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TOTAL PHOSPHORUS, PHYTATE PHOSPHORUS CONTENTS AND THE CORRELATION OF PHYTATES WITH AMYLOSE IN SELECTED EDIBLE BEANS IN SRI LANKA

Keerthana Sivakumaran, Jagath Wansapala, Theja Herath

ABSTRACT

Phytate a major anti nutritional factors in legumes and it accounts for larger portion of the total phosphorus, while limiting the bioavailablity of certain divalent cations to the human body. Legumes of eleven varieties cultivated in Sri Lanka, Mung bean (MI5, MI6), Cowpea (Waruni, MICP1, Bombay, Dhawala, ANKCP1), Soybean (MISB1, Pb1) and Horse gram (ANKBlack, ANKBrown) were analyzed for phosphorus content and phytate content. Total phosphorus content was quantified by dry ashing followed by spectrophotometrical measurement of the blue colour intensity of acid soluble phosphate with sodium molybdate in the presence of ascorbic acid while phytate phosphorus using anion exchange chromatographic technique followed by spectrometrical measurement of the digested organic phosphorus and amylose content by Simple Iodine-Colourimetric method. Where the least value for phosphorus was observed 275.04 ±1.44 $mg.100g^{-1}$ in ANKBlack (Horse gram) and the highest in MISB1 (Soyabean) with 654.94 ±0.05 $mg.100g^{-1}$. The phytate phosphorus content (which is a ratio of phyate to total phosphorus) was highest in Dhawala (Cowpea). The phytate phosphorus (which is a ratio of phyate to total phosphorus) was highest in Dhawala with 67.42% and least in Bombay (Cowpea) with 24.87%. The amylose content of the legumes was least in Pb1 with 8.71 ±0.13 $mg.100mg^{-1}$ and the highest in MI6 22.58 ±0.71 $mg.100mg^{-1}$. The correlation between phyate and total phosphorus was significant ($p \leq 0.05$) and positive (r = 0.62). Similarly the correlation coefficient for phytate phosphorus and total phosphorus was significant ($p \leq 0.05$) and positive (r = 0.63). Amylose content of legumes was significantly correlated negatively ($p \leq 0.05$) with the total phytates content (r = -0.82).

Keywords: phytates; phosphorus; amylose; phytate phosphorus; legumes

INTRODUCTION

Phosphorus which is an essential mineral is important for human health and optimal livestock production. Phytic acid (phytate; myo-inositol 1,2,3,4,5,6, hexakisphosphate) is one of the anti-nutritional factors (ANFs) among naturally occurring constituent of plant seeds, roots, tubers, and some fruits and vegetables and it acts as a storage form of phosphate **(Reddy and Sathe, 2002)**. In seed and grains phytate is accumulated within subcellular single membrane particles, aleurone grains or protein bodies. Legumes are the richest source of macro nutrients such as protein, starch and micronutrients minerals and vitamins while they contribute to important health protective compounds such as phenolics, inositol phosphates and oligosaccharides. **Lolas and Markakis (1975)** stated that phytate accounts for 80% of the total phosphorus in most legumes. The recommended average daily intake of phytate for humans on vegetarian diets, is 2000 – 2600 mg, for inhabitants of rural areas in developing countries, on mixed diets, it is 150 – 1400 mg **(Greiner, 2006)**. Presence of phytates is of a major concern in the foods and animal feeds industries because the phosphorus in this form is unavailable to monogastric animals due to a lack of endogenous intestinal phytases; enzymes specific for the dephosphorylation of phytic acid **(Greiner, 2006)** .In poultry rearing where sufficient dietary intake of phosphorus is maintained for reducing phosphorus intake in poultry manure. In addition, the strong chelating characteristic of phytic acid which works on a broad pH range reduces the bioavailability of other essential dietary nutrients such as minerals (e.g. Ca^{2+} ,Zn^{2+},Mg^{2+}, Mn^{2+}, $Fe^{2+/3+}$, proteins and amino acids **(García-Estepa et al., 1999; Azeke, 2010)**. Phytate occurs primarily as Potassium-Magnesium salt in rice, beans, sesame seeds and as a Calcium- magnesium-Potassium salt in soyabeans. Phytic acid is hydrolysed enzymatically by phytases. Apart from the binding divalent cations dietary

phytic acid has beneficial effects by acting as antioxidant or anticancer agent **(Raboy, 2001)**.

Scientific hypothesis

Usually legume based food (cooked) items contain higher amounts phytate than the cereal-based food items. Few food items, such as sesame seeds (toasted), soy protein concentrate, rice (unpolished and cooked), maize bread (unleavened) and peanuts have exceptionally high amounts of phytate **(Dahiya, 2016)**. As such the aim of this study is to determine the correlation between Phosphorus content and the phytate contents in legumes, correlation between the phytate phosphorus and the total phosphorus content as well as the correlation between Amylose and total phytates in some commonly consumed legumes in Sri Lanka.

MATERIAL AND METHODOLOGY

Chemicals

Anion exchange resin (AG 1- X 4 Chloride form, 100-200 mesh) and the other reagents with analytical grade.

Materials

In this study, two varieties of mung bean (MI5 and MI6), five varieties from cowpea (ANKCP1, MICP1, Bombay, Wauni and Dhawala), two varieties from soybean (Pb01and MISB1) and two varieties from horse gram (ANKBlack, ANKBrown) recommended by the Department of Agriculture, Sri Lanka were selected. These eleven legume varieties were obtained by random sampling method under same field and similar environmental conditions from Angunakolapelessa, Grain Legumes and Oil Seed Crops Research and Development Centre, which is the main agriculture research centre located in Southern Dry Zone of Sri Lanka. Samples were stored in the cold room at 10 ˚C till further usage.

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 till further usage.

Determination of phytate phosphorus content

Anion exchange method described by **AOAC (2012)** in method 986.11 was used in determining the phytate content in the legumes.

A glass column about 0.7 mm x 30 mm with a valve with anion exchange resin AG 1 – X 4 Chloride form, 100 – 200 mesh was used in the determination of phytate phosphorus content.

Phytate extracted from duplicate test portions of dried legume flour using dilute HCl (1 mL), mixed with 1 mL Na_2EDTA-NaOH solution and placed on an ion exchange column, the elute was discarded. Then the column was eluted with 15 mL of distilled water followed by 0.1 M NaCl respectively. Both elutes were discarded. Finally the column was eluted with 15 mL of 0.7 M NaCl and the fraction was collected to a digestion tube. 0.5 mL of concentrated H_2SO_4 and 3 mL of concentrated HNO_3 were added to the tube and digested on a kjeldhal block at 250 ˚C until yellow fumes evolved. The boiling was continued until clear solution was obtained. When the flask was cooled 10ml of distilled water was added and heated for 10 minutes at low heat. After cooling the contents of the tube was transferred to a 50 mL volumetric flask followed by addition of 2ml of molybdate solution and 1ml sulfonic acid make up to the mark and mixed well. After 15 minutes absorbance was measured at 640nm.

The recovery of the column has been tested using standard Sodium phytate solution of concentration 2.8 µg.mL^{-1}. Triplicate samples were done with the standard phytate solution to test the recovery of the column. Standard curve plotted using Standard phosphate solution (Primary standard, 80 µg.mL^{-1} KH_2PO_4) was used in determining the phytate phosphorus content.

Determination of total phosphorus

Initially a standard curve for Phosphorus was plotted using 0.01 mg P.mL^{-1} standard phosphorus (KH_2PO_4) solution. Phosphorus (total) in foods method described in **AOAC (2012)** (method 995.11) was used to analyse the phosphorus content in eleven legume varieties. Flour (1.5 g) was weighed into a crucible and 0.5g of Zinc oxide was added and mixed. Then the samples were ashed in the muffle furnace at 550 ˚C for 4 hours. Then the crucibles were removed from the furnace and let to cool. To the cold crucibles, 5 mL of water, and 5 mL of concentrated HCl were added. The crucibles were covered with watch glass and boiled for 5 minutes in a water bath. The contents of the crucibles were filtered into a 100ml volumetric flask and rinsed the crucibles and watch glass with hot water through the filter into the flask. After cooling the flask to room temperature, 50% KOH was added until the solution was slightly opalescent. HCl was added until the opalescent disappears. The solution was cooled to room temperature and diluted to the volume with water. Then 10 mL of the solution was transferred into a 100 mL volumetric flask and diluted to the mark. Then 5 mL of the diluted solution was transferred to a 50 mL volumetric flask and 15 mL of deionized water was added. Then 20 mL of molybdate ascorbic acid solution prepared immediately before use, 25 mL of sodium molybdate solution and 10 mL of ascorbic acid solution was transferred to a 100 mL volumetric flask, the solution was mixed and diluted to markwas added and swirled. The flasks were loosely stoppered and placed in a metal basket. The metal basket was placed in vigorously boiling water bath for 15 minutes. Then the flasks were cooled under the tap water and diluted to the volume with deionized water. Absorbance was measured at 823nm.

Determination of amylose content

Initially a standard curve for Amylose was plotted using Standard potato amylose solution (0.40 mg.mL^{-1}). Powdered sample of the legume variety (particle size 0.5 mm, 100 mg) was precisely measured into an Erlenmeyer flask (100 mL). ethy alcohol (95%, 1 mL), NaOH (1 N, 9 mL) were added to the flask and boiled to gelatinize for 10 minutes in boiling water bath. The solution was cooled to room temperature and was transferred into a volumetric flask (100 mL) with two successive washings. An aliquot (5 mL) was transferred into a volumetric flask (100 mL).

Acetic acid (1 N, 1 mL) and Iodine/Potassium Iodide (2 mL) were added. The solution of each flask were diluted to 100 mL mark with distilled water. Meanwhile blank was prepared without sample with other same conditions. After stabilizing the samples at 30 °C, the absorption was measured 620 nm using UV-spectrophotometer.

Statistical analysis

All the data were analyzed using parametric tests. The data were statistically evaluated by one way ANOVA 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. The correlation between phosphorus and phytate contents were determined using Pearson's correlation test.

RESULTS AND DISCUSSION

Total phosphorus content in legumes

Legume sample is dry ashed to remove any organic compounds. Acid soluble phosphate forms a blue complex with Na_2MoO_4 in the presence of ascorbic acid as the reducing agent. Intensity of the blue colour is determined spectrophotometrically.

The phosphorus content of the legume varieties ranged from 275.04 ±1.44 mg.g^{-1} in ANKBrown to 654.94 ±0.05 in MISB1. There was a significant difference ($p \leq 0.05$) among the phosphorus content of the legume varieties (Refer to Tables). There was a significant difference ($p \leq 0.05$) in phosphorus contents between soya bean varietals of Pb1 and MISB1. There was no significant difference ($p > 0.05$) existing between Waruni and MICP1 varieties of Cowpea, while there was significant difference ($p \leq 0.05$) among Bombay, Dhawala and ANKCP1. According to **Ravindran et al. (1994)** it was reported that the phosphorus content of soyabeans, cowpea and green gram were 600 mg.100g^{-1}, 390 mg.100g^{-1} and 380 mg.100g^{-1} respectively which are in accordance to the results obtained. There was a significant difference ($p \leq 0.05$) between MI5 and MI6, and similarly ANKBlack and ANKBrown. According to **Vitorello et al. (2002)** grain phosphate content can vary depending the dose of fertilizer phosphorus and the difference in genotypes.

Phytate phosphorus contents of legumes

It was shown in the table the phytate phosphorus content of the legume varieties were significantly different ($p \leq 0.05$) from each other. The phytate phosphorus content ranged from 103.056 ±10.255 mg.g^{-1} in ANKBlack to 334.545 ±13.397 mg.g^{-1} in Pb1. There was no significant difference ($p > 0.05$) between the phytate phosphorus contents of Soya bean varieties Pb1 and MISB1 varieties. Meanwhile there was significant difference ($p \leq 0.05$) between the Cowpea varieties Dhawala and ANKCP1. There was no significant difference ($p > 0.05$) between Mung Bean varieties of MI5, MI6, and the cowpea varietals of Dhawala, Bombay and ANKBlack of Horse gram. The study of **Ologhobo and Fetuga (1982)** indicated that generally phytic acid phosphorus represented 31.3 – 59.4% of total phosphorus with an average of 47.2%. These results are partly consistent with a view that phytic acid is the principal form of phosphorus in many seeds and that about 40 – 80% of the total phosphorus contents of dry legume seeds are in the form of phytic acid phosphorus **(Lolas and Markakis, 1975)**.

Phytic acid is the principal form of phosphorus in many seeds and that about 40 – 80% of the total phosphorus contents of dry legume seeds are in the form of phytic acid phosphorus **(Lolas and Markakis, 1975)**. **Ologhobo and Fetuga (1982)** indicated that the soybean dry seeds were the richest source of phytate (1.47% dry weight basis) followed in descending order by cowpeas (1.37%). The ratio of phytate phosphorus as percentage of total phosphorus was highest in soybeans.

There was a significant, ($p \leq 0.05$) positive correlation between phosphorus and phytate contents with the correlation coefficient of 0.62 as shown in Figure 1. According to the findings of **Chitra (1994)**, there was a significant positive correlation (r = 0.99) between phytic acid and total phosphorus content in all the legumes.

Table 1 Phosphorus and phytate phosphorus.

Nameof Variety	Phosphorus mg.100g^{-1} ±SD	Phytate Phosphorus mg.100g^{-1} ±SD	Phytate P as a % of total phosphorus
Soya bean			
Pb1	573.70 ±3.37 ^{b}	334.55 ±13.40 ^{a}	58.31%
MISB1	654.94 ±0.05 ^{a}	286.23 ±0.92 ^{a}	43.70%
Cowpea			
Waruni	443.19 ±0.00 ^{d}	117.79 ±4.63 ^{d}	26.58%
MICP1	441.44 ±1.77 ^{d}	241.26 ±7.65 ^{b}	54.65%
Bombay	544.71 ±1.89 ^{c}	135.45 ±4.58 ^{d}	24.87%
Dhawala	377.70 ±0.31 ^{g}	254.64 ±23.27 ^{b}	67.42%
ANKCP1	427.45 ±0.00 ^{e}	192.14 ±0.95 ^{c}	44.95%
Mung bean			
MI5	373.09 ±0.64 ^{g}	131.83 ±28.48 ^{d}	35.33%
MI6	405.63 ±3.16 ^{f}	119.32 ±7.00 ^{d}	29.42%
Horse gram			
ANKBlack	284.49 ±4.66 ^{h}	103.06 ±10.26 ^{d}	36.23%
ANKBrown	275.04 ±1.44 ^{i}	159.12 ±19.10 ^{cd}	57.85%

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

Table 2 Amylose contents of legumes.

Name of variety	Amylose mg per 100mg sample ±SD
Soya bean	
Pb1	8.71 ±0.13 [e]
MISB1	8.99 ±0.18 [e]
Cowpea	
Waruni	20.85 ±0.16 [b]
MICP1	18.56 ±0.41 [c]
Bombay	21.02 ±0.19 [b]
Dhawala	20.60 ±0.22 [b]
ANKCP1	20.06 ±0.25 [b]
Mung bean	
MI5	22.24 ±0.91 [a]
MI6	22.58 ±0.71 [a]
Horse gram	
ANKBlack	20.10 ±0.04 [d]
ANKBrown	19.23 ±0.04 [d]

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

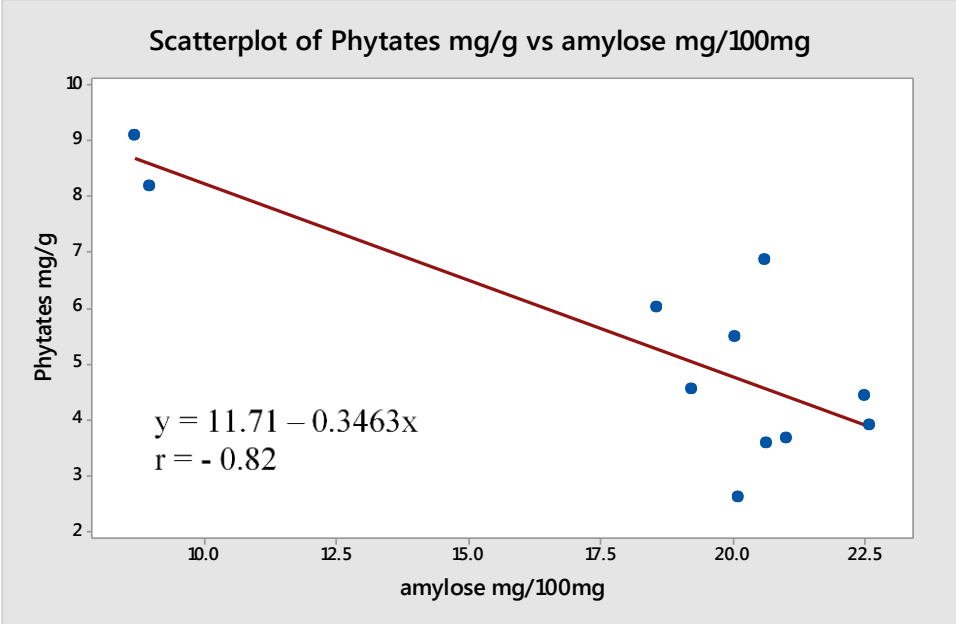

Figure 3 Correlation between total phytates – $mg.g^{-1}$ and amylose – $mg.100mg^{-1}$ in legumes.

According to the finding of **Raboy et al. (1984)** and **Mosenthin (2007)**, phytic acid and seed total phosphorus in soybean gradient stated were highly and positively correlated (r = 0.94)

The magnitude of correlation coefficient obtained in the analysis was low due to one of the reason of prolonged storage of legumes led to activation of phytase enzyme at high humidity and high temperature conditions which can lead to significant loss in phytates. According to **Chitra (1994)** the decrease in phytic acid was the lowest in soybean (29%) after 12 months of storage at 25 °C and 37 °C. The values obtained are in close agreement with the results reported by **Reddy and Sathe (2002)**.

An experiment conducted by **Cossa et al. (1999)** for maize samples phytate phosphorus and phosphorus contents were determined where the correlation coefficient was 0.70. Though some of the results of phytate phosphorus and total phosphorus obtained deviates from that reported in the literature, could be due to certain reasons such as variations in the environmental factors such as locations, irrigation conditions, type of soil, fertilizer applications, year of growing the cultivar etc.

The sample that has been used for the analysis was stored in the cold room till further usage which could had again led to loss in phytates with storage time **(Reddy and Sathe, 2002)**. The population in the developing countries consumes plant foods like legumes on a daily basis, there can be problems in meeting the daily dietary requirement for phosphorus, since it is clear from the experimental results that phytate phosphorus account for 29 to 67% of the total phosphorus which can adversely affect the mineral absorption **(Chitra, 1994)**. As such it is advisable to consume processed (fermented/cooked/germinated) legumes in order to reduce phytate contents **(Reddy and Sathe, 2002)**. There are studies stating that phytate containing foods are rich sources of dietary fiber which have great affinity for minerals at the same time, therefore

it is difficult to state that phyate availability solely affects mineral absorption **(Ravindran et al., 1994)**.

Amylose contents of legumes

The amylose content in legumes ranges from 8.705 ±0.129 mg.100g^{-1} in Pb1 to 22.580 ±0.714 mg.100g^{-1} in MI6. There is a significant difference ($p \leq 0.05$) existing among the amylose content of eleven legume varieties. The amylose content of MI5 and MI6 are significantly higher than the other varieties, which are not in the range of the values obtained by **Kaur et al. (2011)** where mung bean (*Vigna radiata* L) amylose contents were varied between 29.9 – 33.6 mg.100mg^{-1}. Similarly **Sandhu and Lim (2008)** who studied the digestibility of Indian legumes stated the amylose % of mung bean as 31.6 ±0.7 mg.100mg^{-1}. the amylose content of Soyabean are significantly lower ($p \leq 0.05$), which is lower that the values obtained by **Stevenson et al. (2006)**, where the apparent amylose content was 19 – 22 mg.100mg^{-1} and absolute amylose content was 11.8 – 16.2 mg.100mg^{-1} in *Glycine max (L.)Merr*. But according to **Gunathilake et al. (2016)** who observed that the carbohydrate contents in two varieties of Soyabean Pb1 and MISB1 as 18.0% and 15.0% respectively, it is evident that a lower amylose content can be as a result of lower total carbohydrate content. There is no significant difference ($p > 0.05$) between ANKBlack and ANKBrown varieties, the values obtained by **Marimuthu and Krishnamoorthi (2013)** for the amylose content of the South Indian horse gram was 32.14 ±0.10 mg.100mg^{-1}, similarly **Chavan et al. (2010)** stated the amylose content of black horsegram to be 36.30 ±1.40 mg.100mg^{-1} which are not in accordance to the values obtained in the experiment. The amylose content of MICP1 is significantly lower ($p \leq 0.05$) from the other cowpea varieties.

According to Pearsons correlation between phytates and amylose there is a significant negative ($p \leq 0.05$) correation existed between the phytate and amylose content of legumes (r = -0.82), according to **Dayakar et al. (2016)** there was a significant correlation between amylose and phytates contents in Sorghum was -0.26.

CONCLUSION

Soyabean contains the highest amount of Phosphorus of 654.94 ±0.05 mg.100g^{-1} in MISB 01 and least amount of 275.04 ±1.44 mg.100g^{-1} in ANK brown. Phytate phosphorus accounts for major portion of the total phosphorus ranging from 29.42% to 67.42% in legumes. There is a high positive correlation between phytate and Phosphorus as well as phytate phosphorus and phosphorus. While, there is a strong negative correlation between phytates and amylose content in legumes.

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Contact address:

Keerthana Sivakumaran, Food Science and Technology, University of Sri Jayewardenapura, Faculty of Applied Science, Department of Food Science and Technology, Gangodawila, Nugegoda, Sri Lanka, E-mail: keerthana37a@gmail.com

Dr. Jagath Wansapala, University of Sri Jayewardenapura, Faculty of Applied Science, Department of Food Science and Technology, Gangodawila, Nugegoda, Sri Lanka, E-mail: jwansapala@gmail.com

Dr. Theja Herath, Industrial Technology Institute (ITI), Food Technology Section, Bauddhaloka Mawatha, Colombo 7, Sri Lanka, E-mail: theja@iti.lk