# OXALATE CONTENT OF SOYBEAN SEEDS (GLYCINE MAX: LEGUMINOSAE) VARIETIES GROWN IN KENYA.

Erastus S.K. Mwangi[1*], Erastus G. Gatebe[1], Mary W. Ndung'u[1]
[1]Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology
P.O Box 62000 -00200 Nairobi, Kenya
[*]E-mail: eskmwangi@ yahoo.com

***Abstract***
*Consumption of soybeans and food products made from them is increasing because of their desirable nutritional value and associated health benefits. However, soybeans like other legumes contain oxalate; which is considered pathological in the formation of urinary/kidney stones. The main objective of the current study is to determine the amount of oxalate in soybean seed varieties grown in Kenya. The insoluble and soluble oxalate content from nine soybean varieties were extracted using hot acid (80 °C, 2 M HCl) and hot distilled water (80 °C), respectively. The supernatant was determined for oxalates and calcium content by high pressure liquid chromatography (HPLC) and by atomic absorption flame emission spectrophotometer (AAS), respectively, against the oxalate and calcium standard solution. The mean total oxalate concentration in the 9 cultivars of soybean analyzed was 1.665 g/100 g of dry weight, with values ranging between 0.832 and 2.847 g/100 g of dry weight, the greatest proportion being insoluble (average of 61.6%). The mean total calcium content was 1.764 g/100g of dry weight, with values ranging between 0.434 and 4.994 g/100g of dry weight. There was a correlation between total oxalate (soluble and insoluble) and calcium content in the seeds of soybean varieties. The amounts of total oxalate in soybean seeds exceed current recommendations for oxalate consumption by individuals who have a history of calcium oxalate kidney/urinary stones. This is the first report on assessment of oxalate concentration on soybean grown in Kenya. SCS-1 displayed the lowest total oxalate levels and is a strong reason why the adoption of the improved varieties of soybean should be encouraged. The study serves as the basis to find soybean cultivars lower in oxalate, which will have lower risk for kidney stone formation after human consumption.*

Keywords: calcium; crystals; legume; soybean; dietary oxalate; urinary/kidney stones

Submitted: 3.01.2012 Reviewed: 19.03.2012 Accepted: 18.04.2012

## 1. INTRODUCTION

Soybean [*Glycine max* (L.) Merril] is the world's most important legume in terms of production and trade due to its versatility. According to FAO statistics, total world production of soybean increased from 136.5 million MT in 1994 to 189.2 million MT in 2003. In 2003, about 190.1 million MT of soybean (representing 56% of world production of oilseed) was produced in the world with Africa accounting for 0.4 – 1% of total world production of soybean. World soybean trade (in terms of imports and exports) is a big business amounting to nearly US$11 billion in 2002 (Chianu and Vanlauwe, 2006).

Soybeans are regarded as a highly nutritious food source because of their excellent oil (61% polyunsaturated fat and 24% monounsaturated fat) and protein (eight essential amino acids) contents (asadg@ pacific.netsg, 1999). Of all grain legumes, soybean has the highest concentration of protein. While most other grain legumes contain about 20% protein by volume, soybean contains about 40% protein (Greenberg and Hartung, 1998). It is important to note that beef and fish contain about 18% protein. Soybean products are cholesterol free and high in calcium, phosphorus, and fiber (Greenberg and Hartung, 1998). Besides these nutritional benefits, soybean seeds are rich in isoflavones, compounds that are being studied for their potential of reducing bone loss and breast cancer (Murphy *et al.*, 1999). They are also processed to extract oil for food and for numerous industrial purposes. As edible oil, it enters the market as salad oil, cooking oil, margarine and shortening. Soybean meal is extensively used as an ingredient in livestock feed.

As a legume, soybean improves soil fertility by fixing atmospheric nitrogen (one of the plant nutrients lacking in most of Kenya's soils) into the soils and this is another strong reason why soybean should be promoted. In addition, Soybean also presents the farmers with the much needed alternative cash income source. In the Economic Recovery Strategy (ERS), the government's vision is to transform Kenya's agricultural sector into a profitable economy (Government of Kenya, 2004). This transformation calls for fundamental shift to market-oriented production, diversification of agriculture such as soybean and adoption of greater use of appropriate farming practices. Soybean is one such crop that has the potential to make significant contributions to healthcare (Government of Kenya, 2002; Ohiokpehai and Osborne, 2003), income and livelihood security.

However, despite the soybean associated benefits, just like other legumes it contains oxalate. Oxalic acid is one of the most highly oxidized organic compound widely distributed in plants, which occurs in two forms: soluble and insoluble oxalates. Soluble oxalate usually forms with sodium ($Na^+$), potassium ($K^+$) and ammonium ($NH_4^+$) ions, and insoluble oxalate forms with calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$) and iron ($Fe^{2+}$) ions (Holloway *et al.* 1989; Savage *et al.* 2000). The accumulated oxalates in plants are in a range of 3-15% (w/w) of their dry weight (Franceschi and Nakata, 2005). The physiological role of oxalate in plants is not precisely known, but it has been suggested that it is involved in seed germination, calcium storage and regulation, ion balance (Na and K), heavy metal detoxification, structural strength, and insect repulsion (Libert and Franceschi, 1987; Franceschi and Nakata, 2005). Despite the broadly suggested oxalate functional roles in plants, high oxalate content can also be a concern to human health and nutrition.

The main sources of dietary oxalate are plants and plant products, principally seeds and leafy plants related to leguminosae (Savage et al. 2000). In contrast, negligible amounts of oxalate occur in foods of animal origin. The catabolic pathway of oxalic acid is present in bacteria, fungi, and plants but not in vertebrates. After oxalate has been absorbed from the diet, it cannot be catabolized and is excreted by the kidney into urine, where it binds to calcium forming an insoluble salt that may precipitate to form kidney stones (Massey *et al.*, 2001). About 75% of all kidney stones are composed primarily of calcium oxalate and hyperoxaluria is a primary risk factor for this disorder. The formation of calcium oxalate stone in the urine is dependent on the saturation level of both calcium and oxalate. Kidney stones not only are very painful but also caused an estimated $1.83 billion in direct medical costs in 1995 (http://vm.cfsan.fda.gov/_lrd/tpsoypr2.html, 1999)**,** not including the value of time lost from work. However, data on oxalate content in commonly consumed plant foods are limited; especially no published data are available on oxalate content in soybean varieties grown in Kenya.

In view of increased nutritional and health awareness coupled with challenges in treatment of ailments associated with plant toxins, it was prudent to investigate the amount of oxalate and calcium in soybean varieties grown in Kenya for soybean to continue attracting attention due to its versatility.

## 2. MATERIALS AND METHODS

### Sources of Soybeans

Nine soybean varieties/cultivars grown in Kenya were collected from KARI, oil crop centre Njoro, Kenya on September 2010.

### Extraction of Oxalate

Soluble and insoluble oxalates in the sample (0.5 g dried in a 37°C oven) were extracted with 15 ml of distilled water and 2 M HCl, respectively, (Ilarslan *et al*,. 1997). The suspension of sample was heated in a bath of boiling water for 18 min (Huang and Tanudjaja, 1992). After cooling to room temperature, the mixture was filtered through whatman no.1filter paper, washed with water and made up to 50 ml.

The filtrate of the acid extract was adjusted to pH 3.0 with 4 M KOH. The filtrates were filtered through a hydrophilic membrane (0.45 μm) prior to HPLC analysis.

**Chemical Analysis**

A 10-μL sample was injected into an HPLC system (Shimadzu Co., Ltd., Kyoto, Japan) and chromatographic separation was carried out on a column of Shodex IC SI-90 4E (size: 4.0 mm ID × 250 mm L) at a flow rate of 1.0 mL $min^{-1}$ using 0.6 mmol $L^{-1}$ $H_2SO_4$ as eluent. Oxalic acid was determined at 210 nm using authentic oxalate. Total oxalate were obtained by adding the insoluble oxalate and soluble oxalate and expressed as g/ 100g dry matter.

**Calcium Analyses of Soybean varieties.**

The calcium concentrations of soybean varieties were determined by blending 0.5 g of the soybeans in 15 ml of distilled water and of 2 M HCl. The supernatant calcium content was determined against the calcium standard solution by atomic absorption flame emission spectrophotometer (Shimadzu model AA-6200).

The oxalate and calcium analyses were carried out in the Department of Food Science and Technology, Jomo Kenyatta University of agriculture and technology (JKUAT).

## 3. RESULTS AND DISCUSSION

In general, oxalate content is highest in the leaves, then in the seeds, and lowest in the stems (Singh *et al*., 1972). It must be noted that the leaves and stem of soybean are rarely eaten and therefore the oxalate content of its leaves and stem are of no concern in human nutrition. Since, oxalate in human health diet has been demonstrated to act as an anti-nutrient, as a toxin, and in calcium oxalate kidney stone formation which is on rise in human. The amount of oxalate absorbed from a food is the critical aspect of dietary choice. This is influenced by three major factors: the amount and form of oxalate in the food as consumed, the amount of calcium in the oxalate-containing food and/or meal, and the presence or absence of oxalate-degrading bacteria in the gut (Chai and Liebman, 2004).

Human consumption of soybeans and products made from them is increasing, due to the reported health benefits of eating them in various prepared forms (Messina, 1997). Therefore, it was prudent to analyse the amount, form of oxalate and calcium in the different varieties of soybean seeds grown in Kenya; which would serve as the basis to select cultivars lower in oxalate. From the nine soybean cultivars investigated, the results indicate that, among genetically different cultivars grown in the same agro-ecological zone, the variation in soluble, insoluble and total oxalate was substantial (Figure 1).

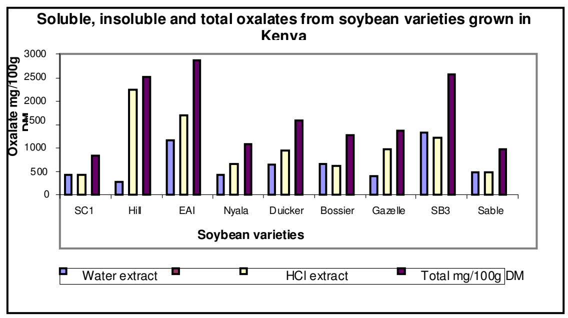

**Fig. 1: Soluble, insoluble and total oxalates from soybean varieties grown in Kenya**

An analysis of nine soybean varieties for soluble oxalate exhibited a ranged from 268.65 to 1332.29 mg/100g DM representing 10% to 52% of the total oxalate, for Hill and SB3, respectively (table 1). The mean of the soluble oxalates was 639.56 mg/100g DM representing 38.4% of the total oxalate. This is in agreement with earlier reports that boiling markedly reduced soluble oxalate content by 30–87% (Chai and Liebman, 2005). Soluble oxalate is extracted from plants using water; it is not surprising that soaking and cooking foods with water reduces the oxalate content by leaching. Soluble oxalates leached out into the cooking water when consumed, have the ability to bind to calcium in the soybean and any calcium in foods consumed with the soybean, reducing the absorption of calcium. Boiling soybean may be an option to reduce soluble oxalate, if the cooking water is not consumed.

The mean of insoluble oxalates in the varieties was 1025.86 mg/100g DM representing 62.6% of the total oxalate; which ranged from 422.57 to 2241.61 mg/100g DM, representing 51% to 89% of the total oxalate for SCS-1 and Hill varieties, respectively (figure 1). Oxalate forms insoluble salts with calcium, magnesium, iron, and copper; these salts have solubilities only from 3 to 22 mg/100 mL (Gélinas and Seguin, 2007). This reduces the bioavailability of these metal ions in animals and human (Savage *et al.,* 2000). Oxalate may be the second compound in legumes that reduces that iron absorption, as phytate binding apparently only accounts for about half of the inhibitory effect of soy on iron absorption (Hurrell *et al.*, 1992; Horner *et al.*, 2005).

The calcium bioavailability is important when one considers the reliance of different population all over the world on plants food as their main source of calcium, as well as the failure to meet the recommended daily allowance of calcium. Calcium is an essential mineral required for the diverse physiological and biochemical functions in the human body (Burton, 1976). Legumes are good source of calcium, in addition to milk and fish with bones. However, oxalates have negative effect on calcium bioavailability. Calcium content varied in different soybean varieties as shown by figure 2.

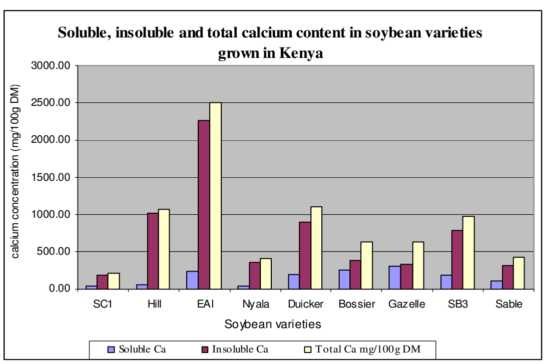


**Fig. 2: Soluble, insoluble and total calcium ion concentration from soybean varieties grown in Kenya**

From water soluble extract, calcium ion concentration of the nine varieties, varied from 35.94 to 299.48 mg/100g DM for SCS-1 and Gazelle, seven folds, respectively, with a mean of 154.86 mg/100g DM. While the insoluble ranged from 181.14 to 2261.4 mg/100g DM for SCS-1 and EA1, 15 folds, respectively, with a mean of 726.96 mg/100g DM. Total calcium content ranged from 217.08 to 2496.99 mg/100g DM for SCS-1 and EAI 3600, respectively, with a mean of 881.81 mg/100g DM. The nine soybean varieties contained calcium higher than 100 mg/100 g DM, which is considered to be a good or excellent source of calcium (Achiraya et al., 2004). Calcium will bind oxalate in the gut, preventing it from being absorbed.

Figure 1, show that SCS-1 had the lowest soluble, insoluble and total oxalate contents of all of the cultivars tested, and it also had the lowest calcium content (figure 2). In contrast, Hill had the highest insoluble oxalate, while EAI 3600 had the highest total oxalate, with higher soluble content than Hill. EAI 3600 exhibited the highest calcium content. There was a correlation between total oxalate content and calcium content in the seeds of soybean varieties, suggesting that as oxalate increases, calcium binding increases.

The simultaneous consumption of calcium with oxalate reduces its absorption because it is virtually insoluble in aqueous solutions, only ~1 mg/100 mL. Liebman and Costa (2000), reported that consumption of 300 mg of either calcium or magnesium with a 198 mg oxalate load reduced absorption by about one half. It has generally been assumed that insoluble oxalate is not significantly absorbed in humans because it is virtually insoluble in aqueous solutions. Some evidence supports that oxalate absorption appears to be proportional to the amount of soluble oxalate (Chai and Liebman, 2004). However, about 2% of calcium oxalate is absorbed intact by rats (Weaver *et al.*, 1997), so a small fraction of insoluble oxalate may be absorbed in human beings as well.

Some cultivars showed much lower total oxalate and calcium contents, suggesting that these, or other high calcium and low oxalate containing cultivars, need to be studied for their suitability in making soyfood products. Bioavailability of food oxalate and, thus, urine oxalate, will also be affected by salt forms of oxalate, food processing and cooking methods, meal composition, and the presence of *O. formigenes* in the patient's gut. The critical factor in a food's effect on urinary oxalate is not the total oxalate but the amount of oxalate that is absorbed from that food and ultimately excreted in the urine (Mandel, 1996).

The present data further reveals that the early maturing varieties and lower yielding Hill and Gazelle exhibited the lowest soluble oxalate levels. While, the old varieties of soybean grown in Kenya (EAI 3600, Hill and Bossier) displayed the highest insoluble, lowest soluble and moderate oxalate, respectively. Gazelle, Nyala and SCS-1 are late maturing, higher yielding and exhibited lower total oxalate. These varieties however, were reported to be eaten by rabbits on farm (data not presented here). Whether this observation may be attributed to oxalate level remains to be investigated. Nyala, Gazelle, and Duicker appear to be the most widespread in Kenya most probably due to seed availability, rather than the choice of farmers. These varieties exhibited moderate oxalate levels in comparison with other varieties with 28%, 38% and 40.3% soluble oxalate, respectively. Sable and SCS-1 displayed the lowest total oxalate levels and is a strong reason why the adoption of the improved varieties of soybean should be encouraged.

These results indicate that, among genetically different cultivars grown in the same general region under the same environmental conditions, the variation in oxalate and calcium content was substantial. These may be attributed to biological variation, including cultivar, time of harvest, and growing conditions; since different cultivars mature at different times. Therefore, identifying germplasms, plant growth conditions and breeding of commercially viable low oxalate cultivars seems a more promising approach to reducing oxalate in soybeans.

All the varieties exhibited higher oxalate levels compared to the recommendations for patients with calcium oxalate kidney stones. Currently, they are advised to limit their intake of foods containing >10 mg per serving, with a total intake not to exceed 50-60 mg per day (Massey and Sutton, 1993; Chicago Dietetic Association, 2000). Under these guidelines, no soybean variety could be recommended for consumption by patients with a history of calcium oxalate kidney stones. Those cultivars with the lowest oxalate and higher calcium content could be used for human feeding studies to determine whether the increases in urine oxalate levels remain below an acceptable level. Research is needed on how to control oxalate synthesis without disrupting normal seed development and quality. With this knowledge, mutants might be found that have little or no oxalate in them, leading to the possibility of their use in making suitable food products with lower oxalate content. All of these issues are of great importance for soybeans to continue to be highly sought after for their nutritional value for human consumption.

## 4. CONCLUSION

In this communication, the initial purposes were to identify soybean variety with lowest oxalate levels for recommendations to patients with high risks of kidney stones or if the patient has been diagnosed with hyperoxaluria, reduction of dietary oxalate may be appropriate i.e. following the blanching or boiling. The result presented provides insights as to the levels of oxalate and calcium in the soybean seeds grown in Kenya and provide dietary advice for reducing urinary oxalate that should include both reduction of dietary oxalate and simultaneous consumption of calcium-rich food or supplement to reduce oxalate absorption. Finally, the total calcium intake for the day should be divided between as many eating occasions as possible.

## 5. ACKNOWLEDGMENTS

The authors are grateful to NCST for financially sponsoring this research (in part) under the grant reference number

NCST/5/003/265 research fund. Thanks to Chemistry, Food Science and Technology Department of JKUAT for allowing us to use their facilities for analysis.

## 6. REFERENCES

[1] Achiraya K.; Prapasri P.; Prapaisri P.; Sirichakwal; Ratchanee K. (2004). In vitro calcium bioavailability of vegetables, legumes and seeds. *Journal of Food Composition and Analysis* 17: 311–320.

[2] asadg@ pacific.netsg (accessed Feb 2001). *Technical Bulletin of the American Soybean Association on Soybean Nutrition*; 1999; Singapore, 23881.

[3] Chai W.; Liebman M. (2004). Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. *Journal of Urology*. 172: 953-957

[4] Chai W. and Liebman M. (2005). Effect of Different Cooking Methods on Vegetable Oxalate Content. *Journal of Agricultural and Food Chemistry* 53 (8): 3027–3030

[5] Chianu and Vanlauwe, (2006). CIAT Africa Highlights No. 35, CIAT Africa, Uganda.

[6] Chicago Dietetic Association. (2000). *Manual of Clinical Dietetics*; American Dietetic Association: Chicago, IL, p 475.

[7] Franceschi, V.R.; Nakata, P.A. 2005. Calcium oxalate in plants: formation and function. *Annual* Review of *Plant Biology*. 56: 41–71.

[8] Greenberg, P.; Hartung, H.N. (1998). The whole soy cookbook: 175 delicious, nutritious, easy-to prepare recipes featuring tofu, *tempeh*, and various forms of nature's healthiest bean. Three Rivers Press, New York.

[9] Horner, H.T.; Cervantes-Martinez T.; Healy R.; Reddy M.B.; Deardorff B.L.; Bailey T.B.; Al-Wahsh I.; Massey L.K.; Palmer R.G.V.(2005). Oxalate and phytate concentrations in seeds of soybean cultivars (*Glycine max* L.). *Journal of Agricultural and Food Chemistry*. 53:7870-7877.

[10] Huang A.S.; Tanudjaja L.S. (1992) Application of anion-exchange high-performance liquid chromatography in determining oxalates in Taro (Colocasia esculenta) corms. *Journal of Agricultural and Food Chemistry*. 40: 2123–2126.

[11] Hurrell, R.F.; Juillerat, M.A.; Reddy, M.B.; Lynch, S.R.; Dassenko, S.A.; Cook, J.D. (1992). Soy Protein, phytate and iron absorption in humans. *The American Journal of Clinical Nutrition* 573-578.

[12] Ilarslan, H.; Palmer, R.G.; Imsande, J.; Horner, H.T. (1997). Quantitative determination of calcium oxalate in developing seeds of soybean (Leguminosae). *American Journal of Botany* 84: 1042-1046.

[13] Libert B.; Franceschi, V. (1987). Oxalate in crop plants. *Journal of Agricultural and Food Chemistry*. 35: 926-938.

[14] Liebman M.; Costa G. (2000). Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *Journal of Urology*. 163: 1565-1569.

[15] Mandel, N. (1996). Mechanism of stone formation. *Semin. Nephrol.* , 16: 354-374.

[16] Massey L.K.; Sutton R.A.L. (1993) Modification of dietary oxalate and calcium reduces urinary oxalate in hyperoxaluric patients with kidney stones. *Journal of American Dietetic Association*. 93: 1305-1307.

[17] Messina, M. (1997). Soyfoods: Their role in disease prevention and treatment. In *Soybeans. Chemistry, Technology and Utilization*; Liu, K., Ed.; International Thomson Publishing: New York, pp 442-477.

[18] Murphy, P.; Song, T.; Buseman, G.; Barua, K.; Beecher, G.; Trainer, D.; Holden, J. (1999). Isoflavones in retail and institutional soyfoods. *Journal of Agricultural and Food Chemistry*. 47: 2697-2704.

[19] Savage, G.P.; Vanhanen, L.; Mason, S.M.; Ross, A.B. 2000. Effect of cooking on the soluble and insoluble oxalic acid content of some New Zealand foods. *Journal of Food Composition and Analysis* 13: 201–206.

[20] Singh P.P.; Kothari L.K.; Sharina D.C.; Saxena S.N. (1972). Nutritional value of foods in relation to their oxalic acid content. *The American Journal of Clinical Nutrition 25:* 1147-1152.

[21] Weast, R.C. (1968). Ed. *CRC Handbook of Chemistry and Physics*, 49th ed.; CRC: Cleveland.

[22] Weaver C.M.; Heaney R.P.; Nickel K.P.; Packard P.I. (1997). Calcium bioavailability from high oxalate vegetables: Chinese vegetables, sweet potatoes and rhubarb. *Journal of Food Science*. 62: 524– 525.