This chapter deals with review of literature of the composition of amaranth flour, effect of germination on amaranth composition, its utilization in different food products and nutritional evaluation of amaranth based food products.

Amaranthus collectively known as amaranth belongs to Amaranthaceae family and cosmopolitan genus of annual or short-lived perennial plants. The scientific plant name – amaranth signifies in Greek “immortal”, “everlasting” or “non-wilting” (Mlakar and others, 2010). Amaranth was used as a staple food throughout history in Inca, Maya, and Aztec civilizations. At present amaranth is grown for commercial purposes in Mexico, South America, the United States, China, Poland, and Austria (Milán-Carrillo et al., 2012). In India amaranth is grown in Himalaya regions from Kashmir to Bhutan, and also in south Indian hills. There are approximately 60 species, which according to the uses for human consumption can be divided into grain and vegetable amaranths. The leaves of the young Amaranthus blitus, Amaranthus tricolor, Amaranthus cruentus, Amaranthus dubius, Amaranthus edulis, and Amaranthus hypochondriacus plants are used in salads and soups. The grains of Amaranthus caudatus, Amaranthus hypochondriacus, Amaranthus cruentus, Amaranthus hybridus, and Amaranthus mantegazzianus are used into breads, cakes, cookies, confectionary and soups, whereas some species are not safe for consumption by either humans or livestock such as Amaranthus retroflexus, Amaranthus viridis and Amaranthus spinosus (Caselato-Sousa and Amaya-Farfan, 2012). Nowadays, amaranth classified as a new, forgotten, neglected and alternative crop of great nutritional value. In India amaranth used in beverages, sauces, porridges, tortillas (also with maize flour),
popped grains like maize, and for various medicinal uses. Besides in the diet, amaranth had an important position also in Indians’ religion (Mlakar et al., 2009).

In botanical terms, amaranth is not true cereals; it is dicotyledonous plants as opposed to most cereals (e.g. wheat, rice, barley) and referred as pseudocereal, as their seeds resemble in function and composition those of the true cereals. The amaranth grains are very small in size and they occur in huge numbers, sometimes more than 50,000 to a plant (Berghofer and Schoenlechner, 2010). Amaranth seeds are small (1 - 1.5 mm diameter), they are lenticular in shape and weigh per seed is 0.6 - 1.3 mg. In addition, the percentage of bran fraction (seed coat and embryo) in amaranth seeds is higher in comparison with common cereals, such as maize and wheat, which explains the higher levels of protein and fat present in these seeds (Bressani, 2003).

The yield of amaranth grain strongly depends on environment, weather conditions, species, genotype, and production techniques, and varies in a wide range from 500 to 2,000 kg grain per ha (Mlakar et al., 2009). With appropriate varieties and production techniques yields of 1,500 to 3,000 kg grain per ha can be expected (Williams and Brenner, 1995). According to Jamriska (1990) and Kaul et al. (1996) grain yields in Europe ranged between 2,000 in 3,800 kg per hectare.

2.1 Characterization of amaranth grain

2.1.1 Chemical composition

A seed of grain amaranth is on average composed of 13.1 to 21.0 % of crude protein; 5.6 to 10.9 % of crude fat; 48 to 69 % of starch; 3.1 to 5.0 % (14.2 %) of dietary fibre and 2.5 to 4.4 % of ash (Mlakar et al., 2009).

2.1.2 Protein

Amaranth contains generally higher protein content than common cereals such as wheat (Bressani, 1994; Koziol, 1992). Among pseudocereals list (amaranth, quinoa, and
buckwheat) usually, amaranth shows highest protein content followed by quinoa and buckwheat. In pseudocereals, most of the protein is located in the embryo (Valencia-Chamorro, 2003). Contrarily to most common grains, amaranth protein is composed mainly of globulins and albumins, and contain very little or no storage prolamin proteins, which are the main storage proteins in cereals, and also the toxic proteins in celiac disease (Drzewiecki et al., 2003; Gorinstein et al., 2002). Amaranth protein has unique characteristics because its amino acid balance is close to the optimal balance required for human nutrition (Drzewiecki, 2001). Furthermore, lysine content is particularly high in relation to common cereals and can, therefore, complement the amino acids present in other cereals such as wheat (Duranti, 2006).

Amaranth proteins consist of about 40 % albumins, 20 % globulins, 25 - 30 % glutelins, and 2 – 3 % prolamins (Schoenlechner et al., 2003). Two main classes of globulins can be differentiated in amaranth: 7S (conamaranthin) and 11S (amaranthin) storage globulins (Marcone and Rickey, 1997; Marcone, 1999). The amino acid composition of globulins and albumins differs significantly to that of prolamins, which has implications in relation to their nutritional quality. Globulins and albumins contain less glutamic acid and proline than prolamin, and an essential amino acids such as lysine (Gorinstein et al., 2002; Koziol, 1992). Therefore, the amino acid composition of pseudocereal proteins is well balanced, with a high content of essential amino acids, and is thus superior to that of common cereals (Aubrecht and Biacs, 2001; Drzewiecki et al., 2003; Gorinstein et al., 2002). Kaur et al. (2010) studied on grain and flour characteristics of 48 Amaranthus hypochondriacus and 11 Amaranthus caudatus lines. The A. caudatus lines had a higher protein content, fat content and tendency for retrogradation and lower a-amylase activity as compared to A. hypochondriacus lines.
The nutritional value of the amaranth protein has been studied by various researchers. To evaluate protein quality, several indexes are used, like protein efficiency ratio (PER), net protein ratio (NPR), net protein utilization (NPU), true protein digestibility and protein biological value. Afolabi et al. (1981) revealed that the nutritional value of the protein of *A. hybridus* was low. They got a negative protein efficiency ratio, PER-value (-0.4). This was due to the high tannin content. However, Osuntogun and Oke (1983) found a very high PER-value for the same species of amaranth. It was comparable to the value of PER of casein, 2.3 and 2.5, respectively. This variety had very low tannin content, so it can be concluded that the nutritional value of the proteins of amaranth depends on the presence or absence of antinutritional factors in the seed. Amino acid profile of amaranth was also studied (Bejosano and Corke, 1998) and leucine was detected as the first limiting amino acid in whole meal flour. The overall amino acid profile of amaranthus protein was extremely favorable. This attribute and its fairly good digestibility showed that Amaranthus is indeed a source of high-quality proteins.

### 2.1.3 Fat

Lipids are very important nutritive constituents of amaranth seeds, with triacylglycerols (TAGs), phospholipids, squalene, and lipid-soluble vitamins such as tocopherols being the main components in the lipophilic fraction. Various minor components, such as phytosterols, waxes, and terpene alcohols have also been reported in different Amaranthus species. The content of all these components in amaranth seeds primarily depends on plant species and cultivar, whereas the extractable amount of lipids also depends on their isolation procedure and the applied solvent. Lipid content in amaranth and quinoa is between 2 and 3 times higher than in buckwheat and common cereals such as wheat (Alvarez-Jubete et al., 2009). Some earlier performed studies reported the content of lipids in amaranth seeds between 4.8 and 8.1% (Saunders and
Becker, 1984). Budin and others (1996) reported crude fat percentages 5.2 to 7.7% on the dry basis of the seeds in 21 accessions of 8 amaranth species. The content of lipids in amaranth seeds may be as high as 17.0 and 19.3%, as it was reported for A. spinosus and A. tenuifolius, respectively (Opute, 1979; Singhal and Kulkarni, 1988). According to Gimplinger et al. (2007) crude fat content in adapted grain amaranth genotypes “Neuer Typ” and “Mittlerer Typ” (A. hypochondriacus) and “Amar” (A. cruentus) in the Pannonian region of eastern Austria was 5.4 to 8.6%.

Pseudocereal lipids are characterized by a high degree of unsaturation, which is desirable from a nutritional point of view. Linoleic acid is the most abundant fatty acid (50% of the total fatty acids in amaranth and quinoa, and approximately 35% in buckwheat) followed by oleic acid (25% in amaranth and quinoa and 35% in buckwheat) and palmitic acid (Alvarez-Jubete et al., 2009; Bonafaccia et al., 2003; Bruni et al., 2001; Ruales and Nair, 1993). The fatty acids palmitic (19%), oleic (26%), and linoleic (47%) appear in higher amounts, and the linolenic fatty acid (1.4%) is also found (Berger and others, 2003). The amaranth oil was compared with many other edible oils. According to Lyon and Becker (1987) reported yellow oil from the seeds of A. cruentus and was similar to corn oil in appearance and composition. Later, fatty acid profiles of amaranth triacylglycerols isolated from 21 amaranth accessions (8 species) were compared to those of barley, corn, buckwheat, oat, lupin, and wheat and it was concluded that amaranth oil was most similar to buckwheat and corn oils (Budin and others, 1996).

2.1.4 Starch and Carbohydrates

The main compositional part of amaranth seeds are constituted by polysaccharides, starch being the main component in this fraction. Amaranth seeds contain almost 65 to 75% starch, 4 to 5% dietary fibers, 2 to 3 times higher content of sucrose in comparison to wheat grain, and non starch polysaccharide components (Burisova and others, 2001b). In
amaranth, starch comprises the main component of carbohydrates but is found usually in lower amounts than in cereals. Amaranth starch is located in the perisperm, this can also be seen in the Figure 2.1 where typically compounded starch particles that can reach a length of 90 μm in diameter are generated in the amyloplasts. The amylose content of amaranth starch varying from 0.1 % to 11.1 % is lower than that in other cereal starches (Schoenlechner et al., 2008). Amaranth seed is one of the few sources of small-granule starch, typically 1 to 3 μm in diameter, and having regular granule size in comparison of commercially available starches have a medium (10 to 25 μm) or large (>25 μm) granule size (Hoseney, 1994).

The small size of the amaranth starch granule as well as its high amylopectin content are responsible for various physical properties such as freeze-thaw and retrogradation stability, lower swelling power and amyllograph viscosity, higher solubility and gelatinization temperature range, higher sorption capacity at high water activity range, higher solubility, swelling power, water-binding capacity, and enzyme susceptibility (Baker and Rayas-Duarte, 1998; Becker et al., 1981; Choi et al., 2004; Schoenlechner et al., 2008).

**Figure 2.1** Illustration of amaranth (*Amaranthus spp.*) starch and seed in cross and longitudinal section obtained from Irving et al. (1981) and Kong et al. (2009)
Amaranth carbohydrates can be considered nutraceutical foods because they have hypocholesterolemic effects (Danz and Lupton, 1992; Qureshi et al., 1997-1978, 1996), beneficial hypoglycemic effects, and induce lowering of free fatty acids (Berti et al., 2004). Becker and others (1981) analyzed amaranth seed samples they found sucrose was the major sugar followed by raffinose, whereas inositol, stachyose, and maltose were found in small amounts. Later, Gamel and others (2006a) reported the range of carbohydrates (g/100g) viz. sucrose (0.58 to 0.75), glucose (0.34 to 0.42), fructose (012 to 017), maltose (0.24 to 0.28), raffinose (0.39 to 0.48), stachyose (0.15 to 0.130, and inositol (0.02 to 0.04) in A. cruentus and A. caudatus.

2.1.5 Dietary fiber

Dietary fibers are very beneficial for human health. Usually, dietary fiber is presented as total (TDF), insoluble (IDF), and soluble (SDF). Studies have shown that the pseudocereals amaranth, quinoa and buckwheat represent good sources of dietary fiber (Alvarez-Jubete et al., 2010). According, Repo-Carrasco-Valencia and others (2009) Centenario” variety of A. caudatus showed higher dietary fiber content (16.4%) than “Oscar Blanco” variety (13.8%). Valcárcel-Yamani, and da Silva Lannes (2012) reported a high variation in fraction of dietary fiber for amaranth within different species, varies between 11.14 and 20.6 %. According Schnetzler and Breene (1994), the pale-seeded amaranths contain 8 % of dietary fibre and black coloured 16 % with soluble fibre rate of 30 to 40 % and 18 %, respectively. Tosi et al. (2001) reported 14.2 % of dietary fibre in the A. cruentus flour (8.1 % soluble, 6.1 % insoluble).

2.1.6 Vitamins

Amaranth is a good source of riboflavin (0.19 - 0.23 %) and ascorbic acid (4.50 %). Becker et al. (1981) analyzed some amaranth samples for ascorbic acid content and found amounts ranging from 3.36 to 7.24%. Furthermore, amaranth is an excellent source of
vitamin E, which contributes to the prolonged stability of the oil. Tocopherols, the major vitamers of vitamin E, are fat-soluble antioxidants that act as scavengers of lipid peroxyl radicals (Ryan et al., 2007). Total vitamin E content in amaranth seeds has been reported to be 5.7 mg/100 g dry weight basis, respectively, (Bruni et al., 2001; Ruales and Nair, 1993; Zielinski et al., 2001). Among the tocopherols, in amaranth, α-tocopherol is the most abundant and was found in amounts of 248 mg/kg amaranth oil. It was also found a high concentration of β-tocopherol (546 mg/kg amaranth oil) (Leon-Camacho et al., 2007).

2.1.7 Minerals

Amaranth grains are a good source of many essential minerals as shown in Table 2.1. Amaranth can fully satisfy the recommended daily intake of iron and one gram of amaranth seed may contribute 46% of the recommended daily intake of calcium (Guzman-Maldonado and Paredes-Lopez, 1998). Berghofer and Schonelechner (2002) explained the calcium/phosphorus ratio is very good in amaranth grains, 1:1.9-2.7 nutritionists recommend a calcium/phosphorus ratio around 1:1.5. The variations in mineral content are influenced by environmental conditions during plant growth and seed set, especially in soil mineral availability (Alvarez-Jubete et al., 2009).

<table>
<thead>
<tr>
<th>Seed</th>
<th>Ca</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranth</td>
<td>180.1 ± 6.1</td>
<td>279.2 ± 1.1</td>
<td>1.6 ± 0.0</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>Quinoa</td>
<td>32.9 ± 3.3</td>
<td>206.8 ± 6.4</td>
<td>1.8 ± 0.0</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>60.9 ± 3.3</td>
<td>203.4 ± 8.8</td>
<td>1.0 ± 0.0</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>Wheat</td>
<td>34.8 ± 0.0</td>
<td>96.4 ± 3.7</td>
<td>1.2 ± 0.1</td>
<td>3.3 ± 0.1</td>
</tr>
</tbody>
</table>

From Alvarez-Jubete et al., (2009). Data presented as mg/100 g dry-weight basis ± standard deviation.

Mustafa et al. (2011) analyzed the chemical composition of 28 white and colored grain amaranth (Amaranthus spp.) genotypes. They documented that colored seeds
genotypes contained higher Mg and Ca concentrations than white seeds genotypes. However, seed color had no influence on K, Na and P concentrations. Copper and iron were the most variable micro-minerals in the evaluated genotypes with no significant effect of seed color on the concentration of either mineral.

In amaranth seeds, the high calcium content (180.1-217.0 mg/100 g) is of special relevance in relation to the well-known prevalence of osteopenia and osteoporosis among newly diagnosed celiac patients (Alvarez-Jubete et al., 2009; Alvarez-Jubete et al., 2010). Kachiguma et al. (2015) indicated that the amaranth grains had calcium (78.3 to 1004.6), iron (3.61 to 22.51), magnesium (44.31 to 97.38), potassium (267.8 to 473.6) and zinc (0.53 to 1.20) in mg/100 grams on dry weight basis.

2.1.8 Bioactive Components

Squalene, a highly unsaturated open-chain triterpene, which is the biochemical precursor of the whole family of steroids, is present in high levels in amaranth (1.9 to 11.19 %) (Jahaniaval et al., 2000; Qureshi et al., 1996). The amount of squalene in amaranth seed oil is considerably higher amount than usually found in oils from other cereal grains (Becker, 1994). As a food constituent, squalene lowering the cholesterol levels by inhibits the cholesterol synthesis in the liver amaranth squalene exerts a cholesterol-lowering effect by increasing fecal elimination of steroids through interference with cholesterol absorption (Qureshi et al., 1996; Shin et al., 2004). This compound is principally used as an oxidation-resistant industrial lubricant, as an intermediate in many pharmaceuticals, organic coloring materials, rubber chemicals, and as a bactericide (Ahamed et al., 1998; Berghofer and Schoenlechner, 2002; Schoenlechner et al., 2008).

Plant sterols (phytosterols) are another group of biologically active components present in pseudocereal lipids. According to Bruni et al. (2001) total sterols in amaranth lipids can represent approximately 20% of the unsaponifiable fraction with the
predominant sterol present being chondrillasterol. Phytosterols are natural components of plant cells are abundant in vegetable oils, seeds, and grains, have antiinflammatory effects, antioxidative and anticarcinogenic activity, and cholesterol-lowering capacity (Abugoch, 2009; Moreau et al., 2002; Ryan et al., 2007). Phytosterols have also shown antiviral and antitumor activity (Li and Zhang, 2001).

2.1.9 Anti-nutritional factors

The grain amaranth contains some antinutritional factors which can have a nutritional impact or can limit their food application. The antinutritional factors namely trypsin inhibitor, phytates, saponins, and tannins are found in grains (Souci et al. 1994, Berghofer and Schoenlecher, 2002). Berghofer and Schonelechner (2002) demonstrated 0.3-0.6 % phytic acid in amaranth grains. Phytic acid serves the plant as a form of phosphorus storage. Cereals and legumes contain higher amount (1-3 %) of phytic acid (Bock, 2000). McKevith (2004) reported range of phytic acid in different grains on a dry weight basis like corn contains 0.9%, soft wheat 1.1%, brown rice 0.9%, barley 1.0% and oats 0.8%. Phytate can bind minerals such as iron, calcium, and zinc, and there is some evidence showing decreased absorption of these minerals in the presence of phytate. The amount of phytates in the amaranth seed was 21.1 μmol/g (Sanz-Penella and others, 2012). According to Teutonico and Knorr (1985) A. hypochondriacus seeds showed higher phytate 5.4 to 6.2 g/kg than the A. cruentus seeds 5.0 to 5.8 g/kg.

The dark seeds of amaranth contain more tannins than the light ones (Bressani, 1994). Becker et al. (1981) evaluated 10 different samples of amaranth and found a range of 80-420 mg/100 g of tannins. Venskutonis and Paulius Kraujalis (2013) reported a very wide range of tannins in various Amaranthus cultivars, ranged from 0.4 to 5.2 mg/g the variation in tannin content might be due to the differences between plant species and cultivars and due to the differences in measurement method. Bressani (1994) reported
different trypsin inhibitor levels from 3.07 to 5.46 TIU/mg protein in amaranth grains. The contents of trypsin inhibitors in raw *A. caudatus* and *A. cruentus* was estimated to be 4.34 and 3.05 TIU/mg, respectively (Gamel and others, 2006b).

### 2.1.10 Total phenolic content and antioxidant activity

Polyphenol compounds possess health promoting properties such as their role in the prevention of degenerative diseases which include cancer and cardiovascular disease (Scalbert *et al*., 2005). In amaranth, polyphenols can be found in amounts ranging from 14.72 to 14.91 mg/100g. Tannins are polyphenolic secondary plant metabolites of higher plants, which can be found in high concentrations in the hulls of cereals and legumes (Schoenlechner *et al*., 2008). According to Klimczak *et al*., (2002) the main phenolic compounds present in amaranth seeds are caffeic acid, p-hydroxybenzoic acid, and ferulic acid. According to Repo-Carrasco-Valencia and others (2010) the total phenolic acids in *A. caudatus* grains varies from 16.8 to 59.7 mg/100 g. The antioxidant defense mechanisms of plant species are based mainly on the chemical properties of various secondary metabolites, particularly polyphenolic compounds. Amaranth seeds could be a source of antioxidative valuable phenolic compounds, particularly in those arid zones where commercial crops cannot be grown (Barba de la Rosa and others, 2009). Gorinstein and others (2008) showed that pseudocereals, including amaranth cultivars, had higher antioxidant activity than some cereals (rice and buckwheat). Conforti and others (2005) evaluated the antioxidant activity of two varities of *A. caudatus* “Oscar blanco” and “Victor red” they found IC50 values of ethyl acetate extracts were 0.50 and 0.62 mg/mL, respectively.

### 2.2 Effect of germination on amaranth grain composition

To improve the nutritional quality of cereals germination is an effective and common process (Lee *et al*., 2007a). During germination, complex polysaccharides, fats
and proteins are converted into their simpler counterparts such as olig and monosaccharides, free fatty acids, and free amino acids. Because of this reason, the germination is considered a type of pre-digestion procedure to turn complex compounds into simpler molecules (Marton et al., 2010). Thus, germination improves the nutritional attributes of the food and allows easy absorption of the simpler molecules, thereby improving the human health (Sangronis and Machado, 2007). The inclusion of germinated grains into the diet improve nutritional value, decrease anti-nutritive factors, and increase digestibility and bioavailability of some nutrients.

2.2.1 Effect of germination on protein content

The increase was observed in crude protein (41%) and true protein (22%) of amaranth after soaking for 10 min, germinating for 72 hr at 35°C, and forced-air drying at 45°C (Paredes-López and Mora-Escobedo, 1989). Gamel and others (2005) worked on the seeds of two amaranth species (Amaranthus caudatus and Amaranthus cruentus). They showed that during germination, the amounts of Asp acid, Ser, and Ala in A. caudatus and A. cruentus seeds increased, while those of Thr, Arg, and Tyr decreased; however, the treatments did not affect the polypeptide bands of the high protein flour compared with the raw seed flours.

Colmenares de Ruiz and Bressani (1990) observed increase in albumin and prolamin while globulin decreased when amaranth seeds were soaked for 5 hr in distilled water, germinated at 32°C for 72 hr, and dried at 40°C for 18 hr in an air oven. The total albumin plus globulin fraction, however, remained constant. According to Lee et al. (2004) crude protein increased 26% after soaking in water and germinating at 25°C for 168 hr followed by freeze-drying in buckwheat. Omary et al. (2012) found an increase in free amino acids and glutamic acid (109%) of buckwheat, when buckwheat was steeped for 12 hr, germinated for 108 hr, and malted for 119 hr. They also observed increased (48%)
proteolytic activity during germination. Moueium et al. (1996) reported a 12% increase in the total protein of corn soaked overnight in distilled water at room temperature, germinated 72 hr at 30°C, and dried at 50°C.

2.2.2 Effect of germination on carbohydrates

Colmenares de Ruiz and Bressani (1990) observed an increase in reducing sugars, total sugars and damaged starch of three species (*Amaranth hypochondricus, cruentus and caudatus*) of amaranth during germination. Gamel et al. (2006) have also shown similar results for germinated amaranth. Balasubramanian and Sadasivam (1989) found a decrease in the starch content of amaranth of 41% over 192 hr of germination. Starch content decreased in two studies on millet germinated at 30°C (Sripriya et al., 1997; Mbithi-Mwikya et al., 2000). Sripriya et al. (1997) soaked and germinated millets for 12 h and 24 h at 30°C, they found starch decreased up to 12%. Mbithi-Mwikya et al. (2000) reported 51% decrease in starch of millets after 96 hr of germination. The high germination temperature and the extended germination time may have contributed to the high rate of starch hydrolysis. Nirmala and Muralikrishna (2002) steeped and germinated finger millets for 24 hr and 96 hr at 25°C they reported a 34% decrease in starch content of finger millet.

2.2.3 Effect of germination on minerals

Gamel et al. (2006) evaluated *A. caudatus* and *A. cruentus* flour they found no change in the ash content of *A. caudatus* flour while a decrease of 11.0–14.0% was observed in *A. cruentus* flour due to the germination process. This may be due to the removal of some seed coats of the black weedy seeds present in the normal *A. cruentus* seeds. Similarly, Colmenares de Ruiz and Bressani (1990) reported no effect on the ash content of *A. caudatus* seeds flour due to germination, while a 5.9% reduction was observed for *A. cruentus* seeds flour compared with the raw one. Lee et al. (2004) found a
115% increase in crude ash in buckwheat after soaking in tap water and seven days germination.

Finney (1982) concluded that the decrease and increase in mineral content of germinating seeds depending on the methods of steeping and germination. Some minerals are leached or absorbed by the hydrating and germinating seeds. In general, increase in total minerals and ash with increasing germination if harder, mineral-containing water is used to steep and germinate the grains. Conversely, minerals will invariably be leached out if distilled water is used. Gamel et al. (2006) worked on two species of amaranth seeds, *Amaranthus caudatus* and *A. cruentus* seeds were germinated at 32 °C for 48 h. and dried at three different temperatures (30, 60, and 90 °C) they found increase in calcium and zinc in both species. These findings may be attributed to a decomposition of phytate or tannins that bind those minerals by enzyme activity such as phytase.

### 2.2.4 Effect of germination on crude fiber

Elkhier and Hamid (2008) found increase in fiber 361 and 199% in two cultivars of seven-day-old malted sorghum. El-adawy et al. (2003) also reported same trend for crude fibre in germinated mung bean, pea and lentil seeds. Similarly, Moongngarm and Saetung (2010) reported 8% increase in crude fiber after germination of rice.

### 2.2.5 Effect of germination on lipids

Several author reported a decrease in lipids 5–59% in three species of amaranth during germination (Paredes-López and Mora-Escobedo, 1989; Colmenares de Ruiz and Bressani, 1990; Gamel et al., 2005). The decrease in lipids may be due to their use as an important source of energy for the developing plant embryo during germination (Gamel et al., 2005). Lee et al. (2004) reported a 10% decrease in crude fat of buckwheat after soaking in tap water and germinating for seven days. Perales-Sánchez et al. (2014) also found decline in amaranth lipid content upto 30%.
2.2.6 Effect of germination on dietary fibre

The soluble, insoluble and total dietary fibre or fiber contents in amaranth seeds increased in 655, 99 and 124 %, respectively, after germination bioprocess (Perales-Sánchez et al., 2014). The germination process tends to modify the structure of cell wall polysaccharides of the seeds, possibly by affecting the intactness of tissue histology and disrupting the protein carbohydrate interaction. This will involve extensive cell wall biosynthesis and therefore the production of new dietary fiber. Germination produces an increase of cellulose, hemicellulose, accompanied by an increase of pectic polysaccharides (Martin-Cabrejas et al., 2013). Ohtsubo et al. (2005) studied pregerminated brown rice they found 45 and 50% increase in total and insoluble dietary fibre after 72 hr soaking and germination at 30°C.

2.2.7 Effect of germination on polyphenols and antioxidant activity

Alvarez-Jubete et al. (2010b) examined the germinated amaranth, quinoa, and buckwheat for total phenolic content and antioxidant capacity. They found increase of total phenols and antioxidant activity in the grains. Subramanian et al. (1992) studied nine cultivars of sorghum (Sorghum bicolor) during 144 hr germination they showed large increases (90, 64, and 105%) in total polyphenols in three cultivars. The large increases in polyphenols were potentially attributed to both de novo synthesis and polymerization (Omary et al., 2012). Perales-Sánchez et al. (2014) optimize the germination conditions (germination temperature, 30°C and germination time, 78 h) for amaranth seeds for producing optimized germinated amaranth flour. At these conditions the germination bioprocess increased antioxidant activity, total phenolic content, and total flavanoid content 300–470, 829, and 213 %, respectively.
2.2.8 Effect of germination on antinutritional factors

Moongngarm and Saetung (2010) observed 13% decrease in phytic acid content in 24 hr germinated rice. Several authors observed decrease in phytate and tannin content of germinated pearl millet (Sripriya et al., 1997; Mbithi-Mwikya et al., 2000; Abdelrahaman et al., 2007). These findings were also confirmed by Khetarpaul and Chauhan (1989) who reported a 40% reduction in phytate in 24 hr germinated pearl millet. According to Khalil and Mansour (1995) a 29.0% decrease in the tannin content of faba bean seeds due to germination. Ruiz and Bresani (1990) also reported a decrease in phytic acid of amaranth grains with respect to germination time.

2.3 Amaranth milling

The small structure of amaranth grains become challenge to the millers. Several researchers (Becker et al., 1981; Berghofer and Schoenlechner, 2002) reported that amaranth is milled using disc, hammer and pin mills and the whole flour is used for preparation of different products. Milling the grain amaranth using roller mill was explored by Nanka (1998) wherein a vario-technical rollermill was used to obtain a protein rich and starch-rich fraction. Schoenlechner (2000) used a technical scale roller mill in combination with a plansifter. However, the focus was limited to use a single break system of roller mill and the seed coat fractions of the grain were not separated. Milling of amaranth using multiple break system of the roller mill and preparation of different grain fractions including the coarse and fine seed coat fractions.

2.3.1 Functional properties

The utilization of amaranth flour in food system greatly depends on its functional properties. Some of the important functional properties of flour are hydration, fat absorption, emulsification, foaming, and viscosity that can decide their utilization for different foods applications. The functional behavior of proteins in food is influenced by
some physicochemical properties of the proteins such as their size, shape, amino acid composition and sequence, net charge, charge distribution, hydrophobicity, hydrophilicity, type of structures, molecular flexibility/rigidity in response to external environment such as pH, temperature, salt concentration or interaction with other food constituents (Damodaran, 1997). Functional properties are those parameters that determine the application and use of food material for various food products (Abedowale et al., 2012).

The water absorption capacity of amaranth flour ranged between 2.09 and 2.43 g/g that were higher than soybean (1.3 g/g), chick pea (1.33 to 1.47 g/g), and was comparable with dry bean (2.23 to 2.65 g/g) reported by (Oshodi and Ekperigia, 1989; Kaur and Singh, 2005; Siddiq and others, 2010). Improved water absorption capacity makes them suitable for applications in the bakery products, where a high water absorption capacity of flour would enable baker to add more water to the recipe so as to improve dough handling characteristics and maintain freshness in the bread (Wolf, 1970). Baljeet et al. (2010) observed that the water absorption capacity of buckwheat flour was lower than that of refined wheat flour (p ≤ 0.05), but oil absorption and foaming capacity of buckwheat flour were significantly higher than that of refined wheat flour (p ≤ 0.05). The buckwheat flour had higher least gelation concentration (32%) as compared with wheat flour (20%). Fat absorption capacity of flours determines mouthfeel and flavor retention of products. Fat absorption capacity of amaranth flour ranged between 1.88 and 1.95 g/g that compared favorably with that of chick pea (1.05 to 1.24 g/g) and sorghum flours (1.72 to 1.85 g/g) (Elkhalifa and others, 2005; Kaur and Singh, 2005).

2.3.2 Physical properties

Physical properties were used to design a new and retrofit existing bins, hoppers and feeders and to determine the basis for flow problems and understand differences between various bulk materials or grades of the same material (Fitzpatrick et al., 2004).
Physical properties like bulk density of granular and powdery materials which is used in describing the flowability of the materials were affected by particle size and moisture content (Oginni, 2014). Raigar and Mishra (2014) showed increase in the bulk density of roasted bengal gram flour with moisture content (3.19–13.06%, w.b.). Mulinda (2010) in bean flour and Adapa et al. (2011) in barley, oat and wheat also found the increase in bulk density with increase in moisture content. Similar results presented by Kibar et al. (2010) they showed a decrease in bulk density of rice grains from 595.5 to 560.5 kg m⁻³ with an increase in moisture content from 10 to 14% d.b.

**True Density**

Raigar and Mishra (2014) indicated the increase in true density of roasted bengal gram flour with an increase in moisture content from 3.19 to 13.06%, w.b. Kibar et al. (2010) also reported increase in the true density of rice grain was found to increase from 939.0 to 962.1 kg m⁻³ with increase in the moisture content.

**Porosity**

Porosity is a measure of the void spaces in a material and can be a good prediction of the sphericity or irregularity of the particles in a bulk solid. High porosity values are a sign of logistical and economic problems that can be encountered during the storage and transportation of flour, unless some form of densification is utilized (Fasina, 2007). Raigar and Mishra (2014) indicated the decrease in porosity of roasted bengal gram flour with an increase in moisture content from 3.19 to 13.06%, (w.b.). Kibar et al. (2010) revealed the linear relationship of porosity and moisture content in rice grains. In addition to flour composition, flour quality is also influenced by flour particle size distribution. The Higher amount of smaller flour particles leads to a less extensible and less fluidable dough. Moreover, narrow flour particle size distribution is correlated with higher water absorption capacity and enzymatic susceptibility (Rukshan, 2001). Besides the abovementioned, the
implementation of small particle size flour results in final cookie products characterised by increased volume (Gaines, 1990). Therefore, in a cookie and similar products it is more appropriate to use flour characterized by larger particle size.

2.3.3 Pasting properties

Pasting properties and gelatinization are the most important characteristics of flours and starch, determining its application in food processing and other industries (Bao, 2008). Pasting properties have been used to predict the end-use quality of various products, for example cooked rice texture and noodles (Shu et al., 1998; Bhattacharya et al., 1999). According to Stone et al. (1984) the Viscosities of amaranth starch was higher than that of corn starch. Shevkani et al. (2014) analyzed 6 lines/cultivars of amaranth seeds (Amaranthus hypochondriacus) for pasting properties they found peak viscosity, breakdown, final viscosity and pasting temperature ranged from 1050 and 1459 cP; 282 and 475 cP; 966 and 1286 cP; 71.3 and 72.1 °C.

2.4 Amaranth flour products

The gluten-free products available in the market are considered of low quality and poor nutritional value (Alvarez-Jubete et al., 2009). In this way, there is an increasing interest in the pseudocereals application in the production of nutrient-rich gluten-free products. The fact that amaranth does not contain gluten could be beneficial for people suffering from celiac disease and wheat allergy or intolerance (Taylor and Parker, 2002). There are many studies in the area of gluten-free cereal-based products which have mainly concentrated on the improvement of the structural properties and also in the viability of pseudocereals such as amaranth, quinoa and buckwheat as ingredients in gluten-free breads, crackers etc. with the aim of improving the nutritional quality of these products (Alvarez-Jubete et al., 2009; Alvarez-Jubete et al., 2010; Caperuto et al., 2000; Hozová et al., 1997; Park et al., 2005; Schoenlechner et al., 2010 and Tosi et al., 1996).
A growing number of studies have investigated the application of pseudocereals in the production of nutrient-rich gluten-free products. However, availability of these products in the market is still quite limited. More research is required to fully exploit the functionality of these seeds as gluten-free ingredients in the production of palatable products which are also nutritionally balanced.

2.4.1 Gluten free cookies

Cookies are the easiest to formulate without gluten because it plays a secondary role in their making and end-product quality because texture mainly depends on sugar and fat to assure crispness and friability (Engleson and Atwell, 2008). Whole amaranth flour has been used to develop gluten free biscuits with higher protein content than the average for gluten-free biscuits (Tosi et al., 1996). The authors also found that the addition of 0.1% butylated hydroxytoluene (BHT) extended the shelf-life without affecting the flavour.

Sindhuja et al. (2005) prepared composite flour cookies by incorporating 0–35% levels of amaranth seed (Amaranthus gangeticus) flour. They found 25% incorporation of amaranth flour to be optimum for the colour, taste, flavour, surface appearance of the cookies. Inglett et al. (2015) used amaranth - oat composites in sugar cookies for improving their nutritional and physical qualities. The study showed amaranth and its composites had more viscous properties and improved water holding capacities compared to wheat flour. Also, the cookies using amaranth - oat composites had enhanced nutritional value with gluten free uniqueness that could be useful for functional foods. Bhat et al. (2015) prepared cookies using the amaranth grains, oats, and the refined wheat flour, and analyzed for nutritional parameters. They observed that the cookies can act as a good source of protein, carbohydrates, and dietary fiber and hence a potential source of energy. Gambus et al. (2009) prepared gluten free cake and cookies with different supplements such as linseed meal, amaranth and/or buckwheat. All supplemented gluten-free products
received high consumer scores, exceeding in some cases those of control samples. Supplementation of gluten-free confectionery products with linseed meal, amaranth, buckwheat flours enhanced their final nutritional quality as compared with the control.

De la Barca et al. (2010) prepared gluten-free breads and cookies by using raw and popped amaranth flours. The best formulation for bread included 60–70% popped amaranth flour and 30–40% raw amaranth flour which produced loaves with homogeneous crumb and higher specific volume than with other gluten-free breads. While, the best cookies recipe had 20% of popped amaranth flour and 13% of whole-grain popped amaranth. The functionality of amaranth-based doughs was acceptable although hydrocolloids were not added and the final gluten-free products had a high nutritional value.

2.4.2 Gluten free pasta

The world pasta production is dominated by the United States and Canada followed by the countries of the European Union (http://www.pasta.unfpa.org). For pasta preparation durum wheat (Triticum durum) is ideally suitable because of its unique properties like relatively high yellow pigment content, low lipoxygenase activity, and high protein content favorable for good cooking quality (Aalami et al., 2007). In addition to the conventional pasta, some cereals (barley, rye, etc.), pseudo-cereals (buckwheat, amaranth, quinoa), and legume flours (pea, chickpea, etc.) are used to provide sources of fibre, minerals, antioxidants, and polyphenols. The most challenging products to formulate and produce are gluten-free bread and pasta, as gluten is their architectural key. In the last few decades, the gluten-free pasta is being consumed not only by the growing number of celiacs but also by health conscious peoples. Moreover, as the celiac disease can occur at any age, the production of good quality gluten free products is the only treatment to celiac disease patient. Pasta prepared only from non-gluten flour is generally considered to be
inferior in textural quality compared to semolina pasta: it does not tolerate overcooking, it is sticky, and, above all, it is characterized by relevant cooking losses. To improve pasta cooking behaviour texturing ingredients are required.

Huang et al. (2001) produced gluten-free pasta with characteristics most similar to wheat-based pasta containing higher levels of modified starch, xanthan gum and locust bean gum. Cabrera-Chavez et al. (2012) prepared gluten free pasta with incorporation of amaranth to rice flour (25:75 ratio), they observed improved nutritional quality of pasta with maintaining good cooking behaviour. Rosa et al. (2015) studied on the influence of the different addition levels of amaranth and rice flour on buckwheat flour pasta. They revealed that the best quality attributes were observed in the pasta made from 100% buckwheat flour, followed by the pasta with 15% amaranth flour and 15% rice flour in place of buckwheat flour. Hymavathi et al. (2014) prepared vermicelli by using pearl millet, semolina, defatted soya flour and three different types of hydrocolloids (Gum Karaya, Guar Gum and Carboxy methyl cellulose) at 2% level and tested against the control (refined wheat flour based) vermicelli. The study demonstrated that incorporation of hydrocolloids not only helped to improve the textural quality of the gluten-free formulations but also enhanced the dietary fiber content and lowered the in vitro starch digestibility.

Ansari et al. (2013) prepared spaghetti by replacing wheat flour with defatted soy flour at 0, 10, and 20% levels (w/w). In addition, xanthan gum was added at three levels (0.0, 0.2, and 0.4%) to spaghetti dough. They showed that spaghetti dough containing 20% defatted soy flour and xanthan gum shows positive properties of spaghetti. Han et al. (2011) were produced starch noodles using a medium grain rice starch in the presence of various gums (xanthan, locust bean gum, curdlan, gellan gum, and κ-carrageenan). They found by the addition of locust bean gum and curdlan cooking loses were reduced and
also increased the hardness of the rice noodles. Padalino et al. (2013) studied the effects of different hydrocolloids (gellan gum, carboxymethylcellulose, pectin, agar, tapioca starch, guar seed flour and chitosan) on chemical composition and cooking quality of spaghetti based on maize and oat flours. Results revealed that most hydrocolloids improved cooking quality and texture properties of spaghetti (adhesiveness, cooking loss, hardness), thus supporting their application in gluten-free pasta.

Pasta based on maize and oat flours with hydrocolloids that increased the insoluble-water fibres content (i.e. chitosan) could be used for reducing the glycemic index; spaghetti with hydrocolloids that increases the soluble-water fibre content (i.e. carboxymethylcellulose and agar) could be used for reducing the blood cholesterol. Rayas-Duarte et al. (1996) substituted amaranth flour to extra fancy and fancy durum wheat flours at 5% to 30% levels to produce multigrain pastas with higher contents of Lysine, acceptable cooking quality, and sensory characteristics. Kovacs et al. (2001, 2002) prepared the best pasta with good cooking and sensory properties was produced from A. cruentus with 1.2% of diacetyl-tartaric ester of monoglyceride.

2.5 Nutritional evaluation of food products

The results of clinical studies have demonstrated that there was a risk of nutritional deficiencies in the group of people with celiac disease that were following the gluten-free diet. The risk was applied mainly to insufficient intake of calcium, iron, zinc and selenium (Kupper, 2005). The main purpose of amaranth application in cereal products is to obtain gluten-reduced and gluten-free products. Cabrera-Chavez et al. (2012) reported that enrichment with amaranth improved mineral and fiber contents, and protein digestibility of rice-based pasta, while a novel extrusion-cooking process was applied prior to pasta-making for improving the textural characteristics. Gambus et al. (2009) prepared gluten-free confectionery products with different supplements such as linseed meal, amaranth
and/or buckwheat. Supplementation of gluten-free confectionery products with linseed meal, amaranth and/or buckwheat flours enhanced their final nutritional quality. A significant rise was observed in the protein content and dietary fiber, and in the case of linseed meal also α-linolenic acid. All of the supplemented gluten-free confectionery products contained more macro-elements and microelements (i.e. potassium, phosphorus, magnesium, calcium, iron, manganese, zinc and copper), as compared with the controls. Taking into account the amino-acid composition, amaranth proved a more beneficial supplement of gluten-free products than linseed. The presence of easily available iron ions makes the amaranth flour especially valuable additive for gluten-free products (Paredes-Lopez, 1994).

2.6 Storage stability of food products

There are different types of product changes that can limit the shelf life of food. Essentially, the shelf-life of a food, i.e. the period it will retain an acceptable level of eating quality from a safety and organoleptic point of view, depends on four main factors, namely formulation, processing, packaging, and storage conditions (Figure 2.2) (Galic et al., 2009).

Cookies belong to the group of food products that are very popular in the daily diet of almost all profiles of consumers (Popov-Raljic, 2007) having not only the nutritive purpose but influencing also on the emotional status of consumers with the effects even on the positive mood enhancement (Turner et al., 2010). Cookies are characterized with quite a long shelf life, which results in their availability almost everywhere at any time (Popov-Raljic, 1999; Sharif et al., 2003). Storage stability or the shelf-life of baked products could be defined as maintenance of the sensory and physical characteristics associated with freshness (Baixauli et al., 2008). Crackers, biscuits, and wafers have a much longer shelf life owing to their low moisture content. The wrapping must provide an excellent moisture
barrier to prevent loss of crispness and texture. The increased fat content of these products imparts a shorter shelf life but the wrapper must act as an efficient grease barrier. The traditional material used for the packaging of biscuits has been regenerated cellulose films (RCF) coated with either PE-LD or PVC/PVDC copolymer, and often with a layer of glassine in direct contact with the product if it contained fat (Robertson, 1993).

![Diagram of factors influencing shelf life of food]

**Figure 2.2  Factors influencing shelf life of food**

Rajiv *et al.* (2012) analyzed the storage characteristics of wheat flour cookies and cookies with 15% roasted and grounded flex seed flour. They showed no significant change in free fatty acid and peroxide values of cookies up to 90 days in metallised polyester pouches at ambient conditions. Peter *et al.* (2012) prepared cookies rich in protein and low in calories were developed by substituting wheat flour (Maida) with defatted soy flour (DSF) and cane sugar with Stevia leaves powder (SLP). These cookies were then packed in LDPE, HDPE and PP films and stored for a period of 90 days under ambient conditions to assess the quality of the cookies based on sensory and storage
parameters. Storage studies revealed that cookies stored in HDPE package to have good sensory acceptability.

Nadarajah (2015) prepared nutritive biscuits by adding different levels of defatted coconut flour to wheat flour. There is no remarkable changes in organoleptic characters were observed in biscuits packed in metalized polypropylene for a period up to 12 weeks of storage in ambient conditions of temperature 30°C and the RH of 75–80%. They suggested that the 40% defatted coconut flour added biscuits could be stored for 12 weeks without any significant changes in quality.

Romani et al. (2015) studied the effect of innovative multilayer packaging materials versus a standard one on biscuit quality was studied during accelerated storage at 25, 35, 45 °C and 50% relative humidity for 92 days. Three different packaging materials were used: metalized orientated polypropylene (OPP)/paper (control) metalized poly-lactic acid (PLA)/paper; metalized OPP with ethylene vinyl acetate pro-oxidant additive (EVA-POA)/paper. EVA-POA additive is used to make the plastic layer biodegradable. No remarkable differences in the evolution of primary and secondary lipid oxidation were observed among differently packed biscuits during storage. All samples maintained PV levels between 4 and 14 meq O₂ kg⁻¹ oil. The product in flexible packaging with PLA reached the highest moisture and aw levels, but they did not significantly and adversely affect the other quality characteristics.

Sharif et al. (2005) studied on rice bran oil (RBO) was applied to baked products such as cookies at various levels i.e. 0, 25, 50, 75 and 100% by gradually replacing normal shortening to improve the quality of cookies in term of shelf life due to natural antioxidants present in RBO. Five treatments of RBO and normal shortening (NS) were used to prepare cookies and 45 days storage study was conducted to investigate improvement in shelf life. Based on the results of the proximate analysis, sensory
evaluation and TBA number. He concluded that by increasing the percentage of rice bran oil (RBO), the TBA number decreases and the onset of rancidity is delayed. Moreover this study suggests that T3 (50% RBO + 50% NS) can produce superior quality cookies to prove effectiveness of RBO as bakery shortening. Nasehi et al. (2011) evaluated the influence of full fat soy flour (FFSF) and different extrusion conditions on the acidity content and color characteristics of spaghetti during 7-month storage. Samples were packed in polyethylene bags and were kept at room temperature during storage. The addition of full fat soy flour and extrusion processing conditions influenced the acidity content of spaghetti. They showed the shelf life of this new product was lesser than traditional spaghetti.

Devi et al. (2013) used LDPE and PP to conduct a shelf life study of pasta prepared from polished proso millet blended with wheat flour. Based on the results obtained in the study it was concluded that pasta could be best preserved up to 3 months at ambient atmospheric condition under LDPE without appreciable quality loss. Kaur et al. (2012) assessed the effect of cereal bran enrichment on the colour, cooking, sensory quality and shelf life of enriched pasta at ambient temperature. Cooking quality of pasta remained constant during storage. Non significant effect of storage was found on water activity, free fatty acids. Enriched pasta (15 per cent level of wheat, rice and oat bran and 10 per cent barley bran) was highly acceptable upto 4 months of storage with respect to quality.