



Calcium Oxalate Crystals as Raw Food Antinutrient: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The nutritional value of food can be altered by the presence of antinutrients such as oxalates present abundantly in plants as ergastic crystals. High oxalate content in plant-based raw food can lead to oxalonephropathy, nephrolithiasis and renal dysfunction. Presence of oxalate can hinder the absorption of other minerals such as calcium and magnesium present in food. CaOx antinutrient intake can be minimised by avoiding plant with high oxalate content or by decreasing oxalate levels through cooking practices like, boiling, fermenting, treating with baking soda, adding yoghurt and milk etc. More sophisticated ways of minimising antinutrient intake through raw diet is by choosing genetically modified crops which are bred for low oxalate content or by choosing mutant varieties which are devoid or low in oxalate content in food crops. The future for sustainable antinutrient management and nutritional value enhancement is by incorporating genes from bacteria or fungi which are capable of breaking down oxalate using the enzyme oxalate decarboxylase, on to plants and thereby modifying them to have less antinutrient effects in raw consumption.

Keywords: Calcium oxalate crystals; ergastic crystals; antinutrient; raw food; CaOx crystals; nephrolithiasis.

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1. INTRODUCTION

Calcium oxalate crystals or ergastic crystals are present in most plants in all organs. Some families of plants such as Amaranthaceae, Oxalidaceae, Chenopodiaceae, Liliaceae, Agavaceae etc. a higher amount of crystal accumulation is noticed. Many of the leafy vegetables, fruits and drinks used by man belong to these families of plants. The presence of CaOx crystals in more than 215 families of higher plants were reported including angiosperms and gymnosperms [1]. Calcium oxalate crystals have many physiological functions in plants, but it acts as an antinutrient when consumed in excess through raw food or raw plant-based drug. Of the different mineral calculi came across in nephrolithiasis 70% is constituted by calcium oxalate [2].

Case studies on Angus cows and sheeps reported rapid death of livestock due to oxalonephropathy after grazing on a weed *Halogeton glomeratus* which accommodates 30% oxalate to its dry weight [3]. Many vegetables like spinach, rhubarb, amaranth, purslane, parsley, soy bean etc. exhibit high oxalate levels when consumed raw [4]. This review aims to give a picture about the antinutrient effects of calcium oxalate in plants and the methods available to minimise it.

2. ERGASTIC CRYSTALS AS RAW FOOD ANTINUTRIENT

2.1 Physiological Significance of Calcium Oxalate Crystals in Plants

2.1.1 Calcium regulation

Calcium oxalate crystals are often seen deposited near vascular bundles near to xylem elements. Sequestering, partitioning and transporting calcium to edible organs and seeds is thereby achieved by plants [5]. Sequestering excessive calcium in an inactive form as calcium oxalate crystals in an effective measure of minimising toxicity. Calcium oxalate crystals have an important physiological function of calcium regulation [6] as they store the excess calcium as calcium oxalate crystals. Calcium oxalate crystals serve as an internal provider of carbon even when the stomata are closed leading to a new photosynthetic pathway called alarm photosynthesis [7].

2.1.2 In the release, germination, and tube growth of pollen grains

In a study on the anthers of *Capsicum annum*, it was found that calcium oxalate crystals are deposited on the hypodermal stomium between the adjacent locules *and* on the connective tissue of it. When the pollen matures it was showed that the calcium is sequestered into CaOX crystals from the connective tissue and thereby degrading the walls between the locules dehiscing the anther and releasing the pollen [8]. In studies on *Petunia hybrida* [9] CaOX crystals are found to be adhering to pollen grains adjacent to stomium and that the calcium needed for pollen germination and tube growth is provided by the crystal and not by the stigma of the flower.

2.1.3 Light adjustment during photosynthesis

Studies on *Peperomia glabella* exhibited that CaOX crystals have an interesting function of reflecting light evenly to chloroplast preventing photo oxidation, it is established by the presence of a druse crystal in every palisade cell and not in idioblast [10].

2.1.4 Herbivore deterrence

Leaves of arrow-leaf sida (*Sida rhombifolia*) when subjected to herbivory [11], CaOX crystal production was found to increase, indicating the formation of CaOX crystals in some plants as a defense response to herbivory. The formation of CaOX crystals in some plants is constitutive rather than induced as simulated herbivory by clipping bulbs of the Negev desert lily, the number of CaOX crystals did not increase significantly (Ruiz, 2002). Increased CaOx crystal accumulation in the secondary phloem in some conifers appears to be antagonistic to beetle attack, suggesting that CaOX crystals functions as a constitutive defense against small bark-boring insects [12].

2.2 Significance of Study of Calcium Oxalate in Diet

The food habits of modern man have changed enormously from past that lifestyle diseases and diseases related to diet imbalances have become very prominent. Calcium oxalate crystals in plants have significance in modern diet as high oxalate levels in certain plant-derived foods can cause serious health problems [13].

Averrhoa carambola (star fruit) belonging to Oxalidaceae family causes serious nephrotoxicity as they are filled with oxalates. Case studies prove that acute renal injury and chronic kidney impairment results from consuming star fruit juice as a health stimulator [14]. It was believed that dietary calcium oxalate has very little contribution to kidney stone formation. But it was experimentally proven that dietary oxalate contributes to 24-53% of urinary oxalate from an intake of 10 to 250 mg oxalate per day [15]. Dietary oxalate was found out to be an important factor in nephrolithiasis or kidney stone formation [16] and a slight increase in its concentration can lead to crystal precipitation. It was also found out that calcium and oxalate have an equal contribution towards the precipitation of calcium oxalate crystals in urine [17].

High-oxalate diet leads to secondary hyperoxaluria whereas primary hyperoxaluria is an autosomal disorder. The dietary recommendation of oxalate is 40-50mg/day [18]. In western countries, the average daily dietary oxalate intake ranges between 44-351 mg/day [19]. When oxalate-rich foods, such as spinach or rhubarb, are consumed, daily intake may even exceed 1000 mg/day [20]. Seasonal rural diets of India even raise the values up to 2000mg/day [21].

2.3 Calcium Oxalate Crystals Present in Plants used as Food

High concentrations of oxalate are accumulated by a number of plants used as food. Polygonaceae, Amaranthaceae and Chenopodiaceae contain most of the plant species with excessively high oxalate concentration. Polygonaceae include buckwheat, rhubarb, and sorrel, whereas beetroot, mangold, spinach, and quinoa are species of the Chenopodiaceae family. Studies show that oxalate is accumulated in plant tissues namely leaves, stems, hypocotyl-root and nuts. Leaves and stems show higher soluble and total oxalate contents than roots and nuts. The highest oxalate content was found in leaves and stems of plants in these families. Soluble oxalate ranged from 59 - 131 mg/100 g in roots and nuts, and from 258 -1029 mg/100 g in leaves and stems. Total oxalate ranged from 143 - 232 mg/100 g in roots and nuts, and from 874 - 1959 mg/100 g in leaves and stems [22]. Analysis of the oxalate content revealed low to medium oxalate concentrations in species of the Brassicaceae and Solanaceae families in radish, kohlrabi,

broccoli, brussels sprouts, cauliflower, cress, sauerkraut, and savoy cabbage, plants of the Brassicaceae family [4].

The raw soybean seed was found to contain a high level of total oxalate (370.5 mg/100 g) and soluble oxalate (200.7 mg/100 g). Total oxalates were variable, ranging from 244.7–294.0 mg/100 g in peas, 168.6–289.1 mg/100 g in lentils, 241.5–291.4 mg/100 g in fava beans, 92.2–214.0 mg/100 g in chickpeas and 98.86–117.0 mg/100 g in common beans [23].

Some of the foods having a high oxalate load are (all values are mg oxalate/100 gm or 3.5 oz of food consumed) chocolate -117mg/100gm, tofu- 275, soy yoghurt-113, black pepper- 419, cocoa powder- 623, buck wheat flour- 269, wheat germ flour- 269, soy flour- 183 [24]. Many green vegetables used widely in green smoothie diets have high oxalate levels such as amaranth leaves- 1090 mg/100gm, chives-1480/100gm, purslane- 1310/100gm, spinach- 970/100gm, lettuce- 330mg/100gm [25]. These foods should only be used in moderation by renal patients.

2.4 Methods Practiced for Minimising Crystals in Food

Studies on two species of taro in Central Vietnam showed that wilting edible parts for 18 hrs resulted in a 5.9% reduction of soluble oxalate content. Soaking in water with 36-38 degree centigrade temperature resulted in 26.2% reduction of soluble oxalate. The most effective method to reduce soluble oxalate in cooked taro was by boiling it for 60 minutes, with an 84.2% reduction in soluble oxalate levels [26].

Another significant reduction in soluble oxalate was observed by boiling and baking taro with cow's milk. The experimental results showed that soaking in baking soda for 2 hrs followed by boiling at 90 degrees for 60 minutes can lower soluble oxalate in taro corm chips [27].

Alcohol fermentation with *Saccharomyces cerevisiae* from 1 to 5 weeks reduced 37–58% of total oxalate and 39–59% of soluble oxalate contents in the juice processing of carambola fruits which is very high on CaOX content. Prolonged fermentation also demonstrated better reduction of oxalate contents [28].

Combining the leaves of Purslane (*Portulaca oleracea L.*) used in raw salads with yoghurt has notable decrease in the soluble CaOX levels from 53% to a very low 10.7 %. [29].

Studies on breeding spinach devoid of CaOx crystals has revealed the scope and significance of genetic engineering in producing modified crops with desired effects. The SNP markers are useful for breeders to select germplasm for reduced oxalate concentrations in spinach breeding programs through marker-assisted selection [30].

Recent studies prove that amino acids including glutamic acid and aspartic acid have an inhibiting action on the nucleation and growth of CaOx crystals [31]. This effect of amino acids has a potential to be genetically utilised for minimisation of CaOx crystals in food crops. Isolation and purification of a novel dimeric protein (98kDa) similar to calnexin from the seeds of *Dolichos biflorus* (L.) exhibit calcium oxalate crystal inhibition in invitro studies [32] is promising in molecular level intervention by gene regulation and thereby eliminating the antinutrient effect of CaOx crystals from plants.

Studies on chemically mutagenized *Medicago truncatula* plants show a variety of cmd (crystal morphology defective) and cod (calcium oxalate defective) mutants [33] which are highly explanatory of formation of CaOx crystals. Genetic analysis of the isolated morphology mutants revealed that a single mutation could result in dramatic alterations in crystal shape and size. Cmd plants showed varied size and shapes of CaOx crystals whereas cod plants showed over expression and under expression of oxalate content in different point mutations.

Genetically modified transgenic tomatoes engineered by introducing a gene expressing oxalate decarboxylase which converts oxalate to formic acid and CO₂ isolated from the fungus *Flammulina velutipes* expressed 90% reduction in oxalate in the fruits [34]. Another important discovery of oxalate catabolism was an anaerobic gram-negative bacteria called *Oxalobacter formigenes* inhabiting the colon of humans and many vertebrates. They are capable of breaking down oxalate to formic acid and CO₂ by secreting the enzyme oxalyl Coenzyme A decarboxylase [35]. Rat model studies confirmed reversal of hyperoxaluria by feeding them with *Oxalobacter formigenes* supplement [36].

2.5 Significance of Minimising Calcium Oxalate in Plants used as Food

Minimising the concentration of calcium oxalate from food crops is a necessity as this acts as an

antinutrient. High oxalate content hinders the absorption of calcium and magnesium [37] from the food consumed decreasing the nutritional quality of the food despite its high nutritional value. An experimental analysis comparing the calcium absorption from kale which is a low oxalate vegetable and spinach which is a high oxalate vegetable showed that high oxalate levels diminish the availability of calcium for nutritional absorption [38].

Increased bioavailability of calcium was demonstrated by feeding mice with genetically engineered *Medicago truncatula* cod5 mutant plants lacking CaOx crystals [39] It showed inversion of adverse effects of CaOx on Calcium absorption from food.

2.6 Other Application of Calcium Oxalate Crystals in Plants

Calcium oxalate crystals also play a role in the taxonomical identification of plant species in the archaeological remains or fossil remains of paleontological interest [40]. CaOX crystals play an important role in species level identification of plants leading to differentiation of adulterants in raw drug analysis [41].

2.7 Future Research Possibilities

Transcriptome analysis of differentially expressed genes for finding the genetic make up of calcium oxalate expression in plants and thereby utilising it in genetically modifying plants can be the future of antinutrient calcium oxalate management in the food crops. Extensive research is needed in finding strains of bacteria and fungi capable of oxalate degradation using the enzyme oxalate decarboxylase. Such isolated genes responsible for oxalate decarboxylase production when incorporated in target plant genomes can lead to minimised oxalate load in plants used as a food and drugs.

The gut bacteria *Oxalobacter formigenes* which can degenerate oxalate can be utilised in making endophytic associations with plants and thereby the calcium oxalate load regulation studies are a promising hypothesis.

3. CONCLUSIONS

Calcium oxalate crystals or ergastic crystals are potential antinutrients capable of causing nephrolithiasis and renal toxicity and hence plants with high oxalate content should be

avoided by renal patients. An exclusive diet on green leaf juices or smoothies high on oxalate for weight management can have negative effects on renal health. Vegetarians following a high oxalate diet can have calcium deficiency as oxalate diminishes calcium absorption. Therefore, an antinutrient analysis is highly recommended in diet plans as an intake of more than 40mg oxalate per day can promote hyperoxaluria and can seriously influence the nutritional quality of foods even if the nutritional value is high.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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