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Raphides in Food – An Unsafe Menu

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Abstract

Calcium oxalate in plant bodies lead to stones in kidney, upon consumption. Calcium oxalate is frequently found in plants in the form of tiny needle like raphides. Out of the 5 types of calcium oxalate crystals, raphides are the predominant ones. Calcium oxalate gets incorporated in our body through plant derived food that contains them; a little amount of them is also synthesized in humans endogenously. Both the sources contribute to kidney problems. Occurrence of calcium oxalate is not limited to higher plants only but also extends to algae, fungi and lichens. Out of all the 3 forms of calcium oxalate, monohydrate form is the one widely reported to cause kidney problems. In this study, we review raphides and explore their possible remediation in order to utilize plants of food and medicinal importance the better way. We also review traditional knowledge of raphide neutralization and point to the methods of removal of calcium oxalate and raphides.

Introduction

Plants are the key sources of food and medicine. Calcium oxalate, a potential causative agent of human kidney stones, can range from 3 to 80% of the dry weight of various plants [1-3]. Calcium oxalate exists in varying crystal shapes and sizes in plants, with raphides being the predominant crystal form [2,4-8]. Deleterious influence of raphides, in addition to promoting kidney stone formation, include irritation to throat, mouth and skin [7,9-18]. Excess presence of raphides, in conjugation with cytotoxic compounds [5,19], can render the food poisonous and is responsible for mentionable fatalities every year [20-22]. Calcium oxalate can contribute up to 70% or 75% of the composition of kidney stones [23,24]. It is present in mono, di and tri-hydrate forms [25-28]. Monohydrate form is the least soluble and the main constituent of nephroliths [23,24,29]. Monohydrate form readily attaches to the cell surface of the renal tubules [24,30]. In addition to major absorption of calcium oxalate from plant food sources, smaller amount of calcium oxalate can be synthesized endogenously from free oxalic acid or directly from several other biosynthetic precursors [23,31-33]. Liver is the main site of endogenous synthesis of oxalic acid [34]. Inter-conversion rates between monohydrate and dihydrate state governs the attachment of calcium oxalate to renal membrane [24]. Oxalate formation can deplete the human body of divalents, including calcium [35,36]. This depletion of calcium due to calcium oxalate formation aids in osteoporosis [37-39].

In addition to medicines [40,41], dietary control is frequently recommended in the treatment of kidney stones[33,37]. Some proteins that can regulate kidney stone formation [24,42,43] have been identified, which raises a hope for novel drug development. Preclinical studies indicate that few plant extracts can inhibit the growth of calcium oxalate crystals [44], as well as block their adhesion to renal epithelial cells [45,46]. Given that the removal of calcium oxalate from diet can have such a large impact on human health, we review crystals of calcium oxalate in plants, calcium oxalate biosynthesis, and possible ways of calcium oxalate neutralization.

Crystalline Calcium Oxalate; Importance of Raphides

Calcium oxalate is found in several crystal shapes in plants. In some plants presence or absence of a certain kind of crystals can help in taxonomic identification [9] and evolutionary studies [47]. The major kinds of crystals include: needle shaped “raphides” [5-7,48,49], cuboidal or pencil shaped “styloids” [6,7], block shaped aggregates called “crystal sand” [2,11], prism shaped structures [8], and mace-head or rosette shaped aggregates called “druses” [4]. Raphides are found to have subdivisions in their shapes. According to their shapes, they are classified from type 1 to 4 [9] and a recent study has reported 2 more additional kinds [50]. The calcium oxalate crystals are formed in specialized vacuoles of idioblast cells. The shape of idioblasts, which is under genetic influence, governs the shape of the crystals [51] and is species specific [52]. There can be several kinds of crystals present in the same plant [4,9]. Figure 1 shows the common kinds of Calcium Oxalate crystals [4,17,22,53-59].

Raphides provide plant defense against herbivore [60,61]. Herbivory has been demonstrated to increase the amount of raphides in plants [60]. Frequently raphides are co-present with cysteine proteases and other chemical defense [5]. The needle shaped raphides bruise the lining of the throat, gut, and intestine, while cysteine proteases add to the irritation [15]. Studies using larvae and caterpillars have shown additive effect of the irritants such as proteases and raphides [62]. Raphides can act as needles or syringes to deposit cysteine proteases in herbivore cells [4]. More acridity measurement studies are needed to evaluate the synergistic action of raphides and cysteine proteases on human taste perception [5].

Raphide crystals also play a role in reducing metal toxicity. This suggestion has largely been based on the observation that such crystals can have many other divalents [63-65]. Raphides have also been implicated in light scattering and increasing efficiency of photosynthesis [12,18] but work is required on other crystal types to see if it is exclusively a phenomenon limited to raphide containing plants. Some studies have also suggested a clear role of raphides in

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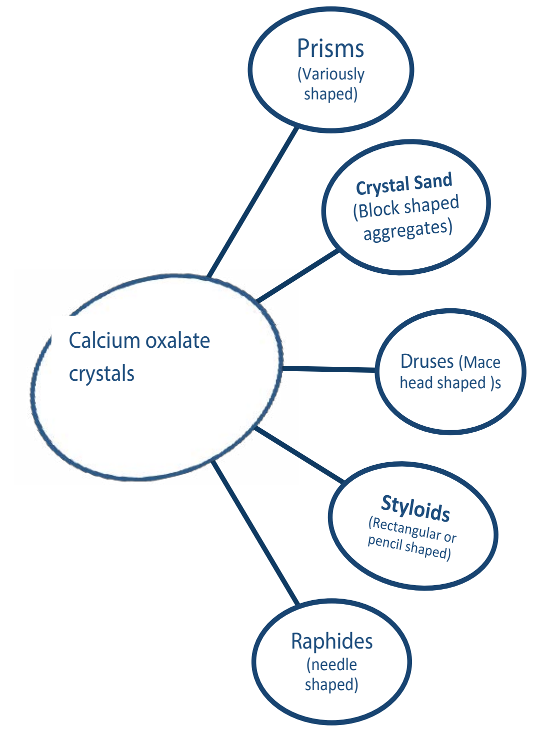

Figure 1: Various kinds of common calcium oxalate crystals.

structural support to the plant [7,66]. So far, other crystal shapes have not been explored much in defense. Several crystal shapes have been implicated in calcium regulation [67-70] and play an important role in ion regulation.

Biosynthesis of Calcium Oxalate

Many recent studies have established that several precursors can be used to synthesize calcium oxalate. The major precursors are ascorbate, glycolate, glyoxylate, oxaloacetate and isocitrate [53,71-75]. Different precursor use by the different parts of a plant remains to be explored. Biosynthetic pathways have been very well reviewed at length in several recent works [53,71-76] so we are merely summarizing the key findings and focusing on amelioration in the next section. The synthesis of ascorbate, a precursor of calcium oxalate takes place in the vacuoles of idioblast cells itself. Concentration of oxalate is higher in idioblasts and calcium is incorporated to make calcium oxalate [18,25]. This higher oxalate in idioblasts results in predominantly the monohydrate form and not the di or trihydrate form [2]. The process of calcium oxalate crystal formation takes place in a matter of hours [77], raising hopes of its possible quick removal. Figure 2 summarizes the common biosynthetic pathways and possible amelioration approaches.

Traditional Remediation of Raphides

Not much modern analysis has been conducted of the traditional approaches, creating a problem of lack of peer-reviewed scientific material on the particular topic of traditional methods. We are bringing forward such information with the hope that modern analysis of these approaches follow soon. Traditional approaches work largely by neutralizing the cysteine proteases. Milk and coconut milk have been used in India to ameliorate the acridity of raphides and they likely work by providing protein substrates to interact with the active poisons present along with raphides. Tamarind [78], lime [79] and various other acidic treatments have also been used because acid can neutralize toxins. Heating, boiling, frying [80], baking [81], battering, mashing, fermentation and sun drying [82] have also been used for the neutralization of cysteine proteases and release of raphides from idioblasts. Changes in crystal structures of calcium oxalate itself due to these traditional approaches should be explored systematically but this topic has not received attention yet.

Removal of Calcium Oxalate

While several methods neutralize raphides, very few have addressed the neutralization of the long-term damage of kidney stones that increases by eating foods rich in calcium oxalate. There are few traditional approaches that offer hope and few established methods that are yet to gain popularity. Peeling of foods where plant pericarp has high calcium oxalate [83] seems to be an effective and economical way of reducing calcium oxalate. In addition, there are emerging pieces of evidence that raise the hope that several plants with huge drug and food potential can be better utilized by reduction or removal of calcium oxalate. Few approaches, such as treatment with strong acid, sodium bicarbonate and tetracycline treatment have been shown to work but it is not clear as of now that any of these approaches can be applied at mass scale due to unacceptable alteration of food and the associated costs. Some fungi have been shown to be able to degrade calcium oxalate [84] and they offer a hope for future biotech applications to remove calcium oxalate. The *Flamnulina velutipes* study shows formation of formic acid but formic acid is extremely unpleasant and additional enzymatic steps might be required to use the enzymes from this fungus [85]. Transgenic tomatoes have been made using OXDC gene from *Flamnulina velutipes* like fungi, are port from bacteria also suggests break down of calcium oxalate [86-91]. CoD genes, as observed in *Medicago trancatula*, regulate the formation of calcium oxalate crystals and this gene might also have good biotechnology potential [14]. Heat at the levels used in traditional cooking removes only a small fraction of calcium oxalate, if any, [92] but we suspect that its potential might lie in combination with other approaches. Fast synthesis of calcium oxalate in idioblasts [77] raises hope that an equally fast removal of calcium oxalate might be a possibility. We would expect next few years of research to provide us with signaling and extrinsic factors.

Conclusion

To summarize, we have briefly evaluated the kidney stone formation due to calcium oxalate, explored the various crystalline forms of the compound, with a focus on raphides and looked at its biosynthesis, with the end goal of evaluating novel possible calcium oxalate amelioration methods for better utilization of plants by humans. Modern evaluation of traditional methods has been largely lacking but from knowledge of known ingredients and limited published work, one can infer that milk, heating, boiling, frying, baking, battering, mashing, fermentation

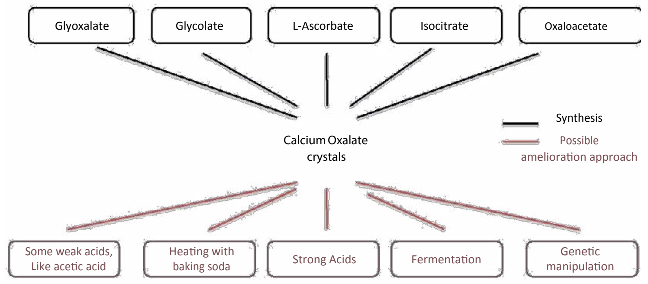

Figure 2: Calcium oxalate biosynthetic pathways in plants and possible approaches to remove calcium oxalate from food.

and sun drying, likely work by neutralization of cysteine proteases or through release of raphides from idioblasts or both. Neutralization of calcium oxalate is a bigger health question than the neutralization of specific crystal form of raphides. A traditional approach of peeling plant pericarp rich in calcium oxalate has beneficial effects. Discovery of fungi and bacteria that can break down calcium oxalate and plant genes that regulate calcium oxalate formation have all offered hope and some initial promising results in genetic engineering to counteract calcium oxalate toxicity. We expect the next few years to witness more chemical, physical and genetic engineering approaches to make food safer for the health of kidneys.

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