CHAPTER II

REVIEW OF LITERATURE

Cereals and legumes are the important and primary source of energy in human diet for long time. They are also the important source of protein in daily diet for millions of people in the world. Poor digestibility and presence of anti-nutritional factors however limits the contribution of nutrition to the consumer. In an independent studies, it was reported that 3 billion people around world suffered from malnutrition (ACC/SCN, 2000). But unknowingly, from long time practices like soaking, germination, fermentation and cooking are involved before consumption or product formation without the knowledge of their benefits, only to increase the flavor and palatability. These process and practices affect anti-nutritional factors significantly (Shahidi, 1997). Recent studies reveal the effect of these treatments on the anti-nutritional factors, bioavailability of nutrients and enhancement in nutrient digestibility. Of all these treatments germination and cooking effectively lowers anti-nutritional factors and increases the digestibility and bioavailability of nutrients. Germination proves to be the most effective and simpler method in improving nutritional aspect of cereal and legumes due to ease of handling (El-Hag et al., 1978).

2.1. Germination

“From a small seed a mighty trunk may grow: Aeschylus”

Every grain is seed and consequently is a living substance. It has all the necessary characteristics of living organism provided it has not been damaged and is viable. Every grain contains its own particular hereditary data and all complex biochemical substances in germ cells required for growth and further generations (Han and Yang; 2015).
As per definition, germination is the process of emergence of plant or fungus from seed or spores respectively, which leads to fully functional organism or structure. The most common examples are sprouting of seeds of angiosperm or gymnosperm. In more or less common sense it defines the anything into greater being from small existence or germ. Germination a main part of growth period of seed gain in which seed undergoes important metabolic processes during the optimum condition for growth and further development (Sangronis et al., 2006). This period is characterized by the utilization of seed reserves for respiration and formation of new cell material (Vidal et al., 2002). In more technical term it is growth of an embryonic plant from germ part of the seed. The seed of a plant is small unit which is produced in fruit after union of opposite sex cells or gametes. Under favorable conditions growth of embryo in the covering layers and emergence of the radical takes place. Emergence of radical is indication of germination completion. Germination process begins with the absorption of water by the dry seed and end at the elongation of embryonic axis and further is the growth of seedling into plant (Bewley and Black 1994). Water is one of the most important factors for germination, and acts as a medium for various biological reactions. Another important climatic factor required by germinating seed for metabolism and respiration process is oxygen. Seeds are considered as living grains, because they perform aerobic respiration by uptake of oxygen for energy for metabolic processes until it grow leaves. Temperature is one of the limiting factors for seed germination, seeds from different species and varieties germinate at different range of temperatures (Wilcox and Pfeiffer, 2008).

In its initial stage germination is a degenerative (catabolic) process as the reserved substances present in the grains are used for development and growth of the embryo. Simon (1984) reviewed various changes (biochemical, morphological and hormonal) in germinating
grains. The process of germination is affected by various factors like climatic changes, abiotic factors (Neumann 1997, Chinnusamy et al., 2006), and chemicals, present in the soil at the time of germination. Presence of minerals and mineral-salt concentration in medium (either soil or synthetic media) containing seed is an important factor affecting germination. Seed grains conclude the germination process after the protrusion of radicle, and further is the seedling growth. Up to this point seed perform autonomously (autotrophic) by utilizing the reserve food, once the radicle comes out it depends on the more complex processes like photosynthesis (photoautotrophic).

2.2. Germination: A traditional approach to improve nutritional characteristic

In developing countries, foods are rarely processed or improved to increase nutrient density. Direct consumption of grains has been reported throughout the history, without knowing the actual composition of grains. However fewer products with germination or fermentation are also available but they are not recognized as main food articles. Products like porridges from cereals are main source of diet for infants in many developing countries. Enyiokwolla production involves milling of the cleaned grains into flour which is then made into slurry, boiling water is added to the slurry to produce a gelatinized product; enyiokwolla (Ocheme, 2007). It may be further heated if the thickness is not satisfactory. The traditional method of enyiokwolla preparation does not take into account the presence of anti-nutrition factors in the millets, which affects its nutritional value. The products like enyiokwolla can be modified for more nutrition intake by the modifying processing method by incorporating the soaking and germination of the grains before milling into flour, the nutritional quality of the porridge (enyiokwolla) will be improved (Ocheme and Chinma, 2008). Germinated grain was used in China not only for food but for medicine 5000 years ago. Samanū, a sweet paste is made from germinated wheat, is quite
popular traditional food item in Persian countries. Malting has been used from ancient times to convert grains into malt by germination for the production of alcoholic products. Mämmi: an easter porridge, is a traditional Finnish food cooked from malt prepared from rye. A thick syrup of Malt known as Liquid malt extract is used in variety of purposes like baking and brewing.

2.3. Effect of germination on composition and nutrition profile of cereal and pulses

Seed germination is accompanied by the change in proximate composition. There is significant increase in the value of simple molecule as the complex molecules break down during germination to simpler ones. There are certain changes during germination that could occur within the seed; those changes can vary depending on the type of crop, the variety of the seed and the conditions of germination (Bau et al., 1997; Dhaliwal and Aggarwal, 1999). It was observed that grain sprouts increase utilization of proper food value i.e. human body can promptly assimilate substances (Lintschinger et al., 1997 and Lorenz et al., 1981). Upon imbibition, the quiescent seed rapidly resume metabolic processes like respiration, enzyme synthesis, organelle formation activity, and RNA -protein synthesis (Bewley and Black, 1994). Enzymes are synthesized and activated to utilize storage macromolecules. These reactions lead to higher bioavailability and increase in the nutritional status of grain by structural modification and formation of new compounds. In a study conducted by Desai et al., (2010) malted ragi flour results in product with higher nutritional value and significant sensory attributes. The nutritional value of cereal and legume is also improved, through an increment in, protein digestibility, essential amino acids (Nakamura et al., 2011; Sangronis and Machado, 2007), vitamins (Frias et al., 2000) and by decrease in some anti-nutritional factors (Ghavidel and Prakash, 2007).
2.3.1. Effect of germination on proteins

Although legumes and cereals are good in their protein content, but utility of protein is limited because limited protein bioavailability and digestibility. Various antinutritional factors binds to the protein and thus decrease its nutritional potential. Proteinaceous anti-nutritional factors such as amylase inhibitors and trypsin inhibitors protect grains against predators. By combining the processes like sprouting and cooking better digestibility of protein in legumes can be attained (El-Hag et al., 1978). Morgan et al. (1992) concluded the fact that the protein content of sprouts increases after germination due to the utilization of nitrogenous compounds, by the facilitating the absorption of nitrates from grain reserves. Germination is the only traditional method that causes the increase in protein content in contrast to roasting and autoclaving etc. that reduces the protein content of grains (Magdi A. Osman, 2007).

Presently many studies have been conducted to get the maximum nutrition from grain by controlled germination process under controlled extrinsic and intrinsic factors. During germination, albumin content increases and globulin content decreases, since the total albumins and globulins content remained constant, it is assumed that globulin breakdown explains the increase in fraction of albumins. Increment in crude protein content is also attributed to an increase of proteases activity; however the degradation of storage protein is also necessary to form amino acids and peptides to stimulate seed growth. Bliss (1975), reported that increase in protein during germination was due to release of free amino acid as a result of enzymatic hydrolysis for synthesis more proteins. Subbulakshmi et al. (1976) observed trypsin inhibitory activity and increase in protein digestibility in beans after germination. The processing conditions and types of legume determine the effects of germination on the free non-protein amino acids (FNPA) and free protein amino acids (FPA). In lentils and peas the amount of free
protein amino acids increases during germination. In beans, germination causes reduction of α-amino adipic acid and an increase of γ-aminobutyric acid (Yu-Haey Kuo et al., 2004).

2.3.2. Effect of germination on carbohydrates

Carbohydrates are the main food reserve of seed grains and are utilized for energy production during germination. Carbohydrates in the form of starch contribute more than 60-70% of grain composition, depending on the type of seed/variety or species and about 50% of it may hydrolyze after germination. Germination is accompanied by decrease in storage carbohydrates which breaks down to form soluble and reducing sugars. This breakdown of carbohydrates releases energy which is required for plant growth and metabolic activities (Da Silva Ferreira et al., 2009). During early growth stages, the complex carbohydrates are converted by hydrolyzing enzymes into more soluble molecules and then are transported from endosperm to embryonic part where they can be immediately used by embryonic axis. The polysaccharides (like raffinose, stachyose and verbascose) are the primary forms of polysaccharides present in the seed grains and are used as easily available sources of energy during formation of embryonic axis. Starch and oligosaccharide levels decreases and consequently the level of reducing sugars increases. As observed during the malting and other processes, increase in alpha-amylase activity during germination is responsible for total starch loss (Uriyo, 1999). The amylose proportion of the starch increases during germination but concentration of hemicellulose decreases. These changes have a favorable effect on the nutritional value of grains and also enhance some of their physical properties like swelling ability etc., which is directly influenced by starch. Decomposition of high molecular weight polymers cause release of smaller bio-functional molecules which aids in the improvement of organoleptic qualities accompanied by generation of flavor and altering texture properties (hard to soft) (Beal
and Mottram, 1993). Higher swelling power of the flour after germination may be due to the reduced lipid content of the flour (Ocheme and Chinma 2008). Therefore reduction of complex carbohydrate results in increase in total and reducing sugars due to enhanced action of hydrolytic enzymes which results in the degradation of starch into simpler compounds (simple sugars, oligosaccharides).

2.3.3. Effect of germination on crude fat (lipids)

The values for fat content of cereal and legume are generally low, however small amount of fatty acids contributes to the total fat content of grain. The low fat levels for legume grains are because the major function of fat/lipid in grains is to maintain the integrity of cell wall mainly (Chikwendu, 2003). Germination does not have any significant effect on the fat content but in some cases it causes slight changes. Changes in fat content during germination vary seed to seed and the extent of β-oxidation required for energy production, however most of the energy production is through the breakdown of carbohydrates and other polysaccharides. Previous studies shows mixed results in value of fat content of grains i.e. it sometimes increases and sometime decreases during germination. Afam-Anene and Onuoha, (2006) suggest that the slight increase might be due to retention of free fatty acids, which are not converted into carbohydrates or not utilized during energy production for the metabolic processes and may lead to increase in total fat content on dry weight basis during germination. Alternatively in the oil seeds with high lipid content the decrease in the value of fat might be due to leaching of lipid content of cell wall components whereas, germination break down the fat to compensate the energy requirement during metabolic processes by the involvement of lipolytic enzymes. The main lipid storage structure in some oil seeds like corn is scutellum, where the most of lipid hydrolysis through β-oxidation occurs to generate energy.
2.3.4. Effect of germination on fibers

Fibers are the carbohydrates, which are indigestible part of plant or grains. Grains are good source of dietary fibers and are main constituents of bran. Grains along with fibers contain several important nutritional components like: resistant starch, soluble fibers, minerals and phytochemicals (like antioxidants), arabinoxylan and β-glucan. According to American association of cereal chemists (AACC) “Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine”. Depending on the solubility, there are two types: insoluble and soluble. Germination significantly increases the total dietary fibre content. Sieving and dehulling of grains reduces the fiber content by great extent. Dietary fiber serves as food for colon bacteria and thus maintains colon health by promoting the growth of beneficial microbes.

Indirectly dietary fibers influence the other physical characteristic like particle size, hydration capacity and oil binding capacity, which determines the food texture quality and have influential effect in maintaining the digestive system (Raghavendra et al., 2006; Nassar et al., 2008; Dhingra et al., 2012). Addition of fibers to the food products like cookies, biscuits and pasta, not only enhance their nutritional value but also contributes to improved sensory attributes and textural properties (Tudoric et al., 2002; Nassar et al., 2008). On health point of view crude fiber consumption of fiber is useful in reduction of obesity, diabetes and cardiovascular disease (Park et al., 2009).

2.3.5. Effect of germination on vitamins

Thiamine, ascorbic acid, niacin, folic acid and riboflavin are the common vitamins present in most grains. Germination is the only process to enhance the vitamin content of grain
especially Vitamin C. Water soluble vitamin B-complexes also shows significant increase. Barley malts reportedly contain as many as two to three fold of riboflavin as compared to raw barley. Similarly thiamine content also increases as result of germination. Ascorbic acid plays important biological functions during seed germination (Ginter, 1989 and Jimenez, 2002). Addition of ascorbic acid enhances the growth of seedling. Ascorbic acid serves as co-factor for synthesis of Gibberellins-a plant hormone required for germination, elongation of seedling and flowering like developmental processes. Ascorbic acid affects the phyto-hormone mediated signalling processes during the developmental stages (Carina et al., 2006). Seed grains pretreated with ascorbic acid shows enhanced seedling growth by several folds even in salt stress conditions and increased germination percentage. Ascorbic acid plays an important role in cell division, developmental process of seedling and acts as plant growth regulator. (Asada, 1999; Conklin, 2001; Pignocchi and Foyer, 2003). L-galactono-1,4-lactone dehydrogenase (GLDH) is the key enzyme for ascorbic acid synthesis in Smirnoff-Wheeler pathway. This enzyme oxidize the L-galactono-1,4- lactone and convert it into l-ascorbate (Wheeler et al.,1998). In addition to it, vitamin-C serves as co-factor for various enzymes and exhibits anti-oxidant properties to some reactive oxidative stresses. Humans being unable to synthesize ascorbic acid, are entirely dependent on dietary sources (Nishikimi et al, 1992).

2.3.6. Effect of germination on trace elements (Ash/Mineral)

Ash content of grain is generally a measure of mineral content of grain which is present in traces (in ppm) and hence also known as trace elements in case of food grains. Ash content of seeds or grains decreases significantly with germination, soaking and dehulling (Ghavidel et al., 2007) whereas, autoclaving increase the ash content of grains. Leaching out of macro and micro elements during soaking could be the reason for significant reduction of mineral matter on
germination. (Magdi A. Osman, 2007) other than that some enzymes uses minerals ions as their co-factors. The main site of these essential trace elements (co-factors) is inside the reserve food material. In plants the bioavailability of minerals depends on the presence or absence of various anti-nutritional factors. Phytic acid inhibits the bio-availability of divalent cations such as Fe$^{2+}$, Ca$^{2+}$,Mg$^{2+}$ and Zn$^{2+}$. Reduction in the phytic acid and other anti-nutritional factor after germination causes the increase in the availability of minerals to some extent (Badau et al., 2005).

2.3.7. Effect of germination on anti-nutritional factors

Anti-nutritional factors are those food components which limit the utilization of nutrition provided by food grains. Various types of anti-nutritional factors present in cereals and legumes are polyphenols, flavonoids, phenols, enzyme inhibitors (lipase or trypsin inhibitors) and mineral binding agents (phytates, glucosinolates). Activity and substrate for these factors depends on the functional group present in their molecules.

In vivo studies demonstrate the effect of tannins, saponins, and lectins on growth of testing animals, when fed with raw legume diet (Liener, 1994). Raw legume grain diet comprises of about 20-30% of lectins. Lectins are the proteins which binds carbohydrates, and due to their binding properties they cause indigestion and nutrient deficiency in diet. They are most common agglutinins and agglutinate RBCs (Vojdani A, 2015). Some protein inhibitors negatively affect the nutrients availability and digestibility by binding to proteins and decrease their solubility and digestibility. Protein inhibitors are present in almost all plant parts especially in grains. Protein inhibitors like trypsin inhibitors are degraded during germination due to utilization as a source of energy during the early stages of germination (Burbano et al., 1999). Bioavailability of minerals is affected by the presence of various mineral binding complexes like
phytic acid. Phytic acid also known as inositol polyphosphate has a mineral binding property. The main function of phytic acid is to store phosphorus (Lopez et al., 2001), but it has strong affinity towards other material as well, like calcium, iron and zinc. Iron and zinc bounded phytates are insoluble and indigestible causing their deficiency in diet (Hurrell, 2003). Germination promotes the phytase activity and reduces the phytic acid content of grains (Egli, 2002). Cereal contains certain bi-functional inhibitors that are able to inhibit the activity of more than one type of enzymes. Xavier-Filho (1991), reported bi-functional inhibitors in salt stressed ragi crops that inhibits the activity of both serine proteinase and alpha amylase. Other than serine inhibitors plant cysteine proteinase inhibitors are also of interest in research studies. They are isolated and characterized, both from animal and plant origin, belong to a protein super-family, the cystatins (Evans and Barrett, 1987).

Polyphenol like tannins are mainly concentrated on the hull of the seeds and provides dark coloration to seed coat. This polyphenols precipitates various proteins, amino acids and alklaoids and thus reduce the protein proportion of diet. Germination reduces the tannin content in a wide variety of legumes; some loss may also be attributed to leaching of tannins during soaking (Liener, 1994) and due to enzymatic hydrolysis by polyphenol oxidase (Alonso-Sanchez, 2008).

2.3.8. Effect of germination on amino acid composition

Sprouted seeds showed a higher content of almost all amino acid than raw grains, although this change might vary. Germination enhances the amino acid content of grains by several folds. The change in the level is attributed to time period for sprouting. This could be due to the fact that, during the germination process, several enzymes are activated and some non-protein nitrogen substances, such as nucleic acids, are produced; therefore, these can cause
protein levels to be increased (Sibian et al., 2016). The increase of total free amino acids, after germination, was a result of the degradation of some proteins by protease and a synthesis of new enzymes, which helped to liberate the free amino acid. For example the germination significantly increased the content of almost all the amino acids in germinated brown rice except histidine, methionine and threonine, glutamic acid, aspartic acid and serine, however the most significant changes, in γ-amino butyric acid, glycine, lysine and leucine, were observed in the germinated rough rice and the germinated rice extracted powder (Moongngarm, 2013). Decarboxylation of glutamic acid yields γ-amino butyric acid. γ-amino butyric acid (GABA) is the one of the important amino acid extensively found in germinated brown rice as compared to un-germinated grains (Shu et al., 2008).

2.3.9 Effect of germination on *in vitro* digestibility

*In vitro* digestibility is important regarding the proper utilization of proteins and carbohydrates by digestive system using enzymes. Germination enhances the *in vitro* digestibility of both protein and carbohydrates (Archana et al., 2001). *In vitro* digestibility is the function of anti-nutritional factors present in legumes. As discussed above, various anti-nutritional factors interact with food components and enzyme system to lower their digestibility (Negi et al., 2001).

Negative correlation between phytic acid and tannin with *in vitro* digestibility of protein has been reported in various studies (Kataria and Chauhan, 1993). Protein and starch digestibility increases during germination (Preet and Punia, 2000). Alonso-Sanchez et al. (2000) reported the *in-vitro* protein digestibility of the *P. vulgaris* increased to 10.3% as a result of germination.
Sangronis et al. (2006) observed the effect of germination and cooking in *P. vulgaris* (both black and white beans). Cooking along with germination provides more effective results as compared to germination alone. Decrease in the anti-nutritional factors makes proteins more susceptible to proteolytic enzymes for better utilization and digestibility. Malted sorghum, barley and other grains exhibit high α-amylase and proteolytic activity, which is absent or low in raw grains. The activation of α-amylase and proteolytic enzymes promotes breakdown of complex molecules into simpler one and thus ease in their digestibility (Kaur et al., 2013).