Chapter 6
Formulation and quality evaluation of chitosan enriched functional foods

6.1 Introduction

Cereals form the dietary staple for about 95% of the population in the developing countries. Being relatively inexpensive, their consumption is seen in almost all the economic classes worldwide. Nearly all the nations were found to use around 600 million metric tons of wheat and maize flours for commercial milling and consuming their products (FFI, 2008).

Being one of the staple food of Indian population, wheat flour provides more than 50% of total energy intake. It is a major source of carbohydrates, protein, dietary fiber, minerals and vitamins in the diet (Winters, 2009). Due to its physicochemical and rheological properties like water absorption capacity, kneadability, elasticity, raisability and others, wheat flour finds its applications in both leavened and unleavened products.

Flat breads are one category of wheat flour based product. They have been popular amongst the Asian people and now spreading to Western countries. Their popularity and demand is growing owing to their versatile form, taste, texture and ease of use. Chapati is one of the traditional unleavened whole wheat based flat bread, a common staple food consumed in India and other Asian countries. About 80-85% of the total wheat produced in the country is used for the preparation of chapati (Misra, 1998).

Chapati has been successfully used as a model for an array of studies that have determined the effect of additives, storage and processing on the physico-chemical and quality characteristics (Shenoy and Prakash, 2002; Gujral and Pathak, 2002; Gujral and Gaur, 2005). Various investigations have also focused on enhancing its health functionality by adding suitable functional ingredients. One approach has been to add dietary fiber from various sources such as oat fiber and wheat bran to whole wheat flour and chapati (Yadav et al., 2010).
Chitosan is a functional ingredient classified as a dietary fiber as per the definition given by Codex Alimentarius Commission in 2009. Chitosan nanoparticles have been also found to increase gastric emptying due to its action as a dietary fiber (Huang et al., 2007). It is an emerging bioactive polymer produced primarily from the crustacean shells obtained as a marine food industry waste. Japan and United States are the main producers of chitosan followed by India, Italy and Poland (Dutta et al., 2004).

Chitosan has both health and economic potential to be a promising component of functional foods. Chitosan has been added in foods such as bread (Kerch et al., 2008), yoghurt, fruit juices; and used as a coating on fruits, vegetables, cheese, and meat products to name a few (Friedman and Juneja, 2010) for its antimicrobial and antioxidant activity. Thus, it could also be used as a natural food additive.

The Japanese Ministry of Agriculture, Fisheries and Foods have approved several products ranging from biscuits to fishcakes containing chitosan as “Food – for specified Health Use” (FOSHU) (Prashanth, 2010). Nissin group, Japan, is marketing noodles with chitosan as a health food (http://www.nissinfoods.co.jp).

The potential use of this polysaccharide has not yet been explored in Indian foods. Wheat and wheat based traditional Indian foods can serve as an ideal vehicle for the development of functional foods. This could improve the health of millions of consumers through the wide variety of products prepared from wheat.

Chapati is also emerging as a convenience food in great demand. However, its short shelf life is a hindrance for its marketing. Chitosan has been shown to exhibit antimicrobial activity in different food systems (Friedman and Juneja, 2010) and antistaling properties in bread (Kerch et al., 2008). Both these properties could help to extend the market shelf life of fresh chapatis.

The present chapter thereby discusses the studies carried out to evaluate the effect of chitosan enrichment on the functional, nutritional and rheological quality of whole wheat flour. The objective also includes evaluation of chitosan addition on the sensory and shelf life quality of chapati.
6.2 Review of literature

6.2.1 Whole wheat flour

6.2.1.1 Functional characteristics

The popularity of wheat flour can be attributed to its functional properties like water absorption capacity, oil absorption capacity, foamability, raisability, etc.

- Water absorption capacity

Water absorption capacity (WAC) is the ability to form flour – water association under limited supply of water. The extent of water retention is an important indicator of the performance of flour in several food formulations (Circle and Smith, 1972). Islam and Johansen (1987) have reported the water holding capacity of commercial wheat flour as 60-70%.

An important factor affecting water absorption capacity is the protein content of the flour. Tipples et al., (1978) have reported that the water absorption property is influenced by not only the quality but also the quantity of protein present in the flour. An increase in the amount of legume flour, a good source of protein, in composite flour was found to increase the WAC (Shahzadi et al., 2005).

Chauhan et al. (1986) and Iwe (2000) have observed that water absorption index decreased with increase in carbohydrate, thus creating an inverse relationship between water absorption index and water solubility index. The damaged starch content of the whole wheat flour is also reported to be a determining factor of WAC of flour (Rao et al., 1989).

Fibre content is also known to influence WAC. The number of hydroxyl groups existing in the fiber structure result in variations in the WAC which allow more water interaction through hydrogen bonding (Rosell et al., 2001). Sudha et al., (2007) studied the effect of addition of different cereals bran as a source of fibre to the flour. The study reported increased water absorption with the addition of bran complex obtained from different sources.
• **Oil absorption capacity**

Oil absorption capacity is another important functional property of flour with respect to flavor retention and also for enhancing the mouthfeel of the food products. Peters *et al.*, (2003), in their studies on plantain and cowpea flour blend, have reported a high oil absorption capacity of blends. Presence of non-polar side chains (numerous in plantain flour) in flour bind to the hydrocarbon side chains of the oil, thereby increasing the oil binding capacity of the flour (Adebowale and Lawal, 2004). Hence, lower the extent of non-polar side chains (as in the case of hydrophobic proteins) in flour, lower is the oil binding capacity.

• **Foamability**

Effect of protein content on foamability, another functional property of flour, has been studied by Okaka and Potter (1977) which indicated the superiority of soya flour. Elkhalifa *et al.*, (2004) reported sorghum flour proteins to reduce the foamability of wheat flour by increasing the surface tension. Narayana and Rao (1982) have also related foamability to the amount of polar and non polar lipids present in the flour.

6.2.1.2 **Nutritional composition**

Whole wheat flour is the major source of carbohydrates and proteins to the majority of the population. Starch makes up the bulk of the wheat flour followed by protein which acts as cement to hold the starch granules. Gluten is the wheat protein which consists of glutenin and gliadin. Moisture content of the flour generally ranges from 11-14 percent. Carbohydrates are present in abundance (72.5g/100g) and apart from starch, 2-3 percent of gums, especially pentosans are also present. These gums add to the water absorption capacity of the flour. Also it contains around 12g of dietary fiber (Šramková *et al.*, 2009).

Lipid content of wheat flour is very low, around 1.87g, which is of great importance for proper gluten development. The creamy, off-white colour of the wheat flour is due to the small amounts of carotenoids present in it. The minerals
provided by whole wheat flour include iron, copper, magnesium, calcium and zinc (Šramková et al., 2009).

Fortification of wheat flour, which is limiting in lysine, with other cereals and millets has been reported to enhance its protein content and its nutritional quality. The moisture content of the developed composite flour was also higher compared to the whole wheat flour (Khetarpaul and Goyal, 2009). Blending of corn by products and wheat flour brought about an increase in the protein content (10.07 to 10.5 at 0% level), crude fiber, minerals like phosphorus and iron, although there was a decrease in the calcium content probably due to its fixation by fiber (Sudha et al., 2007).

One of the problems with whole wheat flour is their high phytate content. Phytates, mostly present in the aleurone layer hinder the absorption of several minerals from the digestive tract, by forming insoluble complexes with them. Calcium has been found to increase the binding effect of phytin on zinc (Matz, 1996).

6.2.1.3 Storage studies in wheat flour

Storage behavior of flours is a key factor in determining its full utilization. Storage flours exhibit several hazards including microbial attack, insect infestation, oxidative rancidity and many other physico-chemical and nutritional changes (Alam et al., 2007).

Moisture content is an important consideration for studying storage stability of flours. Lower the moisture content, lesser is the deterioration of the baking quality due to microbial activity (Staudt and Zeigler, 1973).

In a study conducted by Butt et al. (2004) an initial decrease in moisture content and further increase during storage was reported in flour packed separately in the polypropylene and paper bags for a period of 60 days. This was related to an increase in the relative humidity of the atmosphere. The study also reported decrease in crude fat and protein content on storage.
6.2.1.4 Rheological properties of wheat flour dough

Several researchers have studied the flour properties and dough characteristics. In the experiments conducted by Rao et al., (1989), dough adhesiveness showed an increase when the flour had higher levels of damaged starch due to more number of dextrins and sugar formation by the action of amylolytic enzymes on the damaged starch. Shahzadi et al., (2005) reported that composite flours containing more of legume flours required more dough development time.

Several studies have recorded the effect of adding different bran sources on dough characteristics, as the high moisture content of bran in the flour is known to hinder quick gluten development.

Shenoy and Prakash (2002) studied the effect of adding wheat bran on the farinograph characteristics of wheat flour dough. They reported an increase in dough development time and a decrease in mixing tolerance index with the increase in the level of bran incorporated (5-15%). The dough with 5% added wheat bran was reported to have good rolling property and dough elasticity when compared to the control flour (0% bran). Addition of defatted rice bran to wheat flour has been reported to significantly reduce the pasting properties (Yadav et al., 2012). This effect has been attributed to higher water absorption capacity due to availability of more surface area and better mixing. The insoluble nature of fiber and the dilution of wheat starch are opined to be other contributing factors. The study also developed a direct relationship between the textural properties of dough and addition of defatted rice bran to wheat flour. There was a marked decrease in the dough extensibility due to gluten dilution, although it varied with the size of the bran.

Yousif (2012) reported the effect of incorporation of different levels white or red sorghum flour (30-50%) in wholegrain flour. Reduction in water absorption, stability of dough and increased breakdown was reported for sorghum enriched doughs.
Storage of the flours also affects the dough characteristics. Leelavathi et al., (1984) found through their studies that after storage, the requirement of water for dough preparation decreased by 3%. Effect of storage on dough development has also been studied by Shahzadi et al., (2005) who reported that storage of flours for 60 days increased the dough development time from 4.73 to 6.63 minutes.

6.2.2 Whole wheat flour based products

Wheat flour has been used in the preparation of a wide variety of products ranging from baked items like bread, cookies, tortillas, etc., to indigenous products like chapati, khakra, and others. This section reviews studies related to chapati, traditional Indian unleavened flat bread prepared from whole wheat flour. It is the staple diet of majority of population in the Indian sub-continent and parts of Middle-East (Islam and Johansen, 1987). About 80-85% of the total wheat produced in India is used for the preparation of chapati (Misra, 1998).

6.2.2.1 Functional quality characteristics of chapati

The quality of chapati is assessed based on its softness and flexibility, the characteristics that are governed by flour protein quality and quantity. Dough consistency, which is a function of quantity of water added, is also an influential factor (Austin and Ram, 1971).

The effect of damaged starch content in whole wheat flour on the quality of chapati has been studied. Rao et al. (1989) observed a desirable dough handling, surface colour, texture and taste in the chapatis when damaged starch contents ranged between 14% and 16.5% in the flour.

There are several factors that influence the puffing of chapati like the moisture content. Dough with higher moisture content resulted in chapati that puffed more compared to those with less moisture. The extent of mixing was also found to be a determining factor in assessing the quality of chapati. Dough that was not
developed fully had inadequate hydration causing incomplete gluten development. Mixing time, thickness of chapati and particle sizes in the flour are the other factors which govern the dough characteristics and chapati quality (Rao et al., 1986).

6.2.2.1 Value addition to chapati: Effect on sensory and shelf life quality

Various ingredients such as different hydrocolloids, enzymes, other flours and fibre have been incorporated in chapati for value addition, with variable effects on its quality.

Chapati has a compact structure (less free volume) because of which when it stales, it becomes hard in texture and its eating quality is lost. This staling on storage has been attributed to a decrease in the moisture content, water soluble starch and in vitro enzyme digestibility (Shaikh et al., 2007). Retrogradation of gelatinized starch is the primary cause of staling in chapatis (Indrani and Rao, 2003) leading to adverse changes in texture such as loss of softness and pliability.

Chapati with high fibre content was prepared by incorporating wheat bran at different levels and evaluated by Shenoy and Prakash (2002). The authors reported lower sensory acceptability in chapatis enriched with higher content (10% and 15%) of bran, whereas, 5% bran incorporated chapati was reported to exhibit good sensory acceptability.

Improvements in chapati texture and antistaling effect on storage have been reported with the addition of sorbitol (Becktel, 1990), α amylase and maltodextrin (Nanjappa et al. 1999) and guar gum (Shaikh et al., 2007).

Nuessli et al., (2000) reported retardation of staling in chapati with 0.75% of glycerol monostearate (GMS) or 0.75% (w/w) sodium stearoyl lactate (SSL), which were stored at 29°C and refrigerated temperature. The authors also reported better moisture retention in the GMS enriched chapati compared to control.
Gujral and Pathak (2002) reported beneficial effect of addition of rice flour at lower concentrations along with additives on the textural quality of chapati. A study by Gujral and Gaur (2002) reported improved texture and quality of fresh and stored chapatis prepared with addition of barley flour, wet gluten and liquid shortening in different proportions.

Gujral and Gaur, (2005) reported anti staling effect of 10 - 20% w/w barley flour, 0.25-0.5% w/w GMS and 0.5-1% w/w sodium chloride in 24h stored chapati as evidenced by higher extensibility values. A study by Shaikh et al., (2008) reported incorporation of various additives like sodium stearoyl-2-lactylate (SSL), glycerol monostearate, propylene glycol, sorbitol, α-amylase, xylanase, maltodextrin and guar gum into the dough to delay staling of chapatis stored for 10 days at room and refrigerated temperatures.

Hemalatha et al., (2010) studied the effect of incorporation of different enzymes like fungal and bacterial α amylase, xylanase and combination of bacterial α amylase and xylanase on chapati making quality of wheat flour. The authors reported the chapatis prepared from enzyme enriched dough to have better pliability, softer texture and also greater sensory acceptability compared to control.

Microbial quality of chapati is another important storage criterion. Chapati incorporated with 4% sodium chloride and stored at 24- 35°C and 70-80% relative humidity (RH) in polyethylene pouches could be satisfactorily preserved for 7 days (Rao et al., 1984).Abu-Ghoush (2008) reported addition of three preservatives, namely fumaric acid (0.2%, F), sodium propionate (0.3%, P), and sodium propionate-fumaric acid mixture (PF) on the shelf life quality of Arabic flat breads. The authors reported F–P to inhibit mold growth 320% longer compared to the control.
6.2.3 Application of chitosan as a functional food ingredient

Interest in the application of chitosan as a functional food ingredient arises from its numerous health related beneficial effects, including strong antimicrobial and antioxidative activities in foods as powders, in solutions, and in edible films and coating against food borne pathologies, spoilage bacteria and pathogenic viruses and fungi in fruit juices, eggs, dairy, cereal, meat and sea food products (Friedman and Juneja, 2010). The wide food applications of chitosan have led to the possibility of its addition to the generally recognized as safe (GRAS) list in the United States (U.S. Food and Drug Administration, 2005).

6.2.3.1 Cereal products

Tsai et al. (2006) have studied the application of chitosan in the preservation of cooked rice, and found out that addition of 2000 ppm low molecular weight chitosan (LMWC) to raw rice water before steam cooking was capable of effectively inhibiting any increase in total aerobic bacterial count and *B. cereus* in cooked rice that was stored at 37°C and 18°C. LMWC was preferred over chitosan since it is more water soluble and hence more useful for the preservation of rice.

In another study conducted by Nobile et al. (2009), the combined effects of MAP and chitosan was found to improve the microbiological quality of amaranth-based home-made fresh pasta, monitored for 2 months at 4°C for the growth of *Staphylococcus* spp., yeasts, molds, and total coliforms.

Other studies on the antimicrobial activity of chitosan include shelf life extension of fresh noodles for 6 more days by addition of 0.05g chitosan per 100ml of acetic acid during storage at 4°C and shelf life extension of rice cakes & rice noodles by treatment with 1% & 2% solutions of 37 KDa chitosan prior to vacuum packaging (No et al., 2007).
6.2.3.2 Baked products

Rodriguez et al. (2003) have studied the antifungal capacity of chitosan when used as an edible film and as dough ingredient in precooked pizza. When used in acetic acid as an edible coating (0.079g/100g pizza), the coating delayed the growth of Alternaria sp., Peniicillium sp. and Cladosporium sp., although its use in dough was not effective.

In a study conducted by Kerch et al. (2008), the effect of chitosan on physical and chemical processes during bread baking was studied. It was observed that chitosan had an influence on the structure and properties of bread crumb and crust due to the changes in the moisture migration processes. The results pointed out that the water state and its redistribution in the bread crust and crumb was affected by chitosan. The freezable and total water content was found to be decreased during staling, more rapidly in the presence of chitosan. Chitosan also facilitates dehydration from starch and gluten and moisture migration from crumb to crust thereby increasing the crumb firming and dense texture formation.

6.2.3.3 Milk and milk products

The antimicrobial activity of chitosan in milk has been studied by Lee et al. (2004) and was found that the growth of aerobic bacteria in milk was suppressed by chitosan–containing antimicrobial paper board.

Krasaeoopt et al. (2006) have found that chitosan encapsulated probiotics when added to yoghurt during storage increased the survival of the probiotic bacteria. The popular milk product, cheese has been used to study the bactericidal effect of chitosan by several researchers. It was observed that chitosan-lysozyme films and coatings inhibited the growth of Escherichia coli, L. monocytogenes, Pseudomonas fluorescens as well as molds and yeasts in mozzarella cheese (Altieri et al., 2005). It is suggested that the microbial lag phase was increased by chitosan, thereby decreasing their density which gives them the potential to be used as a dairy preservative.
Chitosan-based edible coatings have also been studied for their impact on cheese shelf life by Coma et al. (2003) and Nobile et al. (2009) and were found to inhibit coliforms, *Pseudomonas* spp. and *S.aureus* bacteria.

6.2.3.4 Fruits and vegetables and their products

Owing to its varied functionality (Austin et al., 1981) chitosan can be used in fruit and vegetable juice production. Its gelling properties were reported to be employed for developing an immobilized cell reactor for continuous production of fermented fruit and vegetable juice products (Knorr and Klein, 1986).

Production of β-carotene has been reported from cultured carrot cells using chitosan containing complex co-acerbate systems (Beaumont and Knorr, 1987).

Sapers (1992) has studied the use of chitosan in enhancing the control of enzymatic browning in apple and pear juice by filtration even at the levels as low as 1000ppm. At this level, chitosan could prevent browning at the same time, clarified the juice. This property of chitosan has been attributed to its ability to coagulate the suspended solids bound by polyphenol oxidase.

As an antimicrobial agent, chitosan has been extensively used in fruit and vegetable industry against several microbial species like spoilage yeasts and lactic acid bacteria, *Saccharomyces* sp., *Streproccocus* sp., etc., in fermented vegetables (Savard et al., 2002).

6.3 Materials and methods

6.3.1 Sample description

The whole wheat grains for the present study were purchased in one lot from SR Trading Company, Bangalore. The grains were cleaned to remove the extraneous dirt and stored in air-tight containers. Raw chitosan was purchased from Panvo Organics, Chennai. The purification of chitosan was done as per the procedure given by El Ghaouth et al. (1992) as described earlier in chapter 2.
6.3.2 Experimental design

6.3.2.1 Whole wheat flour study

Wheat grains were milled in one lot by means of hammer mill and the resultant flour was divided into four portions. The four flour portions were mixed with purified chitosan at the level of 0%, 3%, and 5%, and the samples were designated as 0%CHWF, 3%CHWF and 5%CHWF, respectively. They were stored in air tight containers in refrigerated temperature until analysis (Fig 6.1).

6.3.2.2 Whole wheat flour chapati study

Chapati, popular flat bread, was selected to study the impact of incorporation of chitosan into the whole wheat flour on its quality characteristics. Thirty grams of unincorporated and chitosan incorporated whole wheat flour samples was weighed and taken in a container. To that 20-25mL water was added and kneaded to soft dough. The dough was covered with a moist cloth and kept for thirty minutes as resting time. The dough was then made into a round ball and sheeted on a clean plain rolling board to a diameter of 15 cm and thickness of approximately 30mm. The sheeted chapatis were baked on both the sides on a hot girdle till the chapatis were cooked on all sides and had a creamy appearance. The fresh chapati (0%CHCh, 1%CHCh, 3%CHCh and 5%CHCh) were analyzed for their nutritional content. Shelf life and sensory quality on storage for 0 h, 24 h, 48 h, and 72 h in LDPE pouches at 28 ± 2°C (Fig 6.2) was also evaluated.
Figure 6.1 Experimental design for whole wheat flour study

**Whole wheat flour (WWF)**

Addition of purified chitosan (CH) powder at different concentrations

- 0% level (0% CHWF)
- 1% level (1% CHWF)
- 3% level (3% CHWF)
- 5% level (5% CHWF)

**Flours analyzed for the following parameters**

**I. Functional quality parameters**
1. Water and oil related functional indices
2. Least gelation concentration
3. Bulk density
4. Instrumental color

**II. Nutritional quality parameters**
1. Moisture
2. Protein
3. Fat
4. Carbohydrates
5. Crude fibre

**III. Dough characterization studies**
1. Sensory characterization
2. Rheological evaluation
   a) Farinograph
   b) Extensograph
Figure 6.2 Experimental design for the study of chapati made from chitosan incorporated whole wheat flour

0% CHWF → 1% CHWF → 3% CHWF → 5% CHWF

30g each of the above flours kneaded into a dough and rested for 30 min

Dough sheeted into a chapati of 15 cm diameter and baked on both sides on a hot griddle till doneness

Obtained chapatis (0%CHCh, 1%CHCh, 3%CHCh and 5%CHCh) stored at 28 ± 2°C in LDPE pouches and analyzed at various intervals

I. Antistaling characteristics
   1. Moisture content
   2. Water soluble starch
   3. In vitro enzyme digestibility
   4. Differential scanning calorimetry (DSC)

II. Microbial quality (visual count of fungal colonies)

III. Sensory acceptability
   1. Texture
   2. Flavor
   3. Overall acceptability
6.3.3 Analysis of functional properties of whole wheat flour

6.3.3.1 Water and oil related functional indices:

One gram flour and 10ml distilled water or refined vegetable oil (density 0.98 g/ml) was taken in a centrifuge tube (Abbey and Ibey, 1988). The determinations were carried out in triplicates in room temperature and various indices were calculated as given below:

- **Water / Oil Absorption Capacity (WAC/OAC)**

  \[ \text{WAC/OAC} = \frac{W_1 - W_2}{W_1} \]

  \[ W_1 = \text{weight of centrifuge tube + flour + water/oil} \]

  \[ W_2 = \text{weight of centrifuge tube after removing the supernatant} \]

- **Water and oil absorption index (WAI/OAI)**

  \[ \text{WAI/OAI} = \frac{\text{Weight of the supernatant}}{\text{weight of the sample}} \]

- **Water and oil holding capacity (WHC/OHC)**

  \[ \text{WHC/OHC} = \frac{\text{mass of water or oil added to the sample} - \text{mass of water or oil removed from the sample}}{\text{mass of the sample}} \]

6.3.3.2 Least gelation concentration

Least gelation concentration of samples was determined by the method of Iyer and Singh (1997). Suspension containing 8-15% (w/v) sample in 0.5% increments were prepared in 15ml of distilled water. The test tubes were heated for 1h in boiling water, rapidly cooled under running tap water and refrigerated for 3h at 5°C. The least gelation concentration was determined as the concentration at which the sample did not fall down or slip from inverted test tube.
6.3.3.3 Bulk density

The method of Akapapunam and Markakis (1981) was used to determine the bulk density of the flour samples.

Ten grams of the flour samples were weighed into a 50ml measuring cylinder and packed by gently tapping the cylinder, ten times, from a height of 5-8 cm. The final volume of the flour was measured and expressed as g/ml.

6.3.3.4 Instrumental color values and whiteness index

The color values in terms of L* (lightness or darkness), a*(redness or greenness) and b* (yellowness or blueness) and whiteness index of the flour samples were determined as per the procedure given in chapter 4, section 4.3.5.2.

6.3.4 Nutritional evaluation of chitosan enriched flours

The control and chitosan enriched wheat flour samples were analyzed for moisture, protein, fat, carbohydrates, minerals (Ca, Mg and Na) and crude fibre, as per standard methods described in detail in chapter 2.

6.3.5 Dough characterization studies

6.3.5.1 Sensory characterization

Thirty grams of whole wheat flour was weighed and taken in a container and to it 20-25 mL of water was added and kneaded to soft dough weighing around sixty grams. The dough was then used for studying its various characteristics. ‘Kneadability’ was used to describe the ease of handling the dough. The elasticity of dough was determined by pressing the dough with forefinger and observing the time taken by it to come back to its original shape. Stickiness was judged based on the amount of dough that stuck to the palms while kneading. The rolling property included the ease as well as extent of rolling. All parameters were rated using ‘+’ to ‘++++’ sign for poor to very good.
6.3.5.2 Rheological evaluation

The deformation and flow of matter in response to an applied stress or strain is studied through rheology. Dough is a viscoelastic system which has been reported to exhibit shear-thinning and thixotropic behaviour due to its complex structure wherein a three dimensional protein network (20-25%) is encompassed by starch granules (75-80%) (Weipert, 1990). Rheology is considered to be one of the most important tools employed for assessment of flour quality and helps to reveal the influence of various ingredients and additives on the behaviour of dough during processing into products.

a) Farinograph characteristics

Brabender farinograph is one of the most widely used devices for assessment of physical properties of the dough, especially the mechanical resistance of the dough while mixing and kneading. A defined mass of flour is placed in a tempered mixing bowl (30°C) that is equipped with two Z type kneaders. Sufficient water is added to obtain a desired consistency of 500 Brabender Units (BU) (Hadjadev et al., 2011). Water absorption (WA), dough development time (DDT) (time elapsed from the beginning of the kneading until maximum dough consistency is achieved), dough stability (DS, time during which there is minimal or no change in dough consistency), degree of softening (S, distance between centre of the curve after the analysis and the central line passing through the maximum point of the curve) and Farinograph quality number (FQN) were noted and the resulting curves were interpreted as per the official methods (AACC, 2000)

b) Extensograph characteristics

Brabender extensograph is another internationally accepted method used for measuring physical properties of the dough during mechanical handling and resting. The force required to pull a hook through a cylinder-shaped piece of dough is measured, which in turn provides information about resistance of dough
to stretching and extensibility. Extensograph was used to study the resistance to extension (maximum resistance that usually corresponds to the height of 55mm on the curve from the beginning of stretching, R), dough extensibility (distance of stretching before rupture, expressed in mm, E), ratio between resistance and extensibility (R/E) and area under the curve (proportional to the energy required to stretch the dough test piece to the point of rupture, cm$^2$) (Hadnadev et al., 2011).

### 6.3.6 Shelf life studies on chapati

Shelf life quality of the chapati samples was assessed in terms of staling characteristics, sensory acceptability and microbial quality.

#### 6.3.6.1 Staling characteristics

- **Moisture content**

  Moisture content of the samples was evaluated using AOAC protocol (1990).

- **Water soluble starch**

  Total WSS was determined by a modified procedure of Morad and D’Appolonia (1980).

  A 200 mg chapati sample was extracted with 15 mL of distilled water by agitating the mixture on a shaker for 20 min. The slurry was centrifuged at 5000 rpm for 5 min, and the supernatant was filtered. Ten mL of the filtrate was treated with 2mL of standard iodine solution (2mg of iodine and 20 mg potassium iodide [KI] in 100 mL water), and optical density (OD) was measured at 680 nm. A standard curve was plotted of OD at 680 nm versus the concentration of starch (a mixture of 25% amylase and 75% amylopectin) by taking varying amounts of the starch mixture and treating it with the standard iodine solution as described earlier.
● In vitro enzyme digestibility (IVED)

Enzyme digestibility of starch in chapati was determined by a modified procedure of Lucia et al., (1995).

A 200 mg chapati sample was extracted with 15 mL of 0.1 M Na-acetate buffer (pH- 4.75) by agitating the mixture on a shaker for 20 min. The slurry was centrifuged at 5000 rpm for 5 min, and the supernatant was filtered. A 1.9 mL filtrate was taken and heated at 60ºC, and 0.1 mL of amyloglucosidase solution (150 mg of the amyloglucosidase in 100 mL of the same buffer) was added. After 10 min of incubation at 60ºC, the reaction was stopped with 2 mL of di-nitro salicylic acid (DNSA) reagent, and the liberated glucose was determined by the DNSA method. A standard curve was plotted of OD at 540 nm versus the concentration of glucose solution. The amount of glucose released because of the action of amyloglucosidase on starch (chapati) was calculated from the standard curve. IVED was then expressed as percent of glucose liberated.

● Differential scanning calorimetry (DSC) analysis

Retrogradation of starch and bread staling have been extensively studied through thermal analysis (Vodovotz et al., 1996). Differential scanning calorimetry has proven to be one of the most useful methods for providing basic information on starch retrogradation. Hence, the present investigation employed DSC for studying retrogradation/staling characteristics of the stored chapati samples.

A 5-mg chapati was weighed in the DSC pans, which was then sealed hermetically, using a sealing machine, reweighed, and allowed to stand for 1 h before DSC analysis. Duplicate sample pans were prepared, and each was heated at a rate of 10C/min from 25 to 200C in the Dupont pressure cell maintained in an inert atmosphere by passing dry nitrogen through the DSC head at a flow rate of 60 mL/min. In all measurements, the thermogram was recorded with an empty
aluminum pan as the reference. The transition temperatures reported in the tables are the onset ($T_0$), peak ($T_p$) and conclusion ($T_c$) of the gelatinization endotherm. The enthalpy of the gelatinization ($\Delta H$) was estimated by integrating the area between the thermogram and a base line connecting the points of onset and conclusion temperature, and was expressed in terms of J/g. All DSC experiments were replicated twice.

6.3.6.2 Microbial quality

The microbial examination of fresh and stored chapati samples was performed visually by counting the number of fungal colonies at the end of each storage period.

6.3.6.3 Sensory acceptability

The sensory acceptability was carried out for the chapati samples using hedonic rating scale from 5 for very good to 1 for poor acceptability. Appearance was rated based upon the degree of doneness and creamy roasted appearance. For texture, the panelists rated their acceptability based on pliability and softness. The eating quality of the chapati was decided based on the flavour of the sample. Overall acceptability of the product was also judged based on the above organoleptic characteristics. The mean values were calculated based on the individual scores of all the panelists.

6.3.7 Statistical analysis

Results are expressed as means of three independent trials. Experimental data were processed by one-way ANOVA using the least significant difference (LSD) as a multiple range test, by setting the statistical significance at 95% level. Analysis was conducted using SPSS software (SPSS Student Version 16.0 for windows).
6.4 Results and discussion

6.4.1 Effect of incorporation of chitosan on the quality of whole wheat flour

6.4.1.1 Functional properties of whole wheat flour

The functional properties of the chitosan incorporated whole wheat flour are given in table 6.1.

- Water and oil absorption capacity (WAC and OAC)

Water absorption capacity of the whole wheat flour, in the present study, was found to be 1.41 ml/g. Kamaljit et al., (2011) reported WAC value of 1.7 ml/g in refined wheat flour. Incorporation of chitosan resulted in a significant increase in water absorption capacity. The WWF sample with 1% chitosan showed a water absorption capacity value of 1.42 ml/g which increased to 1.46 ml/g and 1.52 ml/g in 3%CHWF and 5%CHWF, respectively. Akubor et al. (2003) have reported a significant increase in water absorption capacity as the amount of plantain flour in a cowpea and plantain flour blend increased from 10% to 90%.

Sudha et al., (2007) also reported an increase in the WAC in wheat flour enriched with different cereal bran as sources of fibre.

Oil absorption capacity of the flour samples, in the present study, has shown an inverse relationship with respect to water absorption capacity. A significantly lower OAC value was observed in 5%CHWF (1.73 ml/g) compared to 0%CHWF (1.89 ml/g) which recorded the highest OAC.

Ikpeme et al. (2010) have reported a decrease in oil absorption capacity of composite flour comprising of whole wheat flour and taro flour with an increase in the level of blanched taro flour.
Table 6.1 Effect of chitosan incorporation on the functional properties of whole wheat flour

<table>
<thead>
<tr>
<th>Parameter</th>
<th>*0% CHWF</th>
<th>1% CHWF</th>
<th>3% CHWF</th>
<th>5% CHWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water absorption capacity (ml/g)</td>
<td>1.41 ± 0.27b</td>
<td>1.42 ± 0.20b</td>
<td>1.46 ± 0.28a</td>
<td>1.52 ± 0.30a</td>
</tr>
<tr>
<td>Water absorption index</td>
<td>1.95 ± 0.29b</td>
<td>1.94 ± 0.39b</td>
<td>2.05 ± 0.36a</td>
<td>2.14 ± 0.34a</td>
</tr>
<tr>
<td>Water holding capacity (ml/g)</td>
<td>1.45 ± 0.49b</td>
<td>1.46 ± 0.58b</td>
<td>1.49 ± 0.39b</td>
<td>1.58 ± 0.51a</td>
</tr>
<tr>
<td>Oil absorption capacity (ml/g)</td>
<td>1.89 ± 0.52a</td>
<td>1.87 ± 0.55a</td>
<td>1.80 ± 0.59b</td>
<td>1.73 ± 0.49b</td>
</tr>
<tr>
<td>Oil absorption index</td>
<td>2.59 ± 0.19a</td>
<td>2.52 ± 0.09a</td>
<td>2.47 ± 0.13b</td>
<td>2.32 ± 0.11b</td>
</tr>
<tr>
<td>Oil holding capacity (ml/g)</td>
<td>2.92 ± 0.06a</td>
<td>2.95 ± 0.03a</td>
<td>2.79 ± 0.01b</td>
<td>2.78 ± 0.12b</td>
</tr>
<tr>
<td>Least gelation concentration (g/ml)</td>
<td>8.25 ± 0.25a</td>
<td>8.01 ± 0.15a</td>
<td>7.80 ± 0.25b</td>
<td>7.50 ± 0.25b</td>
</tr>
<tr>
<td>Bulk density (g/ml)</td>
<td>0.43 ± 0.01a</td>
<td>0.44 ± 0.01a</td>
<td>0.44 ± 0.01a</td>
<td>0.45 ± 0.03b</td>
</tr>
<tr>
<td>Colour L*</td>
<td>86.0 ± 0.55a</td>
<td>86.2 ± 0.09a</td>
<td>85.9 ± 0.08a</td>
<td>85.2 ± 0.61a</td>
</tr>
<tr>
<td>a*</td>
<td>-1.93 ±0.12a</td>
<td>-1.90 ±0.08a</td>
<td>-1.86 ±0.12b</td>
<td>-1.73 ±0.09b</td>
</tr>
<tr>
<td>b*</td>
<td>9.76 ± 0.12b</td>
<td>9.86 ± 0.12b</td>
<td>10.6 ± 0.32a</td>
<td>10.1 ± 0.20a</td>
</tr>
<tr>
<td>Whiteness index</td>
<td>83.0 ± 0.41a</td>
<td>82.9 ± 0.21a</td>
<td>82.5 ± 0.34a</td>
<td>80.0 ± 0.41a</td>
</tr>
</tbody>
</table>

*0%, 3% and 5% CHWF – Wheat flour incorporated with 0%, 3% and 5% Chitosan. Values expressed as Mean ± S.D. Different alphabets in a row indicate significant difference (P<0.05).
Water and oil absorption index

Water absorption index of 0%CHWF was found to be 1.95. Though chitosan incorporation at lower concentration did not show any significant effect, at 3% and 5% level, chitosan incorporated whole wheat flour exhibited an increase in water absorption index with a value of 2.05 and 2.14. Nwabueze (2006) has reported an increase in water absorption index with an increase in African breadfruit flour from 40% to 100% in a soya bean-African breadfruit flour blend.

The oil absorption index decreased significantly from 2.59 for 0%CHWF to the lowest value of 2.32 for 5%CHWF.

- Water and oil holding capacity (WHC and OHC)

The WHC of whole wheat flour samples increased with increase in level of chitosan incorporation. The lowest value was exhibited by 0%CHWF (1.45 ml/g). It increased from 1.46 ml/g in 1%CHWF to 1.49 ml/g in 3%CHWF and to 1.58 ml/g in 5%CHWF. Chen et al., (1988) reported a non-linear relationship of WHC with increasing concentrations of apple fiber and cellulose. In case of OHC, chitosan incorporated whole wheat flour showed a decreasing trend with increasing level of chitosan addition. Highest OHC was observed in 0%CHWF (2.92 ml/g) which decreased to 2.78 ml/g in 5%CHWF. Interaction between gluten and chitosan, a fiber, could have reduced the OHC of flour mixtures.

- Least gelation concentration (LGC)

Least gelation concentration denotes the least concentration at which gelation of the substance takes place (El Khalifa, 2005). LGC of 0%CHWF in the present study was observed as 8.25 g/ml which is similar to the value (8.0 g/ml) reported for wheat flour by Kamaljit et al. (2011).

A significant decrease in the LGC was observed in chitosan incorporated whole wheat flour, compared to control. LGC of 8 g/ml was observed in 1%CHWF, which further reduced significantly to 7.5 g/ml in 5%CHWF. The
Applications of Aloe vera gel and chitosan biopolymers

LGC of wheat flour has been reported to reduce from 10% to 6% on addition of taro flour (Ikpeme et al., 2010).

Gelling properties of the flour is dependent on the relative ratio of its proteins, carbohydrates and lipids and the interaction between them (Sathe et al., 1982). The lower the LGC, the greater is the swelling ability of the flour. Presence of chitosan seems to have lowered the least gelation concentration value of whole wheat flour suggesting its use in the foods where thickening and gelling is required, such as, puddings, gravies, etc.

- **Bulk density**

  Addition of chitosan to the whole wheat flour in the present study caused a non-significant increase in bulk density. A value of 0.43 g/ml was recorded in 0%CHWF which increased slightly to 0.44g/ml in 1% and 3%CHWF, and to 0.45 g/ml in 5%CHWF.

- **Whiteness index**

  The chitosan incorporated whole wheat flour samples showed a slight decrease in ‘L*’ value. Similar trend was observed for ‘a*’ values. The ‘b*’ values on the other hand showed an increase. Consonant to the slight decrease in L* values, an insignificant decrease in the whiteness index was observed in chitosan incorporated flour. This low colour difference was also not discerned visually in the fresh flour samples.

### 6.4.1.2 Nutrient composition of whole wheat flour

Effect of chitosan incorporation on the proximate composition and mineral composition of whole wheat flour is given in table 6.2 and 6.3, respectively.

No significant difference was detected between the wheat flour samples for moisture content (11.1%-11.7%). Samples with higher content of chitosan i.e. 3% and 5%CHWF showed significantly higher protein content of 11.9g/100g and
12.5g/100g, respectively, compared to control (11.4g/100g). Chitosan incorporation also resulted in significantly higher crude fibre content of 2.6g/100g in 5%CHWF compared to control (1.75g/100g).

With regard to mineral composition, 3% and 5%CHWF, recorded significantly higher content of all the minerals evaluated, compared to control. The content of Fe, Ca, Zn and Na was found to increase significantly by 80%, 48.4%, 15.8% and 88%, respectively, in 5%CHWF compared to 0%CHWF.

Chitosan, being a rich source of fibre as well as being an aminopolysaccharide increased the protein and fibre content. Being obtained from marine source, it also has good amounts of various important minerals as reported in chapter 2.
### Table 6.2 Effect of chitosan incorporation on nutritional quality of whole wheat flour

<table>
<thead>
<tr>
<th>*Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Total carbohydrate (g/100g)</th>
<th>Protein (g/100g)</th>
<th>Total lipids (g/100g)</th>
<th>Crude fibre (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% CHWF</td>
<td>11.1 ± 0.13b</td>
<td>0.82 ± 0.19b</td>
<td>85.4 ± 0.45</td>
<td>11.4 ± 0.11c</td>
<td>1.83 ± 0.11</td>
<td>1.75 ± 0.15d</td>
</tr>
<tr>
<td>1% CHWF</td>
<td>11.3 ± 0.13b</td>
<td>0.89 ± 0.19b</td>
<td>84.3 ± 0.45</td>
<td>11.6 ± 0.11c</td>
<td>1.87 ± 0.13</td>
<td>1.91 ± 0.15c</td>
</tr>
<tr>
<td>3% CHWF</td>
<td>11.6 ± 0.15a</td>
<td>1.02 ± 0.21a</td>
<td>83.1 ± 0.45</td>
<td>11.9 ± 0.14b</td>
<td>1.89 ± 0.14</td>
<td>2.36 ± 0.13b</td>
</tr>
<tr>
<td>5% CHWF</td>
<td>11.7 ± 0.23a</td>
<td>1.05 ± 0.14a</td>
<td>82.5 ± 0.45</td>
<td>12.5 ± 0.16a</td>
<td>1.91 ± 0.12</td>
<td>2.60 ± 0.11a</td>
</tr>
</tbody>
</table>

### Table 6.3 Effect of chitosan incorporation on mineral profile of whole wheat flour

<table>
<thead>
<tr>
<th>*Sample</th>
<th>Iron (mg/100g)</th>
<th>Calcium (mg/100g)</th>
<th>Zinc (mg/100g)</th>
<th>Sodium (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% CHWF</td>
<td>3.93 ± 0.14d</td>
<td>48.7 ± 0.21d</td>
<td>1.96 ± 0.14c</td>
<td>10.6 ± 0.21d</td>
</tr>
<tr>
<td>1% CHWF</td>
<td>4.05 ± 0.14c</td>
<td>57.9 ± 0.21c</td>
<td>1.97 ± 0.14c</td>
<td>21.5 ± 0.21c</td>
</tr>
<tr>
<td>3% CHWF</td>
<td>13.2 ± 0.22b</td>
<td>76.5 ± 1.23b</td>
<td>2.01 ± 0.15b</td>
<td>71.2 ± 0.25b</td>
</tr>
<tr>
<td>5% CHWF</td>
<td>19.7 ± 0.31a</td>
<td>94.6 ± 1.56a</td>
<td>2.33 ± 0.24a</td>
<td>91.2 ± 0.34a</td>
</tr>
</tbody>
</table>

Values expressed as Mean ± S.D.
Different alphabets in a column indicate significant difference (P<0.05)
6.4.1.3 Dough characteristics

Table 6.4 shows the effect of chitosan incorporation on physical attributes of whole wheat flour dough. Sensory analysis in terms of kneadability, elasticity, stickiness and rolling properties revealed that addition of chitosan to whole wheat flour had a favorable effect on its rheological properties.

With 1% chitosan incorporation, no significant differences were observed in the physical attributes of the dough. However, incorporation of chitosan at 3% and 5% levels improved kneadability, elasticity and rolling property. A lower degree of stickiness was observed in all the dough samples.

The improvement in some of the dough characteristics can be attributed to the hydration properties of chitosan. Incorporation of fiber to the whole wheat flour, as reported in earlier researches, had shown certain negative effects on the dough properties like extensibility (Yadav et al., 2012) and mixing characteristics (Yadav et al., 2010). When compared to other fibers, chitosan could be a better functional ingredient of the doughs.

6.4.1.4 Rheological characteristics

Instrumental characterization of dough rheology was carried out to confirm the improvement in sensory dough characteristics observed with 3% and 5% chitosan incorporation. The farinogram obtained for the various samples is depicted in fig. 6.3a, b & c. The farinograph characteristics derived from this is given in table 6.5a.

The farinograph water absorption was found to be significantly higher in 5%CHWF (78%) compared to 0%CHWF (70.7%) and 3%CHWF (72.6%). This could be attributed to chitosan which functions like a fibre. More number of hydroxyl groups present in the fibre structure has been reported to allow higher water interactions due to hydrogen bonding (Rosell et al., 2001). Increase in water absorption has also been reported on addition of wheat bran (Pomeranz et al., 1976), carob and pea fibre (Wang et al., 2002) and carob fibre and oat wholemeal (Mis’ et al., 2012).
Table 6.4 Effect of chitosan incorporation on physical attributes of whole wheat flour dough

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kneadability</th>
<th>Elasticity</th>
<th>Stickiness</th>
<th>Rolling property</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% CHWF</td>
<td>++ +</td>
<td>++ +</td>
<td>+</td>
<td>++ +</td>
</tr>
<tr>
<td>0% CHWF</td>
<td>++ +</td>
<td>++ +</td>
<td>+</td>
<td>++ +</td>
</tr>
<tr>
<td>3% CHWF</td>
<td>++ + +</td>
<td>++ ++</td>
<td>+</td>
<td>+++ +</td>
</tr>
<tr>
<td>5% CHWF</td>
<td>++ + +</td>
<td>++ ++</td>
<td>+</td>
<td>+++ +</td>
</tr>
</tbody>
</table>

*0%, 1%, 3% and 5% CHWF – Wheat flour incorporated with 0%, 1%, 3% and 5% Chitosan.
Fig 6.3 Farinograms of 0% CHWF (a), 3% CHWF (b) and 5% CHWF (c)

0%, 3% & 5% CHWF – Wheat flour incorporated with 0%, 3% and 5% chitosan, respectively
Table 6.5 Effect of chitosan incorporation on farinograph (a) and extensograph (b) characteristics of whole wheat flour

<table>
<thead>
<tr>
<th>*Sample</th>
<th>FWA(%)</th>
<th>DDT(min)</th>
<th>DS(min)</th>
<th>S(BU)</th>
<th>FQN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% CHWF</td>
<td>70.7</td>
<td>2.2</td>
<td>1.1</td>
<td>173</td>
<td>29</td>
</tr>
<tr>
<td>3% CHWF</td>
<td>72.6</td>
<td>3.2</td>
<td>1.3</td>
<td>148</td>
<td>35</td>
</tr>
<tr>
<td>5% CHWF</td>
<td>78.0</td>
<td>4.9</td>
<td>1.4</td>
<td>65</td>
<td>57</td>
</tr>
</tbody>
</table>

(a)
FWA-Farinograph water absorption, DDT-Dough development time, DS-Dough stability, S-Softening of dough, FQN-Farinograph quality number

<table>
<thead>
<tr>
<th>*Sample</th>
<th>R(BU)</th>
<th>E(mm)</th>
<th>R/E</th>
<th>Area(cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% CHWF</td>
<td>205</td>
<td>118</td>
<td>1.73</td>
<td>36.1</td>
</tr>
<tr>
<td>3% CHWF</td>
<td>265</td>
<td>128</td>
<td>2.07</td>
<td>52.4</td>
</tr>
<tr>
<td>5% CHWF</td>
<td>430</td>
<td>96</td>
<td>4.47</td>
<td>52.9</td>
</tr>
</tbody>
</table>

(b)
R-Resistance to extension, E-Extensibility, R/E-Ratio figure

*0%, 3% and 5% CHWF – Wheat flour incorporated with 0%, 3% and 5% Chitosan.
Higher values of dough development time (DDT) and dough stability (DS) are indicative of stronger doughs (Wang et al., 2002). An increase in the dough development time (DDT) and dough stability (DS) was observed with an increase in chitosan concentration. A 55% higher DDT and 21.4% higher DS was seen in the 5%CHWF samples, compared to 0%CHWF.

An increase in DDT has been similarly reported (Shenoy and Prakash, 2002) on addition of wheat bran to wheat flour at 5-15% concentrations.

Incorporation of various seed flours as fibre sources to wheat flour have been reported to reduce dough stability (Indrani et al., 2011). This has been attributed to weakening of the dough because of reduction in wheat gluten and competition between the seed flours and wheat flour (Indrani et al., 2011) for water. An increase in dough stability observed in the present study, could be attributed to the mucilaginous gel forming property of chitosan and its good protein content which supports the gluten structure. Similar increase in DDT and DS has been reported for wheat flour doughs enriched with fenugreek flour, (Hooda and Jood, 2005). The authors attributed this to the protein and gum content of fenugreek which helps in the formation and stabilization of gluten network.

Increasing concentration of chitosan also brought about a reduction in the dough softening (S) and an increase in the farinograph quality number (FQN). Dough softening of 65BU was recorded by the 5%CHWF samples as against 173BU recorded by the 0%CHWF samples.

It has been reported that higher dough stability and decreased softening are indicative of doughs having increased sustainability towards longer mechanical processing treatments (Hadnadev et al., 2011). Hence, it could be inferred that chitosan enriched doughs could be more suitable for mechanized industrial processes, compared to control doughs. Highest FQN of 57 was observed in the 5%CHWF samples compared to 0%CHWF (29) and 3%CHWF (35).
The extensograph characteristics (Table 6.5b) of the samples analyzed revealed a greater resistance to extension (R) in 3%CHWF (R=265BU) and in 5%CHWF (R=430BU) samples compared to 0%CHWF samples (R=205BU). With regard to extensibility (E), 3%CHWF (128 mm) recorded higher values compared to 0%CHWF (118 mm). However, a reduction in extensibility was observed with chitosan incorporation at higher concentration of 5% (96 mm).

An increase in resistance to extension in wheat flour has also been reported with the addition of carob fibre (Mis´ et al., 2012). This increase in dough resistance was attributed to the strong interactions between the dough gluten matrix and the carob fibre, which counteracts the weakening effect of higher hydration. The study also reported differential effect on extensibility. The dough with carob fibre exhibited lesser extensibility compared to that with oat whole meal.

An R/E ratio of 1.73 and area of 36.1cm$^2$ was recorded by 0%CHWF in the present study, significantly lower than 5%CHWF (R/E ratio of 4.47 and area of 52.9cm$^2$). A lower R, E and area indicating lower elasticity, extensibility and strength of dough was observed in a study on parotta dough enriched with multigrains (Indrani et al., 2011), which in turn affected flat bread making quality.

Addition of dietary fibre from various sources such as orange, pea, wheat, and microcrystalline cellulose has been found to increase absorption of water, tenacity, and mixing tolerance of dough (Gomez et al., 2002). The addition of these fibers, however, resulted in lower extensibility compared to bread flour without fibre addition. The study also reported a concentration dependent effect with 2% fibre addition being optimum. Addition of apple pomace flour has been reported to improve DDT and DS, but at higher concentrations reduced volume, width, thickness and spread ratio of biscuits (Kohajdová, 2014). Hence, the rheological properties of dough are reported to vary with the type of fibre source and flours used, as well as their concentration.

In the present study chitosan incorporation at 3-5% levels produced desirable changes in most of the rheological characteristics of the dough, suggesting its
effective utilization in the development of flat breads and similar products where extensibility is not a major criterion such as in leavened products.

6.4.2 Effect of incorporation of chitosan on shelf life quality of chapati

6.4.2.1 Antistaling characteristics

- **Moisture content**

As is evident from the results (Fig 6.4), the moisture content of the fresh chapatis showed an increasing trend with an increase in the level of chitosan incorporation. The moisture content of 0%CHCh was 29.3%, which increased to 33.2% in 5%CHCh. Similar to the observation in the present study, Gujral et al. (2004) also reported an increase in the moisture content of fresh chapati from 35.84% to 37.59% with the addition of hydrocolloids.

A reduction in the moisture content was observed on storage in all the samples. The moisture content dropped to 21.6% for 0%CHCh and to a lesser extent to 27.8% in 5%CHCh samples at the end of storage period. In a study conducted by Yadav et al. (2008), the moisture content of ready-to-bake frozen chapatis decreased from 45.8% to 43.0% on storage. Shaikh et al. (2008) also observed a reduction in moisture content of chapati from 32.35% to 21.79% after 10 days of refrigerated storage. They further reported that the addition of emulsifiers like glycerol monostearate resulted in a lesser reduction in the moisture content, compared to control. These results are similar to those obtained in the present study wherein, addition of chitosan improved the moisture retention of stored chapati.

- **Water soluble starch (WSS)**

The highest WSS of 6.19% was observed in fresh 0%CHCh and that of 5%CHCh was found to be the lowest (3.19%). Thus, an inverse relationship was seen between the addition of chitosan and WSS content of chapati. A reduction in WSS in chapatis from 5.23% to 2.36% and 3.29% has been reported with the addition of 0.75% glycerol monostearate and 1.5% guar gum (Shaikh et al., 2008).
Fig 6.4 Effect of chitosan incorporation on the moisture content (%) of whole wheat flour chapati on storage

Fig 6.5 Effect of chitosan incorporation on the water soluble starch content (%) of whole wheat flour chapati on storage

0%, 1%, 3% and 5%CHCh – Chapati prepared form flour incorporated with chitosan at 0%, 1%, 3% and 5% levels, respectively
As the storage period advanced to 72hrs, there was a reduction in WSS levels in all the samples. The values reduced from 6.19% to 4.25% in the control chapati (0%CHCh), while in 5%CHCh, it reduced from 3.19% to 2.04%. Similarly, a reduction in WSS from 5.23% to 1.64% has been reported (Shaikh et al., 2008) after 4 days of room temperature storage in plain chapati and from 3.29% to 1.33% in chapati wherein 1% guar gum was used as an antistaling agent.

• *In vitro enzyme digestibility (IVED)*

IVED of starch of the chitosan incorporated whole wheat flour estimated in the present study was expressed in terms of percent glucose liberated. IVED of starch of fresh chapati samples showed a similar trend as WSS, i.e., as the level of chitosan incorporation increased, the amount of glucose liberated decreased significantly from 3.43% in 0%CHCh to 2.85% in 5%CHCh. Over a period of storage for 72 hrs, there was a decrease in the IVED within each sample.

Shaikh *et al.* (2008) have also examined the effect of addition of various antistaling agents, like α-amylase, xylanase, maltodextrin and others on IVED and reported a decrease in IVED in chapatis with added antistaling agent when compared to the control chapatis (4.63%). For instance, chapatis containing maltodextrin, recorded an IVED value of 4.38% on 0 day, which reduced to 4.01% after 4 days storage.

• *Differential scanning calorimetry characteristics*

Differential scanning calorimetry was carried out for the stored chapati samples and recorded in terms of the onset (T<sub>O</sub>), peak (T<sub>P</sub>) and conclusion (T<sub>C</sub>) temperatures.

The T<sub>O</sub>, T<sub>P</sub> and T<sub>C</sub> of all samples were found to range from 59.9 - 62.7°C, 96.9 – 98.7°C, and 114.6 – 117.4°C, respectively, with no significant differences between the samples (Appendix I). A decrease was seen in the T<sub>O</sub>, T<sub>P</sub> and T<sub>C</sub> on storage for all the samples, with the percentage reduction being significantly higher for control (0%CHCh) and 1%CHCh. Similar decrease in T<sub>O</sub> and T<sub>C</sub> on storage has been reported by Shaikh *et al.* (2007).
Fig 6.6 Effect of chitosan incorporation on the *in vitro* enzyme digestibility (% glucose liberated) of whole wheat flour chapati on storage

Fig 6.7 Effect of chitosan incorporation on the retrogradation enthalpy $\Delta H$ (J/g) of whole wheat flour chapati on storage

0%, 1%, 3% and 5% CHCh – Chapati prepared from flour incorporated with Chitosan at 0%, 1%, 3% and 5% levels, respectively.
The retrogradation enthalpy ($\Delta H$) values of the chapati samples ranged from 0.38-0.45 J/g. An increase in $\Delta H$ was seen in all samples after 24h storage, with the values being significantly higher for 0%CHCh (0.98) compared to other samples (0.54-0.49). Similar $\Delta H$ value of 0.38 has been reported in fresh conventionally baked chapati (Gujral et al., 2008), which increased to 1.10 on 24h storage. Shaikh et al., (2008) also reported an increase in $\Delta H$ from 129.8 Cal/g to 136.5 Cal/g in chapatis after 48h storage at room temperature, which increased to 141 Cal/g after 5 days. Further increase in $\Delta H$ was observed after 72h storage period in the present study too.

This increase is attributed to the formation of crystalline structure of starch on storage, which requires greater energy for melting the starch. A lower $\Delta H$ value recorded in the chitosan enriched samples indicates lower retrogradation enthalpy. This could be due to the action of chitosan as a hydrocolloid. Hydrocolloids have been reported to have stabilizing effect on starch retrogradation due to their favorable interactions with water as well as the starch chains in the dough (Lee et al., 2003). Similar beneficial effect has been reported in chapatis with the incorporation of hydrocolloids like xanthan and guar gum and enzymes like $\alpha$ amylase, due to reduction in retrogradation enthalpy resulting in delayed staling.

Several techniques have been used to retard staling in chapati, some of which are: use of different packaging materials, heat treatment, refrigeration and antistaling agents. In the present study, changes in the staling parameters of chapati was retarded considerably by the addition of chitosan, in spite of being stored in ordinary LDPE pouches which have relatively high water vapour permeability.

Similar antistaling effect of chitosan has been reported by Kerch et al. (2008) in bread where chitosan is postulated to have been adsorbed on to the starch molecules due to its hydrogen bonding capacity. As a result, starch granules are prevented from taking up the water released by gluten and this in turn inhibits the cross linkage between starch granules and gluten, which is responsible for staling. The results thus indicate the potential of using chitosan also as an antistaling agent in products such as chapati, helping to maintain their shelf life quality for a longer time.
6.4.2.2 Microbial quality

Mold growth was observed in 0%CHCh samples after 24 hours showing 10 visual colonies. Addition of chitosan at 3% and 5% level could extend the shelf life by another 48 hours. After 72 hours, mold growth started in 3%CHCh showing (8 visual colonies, with initiation of mold growth seen in5%CHCh. This effect is of significance as no salt or other preservatives were added and the samples were stored at room temperature in ordinary packing material (LDPE) normally used for chapati sold in most markets.

The antifungal activity of chitosan has been reported in many studies (Park et al., 2008; Pena et al., 2013). Rodriguez et al. (2003) reported the antifungal activity of chitosan in acetic acid used as an edible coating (0.079 g/100 g pizza) against Alternaria sp., Penicillium sp., and Cladosporium sp., (Deuteromycetes) on precooked pizza.

Ready to eat chapatis are increasing in demand owing to rapid urbanization and changing lifestyles. However, these RTE products have to be preserved for more than 24h unlike home consumed chapatis. This has led to various studies investigating the use of many chemical additives such as fumarates, sodium propionates and ascorbic acid (Abu-Ghoush et al., 2008; Khan et al., 2011). Sodium chloride is also widely used to extend the shelf life of flat breads. However, the ill effects of NaCl on health and the demand for natural preservatives makes chitosan an ideal candidate for extending microbial shelf life of flat breads such as chapati. Presence of natural salts such as sodium (34mg/100g of 5%CHWF) and calcium (3.8mg/100g of 5%CHWF) in chapati could have also helped its preservative role apart from its established antifungal activity.

6.4.2.3 Sensory acceptability

The effect of addition of chitosan to whole wheat flour chapati revealed that chitosan incorporated chapatis were of better quality compared to control chapati, particularly for flavor. The panelists described a better taste and aroma of chitosan enriched chapati.
Fig 6.8 Sensory acceptability of chapati at 0h (a) and after 24h (b), 48h (c) and 72h (d) of storage

0%, 1%, 3% and 5% CHCh – Chapati prepared from wheat flour incorporated with chitosan at 0%, 1%, 3% and 5% levels, respectively
This may be partially attributed to the natural salts present in chitosan (chapter 2) which includes Na and Ca among others, which could have contributed to the flavor.

With respect to texture, 0%CHCh had become hard and brittle within 48h of storage indicative of staling of chapati. Also these samples had developed off-flavavour and became inedible also due to microbial spoilage. Hence, the control chapatis were not evaluated after 48h storage period.

Compared to 0%CHCh, 3%CHCh and 5%CHCh maintained better texture and flavor. The overall acceptability, judged at the end of 48 hours, for various chapati samples showed highest scores for 5%CHCh (4.3), followed by 4.0 for 3%CHCh, and 3 for 1%CHCh. The 5%CHCh chapati remained acceptable up to 72 h storage at room temperature.

Addition of wheat bran at 5%-15% level in chapati has been reported to significantly reduce the sensory quality compared to control, though at 5% level the acceptability was good (Shenoy and Prakash, 2002). Similarly addition of multigrain flour beyond 10% was found to significantly reduce the overall acceptability of north Indian parotta (Indrani et al., 2011).

Chitosan incorporation, unlike few additives and fibre sources such as bran, did not negatively affect sensory quality of chapati. On the other hand, it was found to enhance the same, particularly with respect to flavor and texture. The panelist gave a higher overall acceptability score also for the better and uniform creamy colour as well as for the well baked appearance with desirable light brown spots indicating doneness. Chapati prepared with 3% guar gum, a source of viscous fibre, has been reported to improve the sensory quality in terms of colour, flavor and texture (Shahzadi, 2005).
6.5 Conclusions

Chitosan incorporation in the wheat flour led to improvement in the functional properties and nutritional composition. Higher mineral profile in chitosan enriched flours with particular reference to Ca and Fe is promising in terms of improving the micronutrient status of the population.

Chitosan enriched flours exhibited suitable rheological characteristics for extruded and flat bread type of products (eg: chapati). The study indicates that chitosan incorporated wheat flour could be used in products such as chapatis for enhanced shelf life and sensory quality. The study has confirmed the suitability of using chitosan as a natural antistaling and preservative agent in cereal based products, not explored earlier in flat breads/chapati. The use of chitosan as a potential salt substitute as indicated in the present study needs to be further explored and confirmed.