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ANTI-DIETETIC FACTORS IN LEGUMES - LOCAL METHODS TO REDUCE THEM

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ABSTRACT

Legumes are the most important crops cultivated for human and animal nutrition in Asia, Africa and Caribbean regions. Legumes are a rich source of protein and dietary fiber in our diet. On the other hand, a wide variety of antinutritional factors such as raffinose family oligosaccharides (RFO's), neurotoxin; proteinaceous compounds, lectins, goitrogenic factor, amylase inhibitors, and phytic acid are present in them. These factors influence the bioavailability and absorption of nutrients by humans and also in animals when used as a feed. The possible methods to reduce the anti-nutritional factors are; use of enzyme to reduce the concentration, breeding of crop varieties with reduced concentration of antinutritional factors, and through local methods like cooking, germination and soaking. Even before the advent of science and technology, humans have employed this traditional knowledge to reduce the amount of antinutritional factors from plant sources. However, recent advances in crop genetics and breeding bring the opportunity to reduce the concentration of these anti-nutritional factors genetically.

Keywords: legumes, flatulence, cooking, soaking, germination, antinutritional factors.

INTRODUCTION

Legumes occupy an important place in human nutrition and considered as poor man's meat especially by those who are in developing countries. This is due to the reason that legumes are a good source of protein and slowly digestible carbohydrates. They are very important in human and animal nutrition. Ideally the basic protein requirement is met by consuming proteins of plant and animal origin. Above all these facts, legumes contain more protein than any other plant proteins. They also have unique property of maintaining and restoring soil fertility.

Legumes are a rich source of nutrients such as protein, starch, minerals and vitamins and also have important health protective compounds (phenolics, inositol phosphates and antioxidants). This advantageous composition of legume seeds, not only make them a meat replacer for vegetarians but also as a component of rational nourishment. They serve as a low-cost protein to meet the needs of the large section of the people. However, several anti- nutritional factors present in legume seeds are a major limiting factor for the increased consumption of legumes, whose presence degrades the nutritive value of legumes. This may even lead to health problems which could eventually become fatal to humans and animals if taken in larger amount. In spite of the increasing interest concerning cultivation of pulses, the growth in area and production of seeds, and their application is relatively small (Kozlowska *et al.*, 1998).

Legumes contain a wide variety of antinutritional factors such as raffinose family oligosaccharides (RFO's), neurotoxin, proteinaceous compounds, lectins, goitrogenic factor, amylase inhibitors, and phytic acid (Maia et al., 2000; Preet and Punia, 2000; Enneking, 2011). Food processing methods including soaking (Frias, 2000; Vidal-Valverde *et al*., 2002) germination, decortications, fermentations, cooking and addition of enzymes have been suggested to reduce the concentration of anti-nutritional factors in pulses which greatly influence their nutritive values. The amount of antinutritional factors in legumes is shown to reduce at varying degrees based upon the processing and food preparation methods involved in it. These processing methods are sometimes, time consuming, expensive and result in loss of energy and nutrient content or have consumer acceptability issues. For this reason, breeding programmes at various national and international research centres are working to reduce the content of antinutritional factors to a safe extent leading to an increased proportion of grain legumes in diets in human and animals (Matric *et al.*, 2005). The objective of this review is to discuss about the antinutritional factors present in different legumes and local processing techniques used to reduce them.

MECHANISMS OF ANTINUTRIENT FACTORS

Generally, there are two mechanisms that act as toxicants in human body: 1. the bioavailability of the nutrients in food is affected by some naturally occurring chemical compounds present in it. These compounds include plant phenolics, dietary fiber, phytates, and oxalates. The bioavailability of minerals, vitamins and proteins are limited by dietary fiber. Plant phenolics bind with proteins and make them unavailable for digestion (Veenstra *et al*., 2010) 2. The presence of chemicals in plant sources decreases the absorption and utilization of one or the other nutrient (Awan and Anjum, 2010).

RAFFINOSE FAMILY OLIGOSACCHARIDES (RFOS)

Recent findings showed that, considerable amount of Raffinose family oligosaccharides (RFO's) are present in chick pea, pigeon pea, and black gram. Consumption of large quantities of pulses containing RFOs leads to negative effects such as stomach discomfort, flatulence, bloating, diarrhea and cramps (Reddy et al., 1984; Guzman-Maldonado et al., 1998). However, RFOs also showed some positive health benefits acting as a dietary fiber (Chibbar *et al.,* 2010). The negative impacts are mainly due to the absence of α- galactosidase enzyme in small intestine, which is necessary for the hydrolysis of α-1, 6 linkages present in RFOs. The negative effect depends on the consumption pattern and α- galactosidase sensitivity of the consumer.

NEUROTOXIN

Higher consumption of grass pea (*Lathyrus sativus* L, *L. cicera*, *L. Clymenum* and *L. ochrus*.) containing high concentrations of a BOAA (β-*N*-oxalyl-amino-L-alanine,) causes neurolathyrism, a disorder and leads to paralysis in humans and animals, the mechanism of which is not well understood (Barennes et al., 2004; Barceloux, 2009; Enneking, 2011). Further investigations in this area are required to understand the pathway and mechanism related to ODAP in grass pea.

PROTEINACEOUS COMPOUNDS

The proteinaceous compound such as haemagglutinins and trypsin inhibitors are widely distributed in chickpea, soybean, cowpea, mungbean, lentil, kidney bean and also in some cereals. These substances reduce the absorption of protein by human body and ultimately poor food utilization leads to impaired growth of the individual. Trypsin inhibitors present in soyabean is responsible for reduced digestibility (Ndubuaku *et al*., 1989; Radha *et al*., 1989).

Favism is a disease which is caused by higher consumption of fava bean (Vicia faba, also known as broad bean, horse bean and Windsor bean), especially in the Mediterranean region. Haemolytic anaemia, haemoglobinuria, jaundice, and high fever are associated with this disease. The sickness will begin after few hours of consumption. The recovery in adults will take 1 to 2 days after sickness and it may even cause death in children. This disease is recognized as a spontaneous disorder in our metabolism. The susceptible people have a deficiency of the enzyme glucose-6-phosphate dehydrogenase in the erythrocytes that leads to differences in the circulation of blood (González de Mejía *et al*., 2002).

Based on the molecular weight and cystine contents, protease inhibitors are classified as two different families, Kunitz and Bowman-Birk. Whole soybean has been reported to contain 17–27 mg of trypsin inhibitor per gram of seed. Kunitz have a molecular weight of c. 20 kDa with 2 disulphide bridges. Bowman-Birk has a molecular weight of 8–10 kDa with seven disulphide bridges. The protease inhibitors from lima bean, cowpea, and lentil are comes under Bowman-Birk family (Belitz and Weder, 1990; Liener, 1994).

LECTINS

Pulse lectins are used for clinical and immunological studies and used for various other purposes. In pulses, lectins ranges from 0.6% in garden pea, 2.4 to 5% in kidney bean, and 0.8% in lima and soya bean with respective to total protein content (Zhang *et al*., 2009). Apart from other uses higher consumption of lectins from the above mentioned pulses results in nutritional deficiencies, and immune (allergic) reactions. Possibly, most effects of lectins are due to gastrointestinal distress through interaction of the lectins with the gut epithelial cells (Oliveira *et al.,* 1989).

GOITROGENIC FACTOR

The goiterogenic effect of soy beans were reported by McCarrison (1933) and further confirmed by other researchers in both rats and chickens. Furthermore, several researchers have reported the effect of soy milk on goiter. This can be alleviated by increasing iodine intake. Pea interferes with the absorption of iodine through the mechanism on the intestinal mucosa. Presence of arachidoside in the outer skin of the peanuts showed goitrogenic effect. Among the entire pulses, pea, common bean, soybean, and peanut inhibited the absorption of radioactive iodine by thyroid in human subjects. These factors are present in soybean and groundnut. Livestock feeding on such legumes secretes goitrogen in milk and consumption of this milk leads to goitre in children (Rosa, 1997).

AMYLASE INHIBITORS

Amylase inhibitors are found in pigeon pea. It is showed decreased broken down of the digestive enzyme. These inhibitors have been found to be active within a pH range of 4.5-9.5 and heat labile. This inhibitors form complex with amylase that depends on ionic strength, pH, temperature, time and concentration of the inhibitors. These complexes inhibit bovine pancreatic amylase but fail to inhibit fungal and bacterial amylase. This enzyme is synthesized during late seed development and degraded during late germination in pigeon pea (Giri and Kachole, 1998). Among the pulses, field bean and chickpea contain very low concentrations of amylase inhibitors. Winged bean, adzuki bean, soybean, lima bean, lentil and pea showed no amylase activity (Grant *et al.*, 1995). This enzyme plays a vital role in the breakdown of starch to release energy in the form of glucose and maltose. Presence of this inhibitors leads to reduced hydrolysis of polysaccharides such as starch and glycogen. In chickens, due to reduced hydrolysis of polysaccharides a reduction in growth was observed. Human consumption of these inhibitors as starch blockers reported to cause symptoms like diarrhoea, nausea and vomiting because of reduced or no digestion.

PHYTIC ACID

Phytic acid, which is abundantly present in seeds, also exists in roots, tubers, pollens and spores of many plant species. Grain crops typically contain about 10 mg phytic acid per gram seed dry weight, representing about 65% to 85% of seed total P (Raboy 1990). In legumes phytic acid is present in chick pea, pea, lentil, and soybean. Total P concentration typically ranges from 3.0 to 4.0 mg per gram in seed produced by grain crops, with phytic acid P ranging from 2.0 to 3.0 mg per gram. In dry pea, 99% of phytic acid is present in cotyledons and 1% is present in embryo axis. 65% of the total phosphorus in pea cotyledons and 10% of total phosphorus in embryo axis arise from phytic acid phosphorus (Ferguson and Bollard 1976). More than 88% of phytic acid is present in pea cotyledon and seed coats contained almost no phytic acid. In peas, the phytic acid content increased from 0.16 to 1.23% during maturation (Welch *et al.*, 1974). Phytic acid content in soybean during maturation increased from 0.87 to 1.26% (Yao *et al.,* 1983). In winged beans there was a proportional increase in phytic acid at four developmental stages of seed maturity (Kadam *et al.,* 1982). It is a strong chelator of mineral cations such as calcium, iron and zinc forming mixed salts that are largely excreted by humans and other non-ruminant animals such as poultry, swine and fish (Sharply *et al.,* 1994). Phosphorous and inositol in phytate are not available to non-ruminant animals, since they lack the enzyme phytase to remove the phosphate from inositol in phytate. On the other hand ruminants readily digest phytates because of the presence of microorganism in rumen. Low absorption of minerals ultimately results in reduced weight gain in livestock. Bioavailability of minerals can be increased by phytase enzyme supplementation or reduction of phytate through breeding of legumes with reduced phytic acid content.

HEALTH BENEFITS

As we discussed earlier, legumes contain considerable amount of various antinutritional factors and have a negative effect on human and animal nutrition. However, legumes play an important role in maintaining human health. Garbanzo beans can be used to improve digestive functions. Bacteria present in our colon can break down the garbanzos insoluble fiber into short chain fatty acids (SCFAs). The extra energy released from SCFAs increases the colon health and reduce the incidence of cancer (Chibbar *et al*., 2010). Many of our body system are prone to oxidative stress and damage from reactive oxygen molecules. Legumes contain bountiful amount of antioxidants. Chickpea contains a remarkable amount of antioxidants which play a crucial role in supporting the body systems. Excellent source of antioxidant present in the legumes showed reduced risk of heart diseases upon regular consumption. Apart from these legumes also provide support for blood and blood vessels. Legumes are not a fatty food and they do contain valuable amount of polyunsaturated fatty acids (PUFAs), including omega-3 fatty acid, and α-linolenic acid. Risk of coronary heart disease is reduced by regular consumption of chickpea and other legumes (Hu, 2003). Legumes contain good source of vitamins, minerals, and have a strong antioxidant composition, which can help to improve blood sugar level and overall insulin function (Jacobs and Gallahar, 2004).

ROLE IN PLANT GROWTH AND DEVELOPMENT

Antinutritional factors accumulate in seeds, stems, leaves, and tubers of plant (Avigad and Dey, 1997). These factors play important role in seed germination, preservation of seed longevity and desiccation tolerance. Stored carbon reserves in seeds will be released during seed germination. These reserves are mostly in the form of polysaccharides, sugars, oil and proteins. Moreover, stored RFOs may play an essential role in the early stages of germination when the breakdown of all the stored reserves is not sufficient to meet out the energy requirement (Blochl *et al*., 2007. Cowpea, jack bean, and soybean showed reduction in RFOs concentration upon germination (Martin-Cabrejas *et al.,* 2008). On the other hand, recent findings from Dierking and Bilyeu (2009) suggest that utilization of RFOs in not required for seed germination. Dessication tolerance in legumes could be the result of antinutritional factors protecting membrane-bound proteins Jones *et al*. (1999). This is because of viscosity of oligosaccharides present in seeds, which might be responsible for the protection of membranes. Abiotic stresses in legumes produces excess concentration of reactive oxygen species (ROS). The generation of ROS such as the superoxide radical anion (O2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (-OH) beyond the antioxidant capacity of a biological system causes oxidative stress (Aruoma *et al.,* 1997; Shigeoka *et al.*, 2002). Antinutritional factors act as antioxidants by scavenging hydroxyl radicals and protecting plants from oxidative damage (Nishizawa *et al*., 2008).

LOCAL METHODS TO REDUCE THE ANTINUTRITIONAL FACTORS IN LEGUME

The reduction or removal of undesirable nutritional factors is very essential to improve the nutritional status of legumes and effectively utilize their full potential as animal and human food. Possible approaches used to reduce the antinutritional factors are: breeding of varieties low in antinutritional factors, use of enzymes to reduce the antinutritional concentration (Reynolds, 1974) finally the local methods such as soaking, and germinate the seeds before cooking (Iyer, *et al*., 1980; Jood, *et al*., 1985). This review is mainly focussing on antinutritional factors and local methods to reduce them and discussing breeding approaches and processing methods such as enzyme treatment is beyond the scope of this review. Local methods to reduce the antinutritional factors are discussed below.

SOAKING

Overnight night soaking of pulses results in sizable reduction of α-galactoside concentration (Okolie and Ugochukwu, 1988). Soaking of cowpea flour for 16hr reduces 26% and 28% of stachyose and raffinose concentration respectively. Overnight soaking of chickpea reduces the concentration of α –galactosides by 16 - 27% (Frias *et al*., 2000). The efficiency and effectiveness of soaking process can be enhanced by the addition of sodium bicarbonate, soaking time, temperature or seed water ratio (Jood *et al*., 1985; Vijayakumari *et al*., 1996; Abdel-Gawad, 1993; Ibrahim *et al*., 2002). In addition to these, differential solubilities of antinutritional factors and their diffusion rates are the two important parameters that influence the losses of antinutritional factors.

COOKING

Cooking was used to reduce or eliminate anti-nutritional factors in legumes. Cooking for 60 min resulted in mean decrease of 49.6% and 46.3% reduction of RFOs in horse gram and green gram respectively. In soybean, 52.3% removal of raffinose and 20.7% removal of stachyose reported during cooking (Mulimani *et al*,. 1997).

Steeping followed by boiling in khaseri dhal reduce the BOAA content. Autoclaving and limewater treatment of seeds for 10 minutes at 15 lb mm^{-2} destroyed the trypsin inhibitors. In contrast to above findings, Rao and Belavady (1978) showed an increase in the level of oligosaccharides after cooking of pulses. Price *et al.* (1988) reported that treatments such as soaking and cooking could change the physicochemical properties of legumes. Moreover, soaking and cooking alone will not be sufficient to bring any significant reduction in the flatulence-inducing activity of legumes.

GERMINATION

Germination is reported to reduce the α-galactosides concentration in pulses. During germination process, complex sugars are converted into simple sugars. Three days after germination of lentils reduces RFOs concentration by 18-40% (Frias *et al.*, 1996). Eighty three percent reductions of α-galactosides are noticed in pigeon pea after four days of germination. Similarly, drastic reductions in RFO contents are also observed for several non-conventional legumes such as cowpea (*Vigna unguiculata*), jack bean (*Canavalia ensiformis*), mucuna (*Stizolobium niveum*) and dolichos (*Lablab purpureus*) (Martin-Cabrejas *et al.*, 2008) after germination. During germination process phytate is degraded by native phytase.

CONCLUSION

Foremost objective of any crop improvement program is to increase the yield. With this objective, quantum jumps in yield of almost every crop had been achieved by plant breeders. Now, there is a shift towards to the quality of foods. The need of the hour is the improvement of grain quality traits. Further, general public have also become highly health conscious and prefer only high quality food products. Varieties with reduced antinutritional factors were developed in brassica (single, double and triple zero varieties) and khesari dhal (Pusa-24 by IARI in India, Quila-blanco in Chile, Line 8612 in Bangladesh) Hence, it is the responsibility of the plant breeders to address the needs of people and concentrate on crop improvement activity which focuses on enhancement of quality of legumes by reducing the anti-nutritional factors.

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