INTRODUCTION AND REVIEW OF LITERATURE
MODIFIED ATMOSPHERE PACKAGING OF READY TO COOK IDLI BATTER

1.1 INTRODUCTION

Fermented cereals, pulses and meat are consumed throughout the world both as means of preservation by identifying their texture, aroma and flavour in addition to their health benefits. Idli, one of the most common traditional cereal-pulse based fermented breakfast product is consumed mostly in the southern part of India and Sri Lanka. Idli is the most preferred breakfast product due to its soft texture, mild pleasant flavour and aroma, easy digestibility and known health and nutritional benefits. Even with rapid social transition, idli still remains to be the choice of breakfast for the population either at home or home-away. With rapid urbanization, idli is one of the most served products in the restaurants and catering establishments. Idli being a lactic acid bacteria fermented product, is traditionally prepared by rice and dhal soaked, ground and fermented before steamed and consumed. With rapid urbanization, Ready to cook, packaged fermented batter is made available in the cities by household vendors, frequently with quality and safety problems. In spite of heavy demand there has not been proper commercialization of the product due to lack of set quality parameters as well as issues with the shelf life of the product. There has also been an effort made to develop starter cultures aimed at preparing the final product with consistent sensory parameters. However these starter cultures did not become popular due to their inferior sensory characteristics. Efforts are being made by various research groups to develop appropriate and acceptable starter cultures for idli.

In spite of heavy demands organized food industries have not taken up the commercialization of ready to cook idli batter in view of short shelf life. Even the commercial prospects of scientifically developed starter cultures will remain curtailed till the shelf life of ready to cook batter is considerably extended.

In the pursuit of extension of shelf life of ready to cook idli batter, a microbiologically dynamic fermentation medium with other several factors needs to be kept in mind. The product should be close to the natural as any change in the dynamics of the fermentation flora could lead to unacceptable product characteristics. Although only a few fermented
products or preserved with Modified Atmosphere Packaging (MAP), most of them are packaged after the fermentation/maturation process.

In this work an effort has been made to scientifically optimize the process of preparation of ready to cook idli batter and packaging the ground product in the initial stages of fermentation with optimized gas combinations supporting slow but desirable fermentation process extending the product shelf life without affecting texture and sensory characteristics.

1.2 REVIEW OF LITERATURE

The review of literature of the current study is done under the following headings:

1.2.1. Significance of fermented foods

1.2.2. Positive health outcomes of breakfast consumption

1.2.3. Idli and its properties

1.2.4. Rice - A staple food grain in idli making

1.2.5. Black gram - A protein source in idli making

1.2.6. Oligosaccharides in foods

1.2.6. Shelf life of fermented foods

1.2.1. SIGNIFICANCE OF FERMENTED FOODS

Fermented foods are those foods which have been subjected to the action of microorganisms or their enzymes to produce desirable biochemical changes and results in significant modification to the food. Fermented foods provide variety to the diet supplying nutrients predominantly proteins and amino acids. The process of fermentation also aids in detoxification (Campbell-Platt, 1994). Fermentation plays diverse roles like enhancing the diet with wide range of flavors, aromas and textures, preserving substantial amounts of food through lactic acid, alcoholic, acetic acid, alkaline fermentations, enriching food substrates with nutrients and also reducing cooking times.
and fuel requirements (Steinkraus, 1994). Lactic acid fermented foods are common in tropical countries and these foods give improved organoleptic qualities (Cookey et al., 1987).

Fermentation affords a natural way to reduce the volume of the material to be transported, abolish undesirable components, enhance the nutritive value and improve appearance of the food, decrease the energy required for cooking and make a safer product (Simango, 1997). Fermented foods are produced worldwide by various manufacturing techniques, raw materials and microorganisms. However, there are only four main fermentation processes which include alcoholic, lactic acid, acetic acid and alkali fermentation (Soni and Sandhu, 1989). Alcohol fermentation results in the production of ethanol, and yeasts are the predominant organisms (e.g. wines and beer), fermented milks and cereals are mainly conceded out by lactic acid bacteria. A second group of bacteria significant in food fermentation is the acetic acid producers (Acetobacter species). Acetobacter sp. converts alcohol to acetic acid in the presence of excess oxygen (McKay and Baldwin, 1990). Likewise, fermentation significantly improves the protein quality as well as the level of amino acid particularly lysine in maize, millet, sorghum, and other cereals (Hamad and Fields, 1979). Fermentation also leads to improvement in the shelf life, texture, taste and aroma of the final product. During cereal fermentation a number of volatile compounds are formed, which contribute to a composite blend of flavours in the products (Chavan and Kadam, 1989).

The presence of aromas representative of acetic acid and butyric acid make fermented cereal based products more appetizing. Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in various parts of the world. Some are utilized as colorants, spices, beverages and breakfast or light meal foods, while a few of them are used as key foods in the diet. The microbiology of many of these fermented products is quite complex and not known. In most of these products, fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may participate in parallel, while others act in a sequential manner with exchanging dominant flora during the course of the fermentation. The common bacteria involved in fermentation are species of Leuconostoc,
Lactobacillus, Streptococcus, Pediococcus, Micrococcus and Bacillus. The fungi genera Aspergillus, Paecilomyces, Cladosporium, Fusarium and Saccharomyces (yeast) are most often found in certain products (Blandino et al., 2003).

1.2.2. POSITIVE HEALTH OUTCOMES OF BREAKFAST CONSUMPTION

Studies done by Agostoni, et al., (2010) disclose that breakfast represents a healthy habit and association with positive health outcomes proves breakfast should be consistent with local and family dietary behaviours. Policies and interventions supportive of breakfast consumption are therefore encouraged. According to neurobehavioral data, the good example of parents and access to a variety of palatable and pleasant breakfast foods should drive children to choose self select breakfast models with balanced composition, while respecting recommended dietary allowances. A balanced macronutrient composition, the proposition of a variety of models leading to a total energy density preferably within lower ranges (< 1 to 1.5), as well as glycemic indices in the lower range for the same food class, could emphasize the positive short and long term health outcomes which is now attributable to breakfast.

Regular breakfast consumption can have a multitude of positive health benefits, yet young people are more likely to skip breakfast than any other meal. Given the evidence that dietary behaviours established in childhood and adolescence track into adulthood along with evidence that breakfast skipping increases with age, identifying correlates of children's and adolescent's breakfast behaviour is imperative. Few studies have examined the same specific family correlates of breakfast consumption, limiting the possibilities of drawing strong or consistent conclusions. Parental breakfast eating and living in two-parent families were the correlates supported by the greatest amount of evidence in association with adolescent's breakfast consumption. The results suggest that parents should be encouraged to be positive role models to their children by targeting their own dietary behaviours and that family structure should be considered when designing programmers to promote healthy breakfast behaviours (Pearson et al., 2009).

Eating breakfast is important for the health and development of children and adolescents. Reports on the findings of an Australian survey of 699 thirteen year old concerning the
extent of skipping breakfasts, indicated approximately 12 percent of the sample skipped breakfast. Gender was the only statistically significant socio demographic variable, with females skipping at over three times the rate of males. Skippers were more likely to be dissatisfied with their body shape and to have been on a diet to lose weight than were those who ate breakfast (Shaw, 1998).

Wesnes et al., (2003) reported in their study that a typical breakfast of cereal rich in complex carbohydrates can help maintain mental performance over the morning. Frequency of breakfast and cereal consumption decreased with age. Days eating breakfast were associated with higher calcium and fiber intake in all models, regardless of adjustment variable. After adjusting for energy intake, cereal consumption was related to increased intake of fiber, calcium, iron, folic acid, vitamin C, zinc, and decreased intake of fat and cholesterol. Cereal consumption as part of an overall healthful lifestyle may play a role in maintaining a healthful Body Mass Index (BMI) and adequate nutrient intake among adolescent girls (Barton et al., 2005).

1.2.3. IDLI AND ITS PROPERTIES

1.2.3.1. Nutritional composition of idli

Idli, a very popular fermented breakfast food consumed in the Indian subcontinent, consists mainly of rice and black gram. Idli fermentation was carried out in the conventional way in a batter having rice to black gram in the ratios of 2:1, 3:1 and 4:1 at room temperature. It makes an important contribution to the diet as a source of protein, calories and vitamins, especially B-complex vitamins, compared to the raw unfermented ingredients. It can be produced locally and used as a dietary supplement in developing countries to treat people suffering from protein calorie malnutrition and kwashiorkor (Nagaraju and Manohar, 2000).

Adding Saccharomyces cerevisiae, along with natural bacterial flora of the ingredients, was the best method for standardizing idli fermentation in terms of improved organoleptic characteristics, leavening and nutritional constituents. Traditional idli fermentation involves several bacteria and yeasts, contributed by the ingredients rice (Oryza sativa), black gram (Phaseolus mungo) and the environment, with overall
dominance of the former in bringing about various changes. Idli fermentation is accompanied by an increase in total acids, batter volume, soluble solids, reducing sugars, non protein nitrogen, free amino acids, amylases, proteinases and water soluble vitamins B$_1$, B$_2$ and B$_{12}$ contents, thus accounting for improved digestibility and nutritional value of the staples. Novel idli batter prepared by replacing conventional black gram with other legumes, revealed significant change but with difference in the levels of some biochemical constituents (Soni and Sandhu, 1989).

Idli, Dhokla, Nan, Kulcha, Bread, Jalebi, Bhatura, Bhalla, Dosa, Gulgule and Wadian were prepared in the laboratory using traditional fermentation techniques. The fermented batter of idli and dosa contained higher amount of available lysine, cystine and methionine. After processing, maximum retention of lysine, methionine and cystine was observed in steamed idli (Riat and Sadana, 2009).

Growth and nitrogen balance feeding trials were conducted with rats to determine the protein quality of idli, a fermented steamed cake prepared from beans (*Phaseolus vulgaris*) and rice. Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER) and Relative PER (RPER) of fermented idli diets were significantly lower (p<0.05) than the FER, PER and PER of unfermented idli diets. The Digestibility Coefficient (DC) and Net Protein Utilization (NPU) of fermented idli diets were significantly lower (p<0.05) than the DC and NPU of unfermented idli diets. Biological Value (BV) of fermented and unfermented idli diets was similar to the BV of a caesin control diet. Fermentation does not improve the protein quality of idli prepared from beans and rice (Joseph and Swanson, 1994).

1.2.3.2. Physico chemical parameters of idli

Balasubramanian and Viswanathan (2007a) has shown that idli batter was prepared from soaking polished parboiled rice and decorticated black gram. The blend a ratio of 2:1, 3:1 and 4:1 (v/v) and the batter was allowed for fermentation (0, 6, 12, 18 and 24 h) adding two percent of salt. Other legumes such as soybeans and Great Northern beans could be substituted for black gram in preparation of idli (Reddy *et al.*, 1981). Fermentation time of the batter varies from 14 to 24 h with overnight fermentation being the most frequent
time interval. The ingredients for idli are carefully washed, soaked in water separately, grounded, mixed, and finally allowed to ferment overnight. When the batter has been raised sufficiently, it is cooked by steaming and served hot. The product has a very soft and spongy texture and a desirably sour flavour and taste. The black gram was washed several times, first with tap water and finally with distilled water to remove the surface microorganisms. These were found to produce off flavour in the idli unless they were washed out (Mukherjee et al., 1965).

Mukherjee et al., (1965), studied the fermentation of idli batter. The microorganisms responsible for the characteristic changes in the batter were isolated and identified. Although there is a sequential change in the bacterial flora, the predominant microorganism responsible for souring, as well as for gas production, was found to be Leuconostoc mesenteroides. In the later stages of fermentation, growth of Streptococcus faecalis and, followed by Pediococcus cerevisiae became significant. The fermentation of idli demonstrates a leavening action caused by the activity of the hetero fermentative lactic acid bacterium, L. mesenteroides. As far as is known, this is the first record of a leavening action produced exclusively by the activity of a lactic acid bacterium.

Idli is traditional fermented rice and black gram based breakfast food of South India. Idli batter was prepared from soaking polished parboiled rice and decorticated black gram for 4 h at 30 ± 1°C in water. The soaked mass was ground to 0.5 to 0.7 mm particle size batter using wet grinder with adequate amount of water. The idli batter parameters such as bulk density, pH, total acidity, flow behaviour index and consistency coefficients were studied for different fermentation times and blend ratios. The bulk density, pH and percentage total acidity of batter during different fermentation times and blend ratios ranged between 0.94 and 0.59 g/cm³, 5.9 and 4.1 and 0.443 and 0.910%, respectively. The consistency coefficient at any fermentation time shows increasing trend as the rice to black gram ratio increased. The flow behaviour index indicated strong non-Newtonian fluid behaviour (pseudoplastic) of idli batter at different fermentation times and blend ratios (Balasubramanian and Viswanathan 2007a).

The rheology of the idli batter was assessed using a Brookfield viscometer having disc spindles. Power law model with yield stress adequately fitted the data. Yield stress values
were in the range of 13-43 Pa and reached a maximum value at 7 h of fermentation. Flow behaviour indices were in the range of 0.287-0.605. Flow behaviour indices at 23 h were significantly lower than those at 0 h. Consistency index values, at any fermentation time, increased as the rice to black gram ratio increased. Mean particle size ranged from 500 to 600 micro meter and there was no definite trend noticed with respect to time of fermentation and rice to black gram ratio. There was a steep change in volume increase after 4-h fermentation (Nagaraju and Manohar, 2000).

The idli batter comprises lactic acid bacteria and yeasts and causes an improvement in the nutritional, textural and flavour characteristics of the final product. The desirable flavour compounds such as ketones, diols and acids were found to be present up to eight days of storage, whereas undesirable flavours like sulphurous and oxazolidone compounds, ethanone and thiazole appeared in the batter subsequent to six days of storage. The sensory attributes of idli (final product) prepared from the stored batter related well to the determined flavour profile (Agrawal et al., 2000).

The work done by Nisha et al., (2005) stabilized the idli batter at room temperature (28-30°C) and refrigerated storage (4-8°C) by using various hydrocolloids and some surface-active agents. The batter was evaluated in terms of decrease in volume, and whey separation. While hydrocolloids gave good stabilization, surface-active agents failed to stabilize the batter and they reduced whey separation. Among the various hydrocolloids, 0.1 percent guar gave best batter stabilization, and idli made after ten days of room temperature and 30 days of refrigerated storage of batter were found to be of acceptable quality.

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 study done by Iyer and Anathanarayan, (2008) was undertaken to explore the possibility of expediting 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 was allowed to ferment. Different parameters were monitored and sensory attributes were also studied and compared with that of the control set. The fermentation
time was reduced from a conventional 14 h to 8 h and the sensory attributes of the final product were also successfully maintained.

Texture Profile Analysis (TPA) test was performed for idli, making cylinder samples (13.5 mm diameter, 10 mm long) of idli. In Pearson correlation matrix, majority of the parameters were positively correlated at \( p<0.01 \) and \( p<0.05 \). The firmness value positively correlated with gumminess and chewiness, which depicts the soft nature of idli (Balasubramanian and Viswanathan 2007b).

1.2.4. RICE - A STAPLE FOOD GRAIN IN IDLI MAKING

Cereal grains particularly rice, form a major source of dietary nutrients for all people, particularly those in the developing countries. However, compared with animal foods, nutritional quality of cereal grains is inferior due to lower protein content, deficiency of certain essential amino acids, lower protein and starch availabilities, and the presence of some antinutritional factors. Fermentation of cereals for a limited period of time improves amino acid composition and vitamin content, increases protein and starch availabilities, and lowers the levels of antinutrients. The traditional foods prepared by fermentation of cereals in different parts of the world are briefly described and future research needs to improve their nutritional contribution are addressed (Chavan et al., 1989).

Cereals are deficient in lysine, but are rich in cysteine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulphur containing amino acids. Thus, by combining cereal with legumes, the overall protein quality is improved (Campbell-Platt, 1994). Fermented foods prepared from cereals and legumes are an important part of the human diet in Southeast Asia and parts of East Africa. The popularity of legume based fermented foods is due to desirable changes including texture and organoleptic characteristics. Improvement in digestibility and enhancement of keeping quality, partial or complete elimination of anti-nutritional factors or natural toxins, increased nutritive value, and reduced cooking time (Joseph, 1994).

Cereal grains constitute a major source of dietary nutrients all over the world. Although cereals are deficient in some basic components, fermentation may be the most simple and
economical way of improving their nutritional value, sensory properties, and functional qualities. Products produced from different cereal substrates (sometimes mixed with other pulses) fermented by lactic acid bacteria, yeast and/or fungi (Blandino et al., 2003).

Rice colour changes from white to amber during parboiling (soaking and steaming). Colour parameters indicated that, during soaking, yellow bran pigments leaches out in the water. The levels of the Maillard precursors (i.e., reducing sugars and free α-amino nitrogen (FAN)) depends on soaking temperature and time: leaching of RS was compensated by enzymatic formation for long soaking times (>60 min), while proteolytic activity was too low to compensate for FAN leaching. Parboiled rice soaking under nitrogen, oxygen, or ambient conditions and determination of polyphenol oxidase activity allowed to conclude that the effect of enzymatic colour changes on the soaked rice colour was rather small. Colour measurements of brown and milled mildly, intermediately, and severely parboiled rice samples showed that both brown and milled rice samples were darker and more red and yellow after parboiling and that the effect depended on the severity of parboiling conditions. Furthermore, steaming affected the rice colour more and in a way opposite to that observed in soaking (Lamberts et al., 2006).

Parboiled brown rice contained considerably more Reducing Sugars (RS) but less sucrose and Free Amino Acids (FAA) than raw brown rice. On milling, there was considerable loss of sucrose and FAA from raw rice, but very little from parboiled rice; reducing sugars changed little in either. Processing conditions affected the contents of sugars and FAA. Maximum increase in RS and decrease in sucrose content occurred after soaking at 60°C. Controlled incubation of rice flour, intact grain, separated germ and deemed rice in water showed that considerable changes in sugars and FAA occurred in all cases, the magnitude depending on the circumstances, but a greater part of the sugars leached out into the water during soaking (Ali and Bhattacharya, 1980).

Grinding characteristics of raw and parboiled rice were evaluated in various wet grinding systems like, mixer grinder, stone grinder and colloid mill. The duration of grinding had inverse effect on the particle size and direct impact on the starch damage as well as energy consumption in batch grinders. Stone grinder was the least energy efficient and specific energy consumption for grinding raw rice (160.6 kJ/kg) was nearly twice as that
of mixer grinder (74.9 kJ/kg). Parboiled rice required longer duration of grinding compared to raw rice, consequently specific energy consumption was higher (~220 kJ/kg) (Sharma et al., 2008). Wet grinding is a critical step in the preparation of batter based traditional food products. It involves both physical and chemical changes while dry grinding is a mere size reduction operation. In wet grinding of cereals, the protein matrix holding the starch granules is destroyed, releasing the starch granules from the protein network (Kent and Evers, 1994). A colloid mil was comparatively evaluated with domestic wet grinding systems, namely, a mixer grinder and a stone grinder for grinding of raw rice, parboiled rice and black gram. The wet ground samples were finer in particle size compared with dry ground samples. The starch damage was the least in black gram followed by raw rice and parboiled rice in dry grinding. In wet grinding, the starch damage in black gram as well as raw rice remained more or less same whereas the parboiled rice showed greater damage. Parboiled rice required 2.5 to 3 times more energy (216-252 kJ/kg) as that of raw rice (72-108 kJ/kg) for grinding in the mixer grinder and the stone grinder (Solanki et al., 2005)

The nutritional quality of wild rice tends to be comparable with other cereals characterized by a high content of starch and protein and a low fat content. As a whole grain, wild rice is also a good source of dietary fibre (Qiu et al., 2010). The presence of Streptococcus faecalis in the fermented batter, the presence of pharmacological active amines such as thiamine was expected but they were not detected (VanVeen, et al., 2008).

Parboiled brown rice contained considerably more (RS) but less sucrose and FAA than raw brown rice. On milling, there was considerably loss of sucrose and FAA from raw rice, but very little from parboiled rice; reducing sugars and FAA. Maximum increase in RS and decrease in sucrose content occurred after soaking at 60°C (Ali et al., 2007). Larsen et al., (2000) opines that rice is an important crop, forming a staple food for many of the world’s population. A study showed there was an effect of severely pressure parboiled rice reduced the glycaemic index.

Brown rice malt from Indica and Japonica type rice were prepared and their nutrient composition as well as Non-Starch Polysaccharide (NSP) contents and also some of the
physicochemical characteristics were determined. The activity of α- and β-amylases in the un-germinated (native) rice was negligible but increased considerably on germination. Malting altered the chemical composition of both Indica and Japonica rice to a small extent but caused noticeable changes in the pasting characteristics. Controlled germination or malting causes considerable changes in the physicochemical and biochemical properties of both Indica and Japonica rice (Mohan et al., 2010). Whole grain rice is rich in phenolic compounds. The effect of γ-irradiation on the main phenolic compounds in the rice grains of three genotypes (black, red, and white) was investigated. Three phenolic acids (p-coumaric acid, ferulic acid, and sinapinic acid) were identified as major phenolic compounds in all rice samples, while two anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) were identified in pigmented grain samples (Zhu et al., 2010).

1.2.5. BLACK GRAM A PROTEIN SOURCE IN IDLI MAKING

Blackgram (Phaseolus mungo) is a pulse traditionally used in the preparation of South Indian breakfast foods, such as idli, which is relished for its soft and spongy texture (Susheelamma and Rao, 1979a). The components responsible for these properties are the surface active proteins that generate a foam and as a result impart a porous structure to the food, and the viscogenic mucilaginous polysaccharide (~6%) that stabilizes the porous structure against thermal disruption during steaming. The overall carbohydrate composition (Bhat and Tharanathan, 1986) and the structure function characteristics of the total polysaccharides of black gram have been reported. During fermentation of black gram, for the preparation of leavened foods, it was found that the mucilaginous polysaccharide undergoes compositional and rheological changes (Muralikrishna et al., 1987). Here, the fermentation is due to the activities of endogenous microflora (endophytes) in black gram, in particular Leuconostoc mesenteroides, yeasts, lactic acid bacteria and coliforms. More than one oligosaccharide was observed as in green gram (stachyose, maltohexaose), sorghum (stachyose, maltotriose), barley (stachyose, raffinose), wheat (stachyose, raffinose) and black gram (stachyose, raffinose). In ragi, bajra and rice malt oligosaccharides were absent. Germination of seeds for 48 h resulted in complete loss of
stachyose and raffinose in cereals and pulses. The maltotriose content in pulses completely disappeared on germination but among cereals, 45.1 and 57.3 percent loss was observed in sorghum and maize, respectively (Sampath et al., 2008). In black gram after fermentation, apparent viscosity of cold paste increased. Some of the properties such as intrinsic viscosity, swelling and solubility after fermentation were reported by them. Fermentation and steaming approximately 40 per cent reduction in oligosaccharides resulting in reduced flatulence in the body (Koh and Singh, 2009).

Nutritional benefits are produced in legume fermentations, when microorganisms break down the flatulence causing indigestible oligosaccharides, such as stachyose and verbascose are broken down into the absorbable monosaccharaides and disaccharides. Biosynthesis of B vitamins in food fermentations has been recognized to be of major nutritional significance, particularly in Africa where high-carbohydrate diets, particularly maize diets can be deficient in essential B vitamins, the significance of B vitamin synthesis during fermentation to maize and sorghum beers in southern Africa was recognized by the use of the term ‘biological ennoblement’ by Platt (1964).

1.2.6. OLIGOSACCHARIDES IN FOODS

Carbohydrates are classified into monosaccharide, disaccharides, oligosaccharides and polysaccharides. Oligosaccharides are low molecular weight carbohydrates consisting 3 to 10 sugar monomers (Voragen, 1998). Oligosaccharides withstand salivary hydrolysis and digestive enzymes of human animal intestine so these oligosaccharides are not absorbed in the upper digestive tract and are able to reach the colon unaltered. In colon, oligosaccharides interact with the microflora and affecting immunomodulation (Reiffova and Nemcova, 2006). The non-digestible oligosaccharides promote the growth of beneficial bacteria in the colon, chiefly the Bifidobacteria sp., and are thus recognized as prebiotics (Mussatto and Mancilha, 2006). Most of the known prebiotics and prebiotic candidates are nondigestible oligosaccharides, obtained by extraction from plants (e.g., chicory inulin), followed by enzymatic hydrolysis (e.g., oligofructose from inulin) and by synthesis (by trans-glycosylation reactions) from mono and/or disaccharides such as sucrose (fructooligosaccharides) and lactose (trans-galactosylated oligosaccharides/galactooligosaccharides) (Crittenden and Playne, 1996).
prebiotics, inulin and oligosaccharides are the most studied and have been recognized as dietary fibre worldwide (Moshfegh et al., 1999).

1.2.6.1 Conversion of polysaccharides into oligosaccharides

Polysaccharides are the major source of bioactive oligosaccharides and around twenty different types of non-digestible oligosaccharides (NDOs) are described for prebiotic activities. Fructooligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides and galacturono-oligosaccharides are predominant NDOs with prebiotic characteristics. Oligosaccharides are recognized as non-cariogenic, non-digestible and low-calorie molecules and can be used as natural food preservatives. Chitosan and oligochitosans, inhibits growth of pathogens and extends shelf-life of food products (Barreteau et al., 2006).

Glycosyl-hydrolases and polysaccharide lyases, are used to obtain oligosaccharide from polysaccharides. Polysaccharide chains were broken down by polysaccharide lyases and form a double bond at new reducing end (Michaud et al., 2003). Polysaccharide hydrolase cleave glycosidic bond by transfer of a H₂O molecule and act as exohydrolases/endohydrolases (Boels et al., 2001; Bojarova and Kren, 2007). Enzyme used for the purpose must be specific to the substrate should be more efficient. The solution for having enzyme specificity is to use bacteria to produce particular enzyme. From the bacteria the specific enzyme of the polysaccharide can be isolated, purified and concentrated and used (Sutherland, 1999).

1.2.6.2. Physiological properties of oligosaccharides

Oligosaccharides possess important physicochemical properties. They are used as food ingredients as their physiological properties were beneficial to human health. The NDOs can be used as low carcinogenic sugar surrogates in products like confectionery, chewing gums, yoghurts and drinks (Crittenden and Playne, 1996). Many NDOs are not digested by humans because the human body doesn’t have the enzymes necessary to hydrolyze certain units of monosaccharides. Compounds include carbohydrates where fructose, galactose, glucose and/or xylose are the monosaccharides units. This property of NDOs makes the suitable for use in sweet, low-caloric diet foods, and for consumption
by individuals with diabetes (Crittenden and Playne, 1996; Rivero-Urgell and Santamaria-Orleans, 2001).

Most oligosaccharides were quantitatively hydrolyzed in the upper part of the GIT. The resulting monosaccharides are transported through the portal blood to the liver and subsequently, to the circulatory system. These carbohydrates are important for health as they serve as both substrates and regulators for major metabolic pathways. Nevertheless, some oligosaccharides present specific physicochemical properties resist to the digestive process, reaching the caeco colon. In the caeco-colon, most of the nondigestible oligosaccharides were hydrolyzed to small oligomers and monomers and further metabolized by most of the anaerobic bacteria. Such a metabolic process, known as fermentation, not only serves the bacteria by providing energy for proliferation, but it also produces gases (H₂, CO₂, CH₄), which are metabolically useless to the host, and small organic acids (Short-Chain Fatty Acids – SCFA) such as acetate, propionate, butyrate and L-lactate. Even though they do not provide the body with monosaccharides, the non-digestible oligosaccharides are indirect energy substrates and metabolic regulators (Delzenne and Roberfroid, 1994). The amounts and types of SCFA produced in the colon depend on the type of NDO substrate as well as on the composition of the intestinal flora (Sako et al., 1999).

Oligosaccharides serve as substrate for growth and proliferation of anaerobic bacteria, mainly the Bifidobacteria, which inhibit the growth of putrefactive and pathogenic bacteria present in the caeco-colon (Sangeetha et al., 2005).

NDOs leads to decrease of pH in the colon and consequently, in faeces, resulting from the production of SCFA. Lower pH values inhibit the growth of certain pathogenic bacteria species while stimulating the growth of the bifidobacteria and other lactic acid species (Manning and Gibson, 2004). An increase in faecal dry weight excretion, which is related to the increased number of bacteria resulting from the extensive fermentation of NDOs (Bielecka et al., 2002)

The indigestible quality of NDOs means that they have effects similar to dietary fibre, and thus prevent constipation. The end products of NDOs fermentation by colonic
bacteria, the SCFA, are efficiently absorbed and utilized by the human colonic epithelial cells, stimulating their growth as well as the salt and water absorption, increasing thus the humidity of the fecal bolus through osmotic pressure, and consequently improving the intestinal motility (Rivero-Urgell and Santamaria-Orleans, 2001). NDOs help in inhibition of diarrhea, especially when it is associated with intestinal infections (Roberfroid and Slavin, 2000).

An increase in absorption of different minerals, such as iron, calcium, and magnesium take place due to the binding/sequestering capacity of the NDOs. The minerals that are bound/sequestered and, consequently, are not absorbed in the small intestine reach the colon, where they are released from the carbohydrate matrix and absorbed. The increase on calcium absorption, in particular, reduces the risk of osteoporosis since this mineral promotes an increase in the bone density and bone mass. The hypotheses most frequently proposed to explain this enhancing effect of NDOs on mineral absorption are the osmotic effect, acidification of the colonic content due to fermentation and production of SCFA, formation of calcium and magnesium salts of these acids, hypertrophy of the colon wall (Younes, 1996).

Beneficial effect on the carbohydrates and lipids metabolism is that oligosaccharides lead to a decrease in the cholesterol, triglycerides and phospholipids concentration in the blood, reducing thus the risk of diabetes and obesity. Changes in the concentration of serum cholesterol have been related with changes in the intestinal microflora. Some strains of Lactobacillus acidophilus assimilate the cholesterol present in the medium, while others appear to inhibit the absorption of cholesterol through the intestinal wall. On the other hand, the changes in lipid metabolism were suggested to be a consequence of a metabolic adaptation of the liver that might be induced by SCFA (Daubiol et al., 2000).

NDOs aid in reduction of cancer risk, mainly the gut cancer. This anti-carcinogenic effect appears to be related to an increase in cellular immunity, the components of the cell wall and the extra-cellular components of bifidobacteria. Faecal physiological parameters such as pH, ammonia, p-cresol, and indole are considered to be risk factors not only for colon cancer development but also for systemic disorders. It has been demonstrated in a human study that the intake of trans-galactosylated disaccharides reduces the faecal pH as well
as ammonia, p-cresol and indole concentrations with an increase in bifidobacteria and lactobacilli and a decrease in bacteroid populations. These alterations may be considered to be beneficial in reducing the risk of cancer development. A low colonic pH may also aid in the excretion of carcinogens (Delzenne and Roberfroid, 1994)

1.2.6.3 Animal studies on Oligosaccharides

Feeding mice with diets supplemented with inulin and oligofructose increased activities of natural killer cells and phagocytes and enhanced T-lymphocyte functions compared to mice fed diets with cellulose or lacking fibre. These results are consistent with the observations of heightened resistance to systemic infections with *Listeria* spp. and *Salmonella* spp., the lower incidence and growth of tumours after exposure to carcinogens and transplanted tumour cells and are in agreement with enhanced innate and acquired immune functions provided by Lactobacillus and other LAB. Supplementing diets with FOS should increase production of SCFA, and particularly butyrate, and can be predicted to strengthen mucosal defences and enhance response to health challenges (Buddington *et al.*, 2002).

Colonic fermentation of FOS results in the synthesis of short chain fatty acids, which influences the lipid metabolism in human beings. Feeding male Wistar rats on a carbohydrate rich diet containing 10 percent FOS significantly lowers serum triacylglycerol (TAG) and phospholipid concentration (Delzenne *et al.*, 2002).

FOS reduces post-prandial triglyceridemia by 50% and avoids the increase in serum free cholesterol level occurring in rats fed with a Western type high fat diet. FOS protects rats against steatosis (liver TAG accumulation) induced by fructose, or occurring in obese Zucker fa/fa rats. FOS given at the dose of 10 percent in the diet of male Wistar rats for 30 days reduces postprandial insulinemia by 26 percent (Daubiol *et al.*, 2000).

Animal studies provide strong evidence that FOS inhibit secretion of TAG rich Very Low Density Lipoprotein (VLDL) particles via inhibition of de novo fatty acid synthesis. High levels of fat present inmost human diets mean that rates of hepatic de novo fatty acid synthesis are extremely low, since exogenous dietary fatty acids provide all the required substrate for hepatic triacylglycerol synthesis (Parks, 2002).
Dietary treatment with inulin/oligofructose (15 percent) incorporated in the basal diets for experimental animals resulted in (a) reduction of the incidence of mammary tumours induced in Sprague Dawley rats by methyl-nitrosourea (b) inhibited the growth of transplantable malignant tumours in mice and (c) decreased the incidence of lung metastases of a malignant tumour implanted intramuscularly in mice. It is reported that the dietary treatment with FOS/inulin significantly potentiated the effects of sub-therapeutic doses of six different cytotoxic drugs commonly utilized in human cancer treatment (Taper and Roberfroid, 2002).

Roberfroid and Slavin, (2000) has reported that feeding rats with FOS (10 percent) for a few weeks decreased uremia in both normal and nephrectomized rats. Dietary FOS enhanced faecal nitrogen excretion and reduced renal excretion of nitrogen in rats. This occurs because these fermentable carbohydrates serve as energy source for the intestinal bacteria, which during growth also require a source of nitrogen for protein synthesis.

**1.2.6.4 Applications of FOS in food formulations**

Inulin and oligofructose are ingredients that deliver a number of important nutritional benefits as well as contribute functional properties that enhance shelf life and taste profile of various food products like nutrition bars (Izzo and Niness, 2001). FOS can be used as the sole sweetening agent and gives 34 percent calorie reduction compared with sucrose standard. Organoleptic characteristics of the products are claimed to be very similar, with the test sample having a lower sweetness and a softer texture. FOS can be used with inulin to replace all the sugar and reduce the fat content and give excellent mouth feel characteristics. Since the freezing point depression is less with oligo-fructose than with sugar, the texture can be harder. Hard candies, gums, and marshmallows can be made while achieving significantly reduced energy values (Murphy, 2001).

**1.2.7 MODIFIED ATMOSPHERE PACKAGING**

The common perception that modified atmospheres are useful for improving storability has significant historical precedent. The written history of the use of modified atmospheres can actually be traced back at least 2000 years to the use of underground, sealed silos (Owen, 1800) where atmosphere modification was detected as “foul air” that
was dangerous to enter and was likely a result of O₂ depletion and CO₂ accumulation due to the respiratory activity of the grain. The modified atmosphere was unintentional, although probably beneficial. The foul air in the storages would presumably control rodent and insect pests, thereby acting to preserve the quality and storage life of the grain. The potential for a positive impact from alteration in the respiratory gases O₂ and CO₂ became increasingly known through the early research of Berard (1821), Mangin (1896), Kidd and West (1914, 1927, 1945), and Blackman and Parija (1928).

Gas modification technologies can be segregated into two classes based on the manner in which the atmospheres are generated and maintained. One class of technologies is referred to as Controlled Atmosphere (CA) storage, in which the atmosphere is either manually or mechanically controlled to achieve target headspace gas concentrations. In CA storages, O₂ and CO₂ concentrations can be modulated independently from one another. The second class of technologies is (MAP), in which a package possessing a film or foil barrier passively limits gas exchange by the living produce enclosed in the package, thereby altering the headspace atmosphere. In MAP, both oxygen and carbon dioxide are modified simultaneously and their concentrations at steady state are a function of one another. In MAP, the primary route of gas exchange may be through gas-permeable film, perforations in film, or both. In what is referred to as active or intelligent packaging techniques, packages may be flushed with specific gas mixtures designed to obtain a desired initial atmospheric composition, gases may be actively released or scavenged in the package, a partial vacuum can be imposed, biologically active materials can be incorporated in the package, sensors may be used to respond to the product or package conditions, and so on. The aim of MAP (passive, active, or intelligent in design) is to take advantage of physiological responses of the enclosed plant material or plant or human pathogens to the respiratory gases O₂ and CO₂. Presumably, MAP use is intended to maintain product quality, thereby ensuring appropriate value to the consumer and adequate cash flow back through the marketing and handling chain such that the production and marketing system is sustainable. Knowledge of the physiological responses to atmosphere modification is beneficial in terms of anticipating improved quality retention as a result of technology investment (Beaudry, 2008).
Atmosphere modification in a package requires a barrier through which gas exchange is restricted. Enclosing an actively respiring product within a package composed all or in part of a film that acts as a gas barrier reduces O₂ and increases CO₂, creating gradients across the film barrier. These gradients provide the driving force for gas flux into or out of the package. In passive MAP, a package always tends toward steady state, in which O₂ and CO₂ levels are constant and O₂ uptake and CO₂ production by the product are equal to those permeating through the package, a situation that exists only when the respiratory rate is constant or nearly so. The steady-state levels of O₂ and CO₂ within a package are dependent on the interaction of respiration of the product and the permeability properties of packaging film or micro-perforations (Beaudry et al., 1992; Cameron et al., 1989; Jurin and Karel, 1963; Kader et al., 1989).

MAP should be carefully designed, as a system incorrectly designed may be ineffective or even shorten the shelf life of the product. The design should take into consideration not only steady-state conditions, but also the dynamic process, because if the product is exposed for a long time to unsuitable gas composition before reaching the adequate atmosphere, the package may have no benefit. The design of Modified atmosphere package depends on a number of variables, the characteristics of the product, its mass, the recommend atmosphere composition, the permeability of the packaging materials to gases and its dependence on temperature and the respiration rate of the product as affected by different gas composition and temperature. Since, respiration rate modelling is vital to the design of MAP for fresh fruits and vegetables (Fonseca et al., 2002).

Temperature is exceptionally important in package design, continuous and perforated films differ in their response to temperature changes. The O₂ and CO₂ permeability of continuous films increases with temperature, while the diffusion of gases through perforations is extremely insensitive to temperature changes. O₂ permeation over LDPE increases 200% in 0 to 15°C, an exchange of O₂ over perforations increases only 11% at the same temperature range. Depends on the rate of respiration and transmission, the atmosphere modification can be achieved quickly or relatively slow. At lower temperatures, atmosphere modification will take several days, that some package systems cannot achieve steady-state environments prior to the end of their shelf-life. In many
cases, purging the package atmosphere with CO₂, N₂ or a combination of gases is often desirable during filling and sealing to rapidly obtain the maximum benefits of MAP. Product temperature affects storability more than any other factor. Pre-cooling and temperature maintenance during handling and shipping were critical in preserving quality. Temperature also significantly affects permeability of film and thereby the O₂ and CO₂ content of the package. The elevated rate of respiration at high temperature could be used to rapidly establish the desired package atmosphere, but this would only be useful in the few circumstances in which it would be more important to rapidly establish the atmosphere than to slow physiological processes, eg., to reduce cut-surface browning.

Negative Responses in MAP show that respiration gets reduced as O₂ becomes limiting, but there is usually a limit to which O₂ can be reduced. The lower O₂ limit is frequently considered to be the level of O₂ that induces fermentation. This fermentation threshold is not always the lower O₂ limit in commercial practice, however, because lower O₂ levels may confer benefits that outweigh the loss in flavour or other quality parameters. Ethanol, acetaldehyde, ethyl acetate and lactate are products of fermentation that can contribute to the development of off-flavours as well as physical injury (Kays, 1997; Mattheis and Fellman, 2000).

With regard to MAP study done by Fandos et al. (2000) on Cameros cheese, it was found that packaging in 50%CO₂/50%N₂ and 40%CO₂/60%N₂ were the most effective conditions for extending the shelf life of cheese with good sensory characteristics. MAP studies have been done in iced fresh hake slices (Pastoriza et al, 1996), Fresh cut mangoes (Aguliar et al, 2000), fresh-cut ice berg lettuce (Fan et al, 2003), refrigerated sea bass slices (Masniyom et al, 2002), blueberry (Song et al, 2002), minimally processed mango and pineapple fruits (Martínez-Ferrer et al, 2002), pomegranate (Artes et al, 2000).

From the review of literature and state of the art it is clear that there are no studies on optimization of idli with respect to components and fermentation time taking into consideration, both instrument based texture analysis and 15mm rating scale and combination of RSM and PCA to evolve the optimized parameters for idli. In addition there are no studies which deal with improvement of shelf life of idli batter with modified
atmosphere packaging. In continuation with this, the present study has been planned with an objective to improve the shelf life of ready to cook idli batter using modified atmosphere packaging.

The set objective is achieved through following the three major sub-objectives:

1. To understand the presently followed practices for the preparation of idli.
2. To optimize the process of preparation of the product with respect to ingredient ratios and fermentation time.
3. To improve the shelf-life of ready to cook idli batter by optimized process.