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Impact of Sprouting on the Oxalate Load of Proteinaceous Plant Based Foods

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Abstract

Total oxalate content of plant-based foods has a prominent impact on renal calculi formation in animals and man. The present study was aimed to estimate total oxalate in six selected whole dry seed samples namely Chick pea - *Cicer arietinum* (S1), Red cowpea – *Vigna unguiculata* (S2), Green pea – *Pisum sativum* (S3), Mung bean – *Vigna radiata* (S4), Almond- *Prunus dulcis* (S5) *and* Soybean – *Glycine max* (S6) and their seed coats used as protein rich foods at 0 hrs., 24 hrs. And 48 hrs. Post soaking in distilled water. Total oxalate (TO) levels were found to be decreased in all the sprouted whole seeds at 24 hrs. And 48 hrs. Than 0 hrs. Post soaking. Whole seeds of S2 contained maximum TO (400mg/100g sample) prior to sprouting which on soaking for 48 hrs. Reduced TO (122mg/100g). Maximum reduction in TO after 48 hrs. Was in S4 (292mg-25mg/100g). S1, S3, S5 and S6 exhibited lowering of TO by (243mg-80mg, 93mg-35mg, 343mg-133mg, 232mg-85mg per 100g sample) respectively. Sprouting induced more than 60% reduction of TO in whole seeds. Seed coat analysis of S4 (259mg-404mg/100g sample) and S5 (280mg-134mg/100g sample) projected an increase in TO post 24 hrs. Soaking, but further soaking till 48 hrs. Decreased the oxalate load to 261 mg and102 mg respectively. Seed coats of S1 and S3 shows lowering of TO from 30mg/100g sample and 33mg/100g sample to12mg/100g and 13 mg/100gsample. Sprouting, regulated oxalate levels in plant-based foods and exclusion of seed coats could reduce oxalate intake. Total Oxalate analysis of plant-based foods need to be addressed for safety of renal patients in choosing low oxalate diet.

Keywords: Calcium Oxalate, Seed Coat, Sprouting, Total Oxalate (TO), Soaking, Antinutrient, Plant Based Food.

1. INTRODUCTION

Oxalate content comparison among vegetative parts of plants showed the maximum in leaves followed by seeds and the minimum oxalate content was reported in stem and fruits [1]. The study of oxalate load in seeds stay relevant as they have been inevitable protein source for humans and animals. The dietary recommendation for oxalate in food has been restricted to 40-50 mg per day [2], for renal patients. An increase in oxalate consumption substantially increased oxalate in urine which could trigger renal urolithiasis. Diet plans such as green smoothie cleanses have an adverse effect on health due to the antinutrient effect of oxalate present in it [3]. Significance on the study of dietary oxalate intake by renal patients gained momentum after being experimentally proven that oxalate from food contributed to 24-53% of urinary oxalate from an intake of 10 to 250 mg oxalate per day [4]. Towards the precipitation of renal crystals, both calcium and oxalate had an equal contribution [5].

Plants reported to have calcium oxalate crystals can accumulate the same from 3 to 80% of their dry weight [6]. More than 217 families of plants have been reported to have ergastic crystals in some point of their developmental history [7].

Germination of seeds have proved to improve the nutritional value of it by increase in vitamin C content [8], increase in lysine, tryptophan and methionine [9], increase in calcium and decrease in fat content [10], decrease in phytic acid [11], etc. Calcium oxalate is an important antinutrient present in seeds whose status prior and post sprouting can help renal patients in choosing a low oxalate diet. Manipulation of oxalate metabolism in plant-based foods is the future of food biotechnology in renal health research [12].

Present study was aimed to find out the calcium oxalate load in selected seeds prior and post sprouting as it could throw some light to the effect of sprouting on

antinutrient levels in seeds. The study has also been aimed to find the oxalate load in seed coats in seeds and thereby understand potential of seed coats on regulating oxalate load in seed foods.

2. MATERIALS AND METHODS

Dry seeds of Chick pea - *Cicer arietinum* (S1), Red cowpea – *Vigna unguiculata* (S2), Green pea – *Pisum sativum* (S3), Mung bean – *Vigna radiata* (S4), Almond- *Prunus dulcis* (S5) *and* Soybean – *Glycine max* (S6), were selected for study. All the seeds were Fabaceae family members except almond, used around the world as a protein source. Seeds were purchased from certified supermarket with import license of food grade seeds.

Seeds were subjected to soaking in distilled water for 48 hrs. Individually. Estimation of total oxalate was carried out on the whole seeds and seed coats separately prior to soaking and at 24 hrs. And 48 hrs. Post soaking to compare the oxalate load in samples and study the effect of sprouting on total oxalate. Estimation of oxalate load was done by permanganometric titration with standardized KM_nO_4 following Association of Official Analytical Chemists (AOAC) procedures 2016 [13].

2.1. Sample Preparation

To extract the total oxalate from seeds, 1 g homogenized sample was added to 30 ml of 0.5 N H_2SO_4 and boiled in water bath for 15 minutes. The extract was filtered using Whatman's No1 filter paper and equal volume of deionized water was added to prepare the sample extract.

2.2. Permanganometry

Oxalate ions were extracted from the plant parts by boiling them with dilute H_2SO_4 (0.5N). Then oxalate concentration estimated volumetrically by titrating the extract with standard $KMnO_4$ solution (0.05N).

$$MnO_4^- + + 5C_2O_4 + 8H+ \rightarrow Mn^{2+} + 10CO_2 + 4\ H_2O$$

1 ml of sample extract was added to 40 ml dil. H_2SO_4 in a conical flask and titrated against standard KMnO4 until a permanent pink colouration appeared and stayed for at least 15 seconds. The end point was noted and the amount of oxalate present in mg/sample was estimated stoichiometrically.

3. RESULTS AND DISCUSSION

3.1. Total Oxalate in Seeds and Seed Coat

Total oxalate analysis results of whole seeds tabulated in Table 1 and that of seed coats in Table 2. Figure1 and Figure 2 represented and compared the total oxalate of whole seeds and seed coats of samples S1-S6 respectively.

3.2. Statistical Analysis

ANOVA was performed for analysing the variance between and among the TO of selected seeds. Homogeneity of the variance was tested by Levene test, and found that the group variances were homogenous. The mean values, Standard deviation (SD), coefficient of variation (CV) of total oxalate content in all the selected whole seeds (Table 1) and seed coats (Table2) were calculated prior and post 24 hrs and 48 hrs of soaking. The results were subjected to analysis of variance to assess the difference in the total oxalate content and the means were compared by the Tukey test at the significance level of 0.05 (p value< 0.05). The statistical analysis was performed using the IBM SPSS software package. Superscript 'a' signifies maximum variance and 'e' represents least variance in samples. Analysis of variance groups samples according to their variance in a decreasing order from superscript a-e.

Estimation of Total oxalate in Seeds S1,S2,S3,S4,S5 and S6 revealed that they all fell under high oxalate category food. Foods containing >50mg/100g sample of oxalate are called high oxalate foods [14]. The estimation of oxalate load in the whole seeds studied revealed a lowering of total oxalate content post sprouting (Figure 1). Total oxalate (TO) content of all the seeds studied exhibited more than 60% reduction in the antinutrient post sprouting. Though S2 possessed maximum total oxalate in whole seed analysis (400mg/100g sample) and on sprouting, after 24 hrs. of soaking, showed reduced total oxalate content to133mg/100g sample which was 67% reduction in TO. After 48 hrs of soaking, TO reduced by 69%. S3 exhibited the least total oxalate in whole seeds (93mg/100g sample) which showed a decrease in oxalate load by 55% and 62% after 24 hrs. And 48 hrs of soaking respectively.

S1 and S4 showed 63% decrease in whole seed total oxalate content post 24 hrs. Sprouting. But S4 after 48 hrs. exhibited 91% reduction of TO (292mg/100g-25 mg/100g). S1 after 48 hrs. reduced TO by only 67%. S6 and S5 exhibited 56% and 54% reduction in TO 24 hrs. Post sprouting respectively (Figure 1). After 48 hrs. TO of S6 and S5 came down to 63% and 61% respectively. The reduction of TO to 25mg/100g by S4 and 35mg/100g by S3 after 48 hrs of soaking makes it safe for consumption among renal patients as it is shifted from high oxalate category to dietary recommended moderate oxalate levels.

Total oxalate in seed coats of selected seeds did not follow decreasing trend as whole seeds. The amount of total oxalate in seed coats of S4 and S5 displayed increased total oxalate content post sprouting by 56% and 18% respectively after 24hrs of soaking (Figure 2). But after 48 hrs. the oxalate load of S4 and S5 decreased further, back to the level of non-sprouted seed coats. Further soaking of seeds than 48 hrs. decomposed the seeds and hence it was not practical. Seed coats of S2 and S3 exhibited a reduction in TO by 31% and 33% respectively after 48 hrs. Of soaking, but after 48 hrs. S3 exhibited 61% reduction in TO whereas S2 exhibited

33% reduction only. Maximum reduction of TO in seed coat was expressed in S6 by 78%.

The breakdown of oxalate in plants occurred through oxidation, decarboxylation and acetylation [15]. The enzymes oxalate oxidase (OXO) breaks down oxalate to carbon dioxide and hydrogen peroxide [16]. Oxalate decarboxylase (OXDC) catalyzed the degradation of oxalate to carbon dioxide and formic acid [17]. Another enzyme Oxaloacetate acetyl hydrolase (OXAC) also breaks down oxalate [18]. The reduction in TO in seeds pointed to the action of these enzymes which are abundant in seed endosperms.

Oxalate synthesis pathways utilized Glycolate oxidase (GLO) and Ascorbate precursors in accumulation of oxalate in plant organs [19]. Study of enzymes responsible for oxalate metabolism showed many enzymes and genes responsible for it [20]. The up regulation and down regulation of genes responsible for oxalate metabolism under the influence of external factors like climate, soil mineral concentration, nitrate-ammonia ratio etc. result in the oxalate concentration in plants at a point of time determined by the concentration of OXO, OXDC, OXAC and GLO enzymes. Hence the seed coat accumulation of oxalate and its sharp increase post sprouting exhibited by S4 and S5 may be due to the absence of OXO, OXAC and OXDC which are distributed in the seed endosperm and also the upregulation of GLO enzyme or a combination of both.

4. Illustrations

Table 1: Total oxalate content of whole seeds mg/g sample

Total oxalate	Seed	Minimum	Maximum	Mean ±SD	CV%
Total Oxalate in Whole seed Before Sprouting	S1	2.35	2.5	2.43 ± 0.764^{d}	3.1
	S2	3.9	4.1	4.00 ± 0.10^{a}	2.5
	S3	0.9	0.95	0.93 ± 0.025^{e}	2.7
	S4	2.9	2.94	2.92 ± 0.021^{c}	0.68
	S5	3.4	3.45	3.43 ± 0.025^{b}	0.73
	S6	2.29	2.35	2.32 ± 0.031^{d}	1.3
Total Oxalate in Whole seed After 24 hrs Soaking	S1	0.85	0.95	0.90 ± 0.050^{d}	5.6
	S2	1.3	1.35	1.33 ± 0.025^{b}	1.88
	S3	0.4	0.45	0.42 ± 0.025^{e}	6.0
	S4	1	1.12	1.07 ± 0.064^{c}	6.0
	S5	1.5	1.6	1.57 ± 0.058^{a}	3.7
	S6	1	1.05	1.03 ± 0.029^{c}	2.9
Total Oxalate in Whole seed After 48 hrs soaking	S1	0.75	0.85	0.80 ± 0.050^{b}	6.3
	S2	1.20	1.25	1.22 ± 0.025^{a}	2.0
	S3	0.32	0.38	0.35 ± 0.030^{c}	8.6
	S4	0.16	0.35	0.25 ± 0.096^{c}	38.4
	S5	1.32	1.35	1.33 ± 0.015^{a}	1.1
	S6	0.83	0.86	0.85 ± 0.015^{b}	1.8

Superscripts a, b, c, d and e represent groups which seeds were grouped based on coefficient of variance.

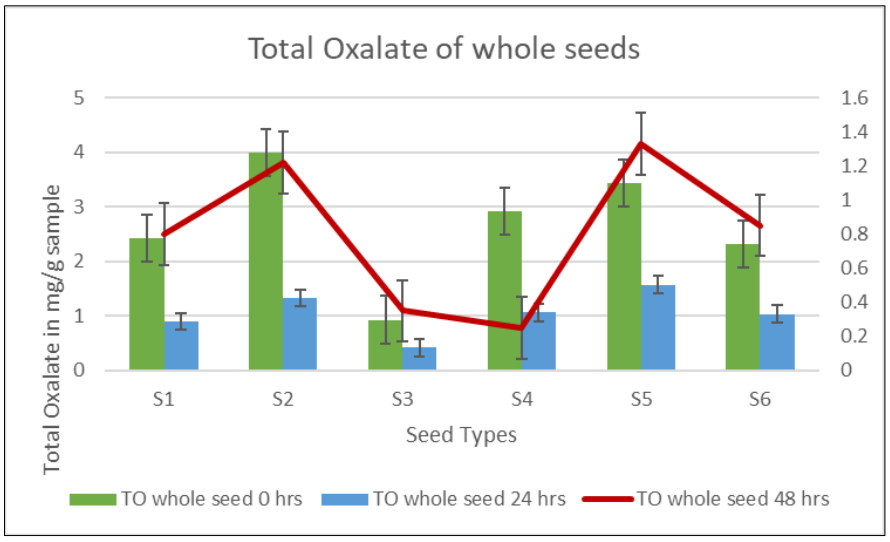

Figure 1: Total oxalate of whole seeds at 0 hrs, 24 hrs and 48 hrs post soaking

Table 2: Total oxalate content of seed coats mg/g sample

Total oxalate	Seed	Minimum	Maximum	Mean ±SD	CV%
Total Oxalate in Seed Coat Before Sprouting	S1	0.25	0.35	0.30 ± 0.050^{e}	16.7
	S2	3.25	3.35	3.30 ± 0.050^{a}	1.5
	S3	0.3	0.35	0.33 ± 0.029^{e}	8.8
	S4	2.56	2.61	2.59 ± 0.026^{c}	1.0
	S5	1.00	1.06	1.04 ± 0.032^{d}	3.1
	S6	2.8	2.81	2.80 ± 0.056^{b}	2.0
Total Oxalate in Seed Coat After 24 hr soaking	S1	0.20	0.25	0.22 ± 0.025^{e}	11.4
	S2	2.20	2.35	2.28 ± 0.076^{b}	3.3
	S3	0.20	0.24	0.22 ± 0.021^{e}	9.5
	S4	4.00	4.07	4.04 ± 0.036^{a}	0.89
	S5	1.20	1.25	1.23 ± 0.025^{d}	2.03
	S6	1.34	1.35	1.34 ± 0.006^{c}	0.45
Total Oxalate in Whole seed After 48 hrs soaking	S1	0.10	0.15	0.12 ± 0.025^{e}	20.8
	S2	2.12	2.25	2.20 ± 0.070^{b}	3.2
	S3	0.12	0.14	0.13 ± 0.010^{e}	7.7
	S4	2.54	2.70	2.61 ± 0.832^{a}	31.9
	S5	1 .00	1.03	1.02 ± 0.015^{c}	1.5
	S6	0.59	0.64	0.61 ± 0.025^{d}	4.1

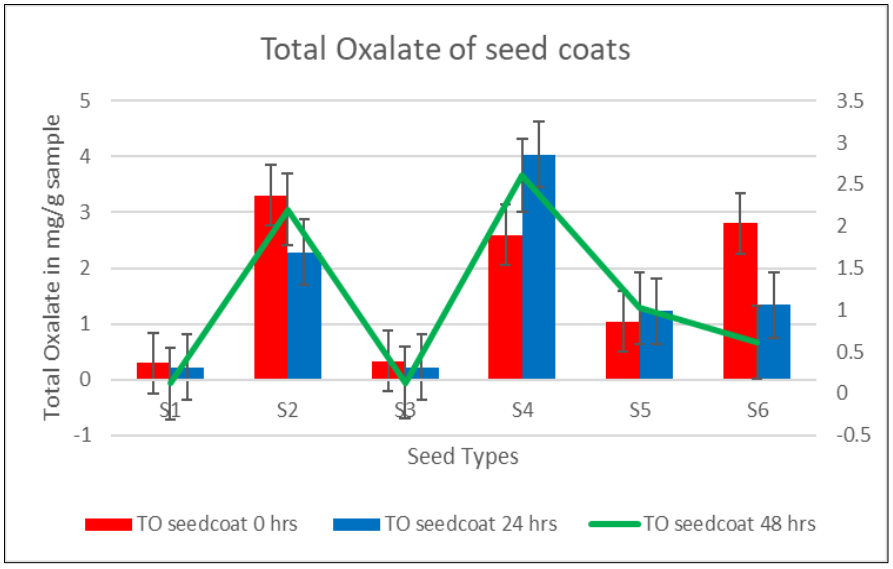

Figure 2: Total oxalate of seed coats at 0 hrs, 24 hrs and 48 hrs post soaking

5. CONCLUSION

Estimation of total oxalate load in seeds prior and post sprouting helped to compare the antinutrient total oxalate levels of seeds. All the selected whole seeds showed decrease in oxalate load post sprouting which points to decrease in antinutrient levels due to sprouting. Seed coats accumulated total oxalate post sprouting, which pointed to the need of exclusion of seed coats of sprouted seeds for considerably reducing total oxalate from diet for renal patients. Consumption of sprouted seeds after seed coat removal can considerably reduce oxalate from diet for renal patients.

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7. Conflict of Interest: The authors declare no conflicts of interest.

8. Author Contribution

Author 2 designed the experiment and supervised the study. Author 1 performed the study, statistical analysis and writing of paper.

REFERENCES

1. Noonan, S. C., & Savage, G. P. (2002). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition*, *8*(1), 64-74.
2. Massey, L. K. (2007). Food oxalate: factors affecting measurement, biological variation, and

bioavailability. *Journal of the American Dietetic Association*, *107*(7), 1191-1194.
3. Makkapati, S., D'Agati, V. D., & Balsam, L. (2018). "Green smoothie cleanse" causing acute oxalate nephropathy. *American Journal of Kidney Diseases*, *71*(2), 281-286.
4. Holmes, R. P., Goodman, H. O., & Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney international*, *59*(1), 270-276.
5. Pak, C. Y., Adams-Huet, B., Poindexter, J. R., Pearle, M. S., Peterson, R. D., & Moe, O. W. (2004). STONE DISEASE Rapid Communication: Relative effect of urinary calcium and oxalate on saturation of calcium oxalate. *Kidney Int*, *66*, 2032-7.
6. Massey, L. K. (2003). Dietary influences on urinary oxalate and risk of kidney stones. *Front Biosci*, *8*, 584-594.
7. McNair, J. B. (1932). The interrelation between substances in plants: essential oils and resins, cyanogen and oxalate. *American Journal of Botany*, 255-272.
8. Laxmi, G., Chaturvedi, N., & Richa, S. (2015). The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. *Journal of Nutrition and Food Sciences*, *5*(5).
9. Bhathal, S., & Kaur, N. (2015). Effect of germination on nutrient composition of gluten free Quinoa (Chenopodium Quinoa). *Food Science*, *4*(10), 423-425.
10. Jan, R., Saxena, D. C., & Singh, S. (2017). Physico-chemical, textural, sensory and antioxidant characteristics of gluten free cookies made from raw and germinated Chenopodium (*Chenopodium album*) flour. *LWT – Food Science and Technology*, *71*, 281–287.
11. Luo, Y. W., Xie, W. H., Jin, X. X., Wang, Q., & He, Y. J. (2014). Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes. *CyTA-Journal of Food*, *12*(1), 22-26.
12. Kumar, V., Irfan, M., & Datta, A. (2019). Manipulation of oxalate metabolism in plants for improving food quality and productivity. *Phytochemistry*, *158*, 103-109.
13. AOAC. (2016). Official methods of analysis, 20th edn. AOAC International, Gaithersburg.
14. Massey, L. K. (2007). Food oxalate: factors affecting measurement, biological variation, and bioavailability. *Journal of the American Dietetic Association*, *107*(7), 1191-1194.
15. Cai, X. F., Xu, C. X., Wang, X. L., Ge, C. H., & Wang, Q. H. (2015). The oxalic acid in plants: Biosynthesis, degradation and its accumulation regulation. *Zhiwu Shengli Xuebao/Plant Physiology Journal*, *51*(3), 267-72.
16. Li, W., Li, W. X., Jia, L., Gang, Y. Z., & Min, D. Z. (2011). Research on oxalate oxidase and its genes in plants. *Agric. Sci. Technol*, *12*, 11–13, 19.
17. Svedružić, D., Jónsson, S., Toyota, C. G., Reinhardt, L. A., Ricagno, S., Lindqvist, Y., & Richards, N. G. (2005). The enzymes of oxalate metabolism: unexpected structures and mechanisms. *Archives of biochemistry and biophysics*, *433*(1), 176-192.
18. Chang, C. C., & Beevers, H. (1968). Biogenesis of oxalate in plant tissues. *Plant Physiology*, *43*(11), 1821-1828.
19. Xiaofeng, C., Chenhui, G., Chenxi, X., Xiaoli, W., Shui, W., & Quanhua, W. (2018). Expression Analysis of Oxalate Metabolic Pathway Genes Reveals Oxalate Regulation Patterns in Spinach. Molecules, 23, 1286-1296.
20. Foster, J., & Nakata, P. A. (2014). An oxalyl-CoA synthetase is important for oxalate metabolism in Saccharomyces cerevisiae. *FEBS letters*, *588*(1), 160-166.