

ANTINUTRITIONAL FACTORS IN PULSES AND THEIR DIVERSITY

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ABSTRACT

In India, legume seeds are identified as a major source of proteins after milk. However, these legume seeds are also known to contain factors which hinder the digestibility and thus nutritionally antagonistic. These may reduce the availability of otherwise good proteins in the diet and cause diseases originating from malnutrition. A comprehensive review on the presence of seed storage proteins and important anti-nutritional factors in food legumes has been carry out. These factors affecting digestibility include proteolytic inhibitors, phytohemagglutinins, phytic acids and tannins etc. These factors are shown to be widely present in leguminous foods which are important constituents of the diet of a large section of the world's population and particularly people in the developing countries.

Keywords: seeds, proteins.

INTRODUCTION

Pulses constitute an important source of dietary protein for a large segment of the world's population particularly in those countries in which the consumption of animal protein is limited by non availability or because of self-imposed restrictions. Pulses are a source of energy, dietary fiber, protein, mineral and vitamins required for human health. Consumption of pulses may have potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol [1, 2, 3]. Such studies have been resulted in increased interests in research and possibly greater use of pulses in the daily diet and in a variety of food products. Pulses have high amounts of edible proteins (18-32%) and provide a major source of essential amino acids and bioactive peptides. Pulse protein possesses functional properties such as water holding, fat binding, foaming and gelation which could expand their potential use in the development of a number of food products [4]. This using well substantiates the need of scrutiny of germplasm available in the country for the search of novel bioactive compounds in the pulses.

Breeders are putting in continuous efforts to develop crops with high amounts of essential amino acids which can be of potential use for nutraceutical industry. The modern agriculture all over the world has increased productivity in logarithmic fashion since 1930s. Besides increased productivity, plant breeding has contributed to remarkable transformations in the seed quality, advancing maturity, associated changes in protein composition and subsequent lowering of antinutritional factors. Incidentally enormous wild germplasm is available especially for resistance to many diseases.

SEED STORAGE PROTEINS

Seed storage proteins are synthesized and accumulated to high levels in seeds during seed development. During seed germination, these proteins are degraded and the resulting amino acids are utilized by the developing seedlings as a source of nutrition. Seed storage proteins are the major proteins in grains and, of the plant proteins, represent those that are the most abundantly consumed by humans and animals. The storage proteins of chickpea seed have been fractionated into globulin (salt soluble), albumin (water soluble), prolamin (alcohol soluble), glutelin (acid/ alkali soluble), and residual proteins [5]. Singh and Jambunathan (1982) reported that globulin comprise the major storage protein (56%), followed by glutelin (18.1%), albumin (12%), and prolamin (2.8%). Globulin is deficient in methionine and cysteine while albumin and glutelin contain slightly higher amount of those amino acids [6].

ALBUMINS

The albumin fraction is more heterogeneous and is more in sulfur amino acids compared to the globulin fraction, thus the albumins in legume proteins may naturally complement the amino acid pattern of globulin. In general, most albumin proteins have some physiological functions, such as enzymatic activities of lipoxygenases, glycosidases, or proteases involved in the degradation of storage proteins. Other albumins, such as protease inhibitors or lectins, are implicated in defense mechanisms [7].

There are two types of albumins, the PA2 albumin and the PA1 or 2S albumins [8]. PA2 albumin from pea has been purified and characterized [9]. Vioque *et al.*, [10] reported that 2S albumin has been described as a lectin which is capable of agglutinating papainized human erythrocytes in pea and chickpea. Chickpea 2S albumin was prepared and purified by solubilization in 60% methanol followed by ion- exchange chromatography. Under denaturing conditions, it has two peptides of 10 and 12 kDa.

GLOBULINS AND GLUTELINS

Globulins represent about 70% of legume seed proteins and are composed of two major groups, characterized by their sedimentation coefficients, the 11S (320- 400kDa) or legumin, and the 7S (145- 190kDa) or vicilin [8]. Legumin is a major storage protein representing a source of energy, carbon and reduced nitrogen for germination and seedling growth in chickpea. Legumin represents around 64% of the total protein content and 97% of the globulins in chickpea seed [11]. The approximate molecular weight of legumin is 360 kDa and is made up of six α - β subunits held together as a triangular antiprism by non- covalent bonds. Each α chain is linked to β chains by disulfide bonds. The β chains are the hydrophobic heart of the protein, and the hydrophilic α chain are at the exterior of the molecule [11]. Glutelins, which are found mostly in cereals, belong to the 11-12S globulin family of proteins; the proportion of glutelins domains is around 18.1% of the total proteins in chickpea [6].

PROLAMINES

The name prolamin was originally based on the observation that these proteins are generally rich in proline and amide nitrogen derived from glutamine. Prolamins are generally defined as soluble in alcohol/ water mixtures (e.g. 60- 70% v/v ethanol), but some prolamin occur as alcohol - insoluble polymers [12]. Nevertheless, all individual prolamin polypeptides are alcohol- soluble in the reduced state; the molecular weights of prolamins vary extensively from 10 to almost 100kDa. Consequently, prolamin storage proteins

are much more variable in structure than those of the 7S and 11/12S globulins, and the major groups of prolamins in the *Triticeae* family (wheat, barley, and rye) and the *Panicoidae* family (maize, sorghum, and millets) may possibly have separate evolutionary origins [13]. Most prolamins share two structural characteristics: firstly, they have distinct regions or domains with different structures and may have different origins; secondly, the amino acid sequences consist of repeated blocks based on one or shorter peptide motifs, enriched in specific amino acid residues, such as methionine. These features result into high proportions of glutamine, proline and other specific amino acids (eg. histidine, glycine, methionine, phenylalanine) in some prolamin groups [14].

The nutritional value of pulses may be adversely affected by the presence of anti physiological or toxic substances such as trypsin and chemotrypsin inhibitors, phytates, lectins, polyphenols, flatulence causing agents, cyanogenic compounds, lathyrogens, esterogens, goiterogens, saponins, anti-vitamins and allergens. These substances reduce the nutritive value of foods by inhibiting digestibility and utilization of proteins. It is therefore necessary to eliminate these substances by processing by genetic manipulation. A brief description of these antinutritional factors is given as follows;

An antinutritional factor is defined as a substance which when present in human or animal feed interferes with assimilation of certain nutrients showing toxic or undesirable physiological effects, such as flatulence. Nevertheless such ANFs provide a protective role against insects [15], predators and pathogens attacks [16]. The variation in ANFs is often resulting in evolutionary adaptations which enable the plant to survive and complete its life cycle. A critical evaluation of such antinutritional compounds can be useful both at quantitations and quantitative levels.

The antinutritional compounds may either be heat- labile or heat stable and in pulse crops they are categorized as: protein ANCs and non-protein ANCs [17]. These compounds range in effect from relatively inoffensive polyphenols to the relatively harmful protease inhibitors. Non-protein ANCs include alkaloids [18], phytic acid, phenolic compounds such as saponins and tannins [19, 20]. Quantification of such ANFs is the prerequisite of the variety release procedures in the context of their market value and adaptability.

TANNINS

Tannins are the compounds present in chickpea which primarily form the basis of pigmentation in the seeds. When insects feed on them, they are highly hydroxylated and can form insoluble complexes with carbohydrates and proteins. The phenolic groups of tannins are bound to enzymes and other proteins by hydrogen bonding to amide groups, and form insoluble tannin- protein complexes which are resistant to digestive enzymes of monogastric animals [21]. The presence of tannins adds to astringency of tannin-rich foods (tea and some vegetables and fruits) especially when immature, because of their precipitation due to salivary proteins.

PHYTIC ACID

Phytic acid (myo- inositol 1, 2, 3,4,5,6, hexakis-dihydrogen phosphate, IP6) is another antinutritional factor and is a major storage form of phosphorus in plants (60-90% of total seed phosphorus). It is ubiquitous in seed comprising 1-3% of all nuts, cereals, legumes and oil seeds [22]. It is present as crystals inside protein bodies in discrete regions of seeds, and predominantly in the aleurone layer of wheat and rice [22]. Phytic acid is considered as one of the antinutrients mainly due to its ability to bind to essential dietary minerals as well as proteins and starch, consequently reducing their bioavailability in humans [23]. However, on food processing, phytates can be dephosphorylated to produce degradation products such as myo-inositol penta kis (IP5), tetrakis (IP4), tris- IP3, bis-(IP2) and monophosphate (IP1) etc.

PROTEASE INHIBITORS

Protease inhibitors are the proteins of low molecular weight forming stable complexes with digestive enzyme therefore irreversibly inhibiting their activity. The most popular and characterized protein

inhibitors of legume seeds are trypsin inhibitor of both, Bowman-Birk type and Kunitz type, and α -amylase inhibitors. The presence of protease inhibitors in food decreases the apparent nutritional quality of proteins in the diet affecting the performance of body digestive enzymes to degrade dietary protein, and limiting intake of amino acids needed to construct new proteins [5].

LECTINS

Plants commonly synthesize some antinutrients as part of their protection against their predators and/or as a means to survive under adverse growing conditions, this includes lectin also. Moreover, two approaches are familiar for lectin classification. They can be classified on the basis of their carbohydrate specificity. They can also be categorized according to the overall structures into merolectins, hololectins, chimerolectins and superlectins, or be grouped into different families (legume lectins, type II ribosome-inactivating proteins, monocot mannose-binding lectins, and other lectins).

Lectins (agglutinins), carbohydrate-binding proteins are also the members of ANFs family, found in a wide variety of crop plants. Lectins are defined as "all plant protein possessing at least one non-catalytic domain that binds reversibly to a specific mono or oligosaccharide" [24]. Plant lectins are commonly found in food consumed without processing, such as fruit and vegetables [25]. Pea, chickpea, lentil, and other pulse crop seeds, contain higher proportions of legume lectins than other lectin groups. The deleterious effects associated with lectins are food poisoning, vomiting, bloating, and diarrhoea in humans [17]. However, recent finding considers lectin as one of the lead molecules in pharmaceutical industry such as antifungal, antiproliferative and HIV-1 reverse-transcriptase activities [26].

POLYPHENOLS

Polyphenols are a diverse class of compounds which occur naturally in a wide range of food plants. More than 8,000 polyphenolic compounds have been identified in various plant species [27]. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenol is also common [28]. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans [29].

Hence, seed protein quantification concerned to ANFs composition and other protein fractions has become present day tool to catalogue the seed genotypes in gene banks. Such stocks are maintained as a part of marker assisted selection. Also this constitutes an effective procedure for seed protein diversity analysis in the present day plant biotechnology research. Breeding programmes also aim to develop the cultivars suitable for food industry where the quantification of ANF'S has become one of the basic requirements. Such information therefore, can be useful for germplasm selection and their subsequent introduction in the market. The nutritional status of the plants and superior quality is of primary importance for breeding programmes which lead to select/identify the elite genotypes.

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