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RESEARCH PAPER

Effect of overnight soaking and boiling on phytic acid, tannins, saponins and proximate composition in legumes

Samawia Qureshi[1]* and AsmaaHamid[2]

[1]Department of Food and Nutrition, University of Home Economics, Lahore, Pakistan
[2]Department of Food and Nutrition, University of Home Economics, Lahore, Pakistan

***Corresponding author: Samawia Qureshi (samawiaqureshi@gmail.com)**

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Key Message: Overnight soaking in water, acidic and basic medium followed by boiling on three legumes was studied and found that overnight soaking prior to boiling in acidic medium was most effective in reducing anti-nutrients and enhancing nutritional quality of legumes.

Abstract: The nutritional value of legumes depends not only on the nutrients but also on anti-nutrients that hinder their absorption. Simple domestic cooking procedures can greatly influence nutrients and anti-nutrients in legumes. The overnight soaking (in tap water, 0.9% w/v $NaHCO_3$ solution and 1.5% w/v vinegar solution) prior to boiling among three legumes was studied; red kidney beans *(Phaseolus vulgaris)*, chickpeas (*Cicer arietinum*) and bengal grams (*Cicer arietinum*) which significantly ($p < .001$) reduced phytic acid, tannins, saponins and ash content, whereas, crude protein, crude fiber and carbohydrates increased significantly ($p < .001$), enhancing the nutritional quality of legumes. The most drastic reduction of phytic acid (33.07% in red kidney beans, 41.13% in chickpeas and 40.47% in bengal grams) was observed after boiling the overnight soaked legumes in 1.5% w/v vinegar solution. The most notable reduction in tannin content was found after $NaHCO_3$ solution soaking followed by boiling (86.81% and 74.10%) in red kidney beans and chickpeas, respectively. Tannin content in bengal gram was reduced upon both vinegar and $NaHCO_3$ solution soaking followed by boiling (84.40 and 83.11%), respectively. Similarly, maximum saponin reduction was noted in bengal grams upon both vinegar and $NaHCO_3$ solution soaking followed by boiling (94.7 and 94.73%). The most noteworthy reduction of saponins among red kidney beans and chickpeas was followed by vinegar solution soaking followed by boiling (96.39 and 85.72%), respectively. Results recommend overnight soaking of legumes in a 1.5% w/v vinegar solution followed by boiling for researchers who rely on plant protein to maximize protein absorption by alleviating anti-nutrients and enhancing the crude protein content of legumes.

Keywords: Anti-nutrients, Legumes, Overnight soaking, Phytic acid, Saponins, Tannins

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Introduction

Legumes belong to the family of leguminosae. There are almost 13,000 species of legumes but only 20 are being consumed. The major types of legumes commonly used are beans, peas, peanuts, soybeans and lentils. Structurally, the beans and peas contain a seed coat or hull, plumule, hypocotyls and the two cotyledons. The hull or seed coat serves for storage, handling and protection (Fabbri & Crosby, 2016). The world survey of per capita legume consumption in 2011 reported almost 2.7–10.8 kg/year. Half of the global production occurs in Asia (Venkidasamy et al., 2019). Per capita consumption of legumes in Pakistan is 15.7 kg/annum (Rahman et al., 2013).

Legumes are consumed widely because of their nutritional significance. They are significant sources of complex carbohydrates, dietary fibers, proteins, minerals (calcium, magnesium, sodium, potassium, manganese, iron, copper, zinc, fluorine, phosphorus and sulfur) and B-vitamins (thiamin, riboflavin and niacin) (Margier et al., 2019; Foschi et al., 2020). The nutritive value and the composition of legumes may differ with the change in processing techniques, variety, climate, species, soil and the exposure to pesticides and fertilizers (Soetan & Oyewole, 2009; Satya et al., 2010). While possessing high nutritive value, the legumes contain some compounds that interfere with the assimilation of these valuable nutrients, these compounds are known as Anti-Nutritional Factors (ANFs) (Kaushik et al., 2010). ANFs are secondary metabolites of plants that are biologically active and act as defense mechanism through metabolism of plants (Gemede & Ratta, 2014).

The ANFs are classified as protein and non-protein categories. Protein ANFs include trypsin inhibitors, lectins and chymotrypsin inhibitors that hinder the protein absorption are heat sensitive in nature while non-protein ANFs include phytic acid, saponins, alkaloids, tannins and phenolics that are heat resistant and are difficult to eliminate only by heat (Nergiz & Gokgoz, 2007; Fereidoon, 2014).

The nutrients and anti-nutrients both are affected by different processing methods like cooking, soaking, fermentation and germination. The processing methods enhance the utilization, palatability, digestibility and bioavailability of the nutrients (Ramakrishna et al., 2008). Literature shows that anti-nutrients leach out in water when soaked and soaking significantly reduces the anti-nutritional factors (Adebayo, 2014). Legumes are of great

importance in vegetarian diets as they contain twice as much protein as cereals and an adequate amount of essential amino acids (Ndidi et al., 2014). The legume seeds contain 21-25% of the protein (Duranti, 2006). These proteins are usually devoid of sulfur containing amino acids known as methionine, cysteine and tryptophan but rich in most essential amino acids, notably lysine. This inadequacy can be overcome if complemented with cereal protein (Ohanenye et al., 2020). The absorption and utilization of protein from legumes is lower than animal protein because of the presence of ANFs like tannins bind to the proteins by hydrophobic interactions, hindering protein absorption. Protease inhibitors suppress pancreatic proteases thus limiting protein utilization. Similarly, lectins have shown to deteriorate hydrolytic and transport functions at the enterocyte site. Moreover, phytic acid impedes the absorption of iron, zinc and calcium and has adverse effects on protein utilization in the gastrointestinal tract (Selle et al., 2000; Nikmaram et al., 2017; Belmiro et al., 2020). Saponins impair the digestion of protein and the uptake of vitamins and minerals in the gut and the toxicity can be reduced by soaking legumes prior to cooking (Francis et al., 2002; Bora, 2014).

Majority of diets in developing countries are cereal based, that needs to be switched to legume based diet to cope with the persisting protein and micronutrient malnutrition (Minde et al., 2020). They are an inexpensive alternative to animal protein and therefore said to be the poor man's meat in the developing countries (Venkidasamy et al., 2019). Legumes are widely consumed in Pakistani diet. It serves as a potential protein source for strict vegetarians, the poor who cannot afford animal protein and the ones who entirely avoid the animal sources due to social or religious causes. Therefore, it is required that the protein availability from legumes should be maximized by reduction or complete removal of anti-nutrients that hinder its absorption. Limited number of studies on the effect of soaking and boiling on the anti-nutrients in indigenous legumes are present in Pakistan. Hence, the present study attempts to find out the effect of simple processing treatments (overnight soaking and boiling) that can be applied on a domestic level on the anti-nutritional factors in selected legumes.

Materials and Methods

Sample

The following three types of legumes were taken as a sample in the study:
1. Red kidney bean *(Phaseolus vulgaris)*
2. Chickpea (*Cicer arietinum*)
3. Bengal gram (*Cicer arietinum*)

Sample preparation

Each legume was subjected to seven treatments; soaking in distilled water, soaking in 0.9% (w/v) sodium bicarbonate ($NaHCO_3$) solution, soaking in vinegar solution 1.5% (w/v), water soaking and boiling, vinegar solution 1.5% (w/v) soaking and boiling, $NaHCO_3$ solution 0.9% (w/v) soaking and boiling, unprocessed seeds without any treatment.

Processing treatments

Each legume was subjected to seven treatments; soaking in distilled water, soaking in 0.9% (w/v) sodium bicarbonate ($NaHCO_3$) solution, soaking in vinegar solution 1.5% (w/v), water soaking and boiling, vinegar solution 1.5% (w/v) soaking and boiling, $NaHCO_3$ solution 0.9% (w/v) soaking and boiling, unprocessed seeds without any treatment.

Soaking

All the three legume seeds were soaked overnight (12 hours) in three different types of soaking mediums as mentioned above. The legume: soaking medium ratio was 1: 5 (w/v). After overnight soaking, the soaking solution was discarded and the legume seeds were rinsed thoroughly. The legume seeds were allowed to be dried. When properly dried, the seeds were ground and stored in plastic zipper bags which were kept in the refrigerator.

Boiling

Half of the seeds following the soaking process were boiled in water. The seed to water ratio was 1: 10 (w/v). Legume seeds were boiled until tender. The seeds were strained and the water was drained off. Strained seeds were allowed to cool at room temperature and then they were ground using pestle and mortar. The prepared sample was placed in a plastic zipper bag and stored at 4 ºC.

Chemicals and reagents

Ethanol, Folin-Denis reagent, Saturated sodium bicarbonate, Tannic acid, Ferric chloride, Sulfosalicylic acid, 0.1M Sodium chloride, 0.7 M Sodium chloride, Sodium hydroxide, Concentrated HCL, Anion exchange resin, 10% HCl, 0.1 N Sulfuric acid, 40% NaOH, 2% Boric acid, Methyl red dye, Sulfuric acid, 10% NaOH, n-Hexane and distilled water.

Equipments

Orbital Shaker, Electro mantle, Spectrophotometer, Electrical balance, Kjeldhal apparatus, Distillation unit, Soxhlet apparatus, Muffle furnace, Desiccators, Drying oven, 500 ml round bottom flask, 250 ml and 100 ml Conical flasks, 10 ml and 1 ml pipettes, micropipettes, 10 ml, 25 ml and 100 ml Volumetric flasks, 100 ml and 500 ml Measuring cylinders, Filter paper, Beakers, Funnels, Rubber bands, Foil paper, Syringes, Cotton, Pestle and mortar, Crucibles, China dishes, Petri-dishes and Test tubes.

Proximate analysis

Proximate analysis of each prepared sample was carried out in triplicate. The crude protein was obtained by the

Kjeldahl method. Fat content was determined by extracting the food samples with n-hexane using soxhlet apparatus. Moisture and ash contents were determined according to the methods described by the methods of (Association of Analytical Chemists [AOAC], 2012)

Analysis of anti-nutrients

Detection of saponins

Methodology of saponin determination followed is reported by Ejikeme et al. (2014). A 5 g sample was weighed in an electrical balance and placed in 250 g conical flask. 20% aqueous ethanol was prepared and 100 g of it was added to conical flask containing sample. The mixture was shaken at orbital shaker for 6 hours. When mixed thoroughly, the solution was filtered via filter paper. The filtered solution was measured by a 25 ml volumetric flask and poured in a pre-weighed china dish. The china dish was placed in a drying oven until completely dried. The dried china dish was placed in a desiccator and pre and post weight of china dish was noted.

Detection of tannins

Tannins were determined according to the method given by AOAC (2012). Extraction of tannins was carried out by weighing 2 g of sample via electrical balance. The weighed sample was placed in an electro mantle with 1oo ml distilled water for half an hour. The solution was filtered using filter paper. 0.5 ml filtered solution was taken in 10 ml volumetric flask, 0.5 ml Folin Denis and 0.1 ml saturated Na_2CO_3 reagent was added. The solution was made up to the mark i.e. 10 ml. Different standards of tannic acid were prepared. Each sample was tested in a spectrophotometer against a blank at 760 nm.

Detection of phytic acid

Phytic acid content in raw and treated samples was determined by the method described by Vaintraub & Lapteva (1988). Phytate was extracted from prepared samples with 3.5% (w/v) HCl solution by shaking the mixture for one hour on an orbital shaker. The mixture was filtered using filter paper. Locally available syringes were used by removing the needle and plunger. The syringes were blocked by small quantity of cotton to avoid leakage. 2 g of anion exchange resin was added to each syringe. 5% NaOH solution was prepared and 10 ml of it was passed with the help of plunger through each syringe. To regenerate phytic acid, distilled water was passed through each syringe. After regeneration, 10 ml of sample was passed through the respective syringe. 0.7 M and 0.1 M NaCl was prepared separately. 15 ml of 0.1 M NaCl was passed through the syringe, while 10 ml of 0.7 M NaCl was passed and collected in the test tube. Wade reagent was prepared by $FeCl_3$ and sulfosalicylic acid and 1 ml of reagent was added to each of the test tubes. The pink colour of the Wade reagent was due to the reaction between sulfosalicylic acid and ferric ion. The iron became bound to the phosphate ester in the presence of phytate and is unavailable to react with sulfosalicylic acid, resulting in a reduction in pink colour intensity. The solution in each test tube was measured against the blank in spectrophotometer at 500 nm.

Statistical analysis

All experiments were carried out in triplicate and the final results were expressed as means ± standard deviation (S.D.). One way ANOVA was used to analyze data using SPSS (version 20). The probability values were considered to be statistically significant differences if $p<0.001$.

Results and Discussion

Effect of processing techniques on ANFs in red kidney beans

Table 1 and 2 show that all the processing treatments significantly ($p<0.001$) reduced the phytic acid content in red kidney beans. The results highlight that boiling of vinegar solution soaked beans resulted in highest reduction (33.07%) of phytic acid. Huma et al. (2008) observed that soaking of kidney beans and faba beans significantly decreased the phytic acid. A study conducted by Yasmin et al. (2008) found that all processing techniques significantly reduced phytic acid in red kidney beans in which citric acid solution soaking reduced phytic acid more than other soaking media. In the present study, the unprocessed red kidney beans contained 713 mg/100 g tannins. It is evident from results that all the soaking and boiling treatments significantly reduced the tannins. Among soaking treatments the highest reduction (67.78%) was caused by $NaHCO_3$ solution soaking. When soaked samples underwent boiling treatment, $NaHCO_3$ solution soaked samples showed maximum reduction among boiling samples (86.81%). While the vinegar soaking and boiling showed 84.95% reduction. A study on red kidney beans from Peshawar supports the claim that cooking after soaking in citric acid or $NaHCO_3$ solution caused drastic reduction in tannins (Yasmin et al., 2008). Khattab and Arntfield (2009) confirmed the results in their study showing that soaking and boiling significantly reduced the tannin content in cowpeas and kidney beans. The saponins in unprocessed red kidney beans were 93.09 mg/100 g. Boiling after $NaHCO_3$ solution and vinegar solution soaking almost eliminated the saponins. These results are in line with a similar study conducted on velvet beans (Nwaoguikpe et al., 2011). A study conducted on soybeans claimed that citric acid soaking prior to cooking resulted in maximum decrease of saponins that might be due to its diffusion into soaking medium (Sharma et al., 2013).

Effect of processing techniques on ANFs in chickpeas

Table 3 and 4 reveal that all treatments significantly ($p<.001$) reduced the phytic acid, tannin and saponin contents in chickpeas. The maximum reduction of phytic acid among different soaking mediums was resulted by vinegar solution soaking that was 30.16%. Huma et al. (2008) support the results claiming that loss is attributed to

the leaching of phytic acid in soaking solution. Like red kidney beans, maximum reduction (41.13%) of phytic acid was observed after boiling the vinegar soaked chickpeas. These results are supported by a study conducted by Sharma et al. (2013) in which they studied the effects of cooking and soaking (in distilled water, 1% citric acid and 2% $NaHCO_3$ solutions) on twenty soybean genotypes. Soaking also speeds up the activity of phytase enzyme present inside the seed (Kumar et al., 2010). Table 3 and 4 present the effect of overnight soaking and subsequent boiling of chickpeas on tannins. It is quite clear from the results that boiling after $NaHCO_3$solution and vinegar solution soaking caused the highest reduction74.10 and 72.89%, respectively. These results are in line with a similar study which reported that maximum reduction of tannins was resulted by $NaHCO_3$soaking followed by cooking (Huma et al., 2008).Overnight soaking in tap water, $NaHCO_3$ solution and vinegar solution following their boiling resulted in significant ($p<.001$) reduction of the saponins from chickpeas but vinegar and $NaHCO_3$ solution soaking prior to boiling caused most notable reduction in saponin concentration. Unlike phytic acid, saponins are heat sensitive in nature so whenever any cooking treatment is used, saponins diminish drastically or removed entirely (Shimelis & Rakshit, 2007). Previous researches are in agreement with the current study showing that cooking significantly decreased the saponins (Alajaji & El-Adawy, 2006).

Effect of processing techniques on ANFs in bengal grams

Table 5 and 6 represent the effects of soaking legumes in water, acidic and basic mediums followed by boiling on phyticacid, tannins and saponins. The maximum reduction of phytatic acid (40.47%) was achieved by boiling the overnight soaked bengal grams in vinegar solution.) Previous literature reported that soaking the legume seeds in distilled water resulted in maximum reduction of phytic acid that could be due to the availability of phytic acid as a water soluble salt (probably potassium phytate) in raw or dried legumes (Vijayakumari et al., 2007; Khattab & Arntifield, 2009). Tannin content in bengal grams was the highest among other studied legumes. All the processing techniques significantly decreased the tannin content. Tables 5 and 6 show that both the boiling after $NaHCO_3$ solution soaking and vinegar solution soaking resulted in drastic decrease of tannin content in bengal grams. A study carried out on black grams reported that soaking in $NaHCO_3$ solution resulted in 2.5 times more reduction of tannins than soaking in water (Shah, 2001). Cooking significantly reduced the tannin content in cowpeas (Kalpanadevi & Mohan, 2013). Soaking in acid or basic solutions showed greatest reduction in tannin (Sharma et al., 2013).This reduction is attributed to the formation of protein-tannin complexes during cooking or leaching of tannin in soaking solution when it is discarded (Khandelwal et al., 2010).The unprocessed bengal grams contained 84.72 mg/100 g saponins. The most drastic reduction was noted among boiling after $NaHCO_3$ solution and vinegar solution soaked bengal grams. A similar study was conducted on pigeon peas which claimed that soaking for 12 hours, dehulling and cooking showed maximum reduction of saponins. This loss is attributed to the diffusion of saponins into the soaking water as well as the thermo-labile quality of saponins (Shi et al., 2009).

Table 1 Effect of soaking technique on three anti-nutrients in red kidney beans

Anti-nutrients	Unprocessed seeds (Control)	Water soaking	Vinegar soaking	$NaHCO_3$ soaking
Phytates (mg/100 g)	630.91 ± 0.200^{a}	629.05 ± 0.614^{b}	516.54 ± 0.205^{c}	591.24 ± 0.708^{d}
% reduction	-	0.30	18.13	6.29
Tannins (mg/100 g)	713.94 ± 0.050^{a}	300.05 ± 0.080^{b}	258.44 ± 0.087^{c}	230.05 ± 0.135^{d}
% reduction	-	57.97	63.80	67.78
Saponins (mg/100 g)	93.09 ± 0.544^{a}	70.35 ± 0.199^{b}	47.89 ± 0.066^{c}	39.75 ± 0.080^{d}
% reduction	-	24.43	48.56	57.30

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 2 Effect of boiling techniques on three anti-nutrients in overnight soaked red kidney beans

Anti-nutrients	Unprocessed seeds (Control)	Water soaking + boiling	Vinegar soaking + boiling	$NaHCO_3$ soaking + boiling
Phytates (mg/100g)	630.91 ± 0.200^{a}	616.55 ± 0.872^{e}	422.28 ± 0.302^{f}	454.62 ± 0.509^{g}
% reduction	-	7.33	33.07	27.94
Tannins (mg/100g)	713.94 ± 0.050^{a}	179.73 ± 0.213^{e}	107.44 ± 0.411^{f}	94.17 ± 0.028^{g}
% reduction	-	74.83	84.95	86.81
Saponins (mg/100g)	93.09 ± 0.544^{a}	10.52 ± 0.187^{e}	3.36 ± 0.134^{f}	4.54 ± 0.270^{g}
% reduction	-	88.70	96.39	95.13

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 3 Effect of soaking technique on three anti-nutrients in chickpea

Anti-nutrients	Unprocessed seeds (Control)	Water soaking	Vinegar soaking	$NaHCO_3$ soaking
Phytates (mg/100g)	604.52 ± 1.471^{a}	549.22 ± 0.759^{b}	422.21 ± 0.390^{c}	503.29 ± 0.942^{d}
% reduction	-	9.15	30.16	16.75
Tannins (mg/100g)	396.16 ± 0.715^{a}	262.21 ± 0.005^{b}	154.71 ± 1.29^{c}	169.54 ± 0.470^{d}
% reduction	-	33.81	60.95	57.20
Saponins (mg/100g)	91.42 ± 0.467^{a}	64.43 ± 0.375^{b}	41.43 ± 0.390^{c}	55.77 ± 0.185^{d}
% reduction	-	29.52	54.68	38.99

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 4 Effect of boiling techniques on three anti-nutrients in overnight soaked chickpea

Anti-nutrients	Unprocessed seeds (Control)	Water soaking + boiling	Vinegar soaking + boiling	$NaHCO_3$ soaking + boiling
Phytates (mg/100 g)	604.52 ± 1.471^{a}	440.05 ± 1.836^{e}	355.89 ± 0.264^{f}	433.61 ± 0.321^{g}
% reduction	-	27.21	41.13	28.27
Tannins (mg/100 g)	396.16 ± 0.715^{a}	159.95 ± 0.757^{e}	107.40 ± 3.54^{f}	102.60 ± 0.330^{g}
% reduction	-	59.63	72.89	74.10
Saponins (mg/100 g)	91.42 ± 0.467^{a}	21.89 ± 0.303^{e}	13.06 ± 0.865^{f}	17.80 ± 0.537^{g}
% reduction	-	76.06	85.72	80.53

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 5 Effect of soaking technique on three anti-nutrients in bengal grams

Anti-nutrients	Unprocessed seeds (Control)	Water soaking	Vinegar soaking	$NaHCO_3$ soaking
Phytates (mg/100 g)	649.52 ± 0.261^{a}	604.35 ± 0.479^{b}	476.94 ± 0.486^{c}	564.03 ± 1.146^{d}
% reduction	-	6.95	26.57	13.17
Tannins (mg/100 g)	847.98 ± 0.062^{a}	229.60 ± 0.155^{b}	173.25 ± 1.156^{c}	204.42 ± 0.043^{d}
% reduction	-	72.92	79.57	75.89
Saponins (mg/100 g)	84.72 ± 0.545^{a}	72.90 ± 0.599^{b}	50.26 ± 0.536^{c}	42.57 ± 0.136^{d}
% reduction	-	13.95	40.68	49.75

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 6 Effect of boiling techniques on three anti-nutrients in overnight soaked bengal grams

Anti-nutrients	Unprocessed seeds (Control)	Water soaking + boiling	Vinegar soaking + boiling	$NaHCO_3$ soaking + boiling
Phytates (mg/100 g)	649.52 ± 0.261^{a}	532.40 ± 0.543^{e}	386.68 ± 1.392^{f}	459.27 ± 7.171^{g}
% reduction	-	18.03	40.47	29.29
Tannins (mg/100 g)	847.98 ± 0.062^{a}	158.61 ± 0.056^{ce}	132.27 ± 4.525^{de}	143.26 ± 0.368^{e}
% reduction	-	81.30	84.40	83.11
Saponins (mg/100 g)	84.72 ± 0.545^{a}	18.52 ± 0.087^{e}	4.49 ± 0.356^{f}	4.47 ± 0.98^{f}
% reduction	-	78.14	94.70	94.73

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Effect of processing on proximate analysis of chickpea, bengal gram and red kidney bean samples

It is evident from the results that soaking in water, $NaHCO_3$ solution and vinegar solution and boiling of respective samples significantly increased the crude protein, crude fiber, crude fat and carbohydrates, thus improving the nutritional profile of legumes while ash content was significantly ($p<0.001$) decreased following the soaking and boiling treatments. The raw or unprocessed samples of all three studied legumes contained the highest ash content that diminished following the processing. These results are consistent with previous studies that, soaking and boiling significantly decreased the ash content (Mubarak, 2005; Osman, 2007). The lower ash content after soaking may be due to the leaching out of minerals and ANFs (Fernandes et al., 2010; Shah et al., 2011). The crude protein content of all three legume samples was enhanced by soaking in tap water, vinegar solution and $NaHCO_3$ solution and subsequent boiling of these soaked samples. The maximum increase in crude protein was recorded by boiling of vinegar solution soaked chickpeas and bengal grams. The crude protein content of red kidney beans was elevated by all boiling

treatments. These results are in line with the literature which reported that soaking followed by cooking improved the crude protein content of selected legume (*B. eurycoma*) (Ramírez-Cárdenasi et al., 2008; Wang et al., 2008). Similar study reports that protein content was increased after soaking and cooking the beans without using the soaking medium (Pujolà et al., 2007). The overnight soaking in water, vinegar solution and $NaHCO_3$ solution followed by boiling significantly ($p<0.001$) boosted the carbohydrates in all three studied samples of legumes as shown in Table 7-12. A study on field peas show similar results that could be due to the diffusion of soluble solids in soaking and boiling medium as it would enhance the starch and protein content of legumes (Wang et al., 2008). A study carried out in Nigeria proved that soaking followed by boiling improves the carbohydrate content of legumes due to the breakdown of complex carbohydrates that were otherwise bound in the raw sample by boiling (Udensi et al., 2010).The crude fiber content in bengal grams, chickpeas and red kidney beans was studied. The results show that all soaking and boiling treatments significantly enhanced ($p<0.001$) the crude fiber content. In current study, unlike chickpeas and bengal grams, all treatments except vinegar solution soaking with its subsequent boiling significantly decreased the crude fat content in red kidney beans. A similar study shows that discarding the soaking water of legumes increased the fiber fractions of all legumes by virtue of soaking (Harsha et al., 2009). In current study, 3.19% and 4.15% crude fat was noted in bengal grams and chickpeas, respectively. All treatments except water soaking significantly ($p<0.001$) increased crude fat content in both seeds. Water soaking in bengal grams and chickpeas significantly ($p< 0.001$) decreased the crude fat content. A study conducted in Nigeria, found that soaking and boiling of legumes causes reduction in crude fat content (Ukom et al., 2009). While, among red kidney beans, all treatments except vinegar solution soaking and its boiling increased crude fat. Alajaji and El-Adawy (2006) found that cooking of pre-soaked chickpeas caused reduction in crude fat content. Similarly, a study conducted on horse eye beans significantly increased crude fat after water soaking (Effiong & Umoren, 2011).

Table 7 Effect of soaking technique on proximate analysis of bengal gram

	Unprocessed seeds (Control)	Water soaking	Vinegar soaking	$NaHCO_3$ soaking
Moisture	10.74 ± 0.01^{a}	ND	ND	ND
Ash	2.44 ± 0.01^{a}	2.41 ± 0.00^{b}	2.00 ± 0.005^{c}	2.16 ± 0.005^{d}
Dry matter	89.26 ± 0.01^{a}	100^{b}	100^{b}	100^{b}
Crude protein	20.68 ± 0.01^{a}	21.37 ± 0.005^{b}	21.34 ± 0.005^{b}	21.43 ± 0.01^{c}
Crude fiber	9.38 ± 0.01^{a}	9.88 ± 0.01^{b}	10.41 ± 0.01^{c}	10.55 ± 0.005^{d}
Crude fat	3.19 ± 0.005^{a}	3.09 ± 0.005^{b}	3.74 ± 0.005^{c}	3.62 ± 0.01^{d}
Carbohydrates	62.94 ± 0.02^{a}	73.11 ± 0.005^{b}	72.90 ± 0.01^{c}	72.38 ± 0.01^{d}

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 8 Effect of soaking and boiling techniques on proximate analysis of bengal gram

	Unprocessed seeds (Control)	Water soaking + boiling	Vinegar soaking + boiling	$NaHCO_3$ soaking + boiling
Moisture	10.74 ± 0.01^{a}	ND	ND	ND
Ash	2.44 ± 0.01^{a}	1.70 ± 0.01^{e}	1.74 ± 0.01^{f}	1.75 ± 0.01^{f}
Dry matter	89.26 ± 0.01^{a}	100^{b}	100^{b}	100^{b}
Crude protein	20.68 ± 0.01^{a}	21.90 ± 0.01^{d}	22.58 ± 0.01^{e}	21.57 ± 0.06^{f}
Crude fiber	9.38 ± 0.01^{a}	9.90 ± 0.005^{e}	10.12 ± 0.005^{f}	10.32 ± 0.005^{g}
Crude fat	3.19 ± 0.005^{a}	4.73 ± 0.005^{e}	5.48 ± 0.005^{f}	5.60 ± 0.01^{g}
Carbohydrates	62.94 ± 0.02^{a}	71.65 ± 0.01^{e}	72.90 ± 0.01^{f}	71.83 ± 0.05^{g}

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 9 Effect of soaking technique on proximate analysis of chickpea

	Unprocessed seeds (Control)	Water soaking	Vinegar soaking	$NaHCO_3$ soaking
Moisture	11.07 ± 0.06^{a}	ND	ND	ND
Ash	3.36 ± 0.05^{a}	2.35 ± 0.01^{b}	2.92 ± 0.03^{c}	1.82 ± 0.005^{d}
Dry matter	88.926 ± 0.06^{a}	100^{b}	100^{b}	100b
Crude protein	15.94 ± 0.005^{a}	17.98 ± 0.01^{b}	18.57 ± 0.005^{c}	18.78 ± 0.01^{d}
Crude fiber	4.65 ± 0.005^{a}	7.43 ± 0.01^{b}	4.88 ± 0.005^{c}	6.37 ± 0.005^{d}
Crude fat	4.15 ± 0.01^{a}	4.04 ± 0.01^{b}	4.16 ± 0.01^{a}	4.32 ± 0.005^{c}
Carbohydrates	65.47 ± 0.052^{a}	75.63 ± 0.02^{b}	74.34 ± 0.03^{c}	75.06 ± 0.01^{d}

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 10 Effect of soaking and boiling techniques on proximate analysis of chickpea

	Unprocessed seeds (Control)	Water soaking + boiling	Vinegar soaking + boiling	$NaHCO_3$ soaking + boiling
Moisture	11.07 ± 0.06[a]	ND	ND	ND
Ash	3.36 ± 0.05[a]	2.28 ± 0.005[e]	2.13 ± 0.03[f]	2.55 ± 0.01[g]
Dry matter	88.926 ± 0.06[a]	100[b]	100[b]	100[b]
Crude protein	15.94 ± 0.005[a]	18.85 ± 0.005[e]	19.18 ± 0.01[f]	18.28 ± 0.01[g]
Crude fiber	4.65 ± 0.005[a]	5.00 ± 0.01[e]	9.03 ± 0.005[f]	4.64 ± 0.005[g]
Crude fat	4.15 ± 0.01[a]	5.15 ± 0.005[d]	5.48 ± 0.005[e]	5.60 ± 0.01[f]
Carbohydrates	65.47 ± 0.052[a]	73.71 ± 0.01[e]	73.20 ± 0.03[f]	73.56 ± 0.01[g]

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 11 Effect of soaking technique on proximate analysis of red kidney beans

	Unprocessed seeds (Control)	Water soaking	Vinegar soaking	$NaHCO_3$ soaking
Moisture	11.71 ± 0.02	ND	ND	ND
Ash	3.97 ± 0.01[a]	3.70 ± 0.005[b]	3.64 ± 0.005[c]	3.67 ± 0.01[d]
Dry matter	88.29 ± 0.05[a]	100[b]	100[b]	100[b]
Crude protein	21.02 ± 0.01[a]	22.37 ± 0.005[b]	23.18 ± 0.005[c]	23.01 ± 0.005[d]
Crude fiber	3.60 ± 0.00[a]	3.70 ± 0.005[b]	4.44 ± 0.005[c]	3.89 ± 0.005[d]
Crude fat	1.20 ± 0.005[a]	1.08 ± 0.005[b]	1.23 ± 0.01[c]	1.18 ± 0.005[d]
Carbohydrates	62.09 ± 0.03[a]	72.83 ± 0.005[b]	71.94 ± 0.005[c]	72.13 ± 0.02[d]

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Table 12 Effect of soaking and boiling techniques on proximate analysis of red kidney beans

	Unprocessed seeds (Control)	Water soaking + boiling	Vinegar soaking + boiling	$NaHCO_3$ soaking + boiling
Moisture	11.71 ± 0.02	ND	ND	ND
Ash	3.97 ± 0.01[a]	2.48 ± 0.01[e]	2.48 ± 0.01[e]	2.48 ± 0.00[e]
Dry matter	88.29 ± 0.05[a]	100[b]	100[b]	100[b]
Crude protein	21.02 ± 0.01[a]	24.08 ± 0.01[e]	24.05 ± 0.005[f]	24.80 ± 0.01[g]
Crude fiber	3.60 ± 0.00[a]	5.10 ± 0.005[e]	4.43 ± 0.005[f]	4.21 ± 0.005[g]
Crude fat	1.20 ± 0.005[a]	0.95 ± 0.005[e]	1.23 ± 0.005[c]	1.20 ± 0.005[a]
Carbohydrates	62.09 ± 0.03[a]	72.48 ± 0.02[e]	72.23 ± 0.02[f]	71.51 ± 0.02[g]

The data are represented as a mean ± S.D of three replications. Mean in the same row followed by the same letters are not significantly different at $p<0.05$.

Conclusion

Considering all the soaking mediums (tap water, $NaHCO_3$ solution and vinegar solution) employed in the present study prior to cooking, the overnight soaking in acidic medium proved to be most beneficial in reducing phytic acid, tannins, saponins. Further, all the processing techniques decreased the ash content, whereas, crude protein, crude fiber and carbohydrates increased significantly, enhancing the nutritional quality of legumes. The saponins being heat sensitive are almost eliminated after boiling. While phytic acid was resistant as compared to tannins and saponins and showed maximum reduction when soaked in vinegar solution overnight prior to cooking. Therefore, overnight soaking in 1.5% (w/v) vinegar solution is recommended before cooking of legumes. It is suggested that the soaking medium should be discarded in order to maximally reduce or eliminate the ANFs and to maximize the utilization of protein from legumes. People from low socio-economic class who cannot afford animal protein, the ones who avoid animal sources due to social, religious or medical issue and vegetarians who totally depend on plant protein can benefit from the study.

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