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*Research Article*

# Characterization of Calcium Compounds in *Opuntia ficus indica* as a Source of Calcium for Human Diet

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Analyses of calcium compounds in cladodes, soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) of *Opuntia ficus indica* are reported. The characterization of calcium compounds was performed by using Scanning Electron Microscopy, Energy Dispersive Spectrometry, X-ray diffraction, and infrared spectroscopy. Atomic Absorption Spectroscopy and titrimetric methods were used for quantification of total calcium and calcium compounds. Whewellite ($CaC_2O_4 \cdot H_2O$), weddellite ($CaC_2O_4 \cdot (H_2O)_{2.375}$), and calcite ($CaCO_3$) were identified in all samples. Significant differences ($P \leq 0.05$) in the total calcium contents were detected between samples. $CaC_2O_4 \cdot H_2O$ content in cladodes and IDF was significantly higher ($P \leq 0.05$) in comparison to that observed in SDF, whereas minimum concentration of $CaCO_3$ was detected in IDF with regard to $CaCO_3$ contents observed in cladodes and SDF. Additionally, molar ratio oxalate : $Ca^{2+}$ in all samples changed in a range from 0.03 to 0.23. These results support that calcium bioavailability in *O. ficus indica* modifies according to calcium compounds distribution.

## 1. Introduction

In Mexico, 21% of people over 20 years have a deficiency in calcium intake. Calcium content in the Mexican diet covers only 50% of the recommended daily intake (1200 mg/day) [1]. Calcium deficiency causes skeletal system diseases, that is, osteoporosis, which is a public health problem, due to the fact that this disease is among the eight leading causes of hospital morbidity with a prevalence of 8% in the Mexican population [2]. For this reason, there is an amplified interest in monitoring and increasing consumption of dietary calcium. Moreover, recently, health professionals have been taking action on the matter [3].

In addition, it has been demonstrated that a high bioavailability of calcium from the diet improves bone health [4], although calcium is distributed in different foodstuffs such as milk and dairy foods that provide more than 80% of calcium to the human diet. Furthermore, the calcium bioavailability in milk and dairy foods is significant, and, due to this, mineral absorption is associated with absorption promoters such as

lactose among other factors [5]. In developing countries, calcium intake from dairy products is limited by the high costs of these foodstuffs, as well as the problems associated with lactose intolerance [6]. These facts restrict the consumption of animal products causing a reduction of calcium in the diet. Consequently, Mexico and Central American countries depend on nixtamalized products as their primary source of calcium in their diet [7]. Thus, it is necessary to propose alternative sources of calcium to improve daily intake of this mineral. *Opuntia ficus indica* cladodes (pads) represent a potential source of calcium in human diet, due to the fact that calcium content in pads increases with the growing stage [8, 9].

McConn and Nakata [10] observed a reduction in calcium availability in prickly pear cactus by using an *in vitro* assay; this was attributed in part to the presence of calcium oxalate crystals. In addition, Contreras-Padilla et al. [11] reported that the concentration of oxalate in *O. ficus indica* in different phases of maturity appears to have a cyclic tendency that could be determined by the presence of calcium content in the soil, the plant's needs during active growth, and seasonal and environmental conditions.

On the other hand, several researches have shown the presence of calcium compounds in mucilage cell and cell walls of *Opuntia ficus indica* [12, 13]. However, there is no previous report about the distribution of these compounds extracted from the matrix of *O. ficus indica* with the purpose of increasing the bioavailability of calcium from this Cactaceae.

The goal of the present research was to characterize the distribution of calcium compounds in soluble and insoluble dietary fiber extracted from *O. ficus indica* cladodes at a late maturation stage (400 g of weight), in order to underlie the potential of this cactus as a source of calcium to help the formation of bone mass. These findings will promote the utilization of powder of *O. ficus indica*, with no commercial value, due to the fact that this cactus is not consumed as a vegetable, which can be used as a dietary supplement with an affordable price to increase calcium intake in the Mexican population.

## 2. Materials and Methods

*2.1. Samples.* *Opuntia ficus indica* cladodes were cultivated in an experimental field in Silao, Guanajuato (Rancho Los Lorenz), Mexico, with organic fertilizer and harvested during the spring of 2014. The *O. ficus indica* cladodes of 400 g (100 days of maturation stage) were washed with distilled water and the thorns were manually removed. Then, *O. ficus indica* cladodes were dehydrated by drying cladode slices (2 × 2 cm) in a force air oven (BG model E102). The dehydration process was carried out at 50°C during 70 minutes, each pan containing 5 kg of *O. ficus indica* slices. The dry material was milled using a hammer mill (PULVEX 200, DF, Mexico) equipped with a 0.8 mm screen.

*2.2. Chemicals and Reagents.* Ethyl alcohol (95% v/v) reactive grade, hydrochloric acid analytical grade, nitric acid ultrapure, and distilled water were obtained from J. T. Baker (DF, Mexico). The Total Fiber Dietary Kit (TDF-1100 A) was purchased from Sigma (St. Louis, MO, USA) as also were oxalic acid and potassium bromide standards.

*2.3. Extraction of Soluble and Insoluble Dietary Fiber from O. ficus indica.* The dried material was mixed with distilled water (4% w/w). This suspension was homogenized using a blender (IKA-WERKE, Mod. Eurostar BSC.S1) (450 rpm for 20 min). Subsequently, the suspension was left to stand for four hours to ensure hydration of the solids. Then, the suspension was placed in the feed tank of a disk centrifuge (DIDACTA Italia, Mod. TAG1/d), which was operated at 450 rpm. The speed of centrifuge was increased gradually until it reached 7000 rpm. Next, the feed valve of the centrifuge was opened to allow the flow of soluble solids through the gravity rings and the upper hopper of equipment, while the insoluble solids (insoluble fiber) were retained in the bowl of the centrifuge. The insoluble dietary fiber was dehydrated in Teflon pans at 80 kPa and 40°C in a vacuum oven (Barnstead International, Mod. 3618) for 35 min, until a humidity content of 4% (w/w). Soluble solids recovered were mixed with ethyl alcohol at 95% (v/v) in a 1 : 2 v/v ratio. This suspension was subjected to vacuum filtration at 4 kPa to remove excess water and alcohol in order to obtain the soluble dietary fiber. Finally, this precipitate was dehydrated at the same conditions as before.

*2.4. Separation of Oxalate Crystals.* Suspensions of dried material (cladodes, soluble dietary fiber, and insoluble dietary fiber) and distilled water (4% w/w) were prepared. These suspensions were processed as was reported by Malainine et al. [14].

*2.5. Chemical Characterization*

*2.5.1. Total, Soluble, and Insoluble Dietary Fiber Content in Dehydrated Cladodes of O. ficus indica.* Total dietary fiber, soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) in samples were analyzed according to methods 991.42 and 993.19 [15], respectively, by using a dietary fiber kit.

*2.5.2. Characterization of Calcium Compounds in Cladodes, Soluble, and Insoluble Dietary Fiber of O. ficus indica by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectrometry (EDS).* The morphology of calcium compounds was analyzed in a Scanning Electron Microscopy (Jeol JSM 6060LV, Japan). Prior to the analysis, the samples were fixed on an aluminum specimen holder with carbon tape and dried under critical point conditions in a Cryo-SEM preparation system (Quorum Technologies, Mod. PP30105, UK) operated with liquid $CO_2$. Subsequently, the mounted samples were then sputter coated with gold. The microcompositional analysis of the samples was carried out using an Energy Dispersive Spectrometer (INCA x-sight) provided with software (Oxford Instrument, UK). Each sample was turned to move the focus position of the microscope. Further, surface views of isolated samples were taken to obtain

the micrographs. The conditions of the analysis were high vacuum, 20 KV electron acceleration voltage, and secondary electron mode. Additionally, standards of pure compounds were observed with comparative purposes.

*2.5.3. Characterization of Calcium Compounds in Cladodes, Soluble, and Insoluble Dietary Fiber of O. ficus indica by X-Ray Diffraction.* Before analysis, samples were calcinated in a furnace (Nabertherm, Mod. L-P 330, GER) at 168°C in order to decompose organic matter and to prevent the formation of new mineral compounds or to avoid decay of calcium compounds commonly present in the Opuntioideae subfamily as was previously reported [14, 16–18]. The samples were ground to a fine powder and passed through a 150 $\mu$m screen. The powder samples were then densely packed into an aluminum sample holder. The X-ray diffraction patterns of the samples were recorded on a diffractometer (Rigaku Miniflex) operating at 35 kV and 15 mA, with a $CuK_\alpha$ radiation wavelength of $\lambda = 1.5406$ Å. The measurements were obtained from 10 to 70° on a $2\theta$ scale with a step size of 0.05°. Spectrum analysis software (Materials Data Inc. Jade V 5.0) was used for the samples analysis.

*2.5.4. Characterization of Calcium Compounds in Cladodes, Soluble, and Insoluble Dietary Fiber of O. ficus indica by Infrared (IR) Analysis.* The IR spectra of dehydrated samples of *O. ficus indica* cladodes, SDF, and IDF were recorded on a IR-Bruker Vector 33 spectrophotometer in the spectral range between 4000 and 400 $cm^{-1}$, using the KBr pellet technique (4 mg of the powdered sample dispersed in 100 mg of KBr).

*2.5.5. Total Calcium and Oxalate Content in Cladodes, Soluble, and Insoluble Dietary Fiber of O. ficus indica Cladodes.* Total calcium and oxalate content was determined according to AOAC Official Method 983.27 and 974.24, respectively [15]. The oxalate concentration was measured with a double beam atomic absorption (Analyst 300 Perkin Elmer), equipped with a deuterium lamp, background corrector, and a hollow cathode lamp. The operating parameters for calcium were a hollow cathode lamp with a wavelength of 422.7 nm, 70 psi of acetylene, nitrous oxide as an oxidant, and slit aperture of 0.7 mm and for oxalates 12 psi of dry air at 70 psi of acetylene with a wavelength of 422.7 nm, 10 mA lamp current, and a 0.7 nm slit width.

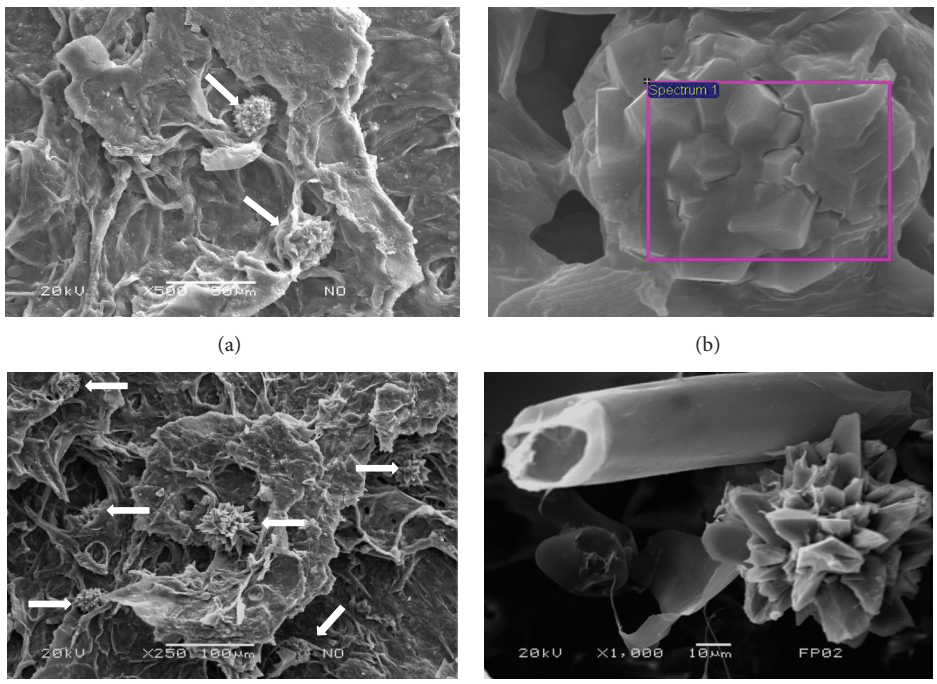
*2.5.6. Calcium Carbonate Content in Cladodes, Soluble, and Insoluble Dietary Fiber of O. ficus indica.* Calcium carbonate content in samples was analyzed by volumetric analysis according to AOAC [19].

*2.6. Statistical Analysis.* Three samples were used for each preparation and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test with $\alpha = 0.05$ and using the Statgraphics procedure (Graphics Software System, Manugistics, Inc., USA).

## 3. Results and Discussion

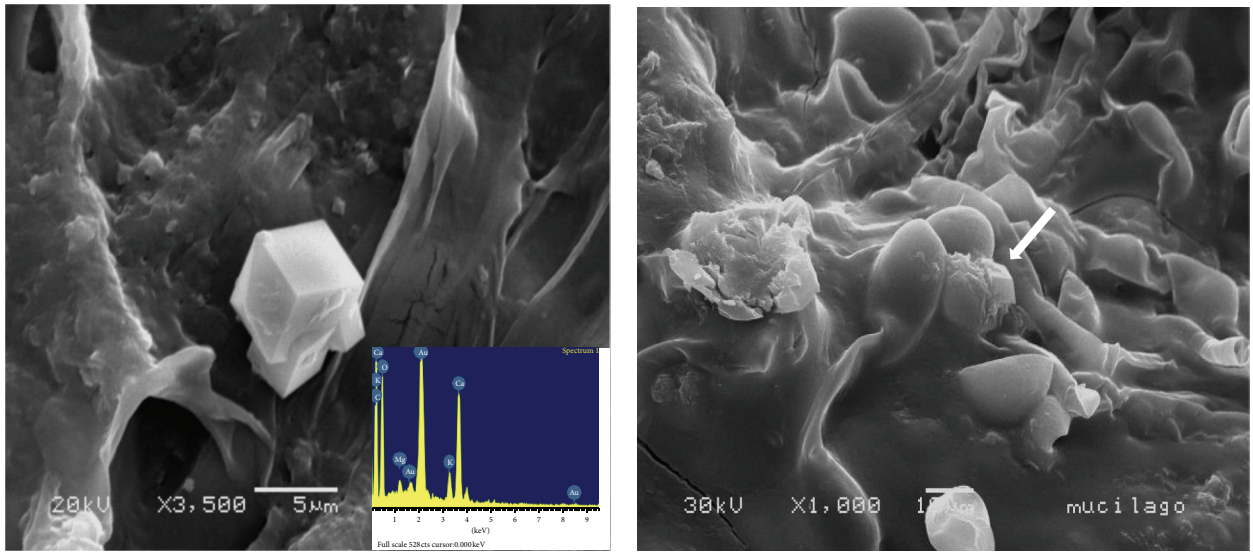
*3.1. Soluble and Insoluble Dietary Fiber Content.* The SDF and IDF contents in samples were 2.53 ± 0.90 and 43.44 ± 1.69%, respectively. These results differ from those reported by Hernández-Urbiola et al. [9]. These authors found that, in nopal pads with 100 days of age (400 g of weight approximately), the SDF and IDF contents were 8 and 52%, respectively. Nutrient profile of cladodes from different harvests and regions varies due to the fact that this profile depends on environmental factors, that is, edaphic factors at the cultivation site, the season, and the age of the plant [8, 20]. A higher content of IDF with respect to SDF is attributed to the process of lignification, where polyphenolic polymer lignin is formed due to maturation of cladodes. On the contrary, young *Opuntia* cladodes lack lignin [21]. The increase of fibers in cladodes involves mainly cellulose and hemicelluloses [22]; these compounds in conjunction with lignin constitute IDF [23]. Total dietary fiber (TDF) content in *O. ficus indica* in the present study is higher than TDF values in cactus pear (fruit) reported by Jiménez-Aguilar et al. [24].

*3.2. SEM and EDS Analysis.* Figure 1 shows representative images of microscopic examinations of samples. Presence of calcium oxalate crystals in *O. ficus indica* cladodes is evident in Figure 1(a) (see arrows) in accordance with previous reports [10, 12, 13, 25]. Biomineralized calcium oxalate crystallites in Cactaceae species were identified either as $CaC_2O_4{\cdot}2H_2O$ (weddellite) or as $CaC_2O_4{\cdot}H_2O$ (whewellite). Whewellite druses differ from weddellite druses principally by their stellate shapes, with individual crystallites having acute sharp points emerging from the center of the druse. On the other hand, weddellite druses are usually made up of individual tetragonal crystals [26]. In this study, also weddellite crystals were detected (see Figure 1(b)) according to Malainine et al. [14] and Saenz et al. [25]. Figure 1(c) shows crystalline calcium oxalate in IDF extracted from *O. ficus indica*. As it can be seen, the quantity of crystals in IDF is more than that detected in cladodes (see arrows). The size of druses ranged from 150 to 250 $\mu$m; these crystals are larger than druses observed by Rodríguez-García et al. [8] and Saenz et al. [25]. These authors reported that the crystal size of whewellite of *O. ficus indica* cladodes (from 60 to 200 g of weight) ranged from 30 to 70 $\mu$m. At this point, it is important to mention that, in the present study, the weight of cladodes was 400 g (100 days of age) and the oxalate crystal size increases as a function of maturation [26]. Figure 1(d) shows a detail of a vessel from the xylem of *O. ficus indica* with a calcium oxalate crystal adhered to the vascular tissue with a high content of lignin. Calcium oxalate crystals are present in all tissues of *O. ficus indica* cladodes [13]; nevertheless, SEM analysis shows that the presence of calcium oxalate crystals in IDF is very noticeable. This result is in agreement with those of Ginestra et al. [13]; indeed, these authors found that calcium oxalate crystals are strongly associated with an alcohol-insoluble residue (constituted by vascular bundles, clumps of parenchyma, and skin) obtained from a cell-wall fractionation of powdered lyophilized cladodes. Figure 2(a) shows a prismatic crystal in SDF extracted from *O. ficus*




(a) (b) (c) (d)

Figure 1: SEM images of cladodes and insoluble dietary fiber of *O. ficus indica*, (a) calcium oxalate crystals (whewellite) in *O. ficus indica* cladodes, (b) calcium oxalate crystals (weddellite) in *O. ficus indica* cladodes, (c) calcium oxalate crystals in insoluble dietary fiber extracted from *O. ficus indica*, and (d) vessel from the xylem of *O. ficus indica* with a calcium oxalate crystal (whewellite).




(a) (b)

Figure 2: SEM images and EDS analysis of soluble dietary fiber extracted from *O. ficus indica*, (a) prismatic crystal in soluble dietary fiber with elemental analysis by EDS and (b) prismatic crystal emerging from a globular structure located in soluble dietary fiber.

*indica*. A qualitative EDS analysis of this material revealed the presence of calcium, oxygen, carbon, potassium, and magnesium (inserted in Figure 2(a)). Contreras-Padilla et al. [18] have reported similar crystalline structures. These authors associate this composition with the presence of calcium carbonate or calcium-magnesium carbonate. Globular structures were detected by microscopic observation of the SDF (Figure 2(b)). Furthermore, a quadratic crystal is emerging from a globular structure (see white arrow). In this regard, Contreras-Padilla et al. [18] found that calcium

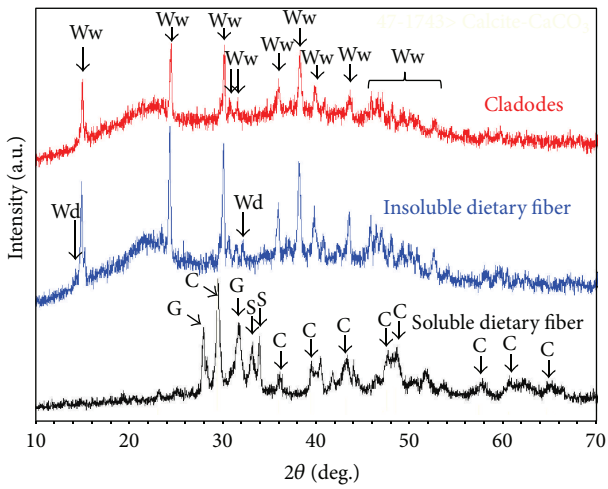



FIGURE 3: X-ray diffraction patterns of *O. ficus indica* samples (cladodes, insoluble, and soluble dietary fiber). Ww: whewellite; Wd: weddellite; C: calcite; G: glauberite; S: spurrite.

carbonate crystals grow into prismatic forms comparable to the crystal observed in SDF extracted from experimental samples. These authors suggest that the growth of these crystal structures can be correlated with the age of the plant.

*3.3. X-Ray Diffraction Analysis.* Figure 3 corresponds to the X-rays diffraction patterns of *O. ficus indica* samples (cladodes, SDF, and IDF). These diffractograms revealed the presence of calcium oxalate monohydrate (whewellite) in cladodes and IDF, which fit with the PDF # 20–0231 of ICDD-JCPDS database. Monje and Baran [17] and Contreras-Padilla et al. [18] reported two characteristic peaks in X-rays diffraction patterns for oxalate monohydrate at 14-15 and 24-25° diffraction angle $2\theta$ in different Cactaceae species belonging to the Opuntioideae subfamily, including *O. ficus indica*; both peaks are evident in cladodes and IDF samples. In contrast, these peaks were not detected in SDF samples. Furthermore, characteristic peaks in X-rays diffraction patterns for calcium carbonate at 29-30, 39-40, and 45–50° diffraction angle $2\theta$ observed by the same authors were identified in SDF only. This indicates the presence of $CaCO_3$ (PDF # 47-1743) in these samples. These findings are in agreement with the distribution of crystalline compounds observed by the SEM and EDS analyses. Two small Bragg reflections located on 14.36 and 32.2° in the IDF sample are indicative of the presence of weddellite crystals ($CaC_2O_4{\cdot}(H_2O)_{2.375}$) (PDF # 75-1314); nevertheless they do not appear in the SDF sample. This result is in agreement with Malainine et al. [14]; these authors also reported the presence of weddellite crystals in *Opuntia ficus indica* cladodes. The other two crystalline compounds containing calcium were detected in SDF sample: spurrite ($Ca_5(SiO_4)_2CO_3$) (PDF # 13-0496) and glauberite ($Na_2Ca(SO_4)_2$) (PDF # 74-2340). These two compounds reveal the presence of other elements such as sodium, silicon, and sulfur. The analysis performed with the MDI Jade software showed no presence of other crystalline compounds. This could be attributed to the fact that peaks of crystalline compounds different to calcite, whewellite, and weddellite are very weak and the strongest calcite and whewellite reflection peaks are superimposed with other compound peaks or possibly they are amorphous.

*3.4. Infrared Analysis.* Figure 4 shows the infrared emission spectra of SDF and IDF isolated from *O. ficus indica* and cladodes. The infrared spectra confirm the existence of calcium carbonate in SDF. This is supported by the presence of bands near 1420 $cm^{-1}$ ($\nu_{as}$ $CO_3^{2-}$) and 875 $cm^{-1}$ ($CO_3^{2-}$ out-of-plane bending vibration). The intensity of these bands indicates a large amount of calcite. Nevertheless, this spectrum shows no evident absorption bands for oxalate, whereas infrared spectra of cladodes and IDF reveal the presence of whewellite and calcite. It is important to denote that calcite is in trace amounts in IDF. Calcium oxalate is responsible for bands at 1625 $cm^{-1}$ ($\nu_{as}$ OCO), 1312 $cm^{-1}$ ($\nu_s$ OCO), and 750 $cm^{-1}$ (OCO deformations). These findings are consistent with the X-ray diffraction results. Finally, the presence of an intense band centered at 1070–1080 $cm^{-1}$ indicates a high content of silicon oxide ($SiO_2$) in all samples as it was observed in the X-ray diffraction results. Biomineralized silicon in plants has been related with a structural role in the cell wall and defense as a mineral barrier to both the invasion of pathogen and insect attacks, as well as the translocation of water and salts [27].

*3.5. Total Calcium, Calcium Oxalate, and Calcium Carbonate in O. ficus indica.* The average total calcium, oxalate, and calcium carbonate contents in *O. ficus indica* samples are shown in Table 1. It is evident that total calcium content in cladodes is significantly higher ($P \leq 0.05$) in comparison to calcium content in SDF and IDF. Nevertheless, it is worth noting that calcium content in SDF is higher than calcium content in IDF. This means that calcium content in SDF is on average 18.24% higher than calcium content in IDF. Calcium oxalate content in SDF significantly decreases ($P \leq 0.05$) with respect to that in cladodes and IDF. In addition, the highest concentration of calcium carbonate was detected in cladodes, while the content of this compound is approximately 50% higher in SDF in comparison with that observed in IDF. These results may be explained as follows: for salts (ionic solids) that dissociate into ions in water, such as the compounds contained in *O. ficus indica*, a solubility product ($K_{sp}$) is typically given. In the case of $CaCO_3$, $K_{sp}$ (25°C) = $3.36 \times 10^{-9}$, while, for $CaC_2O_4$, $K_{sp}$ (25°C) = $2.32 \times 10^{-9}$ [28]. The smaller the solubility product of a substance, the lower its solubility. This means that $CaCO_3$ is more soluble in water than $CaC_2O_4$; consequently, this fact justifies a major concentration of oxalate in IDF compared with that in SDF and a higher content of calcium carbonate in SDF with respect to IDF.

Calcium carbonate in plants has been related to a mechanism to control soluble $Ca^{2+}$ levels within plant tissues [29]. Similarly, calcium oxalate has a role as a calcium regulator and other functions, that is, mechanical support, intracellular pH regulation, ion balance detoxification, and gravity perception between others [30].

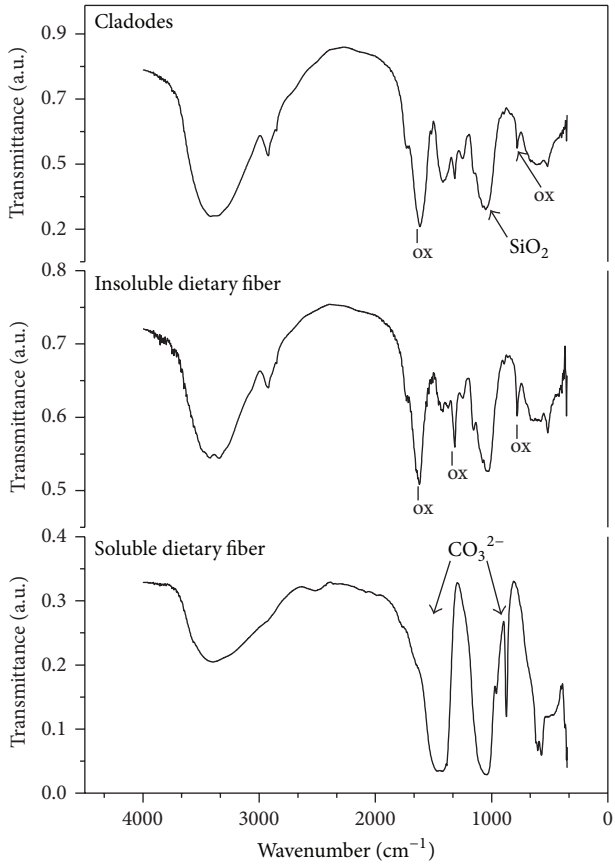



Figure 4: Infrared spectra of *Opuntia ficus indica* samples (cladodes, insoluble, and soluble dietary fiber). ox: $CaC_2O_4{\cdot}H_2O$ (whewellite).

Table 1: Total calcium, oxalate, calcium carbonate content, and molar ratio oxalate : calcium in *Opuntia ficus indica* samples (cladodes, soluble, and insoluble dietary fiber)*.

| Samples | Total calcium content (mg/g) dry matter | Calcium oxalate content (mg/g) dry matter | Oxalate content (mg/g) dry matter | Calcium carbonate content (mg/g) dry matter | Molar ratio oxalate : calcium |
|---|---|---|---|---|---|
| Cladodes | $32.33 \pm 2.90^a$ | $6.71 \pm 0.80^a$ | $8.31 \pm 0.80^a$ | $70.81 \pm 3.40^a$ | $0.12^a$ |
| Soluble dietary fiber | $18.99 \pm 1.30^b$ | $0.27 \pm 0.20^b$ | $1.25 \pm 0.20^b$ | $49.67 \pm 1.87^b$ | $0.03^b$ |
| Insoluble dietary fiber | $13.08 \pm 1.10^c$ | $5.39 \pm 0.17^a$ | $6.61 \pm 0.17^a$ | $21.95 \pm 2.17^c$ | $0.23^c$ |

*Values ± SD followed by the same letter are not significantly different ($P < 0.05$).

In a food evaluation, the chelating agent to mineral chelated molar ratio is an important factor for determining potency of mineral bioavailability. The World Health Organization considers this value as a good index as a preliminary criterion for mineral bioavailability [31].

In order to predict the bioavailability of calcium in samples [oxalate]/[$Ca^{2+}$], ratios were calculated (Table 1). The values obtained from this molar ratio (oxalate : $Ca^{2+}$) are below the critical level of 1, known to impair calcium bioavailability. This means that molar oxalate : $Ca^{2+}$ ratios ≥ 1 are indicative of calcium unavailability [32]. These results are in agreement with those reported by Contreras-Padilla et al. [11]. These authors found that the molar ratio between oxalate and calcium in *O. ficus indica* pads at different maturity stages was lower than 1, suggesting that the bioavailability of calcium is not compromised.

## 4. Conclusions

Calcium carbonates and calcium oxalates were detected in cladodes, IDF, and SDF of *O. ficus indica*. Nevertheless, significant differences in total calcium, calcium carbonate, calcium oxalate contents, and molar oxalate : $Ca^{2+}$ ratio were observed in all samples. This means that calcium bioavailability in *O. ficus indica* varies according to calcium compounds distribution.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

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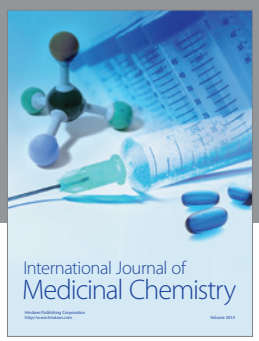

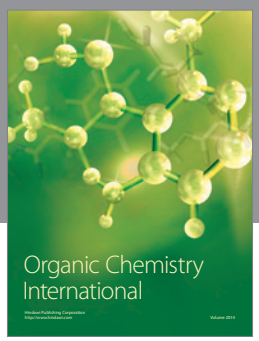

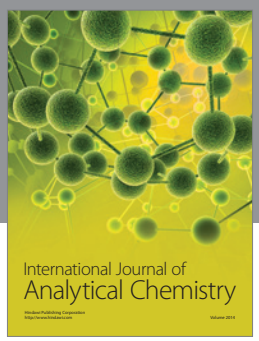

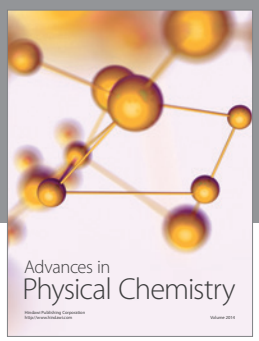

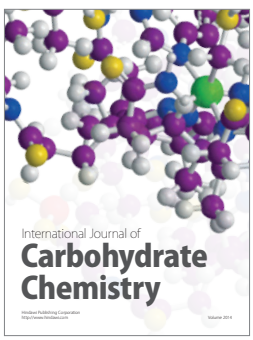

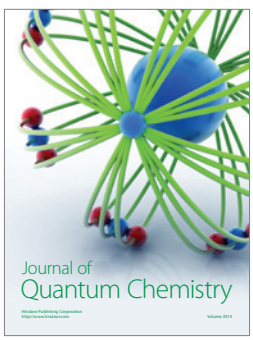

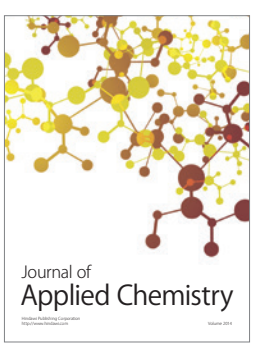

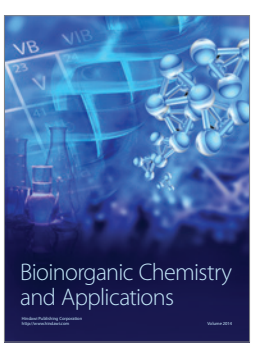

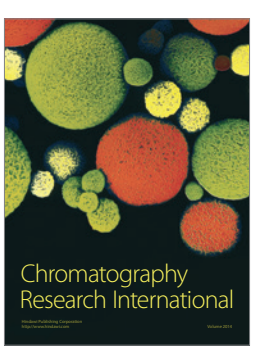

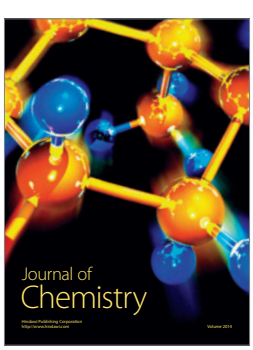

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