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Comparison of contents of phytates and saponins and the effect of processing in some selected edible beans in Sri Lanka

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Abstract
Legumes which are important sources of proteins, minerals and vitamins, comprise significant amounts of anti-nutritional factors (ANFs) leading to limited functionality and bioavailability of proteins and minerals. This study was focused on determining the contents of phytates and saponins in eleven legume varieties in Sri Lanka and the effect of processing on their contents. Eleven legume varieties, Mung bean (MI5, MI6), Cowpea (Waruni, MICP1, Bombay, Dhawala, ANKCP1), Soybean (MISB1, Pb1) and Horse gram (ANK Black, ANK Brown) were analyzed to determine the phytate contents on eluted acid extracted fraction from anion exchange chromatographic technique followed by spectrophotometrical determination while saponins by double solvent extraction gravimetric method. Soya bean (Pb1) had the significantly ($p \leq 0.05$) highest initial phytate content of (9.12 ± 0. 61 mg/g) and horse gram (ANK Black) had the lowest phytate content (2.60 ± 0. 26 mg/g). Saponin content ranged from (8.01 ± 0.70 mg/g) in soyabean (Pb1) to (12.76 ± 1.28 mg/g) in soyabean (MISB1). Processed legumes, soaking followed by autoclaving, showed a reduction in phytate and saponin contents ranging from 8.34% - 33.05 % and 6.35% - 72.02% respectively.

Keywords: Legumes, phytates, saponins, autoclaving, antinutritional factors

1. Introduction
Legumes which are commonly known as the poor man's meat, is consumed by millions of people across the world especially in the developing countries where legumes are used as meat, egg, cheese analogues (Shashi Kiran Misra, 2012) [26]. While being important sources of macro nutrients such as proteins, resistant starch etc, the prominent sources of dietary fiber, essential amino acids, poly unsaturated fatty acids and minerals of legumes contribute to a wide array of phytochemicals or antinutritional factors (Awoyinka. *et al*., 2016) [1]. Phytochemicals with health protective functional properties comprise of dietary fibre, antioxidants, detoxifying agents, immunity potentiating agents and neuro-pharmacological agents (Shashi Kiran Misra, 2012) [26].

There are varieties of legumes cultivated in Sri Lanka; Cowpea (*Vigna unguiculate*), mung bean (*Vigna radiate wilczek*), soybean (*Glycine max L.*), groundnut (*Arachis hypogaea L.*) and black gram (*Vigna mungo L.*).

Phytic acid (phytate; myo-inositol 1, 2, 3, 4, 5, 6, hexakisphosphate) which is one of the major anti nutritional factors present in legumes and a common constituent of plant derived foods, is the primary source of inositol and storage phosphorus in plant seeds contributing to approximately 70% of total phosphorus (Greiner *et al.*, 2006) [13]. Inositols with 4, 5 or 6 phosphate groups are common in the seed of many of our grain legume and lead to concentration higher than 10% of dry matter (Bora, 2014) [5]. Phytate is regarded as a major ANF as it chelates divalent cations such as Ca^{2+}, Zn^{2+},Mg^{2+}, Mn^{2+}, $Fe^{2+/3+}$ thereby reducing their bioavailability (Dahiya, 2016) [8]. Many phytate-mineral complexes are insoluble and therefore they may become unavailable for absorption under normal physiological condition. Also it was reported that phytates can affect the digestibility of protein and solubility of starch. Phytates are ionic in nature (Chitra, 1994) [7] and can react with charged groups in proteins. Sometimes mineral ions like Ca^{2+} can mediate this reaction (Chitra, 1994; Reddy and Sathe, 2002) [7, 23]. The resultant complexes of phytate-protein and phytate-mineral-protein may adversely influence the protein digestion and bioavailability. Phytates can also bind with starch through phosphate groups or can bind indirectly through protein resulting in a decrease in starch solubility and digestibility (Reddy and Sathe, 2002) [23]. Dietary phytate was reported to prevent kidney stone formation, protect against diabetes mellitus, caries, atherosclerosis coronary heart diseases and cancers. Degradation of phytates can occur due to enzymatic and non enzymatic processes. Phytic acid is hydrolysed to lower inositols by phytases or by chemical means (García-Estepa *et al*., 1999) [12].

Saponins are amphiphilic compounds, with the presence of a lipid-soluble aglycone and water soluble chain(s) in their structure. It is found in plant tissues that are most vulnerable to fungal or bacterial attack or insect predation (Cheok *et al*., 2014) [6]. They exhibit surfactant properties as a result they show foaming action upon shaking in an aqueous solution. Saponins are divided into two groups: Steroidal saponins, which occur as glycosides in certain pastures plants and triterpenoid saponins, which occur in soybean (Das *et al*., 2012) [9].

Certain evidences show that saponins provide neuro protective effects on attenuation of central nervous system disorders, such as Parkinson's disease, stroke, Huntington's disease and Alzheimer's disease, along some *in-vivo* studies showing saponins have tumor-inhibitory effects and antifungal activity (Jiayi, 2016) [16]. The presence of saponins in soybean has

attracted considerable interest owing to health benefits while having adverse sensory characteristics (Omizu *et al*., 2011) [21]. There are many chemical and physical processes employed in domestically as well as in industrially to eliminate or to reduce the antinutrtional factors. Some basic processing techniques include soaking, cooking, autoclaving, fermenting, germination etc. individually at many occasions a combination of the above methods are used for effective elimination or the reduction of anti-nutritional factors (Shashi Kiran Misra, 2012) [26].

2. Materials and Methods

2.1 Chemicals

Anion exchange resin (AG 1- X 4 Chloride form, 100-200 mesh) from Bio-Rad Laboratories, Inc. was used for the purification purpose in phytate analysis and the other reagents with analytical grade. KH_2PO_4 was used to plot the Phosphorus standard curve.

2.2 Materials

In this study, two varieties from Mung bean (MI5 and MI6), five varieties from Cowpea (ANKCP1, MICP1, Bombay, Wauni and Dhawala), two varieties from Soybean (Pb1and MISB1) and two varieties from Horse gram (ANK Black, ANK Brown) recommended by the Department of Agriculture, Sri Lanka were selected. These eleven legume varieties were obtained by random sampling method under same field practice and similar environmental conditions from Angunakolapelessa, Grain Legumes and Oil Seed Crops Research and Development Centre (GLOSCRDC). Samples were stored in the cold room at 10^0C until further usage.

2.3 Sample preparation

Cleaned and dried whole legume seeds were ground with a RETSCH S/S CROSS BEATER Hammer Mill Sk1 to 0.5 mm (500 µm) sieve size and the flour was packed in an air tight polythene bag until further usage.

2.4 Determination of moisture content

The moisture content was determined using oven drying method as described by AOAC, 2012. (Method No. 925.09B).

2.5 Determination of phytate content

Anion exchange chromatographic chromatographic technique followed by spectrophotometrical method as described by AOAC 2012 in method 986.11 which has been used in determining the phytate content in the legumes.

A glass column about 0.7mm x 30mm with a valve filled with anion exchange resin AG 1- X 4 Chloride form, 100-200 mesh was used in the analysis. The recovery of the column was tested using standard Sodium phytate solution of concentration 2.8 µg/ml.

Test portions of 1ml of of acid extracted fraction of dry legume flour using dilute HCl (2.4%, 3 h), were mixed with 1ml of Na_2EDTA-NaOH (10.23g of Na2EDTA in 7.5g NaOH in 250ml solution) solution and placed on an ion exchange column. The fraction containing phytate which was eluted with 0.7M NaCl was wet digested with a mixture of concentrated acids H_2SO_4 and HCl to release inorganic Phosphorus which was measured colourimetrically at 640nm using UV-VIS spectrophotometer. Standard curve plotted using Standard phosphate solution (Primary standard KH_2PO_4, 80µg/ml) was used in determining the content of phytates.

2.6 Determination of saponins

Saponin content of legume flour was determined using the double solvent extraction technique followed by gravimetric method as described by Poornima and Ravishankar Rai, 2009. 2.00g of the test sample that was extracted twice using 20ml of 20% ethanol aqueous and the combined extraction was reduced to 5ml over a water bath at about 90ºC, was shaken with 10ml of diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 6 ml of n-butanol extracts were washed twice with 1 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven at 105ºC into a constant weight. The saponin content was calculated in percentage.

2.7 Processing of legumes

20-25g of the legume seeds were weighed into a beaker. The weighed sample was cleaned with tap water and soaked in distilled water of 1:10 ratio (sample/distilled water) for 10 h.

2.7.1 Autoclaving

The sample was rinsed several times with water and autoclaved at 121^0 C for 15 minutes at 15psi. After autoclaving the samples were dried using a domestic dehydrator at 40^0C till constant weight was obtained.

2.7.2 Sample preparation

After complete drying the samples were grounded into powder using hammer mill (0.5mm sieve size). The samples were sealed and stored in the cold room at 10^0C until further use.

Moisture content (AOAC 2012, Method No. 925.09B), phytate content (AOAC 2012 method No. 986.11), saponin contents (double solvent extraction technique followed by gravimetric method) of processed legumes were determined using the respective methods as given in in 2.4, 2.5 and 2.6 as previously discussed.

2.8 Statistical analysis

The calculations were done on dry basis. All the data were analyzed using parametric tests by one way analysis of variance (ANOVA) and all comparisons were done by paired sample t test using Minitab 17 software. All test procedures were made at 5% significant level ($p \leq 0.05$). Microsoft excel 2013 has been used for graphical illustration of data.

3. Results and Discussion

3.1 Determination of Phytates

The amount of phosphorus recovered by an anion exchange column using the working standard solution (2.8 µg/ml KH_2PO_4) at different amounts of anion exchange resin is tabulated in Table 1. Recovery values were ranged from 55.00-96.00 % with varying amount of resins in present study showing a wide range. Results were shown that the amount of resins used were highly dependent on the amount of recovery, while was a significant difference ($p \leq 0.05$) among the recovery values with respect to the amount of resins. Significantly highest ($p \leq 0.05$) result for recovery was obtained at 2.00 g of resin where the recovery was 96.00%. As such 2.00 g of resin has been used throughout the analysis.

Table 1: Determination of Column recovery of Phosphorus

Amount of resin (g)	% of recovery of KH_2PO_4
1.00	55.00 ± 2.36% [d]
1.50	79.41 ± 2.20% [c]
1.70	84.00 ±1.81% [b]
2.00	96.00 ± 0.79% [a]

Note: results are expressed as mean ± standard deviation of triplicates and Means that do not share a same letter are significantly different ($p \leq 0.05$)

In this study, the value for the phytate and saponins contents are tabulated in Table 2. The highest value for phyates was observed in Soyabean Pb1 variety with 9.12 ± 0.61 mg/g and the lowest was observed in Horse gram, ANK Black with 2.60 ± 0.26 mg/g which is in accordance with the range stated by Deshpande *et al*., 1982 where the phytate content of legumes varies from 4.0 mg/g - 20.0 mg/g. The values for phytates in Pb1 and MISB1 are significantly higher ($p \leq 0.05$) than those of other varieties. While the means of Pb1 and MISB1 were not significantly different ($p > 0.05$).

There was no significant difference ($p > 0.05$) of phytates contents between Bombay and Waruni as well as between Dhawala and MICP1, similar observations for phytate contents in Cowpea varieties were shown by Farinu and Ingrao, (1991) [11] where phytic acid content of cowpea showed large varietal differences ranged from 5.1 mg/g to 10.27 mg/g. It was observed that there was no significant difference ($p > 0.05$) between the means of MI5 and MI6 of mung bean varieties and ANK Brown of horse gram. According to Reddy and Sathe, (2002) [23], the phytate content for Soyabean can range from 1.00 – 2. 22%, Green gram 0.59-1.10%, Cowpea 0.37-1.45%. Chitra, 1994 [7] stated that phytic acid was high in soya bean varieties 36.4 mg/g followed by black gram13.7 mg/g and Mung bean 12.0 mg/g. Present finding regarding phytate contents of mung bean, cowpea, soybean were in conformity with values described in previous literature, however slight variations may be due to genotype and environmental conditions (M. M. N. Qayyum *et al.*, 2012) [27] variety or cultivar, climatic conditions, location, irrigation conditions, type of soil and year during which they are grown (Bassiri and Nahapetian, 1977) [3]

In legumes, phytate is distributed throughout the cotyledon and located within the subcellular inclusions of protein bodies. It has been stated that 99% of the phytate in dry peas was in the cotyledons and 1% in the embryo axis. Phytate phosphorus represents about 65% of the total phosphorus in the cotyledons and 20% of the total phosphorus in the embryo axis. The hull or seed coat fraction contain little or no phytate (Chitra, 1994) [7].

3.2 Determination of Saponins

According to the results obtained for Saponins MISB1 had the highest saponin content of 12.76 ±_0.83 mg/g whereas the lowest value for saponin content was found to be in Waruni with 7.06 ± 1.04 mg/g. Values obtained were within the range stated by Santosh Khokhar, (2009) [25] where saponin content can vary between 0.5% and 5% dry weight, with soybean had the most important dietary source for saponins. Further there was significant difference among the saponin contents of all legume verities ($p \leq 0.05$). There was a significant difference ($p \leq 0.05$) existing between the saponin contents of soyabean Pb1 and MISB1 varieties. The values obtained from the present study were in accordance to the values obtained by Price *et al.*, 1986 where the soybean saponin content can range from 5.6 to 56 mg/g but those values were contradictory to the literature stated by Loren Cordain, 2015 where soyabean saponin concentration can range up to 4.04 mg/g, green gram upto 0.50 mg/g and kidney beans upto 3.50 mg/g, Okwu *et al*., 2007, stated that saponin contents of *Vigna unguiculata* and *Glycine max* can be within (0.11-0.23 mg 100 g^{-1}). MISB1 and MI6 varieties had saponin contents which were significantly higher ($p \leq 0.05$) than other varieties. The results obtained for mung bean were contradictory to the results obtained by Price *et al.*, where Mung bean saponin content can range from 0.50 – 5.70 mg/g. There is no significant difference ($p > 0.05$) between cowpea varieties of MICP1, Bombay and Dhawala which were again contradicting with the results obtained by Bala, 2012 where the saponin content of cowpea ranges from 0.2 to 0.8 mg/g.

Since being a gravimetric technique of determination of saponins with a high initial sample weight may had produced more accurate readings. The difference in varieties used, the agronomical climate and location may have an influence over the variation in saponin contents.

Table 2: Phytates and saponin content of legumes

Name of Variety	Phytates mg g^{-1} Mean ± SD	Saponin mg g^{-1} Mean ± SD
Soya bean		
Pb1	9.12 ± 0.61 [a]	8.01 ± 0.70 [bc]
MISB1	8.19 ± 0.03 [ab]	12.76 ± 0.83 [a]
Cowpea		
Waruni	3.56 ± 0.14 [ef]	7.06 ± 1.04 [c]
MICP1	6.09 ± 0.19 [bcd]	9.87 ± 3.56 [abc]
Bombay	3.65 ± 0.45 [ef]	9.25 ± 0.75 [abc]
Dhawala	6.88 ± 1.23 [bc]	8.76 ± 0.60 [abc]
ANKCP1	5.50 ± 0.03 [cde]	8.44 ± 0.96 [bc]
Mung bean		
MI5	4.44 ± 0.96 [def]	11.83 ± 0.66 [ab]
MI6	3.91 ± 0.09 [def]	12.59 ± 1.33 [a]
Horse gram		
ANKBack	2.60 ± 0.26 [f]	11.52 ± 0.78 [ab]
ANK Brown	4.55 ± 0.55 [def]	10.06 ± 0.73 [abc]

Note: results are expressed as mean ± standard deviation of triplicates and Means that do not share a same letter are significantly different ($p \leq 0.05$)

3.3 Determination of moisture content in processed legumes

There is a significant difference ($p \leq 0.05$) in moisture contents of eleven legume varieties after processing. The highest moisture content has been observed in Dhawala with 11.22 + 0.035% and the least from ANK Black with 9.20 + 0.05%. Moisture content of Dhawala is significantly higher ($p \leq 0.05$) than the other varieties.

3.4 Determination of phytate content in processed legumes

The phytate content in processed legume samples ranged from 2.44 ± 0.00 mg/g in ANK Black - 7.51 ± 0.04mg/g in MISB1. There was a significant difference ($p \leq 0.05$) in the phytate contents of the processed legume varieties. There was significant difference ($p > 0.05$) existing among MICP1, Waruni and ANKCP1 varieties. The phytate contents in Pb1 and MISB1 varieties were significantly higher ($p \leq 0.05$). There was no significant difference ($p > 0.05$) between MI5 and MI6 varieties if Mung bean. In this study the highest reduction in phytates was observed in Dhawala with 33.05%. The lowest content of phytic acid was observed to be present in MISB1with 8.34% after processing**.** As dry beans have tough seed coats that are not highly permeable to water. In household situations legumes are soaked in water overnight for a period of 12 – 14 hours. Since phytate is water soluble significant portion of phytate is removed when soaked water is discarded. If salts, alkalies and acids are added to the soaking medium the rate of water imbibition can change significantly. (Reddy and Sathe, 2002) [23].

There was an overall of a significant difference ($p \leq 0.05$) of phytates between processed and unprocessed legumes. It was observed that germination and fermentation were the most effective methods of lowering phytic acid of green gram and soyabean genotypes. In this study the reduction of phytates in soyabean was from 8.34% to 30.01%, in Cowpea 21.01% - 33.05%, Mung bean 15.32% - 19.95%, Horse gram 14.467% - 30.00%. Similar results for reduction of phytates of 30.8% in soyabean after autoclaving was observed by Chitra, 1994 [7]. Reddy & Sathe, 2002 [23] stated that autoclaving of soyabeans for 15 to 20 minutes at 105-121^{0}C led to a loss from 6.4-30.1%, for cowpeas the loss was 5.4 to 27.9% which is in accordance with the results obtained in the study, for Mung beans the loss was 17.6-18.0% and results obtained by Chitra, 1994 [7] where autoclaving and roasting of green gram genotypes decreased phytate by 17-40% and 15-21%. Both, wet-heating and dry-heating also reduced the phytic acid levels in these legumes ranging from 15-51%.

During soaking certain water soluble and nutritionally important minerals and vitamins may also be lost to the soaking water. If soaked water is discarded, certain undesirable components such as flatulence-causing oligosaccharides, phytate and tannins too are removed. (Chitra, 1994) [7]. Since phytate is water soluble significant portion of phytate is removed when soaked water is discarded. If salts, alkalies and acids are added to the soaking medium the rate of water imbibition can change significantly. Soaking temperature too has a significant effect of removal of phytates due to activation of endogenous phytases and acid phosphatases (Reddy & Sathe, 2002) [23].

Phytic acid has been implicated in influencing the cooking quality of legumes. Phytic acid chelates divalent cations (Ca, Mg) and prevents their crosslinking with pectin, thereby facilitating cell wall dissolution during the cooking process (Moscoso *et al.*, 1984) [19]. Because phytate is heat stable significant phytate reduction during cooking is not expected unless either cooking water is discarded or the food receives some additional processing treatment such as soaking, germination, fermentation etc.

3.5 Determiation of saponins contents in processed legumes

The values for saponins in processed legumes ranged from 3.05±0.52 mg/g - 15.12±0.61mg/g in MISB1. There was a significant difference among the saponin contents on processed legume varieties ($p \leq 0.05$). The saponin content in MISB1 was significantly higher ($p \leq 0.05$). In this study the highest reduction in saponins was observed in ANK Black with 72.018% and least in MISB1 with 6.350%. There was a significant reduction ($p \leq 0.05$) in saponins contents of the processed legumes. The results of saponin reduction as a percentage after autoclaving is within the range stated by Raquel G., (1996) where the cooking of legume reduced the amount of saponins by 7 – 53%. And autoclaving of legumes reduced the amount of saponins by 40%, while soaking did not modify the saponin content or composition of chickpeas and lentils regardless of the pH of the soaking solution.

Since saponins are water soluble they can be removed easily during soaking by osmosis. But the amount of saponin removed will vary depending on the toughness of seed coat, hours of soaking, the soaking media, the cultivar etc.

Table 3: Phytates and saponin contents in processed legumes

Variety	Phytates mg g^{-1} Mean ± SD	Reduction in phytic acid %	Saponin mg g^{-1} Mean ± SD	% reduction in saponin content
Soya bean				
Pb1	6.38 ± 0.78 ^{a}	30.01%	8.80± 0.22 ^{c}	14.34%
MISB1	7.51 ± 0.04 ^{ab}	8.34%	15.12±0.61 ^{a}	6.35%
Cowpea				
Waruni	2.49 ± 0.10 ^{ef}	30.00%	3.39 ±1.46 ^{i}	52.22%
MICP1	4.65 ± 0.97 ^{bcd}	23.67%	6.14± 0.01 ^{e}	37.77%
Bombay	2.74 ± 0.14 ^{ef}	25.01%	5.88 ± 0.37 ^{f}	36.48%
Dhawala	4.61 ± 0.08 ^{bc}	33.05%	10.26 ±0.11 ^{b}	14.64%
ANKCP1	4.34 ± 0.43 ^{cde}	21.01%	5.58 ± 0.41 ^{g}	33.83%

Mung bean				
MI5	3.76 ± 0.28 ^{def}	15.32%	5.50±0.68 ^{h}	53.47%
MI6	3.13 ± 0.19 ^{def}	19.95%	6.56±0.46 ^{d}	47.88%
Horse gram				
ANK Black	2.44 ± 0.00 ^{f}	30.00%	3.22± 0.30 ^{j}	72.02%
ANK Brown	4.60 ± 0.08 ^{def}	14.47%	3.05±0.52 ^{k}	69.73%

Note: results are expressed as mean ± standard deviation of triplicates and Means that do not share a same letter are significantly different ($p \leq 0.05$)

4. Conclusion

This study revealed that soaking and autoclaving are appropriate techniques to remove phytochemicals such as phytate and saponins effectively from the edible legume beans. After processing the highest reduction in phytate was observed in Dhawala with 33.05% and lowest reduction in original phytate content in MISB1 variety with 8.34%. Reduction in saponin was highest in ANK Black 72. 02 % and least in MISB with 6.35%.

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6. Author's contributions

In designing the study and revised the manuscript was done under the supervision of Dr. J. Wansapala and Dr. T. Herath. K. Sivakumaran carried out the experimental works and statistical interpretations.

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