Minireview Article

CALCIUM OXALATE CRYSTALS AS RAW FOOD ANTINUTRIENT: A REVIEW

Abstract

The food habits of modern man has 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 like hyperoxaluria, urolithiasis and renal failure. CaOX antinutrient can be minimised by cooking practices like adding yoghurt and milk, boiling, fermenting, treating with baking soda etc. More sophisticated way of minimising antinutrients is by adopting genetic manipulations in food crops which is paved way by knowledge of mutant analysis studies and SNP marker analysis.

KeyWords: Calcium oxalate crystals, antinutrient, raw food

1. Introduction

The presence of calcium oxalate crystals in the tissues of higher plants is a common phenomenon (Arnott and Pautard, 1970). Calcium oxalate crystals are formed in specialized cells called crystal idioblasts (Foster, 1956). Calcium oxalate is found in nature in three different crystalline states determined by the number of H_2O in the unit formula (whewellite $CaC_2O_4.H_2O$, COM; weddellite $CaC_2O_4.2H_2O$, COD and caoxite $CaC_2O_4.3H_2O$, COT). Members of more than 215 plant families accumulate crystals within their tissues (McNair, 1932). CaOx crystals are beneficial to plants but when consumed as food by animals and humans can act as an antinutrient causing serious renal ailments.

2. Ergastic Crystals As Raw Food Antinutrient

2.1. Physiological significance of calcium oxalate crystals in plants

2.1.1. Calcium regulation : Calcium is often accumulated by plants in excess for cytoplasmic requirements which can interfere with several metabolic processes like calcium dependent signalling and energy metabolism. Hence cytoplasmic free calcium levels are strictly controlled (Webb, 1999). Sequestering excessive calcium in an inactive form as calcium oxalate crystals in an effective measure of minimising toxicity. Calcium oxalate crystals are the most common biominerals in plants and ranges from 3-80% of plant dry weight and about 90% of total calcium of a plant. So calcium oxalate crystals are assigned an important physiological function of calcium regulation (Franceschi and Nakata 2005). Accumulation of calcium oxalate crystals at the vicinity of stomatal guard cells are responsible for calcium regulated opening of stomata and gas exchange thereby (Ruiz and Mansfield, 1994).

2.1.2. In the release, germination, and tube growth of pollen grains : In a study on the anthers of *Capsicum annuum*, 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 (Horner and Wagner, 1980). In studies on *Petunia hybrida* (Iwano, 2004) 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 chrloroplast preventing photo oxidation, it is established by the presence of a druse crystal in every palisade cell and not in idioblast (Kuo-Huang, 2007)

2.1.4. Herbivore deterrence : Leaves of arrow-leaf sida *(Sida rhombifolia*) when subjected to herbivory (Molano-Flores,2001), 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 (Hudgins,2003).

2.2. Significance of study of calcium oxalate in diet

The food habits of modern man has 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 (Nguyên and Savage, 2013). Hyperoxaluria a condition of excessive excretion of oxalate through urine can result from the over absorption of oxalate from the gastrointestinal tract (Williams and Wandzilak, 1989). Until recently 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 (Holmes et al., 2001). Dietary oxalate was found out to be an important factor in nephrolithiasis or kidney stone formation (Holmes and Assimos, 2004) and a slight increase in its concentration can lead to crystal precipitation even though urinary oxalate concentration is only $1/10^{th}$ that of calcium (Young, 1987) .It was also found out that calcium and oxalate has an equal contribution towards the precipitation of calcium oxalate crystals in urine (Pak et al., 2004).

High-oxalate diet leads to secondary hyperoxaluria whereas primary hyperoxaluria is an autosomal disorder. In western countries, the average daily dietary oxalate intake ranges between 44-351 mg/day (Holmes and Kennedy, 2000). When oxalate-rich foods, such as spinach or rhubarb, are consumed, daily intake may even exceed 1000 mg/day (Hoppe et al., 2005). Seasonal rural diets of India even raises the values up to 2000mg/day (Siener et al., 2003).

2.3. Calcium oxalate crystals present in plants used as food

High concentrations of oxalate is 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 (Siener et al., 2006.). Patients with calcium oxalate stone disease should avoid these oxalate-rich foods.

A recent 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, total oxalate concentration was very low (<10 mg/ 100 g), ranging from below detection limit to 7.1 mg/ 100 g. In potatoes, tomatoes, and aubergine, plants of the Solanaceae family, total oxalate content was low to medium (<50 mg/100 g), ranging from 8.5 to 17.1 mg/100 g). (Ho¨now & Hesse, 2002).

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 (Shi etal., 2018).

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 (Hang etal., 2013)

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 (Kumoro et al.,2014).

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 (Huynh and Nguyen,2017).

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 %. (Savage and Moreau,2009)

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. It overlaps with the absorption of calcium and magnesium from the food consumed even if it is rich in calcium and magnesium (Weaver etal.,1987). 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 (Benway and Weaver, 1993).

Increased bioavailability of calcium was demonstrated by feeding mice with genetically engineered *Medicago truncatula* cod5 mutant plants lacking CaOX crystals(Morris etal., 2007) It showed interference 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 (Canti, 2003). CaOX crystals play an important role in species level identification of plants leading to differentiation of adulterants in raw drug analysis (Nayagam, 2015).

2.7. Future Research Possibilities

Studies on breeding spinach devoid of CaOx crystals has revealed the scope and significance of genetic engineering in producing modified crops with desired effects. Association analysis indicated that six SNP markers (AYZV02031464_116, AYZV02031464_117, AYZV02031464_95, AYZV02283363_2707, AYZV02287123_2830, and

AYZV02296293_852) were associated with the oxalate concentration. The SNP markers may be useful for breeders to select germplasm for reduced oxalate concentrations in spinach breeding programs through marker-assisted selection (Shi et al., 2016)

Recent studies prove that amino acids including glutamic acid and aspartic acid have an inhibiting action on the nucleation and growth of CaOX crystals (Golovanova et. al., 2006). This effect of amino acids have 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 (Bijarnia etal., 2009) is promising in molecular level intervention by gene regulation and thereby eliminating the antinutrient effect of CaOX crystals from plants

Recent studies on chemically mutagenized *Medicago truncatula* plants show a variety of cmd (crystal morphology defective) and cod (calcium oxalate defective) mutants,which are highly explanatory of role 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 overexpression and under expression of oxalate content in different point mutations .

3. Conclusions

Calcium oxalate crystals are ergastic substances found frequently in plant tissues even in edible or economicaly useful parts of plants. Eventhough calcium oxalate crystals play a role in the taxonomical identification of plant species in the archaeological remains or fossil remains of paleontological interest and in species level identification of plants leading to differentiation of adulterants in raw drug analysis it is a potential antinutrient when it is consumed in raw form. It is proved that high-oxalate diet leads to secondary hyperoxaluria.

Therefore care should be taken for minimising CaOX antinutrient by cooking practices such as adding yoghurt and milk, boiling, fermenting, treating with baking soda, cooking or baking. More sophisticated way of minimising antinutrients is by adopting genetic manipulations in food crops which is paved way by knowledge of mutant analysis studies and SNP marker analysis.

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