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Antinutritional factors of chickpea and pigeonpea and their removal by processing

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Abstract. Protease inhibitors, amylase inhibitors, phytolectins, polyphenols, and oligosaccarides are important antinutritional factors of chickpea and pigeonpea. Research on these factors is reviewed and compared to those in other grain legumes. Both chickpea and pigeonpea are consumed in various forms as processed food. The effects of such processing practices as cooking, germination, and fermentation to reduce the levels of these antinutritional factors are also discussed.

Introduction

Among food legumes, chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.) are valuable sources of protein, minerals, and vitamins, and occupy a very important place in human nutrition in many developing countries. Although most of the world's chickpea production and consumption (>70%) is in India, this crop is of importance in many other countries in Asia, Africa, Europe, and the Americas. Pigeonpea is grown throughout the semi-arid tropics but is of greatest importance in India, where over 80% of the world's recorded production of this crop is grown and consumed [12]. These two legumes are consumed as food after processing that includes such traditional practices as soaking, sprouting, fermentation, boiling, roasting, parching, frying, and steaming.

It is well recognised that the majority of food legume plants including chickpea and pigeonpea, have the capacity to synthesise certain biologically active substances commonly considered to be antinutritional factors since they have been shown to affect animal and human nutrition [21]. A recent review emphasized the role of such factors in determining the nutritional quality of chickpea [34]. But these two food legumes are consumed by millions of people in developing countries without any harmful effect. This indicates that their deleterious and antinutritional effects were partly or wholely removed by processing. This paper is intended to review and summarise the work on this subject with particular emphasis on literature published in the past decade.

For convenience, the topic is presented under the following headings: (1) protease inhibitors, (2) amylase inhibitors, (3) phytolectins, (4) oligosaccharides, and (5) polyphenols. These are the most commonly observed antinutritional factors of these crops. In addition, reports that indicate that these crops contain other toxic factors are also briefly discussed since information on such factors is limited.

1. Protease inhibitors

Protease inhibitors are widely distributed in plants and it has been recognised for many years that the nutritive value and protein digestibility of many plant proteins, particularly those derived from legumes, are very poor unless they are cooked or subjected to some other form of heat treatment [20]. This beneficial effect of cooking has been generally attributed to the destruction of a unique class of proteins called protease inhibitors which otherwise have the ability to combine in the intestinal tracts of humans and animals in a specific manner with the trypsin and chymotrypsin enzymes.

Protease (trypsin and chymotrypsin) inhibitors of legumes have been extensively studied and their mode of action established [21]. In comparison with soybeans, peas, and common beans, chickpea and pigeonpea offer less problem as far as these factors are concerned [7, 25, 43, 11]. Using similar assay procedures, trypsin inhibitor activity was shown to have decreasing importance in soybean, common bean, broad bean, peas, lentils, and chickpea [7] and in blackgram, kidney bean, pigeonpea, mung bean, and chickpea [25]. Both chickpea and pigeonpea contained considerably higher levels of protease inhibitors than the other commonly consumed Indian grain legumes, but much lower than soybean [43]. Hettiarochchy and Kantha [11] showed 33.4 mg trypsin inhibitor/g of soybean sample, 22.1 mg trypsin inhibitor/g of pigeonpea sample, and 1.9 mg trypsin inhibitor/g of chickpea sample when assayed under identical laboratory conditions. Further, when those three legumes were compared by animal feeding trials, the nutritive value of pigeonpea protein appeared to be the poorest indicating the influence of some antinutritional factors [45]. Singh and Eggum [36] indicated that protease inhibitors were important factors that affected protein quality of pigeonpea. According to Ochetin and Bogere [24] the trypsin inhibitor content of pigeonpea was lower than that of common beans and cowpea when compared under similar conditions. A large variation has been reported in trypsin and chymotrypsin inhibitors of chickpea (Table 1) and pigeonpea (Table 2). The levels of these were higher in whole seed than dhal (decorticated split seed) samples of several chickpea cultivars [39]. This same study reported that mean values for trypsin and chymotrypsin inhibitor units were higher in desi (dark seed-coat) than in kabuli (light seed-coat) chickpea cultivars. The low protein digestibility of some wild pigeonpea is attributed to their high levels of protease inhibitors [38]. The developing green seeds of pigeonpea and chickpea are consumed as vegetables in many parts of India. These pigeonpea seeds contained less protease inhibitors than mature seeds indicating their better protein digestibility [37]. Further research is needed to analyse green and mature chickpea seed for presence of protease inhibitors.

The thermo-labile nature of legume protease inhibitors has long been known [20]. The heat stability of chymotrypsin inhibitor activity was greater than that of the trypsin inhibitor activity when assayed using in vitro system [43]. The inhibitory activities of chickpea and pigeonpea are more heat-labile under acidic conditions and are completely destroyed only when subjected to heat under acidic conditions [43]. Trypsin inhibitors of chickpea were

Table 1. Antinutritional factors and toxic substances in chickpea seed and extent of their presence

Constituent	Number of cultivars tested	Range	Mean	Quoted Reference number
Protease Inhibitors				
Trypsin (units/mg)	15	6.7–14.6	10.9	39
Chymotrypsin (units/mg)	15	5.7–9.4	7.1	39
Amylase inhibitor (units/g)	16	0–15.0	8.7	14.41
Oligosaccharides (g/100 g)				
Raffinose	16	0.36–1.10	0.52	13.41
Stachyose	16	0.82–2.10	1.31	13.41
Stachyose + Verbascose	4	1.90–3.0	2.41	29
Polyphenols (mg/g)				
Total phenols	22	1.55–6.10	3.03	26, 36, 30, 39
Tannins	5	Traces		3
Phytolectins (units/g)	1	400	400	11
Cyanogens (Glycosides)	3	Traces		25
Mycotoxins (ppb)	3	Traces–35	18	23

Table 2. Antinutritional factors and toxic substances in pigeonpea seed and extent of their presence

Constituent	Number of cultivars tested	Range	Mean	Quoted number
Protease inhibitors (units/mg)				
Trypsin (units/mg)	9	8.1–12.1	9.9	37
Chymotrypsin (units/mg)	9	2.1–3.6	3.0	37
Amylase inhibitor (units/g)	9	22.5–34.2	26.9	37
Oligosaccharides (g/100 g)				
Raffinose	10	0.24–1.05	0.47	13,37
Stachyose	9	0.035–0.86	0.49	37
Stachyose + Verbascose	4	1.60–2.30	2.0	29
Polyphenols (mg/g)				
Total phenols	14	3.0–18.30	10.67	30.37
Tannins	10	0.0–0.2	0.03	26
Phytolectins (units/g)	1	400	400	11
Cyanogens (Glycosides)	1	Traces		10
Mycotoxins (ppb)	1	Traces		10

inactivated by moist heat at 121 °C for 30 minutes but not by dry heat [4]. Preliminary soaking followed by dry heat treatment resulted in partial inactivation of the inhibitor trypsin activity [4]. Heat treatment partially destroyed trypsin inhibitors in pigeonpea [36]. Antitryptic activity of chickpea and pigeonpea decreased significantly as a result of fermentation [28, 46]. An increase in protein efficiency ratio was attributed to the destruction of trypsin inhibitors as a result of germination in some legumes [16]. But Khaleque et al. [18] reported that germination did not bring about appreciable changes in the trypsin inhibitors of chickpea. There appears to be little doubt regarding the effect of germination on trypsin inhibitors.

It may be stated that most literature related to the nutritional effects of trypsin and chymotrypsin inhibitors are based on experiments with animals [21]. Further most investigators have generally used trypsin and chymotrypsin of bovine origin to measure their inhibitor contents of various foods [7, 25, 43]. Some recent reports have indicated that extracts of several legumes, including chickpea, showed comparable inhibition of human and bovine trypsin enzymes whereas the inhibition of human chymotrypsin was noticeably more than that of bovines [2]. It has also been recently observed that the role of soybean trypsin inhibitor in human nutrition is not clearly understood suggesting additional efforts in this direction [22].

Considering the effect of heat treatment, a remarkable reduction in protease inhibitor activities can be achieved by heating. But excessive heating reduces the nutritive value of legume proteins. Methionine, the most limiting essential amino acid of legumes, has been reported to undergo nutritional damage when heated [32]. Therefore, it is important to establish the optimum heat conditions to realize the maximum nutritional advantages of cooking these pulse crops in respect to their protease inhibitors.

2. Amylase inhibitors

The proteinaceous alpha amylase inhibitors have received considerable attention from biochemists and nutritionists ever since their presence was revealed in legumes [15] and cereals [33]. Jaffe [14] reported that pigeonpea seed extracts showed remarkably higher amylase inhibitor activity (22–45 units/g) in comparison with chickpea (4–6 units/g). However, both these legumes showed lower amylase inhibitor activity compared with other common beans and peas studied under similar assay conditions [14]. Singh et al. [41] observed a small variation in amylase inhibitors of chickpea cultivars and also reported that inhibitor was more active towards pancreatic amylase than human salivary amylase. Pancreatic amylase inhibitors were considerably higher in mature pigeonpea seeds than in the developing green seed and indicated some adverse effect on starch digestibility [37]. Similar to protease inhibitors and phytolectins, the heat-labile nature of amylase inhibitors is well known [8]. Amylase inhibitors of chickpea were inactive when extracts were boiled for 10 minutes [41]. Since chickpea and pigeonpea are usually consumed after boiling, the amylase inhibitor may not be of practical importance except when unheated seeds are eaten, where some inhibition of starch digestion by amylase inhibitors may be expected.

3. Phytolectins

Phytolectins are toxic factors that interact with glycoprotein on the surface of red blood cells and causing them to agglutinate. Food legumes have long been known to contain protein compounds which agglutinate the red blood cells. Chickpea produced a certain amount of agglutinating activity in cow erythrocytes, although not at a toxic level [4]. The phytolectins present in legumes are known to exhibit different degrees of specificity depending on the animals species tested [9]. Using human blood cells, a recent study of the hemagglutinating properties of several legume seeds has indicated that chickpea and pigeonpea have low lectin activity, i.e., below the toxicity level [9]. By comparing several legumes under similar assay conditions, it was reported that phytolectin levels were 100 units/g of sample for green gram and black gram, 400 units/g of sample for chickpea and pigeonpea, and 800 units/g of the sample for cowpea and soybean [11].

Phytolectins are highly sensitive to heat treatment. In chickpea, almost complete reduction of hemagglutinating activity was obtained with moist heat at 100 °C, whereas presoaking alone had little effect [4]. A complete destruction of hemagglutinating activity was achieved in pigeonpea by atuoclaving at 121 °C for 30 minutes [24]. It must be emphasised here that moist heat treatment was essential to inactivate the hemagglutinating activity. Dry heat may not completely destroy lectins as activity in (*Phaseolus*) beans was still detectable even after 18 hours of dry heat treatment at 100 °C [21]). There was a marked decrease in the phytolectin contents of horse gram germinated for 72 hours [42]. Although information is scanty on the influence of other processing practices on lectins of chickpea and pigeonpea, these toxic factors may be of little significance in chickpea and pigeonpea because (1) lectins are present below the toxicity level, and (2) their activity is completely destroyed by moist heat treatment, which is commonly given to these pulses before consumption.

4. Polyphenols

In the past decade, the nutritional importance of polyphenolic compounds has been recognised in food legumes. Polyphenols of dry beans decreased protein digestibility in animals and humans probably by making protein partially unavailable or by inhibiting digestive enzymes [3]. Both chickpea and pigeonpea contain considerable amounts of polyphenolic compounds that are genotypically variable [35]. The polyphenolic compounds that inhibit the activity of digestive enzymes, trypsin, chymotrypsin, and amylase, are higher in chickpea and pigeonpea cultivars with dark seed-coat colour [35]. On the other hand, tannins have not been detected in chickpea but are present at very low concentration (0–0.2%) in pigeonpea [26], although not detected by earlier workers [10].

Such processing practices as decortication, soaking, germination, and cooking have been reported to influence the levels of polyphenolic compounds in these crops. According to Rao and Deosthale [30] nearly 50% of the polyphenolic compounds were lost in chickpea and pigeonpea as a result of overnight soaking in water, and when germination was continued for 48 hours a further 10% was observed. These workers also observed that cooking without prior soaking brought about a 70% decrease in the polyphenolic compounds of chickpea and pigeonpea when cooking water was discarded. The water-soluble nature of the polyphenolic compounds in chickpea seed has been confirmed by Kumar et al. [19]. By analysing whole-seed and decorticated (dhal) samples of chickpea for polyphenolic compounds, it was noticed that seed-coat contributed about 75% of the total phenolic compounds [38] Also, decortication of chickpea and pigeonpea has been reported to reduce polyphenolic compounds by 90% [30]. Khaleque et al. observed no appreciable changes in polyphenolic compounds of chickpea during germination [18].

It seems that polyphenols may not pose a serious problem particularly for people in regions where these pulses are consumed after decortication. Soaking (water discarded) followed by cooking before consumption is suggested as a means of removing harmful effects of polyphenolic compounds in the regions where these pulses are consumed as whole seed [30].

5. Oligosaccharides

Rackis [27] reviewed the literature on oligosaccharides of the raffinose sugars family, and concluded that stachyose, raffinose, and verbascose contribute to flatulence in man and animals. Flatulence is characterized by the production of high amounts of carbon dioxide, hydrogen, and small amounts of methane gas. Flatus production, as measured in albino rats, followed a similar pattern in oligosacharide contents of soybean, chickpea, cowpea, pigeonpea, black gram, horse gram, and green gram [31]. There were noticeable differences in the contents of these sugars among the cultivars of chickpea (Table 1), and pigeonpea (Table 2). The raffinose content of 12 pigeonpea cultivars ranged between 0.3% and 1.8% [29]. This study also reported a large variation in the combined stachyose and verbascose content of these cultivars. The raffinose content of chickpea was higher than that of pigeonpea and the reverse was true for stachyose and verbascose contents [29]. These three sugars together constituted about 53% of the total soluble sugars in pigeonpea [37], whereas stachyose and raffinose accounted for about 37% of the total soluble sugars in chickpea [41]. This study did not reveal any relationship between total sugars and levels of oligosaccharides.

Flatus production as measured in albino rats, was greater from chickpea than other legumes including pigeonpea and this may be due to its higher content of flatulence-causing oligosaccharides [31, 5, 44]. The volume of flatus produced by rats fed with the pulses was in the following decreasing order: pigeonpea $<$ chickpea $<$ black gram $<$ green gram [17]. These studies suggest that chickpea and pigeonpea may cause more discomfort because of their higher flatus production, if consumed in large quantity.

Considerable amounts of chickpea and pigeonpea are consumed as vegetable in the form of green seeds. The levels of flatulence-causing sugars increased as the seed matured in case of both chickpea [40] and pigeonpea [37]. In addition, some processing methods also influence the levels of flatulence-causing sugars. Germinated chickpea and pigeonpea produced less flatus than ungerminated, as measured in albino rats [31]. A significant decrease (61%) was observed in the levels of raffinose and stachyose of chickpea as a result of germination [1]. Germination followed by cooking brought about 60% reduction in the leves of total oligosaccharides in chickpea and 70% in pigeonpea [13]. Kantha et al. [17] reported that cooked dhal of chickpea did not greatly alter its flatus-inducing capacity as compared to the raw dhal. This observation is, however, contradicted by others. Cooking of chickpea and pigeonpea dhal brought about significant increase in their oligosaccharide contents and this might have been due to improved extractability of these sugars after cooking [31, 29], There was also a significant reduction in the raffinose content of chickpea as a result of fermetation [46]. It appears that germination and fermentation reduce the oligosaccharide level, although a complete removal of these sugars may not be possible. However, there seems to be some confusion in understanding the effect of cooking on these sugars and this would require additional efforts.

Finally, such compounds as glycosides, mycotoxins, and saponins constitute the other series of antinutritional and toxic factors of chickpea and pigeonpea. The glycosides from which HCN is released by hydrolysis have been reported in chickpea, although not at a toxic level [25]. Pigeonpea may also contain traces of glycosides [10]. In a recent review, mycotoxins have been recorded in chickpea [23]. Reports indicate that aflatoxin contamination in chickpea increases with storage. No large differences in alfatoxin content were observed when desi and kabuli cultivars of chickpea were compared [23]. Although the nutritional role of saponins remains unclear, the food plants richest in saponins are chickpeas and soybeans and these compounds are not destroyed by processing or cooking [6].

Summary

Of the various antinutritional factors of chickpea and pigeonpea, protease inhibitors are nutritionally more important. These inhibitors are not completely destroyed by heat treatment; and germination does not appreciably decrease their levels. Genotypes of these pulse crops containing low levels of these inhibitors are nutritionally preferable.

Since chickpea and pigeonpea are usually boiled before they are eaten; amylase inhibitors may not be of practical importance because of their heat-labile nature. However, inhibition of some starch digestion is expected due to amylase inhibitors in case of consumption of raw seeds. Phytolectins of these crops are of litle nutritional significance, because of their low toxicity levels and because their activity is completely destroyed by the moist heat treatment commonly given to these pulse crops before consumption. No appreciable amounts of tannins have been reported in chickpea and pigeonpea but they do contain considerable amounts of polyphenolic compounds that inhibit digestive enzymes. However, these compounds are removed to a large extent by soaking and cooking. Flatulence-causing oligosaccharides, raffinose, stachyose, and verbascose are present in chickpea and pigeonpea. Excepting germination, traditional processing practices do not bring about appreciable changes in the levels of these sugars. Identification and selection of genotypes of chickpea, and pigeonpea containing low levels of these sugars may be preferred from utilization point of view.

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