CHAPTER II
REVIEW OF LITERATURE

2.0 IDLI AS A POPULAR FOOD IN SOUTH INDIA

A fermented thick suspension made of a blend of rice (Oryza sativa L.) and dehulled black gram (Phaseolus mango) are used in several traditional foods in Southeast Asian countries. Among them, Idli is very popular in India and Sri Lanka (Sands and Hankin, 1974). Traditionally, for Idli preparation, parboiled rice and black gram dhal 2:1 ratio are soaked separately. After draining the water, rice and black gram are ground independently, with occasional addition of water during the grinding process. The rice is coarsely ground and the black gram is finely ground. Then the rice and the black gram batters are mixed together with addition of 1% to 3 % common salt and allowed to ferment overnight 8 hours at room temperature (about 30°C). Finally the fermented batter is placed in special Idli pans and steamed for 5–8 minutes (Nagaraju and Manohar, 2000).

A starter culture can be defined as a microbial preparation of large number of cells of at least one microorganism to be added to a raw material to ferment food by accelerating and steering the fermentation process. They cause rapid acidification of the raw material through the production of organic acid, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product (Leroy and De Vuyst 2004). The bacteria identified as a part of the microflora for Idli batter fermentation included Leuconostoc mesenteroides, Lactobacillus delbruckii, Lb. fermentum, Lb. lactis, Lb. brevis, Streptococcus faecalis and Pediococcus cerevisiae, which are essential for leavening of the batter and for acid production in Idli and yeasts such as Geotrichum candidum, Torulopsis lolmii, T. candida, Trichosporon pullulans, Candida, C. fragilola, C. kefyr, C. tropicalis, Hansenula anomala and Rhodotorula graminis, which are responsible for pH reduction and may increase the thiamin and riboflavin content (Agrawal et al 2000, Charan and Kadam 1989, Mukherjee et al 1965, Purushothaman et al 1993,
Reddy et al 1981, Soni and Sandhu 1990, Steinkraus 1995, Thyagaraja et al 1992, Venkatasubbaiah et al 1984). In a direction towards reducing the fermentation time of Idli batter and increasing the shelf life of fermented batter, an improved process for the preparation of shelf-stable Idli batter was made available (Varadaraj et al 1999). The purpose and scope of the present study was to develop a suitable combination of starter cultures for Idli batter fermentation and to evaluate the quality of the batter and Idlis for nutritional, textural and sensory quality.

Idli is a low calorie, starchy and nutritious food. Steamed Idli contains about 20.3 percent carbohydrate, 3.4 percent protein and 70 percent moisture (Teniola and Odunfa, 2001). Large-scale production of Idli is carried out in batch compartmental steaming units. This is labour intensive and has limited capacity. With the growing demands for breakfast foods, Idlies are being consumed on a large scale in some Indian institutions such as army, railways, industrial canteens, etc. In recent years there has been a greater interest in making available the traditional foods as convenient foods to meet the growing demands of changing social patterns (Varadharaj et al., 1999).

2.1. IDLI INGREDIENTS

For Idli preparation, the ingredients are thoroughly washed, soaked and finally ground in a stone wet grinder. Major ingredients in Idli are rice, black gram, fenugreek, salt and water. Sometimes castor seeds (Ricinus communis L.) are added as it increases colour and puffiness of the Idli.

2.1.1. Rice

The rice was ground into semolina. Rice semolina contributes microorganisms and also acts as a substrate for microbial activity. The parboiled rice is pre-processed, reducing the time required to presoak before grinding about 4 h on average. Parboiled rice also has gelatinized starch that gives added texture. There are two types of starch in rice, amylase and amylepectin (Chinnaswamy and Bhattacharya 1983). Long grain rice has 22 percent amylase and 78 percent amylepectin, while medium and short grain rice has 18 percent amylase and 82 percent amylepectin (Chinnaswamy and
Bhattacharya 1984). Short to medium grain rice with 80 percent amyllopectin content is used to make soft and fluffy Idlis, which is ideal Idli (Reddy, 1981).

2.1.2. Black gram dhal

After cereals, pulses are the chief sources of proteins in Indian diet. They not only have high protein content but also supplement the low lysine content of cereal proteins. Pulses contain high amounts of protein and carbohydrate, but low level of fat. Protein subunit constitution, amino acid composition and electrophoretic properties of black gram proteins have been reported by Sathe et al. (1983).

The specialty of black gram in Idli preparations is due to the presence of the mucilaginous material, which is generally absent in other edible legumes. The mucilaginous material is a complex carbohydrate containing galactose and arabinose together with small amounts of rhamnose and galacturonic acid. The complex carbohydrate is combined with a protein in mucilage. It is believed that this mucilaginous principle helps in the retention of carbon-dioxide during the fermentation of the thick batter and is thus responsible for the soft spongy honeycomb texture of the Idli. The protein component of the mucilage does not contain the sulphur containing amino acids except traces of methionine (Yajurvedi, 1980). Black gram, the leguminous component of Idli batter, serves not only as an effective substrate but also provides the maximum number of microorganisms for fermentation (Balasubramanian and Vishwanathan, 2007).

Soybean, green gram and chickpea can be substituted for black gram. Wheat and maize can be substituted for rice to make Indian dhokla (Ramakrishnan, 1979 b; Steinkraus, 1996). Idlies prepared by substituting black gram by Bengal gram, green gram, soybean flour or other bean flour will be either less spongy or brittle and may have unacceptable flavours.

2.1.3. Water

Spring water and filtered tap water should be used for soaking of ingredients and Idli batter preparation to avoid chlorine content in the Idli batter. Chlorine in the water can destroy the wild yeast (Caplice and Fitzgerald, 1999).
2.2. PROCESSING OF INGREDIENTS

Processing of ingredients has desirable advantages. Common processing in Idli batter preparation involves washing, soaking, grinding and fermentation.

2.2.1. Washing of ingredients

Raw ingredients are washed in an excess of cold or warm water. A valid concern is that mycotoxins are present in many cereal grains and legume substrates before fermentation. Mycotoxins are produced when the cereal grains or legumes are improperly harvested or stored. During washing of raw substrates before fermentation, many potential toxins such as trypsin inhibitor, phytate and hemagglutinin are destroyed. So, in general, fermentation tends to detoxify the substrates (Steinkraus, 1998).

2.2.2. Soaking of ingredients

Soaking involves extended steeping of raw ingredients in an excess of cold or warm water. Soaking induces the leaching out of water-soluble anti-nutritional factors. Glycosides, alkaloids, phytates, oligosaccharides and tannins are all significantly reduced (Kadam and Salunkhe, 1985), which can be eliminated with the discarded soaking solution, but some metabolic reactions can take place during soaking, affecting the content of some compounds. Although water soluble micronutrients are also lost by leaching, extended soaking has the net effect of enhancing the protein solubility index and the availability of the limiting amino acids of edible grains, as much as 50 per cent or more. Black gram soaked in water has a higher concentration of soluble nutrients to support the growth of lactic acid bacteria.

2.2.3. Grinding of ingredients

500 g of ponmani rice was soaked in 1000 ml of water for 4 h and 150 g of black gram dhal was soaked in 500 ml of water for 4 h. After 4 h of soaking time the black gram dhal was decorticlated and ground to a fine paste by adding sufficient water two an extent of 200-250 % for 30 min. The soaked rice was ground separately to a fine paste by adding sufficient quantity of water to an extent of 50 – 60% for 30 min .The grinding time was found to vary depending upon the type of grinder (table top wet grinder or normal single stone wet grinder) being used. The grinding time also
was found to depend upon the rpm of the grinder and the roughness of the inner surface of the grinder. Normally the grinder time varies between 20 min to 45 min. (Nagaraju and Manohar 2000). Microflora present in wet grinder also contributes to the fermentation (Desikachar et al., 1960).

2.3. FERMENTATION OF IDLI BATTER

The fermentation process mainly depends on the composition of media, microorganisms used for fermentation, temperature of fermentation, pH of the medium, aeration and agitation. A fermentation process is successful when the media contains all essential elements in right proportion with suitable microorganism present in it and when the fermentation is carried out with optimum operating conditions namely (temperature, pH, aeration and agitation). The medium should contain required quantity of carbon sources, nitrogen sources, energy sources, minerals and vitamins (Casida Jr., 1968).

Many commercial important microorganisms are chemoheterotrophs whose energy and carbon needs are satisfied by simple sugars. Instead of purified sugars, crude sources such as beet, cane or corn molasses (50-70% fermentable sugars) are frequently used as carbon and energy sources in industrial fermentation media. A variety of possible nitrogen sources are available including ammonia, urea, nitrate, distiller’s soluble, cereal grains, peptones, meat scraps, soybean meal, casein, yeast extracts and cotton seed meal. Precursors may be added to the medium to improve yield or quality. The fermentation must be operated on the aseptic basis to avoid contamination. The microorganism’s synthesis metabolites simultaneously during the growth period and are called as growth associated fermentation. Sometimes the growth appears in the first step and metabolites are obtained in the second step and are called as non-growth associated fermentation (Peppler and Perlman, 1979; Webb, 1964).

Idli batter fermentation is mixed auto fermentation (Radhakrishna Moorthy et al., 1961). Idli batter fermentation depends on the organisms present in the ingredients and natural contamination by environmental microflora (Desikachar et al., 1960). This contamination occurs during handling of ingredients for batter preparation. Such natural fermentations are not controlled. \textit{Leuconostoc mesenteroides} is the most
commonly encountered bacterium in Idli batter (Nout et al., 1999). Lactic acid bacteria viz., *Lactobacillus lactis*, *Lactobacillus brevis*, *Streptococcus faecalis*, *Pediococcus* and *Leuconostoc mesenteroides* are also involved in the Idli batter fermentation.

Reduction in the fermentation time of the idli batter is of great commercial significance for large-scale idli production and can be potentially achieved by addition of enzymes. The idli batter fermentation process by adding an exogenous source of α-amylase enzyme; 5, 15 and 25 U per 100 g batter of amylase added to the idli batter allowed to ferment. Different parameters were monitored and sensory attributes were also studied and compared with that of the control set (Bharti K Iyer and Laxmi Anathanarayan, 2008)

2.3.1. Factors influencing fermentation of Idli batter

As the fermentation is carried out by microorganisms, the quality and quantity of micro flora is of paramount importance in the Idli batter fermentation. Therefore the factors affecting microbial growth and activity indirectly influences the Idli batter fermentation (Simango, 1997). Rice to black gram dhal ratio of nutrient composition, surface microbes, surface area and soaking time of ingredients used in Idli batter influences fermentation though microbial growth. Rice semolina contributes carbohydrate microorganisms and also acts as a substrate for microbial activity. Chlorinated water or iodized salt used have negative effect on fermentation as it destroys wild yeasts (Caplice and Fitzgerald, 1999).

2.3.2 Starter culture

The higher activity of amylases, and of amounts of B-vitamins and free amino acids attained in yeast-enriched fermentations suggest a positive contribution of yeasts to these constituents. Biochemical characterization of the micro-organisms associated with Idli fermentation (Soni and Sandhu, 1990) confirms the production of amylases by all the yeasts used in the study and the synthesis of vitamins by *Saccharomyces cerevisiae*, *Torulopsis candida* and *Hansenula anomala* as indicated by their ability to grow in vitamin-free medium. Further, most of the yeasts also add to leavening of batters as a result of their ability to ferment several carbohydrates.
The attractive organoleptic qualities attained in some of the yeast-enriched sets are presumably due to the synthesis of aroma-producing compounds by yeasts. Most of the yeasts. These observations corroborate our earlier findings on the fermentation of dosa, another rice to black gram-based food of India (Soni and Sandhu, 1989b). Maximum acidification noted in bacterial-dominated control is perhaps due to the ability of Leuconostoc mesenteroides, Streptococms faecalis and lactobacilli, the principal bacteria associated with traditional natural Idli fermentation, to produce acids from various carbohydrates as indicated by their physiological studies (Soni and Sandhu, 1990).

In a similar study, Venkatasubbaiah et al. (1985) reported that yeasts contribute not only towards gas production resulting in good texture but also towards organoleptic qualities during Idli batter fermentation. They suggested that the role of lactic acid bacteria, including Leuconostoc mesenteroides, is to reduce the pH of batter to a level that is optimum for yeast activity.

2.3.3 Temperature
As temperature increases the rate of Idli batter fermentation also increases sharply up to 40°C - 45°C later on the rate of fermentation may decreases for future increases in the temperature and may be attributed to enzyme denaturation.

For many proteins, denaturation begins to occur at 45°C - 50°C and is severe at 55°C. One physical mechanism for this phenomena is obvious as the temperature increases, the atoms in the enzyme molecule have greater energies and a greater tendency to move. Eventually, they acquire sufficient energy to overcome the weak interactions holding the globular protein structure together and deactivation follows.

A temperature of 25°C – 30°C is favorable for fermentation. Temperature up to 40°C accelerates the rate, but undesirable smell occasionally develops at a higher temperature (Steinkraus, 1983). If the temperature is below 30°C, it will take longer time to achieve acceptable level of fermentation. If the temperature is higher than 32°C, the batter will reach a volume of 250 percentage, two and half time the original volume (Nout and Motarjemi, 1997).
2.3.4 Fermentation time

Idli fermentation is a mixed auto fermentation; organisms present in the ingredients as well as the environment determine the nature of microflora involved (Desikacharr et al., 1960). Black gram is the main ingredient responsible for the characteristic texture of Idli. The surface-active proteins and polysaccharides of black gram are well conditioned to retain a large volume of gases to give soft and fluffy texture (Susheelamma & Rao, 1978). It is observed that, after a certain period of fermentation the batter starts collapsing, and with further days of storage, there is whey separation, resulting in Idlis with a very hard texture. In refrigerated storage, the batter without stabilization or preservatives has a limited shelf life of few days after which there is collapse in batter volume causing whey separation and consequently very hard Idlis.

2.3.5 Addition of Baking Soda

Baking soda is one of the important ingredients necessary for increase the rate of Idli batter fermentation. The Idli batter does not get fermented for a long time in the cold climatic conditions. In such situations baking soda is added to an extent of 0.1% to 1% (w/v) in addition to the common salt in the Idli batter to enhance the rate of fermentation the batter obtained by the addition of baking soda gives Idli with a soft spongy texture and characteristic aroma. Sodium bi carbonate, referred to as “baking soda” is primarily used in fermentation as a leavening agent. Heat causes sodium bi carbonate to act as a raising agent by releasing carbon dioxide.

2.3.6 Whey Protein Concentration

Maltodextrin was found to decrease the viscosity of the batter and was, therefore, not successful in preventing batter collapse, however, probably due to its surface-active properties, it was effective in reducing whey separation (Alexander, 1992).

2.3.7 Additives

Each stabilizer was added at two levels. The stabilizers used in the study were xanthan (0.4 to 2.0 %W), agar (0.4 to 2.0 %W), carrageenan (0.4 to 2.0 %W) and guar gum (0.4 to 2.0 %W). The level of addition of each additive was decided on the
basis of their level of usage in different foods and also considering their regulatory status. The stabilizers were incorporated in the batter before fermentation. Weighed amount of the stabilizer was mixed in a small amount of water and solubilized according to its solubility pattern (Glicksman, 1986b,c), (Bharti K. et. al 2008).

The stabilizer solution was mixed well with the freshly ground batter and homogenized with a hand mixer to distribute the stabilizer uniformly in the batter. The ones that were good in stabilizing batter volume completely eliminated whey separation. At room temperature storage the % whey separation was high compared to that of refrigerated storage. The hydrocolloids are known to stabilize foam by increasing the viscosity of the continuous phase of the foam (Glicksman, 1986a).

2.4. QUALITY OF IDLI

The best quality Idli is defined as the one which is being a maximum sponginess with a high porosity, with uniform consistancey through out the Idli, with completely cooked, with maximum whiteness and having ever lasting taste.

Idli is also served with sambar popularly called as “Idli sambar”. Some times this sambar is served with oil. In star hotels Idli is soaked in sambar for some times and is served as “sambar Idli”. Idli is also served with fish kozhambu, mutton kozhambu and chicken kozhambu. Idli is also served with milagai podi and oil in addition to chutney.

Idli is very soft and spongy and possesses a sour flavour like that of steamed sour dough bread. The effects of processing variables like soaking time, grinding conditions, proportions of ingredients, temperature, time of fermentation and adjuncts on quality of Idli have been reported (Van Veen et al.,1970). The effects of variety, parboiling and aging of rice on the quality of Idli have also been investigated (Sowbhagya et al. 1991). They reported the total and insoluble amylose contents of different varieties of rice, physicochemical properties and sensory attributes of Idli.
2.5. SENSORY ANALYSIS

It is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch and hearing) for the purposes of evaluating consumer products. The discipline requires panels of human assessors, on whom the products are tested and recording the responses made by them. By applying statistical techniques to the results it is possible to make inferences and insights about the products under test. Most large consumer goods companies have departments dedicated to sensory analysis.

Sensory analysis can mainly be broken down into three sub-sections:

- Effective testing (dealing with objective facts about products)
- Affective testing (dealing with subjective facts such as preferences)
- Perception (the biochemical and psychological aspects of sensation)

2.5.1. Effective testing

This type of testing is concerned with obtaining objective facts about products. This could range from basic discrimination testing (e.g. Two or more products differ from each other) to descriptive profiling (e.g. Differentiating the characteristics of two or more products). The type of panel required for this type of testing would normally be a trained panel. Methods of collection and statistical analysis of sensory data include free choice profiling, and Generalized procrustes analysis (GPA).

2.5.2. Affective testing

Also known as consumer testing, this type of testing is concerned with obtaining subjective data, or how well products are likely to be accepted. Usually large (50 or more) panels of untrained personnel are recruited for this type of testing, although smaller focus groups can be utilised to gain insights into products. The range of testing can vary from simple comparative testing (e.g. choice of preference, A or B) to structured questioning regarding the magnitude of acceptance of individual characteristics (e.g. rate the “fruity aroma”: dislike / neither / like).
2.5.3. Perception

Perception involves the biochemical and psychological theories relating to human and animal sensations. By understanding the mechanisms involved it may be possible to explain why certain characteristics are preferred over others. Descriptive analysis as a major branch of the sensory science, and is widely used for collecting people’s sensory opinions on an object being food, cosmetics, apparel items, etc. A typical procedure of descriptive analysis starts from the recruitment of a number of evaluation panellists being either trained experts or native consumers according to the objective of the research. Normally, for descriptive analysis, a minimum of 5 experts are required, while with respect to native panellists, this number should be much bigger. The sensory experiment should be carried out according to standardized techniques and procedures designed before the evaluation. After experiments, statistical analysis is often applied to the interpretation of the sensory results obtained. Consumer testing (sometimes called ‘hedonic testing’) involves having potential consumers of a product evaluate various products and a small number of items on a ballot.

2.6. TEXTURAL CHARACTERISTICS

Ingredients have direct influence on Idli texture. Texture of Idli is an important criterion as it decides the consumer preferences. The use of rice semolina free from flour is found to improve the texture of Idli. The presence of fine rice flour in semolina would induce more adhesiveness or pastiness than is desirable for the Idli texture. For the same reason, parboiled rice, which is relatively hard in texture and would give less flour during grinding, is found to be more suitable than raw rice for making soft and spongy textured Idlís.

Idli batter is foam in which CO₂ molecules are entrapped in a solid-liquid phase. The surface-active proteins from black gram act as surface-active agent and the polysaccharide acts as stabilizing agent. The surface-active agent is found to be the protein globulin and is responsible for the soft and porous texture of Idli. Foams are colloidal systems in which tiny air bubbles are dispersed in continuous phase. Due to the air-water interface, which is a high-energy interface, foam collapses. Surface-
active agents can reduce the interfacial tension, which facilitate the gas-water interface system (Damodaran and Paraf, 1997).

The stabilization of foam is very important with respect to Idli texture. An important way to stabilize the foam is through the formation of a rigid system by means of crystallization, denaturation or gelatinization of the continuous phase. Hydrocolloids are very effective functional agents in making stable foam products. They act as whipping agents to permit aeration and formation of foam and then act to stabilize the interfacial film and thus prevent the leakage of air and collapse of the structure. Addition of 0.1 per cent pre-swollen xanthan to instant Idli batter is known to give desirable Idli texture (Thakur et al., 1995). Idli diet differed not only with regard to fermentation, but also in legume content. Susheelama and Rao (1979) have reported that the special texture of Idli is due to the surface – active principle in the black gram and confirmed to be the nature of a globulin and an arabogalactan type polysaccharide. It is also known to be dialyzable and precipitable with alcohol and acetone. This surface-active extract, if added to other rice – legume batters (containing legume than black gram) could give Idli of similar quality. Gupta and Wagle (1978) have reported the contents of the flatulent materials like stachyose and raffinose in black gram. Investigation on the possible removal of these by the microorganisms in the soaking stages can be envisaged.

2.6.1 Flavour

The presence of aromas representative of diacetyl acetic acid and butyric acid make fermented cereal based products more appetizing.

2.6.2 Biochemical changes during Idli batter fermentation

Biochemical changes during fermentation are brought about by microbial activity. Two significant changes occurring in Idli batter fermentation are leavening and acidification. These two parameters have been used as the criteria for judging the progress of fermentation (Renu Agarwal et al., 2000). As the fermentation progresses, both bacterial and yeast cell numbers increase significantly with a concomitant decrease in pH, and an increased volume of batter, amylase and protease activity (Aidoo et al., 2006).
The process of fermentation increases the nutritive value of Idlis. It also increases the digestibility by virtue of its spongy structure, which facilitates enzyme action during digestion (Salunkhe et al., 1982).

2.6.3 Change in pH and acidity

During batter fermentation, microorganisms produce lactic acid (1.0 %) utilizing fermentable sugars present in the batter so that the acidity of batter increases and pH decreases (5.9–4.1) with time. This is mainly associated with the development of Streptococcus faecalis producing both lactic acid, which lowers the pH and carbon dioxide, which leavens the batter.

With the fermentation time, there is an increase in the percent total acidity value because of the formation of air pockets and leavening action. During 18 and 24 h of fermentation, there is less amount of variation in percent titratable acidity of Idli batter. The percent titratable acidity of Idli batter at different fermentation times ranged between 0.443% and 0.910 %.

2.6.4 Change in volume of batter

Microbes release CO₂ during fermentation. With the progress of fermentation, batter volume sharply increases owing to CO₂ formation. Lactic acid and carbon dioxide make the batter anaerobic and leaven the product (Balasubramanian and Viswanathan, 2007). The volume increases shows a better fermentation and gives a much tastier Idli. The increase in volume of the batter is generally high in summer seasons and low in winter seasons. Additives like baking soda, xanthan and guar gum are added to obtain the required consistancy of the batter in the same fermentation period. (Laxmi Ananthanarayan 2004).

In the later stages of fermentation on the gas present in the batter was found to escape resulting in more leavening and the batter was found to become thinner and thinner. That kind of batter was not suitable for Idli making but proves to give spongy dosas with a better taste. (Soni. S.K.,1985 and 1986)
As fermentation progresses the batter gets contaminated sometimes if it is not preserved properly. Care must be taken to prevent the contamination (Varadaraj et al., 1999).

2.6.5 Change in flavour and aroma compounds

Fermentation also leads to a general improvement in the texture, taste and aroma of the final product (Seo et al., 1996) during cereal fermentations, several volatile compounds are formed, which contribute to a complex blend of flavours in the products (Khetarpaul and Chauhan, 1990). The flavour and taste of acid-fermented foods are believed to be produced mainly by the organic acids together with free amino acids and carbonyl compounds such as acetaldehyde and diacetyl. The desired flavour and aroma may be obtained by selecting various types of Idli rice (Ponmani JR-20 and CO-43) and black gram dhal, varying the intensity of grinding, varying the grinding time and adding additives like fenugreek seeds paste, rava and maida flour etc (Jama and Varadaraj (1999), (Thyagaraja, N., 1991).

The flavour and aroma obtained by mixing rava flour with Idli batter was found to be much tastier and attract all people. The flavour and aroma obtained by mixing fenugreek seeds flour with Idli batter was found to give an elastic nature.

2.7. EVALUATION OF IDLI

The final product Idli analyse is for sensory acceptability and the texture is very important. Both subjective evaluation and objective evaluation of the Idli are used to find the texture and sensory acceptability.

2.7.1 Sensory evaluation of Idli

Sensory evaluation is a scientific discipline used to evoke measure, analyze and interpret reactions to those characteristics of food and food materials, as they are perceived by the sensor of sight, smell, taste, touch and hearing. In general, sensory quality of food is the consumers’ reactions to the physical and chemical constituents of the food in its prepared and formulated form (Ranganna, 1996).
Nisha et al., (2005) reported, based on their study of stabilizer effect on the stability of Idli batter during storage, that the sensory analysis of Idlis made from stored batter stabilized with different stabilizers was done on a 10-point hedonic scale and graded as very good (points 8–10), good (points 5–8), fair (points 3–5) and poor (points 1–3). Idli batter stabilized for 10 days at room temperature and 30 days at refrigerated storage gave good Idlis as evaluated from bulk density, texture, colour and overall acceptability.

2.8. SHELF LIFE OF IDLI BATTER

2.8.1 Storage studies of batter

Batter with stabilizing agents was kept for fermentation for 12 h at 28–30 °C. This batter was then added back to the remaining batter and mixed well without disturbing the foamed batter. The batter was portioned and kept for storage studies at room temperature and refrigerated storage. For room temperature (28–30 °C) and refrigerated (4–8 °C) studies, 40 ml of fermented batter was kept in 50 ml test tubes and sealed with aluminium foil. The batter was studied at the end of 10 days of room temperature storage. For refrigerated storage studies samples were kept for 30 days and at the end of 10, 20 and 30 days samples were withdrawn and evaluated. In each case both the batter as well as Idlis prepared from the batter were evaluated by the methods given bellow.

Lactic acid fermentation contributes towards the safety, nutritional value, shelf life and acceptability of a wide range of cereal based foods (Oyewole, 1997). Renu Agrawal et al. (2000) reported that the desirable flavour compounds such as ketones, diols and acids were found to be present upto 8 days of storage, After 8 days undesirable flavours like sulphurous and oxaloideone compounds, ethanone and thiazole appeared in the batter subsequent of Idlis (final product) prepared from the batter stored at room temperature (30±2°C) upto 3 days.

In refrigerated storage, the batter without stabilization or preservatives has a limited shelf life of few days after which there is collapse in batter volume causing whey separation and gives very hard Idlies. Compared to control batter with no
stabilizers, the batter with stabilizers showed lesser decrease in volume on storage at both refrigerated and room temperature (Puspa and Jha, 2004).

White colour of the Idli decreased and yellowness of Idli increased with time if batter is stored at room temperature. But this rate of colour change was slowed down if the batter is stored in refrigerated condition (Nisha et al., 2005).

2.9. MICROORGANISMS INVOLVED IN IDLI BATTER FERMENTATION

2.9.1. Taxonomy of Lactic acid bacteria (LAB) (Oyewole, O. B., 1997)

According to Aguirre and Collins (1993), the term LAB is used to describe a broad group of gram-positive, non-sporulating, catalase negative, devoid of cytochromes but aerotolerant, fastidious, acid tolerant and fermentative with lactic acid as the major end product of sugar fermentation. The common LAB in food fermentations includes Corynebacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus and Weisella. Of these, Lactobacillus, Leuconostoc, Pediococcus and Streptococcus historically represent the ‘core genera’. The genus Bifidobacterium is often considered to belong to the LAB as it shares some features like fermentative metabolism and lactic acid production (Sabia et al., 2002).

2.9.2. Lactobacillus brevis

The hetero fermentative group of lactic acid bacteria including Lactobacillus brevis produces several compounds namely acetic acid, lactic acid, formic acid, phenylacetic acid, carbonic acid, carbon dioxide, hydrogen peroxide, diacetyl ethanol, bacteriocins, neutrin and reutericyclin. They are present in the fermented products, probiotic milk, cheese and sour dough (Yong and Wood, 1997). Lactobacillus brevis helps in the reduction of the toxic and anti-nutritional compounds.

2.9.3. Lactobacillus lactis

Isolates of Lactobacillus lactis obtained from raw milk samples were compared for the ability to produce hydrogen peroxide (H_{2}O_{2}) at 5°C. The isolates of Lactobacillus lactis from most raw milk samples produced more H_{2}O_{2} than the isolates of other species of lactobacilli from the same samples. Lactobacillus lactis
was prototrophic for alanine, glycine, aspartic acid, asparagine, glutamine, threonine and proline. The lysine requirement was strain dependent. Magnesium was the only essential oligo element (Khetarpaul and Chauhan, 1990).

2.9.4. **Leuconostoc mesenteroides**

These organisms are coccal shaped and they are arranged in pairs and chains. The fermentation of Idli demonstrates a leavening action caused by the activity of the hetero fermentative lactic acid bacterium. They form CO₂ and ethanol or acetic acid in addition to lactic acid, if the glucose is of D (-) type. In Idli batter fermentation, as already indicated, the predominant microorganism responsible for souring as well as for gas production was found to be *Leuconostoc mesenteroides*. These strains fermented glucose, fructose, galactose, mannose, sucrose, maltose and xylose and produce lactic acid as metabolites. However, they failed to ferment arabinose. They are low acid producers, appeared early in the fermentation followed by the high acid producing *Pediococcus cerevisiae* (Mukherjee et al., 1965). *Leuconostoc* are hamless saprophytes and isolated from diverse sources such as grass, silage, grape leaves sauerkraut, batter and spoiled food. They are often used as ‘starter cultures’ for the manufacture of batter, buttermilk and cheese because of the formation of the flavour compound diacetyl (2,3- butanedione) from citrate.

2.9.5. **Streptococcus faecalis**

Gram-positive cocci is a short streptococcus form. The cells are arranged in pairs or chains. They are catalase negative. The organisms are homo-fermentative i.e., the predominant end product of sugar fermentation is lactic acid. They are low acid producers, appeared early in the fermentation followed by the high acid – producing *Pediococcus cerevisiae*. *Streptococcus* is found growing at the start of fermentation, comprising 50 per cent of the total count in 20 h. They are acid producers with no carbon dioxide gas, grew in 6.5 per cent sodium chloride broth at 45°C. They ferment glucose, fructose, galactose, mannose, arabinose, sucrose, maltose, lactose, raffinose, and mannitol (Lewis and Johar, 1953).
2.9.6. Pediococcus cerevisiae

They are coccal shaped and found to occur in pairs and tetrads. They are catalase negative. They grow well at 45°C as homo-fermentative lactic acid bacteria, which from an industrial perspective include species primarily important for meat and vegetable fermentations. These bacteria sometimes dominate populations of nonstarter lactic acid bacteria in ripened cheese and some strains are used as adjunct cultures to improve attributes of cheddar and mozzarella cheese. Unfortunately, *Pediococci* typically are unable to ferment lactose. As an example, lactose positive (*Lac<sup>+</sup>*) *Pediococcus acidilactici* and *Pediococcus pentosaceus* may be suitable replacement cocci for *Streptococcus thermophilus* in Italian starter blends.

2.10 ROLE OF MICROORGANISMS IN IDLI BATTER FERMENTATION

2.10.1. Role of Lactic acid bacteria in Idli batter fermentation (Steinkraus KH., 1983)

In Idli batter fermentation, lactic acid bacteria have three different purposes, namely (i) to improve the safety of the fermented products by inactivating pathogens (ii) to improve the product stability and shelf life by inhibiting undesirable changes brought about by spoilage microbes of biotic reactions and (iii) to provide diversity by the modification of raw material to obtain new sensory properties. Finally, microorganisms other than LAB, such as catalase-positive and yeast are involved in the production and stability of the desired sensory properties (Lucke, 2000).

LAB fermentation has preservative effect on the food products because they produce bacteriocins (Daly, 1991). The bacteriocins from Generally Recognized as Safe (GRAS) lactic acid bacteria (LAB) have arisen a great deal of attention as a novel approach to control pathogens in food stuffs.

2.10.2. Role of yeast in Idli fermentation

The role of yeasts in Idli batter fermentation is controversial. Although the fermentation is reported (Ramakrishnan, 1979) to be entirely due to heterofermentative *Leuconostoc mesenteroides*, later work has shown yeast involvement in the fermentation (Venkatasubbaiah *et al.*, 1984 a). The major functions of the fermentation include the leavening of the batter and the improvement of taste and nutritional value of Idli. The role of lactic acid bacteria is to reduce the
pH of the batter to an optimum level (pH 4.1-4.5) for yeast activity (Hugas and Monfort, 1997). Yeasts help in the degradation of starch (which cannot be carried out by *Leuconostoc mesenteroides*) into maltose and glucose by producing extra cellular amylolytic enzymes. Yeasts also produce carbon dioxide and play a significant role in leavening.

Fermentation of batter by inoculating the ingredients with individual yeasts and in combination with *Leuconostoc mesenteroides* reveals that yeasts contribute not only to gas production, resulting in good texture, but also towards sensory qualities (Jama and Varadharaj, 1999). The higher activity of amylases, levels of B vitamins and free amino acids attained in yeast-enriched fermentations suggest the positive contribution of yeasts to these constituents. It can be said that even if the yeasts are not essential for the leavening of Idli batter, they are certainly important for the desirable organoleptic qualities and on nutritional grounds. The possible synergism between bacteria and yeasts remains to be determined (Aidoo *et al.*, 2006).

There are about thirty species of *Saccharomyces* (Class: Ascomycetes). Yeast flora responsible for gas production during Idli batter fermentation; have been found to consist of *Saccharomyces cerevisiae*, *Torulopsis candida*, *Torulopsis holmii* and *Trichospora pullulans*. Sources of the yeast strains are the surface of stone grinders used for preparation of batter and also parboiled rice used in batter production. Of several yeast strains identified, *Torulopsis holmii* had higher fermentation rate compared to other strains (Venkatasubbaiah, 1984 a). Lewis and Johar (1953) have found a strain of yeast *Torulopsis holmii* besides strains of lactic acid bacteria in the fermenting batter have isolated *Torulopsis candida* and *Trichosporan pullulans*.

### 2.11 RESPONSE SURFACE METHODOLOGY (RSM)

RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize this response. RSM has important application in the development and formulation of new products, as well as in the improvement of existing product. It defines the effect of the
independent variables, alone or in combination, on the responses. In addition to analysing the effects of the independent variables, it provides a mathematical model, which describes the relationships between the independent and response variables (Myers and Montgomery, 1995).

Maximum and minimum predictor values were chosen after carrying out preliminary trails. Five levels of each predictor variable were incorporated into the developed design. RSM reduces the number of experimental trials needed to evaluate multiple parameters very accurately and their interactions (Durgadevi et al., 2012).

The RSM technique was used commonly for optimization of multi parameters processes, particularly for biotechnology applications. Very few researches have employed RSM for food processing applications (Ravi and Susheelamma, 2005).