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Anti-nutritional compounds in pulses: Implications and alleviation methods

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Abstract

Pulses are a rich source of protein and minerals particularly for the vegetarian and vegan population. However, several anti-nutritional compounds, such as trypsin inhibitor, phenolic compounds, phytates, cyanogenic compounds, lectins and saponins are also found in the legumes. Most of the anti-nutritional compounds of the pulses are present in the seed coat. Most of these compounds are sensitive to heat and can be substantially reduced by milling, cooking, germination, fermentation and heat processing. This review paper summarizes anti-nutritional compounds present in different pulses including their fractions, significance and beneficial and adverse effect on human health. The aim of this paper is to enlighten the readers about the anti-nutritional compounds present in the pulses and possible processing methods to enhance utilization of pulses.

KEYWORDS
anti-nutritional compounds, processing, pulses, removal techniques

1 | INTRODUCTION

Pulses are an important protein source for billions of people around the world and are widely consumed as staple food in combination with cereals in many parts of the world. Pulses also provide energy, vitamins, phytochemicals and minerals. They are given importance due to their protein content as well as their low glycaemic index and are part of staple diets of a large population in the developing countries of South Asia and Africa (Khandelwal & Kumar, 2010).

Pulses supplement cereal diets by improving their protein nutritive values (Darmadi-Blackberry et al., 2004). The world production of pulses was 83.46 MT in 2016 (DAC, 2018) in which India alone contributed 18.15 MT. India is the largest producer and consumer of pulses in the world with 25.5% share in total world pulse production (DAC, 2018). The common pulses used as food are chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan*), black gram (*Vigna mungo*), green gram (*Vigna radiata*), lentil (*Lens culinaris* Medic.), cowpea (*Vigna unguiculata*), kidney beans (*Phaseolus vulgaris*), winged beans (*Psophocarpus tetragonolobus*), horse gram (*Dolichos biflorus*), moth bean (*Vigna aconitifolia*), fava beans (*Vicia faba* L.), jack bean (*Canavalia gladiata*) and grass peas (*Lathyrus sativus*).

2 | NUTRITIVE VALUE OF PULSES

The nutritional profile of pulses has many health-promoting properties, such as controlling high cholesterol, diabetes and cancer. Pulses comprise complex carbohydrates, such as oligosaccharides, dietary fibres and resistant starch along with proteins having good amino acid profile. Pulse proteins are higher in essential amino acids, particularly lysine, in comparison with animal proteins. However, it is deficient in sulphur-containing amino acids, such as methionine, cystine and cysteine (Khattab et al., 2009). A combination of pulses with cereals, which are deficient in lysine but rich in methionine, may complement

TABLE 1 Proximate composition of different pulses

S. N.	Common name	Latin name	Energy (Kcal)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrates (%)	Minerals (%)	Fibre (%)	Calcium (mg/100 g)	Phosphorus (mg/100 g)	Iron (mg/100 g)
1	Pigeon pea (tur, red gram, arhar)	Cajanus cajan	301	10	21	1.25	58	4.1	10.4	143	228	3.6
2	Green gram (moong)	Vigna radiata	334	10	24	1	57	3	4	124	326	4
3	Black gram (urad, urd)	Vigna mungo	-	-	23.6	0.45	67		3.5	-	-	-
4	Chickpea (bengal gram, chana)	Cicer arietinum	360	10	18.5	4.3	55	3.5	4	138	364	5
5	Kabuli Chickpea (kabuli chana)	Cicer arietinum	-	10	20.5	5.7	55	3.8	4	68	337	3.8
6	Lentil (masoor)	Lens culinaris	343	12	25	1	59	2	1	69	293	7
7	Field pea (dry pea)	Pisum sativum	315	16	20	1	56	2	4	75	298	7
8	Kidney bean	Phaseolus vulgaris	333	11.8	23.6	0.8	60	2.1	24.9	143	407	8.2
9	Cowpea (black eyed pea, southern pea)	Vigna sinensis	323	13	24	1	54	3	3	77	414	
10	Faba bean (bell bean, horse bean, broad bean)	Vicia faba	341	11	26.12	1.53	58.29	-	2.5	103	421	6.7
11	Horse gram[a]	Dolichos biflorus		5.8	22.6	1.8	66.9		1.6			

Source: Vishwakarma et al. (2017).
[a]Prasad and Singh (2015).

TABLE 2 Major anti-nutritional factors content in different pulses (dry matter basis)

| Pulse | Anti-nutritional compound | | | | | |
| | Enzymatic inhibitors | | | Non-enzymatic inhibitors | | |
	Trypsin inhibitor (TIU/mg protein)	Chymotrypsin inhibitor (IU/mg)	α-Amylase inhibitor (IU/g)	Haemagglutinin activity (HU/mg)	Tannins (mg/g)	Phytic acid (mg/g)
Green gram[a]	15.8			26.70	3.30	5.80–12[j]
Chickpea[b]	8.10–20.89	6.1–8.8	3.1–10.5	6.22[k]	0.04–4.85	5.8–12.1
Pigeon pea[c,m]	8.1–31.28[m]	2.1–3.6	22.5–34.2	25.01	0–6.88[m]	5.01–12.7[j]
Lentil[d]	3.6–7.6	0[k]	0[k]	50[k]	1.28–3.9	4.11–12.5
Black gram[e,j]	7.1–10.6				5.3–9.2	3.7–13.7[j]
Field pea[f]	0.78–6.32	2.73–4.85	16.8[k]	80[k]	2.78–3.09	3.0–13.3 not mentioned
Kidney bean[g]	3.10–31.3	3.97[k]	76–675	74.5[k]	0.03–19.9	9.9–29.3
Cowpea[h]	3.4–67.1	1.6	1.4–89.5	40–640		2.64–15.24
Faba bean[i]	2.31–7.20	3.56[k]	18.9[k]	49.3[k]	0.13–21	6.4–21.7

[a]Mubarak (2005).
[b]El-Adawy (2002).
[c]Singh (1988).
[d]Hefnawy (2011).
[e]Suneja et al. (2011).
[f]Khattab and Arntfield (2009).
[g]Yasmin et al. (2008).
[h]Gonçalves et al. (2016).
[i]Alonso et al. (2000).
[j]Chitra et al. (1995).
[k]Shi (2015).
[l]Siddhuraju et al. (2002).
[m]Solomon et al. (2017).

TABLE 2 Continued

| | Anti-nutritional compound | | | | |
| | Non-enzymatic inhibitors | | | | |
Pulse	Total oxalate (mg/100 g)	Total oligo-saccharide (g/100 g)	Total phenols (mg/g)	Total saponins (mg SBaE/g)	In vitro protein digestibility (%)
Green gram[a]			4.5[l]	2.20	67.2–72.2[j]
Chickpea[b]	70.3[k]	2.94[k]	1.9–6.1	0.91–60	65.3–72.2[j]
Pigeon pea[c,m]		1.84–3.35	3.0–18.3	21.6–34.9	60.4–74.4[j]
Lentil[d]	13.3–144	4.75[k]	-	34[k]	
Black gram[e,j]			1.1–3.2	8.7–21.4	55.7–63.3[j]
Field pea[f]	0–103	5.63–5.73	3.7–5.0	0.01–31.7	83.7
Kidney bean[g]	13.9–946	6–6.19	2.07[k]	0.02–16	68.1
Cowpea[h]	42–770			2.51–37.16	
Faba bean[i]	194[k]		3.92[k]	3.52[k]	70.8

[a]Mubarak (2005).
[b]El-Adawy (2002).
[c]Singh (1988).
[d]Hefnawy (2011).
[e]Suneja et al. (2011).
[f]Khattab and Arntfield (2009).
[g]Yasmin et al. (2008).
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[i]Alonso et al. (2000).
[j]Chitra et al. (1995).
[k]Shi (2015).
[l]Siddhuraju et al. (2002).
[m]Solomon et al. (2017).

TABLE 3 Anti-nutritional compounds content in different milling fractions of pulses (dry matter basis)

Pulses	Milling fraction	Phytic acid (mg/g)	Flatulence factors (mg/g)			Enzyme inhibitors (TIU/mg)			Haemagglutinin activity (HU/mg)	Condensed tannins (g eq cat kt⁻¹)	Total phenolic compounds (mg/g)
			Raffinose	Stachyose	Verbascose	Trypsin inhibitor activity	Chymotrypsin inhibitor (IU/mg)	α-Amylase inhibitor activity			
Horse gram[a]	Seed coat	1.02	0.96	0.70	1.05	1.13		0.01			0.20
	Cotyledon	8.42	6.35	14.84	3.75	9.86		0.06			0.53
	Embryonic axe	3.81	2.86	6.38	1.38	2.02		0.01			0.24
Faba bean[b]	Raw seed	21.7				4.47	3.56	18.9	49.3	1.95	3.92
	Cotyledon	23.8				4.99	3.71	20.7	49.3	0.15	0.72
Kidney bean[b]	Raw seed	15.9				3.10	3.97	248	74.5	3.59	2.07
	Cotyledon	16.2				3.43	4.56	254	74.5	0.24	0.19
Green gram[c]	Raw seed	5.80				15.8			2.67	3.30	
	Cotyledon	4.60				14.6			1.80	2.20	

[a]Sreerama, Neelam, et al. (2010); Sreerama, Sashikala, and Pratape (2010).
[b]Alonso et al. (2000).
[c]Mubarak (2005).

each other. Besides protein, pulses are also a rich source of energy, phosphorus and calcium (Table 1). They also contain important vitamins, such as water-soluble B vitamins (thiamine, riboflavin, niacin and pyridoxine), folates, ascorbic acid and tocopherols, as well as antioxidants, polyphenols and other phytochemicals (Tiwari et al., 2011). Pulses in daily diet may reduce risk of diabetes and cardiovascular disease (Flight & Clifton, 2006) and may protect against breast cancer, colon cancer and rectal cancer (Campos-Vega et al., 2010). The pulses are low in sodium but are a good source of other minerals, such as calcium, copper, iron, magnesium, phosphorus, potassium and zinc (Venter & Eyssen, 2001). The predominant fatty acid in the pulses is linoleic acid, although they also contain α-linolenic acid (Venter & Eyssen, 2001).

3 | ANTI-NUTRITIONAL COMPOUNDS

Pulses contain a number of organic compounds, which are produced through secondary metabolism. These compounds have no role in direct metabolism of plants and hence not required for normal growth and development. However, these have a wide range of biological activities and aid in different biological or ecological functions in plants such as allelopathy, to defend against herbivorous animals, insects and microorganisms and to serve as attractants for pollinators, as seed-dispersing animals and as an agent for plant–plant competition and plant regulation (plant hormones).

However, some of these compounds are toxic, unpalatable or anti-nutritive for human consumption due to their abilities to block nutrients, inhibit metabolism or reduce digestion (Enneking & Wink, 2000) and are termed as 'anti-nutritional factors' (ANFs) or 'anti-nutritional compounds' (ANCs). The common ANCs present in pulses are enzyme inhibitors, such as trypsin inhibitor, chymotrypsin inhibitor and α-amylase inhibitor, lectins, tannins, phytic acid, oxalates, phenolic compounds, saponins and oligosaccharides (flatulence factors) (Table 2). Distribution of these ANCs in different components of pulses also varies (Table 3). Phytic acid, oligosaccharides and enzyme inhibitors are mainly concentrated in the cotyledons of pulses. Tannins and phenolic compounds are mainly located in the seed coat of pulses (Dueñas et al., 2002; Reddy et al., 1985). Interestingly, in recent years, many of these ANCs demonstrated several health benefits and have potential to become active ingredients for food industry (Champ, 2002; López-Barios et al., 2014; Roy et al., 2010). Therefore, scientific knowledge about the ANCs present in the pulses is extremely important for pulse storage, trading and functional food product development.

4 | PHYTIC ACID

Phytic acid or phytate is major storage form of phosphorus representing 50%–85% of total phosphorus in plant (Reddy et al., 1982). Phytates are present in all pulses in varied quantity in the form of inositol phosphate, and myoinositol hexaphosphate is the most abundant

inositol phosphate in dry pulses, accounting for about 83% of the total inositol phosphates. Its concentration is higher in dry beans and pigeon peas than in lentils, field peas and chickpea. Phytates are generally found in the outer layer of the aleurone or endosperm (Deshpande & Cheryan, 1984). The phytates form insoluble complexes with minerals, especially essential minerals such as iron, zinc, magnesium and calcium, exhibiting 'chelating effect' and rendering them biologically unavailable for absorption, which may lead to severe mineral deficiency in human and animals (Thompson, 1993). Phytic acid also forms complex with proteins and decreases protein solubility. Therefore, phytates reduce activities of key digestive enzymes, such as lipase, α-amylase, pepsin, trypsin and chymotrypsin (Thompson, 1993). It is also reported that phytic acid binds with starch through phosphate linkages (Muzquiz et al., 2012) and may also affect the starch digestibility by forming a ternary complexes of protein-phytate carbohydrates (Thompson et al., 1986). Ideal phytate content for healthy intake may be 25 mg or less per 100 g in the food eaten for minimization of micronutrient losses (Onomi et al., 2004). Phytates are considered as ANCs due to their mineral, protein and starch binding properties (Morris & Hill, 1996; Rochfort & Panozzo, 2007). But phytates are also very important in plant development and animal/human nutrition for several molecular and cellular functions like DNA repair, chromatin remodelling, endocytosis and hormone signalling (Zhou & Erdman, 1995). Phytic acid (inositol hexaphosphate, InsP6) demonstrates anticancer properties as it can reduce cell proliferation and tumor abrogation. High-fibre diets rich in phytates are found to be associated with lower incidence of some types of cancer. Phytates also display antioxidant activities and can delay postprandial glucose absorption.

5 | ENZYME INHIBITORS

Pulses contain some bioactive compounds that exhibit enzyme inhibition activity. This class of compounds are proteins by nature, having specific enzyme-inhibitor complex formation, which leads to blocking of the active site or altering enzyme conformation, finally reducing the catalytic function. Some of these present in pulses are protease inhibitors (trypsin and chymotrypsin), α-amylase inhibitors and cholinesterase inhibitors. Protease inhibitors are of two types: (i) Kunitz type (KTI), single-chain polypeptides of about 20 kDa with two disulphide bridges that inhibit the enzyme activity of only trypsin but not chymotrypsin, and (ii) Bowman–Birk inhibitors (BBI), also single-chain polypeptides of about 8 kDa in size with seven disulphide bridges that inhibit the enzyme activity of both trypsin and chymotrypsin. The trypsin inhibitors, which are serine protease inhibitors, are the most studied ANCs in pulses. These are low molecular weight proteins found in a wide range of food sources including pulses (Savage, 1989; Wang & Daun, 2004). They are susceptible to binding with lysine and arginine residues in trypsin (a proteolytic enzyme found in the pancreas) (Mondor et al., 2009; Savage, 1989). Therefore, protein digestion is reduced, and inadequate amino acids are available for healthy nutrition (Savage, 1989). Chymotrypsin inhibitors, just like trypsin

inhibitors, limit the protein digestibility. The major difference in activity of these two inhibitors is that the chymotrypsin inhibitors target hydrophobic residues such as tyrosine, tryptophan and phenylalanine instead of lysine and arginine, which are target site for trypsin inhibitors. The α-amylase inhibitors are proteinaceous compounds present in pulses and adversely affect the activity of pancreatic and α-salivary amylase, thereby hampering the starch digestibility (Jaffé et al., 1973; Savelkoul et al., 1992; Wang et al., 2011). Pulses, especially beans, are the second largest group of seeds after cereals as natural sources of α-amylase inhibitors (Campos-Vega et al., 2010). Interestingly, protease inhibitors have been found to be anti-inflammatory and anti-carcinogenic. Amylase inhibitors slow down the digestion of starch and alter the sugar response to insulin, which can be exploited for product development for the diabetic patients (Roy et al., 2010).

6 | LECTINS OR PHYTOHAEMAGGLUTININ

Lectins, also called as phytohaemagglutinins due to their ability to agglutinate red blood cells, are proteinaceous toxic compounds that are commonly found in legumes. These are defined as carbohydrate-binding proteins, which are of non-immune origin and can recognize and bind simple or complex carbohydrates in a reversible and highly specific manner. Legume lectins are the largest family of carbohydrate-binding proteins and are abundant in the legume seeds. The toxicity of legume lectins depends on the species of legume, animal species and animal strain. In human, upon ingestion, the legume lectins bind to specific receptor sites on the intestinal epithelial cell surface and therefore cause interference in the nutrient's absorption across the intestinal wall, and this interference is non-specific. Once bound to the digestive tract, the lectin may induce changes in some, or all, of the digestive, absorptive, protective or secretory functions of the whole digestive system and affect cellular proliferation and turnover. All pulse lectins have the need for transition metal ion and calcium binding for stabilization of active confirmation in these loops (Bouckaert et al., 1999). Thus, lectins adversely affect the activity of digestive enzymes, thereby reducing the in vitro digestibility of proteins (Thompson et al., 1986). Injection of toxic phytohaemagglutinins has been reported to cause pathological changes such as faulty infiltration of liver, zonal necrosis, oedema and haemorrhages in animal tissues. Hence, legume lectins are considered 'anti-nutrients' for human consumption. But at the same time, lectins have anti-immunomodulatory and anti-carcinogenic activities (Hartmann & Meisel, 2007). Bean lectins can be used as a therapeutic agent for tackling obesity problems due to their ability to resist gastric digestion (Hartmann & Meisel, 2007).

7 | PHENOLIC COMPOUNDS

Phenolic acids, tannins and flavonoids are responsible for the bitter taste and dark colour of the seeds. Dark-coloured and pigmented pulses generally have more phenolic content in comparison to light-

coloured varieties. Upon ingestion, these are reported to reduce bio-availability of minerals, especially of zinc. Tannins, one of the phenolic compounds, are water-soluble compounds having molecular weight in the range of 500–30,000 Da. These are of two types—hydrolysable tannins and condensed tannins (Goldstein & Swain, 1963; Gupta & Haslam, 1979; Marquardt et al., 1977). These have the capability to chelate with metal ions (Carbonaro et al., 1996) and form hydrogen bonds with proteins (Beebe et al., 2000). They form insoluble and enzyme-resistant complexes with protein and carbohydrate (Reddy et al., 1985). Hence, these reduce mineral absorption and digestibility of proteins (Reddy & Butler, 1989). Tannins also contribute towards the decreased nutritional value of pulses by complex formation with starch or its digestive enzymes and reduce palatability because of undesirable astringency and bitterness (Chung et al., 1998). Tannins are usually heat stable and may negatively affect the pH mechanism and reduce the protein digestion. Among the food legumes, black gram, mung bean, pigeon pea, kidney beans, moth beans, horse gram peas and faba beans have the highest tannin contents. Tannins are located primarily in the seed coats of pulses, and hence, the tannin content of pulses varies with the colour of seed coat or testa, the dark-coloured pulses being containing large quantities of tannins.

8 | SAPONINS

Legumes are the major source of dietary saponins, the secondary plant metabolites containing a carbohydrate moiety (mono-/oligosaccharides) that is attached to a lipid-soluble aglycone that is steroidal or triterpenoid in structure. These adversely affect the biological cell membranes as they can enhance the permeability of intestinal mucosal cells, inhibit the transport of active mucosal and facilitate the uptake of substances that are normally not absorbed by human intestine (Couto et al., 2015). These are present in all pulses and is highest in pigeon pea. These are mainly located in the seed coat of pulses. Saponins adversely affect the feed/food palatability and lead to foam formation in different solutions. They are found to have a plasma cholesterol-lowering effect in humans and are prominent in decreasing the risk of many chronic diseases (Singh et al., 2017). They are primarily consumed from the pulses, and they are extremely important in human diet due to their anti-carcinogenic, anti-inflammatory, anti-mutagenic, hypoglycaemic, immunomodulatory and neuroprotective attributes (Singh et al., 2017).

9 | OLIGOSACCHARIDES OR FLATULENCE FACTORS

Legume contains some oligosaccharides such as raffinose, stachyose, verbascose and adjugose, which contain α-galactosidic bonds and are α-galactosyl derivatives of sucrose (Muzquiz et al., 2012). Due to lack of α-galactosidase enzyme in human body, which is required for hydrolysis, these carbohydrates remain undigested in the human intestine and hence constitute the indigestible fibre group. However,

in the colon, anaerobic fermentation of these undigested carbohydrates by the residing microflora leads to the production of gases (H_2, CO_2 and traces of CH_4), thus causing flatulence. These gases cause abdominal discomfort, and excessive consumption of these carbohydrates may lead to diarrhoea (Sefa-Dedeh & Stanley, 1979). Due to these effects, these oligosaccharides are known as flatus-producing carbohydrates. Oligosaccharides are dietary fibres and display physiological benefits like bowel function and immune health, reduce chances of coronary heart diseases, increase lactobacilli and bifidobacteria population and decrease enterobacteria in the intestine (Anderson & Major, 2002; Singh et al., 2017).

10 | GOITROGENS

Goitrogens are sulphur-containing glycosides present mainly in soybean. Upon ingestion, these inhibit iodine intake of the thyroid gland and lead to the enlargement of thyroid glands called as 'goitre'. The effect of goitrogens can be counteracted by addition of iodine to the diet.

11 | ALLERGENS

There are some bioactive compounds in legumes that cause allergic reactions such as diarrhoea and vomiting upon ingestion. The allergic reaction is specific for certain individuals, and its severity depends on the sensitivity level of the individual's body. Glycinin and β-conglycinin are two most important antigenic proteins in soybean.

12 | CYANOGENIC GLYCOSIDES

Some legumes such as lima bean are known to have potential toxicity due to their cyanide-producing capability. The cyanides are originally absent in the legumes but are released by the action of the plant enzyme. Although cooking of the beans is thought to destroy these cyanogenic glycosides, the presence of these cyanides in urine even after ingestion of cooked beans indicates the release of hydrogen cyanide (HCN) by the intestinal microflora.

13 | TOXIC AMINO ACIDS

Some pulses such as lathyrus and broad beans contain certain amino acids that are not of protein but can cause toxic effects and substantially reduce the nutritive value. Dihydroxyphenylalanine (DOPA) is the most common toxic amino acid found in legumes (Onder & Kahraman, 2009). Toxic amino acids are believed to cause metabolic favism. Apart from this group of toxic substances, some pulses may contain minute amounts of oestrogen factors and anti-vitamin substances. These substances are triggered by heat and cause serious harmful effects (Deshpande & Cheryan, 1984).

14 | PROCESSING METHODS TO REDUCE THE ANCS

The ANCs present in pulses generally decrease the palatability and reduce protein digestibility and mineral bioavailability, thereby limiting the biological value and acceptance of pulses in regular diet. Thus, the pulses need to be processed appropriately prior to consumption (Soetan & Oyewole, 2009). Several processing techniques are being used in order to reduce or eliminate the metabolic impediments caused by ANCs. Physical method employs milling of the legumes followed by hull separation. Soaking, germination and cooking are also methods for reduction of ANCs in pulses. Chemical methods are also used in which chemical agents are used for complex formation with ANCs. These techniques not only increase the bioavailability of nutrients by inactivating ANCs but also improve the flavour and palatability of pulses. The selection of appropriate processing techniques for removal/reduction of these ANCs in pulses requires the understanding of the chemical structure, distribution in seed fractions, biological effects, heat sensitivity and solubility in water. Various processing techniques such as heating, extrusion cooking, milling, dehulling, soaking, sprouting, fermentation and cooking are commonly used for reduction/removal of ANCs present in pulses.

15 | DEHULLING

Removal of seed coat of pulses is termed as dehulling, and it is one of the post-harvest primary processes of food grains to improve palatability. However, it also causes loss of minerals and dietary fibre (Goyal et al., 2009). This process however reduce/remove some ANCs such as tannins, saponins and total phenolics but is liable to increase the level of phytic acid, trypsin inhibitor, chymotrypsin inhibitor and α-amylase inhibitor. This may be due to higher concentration of these ANCs in pulse cotyledon as compared with the hull (Table 3). Further, dehulling removes embryo and gummy layer present between hull and cotyledons (Goyal et al., 2010), which may also be responsible for changes in ANC concentration. The wet dehulling method involves soaking of pulses in water for 6–8 h during which some water-soluble ANCs may leach out, though it is not quantified (Vishwakarma et al., 2017).

As phytates are mainly located in the cotyledons, the physical removal of testa by dehulling is reported to increase the phytic acid content of pulses, namely, lentil (Wang et al., 2009), faba bean and kidney bean (Alonso et al., 2000). However, a contrasting effect of dehulling on phytic acid content was also observed by Ghavidel and Prakash (2007) in green gram, cowpea and lentil.

Dehulling of pulses results into an increase in the trypsin inhibitory activity (Alonso et al., 1998, 2000; Mubarak, 2005). This phenomenon might be also due to the fact that trypsin inhibitors are present in the cotyledon fractions of pulses and after the removal of seed coat during dehulling process, the concentration of trypsin inhibitor increases on a unit weight basis. Dehulling is not effective in reducing α-amylase inhibitors (Alonso et al., 2000). Dehulling of pulses has also

been reported to decrease the polyphenols (Alonso et al., 2000) and tannins (Alonso et al., 2000; Wang et al., 2009).

16 | SOAKING

Soaking of pulses before cooking is a conventional process that involves complete submergence of pulses in water at room temperature for certain time period (3–48 h). This process not only saves the cooking time but has also been reported to cause reduction in certain ANCs of pulses either due to their water solubility or activation of some degrading enzymes. Therefore, the soaking water is usually discarded after completion of soaking period. The reduction in the ANC concentration depends on the soaking medium used (water, salt solution or bicarbonate solution) and soaking time. The $NaHCO_3$ and Na_2CO_3 salt solutions are also used for soaking of pulses to reduce the cooking time (Yasmin et al., 2008).

Soaking induced reduction in phytate level in pulses (Alonso et al., 2000; Avanza et al., 2013; Yasmin et al., 2008), which may be attributed to the activation of phytase enzyme upon soaking and diffusion, resulting into hydrolytic degradation of phytates in the soaked grain. Effectiveness of this method is highly influenced by the temperature and pH of soaking solution, and considerable reduction in phytates in temperature range of 45–60°C and pH range 5–6 is reported (Greiner & Konietzny, 2006). Leaching-out effect has also been attributed to the soaking-induced decrease in phytic acid content of pulses (Beleia et al., 1993).

Soaking of pulses in water also decreases the tannin content (Avanza et al., 2013) due to their diffusion into water during soaking (Barampama & Simard, 1994) and causes aqueous extraction of saponins due to their high water solubility (Francis et al., 2001). Reported decrease in polyphenols upon soaking (Avanza et al., 2013; Yasmin et al., 2008) may be attributed to the effect of soaking in creating an ionic environment, which in turn might change the seed coat permeability (Mulimani & Supriya, 1994). Soaking can also induce eliminating effect, although of lower magnitude, on the cyanogenic glycosides of the pulses. Soaking in sodium bicarbonate solution is observed to be more effective (13.9% reduction) than soaking in citric acid solution (8.7% reduction) or in water that showed only 7.7% reduction in cyanogenic glycosides (Yasmin et al., 2008).

Soaking method has been studied for reduction of enzyme inhibitors such as trypsin inhibitor (Shi et al., 2017), α-amylase inhibitors (Alonso et al., 2000; Shi et al., 2017) and chymotrypsin inhibitor (Shi et al., 2017) in pulses. This phenomenon may be due to the leaching of inhibitors into steeping water. The effectiveness of this process is affected not only by the soaking water temperature but also by the cellular structure of the intact seed also (Alonso et al., 2000; Shi et al., 2017). Porosity of the seed coat may have limiting effect on the enzyme inhibitor's extraction. Hence, there may be greater reduction in soaked split (dehulled) materials than the whole seeds. However, the levels of trypsin inhibitors may also be increased after the soaking treatment of pulses, which may be due to comparatively higher loss of other seed constituents to the soaking water than the trypsin

inhibitors (Wang et al., 2008). Moreover, as there are several types of trypsin inhibitors, this may contribute to the noted variation in the soaking treatment results. Hence, detailed investigation into the characteristics of pulse trypsin inhibitors may be the need of near future. During soaking, the reduction in oligosaccharides may be due to the autolysis and extraction in soaked water (Onigbinde & Akinyele, 1983).

17 | HYDROTHERMAL TREATMENT/ COOKING/ROASTING

Pulses are generally consumed after hydrothermal processing/ cooking/roasting. These are usually cooked in boiling water, and their cooking requirement is affected by the seed composition, structure, size, etc. Low molecular weight compounds are leached out into cooking water when cooking is done in water. Cooking (boiling, autoclaving and microwave cooking) is very effective in reducing trypsin inhibitors, haemagglutinin activity, tannins and saponins (El-Adawy, 2002). Cooking process has been reported to decrease both water and acid-extractable phytate phosphorus in pulses, which may be due to formation of insoluble complex of phytate phosphorus with other components (Kumar et al., 1978).

Reduction of tannins content after cooking in various pulses such as lentil (Hefnawy, 2011; Wang et al., 2009), cowpea (Avanza et al., 2013) and kidney bean (Wang et al., 2010) may be due to the binding of tannins with proteins and other organic substances during cooking (Barampama & Simard, 1994). However, an increase in tannin content of lentils is also reported (Vidal-Valverde et al., 1994). Autoclaving and extrusion (140°C) can also be used for reduction of tannins in pulses (Van der Poel et al., 1991). Besides tannins, cooking also causes destruction of polyphenols (Avanza et al., 2013; Yasmin et al., 2008).

Trypsin inhibitors are heat-liable proteins, and hence, these may be inactivated by denaturation to the extent of 67%–100% by cooking (Hefnawy, 2011; Khattab & Arntfield, 2009; Shi et al., 2017; Wang et al., 2010), up to 30%–100% by extrusion cooking (Adamidou et al., 2011; Alonso et al., 2000; Rathod & Annapure, 2016), up to 93.29% by wet heat treatment, that is, autoclaving at 121°C for 15–30 min (Hefnawy, 2011) or by aqueous heat treatment at 100°C for 10 min (Grant, 1991). In one study, the highest reduction of trypsin inhibitor activity is recorded after autoclaving (83.67%), followed by boiling (82.27%), microwave cooking (80.50%) and germination (33.95%) (Vijayakumari et al., 1998). The extent of heat inactivation is dependent on temperature, duration of heating, particle size and moisture content. Although trypsin inhibitors are considered to be heat sensitive and generally thought to be inactivated by cooking due to denaturation (Vidal-Valverde et al., 1994), there have been reports of heat-stable trypsin inhibitors. Two types of soybean trypsin inhibitors, KTI and BBI, show some heat stability because of the presence of disulphide bonds—two in KTI and seven in BBI (Van der Ven et al., 2005). Chymotrypsin inhibitors are more heat sensitive than trypsin inhibitors (Shi et al., 2017). Complete

inactivation of chymotrypsin inhibitors can be achieved by cooking (Martín-Cabrejas et al., 2009; Shi et al., 2017) and extrusion cooking (Alonso et al., 2000). The α-amylase content can be reduced in pulses up to 100% by cooking (Shi et al., 2017) as well as extrusion processing (Alonso et al., 2000). The lectins or phytohaemagglutinins present in food legumes are reported to be inactivated/detoxified by the simple process of cooking or autoclaving (Thompson et al., 1983) and toasting (Solomon et al., 2017).

Cooking of pulses results in the reduction of oligosaccharides such as raffinose, stachyose and verbascose by thermal hydrolysis to simple sugars such as disaccharides and monosaccharaides or due to the formation of other compounds (Onigbinde & Akinyele, 1983). Cooking is more effective against stachyose and verbascose than raffinose (Wang et al., 2010). Cooking, extrusion and autoclaving hold promising results in reduction of polyphenols in pulses (Avanza et al., 2013; Yasmin et al., 2008). Most lectins in pulses are destroyed by wet heat treatment (autoclaving at 121°C for 15–30 min) or aqueous heat treatment at 100°C for 10 min (Grant, 1991). Cooking of lentils can cause 15.3%–25% reduction in the total cyanide content with best results when pre-soaked in sodium bicarbonate (Yasmin et al., 2008). Heating or boiling in water does not affect protein allergens present in the pulses; however, autoclaving at 2.56 atm for 30 min decreases IgE binding capacity of lentil and chickpea (Verma et al., 2013). Lathyrogens are readily removed by cooking in water (100°C) and draining off the excess water (Tacon, 1993).

18 | GERMINATION

Germination is the first stage of a plant's growth during which the primary root and stem come out. In this stage, the reserve nutrients required for plant growth are mobilized by hydrolysing proteins and carbohydrates to obtain the required substrates for the seed development. The seed enzymatic system is activated during its germination. It is considered one of the most effective processing methods for improving the nutritional quality of pulses, enhancing the digestibility of nutrients—protein and carbohydrates. Germination process is studied extensively for degradation of ANCs in pulses. However, the extent of degradation depends on the type of pulses, type of ANCs and germination conditions. It is suggested that the proteases are responsible for inactivation of proteinaceous ANCs such as enzyme inhibitors and lectins. During germination, phytic acid is hydrolysed by an endogenous enzyme, phytase, into inorganic phosphorus, the biologically available form, for plant growth and development. The phytic acid present in pulses therefore converts into a soluble form, and due to this phenomenon, the reduction in phytic acid content of germinated pulses has been demonstrated by many researchers (Camacho et al., 1992).

Germination modifies the quantitative and qualitative phenolic composition of pulses (Lopez-Amorós et al., 2006). This process has shown up to 20.8% reduction in total cyanide content in kidney bean (Yasmin et al., 2008). It also reduces the content of enzyme inhibitors such as trypsin inhibitors, α-amylase inhibitors and chymotrypsin inhibitors in pulses (Alonso et al., 2000).

19 | FERMENTATION

Fermentation is an anaerobic and catabolic process during which complex molecules (protein and carbohydrates) are transformed into simple ones by microorganisms. Fermentation process improves the starch and protein digestibility and bioavailability of the minerals present in the pulses. It has also been reported to be an alternative method for reduction of ANCs in pulses (Maleki & Razavi, 2020; Nkhata et al., 2018).

20 | IRRADIATION

Irradiation of pulses is also being studied as a method for destruction of certain ANCs in pulses. It can inactivate the trypsin inhibitors in pulses because of destruction of sulphydryl (–SH) and disulphide (–S–S–) bonds that are apparently highly susceptible to irradiation process (Siddhuraju et al., 2002).

21 | CONCLUSION

Pulses are very good source of protein, energy, fibre and many essential vitamins and minerals. Besides these nutrients, pulses also contain a number of organic compounds produced through secondary metabolism, which have a wide range of biological activities and aid in different biological or ecological functions in plants. However, some of these compounds act as anti-nutrients for human consumption because of their adverse effect on palatability, digestibility and bioavailability of nutrients. In pulses, enzyme inhibitors such as trypsin inhibitor, chymotrypsin inhibitor and α-amylase inhibitor, lectins, tannins, phytic acid, oxalates, phenolic compounds, saponins and oligosaccharides are the common ANCs. Thus, pulses need to be processed appropriately prior to consumption in order to reduce or eliminate the harmful metabolic impediments caused by ANCs. However, selection of the appropriate processing techniques for removal/reduction of these ANCs in pulses requires the understanding of the chemical structure, distribution in seed fractions, biological effects, heat sensitivity and solubility in water. From the reported literature, it can be concluded that processing methods such as dehulling, soaking, cooking, extrusion, germination and fermentation are among the promising techniques for reduction/removal of these ANCs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

The article did not involve any human or animal ethics issues to be considered.

AUTHOR CONTRIBUTIONS

Original manuscript: Yogesh Kumar, Santanu Basu, Deepika Goswami and Rajesh Kumar Vishwakarma. Manuscript editing: Santanu Basu, Mridula Devi, Umashanker Shivhare and Rajesh Kumar Vishwakarma. Article conceptualization: Yogesh Kumar, Santanu Basu and Rajesh Kumar Vishwakarma. Final manuscript correction and preparation: Santanu Basu and Rajesh Kumar Vishwakarma. Coordination: Rajesh Kumar Vishwakarma. All authors read and approved the manuscript.

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