2. REVIEW OF LITERATURE

2.1. Grain and flour characteristics

Pulses contain approximately 21 to 25% protein and 60 to 65% carbohydrates; however have limiting amount of essential amino acid such as methionine and cystine (Tiwari and Singh 2012). Pulses are also in rich in many vitamins and minerals (iron, zinc, calcium, magnesium). However, due to presence of anti-nutritional factors digestibility of pulse proteins has been reported to be low (Genovese and Lajolo 1996). Pulse proteins contain two major classes of proteins i.e. globulins (salt soluble) and albumins (water soluble). Globulins are most abundant class of storage proteins in pulses and are generally classified as 7S and 11S globulins. Albumins on the other hand are important during seed development as they contain most of the enzymes and metabolic proteins (lipoxygenase, protease inhibitors and lectins). Many health benefits of albumins and globulins including anticarcinogenic, antihypertensive, hypoglycemic, hypocholesterolemic have been identified (Duranti 2006). Pulses are also rich source of oligosaccharides, vitamins, enzyme inhibitors, minerals and phenolics (Rebello et al. 2014). Pulses were reported to have low digestibility owing to the presence of antinutrients which inhibit enzymes involved in digestion and reduces bioavailability of nutrients (Duranti 2006). Processing techniques such as soaking, cooking, extrusion and germination have been reported to reduce these antinutrients and increase digestibility.

2.1.1. Hunter color and minerals composition

Color affects many grain and flour characteristics. The color of grain depends on the presence of phenolics and flavonoids in the grain especially in the hull (Feenstra 1960). Interaction of these phenolics with different grain components (starch and protein) imparts different properties of grain and its products. Parengam et al. (2010) determined mineral composition using neutron activation. Rice and legumes were reported to be good sources of trace elements. Rice bean (Kaur et al. 2013) and chickpea varieties/cultivars (Kaur et al. 2005) have also been studied for variation in color parameters. Singh et al. (2010a) observed positive correlations between color
parameters and seed weight and seed volume in field pea varieties. Sharma et al. (2015) studied color and mineral characteristics in field pea and stated that chlorophyll and carotenoid pigments contribute to color of field pea grains. Positive correlations between Zn and Mn and Fe content, Cu and Mn content and Fe and Zn with $L^*$ value were observed. Kidney bean germplasm has been evaluated for diversity in seed and flour properties by Parmar et al. (2014). Significant correlations were reported between Mn content and $L^*$ and $b^*$ values and Ca and Mg contents and antioxidant activity. Sharma et al. (2015) studied physicochemical, structural and functional properties of Himalayan Kidney bean germplasm. They reported negative correlations between antioxidant activity and $L^*$ and $b^*$ values. Katoch (2013) determined mineral composition in rice bean varieties and reported that potassium, phosphorus and calcium were present in higher concentration in rice bean as compared to other minerals.

2.1.2. Gel electrophoresis

Biochemical data has been used to solve taxonomic problems and to assess genetic variability within and among populations. Electrophoretic analysis of seed proteins can be used for varietal identification. SDS-PAGE is a useful tool for distinguishing and determining genetic similarities among varieties/cultivars. Several reports are available on protein electrophoresis for evaluating genetic diversity in seed storage proteins different species, including Lima bean (Lioi et al. 1999), soya bean (Ram 1996); Phaseolus vulgaris (Ferreira et al. 2000), amaranthus (Zheleznov et al. 1997) and chickpea (Ghafoor et al. 2003). Diversity among cultivars can be identified and characterized by analyses of seed storage proteins and phylogenetic relationship among the accessions can be understood. Diversity in seed storage proteins has been associated with germplasm and its origin (Ghafoor et al. 2003).

Hameed et al. (2009) carried out comparative seed storage protein profiling of kabuli chickpea genotype. They concluded that electrophoresis of seed storage proteins could be used to determine genetic variation and relation in germplasm and the specific bands of seed storage protein profiles may be used for identification of mutants. Asghar et al. (2003) studied variation both intra and inter specific, in total seed protein electrophoretic pattern of chickpea germplasm. Singh et al. (2004), identified paddy and
moong varieties by gel electrophoresis of soluble seed proteins. Tomooka et al. (1992) observed similar patterns in strains of mung bean from Southeast Asia using SDS-PAGE analysis. Ladizinsky and Hymowita (1979) also stated that taxonomic categories below the species level possess same seed protein profiles despite morphological and ecological differences.

Galani et al. (2011) reported electrophoretic analysis of the seed storage proteins had direct relation to the genetic basis of the proteins and exploit genetic diversity and can be used to certify the genetic makeup of germplasm. Mustafa et al. (2007) determined genetic variation among albumins and globulins from Vicia faba L. cultivars using electrophoretic data. Vishwanath et al. (2011) identified of tomato (Lycopersicon esculentum) varieties by examining total seed proteins. Miskoska-Milevska et al. (2008) also observed differences in tomato seed protein profiles obtained by SDS-PAGE analysis. Elham et al. (2010) studied phylogenetic diversity and relationships of some tomato varieties by protein and RAPD analysis. They observed both of SDS-Protein and RAPD markers were equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different varieties.

Gangwar et al. (2006) studied seed protein variation in Pigeonpea. It was stated that the cultivated, intermediate and wild seeds could be identified for high protein rich progenies from successive generation by observing differences in protein band intensities. They also concluded that the interspecific hybridization is important for obtaining variability in seed protein of Cajanus. Shareif et al. (2005) identified maize genotypes biochemically and concluded that PAGE patterns can used to identify and determine genetic diversity among maize genotypes. Valizadeh et al. (2001) studied seed storage protein profile of legumes grown in Iran, using SDS-PAGE. In the study it was concluded that all species were clearly recognizable from their protein banding patterns. Malviya et al. (2005) also characterized total salt soluble seed storage proteins of legumes using SDS-PAGE. Cheema et al. (2010) characterized castor bean genotypes under various conditions using electrophoresis of total seed proteins. The study revealed that it might be useful to distinguish diverse forms of it from one another, but the variation in castor bean genotypes was very limited.
2.1.3. Protein secondary structure

FTIR spectroscopy provides information about the secondary structure content of proteins. Each compound has a specific set of absorption bands in its infrared spectrum. Characteristic bands found in the infrared spectra of proteins and polypeptides include the amide I and amide II. These arise from the amide bonds that link the amino acids. Relationship between digestibility and secondary structure of pulse proteins has been evaluated in many studies. β-sheets have been reported to be the main components of secondary structures of legume proteins (Carbonaro et al. 2012) and were found to play a major role in determining the protein digestibility. Processing techniques have been reported to bring variations in secondary structures of legume seed flour. Carbonaro et al. (2008) reported higher β-sheets and lower random coils in lentil as compared to common bean. Decrease in β-sheets and increase in random coils was observed on dry heating. Heat-induced complexes of legume proteins have high stability due to high content of β-sheet.

Shewry and Halford (2002) made an attempt to understand the structures of gluten proteins and how it effects of the grain for bread making. Wong et al. (2009) studied relationship between starch and protein digestibility and structural features of sorghum. Secundo and Guerrieri (2005) investigated effect of thermal treatment on wheat gliadins structure and conformations in the absence and in the presence of dextrins. Savadkoohi and Farahnaky (2012) reported that major conformations in tomato seed proteins were β-sheets and α-helices. Ellepola et al. (2005) conducted conformational analysis on rice globulins under different conditions using FTIR. High α-helical, β-sheet and β-turn content in rice globulins were reported. Highly acidic and alkaline conditions were observed to influence intermolecular β-sheet structure. Chaotropic agents and structure perturbants influence band intensity and cause shifts in band positions, respectively. Decrease in α-helix and increase in random coils was also observed with heat treatment.

Shevkani et al. (2014a) reported that amaranth protein isolates with higher β-sheets had higher DSC denaturation temperature and gel temperature. Shewry et al. (2002) evaluated structural properties of wheat gluten proteins. Belton et al. (1997) conducted structural studies on hydrated pea proteins. Small structural changes in vicilin and
legumin were observed on hydration as compared to cereal proteins. This may be due to differences in globular structure of the legume proteins. Characterization of structural properties of lotus protein fractions was studied by Zeng et al. (2011). High stability of albumins and globulins was attributed to higher α-helix and β-sheet content as compared to prolamins.

2.1.4. Functional properties of flours

Pulses are used to enrich foods and provide a cheap source of protein in developing countries. Pulse flour has been used as a food ingredient due to its high protein content and functional properties (Kaur et al. 2007). Functional properties vary significantly among different legumes (Du et al., 2013). These variations were attributed to differences in protein to starch ratio and other constituents in the flours. Phaseolus was observed to have highest water (WAC) and oil absorption capacity (OAC) and emulsifying properties. Differences observed in pasting properties were related to starch swelling and water absorption. Appiah et al. (2011) evaluated functional properties of flours from cowpea varieties of Ghana. Water and oil absorption capacities were reported to vary between 1.89 and 2.15, and 1.95 and 2.31 ml/g, respectively and swelling capacity were observed from 265 to 268%.

Deshpande et al. (1982) examined the effect of removing hull on functional properties of Phaseolus vulgaris L. flours. Improvement in WAC, OAC, FC, emulsifying capacity and gelling properties was observed. Seed coat had significant effect on functional properties of flours. El-Adawy et al. (2003) studied the functional properties of germinated mung bean, pea and lentil seeds. They reported improvement in functional properties and nutritional quality of flours due to increase in protein digestibility and decrease antinutritional content. Functional properties of pea proteins were evaluated by Barac et al. (2011). They observed significant differences in concentration of 7S and 11S among different pea varieties and ratio of these proteins influenced protein extractability. Shevkani et al. (2014b) observed significant effect of defatting on functional and pasting properties of amaranth flours. Improvement in FV, WAC, and FS of flours was observed and was attributed to increase in protein content and reduction in lipid content.
2.2. Protein fractionation

Various methods of protein fractionation have been adopted but the most widely accepted method is based on the principle of solubility of protein in different solvents. Using this principle Osborne and Mendel (1914) developed a method and isolated proteins from wheat kernels. Ragab et al. (2004) used Landry and Moureaux (1970) method of protein fractionation and investigated effects of pH and NaCl concentration on functional properties of isolated proteins. Albumin was observed to be the major fraction among different cowpea proteins. Sathe and Venkatachalam (2007) fractionated proteins from moth bean and characterized them biochemically. Most of moth bean seed proteins were observed to be acidic and alkaline proteins were few. Globulin fraction was observed as major seed protein consisting of three major glycopeptides of molecular weight of 45–55 kDa.

Mendoza et al. (2001) fractionated, purified and characterized vicilin type (8S), basic 7S and legumin type (11S) globulins from mungbean chromatographically. Ramos and Bora (2004) characterized globulin from acetylated Brazil nut kernel using method described by Koyoro and Powers (1987). Afify (2012) isolated different protein fractions using method described by Landry and Moureaux (1970) from germinated sorghum varieties and determined solubility and digestibility of these fractions. They reported increase in albumin, globulin and glutelin fractions and decrease in cross linked kafirin and cross linked glutelin fraction after germination. Mundi and Aluko (2012) studied functional properties of albumin and globulin isolated from kidney beans using method of Aluko (2004). Both the proteins were detected with high lysine and arginine content. Globulins were observed to have better foaming and emulsion stability properties as compared to albumins. The higher surface hydrophobicity of globulins from kidney bean was considered the main reason for formation gel at a lower concentration.

Czubinski et al. (2014) tested the digestion susceptibility of globulins from lupin seed to different enzymes. The globulins were isolated using method of Czubinski et al. (2012). The globulins were extracted using pellet left after albumin extraction using buffer containing Tris–HCl pH 7.5, NaCl and Na$_3$N. Both the proteins were purified using anion exchange chromatography.
Sulieman (2008) fractionated proteins from lentil cultivars using Osborne and Mendel (1914) method based on solubility and studied effect of cooking on fraction content, structure and digestibility. Albumins were observed to be major proteins in lentils followed by globulins and quantitative and qualitative changes were noticed in protein fractions with most pronounced effect on prolamins. They observed significant reduction in IVPD and albumin fraction while glutelin level increased. SDS-PAGE of cooked and uncooked proteins fractions showed that lentil protein was altered due to cooking; this effect was most pronounced in prolamin fractions.

2.2.1. Functional properties of protein fractions

Mundi and Aluko (2012) studied functional properties of albumin and globulin isolated from kidney bean. Both the proteins had high lysine and arginine contents making these proteins an important ingredient in food preparations. Foaming, gelation capacity and emulsion stability of globulin was better as compared to albumin. Globulins showed ability to form better gels at a lower concentration and this was attributed to the higher hydrophobicity that increased protein–protein interactions as compared to albumins. Malomo and Aluko (2015) evaluated functional properties of albumins and globulins protein fractions isolated from hemp seed. Globulins were reported to have more disulfide bonded proteins, hydrophobic amino acids and low PS and FC as compared to albumins. The variation observed in their functionalities was attributed to differences in amino acid composition and changes in structural conformation depending on pH. Globulins had rigid conformational structure which reduced exposure of aromatic amino acids. Albumins were considered to have role in the formation of food foams.

Yin et al. (2011) studied various properties of protein isolates, globulins and albumins isolated from soapnut proteins. Globulins were major proteins in soapnut consisting of two polypeptides linked by disulfide bonds. Albumins showed significantly different electrophoretic pattern, amino acids and surface charge as compared to globulins or protein isolates. Disulphide bonds and hydrophobic interactions were considered to play important role in thermal stability of globulins. Close relationships among the physicochemical properties and conformational features of soapnut proteins were also observed. The succinylation of lentil globulins resulted in more acidic proteins with
higher solubility, water absorption capacity, apparent viscosity, emulsifying activity and stability and lower oil absorption capacity and foam stability as compared to native globulins (Bora 2002). However, extent of succinylation was reported to have no significant on functional properties globulins.

The rheological and functional properties of albumins isolated from two Mexican amaranth varieties were evaluated (Silva-Sa´nchez et al. 2004). The solubility of albumins above pH6 was observed to be maximum and comparable to that of egg albumins. Albumins showed excellent foaming stability and foaming capacity at pH 5 and maximum oil and water absorption capacities at acidic pH. Incorporation of albumins improved the dough properties and bread crumb. They suggested the amaranth albumins can be used whipping agents and as an ingredient in preparation of acidic foods. Lawal et al. (2005) compared functional properties of albumin and globulin fractions isolated from full fat and defatted flours of African locust bean and observed maximum solubility at pH 10 for both albumins and globulins; whereas minimum solubility for albumins and globulins were reported at pH 5 and pH 4, respectively.

Guerrero et al. 2007 isolated globulins from Phaseolus lunatus and were characterized to have both acidic and basic polypeptides and lower molecular mass and higher thermal stability than typical vicilins. Functional properties of protein isolates, albumins and globulins isolated from Ginkgo biloba seed were investigated by Deng et al. (2011). They showed better oil-holding capacity, foam stability, solubility, and emulsifying properties, but poor foaming and water-holding capacity as compared to other vegetable proteins. Albumins from Ginkgo were reported to have better emulsion stability, oil-holding, foaming and emulsifying capacity, as compared to its globulin fraction whereas globulins showed better foam stability as compared to albumins.

Baxter et al. (2010) studied the affect of albumins on pasting and textural properties of the rice flour and on rice kernels. They attributed the results of starch-water protein interactions to the water-soluble nature of albumins and excluded the possible role of protein barrier in the rice endosperm in restricting swelling of starch during heating. The effect of addition of 11S globulin from soybean on thermal and retrogradation properties of non-waxy maize starch was investigated (Yu et al. 2015). Increase in onset
and peak temperatures and decrease in thermal enthalpy of maize starch on increase in concentration of globulin-starch ratio was observed. A decrease in starch retrogradation and retrogradation enthalpy was also reported with incorporation of soybean 11S globulin. Therefore, soybean 11S globulin was recommended as a possible ingredient in preparation starch-soybean protein based products to control starch gelatinization and retrogradation properties.

Ogunwolu et al. (2009) studied functional properties of protein isolates and concentrates isolated from cashew nut and observed improvement in these properties with processing of nut along with protein concentrate and protein isolate. Cashew nut protein isolate were reported to show functional properties as compared to its protein concentrate. Concentration of H+ ions/pH significantly affected functionality of cashew nut protein.

2.2.2. Amino acid composition and zeta potential

Dhillon et al. (2014) developed a common HPLC method for analysis of plant and insect amino acids. Tang and Sun (2011a) determined physicochemical and conformational properties of three vicilins from kidney, red and mung beans. The amino acid composition (especially percentage of polar uncharged amino acids and relative ratio of acidic/basic charged amino acid) was reported to determine physicochemical and conformational properties. The surface charge was found to be closely related to protein solubility and emulsification.

Cavada et al. (2011) evaluated various nutritional characteristics Lathyrus species (fabaceae). Leu, Lys, Phe, Thr and Val were reported to be most abundant and Lys was detected in concentration more than recommended by FAO. Trp, Met and Cys were reported to be limiting amino acids. High protein efficiency ratio was also reported.

Karaca et al. (2011) observed that functional properties of protein isolates were by its surface charge, hydrophobicity and solubility. They observed that isolates prepared by salt extraction had lower surface charge compared to those produced by isoelectric precipitation. They suggested that chickpea and lentil protein isolates could be used as an alternative to soy protein isolates for stabilizing emulsions.
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Tang and Sun, (2010) studied physicochemical and conformational properties of mungbean 8S and/or 11S globulins and reported that amino acid composition, free SH and SS contents surface thermal stability, charge and hydrophobicity, protein solubility and emulsifying property varied with their polypeptide constituents. The emulsification index of globulins were observed to be closely related to its surface charge, protein solubility and surface hydrophobicity.

Bhatty et al., (1976) determined the amino acid compositions of six of lentils genotypes. Aspartic acid, glutamic acid, leucine, arginine, and lysine were observed to be the major amino acids present whereas methionine, cystine and tryptophan were present in limiting amount. Bhatty et al., (1982) reported higher lysine, threonine, tryptophan, methionine and valine content and lower leucine, arginine, and phenylalanine content in albumin as compared to globulin fraction of eight legume species. Sathe et al. (1997) Percentage of hydrophobic amino acids was the highest and basic amino acids were observed to be the lowest in globulins. Sulfur amino acids were reported to be the first limiting amino acids in globulin.

Candel et al. (1997) studied the effect of cooking and warm-holding on amino acid composition of legumes. Cooking was observed to decrease methionine, tyrosine, and threonine significantly in kidney bean. Lysine was the only essential amino acid that was affected by the cooking of chickpeas. The other hand, decrease in leucine, isoleucine and valine content and increase in phenylalanine, lysine and tyrosine were reported. Effect of warm holding was observed to be the highest in lentils. Katoch (2013) evaluated rice bean genotypes were for various nutritional constituents including amino acids.

2.2.3. Protein digestibility

Low digestibility of some essential amino acids limits the utilization of pulse proteins which has been attributed to many factors such as presence of anti-nutritional factors, structure of the proteins and interaction of proteins with other components (Nielsen 1991). Hsu et al. (1977) developed quick, easy and sensitive method for predicting protein digestibility using a multienzyme system consisting of trypsin, chymotrypsin and peptidase. It is the most widely adopted method to determine in vitro protein
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digestibility. Saunders and Kohler (1972) also developed an *in vitro* method for the determination of protein digestibility in wheat. Akeson and Stahmann (1964) measured digestibility of alfalfa protein concentrates by both *in vivo* and *in vitro* methods. Tang *et al.* (2009) conducted a comparative study on vicilin-rich protein isolates from Phaseolus varieties and determined its amino acid composition, digestibility and thermal aggregation properties. Digestibility was affected by heating and it was related to the extent of heat-induced aggregation.

Park *et al.* (2010) studied relationship between pea protein composition and digestibility. They identified TI, lectin, lipoxygenase, and PA2 as albumin proteins resistant to thermal treatment. The digestibility of untreated seeds was found to be positively related to globulins content and negatively to albumins content. The ratio of albumins to globulins and variations in PA2, TI, lectin, and lipoxygenase components influenced the digestibility of pea proteins. Effect of germination on content and digestibility of globulins from chickpea was determined by Portari *et al.* (2005). Germination had significant influence only on major globulin fraction as compared to other protein fractions. Decrease in the digestibility of flours was reported after 6 days of germination. Globulins were found to be more readily digestible as compared to albumins and therefore albumins were reported to influence the digestibility of chickpea flour.

Kalpanadevi and Mohan (2013) studied the effect of hydration, cooking, autoclaving and germination on *in vitro* protein digestibility of *Vigna unguiculata*. Protein digestibility increased and it was attributed to the reduction in antinutrients and disintegration of the structure of native proteins. Autoclaving was effected the digestibility of proteins to a greater extent as compared to other treatments. One or two processing methods together could be combined to obtain required improvement in digestibility of seed proteins. Germination was found to reduce phytic acid, tannins and oligosaccharides significantly whereas cooking and autoclaving reduced trypsin inhibitors and phytohemagglutinating activity. Shimelis and Rakshit (2007) evaluated effect of various processing conditions and their combinations on antinutrients and protein digestibility of *Phaseolus vulgaris* varieties from East Africa.
Zhang et al. (2010) determined protein digestibility of Indica and Japonica cultivars using pH-drop method with 3 or 4 enzyme systems. Improvement in protein digestibility in the four-enzyme system was observed and significant correlation between protein content and digestibility were obtained. Barbana and Boye (2013) evaluated in vitro protein digestibility of protein concentrates and flours from different colored varieties of lentil. Protein concentrates from both the varieties were more digestible as compared to their respective flours. Khattak et al. (2008) investigated impact of type of illumination and germination time on carotenoid content and protein digestibility of chickpea. Germination duration and illumination type had significant effect on these properties. Increase in digestibility was observed with increase in germination time and the maximum value was reported under fluorescent illumination after 48 h germination and under yellow illumination after 72 h.

Genovese and Lajolo (1996) examined effect of chemical modification on digestibility of albumin in *Phaseolus vulgaris* L. Digestibility of albumin was low and attributed it to the formation of high-molecular mass aggregates resistant to proteolysis due to formation of disulfide bonds. Carbohydrates attached to protein have also been reported to determine their susceptibility to digestion. Clemente et al. (2000) also studied factors affecting the protein digestibility of chickpea albumins and reported similar results specifying role of disulfide bonds in digestion of albumins. Chitra et al. (1995) determined variation in phytic acid content and its effect on protein digestibility of different legumes. The in vitro protein digestibility of pigeonpea, chickpea, mung bean, urd bean and soybean genotypes was reported between 60.4 to 74.4 %, 65.3 to 79.4 %, 67.2 to 72.2 %, 55.7 to 63.3 % and 62.7 to 71.6 %, respectively. Negative correlation between phytic acid content and protein digestibility were observed.

Vadivel and Janardhanan (2005) determined amino acid composition, protein digestibility, and anti-nutritional factors in wild legumes of South India. They reported that composition of all seven wild legumes was comparable to that of cultivated legumes in addition to higher levels of certain amino acids and higher in vitro protein digestibility of these legumes. Sangronis and Machado (2007) reported improvement in protein digestibility and reduction in trypsin inhibitors, phytic acid and tannins on germination of *Phaseolus vulgaris* and *Cajanus cajan*. Carbonaro et al. (1997) studied
relationship between solubility and digestibility of legume proteins. Hydrophobic forces and basic residues were involved in the stabilization of heat-induced aggregates resulting in low proteins digestibility.

Khattab et al. (2009) studied the effect of different treatments on quality of legume seeds. Autoclaving and water boiling improved amino acid composition. Sulfur containing amino acids content improved to a greater extent with autoclaving, microwave, micronization, cooking and fermentation. Autoclaving was best for improving PER, CS and EAAI. Enhancement in protein digestibility was also observed after treatments. Vijayakumari et al. (2007) also studied the effect of effect of soaking and hydrothermal processing on antinutrients and digestibility of Bauhinia purpurea L. seeds and reported similar increase in digestibility with autoclaving. Luo et al. (2014) reported decrease in crude protein and increase in protein solubility and free amino acids especially valine and phenylalanine after germination.

2.2.3.1. Digestibility of protein fractions

Sathe et al. (1997) studied in vitro digestibility of globulins and observed that pepsin was most efficient in hydrolyzing the globulin. Denadai et al. (2007) reported that sapucaia nuts are a valuable source of proteins, with higher levels of essential amino acids and minerals and low hemagglutinating or inhibitory activities were observed. Digestibility of globulins from sapucaia nuts was observed to be significantly high. Marquez and Lajolo (1981) evaluated effect of heat on digestibility of protein fractions including globulins, albumins and glutelins. They observed improvement in digestibility of globulins and glutelins and reduction in that of albumins with heating. They provided with evidences of presence of heat stable trypsin inhibitor in the albumin fraction. Czubinski et al. (2014) evaluated the digestibility properties of lupin seed globulins and observed that globulins were susceptible to chymotrypsin digestion. However, c-conglutin was reported to be resistant to trypsic and pancreatin digestion. Positively charged lysine and arginine and interactions between partially ionized flavonoids and proteins at pH 7.5 were responsible for the same. Czubinski et al. (2014) tested the digestion susceptibility of globulins from lupin seed to different enzymes. The digestibility of globulins by pancreatin, trypsin and chymotrypsin was examined and globulins were observed to be most susceptible to chymotrypsin digestion.
2.3. Interaction with grain components

2.3.1. Starch-protein interaction

Starch is widely used in many food and industrial applications as a thickener, stabilizer, gelling agent, bulking agent, water retention agent and adhesive. It significantly affects textural properties of many foods preparations (Goel et al. 1999). Functional and mechanical properties of food packages such as edible films are determined to biopolymer interactions (Phan et al. 2009). The properties of food products such as functional, flow, structure, stability, texture and mouth feel are influenced by starch and protein interaction (Li et al. 2007).

Jamilah et al. (2012) reported that interaction between starch and protein resulted in increase in strength of gel and attributed it to the formation of elastic starch globules and increase in the density of protein matrix in food systems. Addition of starch improved the physicochemical properties such as final textural of the final product. Understanding of the interaction between protein and starch is very important to develop food products with stable thermal and rheological properties and improve product quality and shelf life. The effect of blending of starch isolated from cassava with soy protein concentrate on functional properties has been evaluated by Chinma et al. (2013). Improvement in functional properties including gelation capacity, swelling power, and water absorption capacity was reported on incorporation of protein concentrate in starch, however, reduction in syneresis, solubility and pasting properties was also observed. They suggested use of cassava starch and soy protein blends in food preparations such as edible films which require high elasticity gel and strength.

Zhang and Hamakar (2003) studied the effect of interaction among starch, protein, and free fatty acids on functionality of starch. The change in cooling stage peak viscosity was used to identify the changes in functionality of starch with addition of different ingredients. Peak viscosity of cooling stage was observed to be affected by the sequence in which different components were added. Class of free fatty acid had significant affect on interaction between the three components indicating molecular structure of free fatty acid influences the pattern of three way interaction. Specific amount of soluble protein and free fatty was essential for production of the cooling stage viscosity peak. Verbeken
et al. (2006) investigated interactions between milk proteins, k-carrageenan, and modified starch in dairy desserts. Attractive interactions were reported between k-carrageenan molecules and milk proteins. Physicochemical properties of the desserts were dependent on formation of gel network and milk proteins were found to be involved in the formation of the same. Effect of interaction between β-glucan, starch and protein pasting properties of oat flours were investigated by Liu et al. (2010). β-glucan contributed majorly to pasting of flours followed by starch whereas protein was observed to have minimum contribution to pasting.

Galus et al. (2013) reported that interaction between soy proteins and starch increased strength of films of soy protein isolates. Incorporation of protein isolates improved the properties of starch as well as nutrition. Sun et al. (2014) studied the effect of microwave assisted dry heating on waxy and non waxy corn starch. Effect of heating xanthan with waxy starch was more pronounced as compared normal starch. Pasting viscosity of both the starches increased significantly on dry heating with soy protein isolate. Qiu et al. (2015) compared the effect of heating on interaction between waxy and normal starch and soy protein isolates. Increase in storage modulus and decrease in tanδ of starch pastes dry heated with protein isolates was reported. The starch heated in the presence of proteins showed more solid like structure as compared to starch. The interactions between waxy starch and protein were more pronounced as compared to non-waxy or normal starch. Ribotta et al. 2007 also investigated interaction between soy protein isolates and starch in suspension. Soy proteins significantly affected the rheological behavior of starch suspensions.

Interactions between OSA starch and water soluble fraction of egg yolk (a-b-livetin) in solution and at the interface was investigated and its relation with emulsion stability was determined by Magnusson and Nilsson (2011). Interaction between egg yolk protein and modified starch was found to be pH dependent and varied with protein to starch ratio. They concluded that interaction in solution did not necessarily lead to interaction at the interface and adsorption of modified starch on the surface protein did not significantly affect the stability of the emulsion. The effect of genotype and interaction with starch on protein digestibility of sorghum was studied by Elkonin et al.
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(2013). Digestion of starch by amylolytic enzymes reduced digestion kafirin by pepsin indicating complex interactions between storage proteins and starch in sorghum endosperm.

Guerrieri et al. (1997) studied interaction between starch and protein through action of enzyme amyloglucosidase. Starch gelatinization was influenced by protein and quality of protein. The interaction was governed by the structure and size of both starch and proteins. Protein obstructed the enzyme action on starch granules.

2.3.2. Protein-polyphenol

Effect of protein-polyphenol interaction on protein digestibility of beans was studied by Toledo et al. (2013). The polyphenols were observed to interfere with digestibility of proteins by significantly decreasing the hydrolysis of phaseolin. The interference was more in black and brown seed having higher tannin content. The interaction between phenols and protein was observed as variation in electrophoretic pattern of proteins on interaction with both crude phenols and purified phenol fractions. S´wieca et al. (2013) concluded that the interaction between proteins and flavonoids had pronounced effect on food quality. The interaction resulted in increased protein digestibility of the breads and also affected the antioxidant activity of flavonoid. They also reported that bioaccessibility of important food components depend on the kind of interaction between various food components.

Carbonaro et al. (2000) reported that lectins and trypsin inhibitors did affect diegestibility whereas protein-bound polyphenols had a minor role in influencing digestibility bean and faba bean proteins. On the other hand, structural properties of 7S common bean and 11S globulins of faba bean were the major factors limiting digestion of proteins. Ravel et al. (2002) studied interaction between protein and phenols by derivatizing proteins with phenols. Phenolic compounds interact with proteins in a way that lysine, tryptophan and cysteine residues are blocked resulting in their limited availability. The reaction of phenolics and proteins induce change in net charge of the proteins which in turn affects the functional properties, secondary and tertiary structure of the proteins influencing the surface properties making them hydrophilic in nature.
2.4. Extrusion processing

Extrusion results in improvement of quality of proteins and digestibility of both starch and proteins (Day and Swanson 2013). Extrusion conditions including feed moisture, screw speed, screw configuration, pH, particle size and temperature significantly influence the digestibility of proteins. During extrusion disulfide, covalent, electrostatic, hydrogen and hydrophobic bonding develops among different molecules which determine the properties of extrudates. The effect of different extrusion conditions on functional and nutritional properties of whole pinto bean meal was studied by Quintana et al. (1998). Temperature and feed moisture significantly affected various extrudate characteristics. Extrusion temperature of 160˚C and feed moisture of 22% yielded the best products with maximum overall acceptability. Extrusion cooking enhanced digestibility of proteins by inactivating trypsin inhibitors.

El-Hady and Habiba (2003) also reported increase in digestibility of proteins on extrusion cooking due to decrease in antinutrients and percentage of phytic acid phosphorus. Soaking of legumes before extrusion cooking for 16 h was advised for better nutritional value. Valim and Batistuti (1998) studied affect of thermoplastic extrusion on functional properties of defatted chickpea flour. Meng et al. (2010) examined changes in physicochemical properties of chickpea flour-based snack with change in extrusion conditions. They reported medium temperature, low feed moisture and high screw speed resulted in extrudates with high expansion ratio, low bulk density and hardness. These were considered desirable extrudate characteristics. An improvement in protein digestibility of popped horse gram due to decrease in antinutrients was also reported Sreerama et al. (2008). Affect of popping of horse gram on functional properties of the flour such as WAC, OAC and emulsifying properties were studied and variation in expansion of horse gram was attributed to modification of cell wall polysaccharides by enzymatic hydrolysis.

Li and Lee (1997) studied the relationship between extrusion temperature and solubility and distribution of disulfide bonds in wheat proteins. They reported an extrusion between 120 to 160˚C resulted in increase in protein solubility due to formation of extensive disulfide cross-linking among wheat proteins. Martínez et al. (2014) observed
improvement in starch gelatinization and emulsifying capacity of wheat flour with extrusion. An increase in free sugar content and susceptibility to enzymatic hydrolysis and decrease in resistant starch content was also reported with increase in extrusion severity. Alonso et al. (2000a) explained that extrusion resulted protein aggregation resulting in decrease in protein solubility, hydrophobicity and OAC and increase in WAC. Alonso et al. (2000b) reported that extrusion was most effective processing method to reduce antinutrients and to improve in vitro protein digestibility among different methods including soaking, germination and extrusion. Pasting properties of expanded pellets and extruded corn and rice flour were evaluated by de Souza et al. (2011). Corn flour was considered to be more suitable for expanded products as compared to rice flour.

Effect of incorporation of legume flour on nutritional and functional properties of protein enriched sorghum and wheat extrudates was studied by Balasubramanian et al. (2012). The incorporated of legumes resulted in production of extrudates with higher water absorption and solubility index and better pasting properties and lower gelatinization. They concluded that legume flours can be used for production of protein rich extrudates. Drago et al. (2007) suggested that extrusion variables were of utmost importance and to obtain perfectly cooked extrudates such combinations of variables should be avoided through which fluid flow transport mechanism cannot be achieved. So, they recommended that bean grits should be extruded at high temperature and low moisture to obtain high calorie density extrudates if cream soup formula desired.

Abu-Ghoush (2014) developed a novel extruded lentil analog product. Singh et al. (1998) examined effect of processing variables on extrusion of maize grits. Kadan et al. (2003) determined functional properties of extruded rice flours. Kaur et al. (2014) concluded that incorporation of banana flour into corn grit resulted into extrudates with better expansion, lighter color and increase solubility index. Atienzo-Lazos et al. (2011) studied the effect of variation in moisture and temperature conditions during extrusion on functional properties of extrudates from composite flour. Effect of composition, moisture and extrusion temperature on characteristics of extrudate from yam-corn-rice based snack food was studied by Seth et al. (2015). Lazoua (2011) evaluated textural and structural properties of lentil based extrudates obtained by blending corn and lentil.
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The author observed relation between sensory characteristics and extrusion conditions and concluded that sensory characteristics of extrudates can be predicted using these attributes and models. Jozinovic et al. (2012) reported that extrudates prepared at lower moisture content and with screw configuration 4:1 expanded more and extrudates with high expansion ratio had lower bulk density and hardness. Extrusion resulted in increase in WAC and decrease in resistant starch and viscosity of extrudates.

2.5. Germination

Germination results in the improvement of nutritional profile of foods incorporated with germinated flour due to increase in the fibre and decrease in the lipid content; there is also potential production of bioactive peptides during germination (Rumiyati et al. 2012). Increased enzymic activities in the germinating seeds are usually accompanied by interconversion and production of new compounds (Wanasundara et al. 1999). The germination of lentils resulted in reduction of starch content, a-galactosides and trypsin inhibitor activity and an increase in riboflavin, thiamin and niacin content with germination (Urbana et al. 1995). The nitrogen balance, ADC and percentage R/A was reported to decrease in dry heated and germinated lentils; however, nitrogen balance was higher for methionine-supplemented lentils as compared to raw lentils.

An increase in antioxidant activity, sucrose, glucose, resveratrol and total free amino acid content with germination was reported by Wang et al. (2005) in peanut. It was suggested that germinated peanut could be used as functional vegetable because of bioactive and chemopreventive relevance of resveratrol. Ribeiro et al. (2011) investigated how soluble carbohydrates, inositol and proteins levels were altered by germination in Phaseolus vulgaris and Vigna unguiculata. An increase in glucose and myo-inositol content and decrease inositol and raffinose content was observed with germination. Bean sprouts were considered as an important source of proteins, myo-inositol, glucose and sucrose. Low levels of raffinose were also reported in germinated beans. Increase in content of pythochemicals in mung bean with germination was reported by Guo et al. (2012). Significant increase in total phenolics, vitamin C, quercetin-3-O-glucoside and content was observed. Antioxidant activity of mung bean increased six times with germination.
Chau and Cheung (1997) investigated the effect of different processing methods on starch and protein digestibility and antinutrients of *P. angularis* and *P. calcaratus*. They suggested that processing methods involving heat treatment were more effective in reducing antinutrients and increasing digestibility legume proteins. They also observed increase in starch digestibility to a greater extent as compared to protein digestibility with these processing treatments. Effect of various treatments on mineral composition, antinutrients and protein digestibility was evaluated (Afify *et al.* 2012). A decrease in antinutritional, amylose and mineral content after processing and increase in protein digestibility and bioavailability of minerals especially iron and zinc was observed. Urbano *et al.* (2005) concluded that optimal conditions for improving nutritional properties of peas included short germination periods, with or without light. Germination periods of 2 to 4 days were sufficient to improve intake and utilization of carbohydrates and proteins.

Shastry and John (1991) determined changes in biochemical composition and protein digestibility of *Dolichos lablab* during germination. Increase in protein digestibility was observed along with major increase in antinutrients after fourth day of germination. During initial stages of germination, increase in protein digestibility in spite of decrease in protein content was attributed to better availability of proteins. Effect of germination on globulin content and *in vitro* digestibility of chickpea was reported by Portari *et al.* (2005). An increase in digestibility up to 4th day followed by decrease on 6th day of germination was observed with major increase in albumin fraction and a small decrease in trypsin inhibitor activity. Chickpea germination was reported to significantly affect *in vitro* digestibility of globulin fraction.

Khalil and Mansour (1995) evaluated the affect of cooking, autoclaving and germination on nutritive value faba bean. Germination and heat processing reduced tannins, vicine, stachyose, trypsin inhibitor, phytic acid and haemagglutinin and enhanced PER value, protein digestibility, threonine, leucine and histidine content, significantly. Significant increase in Fe and Zn content and decrease Cu, Na, Mn, K and Mg content was also reported.
Chinma et al. (2009) studied changes in functional and pasting properties of flours from brown and yellow varieties of tigernut with germination. Decrease in carbohydrate and fat and increase in was reported and improvement in functional properties was also observed. Rusydi et al. (2012) observed reduction in tannin, phytic acid and phenolic contents with germination with greater reduction in peanut as compared to soy bean.

Bamdad et al. (2009) reported that in early stages of germination the largest subunit of vicilin and acidic subunit of legumin were cleaved by proteolytic enzymes during germination of lentils. Improvement in foaming capacity, protein solubility and water and oil absorption capacity and decrease in foam stability were also observed. Ghavidel and Prakash (2007) observed improvement in protein and starch digestibility and bioavailability of thiamin, Fe and Ca of legumes with germination with greater increase on dehulling the germinated legumes Reduction in tannin by 43–52% and phytic acid by 47–52% was also reported. Combining germination with dehulling improved quality of legumes by enhancing digestibility and bioavailability of nutrients and reducing antinutrients.

Urbano et al. (2005) observed noticeable increase in glucose, sucrose, fructose and vitamin B2 content and decrease in galactoside in germinated peas. Better nutritive utilization of protein and carbohydrates and improvement in palatability pea seeds was reported with germination. Rumiyati et al. (2012) determined protein profile of germinated ASL flour gel electrophoresis and observed decrease in proteins of HMW with germination. Afify et al. (2012) observed decrease in most of amino acids except phenylalanine and valine after germination. Increase in globulin, albumin and kafirin and decrease in cross linked kafirin and glutelin protein fraction after germination was also reported.

2.5.1. Effect of germination on starch

Germination affects the amount of available and resistant starch depending on the type of legume Benítez et al. (2013). Significant decrease in resistant starch and increase in available starch, BD, OHC, WHC, WAC, and gelation capacity was observed. Chu et al. (2014) examined changes molecular structure of starch that occur during germination in barley grains and observed that barley grains containing shorter starch
chains produced sugars readily as compared to those containing long chains. Germination did not significantly affect physicochemical properties of lentil starch, amylose and amylopectin; however, it significantly improved the digestibility of starch (Frias et al. 1997)

Germination resulted in decrease in the ability of the starch to resist swelling in lentil and faba bean. This has been attributed to the increase in $\alpha$-amylase activity during germination (Morad et al. 1980). Negative correlation between peak viscosity and amylose content has been reported by Noosuk et al. (2003) which has been confirmed by Musa et al. (2011). It was observed that peak viscosity of increased whereas gelatinization temperature decreased after germination in rice starches.

Germination decreased amylose and amylopectin content of starch however increased the same in starch from waxy cultivar Wu et al. (2013). Increase in peak viscosity was observed with prolonged germination. SEM pictures showed pits and pores on starch granules as result of the partial starch hydrolysis by enzymes activated during germination.

2.5.2. Germination and phenolic content

Aguilera et al. (2010) reported that germination did not have significant effect on phenolic content however, fermentation increased content of phenolic compounds. Content of gentisic acid, p-hydroxyphenylpropionic acid and tryptophol increased after fermentation in comparison to raw lentils. Sreerama et al. (2012) compared phenolic compounds in cowpea and horse gram flours to chickpea flour they also evaluated antioxidant properties in association with hyperglycemia and hypertension of these flours. Sreerama et al. (2010) also determined variability in phenolic compounds in chickpea and horse gram.

Alvarez-Jubete (2010) determined the affect of sprouting on polyphenol composition of amaranth and quinoa. Germination in the presence of elicitors resulted in important changes in total phenol content and antioxidant activity. Decrease in the concentration of total and individual phenolic compounds was observed in sprouts in the presence of elicitors. Folic acid was found to be the best elicitor to improve phytochemical quality of germinated kidney bean. Ravichandran et al. (2012) observed decrease in 4-hydroxy
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Benzoic acid content and increase in cinnamic acid content with germination. Increase in vanillic acid and p-coumaric acid content with germination of beans was reported by Diaz-Batalla et al. (2006).

Cevallos-Casals and Cisneros-Zevallos (2010) concluded that phenolics acts as free radical scavengers during early stages of germination and with progress in germination they lose their antioxidant potential. Dueñas et al. (2009) germination caused activation of many endogenous enzyme systems and changes in metabolism of seeds which resulted in significant variation in phenolic composition of lupin. Germination resulted in increase in flavonoid content, such as flavones, isoflavones and dihydroflavonols as compared to raw lupin. Germination led to increase in the content of free and bound phenolics as well as flavonoids in germinated brown rice (Ti et al. 2014). However, these changes varied with the stage of germination. The phenolic were present in free form whereas total flavonoids existed mainly in the bound form during germination.

2.6. Wheatgrass

Wheat grass is a rich source of nutrients and vitamins for the human body and has high concentration of chlorophyll, active enzymes, vitamins and other nutrients. Ben-ayre et al. (2002) studied the effect of wheatgrass juice on various gastrointestinal conditions and found it effective for treatment of active distal ulcerative colitis. Dhaliwal et al. (2015) also investigated the free radical scavenging potential of lyophilized wheat seedling juice powder. It was suggested that wheatgrass juice can be used for treatment of various diseases as well as antiaging agent.

Hussain et al. (2014) studied the role of wheatgrass in cancer treatment. Cancer patients that consumed wheatgrass juice showed better recovery as compared to those who did not consume the same. Therefore, wheatgrass juice was recommended as an alternative to chemotherapy for cancer treatment. Wheatgrass was also found to play important role in cancer patients and exhibits tumoricidal effects. It was observed to induce apoptosis and cell cycle arrest resulting in modification of biological response during cancer treatment.

Singh et al. (2012) reviewed various aspects of wheatgrass in depth and gave an overview of therapeutic potential of wheatgrass in prevention as well as treatment of
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diseases such as diabetes, cancer and chronic heart diseases. Kardas and Durucasu (2014) developed a new method for determination of phenolic compounds and their antioxidant activities in different wheat grass varieties. Bar-Sela et al. (2007) investigated the role of wheatgrass juice in prevention and treatment of cancer in patients of breast cancer. Wheatgrass juice taken during chemotherapy was observed to reduce its toxicity, required dose and GCSF support in patients. Antioxidant activity of wheatgrass, spirulina and amalki was determined by Shukla et al. 2009. Alcoholic and aqueous extracts of wheatgrass, spirulina and amalki were analyzed for vitamin C and E content and total phenolic compounds. The highest antioxidant activity and vitamin E was observed for aqueous extract of amalki.

Kulkarni et al. (2006a) evaluated concentration of different elements shoots of wheatgrass under different growth conditions. A linear increase in concentrations of K, Ca, Mg, Mn and Na with increase in days of germination was reported. Wheatgrass tablet available in the market showed higher concentrations of various elements as compared to cultivated wheatgrass. Kulkarni et al. (2006b) also evaluated antioxidant potential of wheatgrass under different conditions. Germinated wheatgrass shoots (7-8 day old) grown in soil with tap water and with or without nutrient solution were rich in antioxidants. Falconi et al. (2002) reported that synthesis of low molecular weight antioxidant molecules occurs during germination of wheat which inhibited oxidative damage caused to DNA by reactive oxygen species in vitro. This molecule was characterized and was reported to be from glycosides family. Peryt et al. (1992) also observed antimutagenic effect in aqueous extracts from wheat sprouts.

2.7. Starch characteristics

Starch is the major component in cereals and legume grains, accounting for 50–80% of the dry matter (Rehman et al., 2001). Starches isolated from various biological sources have different and unique physicochemical and technological properties (Zhou et al., 2012). Legume starches are more viscous than cereal starches, exhibiting high resistance to swelling and rupture, high gelatinization temperature (GT), fast retrogradation, and high RS content (Singh et al., 2004; Ambigaipalan et al., 2011). Singh et al. (2008) evaluated starches separated from different pulses. Blackgram starch
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was reported to have lowest retrogradation tendency as compared to other legume starches. Higher crystallinity was reported in starches with lower proportion of amylose and intermediate fraction. Blackgram and pigeon pea starches were reported to have higher crystallinity as compared to other legume. Higher breakdown in $G'$ was observed in starches with higher crystallinity and lower amylose content.

Singh et al. (2012) determined characteristics of starch isolated from different kidney bean lines. Correlation between pasting properties and amylose content and short-chains amylopectin and thermal properties and granule size and long-chains amylopectin were observed. Starches with low breakdown were considered suitable for products requiring heat stability and mechanical shearing. Singh et al. (2014b) studied the effect of removal of protein from starch during successive purification stage. Whiteness, solubility power and syneresis were observed to decrease in the presence of protein. Decrease in transition temperatures and increase in gelatinization enthalpy was reported with starch purification. Singh et al. (2009) reported negative correlation between swelling power and transition temperatures. Setback was reported to be negatively correlated to thermal characteristics and that retrogradation affected by degree of polymerization and amylose–lipid complexes.

Singh et al. (2010c) evaluated effects of debranning on thermal and pasting properties of starches from wheat varieties. Singh et al. (2011b) investigated the effect of gamma-irradiation on various properties of potato starches. Increase in transition gelatinization temperatures and decrease in pasting properties of irradiated starches was reported.

2.7.1. Scanning electron microscopy

Ambigaipalan et al. (2011) studied the morphology of different bean. Scanning electron microscopy revealed that the shape of starch granules varied from oval to round for all the three starches. Faba bean starches showed numerous cracked granules whereas no cracks or indentations were observed on the surface of pinto bean and black bean starches. Hoover and Ratnayake (2002) examined the microscopic structure of bean starches. The starch granules had smooth surfaces and irregular shape (round, elliptical and oval). No fissures were observed on the granule surface. The size of starch granules varied from 8-35 mm. Morphology of chickpea starches was studied by Miao et al.
The shape of starch granules varied from cobble shape to spherical without presence of any fissures. Granules from both the cultivars showed significant variation in size and shape. The size of granules ranged between 7 and 29 μm. Rupollo et al. (2011) evaluated the morphological properties of bean stored under different conditions (hermetically stored at 5 °C, nitrogen storage at 15 °C and room temperature storage at 25 °C). The granules were oval to spherical and showed smooth surface with absence of fissures.

2.7.2. X-ray diffraction (XRD)

The crystallinity of starches can be depicted using technique of XRD. According to their XRD patterns, crystallinity of starches can be classified into A, B, or C type. Peaks at 2θ = 15°, 17°, 18°, 20°, and 23° have been observed for A-type starches, which are observed in cereal starches. B-type X-ray pattern with diffraction peaks at about 5°, 15°, 17°, 20°, 22°, and 24°, 2θ are exhibited by starches isolated from tubers while C type crystallinity is a combination of A and B patterns (Zobel 1988). The C-type pattern is the observed in starches isolated from legumes and they are the highly resistant to digestion (Tharanathan and Mahadevamma 2003). Huang et al. (2007) investigated the crystallinity of pea starches. Pea starches showed C-type pattern along with peak at 2θ = 15.2°, 17.2° and 23.2°, corresponding to 0.58, 0.52 and 0.38 nm of spacings, respectively. An additional peak at 18.0°, 2θ was observed at d-spacing of 0.49 nm in cowpea starch indicating difference in crystalline structure as compared to chickpea and yellow pea starches.

The review of literature indicated that many aspects of proteins such effect of germination on protein structure, composition of pulse shoots etc has not been investigated in this field. Literature on effect of germination on pulse starch is also limited.