2

Review of literature

2.1. Legume-based traditional foods of India

Cereals and food grain legumes are staple foods of the people of India. Legumes alone or in combination with cereals, like rice constitute the basic ingredient of many traditional fermented foods. A food is considered fermented when microorganisms act upon one or more of the food constituents to produce a considerably altered product acceptable for human use (Van Veen, 1957). Traditional foods are those popular products, whose preparation methods have been handed down from one generation to another since early history and have formed an integral part of the diet of a certain local area, region or country. They are prepared in a small scale either at homes or in the cottage industries using relatively simple techniques and equipment (Aidoo *et al.*, 2006). Traditional foods reflect the culture and heritage of a region and have their imprints on contemporary dietary patterns. The traditional techniques of preparing some fermented foods are fast fading due to the rapid urbanization and changing food habits of the people. Besides this, these techniques are passed on as trade secrets in the families of certain communities, a practice protected by tradition (Tamang *et al.*, 1988), and a break in the transfer of such trade secrets from one generation to another will result in the gradual loss of the knowledge about the food. However, many fermented foods are now receiving global attention for their health-promoting or disease-preventing effects. Besides improving pulse digestibility for humans, fermentation reduces several antinutritional factors in legumes. Other attributes being improving the flavour, nutritional value and appearance, reducing the cooking time and enhancing the shelf-life of the food.

Many important products made from legumes are gaining rapid popularity all over India (Table 1). Some legume-based fermented foods like amriti, kinema, papad and wadi are popular in the northern and eastern parts of the country. With the passage of time, southern and western Indian fermented foods like dhokla, dosa, idli and vada have also become quite popular as nutritious and delicious light midday meal. Besides these, the non-fermented legume-based foods like besan and sattoo are also very popular all over the country. An overview of the various traditional legume-based foods of India along with their methods of preparation is described below.

Table 1. Some legume-based traditional foods of India

Food	Substrate	Functional microbiota	Product marketed as	Nature of the product	Reference
Amriti	Legume		Ready-to-eat	Syrup-filled, ring-shaped confectionery	
Besan	Legume		Raw	Powder	
Dhokla	Legume	LAB, yeasts	Ready-to-eat	Steamed, spongy cake	Desai and Salunkhe (1986); Joshi *et al.* (1989)
Dosa	Legume-rice mixture	LAB, yeasts	Ready-to-eat	Thin, seasoned griddled pancake	Soni and Sandhu (1999); Aidoo *et al.* (2006)
Idli	Legume-rice mixture	LAB, yeasts	Ready-to-eat	Steamed, spongy cake	Soni and Sandhu (1999); Aidoo *et al.* (2006)
Kinema	Legume	Mesophilic bacteria	Raw	Sticky fermented beans	Tamang *et al.* (1988)
Papad	Legume	Yeasts	Raw	Thin, circular wafer	Shurpalekar (1986); Aidoo *et al.* (2006)
Sattoo	Legume		Ready-to-eat	Powder	
Wadi	Legume	LAB, yeasts	Raw	Hollow, brittle balls or cones	Sandhu and Soni (1989); Aidoo *et al.* (2006)

Based on Roy *et al.* (2007)

2.1.1. Besan

Besan or gram flour is finely ground powder (Fig. 1a) made from fried chickpeas and is a staple ingredient in many cuisines of the Indian subcontinent. It is primarily used for breading different types of Indian fritters such as pakodas and onion bhajjis but sometimes also added to the curries to thicken its consistency. It is also used for preparing several sweets like bonda, laddoo, and so on.

Besan is a nutritious legume-based product as it is rich in various nutrients like magnesium, copper, folate and manganese. It also contains iron, zinc, phosphorus, calcium and potassium. Besan is low in saturated fat, cholesterol and sodium; although it contains lesser carbohydrates in comparison to wheat flour, the amount is still good enough at 57 g/100 gram (betteralive.hubpages.com, surfed on July 11, 2014).

2.1.2. Dhokla

Dhokla is originally a traditional Gujarati dish with an appealing taste, but now popularly consumed all over the country. It is readily available at sweet shops and also prepared conveniently at homes. This legume-based fermented steamed product has a mild sour flavour and spongy texture (Fig. 1b) and can be eaten for breakfast, as a main course, as a side dish or as a light mid-day snack. Traditionally, dhokla is prepared (Fig. 2) by the overnight fermentation of a mixture of coarsely ground polished rice and chana dal (Bengalgram) batter at room temperature (Desai and Salunkhe, 1986). Fermented batter is spiced by adding chilli, pepper and ginger paste and steam-cooked for 10-15 min, cut into square or diamond shapes and seasoned with hot refined oil containing curry leaves (*Murraya koenigii* (L.) Spreng.) and cracked mustard seeds. Before serving it is garnished with coriander leaves, deep fried chillies and grated coconut.

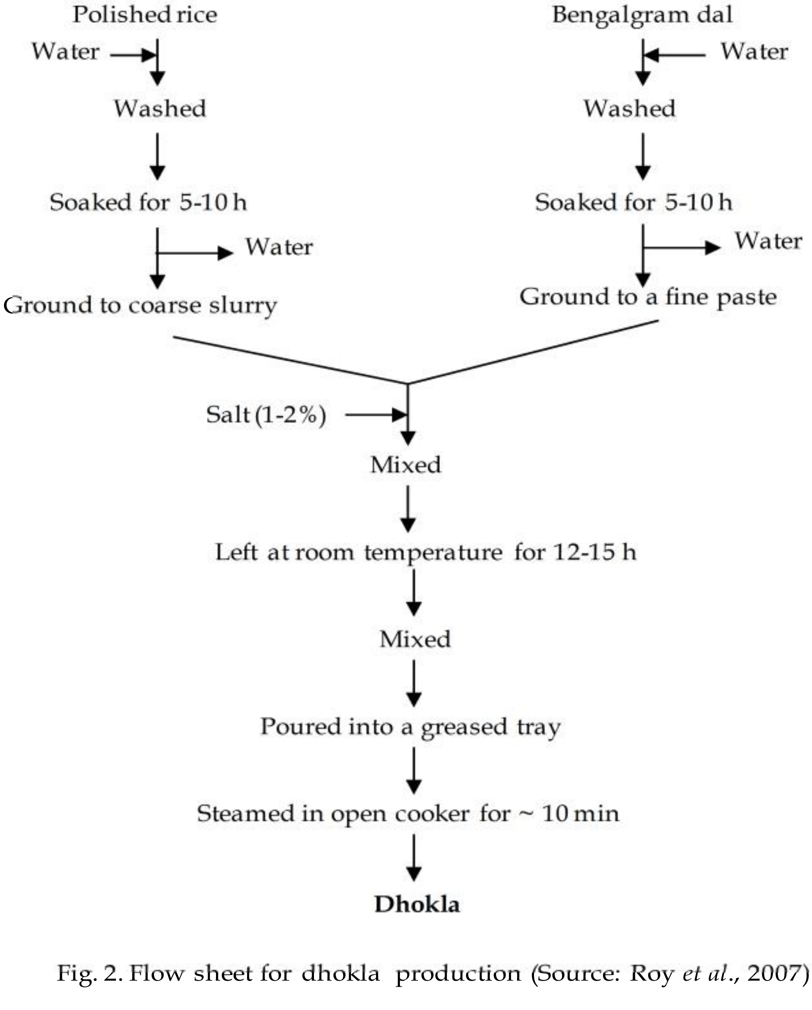

Fig. 2. Flow sheet for dhokla production (Source: Roy *et al*., 2007)

However, there are different types of dhokla prepared with different ingredients such as semolina (suji) and maize (*Zea mays* L.) substituting for rice and blackgram, red gram (*Cajanus cajan* (L.) Millsp.) and soybean (*Glycine max* (L.) Merr.) cotyledons for Bengalgram. For instance, khatta dhokla is prepared by fermenting a mixture of blackgram, rice and yogurt while rawa dhokla is prepared from semolina without fermentation. Mahajan and Chattoapdhayay (2000) have suggested a ratio of 0.6:1.0:0.3 of rice, Bengalgram and blackgram for minimizing the hardness as well as enhancing the taste of dhokla.

The chief fermenting microorganisms found in dhokla are *Lactobacillus fermentum, Leuconostoc mesenteroides*, and *Pichia silvicola* (Joshi *et al.*, 1989). The sour taste and a typical flavour is due to the production of lactic acid and acetoin by the LAB while an increase in the batter volume and sponginess of the product is attributed to the production of folic acid by the yeast (Kanekar and Joshi, 1993; Aidoo *et al.*, 2006).

Due to its organoleptic attributes, nutritional quality and improved digestibility dhokla is well accepted by persons of all age groups and patients with digestive disorder (Ramakrishnan, 1979). Dhoklas are good for diabetic patients as well. Being steamed and not fried, these have no oil content which makes them low on calories and being made from legumes increases the proteins and fiber content.

2.1.3. Dosa

Dosa is indigenous to the south Indian states of Andhra Pradesh, Karnataka, Kerela and Tamil Nadu and also Sri Lanka (Soni *et al.*, 1985, 1986; Soni and Sandhu, 1999; Aidoo, *et al.*, 2006). However its popularity has spread over to other countries as well such as, Malaysia and Singapore where it is popularly called Thosai and in Myanmar where it is called Toshay.

It is a thin, crisp, fried and highly seasoned griddled pancake (Fig 1c) made from fermented batter of finely ground blackgram and rice mixture (Fig. 3). It is a common breakfast dish available as a street food or at restaurants and fast-food outlets. To make a dosa, the fermented batter is spread in a thin layer of 1-5 mm thickness on a flat heated griddle which is smeared with a little oil or fat. Within a few minutes of heating a circular, semi-soft to crisp product resembling a pancake, ready for consumption is obtained (Bhattacharya and Bhat, 1997). These can be stuffed with fillings of vegetables and spices and served hot with chutney (a batter containing fresh ground coconut with spices) along with sambar (a soup prepared from red gram dal, vegetables, tamarind juice and salt).

Both bacteria and yeasts occur in the batter during fermentation and cause the desired acidification and leavening. Among the bacteria, *Ln. mesenteroides* is the most important microorganism during dosa batter fermentation followed by *Streptococcus faecalis*, *Lb. fermentum*, *Bacillus amyloliquefaciens*, *Lactobacillus delbrueckii*, *Bacillus subtilis*, *Pediococcus cerevisiae*, *Bacillus polymyxa* and *Enterobacter* sp. While *Saccharomyces cerevisiae* is the most predominant yeast involved in the fermentation, other yeasts involved are *Debaryomyces hansenii*, *Hansenula anomala*, *Trichosporon beigelii*, *Oosporidium margaritiferum*, *Trichosporon pullulans*, *Kluyveromyces marxianus*, *Candida kefyr* and *Candida krusei* (Soni *et al.*, 1986).

The pH of the dosa batter decreases from 5.1 to 4.1 due to fermentation. The other effects of fermentation being an increase in batter volume, increase in the amount of soluble solids and reducing sugars. However, the proteinase activity does not exhibit anyappreciable change during fermentation (Soni *et al.*, 1985; Soni and Sandhu, 1999).

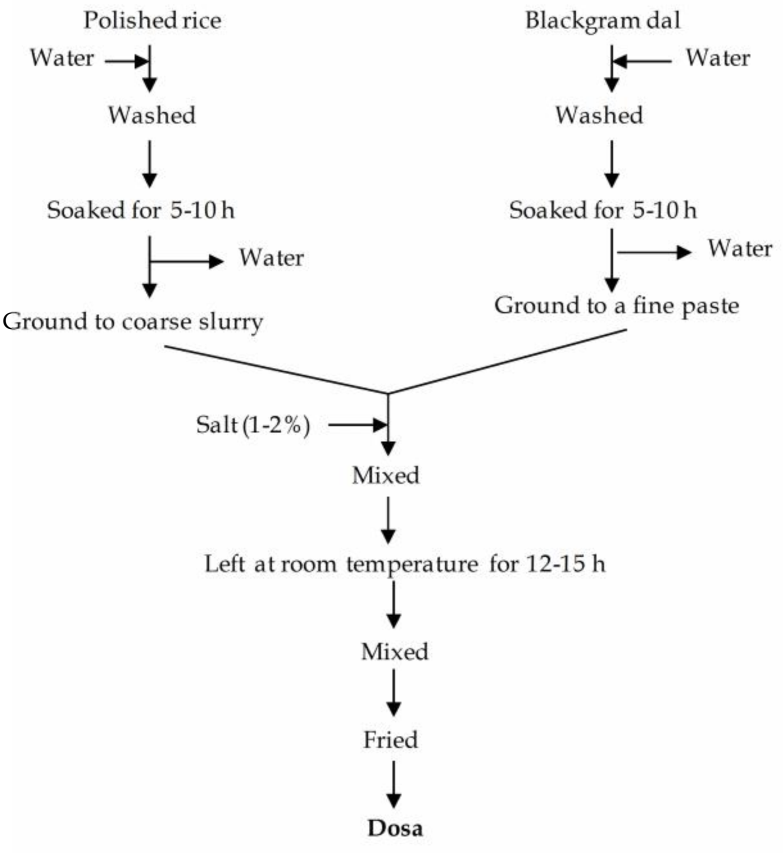

Fig. 3. Flow sheet for dosa production (Source: Roy *et al.*, 2007)

2.1.4. Idli

Idli is a savoury cake of south Indian origin however popular throughout India. These small, white, acid-leavened spongy cakes are usually 2-3 inches in diameter (Fig. 1d). It is a low calorie, starchy and nutritious food, which is now served at institutions such as army, railways, industrial canteens, etc. as a ready-to-eat healthy breakfast food. Traditionally, for idli preparation rice and blackgram are soaked and ground separately, then mixed together in a 2:1 ratio before fermentation (Fig. 4). Finally, the fermented batter is placed in special idli pans and steamed for about 10 min (Nagaraju and Manohar, 2000). The soft spongy texture of idli is due to the presence of a protein and a polysaccharide component in blackgram, namely globulin and arabinogalactin, respectively (Reddy *et al.*, 1982). Arabinogalactin is the mucilaginous principle found in blackgram that helps to retain carbon dioxide during the fermentation process which in turn results in the spongy and 'honey-comb like' appearance of idli.

The LAB, *Ln. mesenteroides*, *S. faecalis*, *Lb. delbrueckii*, *Lactobacillus fermenti*, *Lactobacillus lactis* and *P. cerevisiae* have been found to be responsible for the fermentation process. Among all the microorganisms, *Ln. mesenteroides* and *S. faecalis* cause the leavening of batter and production of acid in idli (Ramakrishnan, 1993). The yeasts *Geotrichum candidum*, *Torulopsis holmii*, *Torulopsis candida* and *T. pullulans* have also been identified in idli production (Chavan *et al.*, 1989; Shortt, 1998).

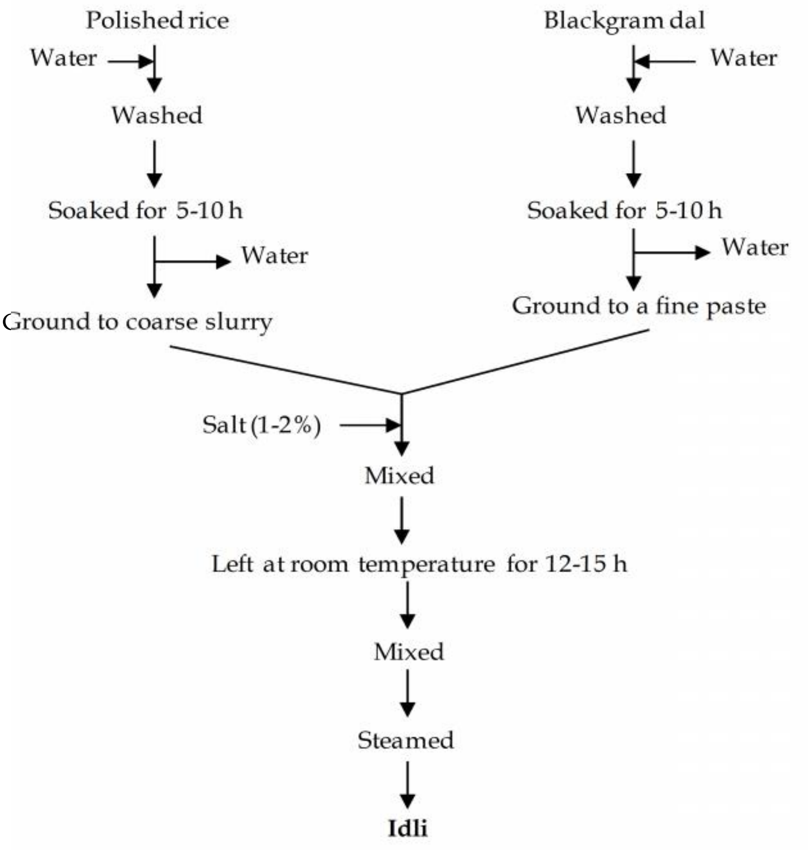

Fig. 4. Flow sheet for idli production (Source: Roy *et al.*, 2007)

2.1.5. Kinema

Kinema is a flavoursome, naturally fermented alkaline traditional food prepared from soybean (Fig.1e). It is a popular savoury dish among the Nepalese, Lepchas and the Sikkimese population of Nepal and the Darjeeling hills of West Bengal and Sikkim in India (Batra and Millner, 1976; Sarkar and Tamang, 1995). It is also a much relished cuisine in some north-eastern states of India and Bhutan. It is known differently in some other states like aakhuni in Nagaland, hawaijar in Manipur, turangbai in Meghalaya and bekanthu in Mizoram.

Traditionally, kinema is prepared by fermenting the overnight soaked yellow seed-coated soybeans for 1-3 days (Fig. 5). The wrapping materials commonly used during the preparation of kinema are either fresh fern (*Athyrium* sp.) or banana leaves (*Musa paradisiaca* L. or *Leucosceptrum canum* Sm.), broad leaves from *Ficus hookeriana* Corner, and *Bauhinia vahlii* Wight and Arn. In some places like Nepal and Manipur, the yellow seed-coated soybeans are replaced by the dark brown coated soybean seeds and *Ficus* leaves are used instead of fern leaves as the packing material. Once the fermentation is complete, a white viscous mass appears on the surface of the grits accompanied with the characteristic sticky texture and typical kinema flavour with a slight ammoniacal odour (Sarkar, 2000). Kinema shows long stringy threads when touched with fingers; the longer the thread, the better the quality of kinema (Tamang, *et al.*, 1988; Sarkar and Tamang, 1994; Sarkar *et al.*, 1994; Nout *et al.*, 1998). Kinema is then cooked to prepare a curry and eaten with boiled rice (Tamang *et al.*, 1988; Sarkar *et al.*, 1993). Kinema imparts a pleasant, nutty flavour to the curry. It is also used to make pickle by frying the dried kinema in edible oil along with salt, onion and chillies. The shelf-life of fresh kinema is 2-3 days during summer and a maximum of one week in winter. The shelf-life of kinema is often lengthened to one month by drying in the sun (Tamang *et al.*, 1988; Sarkar *et al.*, 1994).

Kinema is produced by the natural fermentation of soybeans in which *B. subtilis* is the most dominant bacterium. However, market samples of kinema contained *B. subtilis* as well as *Enterococcus faecium* along with two, yeasts such as *Candida parapsilosis* and *G. candidum* (Sarkar *et al.*, 1994). *Bacillus subtilis* is the sole fermenter during kinema preparation and is not affected by the presence of other opportunistic microorganisms or *E. faecium* in terms of proteolytic activity, ammonia production and final pH of the fermentations (Sarkar *et al.*, 1993). Monoculture fermentation of soybeans by *B. subtilis* produces kinema with superior organoleptic attributes within a much shorter period, compared to the traditional fermentation process (Sarkar and Tamang, 1995).

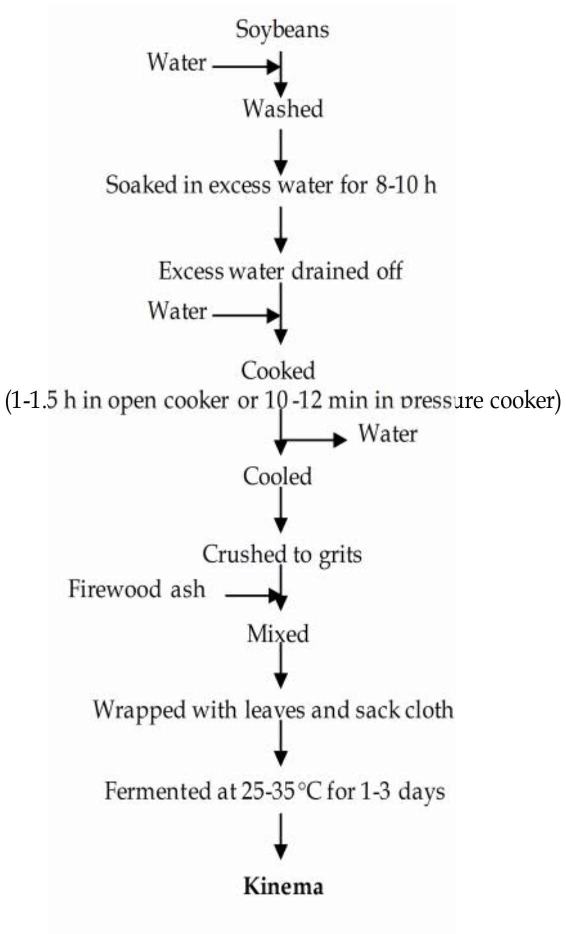

Fig. 5. Flow sheet for kinema production (Based on Sarkar, 2000)

The pH and moisture content of kinema range between 7.7-8.1 and 60.1-63%, respectively (Sarkar *et al.*, 1994; Sarkar and Tamang, 1995).

Fermentation up to 6 h causes a 2.2-fold decrease in the total oligosaccharide content in the soybeans and after another 6 h, there is a further 9.5-fold decrease. The soaked and cooked unfermented soybean contained sucrose, raffinose and stachyose in the ratio of 7.2:1:4.4 which changed to 1.6:1:4.3 after 6 h of fermentation. After 24 h, the levels of all these sugars went below detection limit (Sarkar, 2000). *Bacillus subtilis* was capable of hydrolysing these sugars on its own without any contribution from the accompanying microbiota in the natural fermentation process (Sarkar and Tamang, 1995; Sarkar *et al.*, 1997).

Unfermented, autoclaved soybeans contain a number of volatile compounds like 1-hexanol, 1-octen-3-ol, hexanal, nonanal, decanal, 2-heptanone, 3-octanone, deacyl butanoate, low concentrations of pyrazines, 2-pentylfuran, vinylbenzene, benzaldehyde, 2-methoxyphenol, 4-vinyl-2-methoxyphenol and indoline. The beany flavour of the soybeans is attributed to the presence of a considerable amount of hexanal (Sarkar, 2000).

With fermentation, this hexanal content begins to decrease and ultimately disappeared after 18 h of fermentation by *B. subtilis* (Owens *et al.*, 1997).

A microbiological study of the market samples of kinema revealed the presence of some bacterial pathogens. *Bacillus cereus* s.l. exceeded 10^4 cfu/g in 33% of the market samples. Enterobacteriaceae and coliform bacteria exceeding 10^5 cfu/g was found in 67% of the samples. *Escherichia coli* exceeding 10^5 cfu/g was found in 13% of the samples. *Staphylococcus aureus* was not detected in kinema. However, the diarrhoeal type enterotoxin, produced by many *B. cereus* s.l. strains was inactivated during frying in oil (Nout *et al.*, 1998).

2.1.6. Papad

Papad is one of the Indian traditional food items that can be prepared in advance and served as and when needed. They are staple in every Indian home with most families consuming it daily (Parpia, 2008). It constitutes an important group among traditional foods which are manufactured on a cottage scale (Deshpande *et al.*, 2005). Papad is a favourite item consumed nationwide and also known by several names, such as 'papadam' and 'appalam'. It is a thin crispy wafer-like circular product (Fig 1f) that can be roasted or fried instantly and are served as an appetizer or accompaniment to meal, as a snack or croutons in soups.

Traditionally, papad is prepared from a variety of base ingredients such as blackgram flour alone or a mixture of blackgram flour, lentil flour and rice flour (Fig. 6). Papad

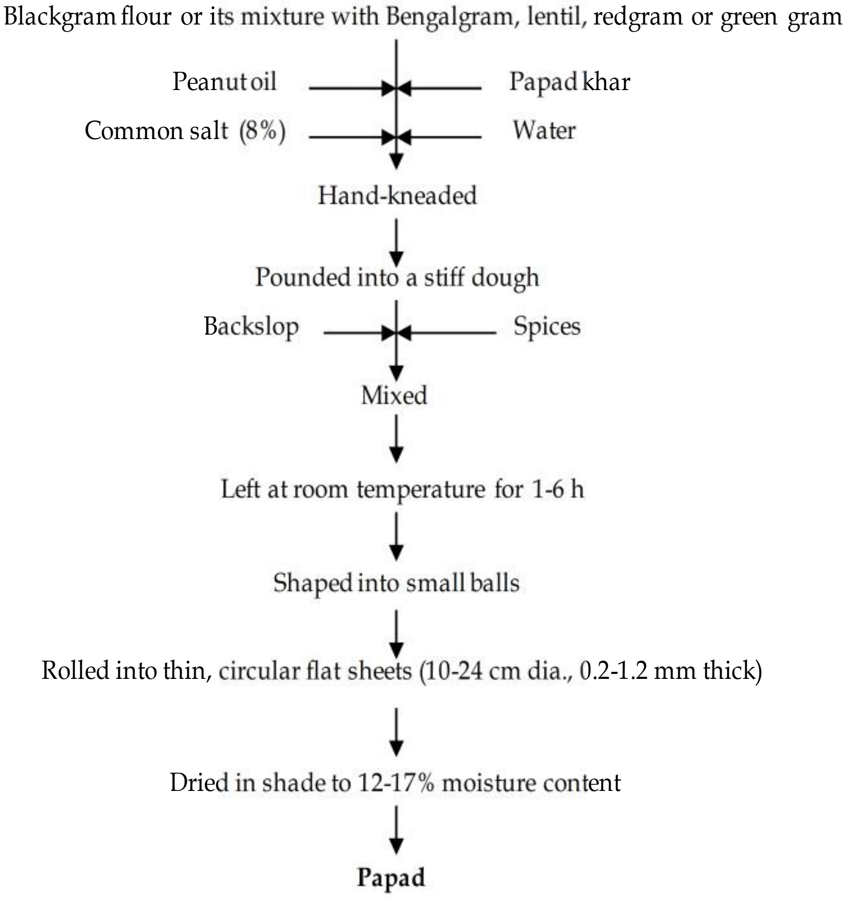

Fig. 6. Flow sheet for papad production (Source: Roy *et al.*, 2007)

khar, a crude carbonating agent, is also added to the flour along with salt and other spices like chillies, cumin, garlic, black pepper, etc. to improve the taste. Papad khar significantly improved the physico-chemical properties of frying medium and also helped to roll the dough well. Patil *et al.* (2005) prepared a substitute for papad khar by combining sodium carbonate and sodium bicarbonate in a ratio of 2:1, and the results indicated that this combination was useful, judged from the quality of fried papad and oil quality after frying.

The chief microorganisms involved during fermentation are *C. krusei* and *S. cerevisiae*. Apart from papads made from pulses, cereal papads are also prepared and known as 'nere happala' or 'mudde happala'. Furthermore, papads are also made from potato, sago, jackfruit, bamboo, tapioca, gelatinized rice flour and wheat flour (Arya, 1990).

A survey of the market samples of papad for their physico-chemical parameters by Shurpalekar *et al.* (1970) revealed wide variations in them. The average weight, diameter, thickness and moisture content varied between 3-25 g, 6-24 cm, 0.2-1.5 mm and 9-17%, respectively.

A sorption study on blackgram papads to study the effect of different additives on the storage stability of papad was conducted by Balasubrahmanyam *et al.* (1973). It was observed that papad adsorbed more moisture in the presence of NaCl in comparison to alkaline additives, thereby limiting its shelf-life. The alkaline salt called happala khar is therefore used but this can be replaced by food grade carbonate which helps in obtaining fried papads of desirable sensory qualities. However, in a previous study by Shurpalekar *et al.* (1972) it was found that common salt helped to soften the dough for easy rolling and proper expansion of papads. Hence a desirable combination of common salt and carbonates at the ratio of 8:1 is suitable for making papad. Use of more than 8% common salt causes it to crystallize on surface at lower relative humidities due to which moisture sorption will increase making the papads susceptible to fungal spoilage.

Papad is traditionally sun dried but with the commercialization of the manufacturing process, various techniques are investigated to increase its output at lesser time. One such innovation is the use of machines to dry the papads. Venkatesh and Shurpalekar (1981) found that drying the papad for 6 h at RH of 66-70% inside a humidity cabinet at 25°C produced pliable papad with a desirable moisture content of 14.5%.

Pruthi *et al.* (1982) conducted a storage study on North Indian spiced urd dal papads at 13-38°C and 30-90% RH. This study revealed that the papads remained in good conditions up to 14.2% moisture level, and mould attacked the papads having 36.5% and 52.1% moisture level at 80% and 90% RH. In order to prevent spoilage, low density polyethylene (LDPE) bags of 200 and 300 gauge were selected for packaging and storage of papad for 3 months.

2.1.7. Sattoo

Sattoo is popularly consumed at breakfast in the eastern states of India such as Jharkhand, Bihar and Orissa. Originally, sattoo is prepared by mixing the powders made from seven cereals, pulses and millets (Fig. 1g). It is used to prepare various dishes and snacks such as "paratha" which is stuffed with a filling of chickpea flour and mashed potatoes. Besides this, sattoo is also used for preparing a refreshing and nutritious drink rich in natural fiber, proteins and carbohydrates but without any added colours or flavours. In the state of West Bengal, sattoo is called "chhatu" and is prepared from Bengalgram dal (Fig. 7).

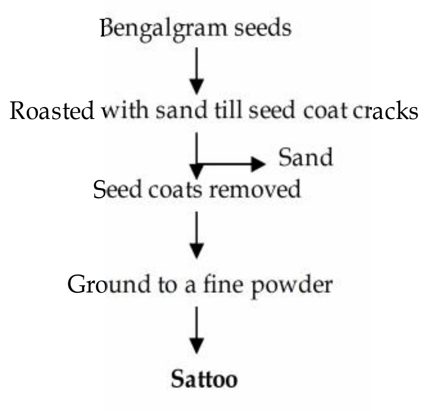

Fig. 7. Flow sheet for sattoo production

2.1.8. Wadi

Wadi is a legume-based ready-to-fry product, popularly consumed in Punjab and West Bengal in India, Pakistan and Bangladesh (Batra 1986; Sandhu and Soni, 1989; Soni and Sandhu, 1990a, 1999; Aidoo *et al.*, 2006; Nout *et al*, 2007). These are dried, hollow, brittle cones or balls (3-8 cm diameter, 15-40 g in weight) (Fig 1h) which are fried briefly in a small amount of oil and used as an adjunct to cooked vegetables and fish curries.

Traditionally, wadi is prepared by soaking and grinding blackgram dal into a smooth batter, and fermenting for 1-3 days at 20-27°C, with or without spices (Fig. 8). However, at present, the batter is not fermented but whisked vigorously immediately after grinding until it becomes light and fluffy due to incorporation of air. Fermentation in such cases accompanies drying. Wadi prepared in Bengal rarely contains any spice except for an occasional addition of black cumin seeds (*Nigella sativa* L.). On the other hand, the Punjabi wadi contains various spices which when added vary from, asafoetida (*Ferula foetida* (Bunge) Regel), caraway (*Carum carvi* L.), cardamom (*Elettaria cardamomum* (L.) Maton), clove (*Syzygium aromaticum* (L.) Merr. and Perry), fenugreek (*Trigonella foenum-graecum* L.), ginger (*Zingiber officinale* Roscoe) and red pepper (*Capsicum annuum L.*) (Sandhu and Soni, 1989). The fermented or whisked batter is hand-moulded into cones or balls and sun-dried for 4-8 days. Dried wadi can be stored in an air-tight container for future use (Batra and Millner, 1974, 1976; Batra 1981; Soni and Sandhu 1999). The gas formed during fermentation is retained by the mucilaginous component of blackgram and a characteristic crust develops on the surface of wadi which looks hollow, with many air pockets (Aidoo *et al.*, 2006; Nout *et al.*, 2007).

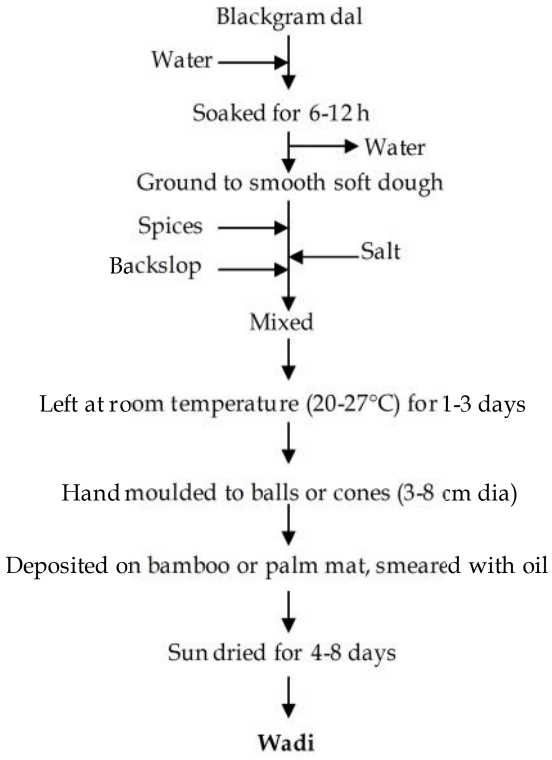

Fig. 8. Flow sheet for wadi production
(Source: Roy *et al.*, 2007)

Leuconostoc mesenteroides is the most abundant bacterium and present in wadi, followed by *S. faecalis*, *Lb. fermentum* and *B. subtilis,* whereas *S. cerevisiae* and *Pichia membranaefaciens* are the most abundant yeasts followed by *Candida vartiovaarai, K. marxianus, T. beigelii, C. kursei* and *H. anomala* (Sandhu and Soni, 1989). Fermentation

Fig. 1. Legume-based traditional foods of India: (a) besan; (b) dhokla; (c) dosa; (d) idli; (e) kinema; (f) papad; (g) sattoo; and (h) wadi

causes an increase in the bacterial and yeast counts in the batter from 10^6 and 10^3 cfu/g to 10^7 and 10^6 cfu/g, respectively (Batra, 1981). With the progress in fermentation, most of the microorganisms, except *Ln. mesenteroides, Lb. fermentum, S. cerevisiae* and *T. beigelii* disappeared (Sandhu and Soni, 1989). The development and prevalence of microbiota are affected by the seasons, summer being more favourable for bacteria and winter for yeasts (Soni and Sandhu, 1999).

The production of acid and gas results in a fall of pH from 5.6 to 3.2 and two-fold increase in the volume of the dough. The LAB are mainly responsible for the acidification of dough, making conditions favourable for the growth of yeasts and for leavening. Acidification is responsible for maintaining the shelf-life of the product (Soni and Sandhu, 1990; Nout *et al.*, 2007).

2.2. Measures to improve safety, quality and shelf-life of foods

2.2.1. Antagonism of LAB against foodborne pathogens

LAB, are microorganisms used widely in the fermentation of different products like meat, vegetables and milk (Makhlouf, 2006; Dortu and Thonart, 2009). They have been used in fermentations because of their ability to bring about desirable changes in the organoleptic qualities of the food as well as inhibit pathogenic and spoilage microorganisms. Since they do not pose any health risk to humans, they are designated as GRAS organisms. These organisms have been isolated from a variety of sources such as grains, dairy and meat products, fermenting vegetables and the mucosal surfaces of animals (Lindgren and Dobrogosz, 1990). The primary inhibition of the undesirable microorganisms in the food is due to pH reduction as a result of lactic acid formation (Daeschel, 1989). In addition, it also produces various antimicrobials, like the low-molecular weight compounds such as hydrogen peroxide, carbon dioxide, diacetyl (2,3-butanedione), and the high-molecular weight compounds such as bacteriocins (Jay, 1982; Klaenhammer, 1988; Piard and Desmazeaud, 1991, 1992). These antimicrobials inhibit the pathogenic and spoilage microorganisms present in the food, thereby enhancing their shelf-life and safety (Aymerich *et al.*, 2000).

Bacteriocins are antimicrobial polypeptides usually with inhibitory activity towards strains closely related to the producer organism (Klaenhammer, 1988). However, in all the cases, the bacteriocin producing cells are not inhibited by their own bacteriocins. The inhibitory spectrum of some bacteriocins includes food spoilage and/or foodborne pathogenic microorganisms such as *Listeria monocytogenes* and *Staphylococcus* (Schillinger *et al*., 1996). Bacteriocins of LAB are considered as safe biopreservatives and differ from most therapeutic antibiotics in being proteinaceous and easily degraded by the proteases in the human gastrointestinal tract (Cleveland *et al*., 2001). Most bacteriocins act on sensitive cells by the formation of transitory pores or ionic channels on the cytoplasmic membrane which allows the free flow of electrolytes, metabolites and water across it. This results in the reduction of the proton motive force which is needed to make ATP by ATP synthase (Cintas *et al*., 2001; Bizani *et al*., 2005).

Bacteriocins are now being explored for their potential utility in agriculture, food and human and animal health sectors (Parada *et al*., 2007; Todorov *et al*., 2011). Some strains of LAB possess the potential to combat gastrointestinal pathogenic bacteria such as *Helicobacter pylori*, *E. coli* and *Salmonella*. There is scope for improving the antibacterial activity and action spectra of bacteriocins as they are ribosomally synthesized peptides, which can be modified and designed accordingly (Parada *et al.*, 2007). Bacteriocins are found to be effective against Gram-negative bacteria such as *E. coli* and *Salmonella*, but usually only when the outer membrane has been destabilized by various agents like detergents or chelating agents and treatments like osmotic shock, low pH, electric field or high-pressure treatment. Several preservation techniques like physical treatments and chemical preservatives increase the permeability of cell membranes, and hence, can be combined with bacteriocins to enhance the effectiveness of some LAB bacteriocins against Gram-negative cells, which are generally resistant (Ananou *et al.,* 2007; Gálvez *et al.,* 2007; Dortu and Thonart, 2009).

In the past few years several bacteriocins associated with LAB have been reported, and some have been extensively characterized. *Listeria monocytogenes* is an emerging foodborne pathogen and the anti-listeria activity of bacteriocins from lactobacilli have also been investigated (Aymerich *et al*., 2000; Messens and De Vuyst, 2002). Bacteriocin-like inhibitory substances have been detected in several bacterial species, including *Streptococcus* sp. (Whitford *et al*., 2001; Yonezawa and Kuramitsu, 2005), *Lactobacillus plantarum* C11 (Moll *et al*., 1999), *Lactococcus lactis* subsp. *lactis* HV219 (Todorov *et al*., 2006), *B. cereus* (Bizani *et al*., 2005) and *Bacillus licheniformis* (He *et al*., 2006). According to Brashears *et al*. (1998) there was a significant decline in the numbers of *E. coli* cells after only 3 days of storage, when the samples were inoculated with *L. lactis* at the rate of atleast 5 × 10^7 cells/ml. The other notable effect of bacteriocin is the decrease in the viable cell numbers of *Lactobacillus sake* from approximately 4 × 10^8 to <10 cfu/ml over a period of 4 h with addition of pentocin TV35b produced from *Lactobacillus pentosus* (Okkers *et al*., 1999). LAB organisms with bacteriocin producing ability have also been isolated from traditional Indian fermented foods such as appam batter and vegetable pickle (Jamuna and Jeevaratnam, 2004). Some species of enterococci like *Enterococcus faecalis* and *E. faecium* are found in dairy products and produce enterocins, a type II bacteriocin with inhibitory activities against *L. monocytogenes*, *S. aureus* and *Clostridium* spp. (Floriano *et al*., 1998; Franz *et al*., 1999; Gelsomino *et al*., 2001). Plantaricins like C19, C and F produced by *Lb. plantarum*, occurring in many cereal-legume fermentations, have shown an inhibitory activity towards a few Gram-positive and Gram-negative bacteria (Atrih *et al*., 1993; Fricourt *et al*., 1994; Gonzalez *et al*., 1994). The addition of plantaricin LP84, produced by *Lb. plantarum* NCIM 2084 to idli batter at 1% (v/w) level was able to retard the growth of pathogens like *B. cereus* and *E. coli* (Jama and Varadaraj, 1999).

Mankind has utilized LAB, generally considered as 'food grade' organisms for thousands of years for preparing various fermented foods. There are many potential applications of protective LAB cultures in various food systems (Holzapfel *et al*., 1995). Bacteriocins from LAB have advantages as the new generation of foods demand minimal processing and discourage the use of chemical preservatives. However, their application in the food preservation processes is limited due to their ineffectiveness against Gram-negative bacteria, and many Gram-positive bacteria associated with food spoilage and foodborne illness.

Food processing methods need to be carefully designed and optimized to ensure safety and quality. RSM deserves special mention in its use as a prime process optimizer. With the advent of sophisticated statistical software, RSM has turned out to be a convenient optimization tool in different fields of science including food science. The other important aspect related to processing of food is a proper knowledge of the sorption characteristics of food. This is important in the designing the equipments and manufacturing processes and understanding the shelf-life and packaging requirements of the food products (Johnson and Brennan, 2000).

2.2.2. RSM: an experimental optimizer to improve food processing methods

RSM is a collection of mathematical and statistical techniques employed for the optimization of a response (output variable) which is influenced by several independent variables (input variables). The response is a function of a set of design variables and can be used in constructing empirical models. Prominent among the models considered are the first-order model and the second-order model.

Originally, RSM was developed to model experimental responses (Box and Draper, 1987). An experiment consists of a series of tests, called runs, in which the input variables are altered in order to identify the reasons for changes in the output response. The response can be represented graphically, either as three-dimensional plots or as contour plots that help visualize the effect of the variables on the responses. Hill and Hunter (1966) outlined four steps for performing a response surface analysis. They are: (a) perform a statistically designed experiment, (b) estimate the coefficients in the response surface equation, (c) check on the adequacy of the equation via a lack-of-fit test and (d) study the response surface in the region of interest. In many experimental situations, a number of responses are measured for each group of design variables. The area of foods and other consumer product research also need to study multi-response data.

The food industry has been a prime user of RSM since the early 1970s. Kahyaoglu and Kaya (2006) optimized the hot-air roasting process for the production of sesame paste by RSM over a range of air temperatures (120–180°C) for various times (30-60 min). The colour parameters, browning index, hardness, fracturability and moisture content of the seeds were used as response parameters to develop predictive models and optimize the roasting process.

Pérez-Francisco *et al.* (2008) optimized the drying process of vegetal pear using RSM and minimized energy resources (cost). Shih *et al.* (2009) used RSM to determine the optimum combinations of three factors, cooking time, inoculated bacterial populations, and fermentation time for producing black soybean natto. Joshi and Rupasinghe (2010) fortifed apple slices with t-resveratrol glucoside by vacuum impregnation of apple slices with a solution containing grape juice using RSM. Chakraborty *et al.* (2011) designed experiments to prepare extrudates from different millet-legume blend ratios of varying moisture content. Second order polynomial models were developed using RSM to understand the effect of the variables on density, sectional expansion index, water absorption index and crispness of extrudates.

Battaiotto *et al.* (2013) optimized the formulation for a cookie sweet cream filling using RSM. The effects of the amount of starch (0–30 %), sugar (40–70 %), gelatin solution (5/55 g water) (0–12 %), and cocoa butter (18–30 %) on the a_w and the textural characteristics (firmness, elasticity, relaxation time, adhesiveness and cohesiveness) of cookie fillings were investigated.

With the recent trends in improvising the product quality of various food products, RSM is gaining the attention of potential users. However, usage lags far behind its potential mainly due to a lack of communication between the researchers and statisticians working in the laboratory and those in industry. The former have little or no access to what is actually going on in industry as far as RSM is concerned. This can be due to the fact that "sometimes, those in industry are dissuaded from publishing for fear of revealing proprietary information" (Hahn, 1984). A better cooperation and communication between academia and industry will definitely ensure better product quality and consumer satisfaction.

2.2.3. Moisture sorption behaviour

Water affects the physical nature and properties of food in a complex way due to their interactions with the different food components (Park *et al*., 2001). The a_w of food reflects the availability of water for its biochemical and microbiological degradation and it is an important control variable in food preservation technology (Van den Berg and Bruin, 1981). The relationship between a_w and the moisture content in a product is often expressed as sorption isotherm. These isotherms determine the stability and quality of the foods. They are important in designing and optimizing operations such as preservation, drying, storing, packaging and mixing (Tsami *et al*., 1990). The typical sigmoidal shaped isotherm has been reported in supplementary foods prepared from cereals and legumes (Baskaran *et al*., 2000), soy proteins (Pan, 2003), dairy products like dudh churpi, milk burfi and sandesh (Hossain *et al*., 2002; Chetana *et al*., 2005; Sahu and Jha, 2008), etc. Various properties of sorbed water are also calculated from moisture sorption data. These properties include the monolayer moisture content (M_c), area of adsorption, amount of sorbed water, density of sorbed water and percent bound water. Iglesias and Chirife (1976) has reported three hundred M_c values of almost hundred different foods and food components. M_c corresponds to the optimal moisture content for storage and for reducing spoilage reactions. They reported that with an increase in temperature, this property showed a declining trend. Ayranci *et al*. (1990) reported M_c-values of 14.0, 17.3, 9.7, 12.6 and 11.7 g/100 g for raisins, currants, figs, prunes and apricots, respectively.

Drying is one of the most widely used method of food preservation, which reduces the water content to a level at which deterioration reactions are greatly minimized (Akpinar and Bicer, 2005; Doymaz, 2007). As a result of this, the shelf-life of foods especially seasonal foods are greatly enhanced. However, improper drying may lead to deterioration of the product quality (Mujumdar, 1997). The moisture sorption isotherm data also help in the thermodynamic analysis and modelling of drying kinetics (Vega-Gálvez *et al*., 2007). The knowledge of the thermodynamic properties can also provide information about the properties of water, food microstructure and physical phenomena on the food surfaces and sorption kinetic parameters (McMinn and Magee, 2003). The sorption isosteric heat, also called latent heat of vapourization in foods, is of practical interest in various processing and storage operations (Mulet *et al*., 2002; Md. Haque *et al*., 2007; Yan *et al*., 2008). It indicates the intermolecular attractive forces between the sorption sites and water vapour (Rizvi, 2005). The total isosteric heat of sorption is the total heat supplied for drying of a material and it may increase to values well above the vaporization of pure water as food is dehydrated to low moisture levels (Rizvi, 2005). Conventionally, isosteric heat of sorption is a positive quantity when heat is evolved during adsorption, and negative when heat is absorbed during desorption. The desorption isosteric energy for reaching a standard moisture content (4%) in the pistachio nut, kernel and powder are 12, 7.6 and 3.8 kJ/mol, respectively (Yazdani *et al*., 2006). The isosteric heat of sorption (ΔH_{st}) of sandesh was found to increase with decreasing moisture content suggesting an endothermic reaction at lower moisture content and at higher moisture contents it approached the value of heat of vapourization of free water (Sahu and Jha, 2008). The sorption heats of Chilean papaya were calculated as 57.35 and 59.98 kJ/mol, for adsorption and desorption isotherms, respectively (Vega-Gálvez and Lemus-Mondaca, 2008). The net isosteric heats of sorption of both peeled and unpeeled ginger powders decreased with increasing moisture content (Alakali and Satimehin, 2009).

Two methods are available for measurement of the heat of sorption. The first is direct calorimetric measurement of the heat evolved, and the second is application of the Clausius-Clayperon equation on the sorption data at different temperatures. The former method is not adopted as precise measurement of the small quantities of heat evolved is required which is difficult (Al-Muhtaseb *et al*., 2002).

Several theoretical, partially theoretical and empirical models have been suggested in the literature for describing the dependence between EMC and RH of the air for a_w of 0-0.95 (Van den Berg and Bruin, 1981). There are some bi-parametric models like: Bradley, Caurie, Day and Nelson, Freundlich, Iglesias and Chirife, Halsey, Halsey simplified, Harkins and Jura, Henderson, Kuhn, Mizrahi, modified Mizrahi, Oswin, Smith, and Wihite and Eiring. Among the tri-parametric models are: Anderson, Anderson-Hall, BET, Chen, Chirife et al. Ferro-Fontan, GAB, Gascoyne-Pethig, Hailwood and Horrobin, Modified Halsey, Haynes, Lewicki, Polinomial, Young and Nelson. Boquet *et al*. (1978) while describing the moisture sorption characteristics of 39 different foods found that the most versatile models were those of Halsey and Oswin. Of all the multi-layer models available for sorption, the BET model, based on the multi-layer adsorption of water, is considered best for interpreting sorption isotherms (Mathlouthi and Rogé, 2003). The equation parameters in BET model have physical significance and the model can fit sorption data up to a_w of about 0.4 (Van den Berg and Bruin, 1981). The GAB model was considered an important improvement on the BET equation, and is applicable up to a_w of 0.9 (Van den Berg and Bruin, 1981; Lewicki, 1997). In spite of a wide number of isotherm equations being reported, only some are widely used, while others have little or no success.

2.3. Foodborne bacterial pathogens

The food that we eat is inextricably linked with microbes. In some cases these microbes produce desirable changes in the quality of foods, while some others spoil the product making it unfit for consumption and also cause illness to humans when they or the toxins they produce are ingested. In spite of this, we tend to accept their ubiquitous presence and have adopted a strategy of containment rather than elimination. Undesirable microorganisms may be present in the raw materials but by employing rigorous processing methods, their population is controlled in foods ready for consumption or sale (Dooley and Roberts, 2000). This is done not only to prolong the shelf-life of the foodstuffs but more importantly to enhance food safety.

Periodic reviews indicate that diseases caused by foodborne bacterial pathogens are a world-wide, and an increasing, public health problem around the globe (CAST, 1994, 1998; POST, 1997; WHO, 1997). Foodborne diseases caused by the ingestion of food contaminated with pathogenic bacteria are a major health problem especially in developing countries. The most common foodborne disease is diarrhoea which varies greatly from mild to severe. It is estimated that around fourteen hundred million cases of diarrhoea occur annually in children under five years, and up to 70% of these cases are due to pathogens transmitted through food (Wood and Adams, 1992; Motarjemi *et al.*, 1993).

Until the mid-1990s, in many countries, 70-80% of bacterial diseases were reported to be caused by salmonellae, *S. aureus* and *Clostridium perfringens* (Dooley and Roberts, 2000). Improved pathogen detection methods have developed concepts like established and emerging pathogens, and their persistence in foods (Varnam and Evans, 1991). In recent years, a number of bacteria, viruses and parasites have been recognized as emerging foodborne pathogens that have caused a number of foodborne disease outbreaks. Some important foodborne pathogens include non-proteolytic strains of *Clostridium botulinum* types B, E and F, *L. monocytogenes* and *Yersinia enterocolitica*, all of which cause little or no deterioration of the food supporting their growth. Some other foodborne bacterial pathogens causing health problems are discussed below.

2.3.1. *Bacillus cereus* group

Bacillus cereus s.l. is a Gram-positive, motile, spore-forming, facultative anaerobic rod. It is ubiquitous in nature, commonly found in soil. It has been isolated from many foods, including cereal and cereal derivatives, spices, milk and dairy products, vanilla sauce, recipe dishes, chicken soup, mashed potatoes, vegetables, rice dishes and dried foods.

Mesophilic strains of *B. cereus* s.l. grow at 10-50ºC with an optimum of 28-35ºC. However, psychrotrophic strains are capable of growing and producing toxin at temperatures as low as 4ºC (Kramer and Gilbert, 1989; Van Netten *et al.*, 1990). The pH range for its growth is 4.3-9.0 (Kramer and Gilbert, 1989) and the minimal a_w required for its growth is 0.912 (Bryan *et al.*, 1981). As it is a sporeformer, it requires quite a severe heat process at about 100°C for 2.7-3.1 min to destroy the spores (Kramer and Gilbert, 1989).

Bacillus cereus s.l., on ingestion, causes two distinctive forms of food poisoning, the emetic form and the diarrhoeal form. Emetic food poisoning is caused by the ingestion of emetic toxin that has been pre-formed in food. It causes general malaise, nausea and vomiting, and occasionally diarrhoea. This type of food poisoning is associated with starchy foods such as cooked rice, pasta and noodles. The emetic toxin is very stable even up to 121°C. So, reheating leftover foods in which these bacteria may have grown and produced toxin does not ensure its safety (Kramer and Gilbert, 1989). On the other hand the diarrhoeal toxin can be destroyed by heating at 56.1°C for 5 min (Kramer and Gilbert, 1989). Diarrhoeal food poisoning is caused from the formation and release of enterotoxin in the small intestine. However, the enterotoxin can also be pre-formed in food. It causes diarrhoea and abdominal cramps, occasionally nausea, vomiting, fever, chills and body aches.

2.3.2. Enteropathogenic *Escherichia coli*

Escherichia coli is a Gram-negative rod. It does not form spores, is motile and is a facultative anaerobe. The organism can grow at 7-43ºC, with an optimum of 37ºC. The conventional detection procedures for faecal *E. coli* in foods use temperatures in the range of 44-45.5ºC. The organism survives well at low temperatures.

The presence of *E. coli* is an indication of faecal contamination of the food and may not cause any illness. However, there are certain serotypes of *E. coli*, known as verotoxigenic *E. coli* (VTEC), which may cause diarrhoeal disease, or more serious forms of illness. They are found in the intestine of dairy cattle and other animals and spread through faecal contamination of water and food, and from person-to-person transmission. The main serotype of verotoxin-producing *E. coli is E. coli* O157. VTEC can cause diarrhoea but in its more serious form, the illness can cause abdominal cramps, bloody stools and vomiting along with acute kidney problems.

2.3.3. *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive coccus. It is non-motile, non-sporing, facultative anaerobe. Humans are an important reservoir of this pathogen. It can grow within at 6.5-50ºC (Halpin-Dohnalek and Marth, 1989) with an optimum of 35-37ºC. The limits for toxin production are, however, narrower than for growth, the optimum being between 40ºC and 45ºC (Tatini, 1973). The pH range and minimal a_w for growth is 4.5-9.3 (Bergdoll, 1989) and 0.83 (Sperber, 1983), respectively.

Some strains of *S. aureus* are capable of producing heat-stable enterotoxins which cause food poisoning. The typical symptoms of *S. aureus* food poisoning are nausea and vomiting with occasional abdominal cramping and diarrhoea. Deaths have occurred amongst children and the elderly, but are rare.

Foods that have been temperature abused prior to consumption are implicated with *S. aureus* food poisoning. Although *S. aureus* is not heat resistant and will be destroyed by pasteurization, the toxin is heat-stable, and has been known to survive a number of commercial sterilization processes.

2.4. Foodborne disease outbreaks

An outbreak of foodborne illness occurs when a group of people consuming the same contaminated food are diagnosed with the same illness. Many outbreaks are local in nature and recognized when a group of people become ill after sharing a meal, but outbreaks are now more widespread, affecting people in many different places and be spread out over several weeks and claiming many human lives and economic losses. Increased virulence of the pathogens due to genetic changes, changes in social attitudes and eating habits of the people, the lack of proper food safety measures, improper food handling and preparation practices in homes or food outlets and an increase in the number of immuno-compromised individuals are some of the several reasons for such foodborne outbreaks. It is essential that a product developer adopts safe-handling and preparation practices while preparing a product as it is only the product developer who has the complete knowledge about the manufacturing process.

In the inter-connected and inter-dependent world of present times, local foodborne disease outbreaks that were once contained within small communities have now become a potential threat to the entire globe (WHO, 2007). In 1991, cholera, caused due to harvesting of contaminated seafood off the coast of Peru, rapidly spread across Latin America resulting in approximately 4,00,000 reported cases and more than 4000 deaths in several countries (Cholera in the Americas. Epidemiological Bulletin of the Pan American Health Organization, 1995, 16 (2) http://www. paho.org/english/sha/epibul_95-98/be952choleraam.htm, accessed on 11 April 2007). According to an estimate made by WHO, microbes cause 25% of the total 57 million annual deaths that occur worldwide and such deaths are higher in the developing countries. In India, foodborne diseases and infections are a serious health hazard claiming many lives every year. Outbreaks of hepatitis, cholera and botulism are reported every year leading to widespread loss of earnings, decrease in work output and physical sufferings. So food safety is an increasingly important public health issue both in developed and developing countries. Most of the foodborne diseases are sporadic and not often reported, but they can take on massive proportions. In India, outbreaks due to *C. perfringens* in which mutton and peas were the food source and *B. cereus* s.l. due to consumption of a bakery product were reported in 1995. In the same year, food poisoning outbreak due to *Salmonella paratyphi A* that affected 33 people, due to vegetarian food was reported from the state of Maharashtra, while outbreaks due to *Salmonella weltevreden* and *Salmonella wein* affecting 34 and 10 people respectively, due to chicken and fish were reported from the city of Mangalore in 2008-2009. An outbreak of *S. aureus* food poisoning due to contaminated "bhalla", a snack made up of potato balls fried in vegetable oil affected more than 100 children and adults in the state Madhya Pradesh in 2007. Enterotoxigenic *B. cereus* s.l. was isolated from 29% of fish samples in a study from the city of Cochin in 2009.

The foodborne disease outbreak, besides taking its toll on the human lives, also adversely affects the economy of a region. With the identification of a single contaminated food ingredient can lead to the recall of tonnes of food products, leading to considerable economic losses for the firms. Coupled with it are the increased medical costs, as well as damage to the tourist industry. In early 2008, an outbreak of bird flu in Bangalore city led to an import ban of Indian poultry products in the Middle East, resulting in losses amounting to thousands of US dollars to the Indian economy (NIDM, 2008).

2.5. Factors leading to foodborne microbial diseases

2.5.1. Contamination

Microorganisms, particularly bacteria, are present almost everywhere and can even survive in extremely inhospitable places such as hot sulphur springs. Hence, the food that we eat is hardly ever free from viable microorganisms. However, certain food processing methods and good hygienic practices adopted during the preparation and handling of the food can reduce the number of organisms present in the food. If all the pathogens come from the same source then it would be easier to control them. Unfortunately, they contaminate the foods from several different sources known as reservoirs of infection, and by a number of routes which are discussed as follows:

The natural microbiota present in plants and animals is important in terms of food safety. For example, the muscle of healthy animals are almost free from microorganisms but the intestines contain a very large and diverse microbiota that can include human pathogens such as *Campylobacter*, *Salmonella* and certain strains of *E. coli*. In the process of butchering these can spread to the other meat surfaces (Adams and Motarjemi, 1999).

Many pathogens occur as natural inhabitants of the soil, water and air. As a result, food produced in the natural environment gets contaminated with various pathogens. For instance, *B. cereus* s.l. spores can be isolated from the soil and the air and can, therefore, contaminate the crops growing in such soils.

Food safety is compromised due to the pollution of the environment with animal or human wastes such as sewage. Raw sewage can contain a wide range of pathogens such as *Vibrio cholerae, Salmonella typhi*, viruses and parasites that are transmitted by the faecal-oral route. These can be transferred to foods if raw sewage is used to fertilize or irrigate the crop fields. Polluted water used in aquaculture can also lead to the contamination of farmed fish and shellfish by pathogens. It can also introduce a wide variety of diseases in people when the water is used for drinking or in food preparation (Adams and Motarjemi, 1999).

Handling of food can introduce and spread pathogenic microorganisms. Food handlers may carry pathogens without experiencing any serious ill-effects themselves. About 20-50% of healthy individuals can carry *S. aureus* which commonly causes skin, nose, throat and skin lesions. Organisms that reside in the gut can be transferred to food if food handlers have poor sanitation habits. The risk is much greater if the food handler is suffering from a gut infection. In many cases, they are not aware of the fact that they are the carriers as they show no symptoms of illness (Adams and Motarjemi, 1999).

The equipments and utensils used in the preparation of food if not properly cleaned can also act as sources of cross contamination, posing a very serious threat to food safety. Raw foods can also contaminate cooked or ready-to-eat foods if they are stored together improperly. Dishcloths left wet can also spread the contaminating organisms as the cloth is used.

From the point of view of the food processor, complete freedom from particular hazardous microbes can never be achieved. Instead, intensive animal production has in some cases worsened the situation considerably, for example with respect to salmonellae. Mechanization of food processing also necessarily does not improve hygiene or the microbiological conditions of foods, partly because the emphasis remains on higher production rather than high standards of hygiene. Hence, food processors should identify the microbes of concern that could be present and consciously take measures to either kill them, or to ensure that they are unable to multiply in their products.

2.5.2. Survival

Bacterial survival is an important aspect of food safety because in many cases, it is only necessary for the bacteria to survive in sufficient numbers in the food to produce illness. For instance, the bacterial pathogen *Campylobacter jejuni* does not normally grow in foods but can survive to cause illness. There are certain conditions that prevent the growth of pathogens but enhance their survival. For example, growth of microorganisms is not possible in dried and frozen foods, but they can survive in them for a long time with only a slight decrease in numbers. With the availability of optimum conditions, like defrosting or hydrating a food for too long at ambient temperatures, will allow these microorganisms to resume growth and multiply very rapidly to levels sufficient to cause illness. However, both growth and survivability of pathogens during storage of a food can be prevented by lowering the pH below the optimum levels required by the pathogens. Heat treatment in the form of cooking is the other most effective method to get rid of the pathogens. The temperature and duration of such treatment varies for different microorganisms but as a general rule, a food should be cooked so that all of it reaches at least 70°C. Although vegetative cells perish with the heat treatment, foodborne toxins like those produced by *S. aureus* and *B. cereus* or most of the mycotoxins and heat-resistant spores produced by *C. perfringens, C. botulinum* and *B. cereus* s.l. survive through it. With the return of favourable conditions like storage of cooked food for too long at an inappropriate temperature, these spores will again germinate and multiply. Therefore, it is difficult to prevent the survivability of pathogens in foods and so it is better to avoid the initial contamination of the foods (Adams and Motarjemi, 1999).

2.5.3. Growth

Foodborne bacterial pathogens have the ability to grow and multiply in a very short period of time in comparison to other pathogens such as parasites and viruses. All these pathogens grow to a sufficient level and cause illness and it is therefore important to understand the factors that influence their growth in food. The factors discussed below constitute a list of intrinsic and extrinsic factors that contribute to the growth of a pathogen.

2.5.3.1. Intrinsic factors

Microorganisms require certain basic nutrients like water, a source of energy, nitrogen, vitamins and minerals for growth and maintenance of metabolic functions (Jay, 2000; Mossel *et al.*, 1995). Foods contain a wide array of nutrients and those microorganisms that can easily utilize them thrive well in the food. In general, the Gram positive bacteria are not able to synthesize certain nutrients required for growth while the Gram negative bacteria can do so from the existing food components (Jay, 2000). In doing so, they first utilize the simple carbohydrates and amino acids, followed by the more complex forms of these nutrients. The highly nutritious foods support the growth of a wide range of microorganisms all competing for the nutrients available. In case of a raw food a pathogen if present may not grow well, but if it is introduced after the food is cooked then it will grow more quickly as cooking will reduce or eliminate the natural microbiota and there will be no competition for growth between them.

Microorganisms need water in an available form to grow in food products. Water availability is often measured or expressed in terms of a_w, which is a scale from 0-1 and describes the degree to which water is "bound" in the food. It also indicates the amount of water available for chemical or biochemical reactions within the food. Most fresh foods have a_w-values between 0.97-0.99 which is close to the optimum growth level of most microorganisms. Hence, bacteria grow at their fastest in such foods. However, their growth can be retarded by modulating the a_w of the foods by either the addition ofsolutes such as salt or sugar or the physical removal of water through drying or baking. Table 2 shows the approximate a_w-values required for growth of pathogens. Total inhibition of growth is however not always necessary as in the case of *S. aureus* that can grow even at a_w as low as 0.83 but it will not produce toxin if the a_w is 0.86 or below.

Increasing the acidity of foods, either through fermentation or the addition of weak acids, has been an age-old preservation technique. In general, pathogens including the spore-formers do not grow, or grow very slowly, at pH levels below 4.6. Table 3 lists the appropriate pH ranges for growth in laboratory media for selected organisms relevant to food. In a study conducted by Byaruhanga *et al.* (1999) the growth and survival of *B. cereus* s.l., a known pathogen commonly found in cereals, during lactic acid fermentation of mageu, a sour maize beverage, has been reported. It was found that in the mageu base inoculated with both the starter culture and *B. cereus*, the acidity developed to pH <4

Table 2. Approximate a_w-values for growth of selected pathogens in food

Organism	Minimum	Optimum	Maximum
Bacillus cereus	0.93		
Campylobacter spp.	0.98	0.99	
Clostridium botulinum types A & B	0.93		
Clostridium botulinum type E	0.97		
Clostridium perfringens	0.94	0.95-0.96	0.97
Enterohemorrhagic *Escherichia coli*	0.95	0.99	
Listeria monocytogenes	0.92		
Salmonella spp.	0.94	0.99	>0.99
Shigella spp.	0.97		
Staphylococcus aureus growth	0.83	0.98	0.99
Staphylococcus aureus toxin	0.88	0.98	0.99
Vibrio parahaemolyticus	0.94	0.98	0.99
Vibrio vulnificus	0.96	0.98	0.99
Yersinia enterocolitica	0.97		

Source: ICMSF (1996)

and 0.10% titratable acidity and the growth of *B. cereus* was reduced from 10^6 cfu/ml to 10^2 cfu/ml within 24 h. In the mageu base to which lactic acid and *B. cereus* were added, the pathogen was inhibited to <10 cfu/ml while in the mageu base to which no starter culture nor lactic acid was added the *B. cereus*, grew to over 10^7 cfu/ml after 12 h. However

Table 3. Approximate pH values permitting the growth of selected pathogens in food

Microorganism	Minimum	Optimum	Maximum
Bacillus cereus	4.9	6.0 -7.0	8.8
Campylobacter spp.	4.9	6.5 - 7.5	9.0
Clostridium botulinum toxin	4.6		8.5
Clostridium botulinum growth	4.6		8.5
Clostridium perfringens	5.5 - 5.8	7.2	8.0 - 9.0
Enterohemorrhagic *Escherichia coli*	4.4	6.0 - 7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2	7.0 - 7.5	9.5
Shigella spp.	4.9		9.3
Staphylococcus aureus growth	4.0	6.0 - 7.0	10.0
Staphylococcus aureus toxin	4.5	7.0 - 8.0	9.6
Vibrio parahaemolyticus	4.8	7.8 - 8.6	11.0
Vibrio vulnificus	5.0	7.8	10.2
Yersinia enterocolitica	4.2	7.2	9.6

Source: ICMSF (1980)

there are exceptions to the notion that low pH does not favour bacterial growth and survival. There are many pathogens like *C. botulinum* that can survive in foods at pH levels below their growth minima. *Clostridium botulinum* was able to produce toxin at a pH level as low as 4.2 (Smelt *et al.*, 1982). Changes in pH can also support the growth of pathogens as observed in case of botulism outbreaks in which the pH increased due to mould growth (ICMSF, 1980).

Redox potential (Eh) is defined as the ratio of the total oxidizing power or the electron accepting capacity to the total reducing power or the electron donating capacity of the substance and is measured in terms of millivolts. Based on their redox potential some foodborne pathogens like *Aeromonas hydrophila, C. botulinum, E. coli* O157:H7 and *C. jejuni,* are classified as aerobes, anaerobes, facultative aerobes and microaerophiles, respectively. Generally, aerobes grow at an Eh range of +500 to +300 mV while facultative anaerobes grow at a range of +300 to -100 mV and the range for anaerobes is +100 to less than -250 mV. The presence of salt and other food constituents can considerably affect the relationship of Eh to growth. For instance, the pathogen *C. perfringens* can grow at an Eh close to +200 mV but in the presence of salt, the limiting Eh-value increases (Morris, 2000).

There are a number of food processing methods like smoking and fermentation that result in the formation of antimicrobial compounds in the food. Maillard reactions between sugars and protein components of certain food can also impart some antimicrobial activity (Mossel *et al.*, 1995).

In food systems, competitive microbiota also affects the growth of microorganisms. Here they compete for nutrition, space for their survival. Organisms that are metabolically more active consume the available nutrients, thereby inhibiting the growth of other organisms. *Staphylococcus aureus* is a poor competitor and gets eliminated whenever there is competition. For instance in raw ground beef, *S. aureus* is often found only in low numbers without enterotoxin production. This is because it cannot compete with the *Pseudomonas-Acinetobacter-Moraxella* association that is always present in this food (ICMSF, 1980). Coliforms, *Pseudomonas* spp. and genera of Micrococcaceae may utilize nutrients more rapidly than staphylococci and make them unavailable. Streptococci can also inhibit staphylocci by exhausting the supply of nicotinamide or niacin and biotin (ICMSF, 1980).

2.5.3.2. Extrinsic factors

Among the extrinsic factors affecting the growth of microorganisms, temperature is a notable factor. All microorganisms grow within a defined temperature range, with a minimum, maximum and optimum level. Table 4 lists the temperature ranges for pathogens of concern (ICMSF, 1996; Lund *et al.*, 2000). Most foodborne pathogens are mesophiles and grow at an optimum temperature of around 37°C. Pathogens do not grow at temperatures above about 60°C and this defines the upper limit of temperature for the growth of foodborne pathogens. Temperature has a dramatic impact on both the generation time and lag period of an organism. It also regulates the expression of virulence genes in certain foodborne pathogens (Montville and Matthews, 2001). For example, the expression of *Y. enterocolitica* proteins controlled by the virulence plasmid is high at 37°C, low at 22°C and not detectable at 4°C.

Besides temperature, time is another important factor determining the growth of microbial pathogens. It is also an important consideration when the shelf-life of a product has to be determined. As per the Uniform Open Dating Regulation, the shelf-life of a perishable food product has to be expressed in terms of a “sell by” date (NIST, 2000). This means that there should be adequate information about the duration for which the product can be consumed. When time alone is used to control the safety of a food product, the duration should be equal to or less than the lag phase of the concerned pathogens. For example, it has been reported that the lag time for growth of *L. monocytogenes* at 10°C is 1.5 days, while at 1°C lag time is approximately 3.3 days. Likewise, the generation time for the same organism at 10°C is 5-8 h, while at 1°C, the generation time is between 62 and 131 h (Mossel and Thomas, 1988).

Table 4. Approximate temperature values permitting the growth of selected foodborne pathogens

Organism	Temperature (°C)		
	Minimum	Optimum	Maximum
Bacillus cereus	5	28 - 40	55
Campylobacter spp.	32	42 - 45	45
Clostridium botulinum types A & B	10 - 12	30 - 40	50
Clostridium botulinum type E	3 - 3.3	25 - 37	45
Clostridium perfringens	12	43 - 47	50
Enterotoxigenic *Escherichia coli*	7	35 - 40	46
Listeria monocytogenes	0	30 - 37	45
Salmonella spp.	5	35 - 37	45 - 47
Shigella spp.	7	37	45 - 47
Staphylococcus aureus growth	7	35 - 40	48
Staphylococcus aureus toxin	10	40 - 45	46
Vibrio cholera	10	37	43
Vibrio parahaemolyticus	5	37	43
Vibrio vulnificus	8	37	43
Yersinia enterocolitica	-1	28 - 30	42

Source: ICMSF (1996); Lund *et al.* (2000); Doyle *et al.* (2001)

In conclusion, food safety during its preparation and storage may differ, depending upon various factors like temperature, time, pH of the product and other conditions. As a general guideline, the product should be prepared under safe and hygienic conditions and well packaged if needed so that the a_w of the product does not get altered in an unfavourable way.