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PHYTIC ACID AND INORGANIC PHOSPHATE IN BLACK GRAM (VIGNA MUNGO) SPECIES AND THEIR EFFECT ON HUMAN BODY

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ABSTRACT Black gram species are fast- growing legume which usually grows in South-Asian region. The seed sample when boiled with an acidic iron solution of known iron concentration form a colored solution. The iron present bound to 2, 2`-bipyridine is inversely related to the amount of phytic acid present which was detected through spectrophotometer. The phytic acid and inorganic phosphate concentration in 34 samples which includes 31 black gram samples, 1 pigeon pea, 1 red lentil and 1 split bengal gram was determined. The phytic acid concentration range from 2.847 to 23.6 mg/g sample whereas inorganic phosphorous concentration range from 56.4 to 121.55 µg/g sample. Considerable variations were observed for both PA and IP content in black gram samples. Consumption of black gram grains with low phytic acid (lpa) will increase the absorption of nutrients in humans.

KEYWORDS : Black gram, Phytic acid, Inorganic phosphorus, Seeds

INTRODUCTION

Black gram species are fast- growing legumes found throughout the Indian sub-continent which act as source of manganese, potassium, magnesium, folate, copper, zinc and various vitamin B in a person's diet. Black gram is also a rich source of phytic acid which is the main phosphorous storage form in the seeds. Phytic acid (PA) is mainly present in aleurone layer and remaining in embryo in plant seeds. PA present in the form of phytate salts in seed is degraded by phytases during germination in order to provide the seedling with phosphate. The inorganic phosphorous(IP) play an important role as storage reserve in the seeds for the synthesis of organic phosphorus compounds (lecithins, phospho- and nucleo-proteins and salts of phytic acid).

PA (also known as inositol hexaphosphate-IP6) due to its antioxidant properties plays supporting role in prevention of cancer, heart diseases, diabetes treatment and formation of renal stone. But same property contributes to PA acting as anti-nutrient or inhibition of absorption of iron, calcium, zinc and magnesium in humans. This may contribute to an iron deficiency especially in vegetarians. The two types of iron in foods: Non-heme iron derived from plant foods highly affected by phytic acid is poorly absorbed while the absorption of heme-iron which comes from animals remain same. High-phytate foods, such as grains, nuts and legumes, can elevate the risk of iron and zinc deficiency. Phytate has been proven to inhibit digestive enzymes such as trypsin, pepsin, α-amylase, and ß-glucosidase [9]**.** Strategies such as soaking, sprouting and fermentation are often employed a lot to reduce the phytic acid content of foods [7].For regular meat-eaters, deficiencies caused by phytic acid are not a concern.

Phytic acid has been known to protect against alcohol-related liver injury by blocking free radicals and elevating antioxidant levels [8]. The anti-oxidative action of phytic acid inhibits Xanthine Oxidase and preventing formation of ADP-iron-oxygen complexes' [9]. Phytic acid was established to be anti-cancer against bone, prostate, ovarian, breast, liver, colorectal, leukemia, sarcomas and skin cancers as it suppresses the expression of matrix metalloproteinases (MMPs) and telomerase [10]. Studies show that phytate reduces blood glucose levels by slowing the rate of starch digestibility as demonstrated in the case of mice and rats [11,12].

In this method, phytic acid and inorganic phosphorous is determined through spectrophotometer. The seed sample when boiled with an acidic iron solution of known iron concentration form a colored solution. The iron present bound to 2, 2`-bipyridine is inversely related to the amount of phytic acid present which is detected through spectrophotometer.

MATERIAL AND METHODS

Reagents for Phytic Acid Measurement

Phytate reference solution: The sodium salt of phytic acid. The reference solutions were prepared by diluting the stock solutions with distilled water in a range from 25 to 500 µg/ml phytate phosphorus. Extraction Media: Dissolve 1g of Na_2SO_4 in 10ml 0.2N HCl. Iron Solution: 0.2g ammonium iron(III) sulfate dodecahydrate in 1000ml of 0.2 N HCl. HL reagent: 5g of 2`2`-bipyridine and 5ml thioglcolic acid in 500ml distilled water.

Reagents for Inorganic Phosphorous Measurement

50% TCA:25mM $MgCl_2$: 25ml TCA in 75mM $MgCl_2$. Chen Regent : 20ml 6N H_2SO_4, 20ml 2.5% ammonium molybdate, 20ml 10% ascorbic acid in 40ml deionized water. Standard Phosphate solution (0.1mg/ml): 0.4394g KH_2PO_4 in 1000ml deionized water.

Measurement of Phytic Acid

In a 2mL microcentrifuge tube, 200 mg of the fine seed powder was thoroughly mixed with 1.5 mL of extraction media. The tubes were shaken overnight at 4˚C in an Incubator Shaker and centrifuged at 6500rpm for 20 minutes in a tabletop centrifuge. 100µl Crude acid extracts were transferred to 1.5 mL microcentrifuge tubes containing 300 µl extraction media and 600 µl iron solution. The tubes were placed in boiling water bath for 30 minutes and allowed to cool at RT for 10minutes. The mixtures were then centrifuged at 6500 rpm for 10 minutes to yield supernatant. The 1ml supernatants were transferred in fresh tubes and 1.5ml HL reagent is added. The resultant solution is used for the determination of PA using the colorimetric method. A series of calibration standards containing 0, 25, 50, 100, 200, 300, 400, 500µg/ml were also prepared from the sodium salt of phytic acid and treated in the same way as above. The absorbance of color reaction products for both samples and standards was measured at 519 nm on a UV/V spectrophotometer.

Measurement of Inorganic Phosphorous

In a 2mL microcentrifuge tube, 100 mg of the fine seed powder was thoroughly mixed with 2mL of 12.5% TCA: 25mM MgCl2 solution. The tubes were shaken for 24 hours at RT in an Incubator Shaker and centrifuged at 5000rpm for 15 minutes at 4˚C in a cooling centrifuge. Crude extracts were transferred to 15 mL test tubes and adjust the supernatant volume to 5ml by deionized water. 300µl Chen's regent is added to the supernatant and incubated for 1 hour at 37˚C. The resultant solution is used for the determination of PA using the colorimetric method. A series of calibration standards containing 0, 5, 10, 15, 20, 25µg/ml were also prepwered from the KH_2PO_4 stock solution and treated in the same way as above. The absorbance of color reaction products for both samples and standards was measured at 820nm on a UV/V spectrophotometer.

RESULTS AND DISCUSSION

The phytic acid concentration in 34 samples which includes 31 black gram samples, 1 pigeon pea, 1 red lentil and 1 split bengal gram were used is given in Fig: 2. The phytic acid concentration range from 2.847 to 23.6 mg/g sample. The IPU2K99-224 contains highest phytic acid concentration while UPU83-35 contains the lowest. The inorganic phosphorous concentration in 34 samples which includes 31 black

gram samples, 1 pigeon pea, 1 red lentil and 1 split bengal gram were used is given in Fig: 4. The inorganic phosphorous concentration range from 56.4 to 121.55 µg/g sample. The PGRU9598 contains highest phytic acid concentration while T-9 contains the lowest.

Table1: The phytic acid and inorganic phosphorous concentration in grain samples

S. No	Name of Cell Lines	Phytic Acid Content (mg/g sample)	Inorganic Phosphorous Content (µg/g sample)
1	T-9	4.463±0.0035	56.4± 0.15
2	Shekhar-2	6.279± 0.005	76.55± 0.125
3	IPU2K99-226	5.368± 0.0015	76.4± 0.15
4	IPU99-150	4.936± 0.001	60± 0.15
5	IPU99-243	6.279± 0.0015	70.55± 0.05
6	IPU99-176	5.149± 0.002	65.65± 0.1
7	PDU-2	5.528± 0.001	94± 0.075
8	UPU83-35	2.847± 0.004	72.65± 0.125
9	IPU99-205	5.162± 0.0015	75.75± 0.05
10	SPS43	5.621± 0.0015	69.4± 0.075
11	IPU99-115	5.295± 0.0005	65.85± 0.075
12	IPU557	5.215± 0.002	80.75± 0.001
13	UPU83-35	5.102± 0.0025	72± 0.05
14	SPS-7	3.771± 0.001	79.1± 0.05
15	PU-31	5.846± 0.665	67.3± 0.05
16	UH82-51	16.775± 0.665	62.4± 0.25
17	IPU2K99-224	23.6± 0.66385	97.3± 0.001
18	NG2119	20.175± 1.6594	96.1± 0.05
19	PGRU95004	18.55± 0.66375	87.65± 0.001
20	PGRU9598	19.35± 0.3319	121.55± 0.1
21	IPU99-243	20.775± 0.6639	89.1± 0.075
22	IPU-25	10.7± 0.99565	84.85± 0.05
23	IPU2K-17	19.4± 0.9951	90± 0.05
24	S9S-38	5.073± 2.66	78.2± 0.05
25	GE154	5.671± 1.330625	83.55± 0.005
26	IPU99-205	5.252± 0.335	68± 0.1
27	IPU99-3	5.989± 0.335	61± 0.1
28	SolNo-2	5.602± 0.3325	66.2± 0.1
29	IPU99-235	5.945± 0.998	84.55± 0.05
30	IPU99-204UH82-14	6.288± 0.331	82.65± 0.05
31	NDU94-10	6.23± 0.665	85.2± 0.05
32	Pigeon Pea	21.125± 2.655	88.3± 0.005
33	Red Lentil	19.9±0.6681	98.2± 0.05
34	Split Bengal gram	19.6±0.8319	86.75±0.075

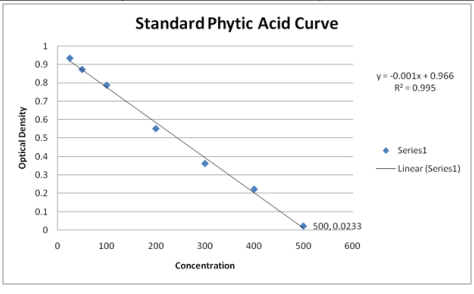

Fig: 1-A series of calibration standards containing 0, 25, 50, 100, 200, 300, 400, 500µg/ml were also prepared from the sodium salt of phytic acid in order to plot standard phytic acid curve

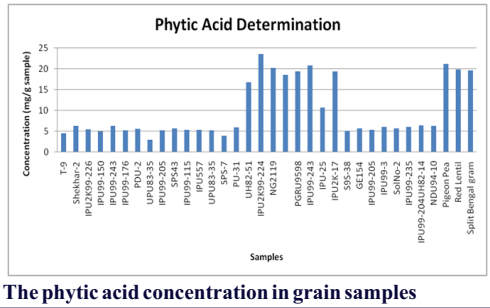

Fig:2 The phytic acid concentration in grain samples

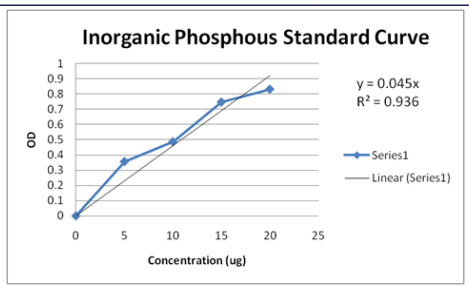

Fig: 3-A series of calibration standards containing 0, 5, 10, 15, 20, 25µg/ml were also prepared from the KH_2PO_4 stock solution in order to plot inorganic phosphorous standard curve

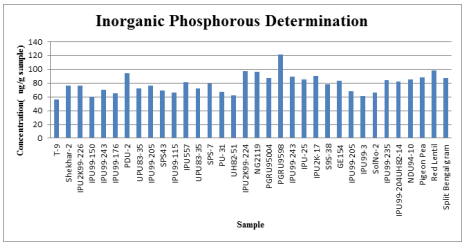

Fig: 4- The inorganic phosphorous concentration in grain samples

CONCLUSION

Some plant foods are labeled unhealthy because of their phytic acid content seems to be a mistake, especially when phytic acid's potential negative effects on mineral assimilation may be counter balance by its health benefits. In black gram, considerable variation was observed for both PA and IP content in samples. Same is also true for the case of comparison between black gram and other cereals. Consumption of black gram species with low phytic acid will increase the absorption of nutrients in humans which can help in overcoming problem of mineral assimilation.

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