Physicochemical characteristics of calcium oxalate crystals in

*Spinacia oleracea* L.

S Subashini & K Sathish Kumar*

Department of Chemical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam-603 110, Tamil Nadu, India

Received 08 June 2017; revised 02 August 2017

*Spinacia oleracea* L. is a leafy green vegetable plant. It contains minerals like potassium, iron, selenium, manganese, and abundance of calcium. The absorption of calcium is affected by oxalate, which converts calcium into calcium oxalates. Calcium oxalate crystals synthesized by plants exhibits specific morphology with unique characteristics. Calcium oxalate crystal was isolated from a different part of *Spinacia oleracea* namely leaves and stems in the present work. Physicochemical characteristics of the crystals isolated from various parts of *Spinacia oleracea* were studied. Crystal size and morphological characteristics of the isolated calcium oxide crystals were studied using Scanning Electron Microscope. Functional groups present in isolated crystals were characterized using Fourier Transform Infrared Spectroscopy and Proton nuclear magnetic resonance. Mineral composition was analyzed using Energy Dispersive X-ray Spectroscopy and confirmed the purity of isolated calcium oxalate crystal. The cubical crystalline structure of the calcium oxalate crystals in *Spinacia oleracea* namely leaves was confirmed using X-Ray Diffraction analysis.

**Keywords:** Calcium oxalate, Edible plants, Plant minerals, *Spinacia oleracea*

Calcium oxalate crystals occur in most organs and tissues of many edible plants. They generally form within plant cells. Edible plants such as spinach, parsley, rhubarb, cranberries, celery, peanuts, soy products, fiber-containing cereals, bran, and chocolate contain a high amount of oxalate. Calcium oxide crystals in plants are formed from endogenously synthesized oxalic acid and calcium from the soil solution in contact with plant roots

Cell mediated crystallization of calcium oxalate in plants was reported by Webb (1999)\(^5\). Calcium oxalate crystals were isolated from seed coats of *Phaseolus vulgaris* and leaves of *Vitis vinifera*\(^6\). Calcium oxalate crystals were extracted from leaves of *Codiaeum variegatum*. The chemical and physical characteristics of extracted crystals were studied. These calcium oxide crystals were reported for very high crystallinity, and crystals were not damaged during the extraction\(^6\). Calcium oxalate crystals were extracted from a foliar tissue of various woods by using different solvents and freezing and thawing.

Calcium was estimated using an inductively coupled plasma emission spectrophotometer and oxalate was estimated using a high-performance liquid chromatography\(^7\).

The calcium oxalate crystals consist of either the monohydrate whewellite or the dihydrateweddellite on the basis of their shape. The presence of calcium oxalate in plants was identified using techniques such as X-ray diffraction, Raman microprobe analysis and infrared spectroscopy and the morphology of calcium oxalate crystals were studied using Scanning electron microscope. But the actual mechanism of controlling shape calcium oxalate is unknown\(^5,8\).

Calcium oxalate in vegetable foods can give toxic effect to the human body and act as an anti-nutrient\(^9\). High calcium oxalate concentrations in vegetable foods can resultin renal problems in human. Humans who form calcium oxalate stones in kidney should avoid eating foods containing high amounts of oxalate\(^10\). Spinach is one of the most commonly used leafy vegetables. It contains calcium oxalate in the range of 540-990 mg/100 g\(^11\).

Thus the present work was focused on

---

*Correspondence:
Email: sathishkumark@ssn.edu.in
identification and characterization of calcium oxalate crystals isolated from leaves and stems of *Spinacia oleracea*.

**Materials and Methods**

**Collection of *Spinacia oleracea* plant**

*Spinacia oleracea* was procured from the local market, Chennai. *Spinacia oleracea* plant was washed thoroughly with tap water and followed by deionized double distilled water to remove soil or other dust. Water present on leaf and stems surface was drained by using the perforated tray. Leaves were separated from stems, cut into small pieces and refrigerated at 4°C for overnight.

**Isolation of calcium oxalate crystals from *Spinacia oleracea***

The refrigerated *Spinacia oleracea* leaves (50 g) was mixed with 50 mL of deionized double distilled water and homogenized using a high-speed mixer to produce juice containing calcium oxalate crystals. The leaf material was separated from plant juice by filtration through a 0.2 mm sieve. The filtrate containing calcium oxalate crystals was collected. The plant material retained on the sieve was ground again with the same volume of deionized water and juice was filtered to collect filtrate containing calcium oxalate. The procedure was repeated until the plant juice becomes clear. The filtrate was centrifuged at 6000 rpm for 30 min to separate remaining plant tissues and calcium oxalate crystals. The supernatant was discarded, and the pellet was dried at 65°C for overnight. The dried pellet was mixed with hydrogen peroxide (30%) and allowed to settle for 24h to remove the organic impurities. Then obtained sediment was washed with deionized water and dried again at 65°C for overnight. The same procedure was followed for isolation of calcium oxalate crystals from *Spinacia oleracea* stems.

**Characterization of calcium oxalate crystals isolated from *Spinacia oleracea***

Calcium oxalate crystals isolated from stems and leaves of *Spinacia oleracea* were characterized. Calcium oxalate crystals were examined by SEM (QUANTR 200) with magnification range 35-30000, equipped with Energy Dispersive Spectroscopy (EDS). The characteristic functional groups present in oxalate crystals were analyzed using Fourier Transform-Infra Red (FTIR) spectroscopy using BRUKER α-T FTIR Spectrometer. The sample was mixed with KBr (binding agent) and made into discs at high pressure using a hydraulic press. The disc was scanned in the range of 400-4000 cm⁻¹ to obtain a FTIR spectrum. The crystalline structure of calcium oxalate was studied using XRD analysis. XRD pattern was recorded in XPERT-PRO diffractometer. The scan step for 2θ was 0.0170° with a scan step time of 38.1 Sec.

**Results and Discussion**

**SEM analysis of calcium oxalate crystals in *Spinacia oleracea***

The Scanning Electron Microscope is used to reveal the morphological and structural characteristics of crystalline materials. The SEM images calcium oxalate from *Spinacia oleracea* stems isolates revealed the presence of a crystalline substance (Fig. 1). The shape of the crystalline material becomes clear as the resolution of the SEM image increased from 20-2 μm (Fig. 1 A-D). The Fig. 1D clearly revealed the crystalline nature and cubical shape of crystals in *Spinacia oleracea* stems. The SEM images of *Spinacia oleracea* leaves isolate also revealed the presence of a crystalline substance (Fig. 2). Though the clarity of the SEM images from Fig. 2 A-D increased with increase in resolution from...
20-2 µm. The concentration of crystals was found very less in *Spinacia oleracea* leaves compare to *Spinacia oleracea* stems.

**EDX analysis of calcium oxalate crystals in *Spinacia oleracea***

The EDX analysis was used to investigate the composition of various components in the sample. Thus it used to identify the type of crystalline materials present in *Spinacia oleracea* isolates from stems and leaves. The peaks in EDX results shown in Fig. 3 & 4 confirmed the presence of calcium oxalate crystals in *Spinacia oleracea* stem and leaf isolate. In addition, carbon represents the major portion of the sample as it is derived from plant material (*Spinacia oleracea* stems and leaves). The higher concentration of carbon confirms the rich carbohydrate content of both *Spinacia oleracea* stems and leaves. The EDX results also confirm the absence of water soluble proteins in *Spinacia oleracea* stem and leaves. The concentration of calcium oxalate crystal in *Spinacia oleracea* stems was found higher than *Spinacia oleracea* leaves isolate.

![EDX pattern of calcium oxalate crystals in *Spinacia oleracea* stems](image1)

![EDX pattern of calcium oxalate crystals in *Spinacia oleracea* leaves](image2)
H-NMR analysis of calcium oxalate crystals in Spinacia oleracea leaves

H-NMR pattern of calcium oxalate crystals in Spinacia oleracea leaves is shown in Fig. 5. Chemical shift of various functional groups confirmed is listed in Table 1. A short narrow peak ranging between 0.5-1 ppm indicated the presence of primary alkyl group. A strong narrow peak between 1-1.5 ppm, particularly at 1.224 ppm showed the presence of secondary alkyl group. The Peak at 1.5 ppm confirmed the presence of tertiary alkyl group. Peaks ranging between 2-2.2 ppm is indicative of the presence of aromatic methyl ketone group. A sharp narrow peak at 2.5 ppm showed the presence of aromatic methyl and alkynyl carbon. Minor and weak peaks ranging between 3-4.5 ppm confirmed that aromatic functional groups such as alcohol, ether, and ester are present in trace amounts along with alkyl fluoride and other inorganic functional groups.

FTIR spectroscopic analysis of calcium oxalate crystals in Spinacia oleracea

The FTIR spectrum is a plot of measured infrared intensity versus wave length of light transmitted or
absorbed. The FTIR spectrum was run for pellets of *Spinacia oleracea* leaves with KBr for the frequency range from 400-4000 cm\(^{-1}\). The percentage transmittance of light in FTIR spectrum *Spinacia oleracea* leaves is shown in Fig. 7.

The presence of inorganic compounds are represented by the peaks at 1072 cm\(^{-1}\), 1384 cm\(^{-1}\), 1454 cm\(^{-1}\), 1548 cm\(^{-1}\), 1644 cm\(^{-1}\) and 1317 cm\(^{-1}\) (Table 3). Transmittance peak at 1072 cm\(^{-1}\) confirms the presence of sulfates and phosphates in *Spinacia oleracea* leaves (NIST). The peak at 1384 cm\(^{-1}\) confirms the presence of nitrates, the peak at 1454 cm\(^{-1}\) confirms the presence of carbonates, the peak at 1548 cm\(^{-1}\) confirms the presence of carboxylate and the peak at 1644 cm\(^{-1}\) and 1317 cm\(^{-1}\) confirms the presence of calcium oxalate in *Spinacia oleracea* leaves. The presence of organic compounds is represented by the peaks at 1454 cm\(^{-1}\), 1644 cm\(^{-1}\), 2926 cm\(^{-1}\) and 3418 cm\(^{-1}\). The peak at 2926 cm\(^{-1}\) representing C-H stretch and the peak at 1644 cm\(^{-1}\) representing C=C stretch confirms the presence of various alkyl and alkenyl organic compounds. The broad spectrum at 3418 cm\(^{-1}\) represents the presence of aliphatic and aromatic compounds in *Spinacia oleracea* leaves. The FTIR spectrum of *Spinacia oleracea* stems represents the presence of following groups (Table 4). The peak at 2927 cm\(^{-1}\) indicates the presence of C-H medium stretch vibration (Fig. 8). Peak at 2857 cm\(^{-1}\) is indicative of the presence of C-H medium stretch vibration. Peak at 1747 cm\(^{-1}\) represents the presence of C=O strong vibration and peak at 1163 cm\(^{-1}\) shows the presence of C-O-C stretch vibration. These results match with the reported presence of calcium oxalate crystals in leaves of Agavaceae, Aizoaceae, and Asphodelaceae was confirmed by FTIR\(^{13}\).

---

**Table 2 — PPM range of various functional groups in calcium oxalate crystals isolate from *Spinacia oleracea* stems by \(^1\)H-NMR analysis**

<table>
<thead>
<tr>
<th>PPM/Chemical shift</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7-0.9</td>
<td>Primary alkyl R-CH(_3)</td>
</tr>
<tr>
<td>1-1.3</td>
<td>Secondary alkyl R(_2)CH(_2)</td>
</tr>
<tr>
<td>1.4-1.6</td>
<td>Tertiary alkyl R(_3)CH</td>
</tr>
<tr>
<td>1.7-2.0</td>
<td>Allylic carbon</td>
</tr>
<tr>
<td>2.1-2.3</td>
<td>Methyl ketone -(\text{C}CH(_3))</td>
</tr>
</tbody>
</table>
The structural characteristics of calcium oxalate obtained from *Spinacia oleracea* leaves and stems were studied using X-ray Diffraction analysis. The crystalline nature was confirmed with respect to the lattice parameters. The study was carried out for bragg angle ranging from 10-100° as shown in Figs. 9 & 10. The diffraction peak obtained at 20°, 24.9° and 35.6° confirms the crystalline structure with respect to the corresponding lattice parameters. The diffraction peak obtained at 24.9° shows the crystalline nature and the sharp peak indicating the cubic structure of calcium oxalate present in *Spinacia oleracea* leaves and stems. Also, the sharpness of the peak indicates there is no change in the nucleation and growth of the crystals. The 2θ value of the peaks was indexed using JCPDS FILE (30-31) and corresponded to following planes of calcium oxalate crystal. The indexed planes corresponded to the cubic structure of calcium oxalate crystals. The sharpness of the peaks in Fig. 10 indicated that the calcium oxalate crystals in pure crystalline phase was characterized using SEM and confirmed by XRD analysis with a larger peak at a 20 value of 24.5°.
Conclusions

The presence of calcium oxalate in *Spinacia oleracea* stems and leaves was confirmed. The SEM analysis has shown the morphology of the calcium oxalate crystals in *Spinacia oleracea* samples. The elemental analysis by EDX analysis confirmed the presence of calcium oxalate in both *Spinacia oleracea* stems and leaves. The $^1$H NMR and FTIR analysis of confirmed the presence calcium and other inorganic functional groups in both *Spinacia oleracea* stems and leaves. The structural analysis by XRD confirmed the crystalline nature and cubical structure of the calcium oxalate in *Spinacia oleracea* stems and leaves. The high density of crystals in SEM image and higher peak height in EDX spectrum were found for *Spinacia oleracea* stems samples. Thus it was confirmed the presence of high concentration of calcium oxalate in *Spinacia oleracea* stems than leaves.

References

10 Canessa LM & Omoloja A, Oxalate Content of Foods, Diet and Oxalate Edited by the Nephrology Department, The Children’s Medical Center, Ohio 45404, 2005.
11 WFBMC (Wake Forest Baptist Medical Center), Medical Center Boulevard, 2015, Winston-Salem, NC 27157.