw substrate was neutral, the mean pH of wadi (5.5) reflects its acidic nature. The reduced moisture content, along with low pH of wadi, could probably enhance its shelf-life. While blackgram dal showed higher ash and protein nitrogen contents, wadi had significantly higher titratable and free fatty acidity, and contents of nonprotein and soluble nitrogen and crude fat.

The microbial analysis of substrate and wadi showed the prevalence of both lactic acid bacteria and yeasts (Table 74). The two types of predominant lactic acid bacteria, isolated from all the positive samples were identified as *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* (Table 71). Similarly,

the four culturally and morphologically distinct groups of yeast isolated from the positive samples were identified as *Saccharomyces cerevisiae, Issatchenkia orientalis, Pichia membranifaciens* and *Rhodotorula minuta* (Table 72). While *S. cerevisiae* was the only yeast isolated from the substrate, laboratory made wadi harboured all the species of yeasts except *R. minuta. Mucor racemosus* was isolated from only 15% of the marketed wadi samples (Table 73).

Fermentation of wadi batter and subsequent drying to wadi showed interesting microbial kinetics. While TAMB cell increased ($P < 0.05$) up to 36 h of drying, the change in their spore count had no significant effect of drying. Occurrence of high level of TAMB cells in the samples generally indicates either the use of highly contaminated substrates or the poor processing practices. Plate count agar (used for the enumeration of TAMB cells) is a nonselective complex medium commonly used for enumerating total microbial content in foods. So, the viable count in the samples was likely of fermenting microorganisms along with associated contaminating microbiota (Roy *et al.*, 2007). Considering that no sign of spoilage was recorded in wadi, it might be assumed that most mesophilic bacterial spores either did not germinate or were not metabolically active during wadi fermentation and drying.

P. pentosaceus and *L. mesenteroides,* which dominated throughout fermentation and subsequent drying of wadi, increased by 5 and 6 log cycles, respectively, during the initial 24 h of drying (Table 76). However, *P. pentosaceus* gradually declined ($P < 0.05$) from 24 h onwards. *L. mesenteroides,* although comprised the major component of the wadi microbiota, showed a sharp decrease ($P < 0.05$) by 2-log cycles during the last 12 h-drying. *S. cerevisiae,* the most predominant yeast encountered during entire fermentation and drying, appeared at the onset of fermentation. Its count increased significantly ($P < 0.05$) during initial 10 h-fermentation and at every 12 h-interval of drying till 24 h, followed by the steady fall in the count. The decrease was significant ($P < 0.05$) after every 12 h-interval till 48 h. *I. orientalis,* which appeared after fermentation of the batter, increased significantly ($P < 0.05$) during the first 24 h of drying. Its count decreased to the final load of 5.2 log cfu g^{-1} fresh wadi at the end of the drying period. The decrease was significant ($P < 0.05$) after every 12 h interval. *Pichia membranifaciens* appeared after initial 12 h of drying, had maximum count of 5.5 log cfu g^{-1} fresh weight at 24 h, beyond which they remained significantly unchanged. *Rhodotorula minuta* and *Mucor racemosus,* though recovered from 12 and 15% of the marketed wadi, respectively, were not encountered during the entire process of wadi preparation under semicontrolled conditions.

The occurrence and role of several bacteria alone or with lesser proportion of yeasts in naturally fermented Punjabi wadi based on blackgram dal has been reported by Soni and Sandhu (1989b). *L. mesenteroides, Lactobacillus fermentum* and *Enterococcus faecalis* were the principal bacteria followed by *Bacillus subtilis, Lactobacillus delbrueckii, Flavobacter* sp. and *Enterobacter* sp. involved in Punjabi wadi dough fermentation causing acidification and leavening. Dahal *et al.* (2003) reported that *P. pentosaceus* constituted 75% of the total lactic acid bacteria recovered from masyaura,. Besides *S. cerevisiae, P. membranefaciens* and *Trichosporon beigelii* the yeast flora generally encountered in Punjabi wadi fermentation comprises *Candida vartiovaarai, Kluyveromyces marxianus, Candida krusei, Pichia anomala, Candida aqiatoca Cryptococcus humicolus* and *Geotrichum candidum.* They were supposed to enhance the acidification and leavening of wadi dough during fermentation (Soni and Sandhu, 1989b). The enumeration of yeast from masyaura samples revealed that *S. cerevisiae* and *Candida versatilis* are the major yeasts involved in fermentation (Dahal *et al.*, 2003). Though the involvement of *Cladosporium* spp., *Penicillium* spp. and *Aspergillus niger* in masyaura, has been reported by Dahal *et al.* (2003), the appearance of *Mucor racemosus* in 15% of the market sample of wadi in the present study could be assumed as mere opportunistic or may be due to cross contamination during marketing.

Soni and Sandhu (1989) further advocated that the development and prevalence of microbial types in Punjabi wadi is affected by seasons; summer being more favourable for bacteria and winter for yeasts. The effect of seasonal variations on the development and prevalence of microbes could be attributed to the appreciable difference in atmospheric temperature during the two seasons. Temperature during summers (37-42°C) probably favoured the rapid multiplication of bacteria, while high prevalence of yeasts during winters can be as due to prevailing favourable temperature for their propagation. However, in present study, the ability of *S. cerevisiae, P. membranifaciens,* and *I. orientalis* to grow at 37°C justified their occurrence, irrespective of the seasonal variations, in both market as well as laboratory-made wadi.

Fermentation of wadi dough is essentially an autofermentation process where inherent microflora contributes a major functional role. Since the defined source of inoculum in the wadi fermentation is not apparent, it is likely that the raw substrates (blackgram dal) used, workers handling the preparations and the aerial environment contribute to the initial inoculation for the fermentation. Moreover, microbial analysis of the substrate exhibited the association of *L. mesenteroides* and *P. pentosaceus*. Some earlier findings have also suggested the role of natural microbial load of ingredients and the environment in the initiation of traditional fermentations. Mukherjee *et al.* (1965) and Ramakrishanan (1979) reported that dehulled blackgram harbours *L. mesenteroides* and other lactic acid bacteria in large numbers which play a significant role in blackgram dal fermentation. The significant role of *L. mesenteroides* has also been reported earlier in blackgram dal fermentations by Batra (1981) and Sandhu *et al.* (1985) who also isolated *L. fermentum* and *S. cerevisiae* from wadi paste.

Yeast in the substrate was represented by *S. cerevisiae* only. The lower frequency of occurrence of yeast in the fermentations carried out by natural microbiota is probably due to their less prevalence on the dry substrates and/or due to low moisture content of the latter. Since the blackgram dal is presoaked in water before ground to smooth batter, yeast species probably developed only after the softening of substrate, presumably, because yeasts require higher moisture content for their survival than bacteria. *I. orientalis* and *P. membranifaciens*, though found below the detection limits in substrate, appeared only after initial stage of fermentation. They probably come from air during drying and produce more acid causing further acidification. The aerial contamination of wadi by yeasts during open air-drying was justified by Sandhu and Waraichi (1981) who isolated seven yeasts, including *S. cerevisiae*, from air. However, the contributions of these yeasts cannot be over looked by considering them as contaminant or opportunist. Their occurrence in the market (54% and 88% of *I. orientalis* and *P. membranifaciens*, respectively) and 100% each in laboratory-made wadi focused on their functional role as a dominant microbiota in wadi fermentation.

Besides successive rise in bacterial and yeast cell counts, the pronounced effect of fermentation of wadi dough was noticed in the decrease in pH (Table 77). The decrease was significant at every 12 h-interval till 36 h of drying. The decrease in pH was in contrast to the load of lactic acid bacteria, yeasts and TAMB. Titratable and free fatty acidity registered significant increase ($P < 0.05$) after fermentation followed by subsequent drying. While the moisture content of the batter during fermentation remained constant, their volume registered a 1.4-fold increase ($P < 0.05$). However, a gradual decrease in the moisture content of wadi was observed during drying. The decrease was significant ($P < 0.05$) at every 12 h-interval. While the contents of protein nitrogen and total protein of the batter reduced ($P < 0.05$) during fermentation, subsequent drying of wadi caused significant increase ($P < 0.05$) in their contents after 12 h-drying. The overall carbohydrate content in wadi batter declined during fermentation followed by drying.

The physicochemical changes are probably the result of microbial activities in the wadi batter involving the production of acid and gas from various carbohydrates thus accounting for the rise in total acid levels and volume during the fermentation. Moreover, the rise in the levels of soluble nitrogen and total protein is presumably due to the production of proteolytic enzymes by the microorganisms and the hydrolysis of insoluble polymers under acidic conditions by these enzymes. Increase in total acidity during fermentation helps in extending shelf-life of wadi. It is apparent that the traditional wadi fermentation is brought about by the microbiota coming from staples and the environment which probably bring about several changes leading to improved digestibility and nutritional value. However, the possibility of various undesirable microorganisms and the production of toxic substances by certain species is a point of serious concern.

The recognition that the fermented foods have generally higher nutritive value and that their flavour and aroma are more attractive than their unfermented precursors has greatly increased the demand for foods produced by fermentation. Improving the nutritive and microbial qualities of traditional fermented foods employing scientific and technological skills would be beneficial to preserve this precious culture. To do this, greater efforts will be required to carry out the comprehensive and systematic studies to explore and preserve the traditional food cultures which have never been reported to the industrial society before. It would also provide a great opportunity to achieve commercial success. Traditional fermented foods then definitely contribute to increase the value of agricultural products and vitalize the rural economy. Moreover, microbial diversity associated with traditional fermented foods may contribute a significant gene pool, and may have potential biotechnological applications. Furthermore, interactions with a general tendency between lactic acid bacteria and yeasts in the cocultures emphasize the need to carefully screen individual yeast and lactic acid bacterial strains for every application to avoid off-flavours and other defects in different types of traditional fermented foods. This will also focus on the indigenous knowledge of rural people to preserve and supplement microorganisms for production of fermented foods. Moreover, people who invented and preserved the age-old traditional food fermentation technology should be reassured about the worth of their indigenous knowledge.