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Effect of Domestic Processes on Chickpea Seeds for Antinutritional Contents and Their Divergence

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Abstract In the present study, effect of some domestic traditional processes such as soaking, germination, boiling and pressure cooking were explored in the seeds of chickpea cultivar for their anti-nutritional composition. The influence of applied water soaking for 12h on chickpea seeds caused maximum reduction in the levels of phytic acid, tannin and trypsin inhibitor by 59.9 %, 10.76 % and 13.98 % respectively as compared to dry seeds. In contrast, total phenolics contents exhibited a conspicuous increase. Boiling of seeds for 40 min caused maximum reduction in contents of phytic acid, tannin and phenolic by 76 %, 90 %, and 77 % respectively. However, cooking for 90 sec completely inactivated the trypsin inhibitor. Autoclaving at 121^0C showed the maximum reduction in the contents of tannin, phenolics and phytic acid by 97.11 %, 80.53 % and 76.56% respectively. The germination of seeds for 72 h showed significant reduction ($P \leq 0.05$) in the antinutritional factors and showed overall 95% reduction. The present study revealed that under different treatment conditions, germination appeared to be the better practice for lowering anti-nutritional contents in chickpea seeds.

Keywords: *chickpea seeds, soaking, cooking; germination, anti-nutritional factors*

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1. Introduction

Legume seeds constitute an important part of human diet which contains moderately high amount of proteins, minerals and vitamins. Chickpea is one such food legume and a preferred source of protein next only to milk. It is the second most important pulse crop of the world and ranks first in the Indian subcontinent [1]. Under domestication, two major forms have emerged termed desi and kabuli types. Seeds of desi chickpea are small, angular with rough brown color testas while kabuli types are relatively large, smooth and cream colored testas. In addition a gulabi, black and green seed forms of chickpea are locally favored and attract market value (Figure 1). Kabuli chickpea seeds are regarded as more advanced by virtue of total protein contents, dietary fibers, complex carbohydrates and minerals [2,3,4].

Most of the legume seeds are known to synthesize certain biologically active substances which devalue the nutritive importance. Utilization of pulse foods including chickpea is inhibited due to presence of anti-nutritional factors (ANFs) like tannins, phytic acid, protease inhibitors and oligosaccharides. These ANFs may cause adverse physiological effects to the humans if consumed raw [2,5]. Plants commonly synthesize these antinutrients as part of their protection against their predators and/or as a means to survive under adverse growing conditions. Protease inhibitors from seed inhibit the actions of trypsin, pepsin and other proteases in the gut preventing the digestion and subsequent absorption of protein. Tannins inhibit the digestive enzymes and thus lower the digestibility of most nutrients especially proteins and carbohydrates. Phytic acid has a strong binding affinity to minerals such as calcium, magnesium, iron, copper, and zinc and thus reduces their bioavailability. An agronomic trait such as ANF increases the crop productivity but decreases the market value and consumer preference. The chemical composition and oligosaccharides of raw and germinated chickpea seeds were reviewed by Singh et al [6]. Published work describe analysis of these antinutritional factors and effect of cooking on various constituents of chickpea seeds [7,8].

Legume foods are cooked employing traditional home processing methods before human consumption. However cooking causes considerable losses in soluble solids, especially vitamins and minerals. To understand the role of domestic processing viz. soaking, autoclaving, cooking and germination etc. on seed composition needs to be explored [3]. Chickpea is consumed in various forms as processed food. The seeds of chickpea containing lower levels of ANFs are nutritionally preferred. The present study was therefore undertaken to ascertain the effect of traditional processes such as soaking, cooking and

germination on the anti-nutritional contents in chickpea cultivars grown in Madhya Pradesh region of India.

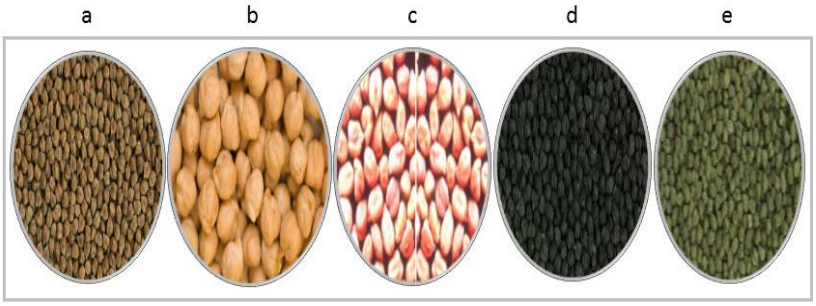

Figure 1. Chickpea seed types a) desi b) kabuli c) gulabi d) black e) green

2. Materials and Methods

2.1. Seed Material

Nineteen chickpea cultivars that are mostly cultivated and consumed in Madhya Pradesh were selected for the analysis. Seeds were collected from agriculture college, Sehore, Madhya Pradesh, India.

2.2. Methods

2.2.1. Soaking, Cooking, Autoclaving and Germination

For each aforesaid treatment, initially seeds were surface sterilized with 10% mercuric chloride solution and then thoroughly washed and finally rinsed with autoclaved deionized water. A seed of each cultivar (100 g) of was soaked in distilled water at a ratio of 1:4 (w/v) for 12h at room temperature. For boiling effect, seeds were boiled in distilled water at 100°C in the ratio of 1:10 (w/v) for 20, 30 and 40 min. Subsequently, rinsed seeds were dried at room temperature (26±2°C) and crushed. Autoclaving at 15 lbs. pressure (121°C) in distilled water (1:10 w/v) for 10, 15 and 20 min. was done at room temperature and seeds were powdered. Germination was stimulated in a petri dish lined with wet filter paper kept in an incubator for 24, 48 and 72 h at 25°C. During germination, deionized water containing sodium azide (0.01%) was sprinkled on seeds every 12h., seeds were air dried overnight and further processed. All these analysis was carried out in each seed samples having normal control and another experimental processed.

2.3. Analysis of Antinutritional Factors

2.3.1. Trypsin Inhibitor (TI) Activity

The inhibitor content was measured using *BAPNA* as a substrate [9]. For measuring trypsin inhibitory activity 10 µg of trypsin was mixed with suitable quantity of the sample (to get 50-60% inhibition) and incubated at 25ºC before measuring the residual trypsin activity. 10 µl of seed extract was mixed with 80 µl of 50 mM Tris-HCl buffer, pH 8.2, containing 20 mM CaCl2, 10 µl of trypsin (in 1 mM HCl) and incubated at room temperature at 30 sec interval between two wells on a microtitre plate. The residual activity was measured by adding 125 µl of *BAPNA* (40 mg/ml dimethyl sulfoxide, freshly diluted 1:100 in 50 mM Tris-HCl buffer, pH 8.2 and 20 mM CaCl2 prewarmed to 37ºC) and then incubated at room temperature for 30 min. Reactions were stopped by the addition of 25µl of 3% (v/v) acetic acid. Liberated p-nitroanilide was measured at 410 nm. 100% trypsin activity was measured from the sample minus the inhibitor extract. One unit of trypsin activity was defined as the amount of enzyme which increases the optical density by one unit at 410 nm due to the release of p-nitroaniline. Further one TI unit was defined as the amount of inhibitor that inhibited 1 unit of trypsin activity [10].

2.3.2. The Total Phenolic Content

Total phenolic contents were estimated using the Folin–Ciocalteu colorimetric method [11]. Defatted sample (1 g) was ground in a mortar pestle with 10 times volume of 80% ethanol (10ml). The homogenate so obtained was centrifuged at 10,000 rpm for 20 min. The residue was re-extracted with 5 times the volume of 80% ethanol. The supernatant was evaporated to dryness. The residue was thereafter, dissolved in 5ml distilled water. Different aliquots ranging from 0.2-2 ml were put into test tubes. The total volume was made up to 3ml in each tube with distilled water followed by addition of Folin-Ciocalteau reagent (0.5ml). After 3 min, 2ml of 20% Na_2C0_3 solution was added to each tube and mixed properly. The tubes were placed in boiling water for exactly 1 min cooled and brought to room temperature then absorbance was measured at 650nm against a reagent blank. To quantify the amount of total phenol content in the seed sample absorbance was compared with standard catechol solution (0.1 mg/ml catechol).

2.3.3. Phytic Acid

Dried chickpea seeds were crushed to a fine meal and powdered 50mg seed samples were extracted overnight in 0.4mM HCl followed by centrifugation for 20 min at 10,000 rpm at room temperature. Supernatant was collected and used as a source for phytic acid analysis. 10 µl of sample was taken in a microtitre plate, diluted with 90µl double distilled water, followed by addition of 100 µl colorimetric reagent (3M H_2SO_4, 2.5% ammonium molybdate, 10% (w/v) ascorbic acid and distilled water in 1:1:1:2 ratio). The contents were incubated for 60 min at room temperature and absorbance was taken at 650 nm using Systronics UV-Vis spectrophotometer [12].

2.3.4. Tannins

Four hundred mg of finely powdered defatted meal was mixed with 40 ml distilled water. The suspension was then boiled for 30 min cooled and subsequently centrifuged at 2000 rpm for 10 min and used as a source for tannin

estimation. Tannins were estimated using Folin-Denis reagent. After extraction, 1 ml of the clear supernatant was used as a source of tannins and to this 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution were added followed by dilution to 100 ml with water. The tubes were incubated at room temperature for 30 min and the color thus developed was read at 700nm using Systronics UV-Vis spectrophotometer [13].

2.4. Statistical Analysis

All work was done in triplicates and the data presented are means ± S.D. of three independent determinations. Significance was accepted at $p \leq 0.05$.

3. Results

For the present experiment seeds were subjected for domestic processing to estimate the amounts of trypsin inhibitor, tannins, phytic acid and phenols. The results of all these investigations are represented in the form of graph (Figure 2, Figure 3, Figure 4 and Figure 5). The highest concentration of trypsin was found in JG11 (0.292±0.03 TIU mg/gm) and lowest concentration was found in JG63 (0.121± 0.03TIU mg/gm). The highest tannin content was present in cultivar JGK1 (10.12±0.04 mg/gm) and lowest concentration was found in BG391 (1.3±0.03 mg/gm). The phytic acid concentration varies widely in cultivars. The highest concentration was found in JAKI 9218 (2.27±0.01 mg/gm) and lowest in JG 315 (0.65±0.03 mg/gm). The total phenol contents also vary in different seed cultivars. The highest concentration was found in JG322 (50.39 ±0.03mg/100 gm) and lowest concentration was found in JG 412 (5.18±0.05 mg/gm).

3.1. Effect of Soaking

The water soaking treatments caused significant decrease in different antinutritional factors which varied with the cultivars. Maximum decrease of 10.76 % in tannin content was found in cultivar BG391 whereas 59.90 % in cultivar JG74 for phytic acid. Trypsin inhibitor contents registered maximum reduction of 13.98 % in cultivar ICCV10.

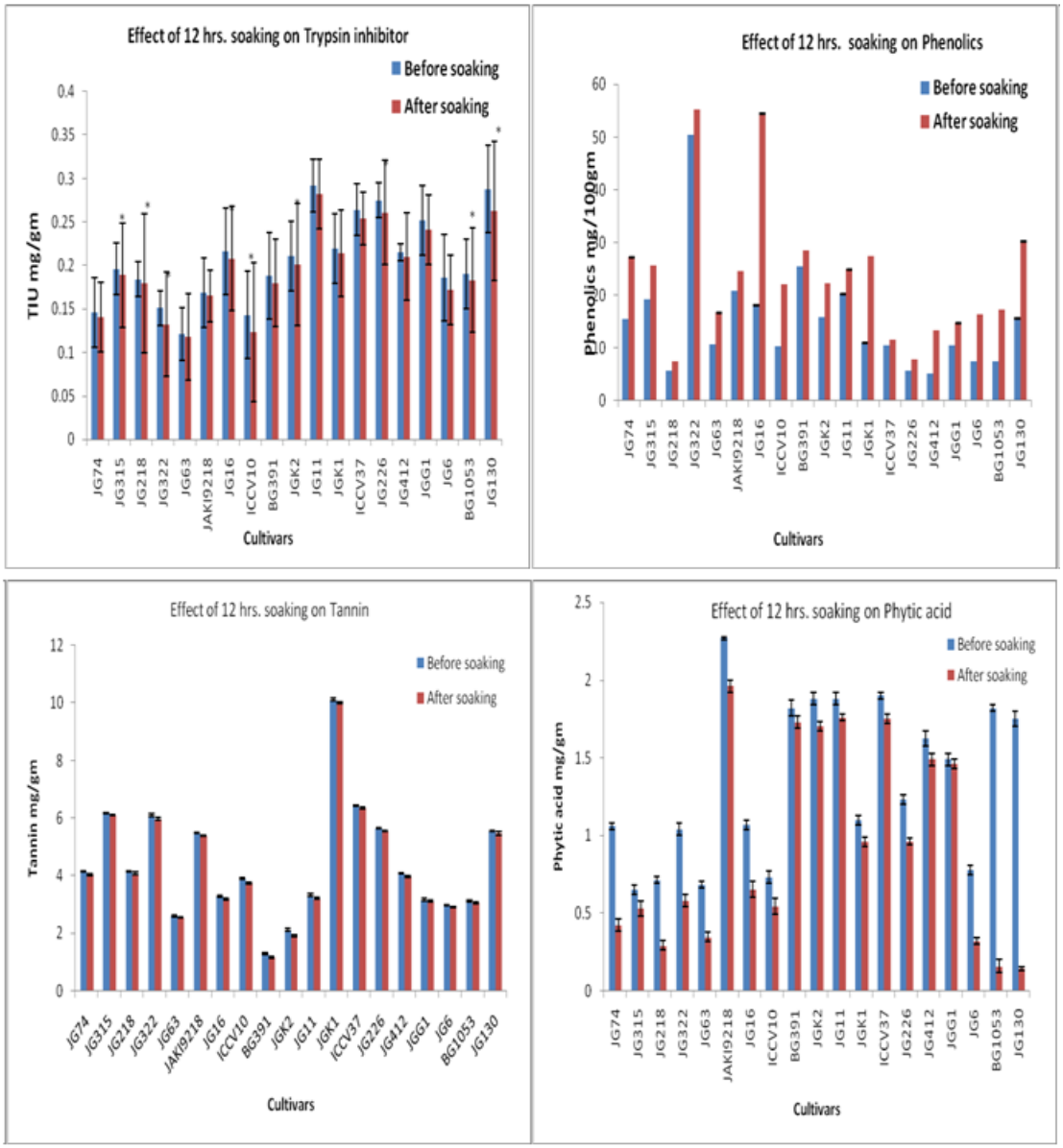

Figure 2. Chickpea seeds as affected by 12 hrs. of soaking

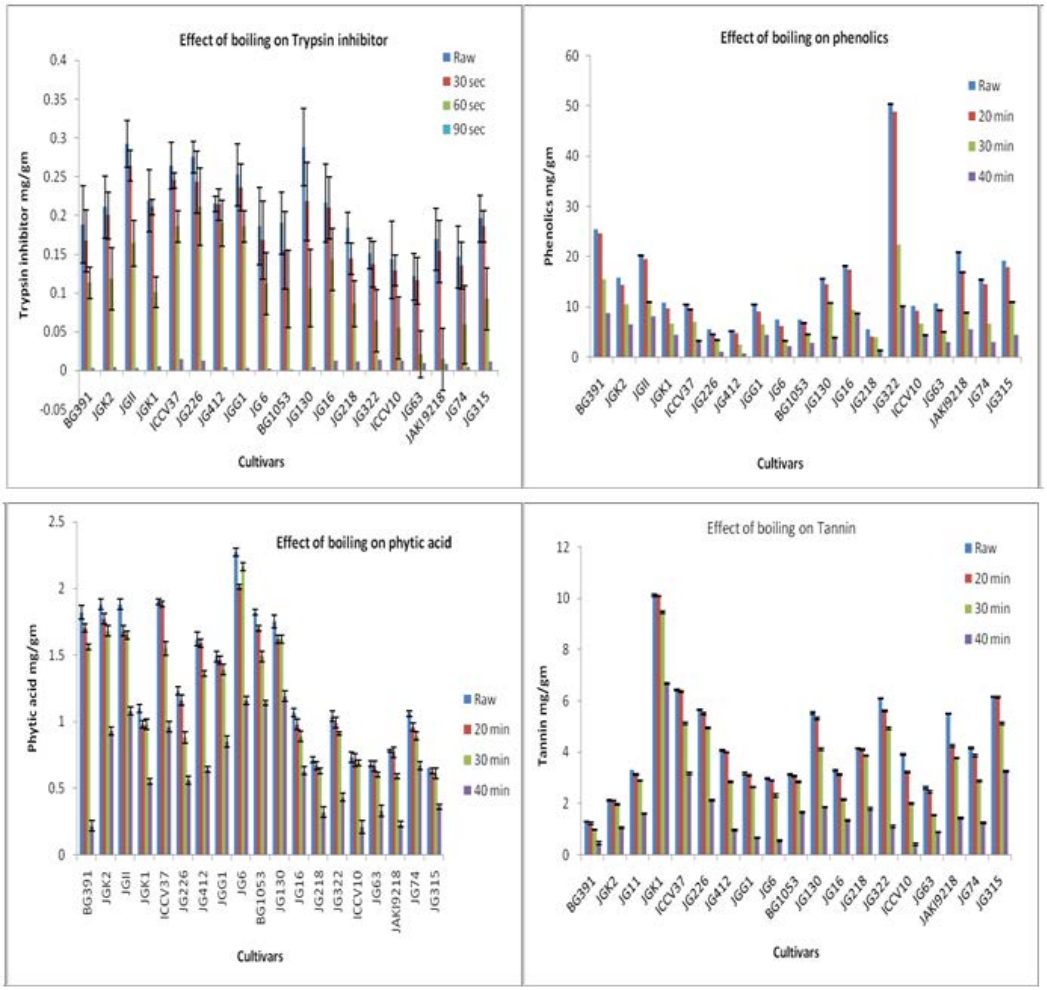

Figure 3. Chickpea seeds as affected by boiling (20, 30, 40 min)

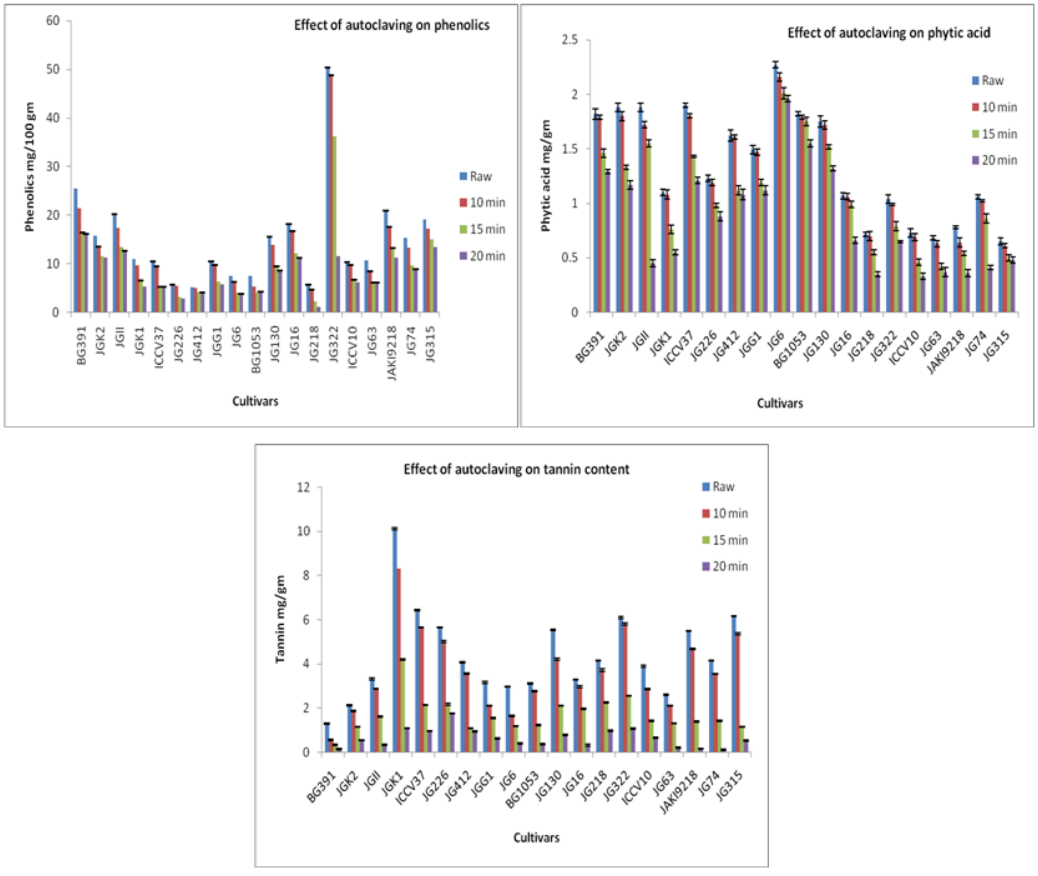

Figure 4. Chickpea seeds as affected by autoclaving (10, 15, 20 min)

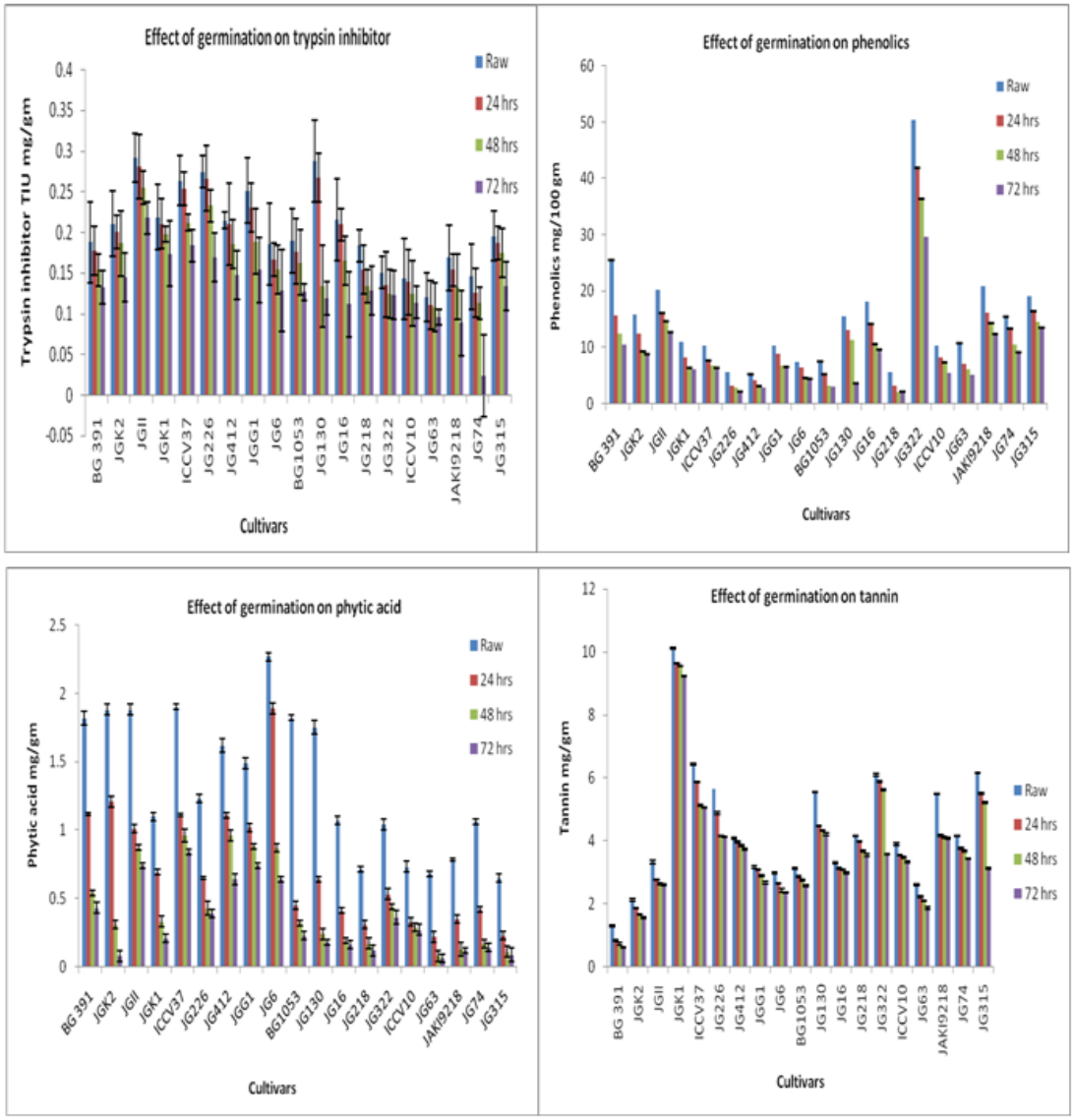

Figure 5. Chickpea seeds as affected by germination (24,48,72 hrs.)

3.2. Effect of Boiling

Boiling at 100ºC in distilled water for 40 min resulted in loss of 49.23 % of tannin contents in ICCV10, 79.93 % of phenolic contents in JG322 and phytic acid up to 87.91% in BG391 cultivars. The obtained data indicated that cooking treatments for 90 sec completely inactivated the trypsin inhibitor. This showed that the trypsin inhibitor of chickpea seeds were heat-labile.

3.3. Effect of Autoclaving

Autoclaving of seeds for 20 min significantly reduced the content of total phenolic, tannins and phytic acid and the effect varied with cultivars under question. Autoclaving at 20 min caused 97.11 % reduction in tannin content in JG74 cultivar and 76.06 % loss in phytic acid in cultivar JG11 whereas 80.53 % decrease phenolic contents in cultivar JG218.

3.4. Effect of Germination

Germination for 72 hours caused the maximum reduction of 53.07 % in tannin content in BG391 cultivar, phytic acid 95.74 % in JGK2 cultivar, phenolic contents up to 77.10 % in JG130 and reduction in trypsin inhibitor to 83.56 % in JG74 cultivar.

4. Discussion

Quantification of ANF'S is one of the requirements for food industry. This information therefore, can be useful variety selection for doing food processing. In the present study different food processing methods like soaking, germination, boiling and autoclaving were employed in chickpea seeds as one of the strategies to minimize adverse effects on ANFs. Chickpea seeds are consumed by human in various forms as a processed food. The present study indicates that seeds of chickpea contain considerable amounts of trypsin inhibitor, tannin, phenolic and phytic acid contents. The removal of these components is essential to improve the nutritional quality of legumes for effective utilization as human food. It is widely accepted that traditional processing are effective methods for achieving the desirable changes in the seed composition. In many instances, usage of only one method may not impart the desired removal of antinutritional compounds and hence a combination of two or more methods is necessary.

Soaking is one of the processes to remove soluble antinutritional factors and some metabolic reactions can take place during soaking which may affect the content of certain anti-nutritional compounds [14]. A soaking period of 12 h may be inadequate for reducing the phytic acid content as revealed in the present study. These results are in agreement with earlier reports [15]. Decrease in tannin might be attributed to leaching into soaking medium [16]. Reduction in the trypsin inhibitor activity after soaking of desi chickpea was reported [17]. Similar results were also noted in kidney beans [18] and fababean [19]. Deshpande and Damodaran [20] supported the finding that there is a loss of certain undesirable components including trypsin inhibitor as a result of soaking. Phenolic compounds are water-soluble in nature [21] and mostly located in the seed coat [22]. In general, water soaking appears effective in reducing the levels of polyphenols. However, in the present study the total phenolic contents estimated in various seeds exhibited increased level of total phenolic contents in water soaked condition as compared to raw seeds. In a similar study by Paramjyothi and Anjali [23] reported significant decrease in polyphenol content of chickpea after soaking. An increase in methanol extractable polyphenols after 72 hours of seed soaking may be attributed to renewed synthesis of polyphenols or degradation of high molecular weight insoluble polymer into smaller molecular weight soluble phenols that might have reacted with the reagents [24].

In the present study boiling of seeds for 90sec completely destroyed the trypsin inhibitor. Our results are in agreement with the findings that cooking for 60 min at 100°C was sufficient to inactivate over 90% of trypsin inhibitor activity in *P. vulgaris* [25] and eliminated completely on heating soaked red gram seeds in boiling water for 5 min [26] and similar findings have also been noticed [27]. The loss of phenolic contents after autoclaving may also be due to the interaction of polyphenols with other components of seeds such as protein to form insoluble tannin protein complexes [28]. In our previous study [29] chickpea seed contains trypsin inhibitor, tannin and lectin at significant levels.

Germination of chickpea brought about significant reduction ($P \leq 0.05$) in the trypsin inhibitor content which was not observed in other legumes like lentil and soyabean. Lentil achieved only 7-18 % during 6 days germination [30] and up 12% trypsin inhibitor contents in soyabean after 12 days of germination [31]. Reduction in phytic acid contents of cereals and legume seeds with sprouting has been frequently reported due to an increase in phytase activity during germination [14]. In present analysis, sprouting has been found to be more effective than other processing methods in reducing phytic acid content. The decrease in total phenol content during germination may be attributed to the polyphenol oxidase based enzymatic hydrolysis [32]. Thus ANFs which are known to cause deleterious effects in humans can be removed by various domestic treatments like soaking, sprouting, cooking out of which germination appears to be the best way to reduce.

5. Conclusion

The regional variation of quality within the chickpea seed growing regions can be attributed to genetic diversity and its interaction with environment. In India, chickpeas are cultivated in different parts and prized for their high protein and fiber content. The globalizing markets demand higher quality seed products that meet new standards of food quality and safety. The results of this study support proposals for the development of technological process for the use of chickpea seeds as a food source. The present study ascertains germination as an effective practice for relieving antinutritional contents for enhancing nutritional quality of chickpea seeds.

Conflicts of Interest Statement

Authors declare that the work carried out by them is original and have no conflict of interests.

Acknowledgment

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