Chapter 1

Introduction
1.0 Wheat

Wheat is a wild grass (Gramineae family) native to arid countries of western Asia (Cornell and Hoveling, 1998). About 600 genera of grasses exist. All wild and cultivated wheat varieties are members of the genus *Triticum*. Economically they are the most important group within the large grass family, the Gramineae (Poaceae) and the Hordeae tribe (Kill, 2001). Genus *Triticum* has some of the main groups listed as below:

- *Aestivum (Vulgare)* - Common wheat,
- *Durum (Duru*m wheat)*,
- *Compactum (Club wheat)*,
- *Turgidum (Poulard wheat)*,
- *Dicocum (Emmer wheat)*,
- *Spelta (Spelt wheat)* and
- *Polonicum (Polish wheat)*.

1.1.0 Classification of wheat

As reported by Kill, 2001, the cytogenetic and cytological work was carried out in 1918, by Sakamura and describes that wheat could be divided into 3 distinct groups based on the number of chromosomes present in each morphologically recognized type. Each group was distinguished by the fact that each of its somatic cells contained 14 chromosomes (seven pairs) or multiples of 14 chromosomes in each somatic cell. The simplest group is the diploid wheat, which has two sets of chromosomes (14 chromosomes). This group has *T.monococcum* as a member. The second group is the tetraploid wheat, the two important members being *T.durum* and *T.dicoccum*. The tetraploid wheat has four sets of seven chromosomes (28 chromosomes). The third group containing 42 chromosomes is the hexaploid wheat. It is the most economically important bread wheat. Each member has six sets of seven chromosomes, the chromosomes being categorized into genomes (Kill, 2001; Bozzini, 1988).

The earliest known grains of domesticated wheat dates back to 7500–6500 BC. Between 6000 and 5000 BC *T.monococcum*, a species of Diploid wheat has been domesticated and used by humans for 8000-10000 years. This type of wheat has tough rachis of the spike and has seeds generally only one per spikelet, almost twice the size of those of the wild *T.boeoticum*. This wheat is still cultivated in the remote mountainous areas of Italy, Turkey and
Transcaucasia. Tetraploid wheat was largely distributed in the Near East. Their general size particularly the head and kernel size made much more worthwhile for domestication than the diploid wheat (Bozzini, 1988). Among all cultivated tetraploid wheat, *T.durum* types are the most important, even though they are grown in only 10% of all the wheat cultivated area, the remaining 90% being represented by the hexaploid wheat, *T. aestivum* (Hanson et al, 1982). Hexaploid wheat (*T.aestivum*) and cultivated emmer wheat (*T. timopheevi var. araraticum* and *T. turgidum var. dicoccoides*) penetrated into the irrigated agriculture of Mesopotamia and Western Iran (Feldman, 1976).

For commercial purpose, wheat is classified as hard or soft, red or white spring or winter. But most of the varieties cultivated today are grouped together under the broad category of common of bread wheat, which accounts for approximately 95% of world production. Nearly all of the remaining 5% of cultivated varieties are durum wheat, used for such products as pasta and couscous.

Based on the suitability for bread making, wheat is normally divided into two quality classes; Hard and soft. Hard wheat has hard kernel which yields flour with high gluten content that is suitable for producing a Western style loaf of bread and some type of noodles. Soft wheat is characterized by a lower protein level and is most suitable for producing cakes and biscuits. There are also semi hard wheat having some combination of the above quality characteristics and utilized in unleavened breads such as chapattis as well as Asian steamed bread and certain noodles (Oleson, 1994).

### 1.1.1 *Durum* wheat- Morphology, physiology and Development

*Durum* wheat is one of the most widely grown food crops in the Mediterranean region and it is the main source of semolina for the production of pasta, couscous, and bread (Belleggia et al, 2009; Bozzini, 1988). Although *T.durum* was initially of Mediterranean origin species has now spread around the world to countries as diverse as Bulgaria, India, Tunisia, Chili, Australia and the Pacific Islands (Kill, 2001). In the first half of this century, Russia was the largest producer and exporter of *durum* wheat and another country where the
durum is grown is India. Currently the major *T.durum* growing areas include Canada, USA, Australia, the Common wealth of Independent States and India.

*T.durum* wheat has a rather strong root system, with a number of fibrous and long roots growing to a depth of more than 1m in good conditions. The stem is cylindrical erect and sometimes solid, but more often hollow, subdivided into internodes (three to nine) by well identified knots, each one being the origin of a leaf. Leaves are composed of a basal portion, the leaf sheath, which envelops the stem and overlaps the margin. The terminal portion of the leaf is linear with parallel nerves and an acute apex. There is a thin transparent membrane at the attachment of the leaf, on the leaf sheath, with two small lateral appendices, called auricles.

Typically, hermaphroditic flowers, cluster in sessile or nearly sessile spikelets that are layered upon each other to form a spike. Each spike consists of an axis, called as rachis subdivided into small portions or fragments, on which rachillae, the axes of the spikelets are inserted. Two bracts are found at the base of each spikelet which are well developed with an acute apex and are called as glumes where in flowers are formed. In durum wheat, flowers vary from 2-6, each formed by 2 glumes.

*T.durum* wheat is typically an annual cereal and is a widely adaptable crop, grown from temperate irrigated to dry and high rainfall areas, and from warm humid to dry cold environments. This wide adaptation is possible due to the complex nature of its genome. The physiological process connected with seed germination can take place at 1-2°C, but the optimal temperature is 20-25°C. Physiologically, various stages of growth may be grouped as, germination to emergence (E); growth stage 1 (GS1) from emergence to double ridges; growth stage 2 (GS2) from double ridges to anthesis and growth stage 3 (GS3), to include the grain filling period, from anthesis to maturity.
1.1.2 Structure of the Wheat Kernel

Botanically known as Caryopsis, wheat kernel is 4-8 mm long. It consists of epidermis (outer layer) and hypodermis, next to a layer of thin walled cells and several other types of cells. This pericarp is about 50 µm thick followed by a thin covering nucellar epidermis, an aleurone layer, and coming to the starch rich endosperm.

The starchy endosperm comprises of starch granules embedded in a matrix of proteins. The protein consists of albumins, globulins, gliadins and glutenins. The combination of gliadins and glutenins are referred to as the “Gluten complex” which is a storage protein, called as prolamines of wheat. The starch is present as lenticular granular varying in size from 10–50 µm diameter (major axis) and smaller, more spherical granules of 2-5 µm diameters (Cornell and Hoveling, 1998). The longitudinal section of the wheat kernel is shown in Fig 1.1.

1.2 Wheat utilization

Wheat is an enormously multi purpose edible grain. It is available in the market for human consumption as breakfast cereals, breads of different types, pasta and noodles. Wheat is also used as a raw material for manufacture of starch and has its application in both food and non food industry. Wheat is the main source of industrial production of vital gluten apart from wheat starch, where, wheat gluten has wide application in baking and cereal industry. Being a valuable commodity in the food industry, because of its ability to form gel, wheat starch is used as a thickener. Wheat starch can be modified to improve its pasting properties and reduced retrogradation. Other uses are, as molding starch in the confectionery industry, as a base for custard powder and as a filler. Wheat derived products have been used as milk and meat substitutes (Cornell and Hoveling, 1998). Small additions are generally considered as beneficial to good texture but in many countries, legislation restricts the use of it being an adulterant of meat.
Wheat is also used as a substitute material for rice called as bulgur has been quite successful in this role. Wheat flour is widely used for the
manufacture of different types of bread, cakes and pastries. Pasta and noodles require *durum* wheat flour, sometimes used in conjunction with flour or meal from other plants to produce new flavors and texture. Potato bread, sourdough bread, oat bread, rye bread are some of the examples of different varieties of bread with health benefits. Flat bread of the Middle East is one of the oldest forms of bread eaten today and is known as Arabic bread. Hallow bread has been used for the Middle Eastern sandwich and makes an excellent envelope for vegetable salad and other nutritious filling. Wheat is one of the major ingredients in breakfast cereal manufacture, with wheat flakes, puffed wheat and wheat biscuits in the front lead. Pasta consumption has increased in Eastern Europe and is being more popular food around the globe. Filled pasta like- *tortellini, ravioli* and *cannelloni* can be filled with meat, cheese, vegetables and even fish (Cornell and Hoveling, 1998).

1.3 Gluten

Gluten may be defined as the cohesive, viscoelastic, proteinaceous matter. The proteins that form gluten are the storage proteins of the mature wheat and may not differ much from those of other grains. Mature wheat grains contain 8-20% of proteins. The gluten proteins, gliadin and glutenins constitute up to 80-85% of total flour protein, and confer properties of elasticity and extensibility. The gliadins and glutenins constitute each around 50% of the gluten proteins. However the distinctive feature that makes wheat unique is the viscoelastic properties of its storage protein. When grain is milled and mixed with water, the ‘storage protein’ forms dough with unique rheological properties, capable of retaining gas bubbles, suiting the dough to the wide range of products. These properties make wheat suitable for the preparation of great diversity of food products- breads, noodles, pasta, cookies, cakes, pastries and many other foods.

Gluten was first prepared from flour almost 300 years ago by *Beccari*, an Italian who conducted a simple water-washing experiment with wheat flour. Gluten as a commodity has a wide range of uses in the food industry. Gluten is traded in the dried state as ‘vital wheat gluten’, its most familiar form. In wheat
based products, gluten is used to fortify flours of lower protein content. Vital wheat gluten’s unique viscoelastic properties improve dough strength, mixing tolerance and handling properties. Its film foaming ability provides gas retention and controlled expansion for improved volume, uniformity and texture. Its thermosetting properties contribute necessary structural rigidity and its water absorption capacity improve yield, softness and shelf life of bakery products (Day et al, 2006).

1.3.1 Gluten proteins

Gluten can be fractionated into the ethanol soluble prolaminines and ethanol insoluble glutenins (Schuppan, 2000). A common feature of the prolamine is a high content of the glutamine (>30%) and proline (>15%) (Schuppan, 2000; Murray, 1999). The wheat prolaminines are subdivided into α, β, γ and ω gliadin, displaying molecular weights between 20,000 and 75,000 Daltons and containing similar or repetitive glutamine and proline rich peptide sequences such as Pro-Ser-Gln-Gln (PSQQ) and Gln-Gln-Gln-Pro (QQQP) that appear to be responsible for the toxicity of gluten in Celiac disease (Schuppan, 2000). Amino acid sequence of the individual prolaminines fall into 3 groups: sulphur-rich (S-rich), sulphur-poor (S-poor) and high molecular weight (HMW) prolaminines. The S-poor prolaminines comprise the ω-gliadins of wheat, hordeins of barley and ω secalins of rye, accounting for about 10-20% of the total fraction in barley, about 11% in rye and probably same proportion in wheat (Tatham and Shewry, 1995).

The amino acid composition of S-poor prolaminines or ω-gliadins are characterized by the high levels of glutamine of about 40-50% mol%, 20-30 mol% of proline and phenyl alanine of 7-9% residues which together account for about 80% of the total residues (Tatham and Shewry, 1995; Shewry, 1986) but few or no residues of sulphur containing amino acids (cysteine and methionine). In contrast, the α, β and γ-gliadins have less proline, glutamine and phenyl alanine but 2-3 mol% cysteine plus methionine. Hence gliadin contains similar or repetitive glutamine and proline-rich peptide sequences such as Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro that are responsible for toxicity (Schuppan, 2000).
1.4 Gluten proteins as allergens

Due to high prolamine content, gluten is highly resistant to proteolytic degradation within the gastrointestinal tract because the gastric and pancreatic enzymes lack post proline cleaving activity. Moreover, high glutamine content makes gluten a good substrate for the enzyme tissue transglutaminase (Catassi and Fasano, 2008). Clinically α-gliadins have been shown to be toxic in patients with Celiac Disease (CD) (Kieffer et al, 1982). Peptides derived from wheat gliadin are found to be more toxic since, amino acid sequence have shown the 4-mer amino acid motifs PSQQ and QQQP present in toxic peptides A(α)-gliadin (Cornell and Johnson, 2001).

Hypersensitivity reactions to ingested wheat protein typically occur within an hour and include cutaneous, gastrointestinal and respiratory symptoms. Adverse reactions to wheat include: 1. Baker’s asthma, an IgE-mediated reaction to inhaled flour, 2. Celiac disease, a non-IgE mediated enteropathy caused by wheat gliadin, 3. Wheat dependent exercise induced anaphylaxis (James et al, 1997) and 4. Atopic dermatitis (Tanabe, 2008).

1.4.1 Baker’s asthma: wheat flour consists of water soluble albumins, salt soluble globulins, gliadins and glutenins. Albumins and globulins appear to be the most important proteins contributing to immediate hyper sensitivity reactions to wheat proteins (James et al, 1997). Baker’s asthma due to wheat allergens is an occupational disease (Gomez et al, 1990; Baur, 1999). It is mainly a type I allergy in which patients has a specific IgE for the allergen. It is caused by immunologic sensitization and also subsequent allergic reactions to airborne allergens in the airways (Houba et al, 1998). Several studies reports that α-amylase inhibitors and trypsin inhibitors have been identified as major allergens (Amano et al, 1998; James et al, 1997). Different research studies have identified nearly 40 different antigens of wheat flour, of which 18 bound specific IgE from sera of sensitized bakers. A 16 kDa wheat protein, a glycosylated subunit of the wheat tetrameric α-amylase inhibitor, has been identified and confirmed as important allergens. One research study implicated 20 kDa and 47 kDa wheat proteins as potential allergens in children with confirmed wheat
hypersensitivity. James et al, (1997) have also identified 15 kDa proteins as wheat α-amylase inhibitor. Other additional bakery allergens comprise of rye flour, and recently reported enzyme xylanase (Baur, 1999).

1.4.1.1 Preventive measures

Avoidance of allergens is the main choice of management of baker’s asthma. This can be achieved by technical dust control, relocation of the baker to less exposed job- task, or by having the baker wear respiratory protection (Brisman, 2007). The total load in most bakeries is high, Total weight average values (TWA) mainly vary between 1 and 10 mg/m³, but peaks of >50 mg/m³ frequently occur. Flour dust concentrations between 0.5 and 2.4 mg/ m³ are associated with significantly elevated risk of sensitization. It is found that powdered enzymes also constitute to the bakery dust. And approximately 0.03% of the total bakery dust was shown to represent α-amylase (Baur, 1999). Hence, the use of granulated or liquid enzyme preparation instead of powdered ones reduces the exposure to allergens. Apart from this, use of closed machinery and exhaust system during production may lessen the exposure. Appropriate protective respiratory devices should be used by susceptible individuals as secondary preventive measures (Baur, 1999).

1.4.2 Atopic dermatitis: According to Leung et al, 2004 written description of atopic dermatitis (AD) dates back to 1800s. AD is an itchy, chronic inflammatory skin condition which is characterized by erythema with edema, vesicles and weeping during the acute stage and lichenification in the chronic stage. AD usually presents during early infancy and childhood. But it can start in adulthood also (Williams, 2005; Leung et al, 2004). Approximately 70% of cases of atopic dermatitis start in children less than 5 years of age, 10% of cases reported in hospital settings start at adulthood (Leung et al, 2004). AD is a complex disease relying on the interplay of several factors. It is the result of several interactions among susceptibility genes, the host’s environment, defects in skin barrier function and systemic and local immunologic responses (Williams, 2005; Leung et al, 2004). There are at least 2 forms of AD: an “extrinsic” form associated with IgE-mediated sensitization and “intrinsic” form without IgE-mediated
sensitization. Extrinsic form involves 70-80% of patients where as intrinsic form involves 20-30% of patients. AD is characterized by dry skin and even involves non lesional skin and increased transepidermal water loss. The impairment of the skin barrier function results in increased antigen absorption contributing to the cutaneous hyperactivity characteristics (Leung et al, 2004).

1.4.3 Celiac disease

Celiac disease (CD) also known as gluten sensitive enteropathy is an immune mediated permanent intolerance to ingested gluten in genetically susceptible individuals (Picarelli et al, 2001; Murray, 1999). The condition is characterized by inflammation of the small intestinal mucosa (Murray, 1999). Researchers established CD as a chronic systemic autoimmune disorder induced by the gluten proteins present in the wheat, barley and rye (Rewers, 2005; Fasano, 2005). CD differs from traditional immunoglobulin E (IgE) mediated food allergies. The condition is unique in that, a specific food component, gluten, has been identified as culprit (Murray, 1999). It is the alcohol soluble protein component of wheat (gliadins), barley (secalins) and rye (hordeins) collectively called as prolamines toxic, whose toxicity remains after peptic-tryptic digestion (Abdulkarim and Murray, 2003). In the continued presence of gluten, CD is self perpetuating. CD represents a unique example of immune mediated disease for which early serologic diagnosis and dietary treatment can prevent severe sometimes life threatening complications (Fasano et al, 2003). Removal of gluten from the diet is an effective way to stop the disease process. A gluten free diet is the only treatment for the disease (Papdopoulous, 2001).

1.4.3.1 Pathogenesis of CD

Murray (1999) describes that, CD is the result of the three processes that end in intestinal mucosal damage: genetic predisposition, environmental factors and immunologically based inflammation. The author also states that, CD may
be the result of an evolutionary collision between the cultivation of the wheat and the human immune system.

1.4.3.1.1 Genetics

CD is a multigenic disorder associated with HLA-DQ2 (DQA1*05/DQB1*02) or HLA-DQ8 (DQ A1*0301/DQ B1*0302) encoded by MHC (Major Histocompatibility Complex) genes (Papdopoulous, 2001) located on chromosome 6. HLA-DQ is expressed in more than 90% people with CD and is encoded either in the cis or in trans (Green and Jabri, 2003; Murray, 1999; Papdopoulous, 2001). It is a heritable condition and is seems to be more common in whites (Murray, 1999). CD is strongly associated with the extended HLA phenotypes (class II α/β heterodimer) (Stugress et al, 2005). Research studies suggest that HLA genes are not the main causative factor in CD. The non HLA chromosome region most consistently linked with CD is 5q 31-33, located on the long arm of chromosome 5. Another potentially interesting region for a susceptibility factor is 11q. The genotypes that encodes DQW2 includes DQ A1*0501 and DQ B1*0201 alleles (Abdulkarim and Murray, 2003). Individuals who are having homozygous for the DQ A1*0501 and DQ B1*0201 alleles may develop the disease at an earlier age (Murray, 1999). These genotypes are found in at least 98% of the patients (Fasano, 2005). It has been shown that gliadin specific CD4 –T cells in the small intestinal mucosa of the CD patients are predominantly restricted by this DQ heterodimer (Stugress et al, 2005). An increased abundance of HLA-DQ heterodimer has correlated with an increased magnitude of invitro gluten-specific T-cell response. HLA-DQ alleles account for only 40% of the genetic susceptibility to the disease (Kagnoff, 2007). HLA-DQ 2 and HLA-DQ 8 heterodimers on APCs (Antigen Presenting Cells) can bind subsequently present ‘gluten’ peptides to populations of CD4+T cells in the lamina propria of the small intestine, because the peptide binding groove of HLA-DQ 2 and HLA-DQ 8 favors the binding of negatively charged residues at key portions (Kagnoff, 2007).

1.4.3.1.2 Environmental factors
The most important environmental factor in CD is gluten (Murray, 1999). Gluten is the protein fraction of wheat, rye and barley that confers the properties of stickiness and thus allows the baking of the bread. As explained earlier the wheat peptides containing similar or repetitive glutamine and proline rich peptide sequences such as Pro-Ser-Gln and Gln-Gln-Gln-Pro that are known to be responsible for the toxicity of the gluten in CD (Schuppan, 2000). The deleterious proteins are gliadins (wheat) hordeins (barley) and secalins (rye) and possibly avinins (oats). Wheat barley rye is related temperate wild grasses are classified in the sub family Festucoideae of the Poaceae (Gramineae) (Tatham and Shewry, 1995). Oats may not be injurious in moderate doses as it is most distantly related to wheat (Murray, 1999; Kagnoff, 2007). Rye is more closely related to wheat than barley (Tatham and Shewry, 1995). These grain plants share a common taxonomy. Glutamine and proline rich peptide sequences are also found in the genetically related prolamines of rye and barley.

It is clear that the gluten contains a relatively large number of T-cell stimulatory sequences (papdopoulous, 2001). Early exposure of immature immune system to gliadin is a prominent cofactor for manifestation of clinically overt celiac disease, probably by skewing the immune system toward a T-cell response (Schuppan, 2000). Large gluten peptides that contain multiple HLA-DQ binding epitopes have greater stimulatory than small peptides containing single HLA-DQ binding sequence (Kagnoff, 2007).

1.4.3.1.3 Immunological factors

In celiac disease there is an increased immunologic reactivity to α-gliadin (Cliona et al, 1983). A gliadin fragment can activate the innate immune system, affecting the in-situ T-cell recognition dominant gliadin epitopes. Gliadin specific T-cell activation is the fundamental step in celiac disease. The immune response to gliadin takes place in two compartments, the lamina propria and epithelium (Green and Jabri, 2003). The intestinal lesion in the intestine is an immunologically mediated inflammation characterized by inflammatory and structural changes of the mucosa of the proximal small intestine. The
inflammatory response due to increased numbers of lymphocytes, macrophages and plasma cells in the lamina propria and also increased number of lymphocytes in the surface layer of the epithelium. Cellular immunity seems to play the major role in the the intestinal mucosa in celiac disease. After 2-4 h of exposure to gluten, there is early increase in the expression of HLA antigen markers on cells in the surface layers of the intestinal mucosa (Murray, 1999).

Untreated CD results in a potent humoral response. IgA, IgM and IgG antibodies directed against toxic peptides and auto antigens to connective tissues are produced by increased numbers of plasma cells in the intestinal mucosa. The antibodies are secreted as secretory IgA into the intestinal lumen and are directed against gliadin may be a vain effort to exclude a harmful antigen, gliadin. Host antigens are targeted by antibodies to connective tissue of the jejunum, reticulin, umbilical cord and endomysium. Tissue transglutaminase (protein–glutamine γ-glutamyl transferase) is native target for the auto antibody. Tissue transglutaminase forms complexes with gliadin, which allows gliadin-responsive T-cells to help tissue transglutaminase responsive but inactivate B-cells to generate a potent self directed antibody response (Murray, 1999).

1.4.3.2 Events of pathogenesis in CD

The initial event observed is an increase in intraepithelial lymphocyte count followed by infiltration of lamina propria with lymphocytes (stage 1), crypt hyperplasia (stage 2), precedes villous atrophy (stage 3) and is only observed in lamina propria lymphocytosis (Schuppan, 2000; Abdulkarim and Murray, 2003). It can be divided into three phases: luminal and early mucosal events and activation of pathogenic CD₄⁺T cells. The entire event of pathogenesis is graphically illustrated in Fig 1.2.

When gluten is ingested, in genetically susceptible individuals, the alcohol soluble protein, gliadin is presented by antigen presenting cells (APC) in association with the human leukocyte antigen HLA-DQ2 or HLA-DQ8 to T cells expressing α/β T cell receptors (Schuppan, 2000; Abdulkarim and murray, 2003; Picarelli et al, 2001). HLA-DQ2 and HLA-DQ8 binds the gluten peptides and forms complexe. Gluten peptides-HLA-DQ complexes can activate T-cells
in the mucosa of the proximal small intestine that recognizes these complexes. These T-cells then become activated and in turn recruit other lymphocyte that produce interferon-γ tumor necrosis factor α- and interleukins -4, -5, -10, and -13 (Murray, 1999; Abdulkarim and Murray, 2003). These cytokines are damaging factors to small intestinal epithelium. The damaged epithelium augments the release of tissue transglutaminase (tTG) (Abdulkarim and Murray, 2003). tTG is a calcium dependent intra cellular enzyme and catalyzes the covalent and irreversible cross linking of proteins resulting in the formation of a ε-(γ-glutamyl)-lysine (isopeptidyl) bond. The enzyme is normally stored intracellularly and is released during cellular wounding due mechanical stress, inflammation infection or during apoptosis (Schuppan, 2000). Gliadins are excellent glutamyldonar substrates for tTG, giving rise to gliadin cross-links and even the covalent incorporation of tTG itself into high molecular weight complexes. Apart from cross linking, tTG can also deamidate glutamyl donor substrates. Deamidation converts certain glutamine residues into a negatively charged glutamic acid. These modified gliadin peptides by deamidation, bind much better to the celiac specific HLA-DQ2 than their non deamidated counterparts and elicit a stronger proliferative response of gliadin specific T-cell clones (Schuppan, 2000; Green and Jabri, 2003; Kagnoff, 2007). In addition, cytokines released by lymphocytes augments the expression of HLA-DQ2 on small intestinal epithelium allowing more gliadin to be presented to sensitized lymphocytes (Abdulkarim and Murray, 2003). This leads to the recognition by CD4 T-cells mainly directed against deamidated peptides (Green and Jabri, 2003).

Intestinal CD4⁺T-Cells that recognize deamidated peptides are presented by DQ 2 and DQ8 produce interferon γ, which in turn provokes inflammation and villous atrophy (Green and Jabri, 2003). The normally tall villi are shortened and flattened. The variable length of the small intestine is affected by the patchy changes (Murray, 1999). The mucosa of healthy individuals is characterized by tall villi lined by a single layer of columnar epithelial cells with nuclei located near the basal surface. The complete loss of villi is accompanied by the presence of markedly abnormal squamoid surface epithelial cells, an increase in
the number of IELs (Intra epithelial leukocytes), a marked increase in the number of plasma cells and lymphocytes in the lamina propria and striking Crypt hypertrophy with increased crypt mitosis (Kagnoff, 2007).

1.4.3.3 Trigger factors

Payer patches are immunologic sampling site. Luminal antigens are taken up by m- cells (a specialized population of thin enterocytes over the Payer patches) by pinocytosis and delivered to the sub-epithelial layer of the payer patch where antigen presenting cells process the antigens. Thus allows a controlled sampling of the antigenic milieu by the intestinal immune system. Primed B cells travel to the mesenteric lymph nodes from Payer patches and return to the jejunal mucosa, where they are terminally differentiated to produce IgA directed against the antigen (Murray, 1999). Active celiac disease is accompanied by mucosal (especially IgA) auto antibodies to reticulin, a common constituent of the extracellular matrix. Anti reticulin auto antibodies are identical to anti endomysial, anti jejunal or anti umbilical cord antibodies. IgA anti endomysial auto antibodies (EMA) allows a screening for biopsy-proven celiac disease with almost 100% sensitivity and specificity (Schuppan, 2000).

1.4.3.4 Prevalence

As reported by Sood et al, 2001, Samuel Jones Gee reported the first case of CD in 1881, but it was only after 1940 that wheat and its close relatives were proposed as the causative factors of celiac disease by Dick a Dutch pediatrician for the first time (Sood et al, 2001; Goddard and Gillet, 2006). A decade ago celiac disease was considered a comparatively uncommon disorder with prevalence rates of 1 in 1000 or lower. However several recent population studies have shown a much highest prevalence and it is estimated that CD may affect 1-2% of individuals (Kocchar et al, 2012). The discovery that CD is a prevalent disorder can be attributed to the judicious use of serological screening tests, which measure the endomysial and anti gliadin antibodies (Feighery, 1999). Among these, antiendomysial test has advanced the diagnosis of CD
owing to its specificity and sensitivity. Study reports that CD is much more common in United States than was previously reported (Feighery, 1999; Fasano et al, 2003). Fasano and Catassi (2001) reported that the prevalence of CD in US to be similar to that reported in Europe ranging from 4.54% among first degree relatives of patients with CD to 0.75% in the subjects who are not at risk of the disease (Fasano et al, 2003). Study also report that the prevalence of CD found among individuals affected by numerous common disorders, including Type 1 diabetes mellitus, anemia, arthritis, osteoporosis, infertility and Down syndrome even in the absence of gastrointestinal symptoms. Among subjects who are not at risk, the prevalence of CD in adults was significantly higher (1:105) than in children (1:320) (Fasano et al, 2003). Rewers (2005) reports the prevalence of preclinical (latent), undiagnosed (largely silent) and diagnosed (mostly active) CD in several European and US populations. Study suggests that, prevalence if diagnosed CD varied widely and estimated of combined undiagnosed and diagnosed (or silent and active) CD were remarkably similar between 0.7%-2.0% in most of the populations including the US. The prevalence of childhood CD has been reported to be 1:285-1:77 in Sweden, 1:99 and 1:67 in Finland and 1:230 and 1:106 in Italian school children. Similar rates have been reported for non-European white populations, such as New Zealand, Australia, Argentina and Israel. Although the disease is believed to be rare in Africa (and in African Americans), the highest prevalence has been reported for Saharawi in North Africa (Rewers, 2005). Catassi and Fasano, 2008 reports 6% prevalence of anti endomisial antibody positively in large sample selected Saharawi children. In Western countries the highest value so far reported is 1% at least five times lower than this. Among other Asian countries Jordan, Kuwait, Iraq, Iran, Saudi Arabia, Oman and Pakistan also reports few cases of CD. Interestingly no cases are reported from other South East Asian countries including Japan, china, Malaysia, Bangladesh and Thailand where rice is the staple diet (Yaccha, 2006).

CD was first reported in India by among both children and adults by Mishra et al, 1966. Thereafter upto 1993 only 3 publications have reported CD among Indian population as reported by Yaccha et al, 2006. A study from India recently revealed the CD prevalence rate of 0.3-2% in adults and 6% in children
(Kocchar et al, 2012). CD constitutes 26% of all mal-absorption syndrome cases in Indian children. Another study reported that CD comprised 16.6% of 266 cases of chronic diarrhea. CD in India is prevalent among Punjab, Haryana, Delhi, Rajasthan, Uttar Pradesh, Bihar and Madhya Pradesh where wheat is predominantly consumed. Study also reports the DR3 allele frequency of 14.9% from North India (Delhi) and is comparable to South India (Tamil Nadu) with 14.34% among yadhavas and 11.6% among piramalai kallar castes (Yaccha, 2006). Major difference in DQ2 allele frequency observed between the two regions in North India, is 31.9% and South India, it is 12.78% for piramalai kallars and 9% for yadhavas. The regional differences of CD occurrence in India are possibly linked with genetic differences along with variations in the staple diet.

1.4.3.4.1 Iceberg model

The concept of celiac iceberg model was originally introduced by Richard Logan in 1991 and demonstrates the clinical variability and helps to understand its systemic nature of celiac disease (Leeds et al, 2008; Fasano and Catassi, 2001). As reported by Goddard and Gillet (2006), over 10 years ago Professor Anne Ferguson first compared the total population of patients with celiac disease with an iceberg having only a small proportion visible above the water line. This highest stratum of the iceberg describes patients with typical gastrointestinal symptoms. The subsequent stratum just under the water line, not immediately visible, contains patients with typical presentations of CD but with no gastrointestinal symptoms. That includes isolated iron deficiency anemia, osteoporosis and persistently abnormal liver function tests. And also includes those with ataxia and/or peripheral neuropathy (Goddard and Gillet, 2006; Leeds et al, 2008). Ferguson defined patients with ‘silent disease’ as those who have characteristic intestinal changes of CD, which return to normal on gluten free diet without manifesting clinical symptoms of disease. The author also describes patients with “latent celiac disease” as those who have normal small bowel mucosa on a normal diet but who have had or will have intestinal changes on biopsy which recovers on gluten free diet (Goddard and Gillet, 2006). Leeds et al, 2008 describes latent celiac disease by two groups of
patients. One those who have at some stage had a normal duodenal biopsy while on regular diet and subsequently develop atrophy (with histological changes) later in life and the other group, as patients who have histological features of villous atrophy but continues on a gluten containing diet and at re-biopsy 2 years later, now have normal duodenal mucosa. Ferguson has defined the patients with “potential celiac disease” (Goddard and Gillet, 2006) the fourth stratum of the iceberg (Leeds, 2008) as those who have had positive serology, morphometrix studies or family history with normal intestinal history on duodenal biopsy (Goddard and Gillet, 2006; Leeds, 2008). The size of the iceberg is influenced by the frequency of the predisposing genotypes in the population. And the dimension of the iceberg also depends to a lesser extent on disease definition that is whether subjects with so called latent or potential CD (Fasano and Catassi, 2001). However, as previously reported screening studies showed that in Western countries, for each diagnosed case of CD, an average of 5-10 cases remain undiagnosed. The “water line” namely the ratio of diagnosed cases depends on several factors such as awareness of CD, availability of diagnostic facilities and variations in clinical intensity. Because of the variable relevance of these factors, the water line is much more unstable than the reported overall size of the iceberg, thus, explains the reported wide fluctuations in space and time of CD incidence (Fasano and Catassi, 2001).

1.4.3.5 Clinical presentations

Clinical manifestations of CD vary greatly according to the age group, the duration and extent of disease and the presence of extra intestinal pathology (Fasano and Catassi, 2001 Green and Cellier, 2007). Infants and young children generally experience the symptoms like diarrhea abdominal distention and failure to thrive, vomiting, anorexia, irritability and even constipation are also common. In older children and adolescents the disease may present as anemia, rickets, short stature, dental enamel defects, poor performance in school or behavioral disturbances or neurological symptoms (Abdulkarim and Murray, 2003). A study reveals that 2-8% of children with unexplained short stature presenting to growth failure clinic had CD. Treatment of CD may improve growth in those treated before growth is complete (Abdulkarim and
Murray, 2003). The clinical spectrum of CD in children is wide as Fassano divides symptoms into classic symptoms and non classic symptoms, silent and potential CD (Fasano, 2005). Classic or symptomatic form is characterized by gastro intestinal manifestations starting between 6 and 24 months of age, after the introduction of gluten in the diet. Infants and young children present with impaired growth chronic diarrhea, abdominal distention, muscle wasting and hypotonia, poor appetite and unhappy behavior (Fasano and Catassi, 2001; Fasano, 2005; Alaedini and Green, 2005). Children may experience classical symptoms or extra intestinal manifestations like short stature, pubertal delay iron deficiency, dental enamel defects abnormalities in liver function tests. Dermatitis herptiformis rarely affects the pediatric population (Fasano, 2005). It is a blistering skin disease characterized by pathognomonic granular immunoglobulin. The most typical sites of the rash are the elbows, knees and buttocks (Fasano and Catassi, 2001). Short stature is well described as only symptom of CD in some older children and adolescents where 9-10% of those with idiopathic short stature have CD. Both bone age and growth velocity is significantly impaired in these patients. Other symptoms like dental enamel hypoplasia, arthritis and arthalgia, chronic hepatitis and hyper transaminasemia, osteoporosis and neurological symptoms are present in some patients (Fasano and Catassi, 2001). Among adults, the prevalence of autoimmune disease is higher in the women than in the men. The classic presentation in adults is diarrhea, which may be accompanied by abdominal pain or discomfort. Iron deficiency anemia, osteoporosis, abdominal pain, constipation, weight loss, neurologic symptoms, dermatitis herptiformis, hypoproteinaemia, hypocalcaemia and elevated liver enzyme levels are other silent manifestations in adults (Green and Cellier, 2007). Patients with asymptomatic or silent celiac disease lack classic or atypical symptoms but have villous atrophy that may be discovered during endoscopy of intestinal biopsy. Images of Small-intestinal mucosal biopsy at normal and CD condition are presented in Fig 1.3.

1.4.3.6 Diagnosis

The diagnostic criteria for CD have changed over the last few decades. The original European society of pediatric gastroenterology and nutrition criteria of 1970 centered on obtaining three small bowel biopsies for the diagnosis of
Current diagnostic criteria for CD in clinical practice are based on the revised guidelines by (ESPGN) in 1990 which requires single biopsy with characteristic CD lesions and unequivocal clinical response (Abdulkarim and Murray, 2003; Alaedini and Green, 2005). Algorithm proposed for the diagnosis of CD is given in the Fig 1.4. Serological testing is simple and minimal invasive, requiring only a blood sample. Serological markers for the CD are very helpful for the practicing clinician and also help in monitoring the patient’s adherence to a gluten free diet (Abdulkarim and Murray, 2003). Typical indications for serologic testing include unexplained bloating or abdominal distress, chronic diarrhea with or without mal-absorption or irritable syndrome (Green and Cellier, 2007). The most sensitive antibody tests for the diagnosis of CD are IgA class.

![Fig 1.2 Pathogenesis of Celiac Disease](Source: Osorio et al, 2012)
The available tests include those for antigliadin antibodies, connective tissue antibodies (anti-reticulin and anti-endomysial antibodies) and antibodies directed against tissue transglutaminase. However, the anti-gliadin antibodies are no longer considered sensitive enough or specific enough to be used for detection. Endomysial antibodies (EMA) are closely associated with gluten sensitive disease and in an appropriate clinical setting CD can be diagnosed with 100% specificity (Feighery, 1999). Endomysium is a connective tissue protein found in collagenous matrix of human and monkey tissue. The rest is based on the immunofluorescence findings of reticular staining when EMA binds to the endomysium (Abdulkarim and Murray, 2003). This test can use either monkey esophagus or human umbilical cord as substrate and its diagnostic utility has shown to be very good with specificity estimated at 99% and sensitivity over 90% (Heel and West, 2006). As the assay is dependent on the detection of IgA antibodies, endomysial antibody test is negative in individuals deficient in IgA (Abdulkarim and Murray, 2003; Feighery, 1999). Selective IgA deficiency affects about 2-5% of patients diagnosed with CD. However, the test is cumbersome to perform requiring a fair degree of expert and expense (Abdulkarim and Murray, 2003). Both IgA and IgG subclass antibodies to gliadin are used as an adjuvant for the diagnosis of CD. But they are no longer used clinically due to their lack of specificity except in children younger than 18 months of age CD (Abdulkarim and Murray, 2003; Green and Cellier, 2007).
It has been demonstrated that tTG is the target of a specific autoimmune response; this enzyme has been used to develop innovative diagnostic tools (Fasano and Catassi, 2001). IgA enzyme linked immunosorbant assay (ELISA) based kits are now available for the measurement of tTG antibody levels, using either pig or human tTG (Abdulkarim and Murray, 2003). Human tTG dot blot test which is based on the detection of anti-tTG antibodies in serum or in one drop of the whole blood opened a new horizon for the diagnosis of CD. Guiney pig based tTG ELISA has been commercialized (Fasano and Catassi, 2001). This ELISA based tests eliminates the disadvantages associated with the use of EMA. The gold standard diagnostic test for CD is a small intestinal biopsy obtained during upper endoscopy, with multiple biopsies taken from the second or third part of the duodenum. Endoscopy is the most convenient method of obtaining biopsies of the small intestinal mucosa (Abdulkarim and Murray, 2003). The characteristic histological feature on the biopsy include partial or complete villous atrophy, crypt hyperplasia and increased intra epithelial lymphocytes of plasma cells (Abdulkarim and Murray, 2003; Alaedini and Green, 2005).

Clear clinical improvement while the patient is on gluten free diet yields a definitive diagnosis when patients do not present with the classic clinical symptoms of CD, a second biopsy that shows histological improvement confirms the diagnosis. In addition, if histology examination yields equivocal results, it is useful to proceed with the HLA typing (Alaedini and Green, 2005). Although HLA screening for DQ2 and DQ8 in CD has very low positive value in difficult cases, when the absence of DQ2/DQ8 essentially rules out the disease (Abdulkarim and Murray, 2003). Yet diagnosis of CD solely based on serologic markers is not accepted and identification of the characteristic mucosal abnormalities on intestinal biopsy is required. However the intestinal biopsy can also yield false negative results, may be because of the intestinal damage or patchy mucosal changes (Alaedini and Green, 2005).

1.4.3.7 Complications of CD
When a patient with CD is adequately treated with a strict gluten free diet, the prognosis is excellent and the patient can probably lead an otherwise normal life. Failure to implement a strict diet or failure to respond to dietary treatment may result in serious complications like intestinal adenocarcinoma, enteropathy associated T-cell lymphoma or refractory sprue, osteoporosis etc (Feighery et al, 1999; Green and Cellier, 2007).

Among these complications, the best recognized is malignancy (Leeds et al, 2008). Patients with untreated CD have an overall relative risk of all types of malignancy twice that in the general population (Green and Cellier, 2007). The types of malignancy include both T-cell and B-cell non- Hodkin's lymphoma that may be either intestinal or extra intestinal; oropharyngeal and esophageal adenocarcinoma; and cancers of the small and large intestine, hepatobiliary system and pancreas. Enteropathy associated T-cell lymphoma occurs in the adults with the incidence peaking in the sixth decade of life. Symptoms may include malaise, anorexia, weight loss, diarrhea, abdominal pain and unexplained fever. Enteropathy associated T-cell lymphoma usually develops in the jejunum but may also be found in the ileum or extra intestinal sites such as liver, brain, chest and bone. The risk of lung cancer and breast cancer appears to be reduced (Green and Cellier, 2007). A report by a longitudinal study, indicate that strict gluten free diet protect against these malignancies. Celiac disease is known to be associated with reduced bone mineral density (both osteopenia and osteoporosis) the prevalence of abnormal bone mineral density is reported to be around 40% and leads to an increased fracture risk (Leeds et al, 2008). The world health organization defines osteopenia as a T-score (number of standard deviations of patient’s below that of young adult man) of between -1 and -2.5 and osteoporosis as a T-score of <-2.5 on BMD (Bone Mineral Density) scanning. Most studies confirm that approximately 1/3rd of adults are osteoporotic, 1/3rd osteopenic and only 1/3rd have normal bone density at diagnosis of CD (Goddard and Gillet, 2005). Several prospective studies agree that there is a marked increase in BMD after starting a gluten free diet. However BMD rarely seems to return to normal and there is a large inter individual variability in response (Goddard and Gillet, 2005). Celiac disease is
also associated with some immune mediated endocrine disorders; most commonly being type 1 diabetes and thyroid disease. Each of these conditions affects 5% to 10% of patients with CD (Alaedini and Green, 2005). Infertility, reduced fertility and an increased risk of an adverse outcome during pregnancy such as miscarriage, low birth weight and intrauterine growth retardation have all been attributed to undiagnosed CD (Leeds et al, 2008). Among the most common neurologic problems associated with CD are peripheral neuropathy, cerebellar ataxia, epilepsy and migraine.

Suscpicion of CD

Serologic test:
- Anti-TG2(anti-endomysial) antibody
- Total IgA

All negative
- Endomysial or anti-gliadin antibody

Positive results for IgA, EMA, anti-tTG2
Or IgA deficient positive results for IgG, anti-tTG2

Low probability of CD

Intestinal biopsy

Probability of CD

Characteristic histologic feature of CD

Provisional diagnosis of CD

Gluten free diet

Definitive diagnosis of CD
In a research study of 26 patients with CD, 31% had abnormalities on neurophysiologic studies, compared with reflux disease (Alaedini and Green, 2005). Treatment with gluten free diet can arrest or improve neurological symptoms (Alaedini and Green, 2005; Leeds et al, 2008). CD is associated with a number of other conditions some of which are autoimmune in origin. Autoimmune disorders arise ten times more often with celiac disease than they do in general population. Such disorders include insulin dependent diabetes, thyroid disease, Sjogren’s syndrome, Addison’s disease, autoimmune liver disease, cardiomyopathy and neurological disorders (Green and Jabri, 2003). Several autoimmune diseases may improve on gluten free diet, including neurological, cardiac and renal disease. There have been some studies suggesting that patients with CD have a high prevalence of other organ specific auto antibodies (Leeds et al, 2008). However this is controversial in perspective with other studies suggesting that development of further autoimmune conditions is dependent on gluten exposure which may be related to specific HLA haplotypes (Leeds et al, 2008).

1.4.3.8 Treatment

The mainstay of treatment of CD is strict lifelong adherence, i.e., permanent withdrawal of gluten from the diet, in which the patients avoids food products containing wheat, rye or barley (Fasano and Catassi, 2001; Green and Jabri, 2003; Alaedini and Green, 2005; Green and Cellier, 2007; Leeds et al, 2008). Studies have shown the less antigen binding property of oats and also its beneficial effects in the gluten free diet (Stosrud et al, 2003). Clinical studies suggest that oats are tolerated by most patients with CD and may improve the nutritional content of the diet and overall quality of life (Green and Cellier, 2007).
Even though various studies have found oats to be generally well tolerated, oats are not uniformly recommended, because most commercially available oats are contaminated with gluten containing grains during growing, transportation and milling process (Alaedini and Green, 2005; Green and Cellier, 2007). Although wheat, rye and barley should be avoided, there are other grains that can serve as substitutes as well as sources of starch. Commonly substituted grains in the gluten free diet include rice, corn, quinova and buck wheat (Alaedini and Green, 2005). Because the substitute flours are not fortified with ‘B’ complex vitamins, deficiencies may occur. Therefore vitamin supplementation is advised. Meats, dairy products, fruits and vegetables are naturally gluten free and help to make for more nutritious and varied diet (Green and Cellier, 2007).

The elimination of gluten usually induces clinical improvement within the days or weeks, though histologic recovery takes months or even years especially in adults whom mucosal recovery may be complete (Green and Cellier, 2007). One of the major controversies in the treatment of CD relates to the amount of gluten allowed in the diet of CD patients. The national Food Authority has recently redefined the term gluten free. Previously <0.02% was considered gluten free, but gluten free now means no gluten and <0.02% is currently labeled low gluten. However, the stringency of gluten restriction is an issue that is far being resolved due to difference in the opinions of the scientists. These controversies are attributed mainly to lack of scientific research to estimate the threshold of gluten consumption, below which no harm occurs (Fasano and Catassi, 2001). Many patients are advised to follow the World Health Organization or Food and Agriculture Organization Codex Alimentarius GFD (Gluten free diet) which allows up to 0.3% per 100 gm of protein in foods, where as others follow a strict GFD with no detectable gluten (Goddard and Gillet, 2006). It is strongly recommended for patients to have a consultation with a dietician who is knowledgeable about gluten free diets. The cost of gluten free products varies by country, but the diet is usually expensive making dietary treatment problematic for patients with limited financial sources. Gluten free products are particularly expensive and hard to find in developing countries. Hence, there is a considerable interest in the development of non dietary
therapies that may either supplement the rigorous gluten free diet or replace it. The use of recombinant enzymes that digest the toxic gliadin fractions in the stomach or the upper small intestine is the current and the most attractive alternative therapy (Green and Cellier, 2007). Treatments like pancreatic supplements might benefit because of reversible pancreatic insufficiency, and course of oral antibiotics in case of co-existing bacterial overgrowth (Green and Jabri, 2003). Therapies that interfere with the immune response by blocking the binding of deaminated gliadin to HLA-DQ or HLA-DQ8 or by blocking action of tissue transglutaminase are likely to be without side effects. Prolyl endopeptidases (PEP) are endoproteolytic enzymes that can cleave proline-rich immune stimulatory gluten peptides. It has been proposed that oral administration of therapeutic dose of a suitably formulated PEP might counter the toxic effects of moderate quantities of ingested gluten (Sollid and Khosla, 2005). Study indicates the use of *F. meningosepticum* PEP in enzyme therapy which meets most criteria for a therapeutically effective PEP. A homologous PEP from *Myxococcus Xanthus* seems to be comparable to *F. meningosepticum* with gluten detoxification. In addition *lactobacillus helveticus* has a zinc dependent PEP that can also cleave long substrates with relatively broad substrate specificity. Protease can also detoxify gluten-containing products before they are ingested (Sollid and Khosla, 2005).

1.5.0 Wheat based food Products

In India, particularly in Northern and Central states of India, wheat is a staple food to a large section of population. Earlier centuries, wheat was ground in chakkis and consumed as whole meal *atta* for chapattis and rotis and other similar products. Now a part of wheat is milled in roller flour mills to produce maida, atta, sooji and bran to cater the growing needs of bakery and confectionary industries of India as well as household requirements (Vithaldas, 1971).

Indian subcontinent uses wheat flour to make unleavened bread, popularly known as roti or chapatti. Chapatti is made from disc milled whole-meal flour, which is made into dough and cut into circular sheets about 2.5 mm
thick and 15 cm in diameter. It is then baked on both sides on hot iron plate and puffed in tandoor oven (Cornell and Hoveling, 1998). In order to prepare good quality chapattis, the dough should be needed well and kept aside for some time to enable the gluten network formation. Medium hard wheat varieties are more suitable for making chapattis, whereas softer wheat is most suitable for cakes, biscuits and other home made preparations such as mathiri, sakkarpura etc. However, a small percentage of wheat is also consumed in the form of bakers’ bread. Sooji or rawa are very popular as a breakfast food used in the preparation of upma, dosa and other snacks.

Refined wheat flour popularly known as maida is cheaper than rice flour and can be substituted for the latter in recipes such as chakli and other fried snacks after pre-treatment. The pre-treatment consists in steaming the maida flour in a steamer after it is placed over a muslin cloth for 5 min. This prevents stretching of gluten strands and preparations made with this will be crisper and of better quality. It requires less shortening than untreated flour for preparing biscuits, short breads and pastries. Wheat can also be parboiled just like paddy before dehusking, popularly known as Bulgar wheat which sometimes used in school meal programme (Vithaldas, 1971).

Refined wheat flour or maida is the major ingredient for most of the bakery products. Products baked from flour, milled either wholly or mostly from soft wheat, include cookies, biscuits, cakes, doughnuts and similar products. They represent a wide range of flour requirement, formulation and processing technique. Soft wheat flour is most suited for these products. The flour gives products that are tenderer, larger in size, greater in volume and superior internal structure as compared to those from other classes of wheat (Yamazaki, 1971).

Biscuits are very significant part of the food industry in most countries of the world. Their success can be attributed to their relatively long shelf-life and great convenience as food products. Wheat flour is the principal component of nearly all biscuits. Emphasis will be on weaker flours as they are most suitable for biscuit making. Most biscuits can be made from flour that have low quantity of protein and gluten that is weak and extensible. Thus flour with protein level
lesser than 9.0% is required. The exceptions are fermented cracker dough and
puff dough where the medium strength flour is required. Sucrose in biscuit
dough dissolves or partially dissolves depending on the amount of water
present and then recrystallizes or forms as amorphous glass (a super cooled
liquid) after baking. In this way it strongly affects the texture of the baked
biscuits. If the quantity of sucrose is high the biscuit is hard. Fat is also one of
the most important ingredients in biscuit making. It adds structure, eating
quality and flavor to the product. Apart from these major ingredients surfactants
(emulsifiers) and anti-oxidants are also added for improved texture and keeping
quality.

Biscuits are classified as crackers, cookies and biscuits based on the
texture and hardness. It is also classified based on the method of preparation of
the dough, e.g., fermented, developed laminated, cut, embossing, moulded,
extruded, deposited, wire cut etc. and also based on the level of enrichment
with fat and sugar. Another classification includes the description of secondary
processing involved in biscuit making. For example, cream sandwiched,
chocolate coated, moulded in chocolate, iced and added jam or mallow or both
(Manley, 2011).

The production of bread from wheat flour has attracted the attention of
scientists for a long time. Technically the difference between bread and biscuit
is the level of enrichment with fat and sugar and the moisture content. In
general biscuits can be baked on flat surface but cakes must be baked in
containers because the dough is softer (Manley, 2011). Bread, in human diet,
provides substantial amount of protein, complex carbohydrates, vitamins and
minerals required. Hence, in recent years much publicity is given to whole meal,
whole grain or multi-grain varieties of bread. High fiber bread made with
bleached fiber was an important development in China in 1990s. Many types of
whole meal flour are made from unbleached flour without the addition of colour,
flavor and preservatives. Wheat flour in conjugation with whole meal or other
non wheat flours are used to make bread to produce new flavors and texture.
For example oat bread, rye bread, bread with the addition of soy flour, oil seeds
like sesame, flax seed, canola seed, sunflower seed etc are developed to cater
the needs of the consumers with health conscious. Some special bread is also made for the people suffering from diseases. Gluten free bread for celiac and bread without sugar for diabetics helps people with disease to maintain their diet pattern and also to enjoy the processed foods (Cornell and Hoveling, 1998).

1.5.1 Pasta products

Pasta is an Italian traditional food, becoming highly popular in other countries also. Its consumption has increased because of its ease of preparation and palatability (Martinez et al, 2009; Petitot et al, 2009a). The word “pasta” is an Italian for “dough”. The basic ingredients are wheat flour or semolina and water (Kill, 2001). Although the durum wheat semolina is usually the raw material, spring wheat and other cereals or legumes can also be used (Wu, 2001). Pasta processing consists of several stages: durum wheat semolina is mixed with water in paddle mixer and fed into extrusion worm conveyer, where it is compressed and extruded through die. Then cut and dried to achieve final moisture content less than 12.5%.

The annual per capita consumption of pasta was highest in Italy at 26.0 kg/person/year followed by Venezuela with 12.3 kg/person/year. Ireland and Salvador had the lowest at 1.0 kg/person/year. It was estimated that in India, pasta production was around 100,000 tonnes in 2011 (IPO Annual Survey on World Pasta Industry -October 2012).

Among cereal products, pasta possesses unique nutritional features. It is considered as slowly digestible food, hence promoting low plasma glucose response. The low glycemic index of pasta is generally attributed to its compact structure (Petitot et al, 2009b). The transformation of semolina into a coherent pasta shape with the potential to deliver the required eating texture is achieved in the process of wetting, mixing and finally extrusion. Semolina has two components that are key importance in pasta preparation; the gluten proteins and starch. The gluten in durum wheat is present in wedge-shaped structures between ovoid starch granules. When dry, the gluten is a flinty or glassy material, but the addition of moderate amount of water allows changes in its physical and chemical nature. Gluten becomes rubbery and elastic and it
acquires the ability to form strands and sheets through intermolecular bonds. These properties are fundamental to its role as the continuous matrix, which traps and encapsulates starch in pasta and holds the product shape during manufacture and cooking. Heating of hydrated gluten leads to the irreversible formation of protein-protein cross links which, when properly controlled, stabilize the structure and eating texture of the final pasta. Some 85% of the solids in semolina are wheat starch. While cold, starch behaves as inert filler which has a strictly limited capacity to absorb water. Upon heating starch loses its rigid structural integrity and can absorb an almost unlimited amount of water. Water absorption is accompanied by a rise in viscosity caused by swelling of the granule and by a release of soluble material from the granule. If the gluten in semolina is at a moisture content of 10% and at temperature of about 25°C prior to wetting and extrusion, it will behave as a glassy material. This will have high viscosity. It does not flow under normal conditions and behaves as a brittle material. If the moisture content is 13-15% at the same temperature, then gluten will undergo glass transition and also has some of the rheological characteristics of the rubbery material. As water is added during and the gluten moisture content rises to 33% it will behave as a flexible material with the ability to flow under applied stress. This is the state in which it can encapsulate starch and be moulded to form the required pasta shape (Kill, 2001). The main purpose of the mixing stage is to distribute water among semolina particles as evenly as possible. Blends of water and semolina submitted to different mixing conditions undergo interlinked physical and biochemical modifications depending on the process parameters like mixing speed, temperature, mixing chamber filling and water content. Hydration and energy input are the two main factors for dough formation (Verniere and Feillet, 1999). Protein modification that occur during the blending stage of the pasta making process, before dough development are sufficient to create enough development, but that further energy is required for complete formation of the dough. After hydration and before dough development, water diffuses slowly into semolina particles and increases the protein mobility through a plastifying effect, allowing the formation of a protein network with increasing solubility in dilute acid and reduction of –SH group content with the formation of new S-S bonds between proteins. During
this stage presence of sufficient water ensure good mobility of the proteins and allows glass transition. The slight modification in the reactivity of the protein components, give rise to new linkages that are responsible for the initiation of the dough development. The slight increase of the blend consistency before dough formation would be responsible for increasing the shearing effect and the energy received by the dough, making possible the formation of protein network, which is a primary stage of the dough development (Verniere and Feillet, 1999).

Pasta is made in different shapes and it can be classified as fresh pasta and dried pasta. Fresh pasta is usually made with fresh ingredients unless it is destined to be shipped, where consideration is given to the spoilage rates of the desired ingredients such as eggs. Fresh pasta is tenderer compared to dried pasta and only takes about half the time to cook. Fresh pastas do not expand in size after cooking. Pasta is normally served with meat, cheese or vegetables with suitable seasoning agents. The ingredients usually used in making dried pasta are semolina flour and water. Eggs can be added as optional ingredient. Dried pasta is dried at a low temperature for several hours to evaporate all the moisture to improve its keeping quality. Dried pasta samples have longer shelf life and suitable for transportation over to farther locations. Dried pasta increases in size by double of its original proportion during cooking. Steps in pasta making and different shapes of pasta are shown in the Fig 1.5a and Fig 1.5b respectively.

1.6.0 New approaches and current trends in the development of hypoallergenic wheat based products.

The current mainstay to treat CD is lifelong strict adherence to gluten free diet (Sollid and Khosla, 2005; Chavez and de la Barca, 2010; Rashtak and Murray, 2012). However consumption of as little as 50 mg of gluten equivalent to 1/100th of a slice of bread may also trigger the mucosal damage (Stoven et al, 2012). Complete gluten free products are not available and many times patients experience accidental exposure to gluten as many gluten free products available may contain an average 5-50 mg of gluten as contaminant (Sollid and
Khosla, 2005; Stoven et al, 2012). Therefore there is a true need to develop safe food for CD patients. In recent years dietary habits have changed dramatically and attributed to improved awareness and understanding of pathogenesis, improved diagnosing techniques of CD. Marked changes in food technology especially wheat technology has paved the way to research new techniques to nullify the immunogenic effect of wheat and wheat based products. These alternative approaches or therapies could free CD patients from restricted diet pattern (Rashtak and Murray, 2012). The possible ways of interrupting the pathogenesis of CD is graphically presented in Fig 1.6.

Gluten removal is one of the novel approaches where breeding and hybridization of different wheat species is carried out to remove the immunogenic peptides of gluten while maintaining satisfactory baking quality (Stoven et al, 2012; Rashtak and Murray, 2012). Level of T cell stimulatory epitopes in the gluten of different wheat varieties vary significantly from each other. Gene encoding most of α-gliadins is located within the Gli-2 locus of the short arm of chromosome 6D. Removal of this locus in the Chinese spring cultivar in the *Triticum aestivum* results in the removal of T-cell stimulatory epitopes (Stoven et al, 2012) but affecting the mechanical properties of wheat. Similarly removal of ω-gliadins, γ-gliadins and LMW-GS loci from chromosome of the D-genome removed T-cell stimulatory epitope but not changing the mechanical properties (Stoven et al, 2012; Rashtak and Murray, 2012). Hence there is a possibility to produce wheat variants without immunogenic properties retaining good baking qualities.

*Lactobacilli* and fungal protease in combination with yeast are the enzymes that are used to render wheat less toxic through fermentation mechanism. *Lactobacilli* culture in preparation of sourdough produces a complex system of peptidases including proline-specific peptidases which completely hydrolyze gluten in wheat flour (Chavez and de la Barca, 2010; Stoven et al, 2012). In recent years there has been a slow and steady increase in consumer interest in wheat free foods (Moore et al, 2004).
Fig 1.5a Steps in pasta making

Fig 1.5b Different shapes of pasta

Fig 1.6 Possible mechanism to avoid T cell activation

A-Adaptive immune pathogenesis mechanism in CD, B, C and D- different ways to avoid the T cell activation by gluten peptides modifications (Source: Chávez and de la Barca, 2010)
Current research on development of wheat based products concentrates on several food processing techniques to reduce allergenic proteins or to eliminate allergens from foods. In the year 1990 Watanabe et al, for the first time developed a process for producing hypoallergenic rice. The product commercially called as “fine rice” was approved as physiologically functional food for specified health use in 1993. Tanabe and Watanabe in 1999 found that amino acid residues of Gln-Gln-Gln-Pro-Pro, is essential for IgE binding, which comprised Gln-X-Y-Pro-Pro, where X and Y are replaceable. With this idea Watanabe et al in 1994 attempted to make wheat flour hypoallergenic by hydrolyzing peptide bonds near the essential residues of the epitope. For this purpose enzymes with low amylase activity were chosen. The ability to hydrolyze peptide bonds near proline residue was determined using 2-furanacryloyl-Leu-Pro-Ala (FALGPA) as substrate. Bromelain and actinase showed high FLGPA hydrolyzing activities and had low amylase activity. Since they found that allergic to salt soluble from wheat flour were also sensitive to bromelian, actinase was selected for producing the hypoallergenic flour (Watanabe et al, 1994a). Team fabricated the hypoallergenic bread and noodles. The wheat flour was mixed with 0.6 fold weight of water dissolving collagenase at 0.1% weight of flour and incubated overnight at room temperature. The resulted batter was mixed with a low sweetness oligosaccharide, a surfactant and salt. The mixture was extruded with heating (Watanabe et al, 1994b). The team also fabricated hypoallergenic bread using hypoallergenic flour prepared with collagenase treatment. The bread making process included mixing of hypoallergenic batter with glucose, a surfactant, a carbon dioxide generator and salt. In 2000, Watanabe et al, proposed two step method using a carbohydrate decomposing enzyme and a protease to produce hypoallergenic flour, in which both carbohydrate decomposing enzyme and protease were mixed in water (60%) with wheat flour and incubated at 50°C. The team also fabricated different products like cup cakes, cookies and wafers using this hypoallergenic flour. The study reveals that actinase, bromelain, collagenase and transglutaminase effectively decreased the allergenicity of gluten and
also successfully manufactured English-muffin like product. Report is also available on attempt to manufacture noodles with hypoallergenic flour (Oishi et al, 2009). Takacs et al, (2008) also studied the effect of transglutaminase (TG) on the wheat based pasta products and its immunoreactivity with anti-gliadin rabbit IgG. In another study, Handoya et al (2008) have applied a new technique to eliminate allergenic proteins from wheat flour where polish-grade method was followed using rice polishing machine. They identified the distribution of proteins with various molecular weights in grains. They found out that allergenic proteins were distributed in all fractions of polished flour but innermost fraction C8 contained lower amounts. And they also observed weaker binding to IgG antibody of wheat allergic patients. They observed that, the protein bands which range 31-52 kDa moved into higher molecular weight range between 80-100 kDa. The protein movements from 11-19 kDa molecular range were also seen. They reported that the cross linking reaction catalyzed by TG results in a polymerization of wheat proteins, bringing protein aggregates of high molecular weight. The protein polymerization catalyzed by TG led to changes in the physicochemical and structural properties of the gluten. But samples when subjected to immune reactive tests, neither high dosages of TG in the range of 100-200 mg per kg nor longer incubation time range from 90-180 min affected the immune reactivity. The soluble gluten fraction components were recognizable as immune-reactive proteins by anti-gliadin rabbit IgG (Takacs et al, 2008). Kaassova et al (2002) studied the chemical and biochemical changes during microwave treatment of wheat. Results showed the changes in the amylolgraphic maximum and also improvement in the baking quality. In one more similar study, Leszczynska et al, (2003) exposed wheat flour and commercial gliadin to microwave with the power distribution of 70, 200 and 500W for different time durations and based on the results they stated that, microwave heating of food could not be applied for the elimination of allergenic properties of wheat gliadins. Research work carried out on development of hypoallergenic wheat flour and wheat based products is summarized in Table 1.1.
Table 1.1 Summary on developments of hypoallergenic wheat flour and products

<table>
<thead>
<tr>
<th>Year</th>
<th>Team</th>
<th>Method</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>Watanabe et al</td>
<td>Hydrolyzing peptide bond Actinase was used</td>
<td>Hypoallergenic wheat flour</td>
</tr>
<tr>
<td>1994</td>
<td>Watanabe et al</td>
<td>Collagenase</td>
<td>Hypoallergenic bread and noodles</td>
</tr>
<tr>
<td>2000</td>
<td>Watanabe et al</td>
<td>Two step method 1st –CHO hydrolyzing enzyme 2nd –Protease</td>
<td>English muffin like product</td>
</tr>
<tr>
<td>2003</td>
<td>Leszczynska et al</td>
<td>Microwave heating</td>
<td>Hypoallergenic flour</td>
</tr>
<tr>
<td>2008</td>
<td>Takacs et al</td>
<td>Transglutaminase</td>
<td>Hypoallergenic flour</td>
</tr>
<tr>
<td>2008</td>
<td>Handoya et al</td>
<td>Step wise polishing grade method</td>
<td>Hypoallergenic flour</td>
</tr>
<tr>
<td>2009</td>
<td>Oishi et al</td>
<td>Enzyme modification</td>
<td>Hypoallergenic noodles</td>
</tr>
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</table>
Scope of the Present Study
As the prevalence of Celiac disease (CD) is increasing worldwide, in recent years there has been increase in consumer interest in wheat free foods driven part by an increased awareness of the CD. The formulation of gluten free (GF) bakery products presents a formidable challenge to food technologists. Presently, researchers have been working significantly more on development of GF products involving a diverse approach which has included the use of starches, dairy products, hydrocolloids, non-gluten proteins, prebiotics and combination thereof, to replace gluten and to improve the quality of the products. With India being the world’s second largest producer of wheat, the actual burden of gluten sensitivity or CD might therefore, be even greater than the current estimate. As the alarming increase in the incidence of wheat allergy and celiac disease invokes fear about the type and quality of wheat being used, only a few reports on modification of allergenicity of wheat proteins are available. However there is no report available on development of modified gluten food products with complete validation including storage studies. Hence, there is a true need for development of suitable cost effective product(s) for the patients suffering with wheat allergy as well as celiac disease. Pasta, a wheat based staple food, is the second most consumed dietary item in the world after bread. Pasta is a highly popular food product because of its convenience, nutritional quality and palatability. Nutritionists consider pasta to be highly digestible providing significant qualities of complex carbohydrates, B-vitamins and iron. Pasta is also known for its low levels of sodium and fat. Against this background, the present study is designed to develop suitable modified gluten pasta using various types of modified gluten flours produced from T.durum by employing enzyme and thermal modification methods or blending with non-cereal flours. Developed pasta samples were validated for immunogenicity and storage stability.
OBJECTIVES

4. Preparation and evaluation of modified gluten flours.
5. Optimization of processing conditions for development of modified gluten pastas.
6. Validation of the developed modified gluten pasta.