2
Review of literature

2.1. General properties and characteristics of antinutritional factors in legumes

Legumes have been a source of food to mankind since early time. These are often considered as a good source of protein, minerals and other nutritional components (Messina, 1999). Legumes are considered as a good substitute of animal protein (Iqbal *et al.*, 2006). However, the antinutritional factors in legumes are a matter of great concern, thus limiting consumption of legumes. The antinutritional factors reduce the digestibility and bioavailability of nutrients in foods, and are also responsible for various physiological abnormalities in humans and animals (Silla Santos, 1996; Messina, 1999; Sandberg, 2002; Kumar *et al.*, 2010; van Buul and Brouns, 2014). General characteristics of major antinutritional factors and their interaction with food matrix have been reviewed below.

2.1.1. Tannins

The term 'tannin' was used for the plant extract used to process animal skin into leather (Evans, 1989). Bate-Smith and Swain (1962) first defined it as a water-soluble phenolic compound (MW 500-3000 Da). Tannins are well-distributed among a large variety of plants used as a source of food. These are responsible for the astringency of foods due their interaction with the protein of saliva or mucous tissue of the mouth. Several phenolic compounds occurring in nature possess similar general properties, but are distinct from one another according to their chemical structure (Amarowicz, 2007). On the basis of chemical structure tannins are divided into two major groups, hydrolysable tannins and condensed tannins (Fig. 1) (Hagerman and Butler, 1989). Hydrolysable tannins possess a central core of carbohydrate and hydroxyl group which are esterified by phenolic acid, such as gallic (gallotannins) and hexahydroxydiphenic acid (ellagitannins). A large number of hydrolysable tannins exists in nature and exhibits diverse variation in their structure. Such a variation is due to oxidative coupling of neighbouring gallic acid units or aromatic rings in their structures (Chung *et al*., 1998). On the other hand, condensed tannins, having molecular weight higher than hydrolysable tannins, are structurally more complex. Those are polymerised products of flavan-3-ol (catechin) or flavan-3,4-diol (proanthocyanidins), or a mixture of the two (Chung *et al*., 1998). While condensed tannins are the main polyphenols present in commonly consumed food products, hydrolysable tannins are present only in small amounts (Salunkhe *et al*., 1990). Tannins exhibit positive human health-related properties, such as anticarcinogenic, antimutagenic, antimicrobial and antioxidant (Amarowicz, 2007). In spite of having these beneficial properties, tannins have been considered as an antinutrient. This is due to the high level of hydroxylation and property of forming insoluble complexes with protein, carbohydrate, metal ions and polysaccharides. The complex formation is associated with reversible interaction of hydrogen and hydrophobic interactions and irreversible interactions of ionic and covalent bonds (McCallum and Walker, 1990; Schofield *et al*., 2001; Gilani *et al*., 2005). Proline-rich protein has a high affinity for tannins in complex formation (Kumar and Singh, 1984). The complex of tannins and protein causes inhibition of enzymes and interferes gastrointestinal digestion, absorption of nutrients, damages intestinal tract and lowers nutrient availability (Serrano *et al*., 2009). Plants possess polyphenol-oxidase which hydrolyses various phenolic compounds (Sandberg, 2002; Saxena *et al*., 2003).

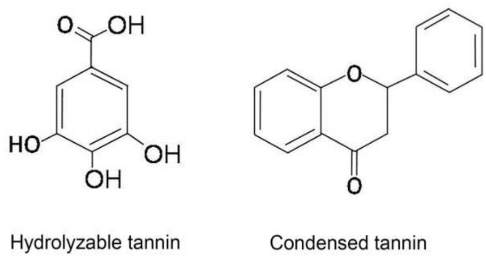

Fig. 1. Tannins

2.1.2. Phytic acid

Phytic acid (Fig. 2), also called myo-inositol hexaphosphoric acid or 1,2,3,4,5,6-hexakis (dihydrogen phosphate) myo-inositol or phytate in salt form, is the principal storage form of phosphorus in many legume seeds (Kumar *et al*., 2010). While it is accumulated during seed ripening and remains in the protein-rich aleurone layer of seeds of monocotyledons, it is uniformly distributed throughout the kernels in the seeds of dicotyledons (Ravindran *et al*., 1999). Phytic acid forms complex at a wider range of pH. The complex formation alters protein structure, making it resistant to digestion by proteolytic enzymes thereby hampering protein solubility and digestibility (Loewus, 2002; Sandberg, 2002). Phytic acid also reduces the availability of divalent and trivalent metal ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Lopez *et al*., 2002; Lönnerdal, 2002; Fredlund *et al*., 2006). Inositol tri-, tetra- and penta-phosphates are also responsible for the reduction of iron or absorption of zinc (Sandberg, 2002). Phytic acid can be degraded by phytase (*myo*-inositol hexakisphosphate phosphohydrolase), a digestive enzyme responsible for the release of phosphorus from the phytic acid molecule. Human beings cannot hydrolyze protein-mineral complex, formed by phytic acid due to the absence of phytase (Iqbal *et al*., 1994). Various possible sources of phytase have been reported, such as plant phytase, microbial (fungal and bacterial) phytase, phytase generated by the small intestinal mucosa and gut-associated microfloral phytase. Phytase activity of small intestine is very low, and activity in the gut is associated with a large number of bacteria present in colon (Kumar *et al*., 2010). However, the International Union of Pure and Applied Chemistry and the International Union of Biochemistry categorized phytase into two types, 3-phytase (myo-inositol

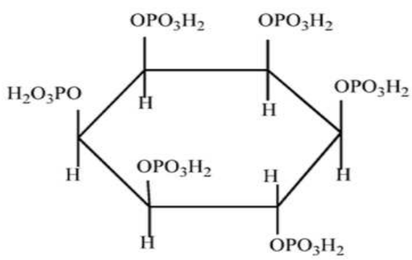

Fig. 2. Phytic acid

hexakisphosphate 3-phosphohydrolase) and 6-phytase (myo-inositol hexakisphosphate-6-phosphohydrolase). While 3-phytase is characteristic of microorganisms, 6-phytase is found in grains and seeds of higher plants (Reddy and Pierson, 1994; Selle and Ravindran, 2007).

2.1.3. Trypsin inhibitors

Legume protease inhibitor gained much attention during 1970s and 1980s due to their interference with digestion and growth in animals (Champ, 2002). Mainly two types of inhibitors exist- Kunitz and Bowman-Birk. These two inhibitors differ in their polypeptide make-up, molecular weight, cysteine content and number of reactive sites (Richardson, 1977). Polypeptide of the Kunitz inhibitor consists of 181 residues with two disulphide bridges and one reactive site, while that of Bowman-Birk consists of 70 residues and seven disulphide bridges (Laskowski and Kato, 1980). Trypsin inhibitor has been reported to function as storage of sulphur amino acids during dormancy. It has a protective potential from predators, such as pathogens and pests. Besides these, it is also used as food additives to prevent proteolysis, especially in fishery products (Kennedy *et al.*, 1993; Izquierdo-Pulido *et al.*, 1994). The negative effect of trypsin inhibitor lies in the fact that it interferes digestion by irreversibly binding with trypsin. The inactivation of trypsin in the digestive tract by trypsin inhibitor induces the release of a cholecystokinin from the intestinal mucosa. This hormone then stimulates pancreatic acinar cells to produce more trypsin. When this negative feedback continues, an important loss of sulphur-rich amino acids takes place. This leads to pancreatic hypertrophy/hyperplasia, growth depression and other carcinogenic effects (Roy *et al.*, 2010).

2.1.4. Haemagglutinins

Haemagglutinins or lectins are abundant in legumes (Chrispeels and Raikhel, 1991). These are glycoprotein that has an ability to reversibly bind specific carbohydrates without altering their covalent structure. Lectin is also known to protect plants from pathogens, such as microorganisms, pests and insects. The structure of lectins has been divided into merolectins, hololectins, chimerolectins and superlectins (van Damme *et al.*, 1998). These are also categorized according to sugar-binding specificities into polyspecific or monospecific. Their interactions with galactose, mannose, mannose-containing glycan and glucose also have been reported (Vijayan and Chandra, 1999; Barre *et al.*, 2001). Lectin finds its important place in many areas of research. The haemagglutinating activity of lectin is used for blood typing. Due to their anti-tumour properties, these are used in preclinical studies in cancer therapies (Liu *et al.*, 2010). These also play an important role in immune function, cell growth, cell death, body fat regulation, glycoconjugate study, cell characterization and cell separation (Sharon, 2008). Molecular weight of lectins ranges from 25,000 to 400,000 Da. In general, lectin molecule consists of one or more units. When the number of units in such a complex reduces, a reduction of agglutinating activity is observed (Kik *et al.*, 1989). Lectin stimulates an immune response and causes various human health-related abnormalities. These bind to mucosal cells of intestine, affecting blood glucose response causing growth depression in animals (Bardocz *et al.*, 1996). Lectins interfere with normal gastric secretion for nutrient absorption by enhancing the shedding of bush border membrane and decrease villus length of small intestine. These are also found to bind with glycan receptors in the intestinal tract causing discomfort. Other toxic effects registered include nausea, bloating, vomiting and diarrhoea (Peumans and van Damme, 1996; van Buul and Brouns, 2014).

2.1.5. Biogenic amines

Biogenic amines are nitrogenous, low molecular weight compounds (Fig. 3) which usually get accumulated in food products as a result of microbial decarboxylation of amino acids or amination, transamination of aldehydes and ketones (Shalaby, 1996; Silla Santos, 1996). These are aliphatic (putrescine, cadaverine, spermine and spermidine), aromatic (tyramine and phenylethylamine) and heterocyclic (histamine and tryptamine) (Silla Santos, 1996), and their prevalence in food is an indication of spoilage (Joosten, 1988). Biogenic amine formation depends on the availability of free amino acids (Joosten, 1988; Marklinder and Lönner, 1992; Soufleros *et al.*, 1998), presence of decarboxylase-positive microorganisms (Holzapfel, 2002) and condition suitable for microbial growth, decarboxylase synthesis and decarboxylase activity (ten Brink *et al.*, 1990; Silla Santos, 1996). Several microbial groups possessing decarboxylase activity include *Bacillus*, *Citrobacter*, Clostridium, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium*, *Lactobacillus*, *Pediococcus* and *Streptococcus* (Rice *et al.*, 1976; ten Brink *et al.*, 1990; Huis in't Veld *et al.*, 1990). The formation of biogenic amines has been reported in various fermented and non-fermented foods (ten Brink *et al.*, 1990; Shalaby, 1996; Silla Santos, 1996). Raw material, manufacturing process, pH variation, salt concentration and temperature are the influencing factors for their formation (Maijala *et al.*, 1995; Eerola *et al.*, 1998). Direct relationship between temperature and microbial proteolytic and decarboxylating activity has been reported by many authors (Joosten and van Boeckel, 1988; Maijala *et al.*, 1995). Microbial decarboxylase activity increases at a low pH and a low salt concentration, resulting their formation (Chander *et al.*, 1989; Silla Santos, 1996). Consumption of foods rich in biogenic amines leads to several human health-related abnormalities.

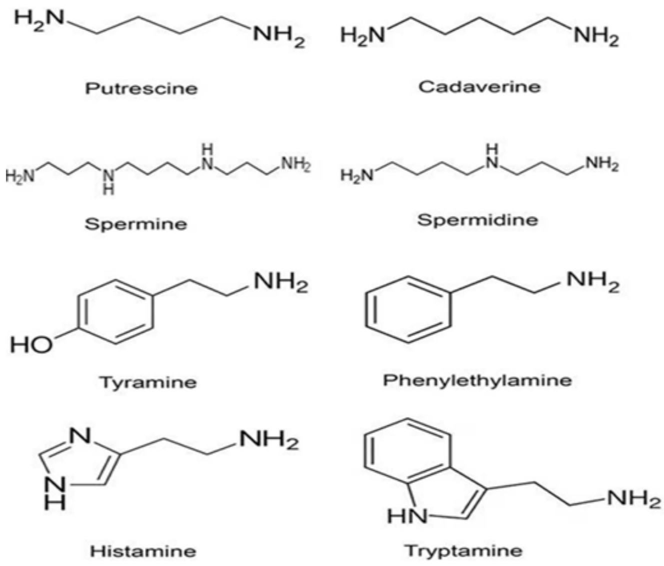

Fig. 3. Biogenic amines

Tyramine and phenylethylamine are responsible for hypersensitivity and migraine, and histamine is related to food poisoning (Parente *et al.*, 2001). Although putrescine, spermine, spermidine and cadaverine do not have health effect, those are responsible for food spoilage (Hernández-Jover *et al.*, 1997).

2.1.6. Oligosaccharides

Oligosaccharides of raffinose family predominate in most legumes (Reddy *et al.*, 1984; Girigowda *et al.*, 2005). These get stored during seed ripening (Guillon and Champ, 2002). In 1970, it was reported that the oligosaccharides present in legumes were responsible for gas production (Rackis *et al.*, 1970). Raffinose is a first member of the series followed by stachyose, verbascose and ajugose (Fig. 4) (Peterbauer and Richter, 2001). Oligosaccharides are synthesized by sequential addition of galactose units to sucrose linked by a a-1,6 bond (Peterbauer and Richter, 2001).

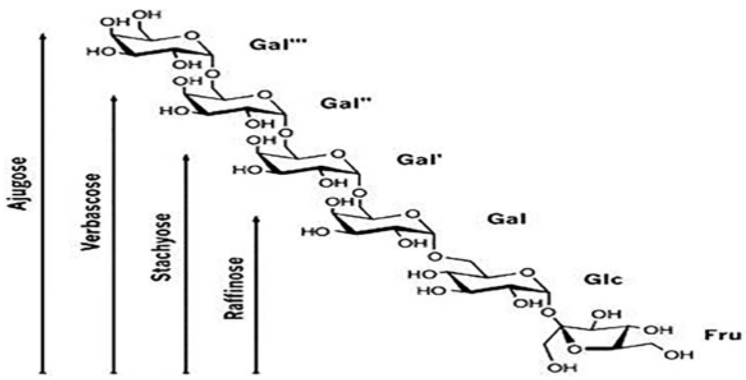

Fig. 4. Raffinose family oligosaccharides

2.2. Dietary intake and biochemical effects of antinutritional factors on human health

Majority of the world population rely on legumes for the basic nutritional requirement, such as protein, calories, minerals, carbohydrates and vitamins. Dietary legume improves nutritional value of the undernourished and promotes human health by reducing the risk of chronic diseases, such as cardiovascular disease, diabetes and cancers (Messina, 1999; Venter and Eyssen, 2001). In addition, legumes when blended with cereals provide a good plant-based supplement with balanced amino acid profile, making legumes a good dietary partner of cereals (Machaiah and Pednekar, 2002). Fermentation improves the nutritional value of foods by synthesizing certain amino acids, increasing the availability of B-group vitamins, essential fatty acids and other nutritional components (Sarkar *et al*., 1996, 1997b, 1998; Giraffa, 2004). A variety of traditional legume-based fermented foods are being prepared and consumed in India. Such foods have several human health-related beneficial effects. Food fermentation improves availability of vitamins and essential amino acids, and combats various nutritional diseases, such as kwashiorkor (a proteindeficiency disease), diarrhoea, growth retardation, muscle wasting, childhood blindness, beri-beri, pellagra, etc. (Steinkraus, 2002). As reported earlier, the presence of antinutritional factors causes nutritional deficiency which leads to serious human health-related abnormalities (Champ, 2002; Holzapfel, 2002).

Tannins reduce protein digestibility, making these partially unavailable for absorption, and inhibit proper functioning of proteins and other digestive enzymes. These damage mucosal lining of gastrointestinal tract, and increase excretion of proteins and essential amino acids. Amino acid availability becomes low in the diets rich in tannins. Moreover, tannins reduce the bioavailability of iron and vitamin B_{12} (Chung *et al*., 1998; Francis *et al*., 2001). Consumption of a high level of tannins (>50 g/kg dry weight) reduces voluntary feed intake, while medium or low consumption (<50 g/kg dry weight) has no effect (Frutos *et al*., 2004). According to Occupational Safety and Health Administration (OSHA), tannins have been listed as a category I carcinogens (OSHA, 1978). Therefore, it is advisable to reduce the excess intake of tannin-rich foods.

Likewise, the presence of phytic acid in food is a matter of concern. The average daily intake of phytic acid in a vegetarian diet is estimated to be 2000–2600 mg. For the population of rural areas of developing countries the intake is estimated to be 150-1400 mg (Reddy, 2002). Ideally, ≤25 mg per 100 g or about 0.035% of the phytic acid-containing food is recommended for better health (Onomi *et al*., 2004; Coulibaly *et al*., 2011). The antinutritional property of phytic acid lies in the fact that it forms complex with minerals, forming insoluble phytic-mineral complex. Among minerals, low Zn^{2+} bioavailability causes most adverse effect to human health. The reduction of Zn^{2+} absorption results in dwarfism and hypogonadism (Lopez *et al*., 2002; Kumar *et al*., 2010). Also, phytic acid forms complexes with proteins and the interaction is pH dependent (Cheryan, 1980). The phytic acid-protein complex is not readily absorbed in the gastrointestinal tract and small intestine of humans, because humans are devoid of phytic acid-degrading enzyme (Iqbal *et al*., 1994). This hampers enzymatic activity, protein solubility and protein digestibility. With carbohydrates, it reduces solubility and digestibility of glucose (Kumar *et al*., 2010).

Trypsin inhibitor is another important antinutritional factor present in various legumes (Guillamon *et al*., 2008). As it is resistant to pepsin and acidic pH of human digestive tract, trypsin inhibitor disturbs normal functioning of digestive enzymes. It stimulates pancreatic hypertrophy, causes growth retardation and decreases amino acid absorption. The retardation of growth is governed by the loss of the sulphur-containing component of trypsin (Messina, 1999). Trypsin inhibitor binds reversibly to trypsin and inhibits its activity. Then, secretion of pancreozymin-cholecystokinin from the gut wall takes place which in turn secretes trypsin from pancreatic tissue and stimulates the gall bladder to empty its contents into the intestine. The synthesis of trypsin in the pancreas results in increased requirement for amino acid cysteine, leading to loss of sulphur-containing amino acids (Krogdahl *et al*., 2010).

Haemagglutinin, due to its globular tertiary structure, remains undegraded by digestive enzymes and causes several health-related problems (van Buul *et al*., 2014). It interacts with enterocytes and lymphocytes, causing persistent antigenic responses. A phenomenon, called molecular mimicry, where haemagglutinin is mistaken as pathogens by antibodies or T-lymphocytes, triggers immunological response (Cordain *et al*., 2000). It has been hypothesized that diets rich in lectins result into obesity (Spreadbury, 2012). When present in the blood stream, those bind to cell membranes, arteries and organs such as joints, kidney, pancreas and brain, causing autoimmune disorders. Other toxic manifestations, caused by the intake of a high dose of dietary lectin, include nausea, bloating, vomiting and diarrhoea (Kumar *et al*., 2013).

Raw food, processed and stored products contain accumulated toxic amines, because of the activity of microorganisms (Silla Santos, 1996). Presence of biogenic amines in foods leads to serious human health-related abnormalities. A level of ≥1000 μg amines/g food is considered hazardous to human health (Taylor, 1985), and an intake of over 40 μg/g per meal has been considered potentially toxic (Nout, 1994). Among the amines, tryptamine increases blood pressure and hypertension (Shalaby, 1996). Amines, such as putrescine, spermine, spermidine and cadaverine react with nitrite present in foods by forming nitrosamines and cause poisoning (Hernández-Jover *et al*., 1997). Histamine consumption higher than 100 μg causes intense poisoning (Parente *et al*., 2001). When the intake of amines is high, the detoxification system present in our body becomes unstable and fails to detoxify these amines effectively. Such an inadequate detoxification makes their easy entry into the circulatory system, where those induce the release of adrenaline and noradrenaline which in turn increase cardiac output, migraine, tachycardia, blood sugar and blood pressure levels (Shalaby, 1996). However, the toxicity threshold of biogenic amines varies from individual to individual as toxic dose is dependent on the efficiency of the detoxification mechanisms of each individual (Halász *et al*., 1994).

Oligosaccharides have been identified as prebiotic agents, a food ingredient beneficial to human health (van Loo *et al*., 1999). These promote the health of colon, increase longevity and reduce the risk of colon cancer (Benno *et al*., 1989; Koo and Rao, 1991). However, oligosaccharides are considered antinutrients because these are a major producer of flatulence. Humans are devoid of α-galactosidase to cleave α-galactosyl linkage. As a result, these intact oligosaccharides are not absorbed by the digestive tract. The undigested oligosaccharides accumulate in the large intestine and are rapidly degraded by colonic microflora having α-galactosidase activity and subsequent anaerobic fermentation results in the production of gases, such as carbon-dioxide, hydrogen and a trace of methane (Guillon and Champ, 2002). The flatulence is usually accompanied by abdominal pain, nausea, cramps, diarrhoea and discomfort (Tanaka *et al*., 1975). Because of the discomfort and social embarrassment associated with flatulence, some people opt to avoid beans entirely (Messina, 1999).

2.3. Strategies for reduction of antinutritional factors

Protein calorie malnutrition is a current problem among the children of many developing nations (Iqbal *et al*., 2006). Legumes being a nutritious food are thought to improve the nutritional status of low-income groups of a population. The presence of a considerable amount of antinutritional factors is responsible for the reduction of food quality and poses negative effect on human health (Rehman and Shah, 2005; Iqbal *et al*., 2006). Several traditional household food preparation methods can be employed to enhance the bioavailability of nutrients in plant-based foods by reducing the levels of antinutritional factors. During processing, it is important that toxic components should be reduced to a minimum level so that those pose no threat to the health of the consumers. Therefore, several approaches may be considered for their removal. The processing treatments can be categorized into physical processing and bio-processing.

2.3.1. Physical processing

Physical processing involves the use of simple equipment in households or small industries. Physical processing includes dehulling, soaking and heat treatment.

2.3.1.1. Dehulling

Dehulling involves removal of seed coat. In a household scale, mortar and pestle are used to dehull seeds, and in industrial scale machine is used. Dehulled seeds are cooked more easily than seeds with intact seed coat, thereby reducing the cooking time (Kon *et al*., 1973; Tharanathan and Mahadevamma, 2003). Further, during dehulling the antinutritional factors confined to the seed coat are removed (Nakitto *et al*., 2015).

2.3.1.2. Soaking

Soaking is routinely followed in a household scale for the preparation of various legume-based foods. The solvent for soaking is water, salt solution or aqueous alkali solution. After soaking, the soaked medium is discarded. The duration of soaking usually varies from minutes to hours depending on the condition of processing steps. Soaking plays a dual role, one is the saving energy cost by shortening cooking time while the other is removal antinutritional factors (Mulimani and Vadiraj, 1994). Soaking increases the permeability of cell membrane and facilitates leaching of the water-soluble antinutritional factors (Ibrahim *et al*., 2002). However, soaking temperature, type of soaking medium and length of soaking are the important factors affecting the removal of antinutritional factors. Soaking also stimulates the endogenous enzyme activity of seeds, such as polyphenol-oxidase and phytase, causing reduction of antinutrients, like tannins and phytic acid (Greiner and Konietzny, 1999; Khandelwal *et al*., 2010). The reduction of phytic acid during soaking depends on pH, soaking time and soaking temperature (Greiner and Konietzny, 1999). Increase in duration of soaking inactivates trypsin inhibitor activity to a considerable extent (Ibrahim *et al*., 2002; Khattab and Arntfield, 2009). Hence, soaking can be considered as an important stage of processing. When legume seeds are soaked, oligosaccharides get solubilized and leached out into the soaking medium (Vadivel and Pugalenthi, 2009). Aqueous or alcoholic extraction of legumes, change in pH, temperature or humidity leads to reduction in flatulence (Calloway *et al*., 1971).

2.3.1.3. Cooking

Cooking is a process which facilitates leaching of antinutritional factors into the cooking medium. Heat treatment reduces the phytic acid content, thereby making the availability of iron, zinc and calcium for absorption. Cooking effectively reduces heat-labile antinutritional factors, such as trypsin inhibitor and haemagglutinins, thereby enhancing protein quality of the cooked product (Shimelis and Rakshit, 2007). Increase in temperature during cooking unfolds the protein structure, and these unfolded proteins are more susceptible to digestive enzymes (Sathe *et al*., 1984). Successive soaking and cooking are more effective in reducing antinutritional factors than cooking alone (Nout *et al*., 1993; Embaby, 2010; Kalpanadevi and Mohan, 2013).

While considering the effect of cooking, it becomes essential to what extent nutritional components get affected so as to improve the nutrition of legume-based product by inactivating the levels of antinutritional factors. To minimize the levels, selection of shorter cooking time and use of steaming rather than simple boiling are necessary. One such example is autoclaving which is a moist heating technique where cooking is done under high pressure. Further, autoclaving also shortens the duration of cooking. High-pressure cooking imparts hydrostatic pressure on foods which sterilizes food, denatures proteins, and controls the enzymes and other chemical reactions without causing any physical damage to the raw material. However, in household scale, pressure cooker instead of autoclave is used. The reduction of antinutritional factors during pressure cooking has been reported by Akande and Fabiyi (2010).

2.3.2. Bio-processing

In the traditional method of legume food preparation, after soaking and cooking, legumes are further processed. Sometimes soaked seeds are germinated or fermented depending on the type of food being prepared. During germination as well as fermentation, there is involvement of enzyme(s) which are either present endogenously in the seeds or produced by microorganisms. Some of the bio-processing strategies used and their possible role in the reduction of antinutritional factors are discussed below.

2.3.2.1. Germination

Germination is a process of intake of water by seeds followed by seed coat weakening, metabolism and growth initiation. In legumes, several enzymes take part in the hydrolysis of seed storage proteins. Many proteinaceous antinutritional factors, such as haemagglutinin, amylase and trypsin inhibitors get reduced during germination (Savelkoul *et al*., 1992; Uwaegbute *et al*., 2000; Kalpanadevi and Mohan, 2013). During germination, the activity of polyphenol-oxidase is high- the condition which is beneficial for the reduction of tannins (Savelkoul *et al*., 1992; Khandelwal *et al*., 2010). Germination induces the synthesis of phytase too. Plant seeds utilize phytic acid as a source of inorganic phosphorus during germination and in turn increase the nutritive value of seeds (Kumar *et al*., 2010). When seeds germinate, reserve nutrients are released, and vitamin and mineral contents are increased. Germination mobilises reserve nutrients such as native proteins, required for the growth of plant seedlings and removal of antinutritional factors (Uwaegbute *et al.*, 2000). Germination plays a positive role in the removal of trypsin inhibitor activity (Savelkoul *et al.*, 1992; Kumar *et al.*, 2006).

2.3.2.2. Fermentation

Fermentation is the oldest method of food processing and preservation (Nout and Rombouts, 1992). It is defined as a biochemical modification of primary food products brought about by the activities of microorganisms and their enzymes (Mortajemi and Nout, 1996). The substrate for fermentation can be whole grains, ground products or processed products. Fermentation can be initiated either by following natural fermentation or by selection of pure culture. In traditional food preparation, fermentation is accelerated by the addition of a starter culture of selected microbes or addition of a small amount of already fermented material (back-slopping). This accelerates the initial phase of fermentation and imparts a desirable change to the product. Fermentation enhances taste, aroma, texture, shelf-life and nutrition, and detoxifies food (Mortajemi and Nout, 1996; Steinkraus, 2002). Legume fermentation is popular as it provides improved digestibility, micronutrient availability, biosynthesis of vitamins, essential amino acids and reduction of antinutritional factors. Reduction of antinutritional factors during fermentation is important because the presence of these antinutrients affects protein and starch digestibility, and reduces bioavailability of calcium, magnesium, iron and zinc in foods (Holzapfel, 2002). Tannins reduce the solubility of iron, zinc and calcium. Fermentation lowers the pH and provides an optimal condition for enzymic degradation of tannins. In addition, low pH favours phytase activity, resulting in the reduction of phytic acid content (Sandberg, 2002). Microbial phytase originates either from the microbiota on the surface of legumes or from the inoculum of the starter culture which helps to hydrolyze phytic acid to inositol phosphates (Sandberg, 1991). Fermentation reduces trypsin inhibitor activity, increases availability of essential amino acids and improves protein digestibility. Trypsin inhibitor activity gets reduced with the increase in microbial growth during fermentation (Holzapfel, 2002). Haemagglutinin is heat-labile and can be destroyed upon heat treatment prior to fermentation. Some traditional Indian fermented foods, such as idli and dhokla, are prepared by soaking and fermentation before final steaming. In such cases, haemagglutinating activity can still be detected (Reddy and Pierson, 1994). The haemagglutinin, present in fermented batter, can be reduced by heat treatment or steaming. During food processing and preparation, toxic amines get accumulated as a result of microbial action during fermentation (Shalaby, 1996). High temperature favours the formation of biogenic amines, because at high temperature proteolytic and decarboxylating reaction rates increase (Maijala *et al.*, 1995). A better understanding of the mechanism of biogenic amine formation during fermentation and its control during food processing becomes necessary. Lactic acid fermentation reduces oligosaccharides of legumes (Azeke, *et al.*, 2005; Refstie *et al.*, 2005; Du *et al.*, 2012).

2.3.2.3. Enzymic processing

Enzymic removal of antinutritional factors depends on the level of endogenous enzymes and supply of extracellular enzymes from any biological source. During food processing, many endogenous enzymes become activated, and the addition of extracellular enzymes further helps in the reduction of antinutritional factors. Polyphenol-oxidase reduces tannins content (Sandberg, 2002). About 60% reduction of phenolic compounds and improvement of iron availability by applying 1500 U/g polyphenol oxidase have been reported by Sandberg (2002). The fungal tannase also has a reducing effect on phenolic compounds (Gustafsson and Sandberg, 1995). Phytic acid gets hydrolyzed by the activity of phytase which is naturally present in plants and microorganisms. Phytase removes six phosphorus groups one by one from the inositol ring of phytic acid (Hurrel, 2003). A complete removal of phytic acid and an increase in mineral availability can be achieved by the addition of extracellular phytase. Nowadays, commercial phytase of microbial origin are available for their use in food processing industries. The commercial phytase isolated from *Aspergillus niger*, named under the trademark Natuphos®, is made into use to improve the availability of phytate phosphorus of corn-soybean meal diets (Yi *et al.*, 1996). Phytase has been isolated and characterized also from a number of plant sources, such as soybean (Hamada, 1996), maize (Maugenest *et al.*, 1999), wheat (Nakano *et al.*, 1999) and rye (Weremko *et al.*, 1997). A number of microorganisms associated with fermentations exhibit the potentiality of degrading biogenic amines through the production of mono- and di-amino-oxidases (Leuschner *et al.*, 1998). Degradation of oligosaccharides could also be achieved by using microbial or plant α-galactosidase (Somiari and Balogh, 1995).

2.4. Some common legume-based fermented foods of India

Indigenous food fermentation is the oldest food biotechnological process where the activity of microorganisms plays an important role. The art of food fermentation might have originated long back through trial and error to standardize the processing conditions. Such a traditional technology is believed to be passed from one generation to the next with the aim to preserve ethnic values. Different types of fermented foods are still being prepared in a household-scale or small-scale industries using relatively simple available resources and facilities. Varied groups of microorganisms are involved in the production of fermented foods. They act either alone or in combination for the production of particular foods (Aidoo *et al.*, 2006; Sharma and Sarkar, 2015). Microorganisms possessing this activity might be indigenously present in substrates or may be added as a starter culture where substrates are transformed biochemically and organoleptically into new upgraded edible products (Harlander, 1992; Campbell-Platt, 1994; Steinkraus, 1996). Some of the popular legume-based traditional fermented foods prepared and consumed all over India are shown in Table 1.

Table 1. Important legume-based fermented foods of India

Food	Substrate	Localilty	Mode of consumption	Reference
Adai	Legume and cereal	South India	Fried food consume as a snack food	Blandino *et al.* (2003); Das *et al.* (2012); Ray *et al.* (2016)
Amriti	Blackgram dal	South India	Syrup-filled, ring-shaped confectionery, consumed as a snack food	Roy *et al.* (2007a)
Dhokla	Rice and bengalgram dal	Gujarat	Steamed spongy cake, taken as snack food	Desai and Salunkhe (1986)
Dosa	Blackgram dal and rice	South India	Fried to thin crispy pancake, taken as a snack food	Soni *et al.* (1985)
Idli	Blackgram dal and rice	South India	Steamed spongy cake, taken as a snack food	Mukherjee *et al.* (1965)
Kinema, Hawaijar, Tungrymbai, Aakhone, Bekang, Peruyyan	Soybean	Darjeeling hills, Northeast India	Sticky with ammonical flavour, made to curry and taken with steamed rice	Sarkar and Nout (2014)
Papad	Blackgram dal	India	Thin circular wafer, taken as condiment or savoury food	Shurpalekar (1986)
Pitha (chakuli, enduri, munha, chhuchipatra, podo)	Blackgram dal and rice	Odisha, West Bengal, Jharkhand, Bihar	Consumed hot as a snack food	Roy *et al.* (2007b)
Vada	Legume and cereal	South India	Fried in edible oil into a donut shape and consumed as a snack food	Blandino *et al.* (2003); Das *et al.* (2012); Ray *et al.* (2016)
Wadi	Blackgram dal	Punjab, West Bengal	Hollow, brittle balls or cones, used as a savoury item	Sandhu and Soni (1989)

Fermented foods are always preferred than non-fermented ones due to their good keeping quality under ambient conditions, safety and acceptability (Holzapfel, 2002). A fermented product enhances food security and income generation to the livelihoods of both rural and peri-urban dwellers residing in different geographical regions of India (Sharma and Sarkar, 2015). Some of the popular legume-based fermented foods and their preparation processes have been discussed below.

2.4.1. Kinema

Kinema is a food of the Nepalese residing in the hilly areas of Indian Himalayan regions. In Darjeeling hills and Sikkim, it is sold in local periodic markets, called 'haat', by rural women belonging to different ethnic communities. They sell by taking known weight of fresh kinema wrapped in a locally available leaf which is tied loosely by straw to make small pouches (Fig. 5). Sun-dried kinema sealed in transparent plastic pouch is sold along with fresh kinema. In India, kinema is popular by different names, hawaijar in Manipur, tungrymbai in Meghalaya, bekang in Mizoram, aakhone in Nagaland, and aagya, chukchoro, peron namsing and peruyyan in Arunachal Pradesh. Their preparation method and consumption mode are more or less similar (Sarkar and Nout, 2014). Some of the kinema-like fermented soybean foods reported in literature are 'natto ' in Japan, 'thua-Nao ' in Thailand, 'douchi ' in China, 'chungkook- Jong ' in Korea, 'tao-si' in Philippines and 'dawadawa' in western Africa (Sarkar *et al.*, 1994; Shrestha and Noomhorm, 2001; Hu *et al.*, 2010)

Traditionally, kinema is prepared by overnight soaking seeds of Small Yellow variety of soybean (*Glycine max* (L.) Merr.) at 10-25°C for 8-12 h. After draining soak water, fresh water is added to soaked beans and cooked for 2-3 h until those become soft and can easily be crushed between finger tips. The cook water is discarded and cooked beans are cooled, placed in a wooden mortar and crushed to make grits of mainly half-cotyledons using a wooden pestle. The split beans are transferred to a bamboo basket, made with internal lining of sackcloth and locally available fern (*Athyrium* sp.) fronds or leaves of banana (*Musa paradisiaca* L.), *Leucosceptrum canum* Sm., *Macaranga pustulata* King ex Hook.f., *Ficus hookeriana* Corner or *Bauhinia vahlii* Wight & Arn. Usually, a small amount (~1%) of firewood ash is added to the crushed grits to maintain the condition alkaline. The whole preparation is kept for about 1-3 days in a warm place, usually above a traditional earthen oven in kitchen. Appearance of a white viscous fluid on beans and a typical kinema flavour dominated by mild smell of ammonia is a sign of successful fermentation. Formation of a characteristic long string when stretched the viscous fluid with fingers indicates a good-quality kinema. Fresh kinema keeps for two to three days during summer and a maximum of one week in winter without refrigeration. However, sun-dried kinema can be stored for several months at room temperature. Fresh kinema, when fried in oil, loses its ammoniacal smell leaving a persistent nutty odour. A thick kinema curry is prepared with added chopped vegetables, spices and salt, and is taken with steamed rice. Sun-dried kinema is reconstituted by adding warm water and made to curry. Microorganisms responsible for the fermentation include *Bacillus subtilis* which is predominant. Other species of *Bacillus*, such as *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thuringiensis* and *B. sphaericus* , are also present (Sarkar *et al.*, 2002). *Enterococcus faccium*, *Candida parasilosis* and *Geotrichum candidum* accompany traditional fermentation processes (Sarkar *et al.*, 1994; Sarkar and Nout, 2014).

Fig. 5. Kinema, as marketed

2.4.2. Idli

Idli is prepared from dehulled blackgram (*Vigna mungo* (L.) Hepper; synonym *Phaseolus mungo* L.) dal and white polished rice (*Oryza sativa* L.). It is indigenous to south India, and over recent times it has gained popularity throughout India and abroad. It is a preferred snack food item in the institutions, army, railways and industrial canteens, etc. (Balasubramanian and Viswanathan, 2007). From nutritional point of view, idli is considered an ideal human food suitable for all age groups and at all times (Jama and Varadaraj, 1999). Traditionally, it is prepared by soaking dal and rice separately in tap water at ambient temperature (Ghosh and Chattopadhyay, 2011). After soaking, soak water is discarded. The soaked dal is ground to a smooth mucilaginous paste and rice is coarsely ground to slurry. The paste and slurry are mixed at different ratios with the addition (1-2%) of common salt to form a thick batter (Balasubramanian and Viswanathan,2007). The batter is taken in a closed container and left to ferment for 14-24 h (Iyer and Ananthanarayan, 2008). The period of fermentation varies depending on the convenience of preparation. During fermentation, the batter volume rises two-fold. Fermentation is achieved by the growth of lactic acid bacteria which produce lactic acid and carbon-dioxide that make the batter anaerobic and leaven the product (Steinkraus, 2002). The fermented batter is thoroughly mixed in order to expel the gas formed due to the release of carbon-dioxide. The batter is dispensed in cups of idli steamer having a batter-holding capacity of about 40 ml and steamed until starch is gelatinized to prepare white, soft and spongy muffins bearing a honey-comb crumb interior and having a pleasant acid flavour (Aidoo *et al.*, 2006; Rakshit *et al.*, 2015). Idli is served hot, and usually eaten with chutney (a batter made of coconut) and sambar (spicy vegetable soup containing tamarind juice) (Fig. 6). Idli batter fermentation is an auto-fermentation process. Microorganisms present in the raw ingredients as well as the environment determine the nature microbiota involved. The major microorganisms involved in the leavening process are hetero-fermentative lactic acid bacterium, *Leuconostoc mesenteroides* and homofermentative lactic acid bacterium, *Streptococcus faecalis*, which regulate the acidity of batter (Mukherjee *et al.*, 1965). Other microorganisms associated during idli batter fermentation include *Leuconostoc mesenteroides*, *Enterococcus faecalis* and *Pediococcus dextrinicus*, and yeasts such as *Saccharomyces cerevisiae*, *Pichia anomala*, *Debaryomyces hansenii*, *Trichosporon pullulans* and *Trichosporon cutaneum* (Nout *et al.*, 2007).

Fig. 6. Idli, ready to serve

2.4.3. Dhokla

Dhokla, a popular food of Gujarat, is commonly available in sweet shops and can be easily prepared at home. The preparation procedure of dhokla is more or less similar to idli; the difference is that bengalgram (*Cicer arietinum* L.), instead of blackgram, is used (Ray *et al.*, 2016). The antioxidative property of dhokla helps to cure oxidative stressed-induced diseases (Moktan *et al.*, 2011).

Fig. 7. Dhokla, ready to serve

Traditionally, dhokla is prepared by soaking bengalgram dal and white polished rice separately in tap water for 5-10 h at room temperature (Reddy *et al.*, 1983; Roy *et al.*, 2007a). Soak water is drained. Soaked dal is ground to soft batter, and rice is ground to coarse slurry. The batter and slurry are mixed with the addition (1-2%) of common salt. The ratio of batter and slurry varies. The mixed batter is left overnight for 12-14 h at an ambient temperature (Joshi, 2016). The two-fold rise in the batter volume indicates completion of fermentation. The fermented batter is poured into a greased tray and steamed in an open condition for 15-20 min to obtain soft and spongy cake (Purushothaman *et al.*, 1993). After cooling, dhokla is cut into different shapes. While serving, it is usually garnished and flavoured by sprinkling grated coconut, fried mustard seeds and chopped curry (*Murraya koenigii* (L.) Spreng) leaves (Fig. 7).

Good-quality dhokla should have a slightly sour taste having a pleasant acid flavour. Enhanced organoleptic quality, nutritional value and improved digestibility have made dhokla a well-accepted food item suitable for all age groups (Ramakrishnan, 1979). Microorganisms associated with the fermentation include lactic acid bacteria, such as *Leuconostoc mesenteroides*, *Lactobacillus fermentum* and *Streptococcus faecalis*. Yeasts, like *Torulopsis* spp. *Pichia silvicola* and *Candida* sp. are responsible for the rise in volume of batter and imparting sponginess to the product (Joshi *et al*., 1989; Steinkraus, 1996; Blandino *et al*., 2003).

2.5. Safety aspects

Traditional food fermentation involves preparation of food by employing simple household techniques without prior knowledge on microbiology, chemistry and food hygiene. Fermented foods are consumed daily by billions of people considering it safe for human consumption. However, the issues related to food safety are also important. Improperly prepared and unhygienic foods cannot be considered as safe. Unhygienic handling during processing poses great threat to the health of the consumers. Contaminated water, environment where foods are being prepared have negative effect on safety of foods (Steinkraus, 2002). Inadequate storage and cooking may reduce the safety of fermented foods (Nout, 1994).

There are various components in food systems which are hazardous to human health. The hazards are due to the property of food itself which includes naturally present antinutritional factors in those or generated during processing, such as biogenic amines (Adams, 2001). Household preparation of fermented products often involves the reduction of processing time. These have serious impact on safety and nutritional quality of food products. Time saved by shortening of fermentation period may affect functioning of beneficial microorganisms or enzymic degradation of antinutritional factors (Motarjemi, 2002). The constraints of processing time can be alleviated by the use of fermentation starter cultures (Holzapfel, 2002). Optimization of household processing stage parameters might be helpful to minimize toxic compounds, like biogenic amines and other antinutritional factors (Nout, 1994; Holzapfel, 2002). Such manoeuveration of processing parameters can be helpful for the production of more nutritious and safe foods.

Food safety in a broader sense can be achieved through production, storage and handling in a safer way to avoid food intoxicants and other detrimental effects associated with consumption (Adams and Nout, 2001). Safety provides commercial reputation to prepare foods. Application of proper safety measures leads to the improvement of overall quality of a food (Motarjemi, 2002). Food safety assurance mainly fall into two category, namely good manufacturing practices (GMP) where safety action is taken during purchase of raw materials, processing, transport and distribution, and good hygienic practice (GHP) which ensures that food products are safe for consumption (Mortarjemi, 2001). Such manufacturing and hygienic practices could be used to improve quality and safety by employing the hazard analysis and critical control point (HACCP) system. The HACCP system is defined as a system which identifies, evaluates and controls hazards during foods processing and ensures safety of foods (Mortarjemi, 2001). While improving safety and nutritional value by reducing antinutritional components present in foods, a regular interaction among the scientists, producers/processors and consumers is necessary so as to ensure acceptable and cost-effective changes of food products.