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Oxalate Reduces Calcium Availability in the Pads of the Prickly Pear Cactus through Formation of Calcium Oxalate Crystals

MICHELE M. MCCONN AND PAUL A. NAKATA*

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030-2600

The pads (nopales) of the prickly pear cactus are considered to be a good source of minerals and other nutrients on the basis of compositional analysis. In this study, this analysis is taken a step further by assessing the availability of selected minerals in nopales using an in vitro digestion and dialysis method. The results obtained suggest that although nopales are enriched in a number of minerals, their tissue calcium is not freely available. Microscopic analysis, energy-dispersive X-ray microanalysis, and oxalate measurements suggest that this reduction in available calcium is a result of its sequestration in the form of calcium oxalate crystals. The issue of mineral availability in plant foods is important when the dependence of many populations around the world on plant foods as their main source of minerals and other nutrients is considered.

KEYWORDS: Availability; calcium; crystals; nopales; oxalate; prickly pear

INTRODUCTION

Prickly pear cactus, from the genus *Opuntia*, is considered to be an important nutrient and food source in many parts of the world (*1*, *2*). Mexico, Chile, Italy, and a number of areas in the United States farm prickly pear for commercial purposes (*2*). Both the prickly pear leaves (nopales) and fruit (tuna) are consumed. In addition to a food source, nopal is also a staple in traditional medicines. The leaves have been consumed as part of traditional treatments of arteriosclerosis, diabetes, gastritis, and hyperglycemia (*1*, *2*). Several studies have investigated the usefulness of this plant in the treatment of various ailments (*3*−*10*).

Edible cactus has been gaining popularity in the United States as well as other parts of the world (*2*). Nopal can be purchased year-round in local grocery stores and ethnic markets. As a vegetable, nopal is eaten fresh in salads, soups, and casseroles as well as grilled and fried. With the increase in production and consumption of prickly pear there has been an interest in establishing the nutrient and mineral composition of the edible tissue (*2*, *11*, *12*). Composition analyses have shown that edible cacti often contain an abundance of protein, vitamins, and minerals (*2*, *11*, *12*). To our knowledge, however, there has not been any investigation into the availability of minerals in this plant. Calcium availability in cacti needs to be investigated as certain cacti have been shown to contain the antinutrient oxalate, which can render calcium and possibly other minerals unavailable for nutritional absorption by humans (*13*, *14*) and other animals (*15*). Thus, the question remains as to how much of the calcium in nopales is actually available for nutritional absorption.

* Corresponding author [telephone (713) 798-7013; fax (713) 798-7078; e-mail pnakata@bcm.tmc.edu].

In this study, we investigate the amount of available calcium present in nopal using a modified in vitro digestion and dialysis method (*16*). Samples of spinach and kale were used as controls for high- and low-calcium oxalate plants, respectively. The in vitro digestion and dialysis method has been utilized in numerous studies to assess the availability of various minerals and compounds in foods (*16*−*23*). On the basis of simply mineral composition, one would expect that nopales would provide the highest amount of available calcium, followed by spinach and kale, respectively. The largest amount of total available calcium, however, was found in kale and the lowest amount in spinach. Nopales had an intermediary amount of available calcium. To determine why nopales had reduced calcium availability, microscopic examination, microanalysis, and oxalate measurements were conducted. The analyses indicated that the reduction in the level of available calcium is attributable to the sequestration of the calcium in the form of crystals of calcium oxalate. This issue of calcium availability in foods needs to be addressed due to the reliance of people in many parts of the world on plant foods as their main source of minerals.

MATERIALS AND METHODS

Mineral Analysis. Mineral content was determined by inductively coupled plasma (ICP) analysis as previously reported (*24*). In brief, dried tissue samples were weighed and wet digested with a nitric/perchloric acid mixture by heating to 150−200 °C while under vacuum until dry. The dry digests were dissolved in 1 M nitric acid, incubated for 1 h, and diluted with distilled water. To determine the amount of calcium, each sample was analyzed by ICP. Each measurement was done in duplicate on three independently purchased sets of plants, the results were averaged, and standard error was calculated.

Oxalate Measurements. Oxalate content was measured as described previously (*24*). In brief, freeze-dried samples were then weighed,

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ground in water, and centrifuged. Total oxalate levels were determined using the Sigma oxalate diagnostic kit (catalog no. 591-D; St. Louis, MO). Crystals were solubilized by the addition of H^+-Dowex in dilute acid. The mixture was heated at 60 °C for 1 h to dissolve the oxalate crystals. The pH of the mixture was then adjusted (pH 5−7), followed by charcoal filtration and centrifugation. The supernatant then was analyzed for oxalate content according to the manufacturer's instructions (Sigma). Standards were prepared from oxalic acid dihydrate (Sigma) and used for total oxalate measurements as recommended by the manufacturer. Measurements were done in duplicate on three independent sets of tissue, the results were averaged, and standard error was calculated.

Dialyzable Calcium. Tissue digestion and assessment of calcium bioavailability were conducted using a modified protocol of Benway and Weaver (*16*). In brief, freeze-dried leaves were ground into small flakes using a coffee grinder. Weighed samples (0.5 g) were ground in 20 mL of water, and HCl was added until the homogenate reached a pH of 2.0. Pepsin (125 μL of a 40 mg/mL in 0.1 M HCl) was added to simulate digestion to give a food weight ratio of 1%. The mixture was shaken in a beaker for 2 h at 37 °C. A dialysis bag, containing sodium bicarbonate, then was placed in each beaker containing the digested mixture and allowed to incubate at 37 °C. After 20 min, a pancreatin/bile solution was added to give a pancreatin-to-food ratio of 0.5%. Water was added to bring the total reaction volume to 35 mL. The mixture then was shaken at 37 °C. After 2 h, the heat was turned off and the mixture allowed to shake at room temperature to allow for equilibration. After 16 h at room temperature, the contents were removed from the dialysis bag and the calcium content was determined (as described above). This was repeated five times, the results were averaged, and the standard error was calculated.

Energy-Dispersive X-ray Microanalysis and Scanning Electron Microscopy (SEM). Mineral microanalysis was conducted on isolated crystals and crystals present in unstained sections of nopales. The crystals were isolated by slicing the nopal with a razor blade and touching the cut surface onto a droplet of water contained on a stub. The preparation of unstained sections of nopales was done by simply cutting small pieces of freeze-dried cactus pad using a razor blade and placing the pieces on a stub. In either case, the stub was allowed to air-dry overnight, carbon coated, and viewed using a JEOL Temscan 100-C scanning electron microscope (SEM). The electron microscope is fitted with a Tracor-Northern (Noran) TN-5500 energy-dispersive X-ray analyzer with a beryllium window (Department of Pathology Service Laboratory, Baylor College of Medicine). The spectra of elements were obtained by focusing the beam at high magnification (>150000×) on the crystal and collecting the emitted X-rays. Spectra were generated from areas of the same section free of crystals for comparison.

For scanning electron micrographs, free-hand leaf sections were cut using a double-edged razor blade, mounted onto stubs, and allowed to air-dry. The mounted samples were then sputter coated with gold−palladium (20/80) and viewed on a JEOL JSM-6100 SEM. Images were taken with Kodak Plus X 620 roll film.

RESULTS AND DISCUSSION

Nopales is valued as a nutritional (*2*, *11*, *12*) and medicinal source (*1*, *2*). The nutritional value is based on the balanced nutrient composition (*1*, *2*, *11*, *12*). Although the nutritional composition of foods plays a major role in determining its nutritional value, there are other factors to be considered. Many foods, particularly plant-based foods, often contain substances that affect the availability of nutrients. In this study we assess the availability of selected minerals in nopales using a proven in vitro method. Although in vivo studies are ideal, this in vitro method offers several advantages as a rapid and economically effective alternative for the assessment of mineral availability in foods.

As a first step in assessing the mineral availability, nopales, spinach, and kale were purchased from the produce section of a local market. ICP analysis revealed each plant food was enriched in a number of minerals (**Table 1**). Among the three vegetables, spinach showed the highest levels of potassium and magnesium, whereas nopales had the highest levels of calcium (**Table 1**). Thus, on the basis of the composition values, it would appear that both spinach and nopales would be a better source of these minerals than kale.

Table 1. Mineral Composition[a] (Milligrams per Gram of Dry Weight)

	K	Ca	Mg
spinach	70.6 ± 6.6	9.0 ± 0.2	14.4 ± 0.8
nopales	50.1 ± 1.6	18.1 ± 1.6	10.9 ± 0.1
kale	22.8 ± 1.5	9.4 ± 0.5	1.6 ± 0.02

[a] Values are the average ± SE of three independent samples done in duplicate.

Table 2. Dialyzable Minerals[a] (Percent)

	K	Ca	Mg
spinach	42.2 ± 8.6	1.9 ± 0.4	35.1 ± 7.5
nopales	43.7 ± 6.2	11.0 ± 1.5	44.9 ± 3.2
kale	51.1 ± 7.5	28.9 ± 4.9	40.4 ± 6.3

[a] Values are the average ± SE of five independent experiments. Percentage of available mineral was calculated by dividing the amount (mg) of the mineral in the dialysis bag by the amount (mg) of mineral in the initial weighed sample.

Although kale generally has lower mineral content, it is known to possess good mineral availability. The high mineral availability of kale has been established using a number of different systems that include human (*25*), rodent (*15*), and in vitro models (*16*). As a part of this study, each vegetable was subjected to an in vitro digestion protocol consisting of a pepsin digestion, bicarbonate neutralization, and pancreatin/bile equilibration. Dialysis tubing was used to measure mineral availability. Of the three minerals tested, calcium showed the largest difference in availability among the three plants (**Table 2**). Available calcium was determined to be 1.9 and 28.9% for spinach and kale, respectively, using this in vitro protocol. These values were found to be similar to the 3.0 and 29.4% calcium bioavailability values determined for spinach and kale, respectively, in animal feeding trials (*15*). Thus, our findings along with the findings of other researchers (*16*−*23*) support the usefulness of this in vitro protocol for assessing mineral bioavailability.

The test plant food, nopales, showed a calcium fractional absorption of 11%. Thus, on a dry weight basis, one would predict that the total amount of calcium obtained from eating nopales would be 12 times greater than that from spinach but 1.4 times less than that from kale. Therefore, even though nopales have double the calcium of kale, ingestion of the kale would provide more total available calcium for uptake and utilization. The large difference in calcium availability between spinach and kale has been attributed to the antinutrient oxalate (*13*−*15*). Oxalate binds calcium in a nutritionally unavailable form known as a calcium oxalate crystal.

To investigate the possibility that oxalate is reducing calcium availability in nopales, oxalate measurements were conducted on nopales and control plants (**Table 3**). As previously reported by other laboratories, we observed a high oxalate content for spinach and barely detectable levels for kale. Nopales also contained substantial amounts of oxalate, although considerably less than spinach (**Table 3**). Microscopic analysis of nopales (**Figure 1**) revealed the presence of numerous oxalate crystals when viewed between crossed polarizers. SEM analysis (**Figure 1G**,**H**) showed that the morphology of the crystals could be classified as druse in shape. Energy-dispersive X-ray mi-

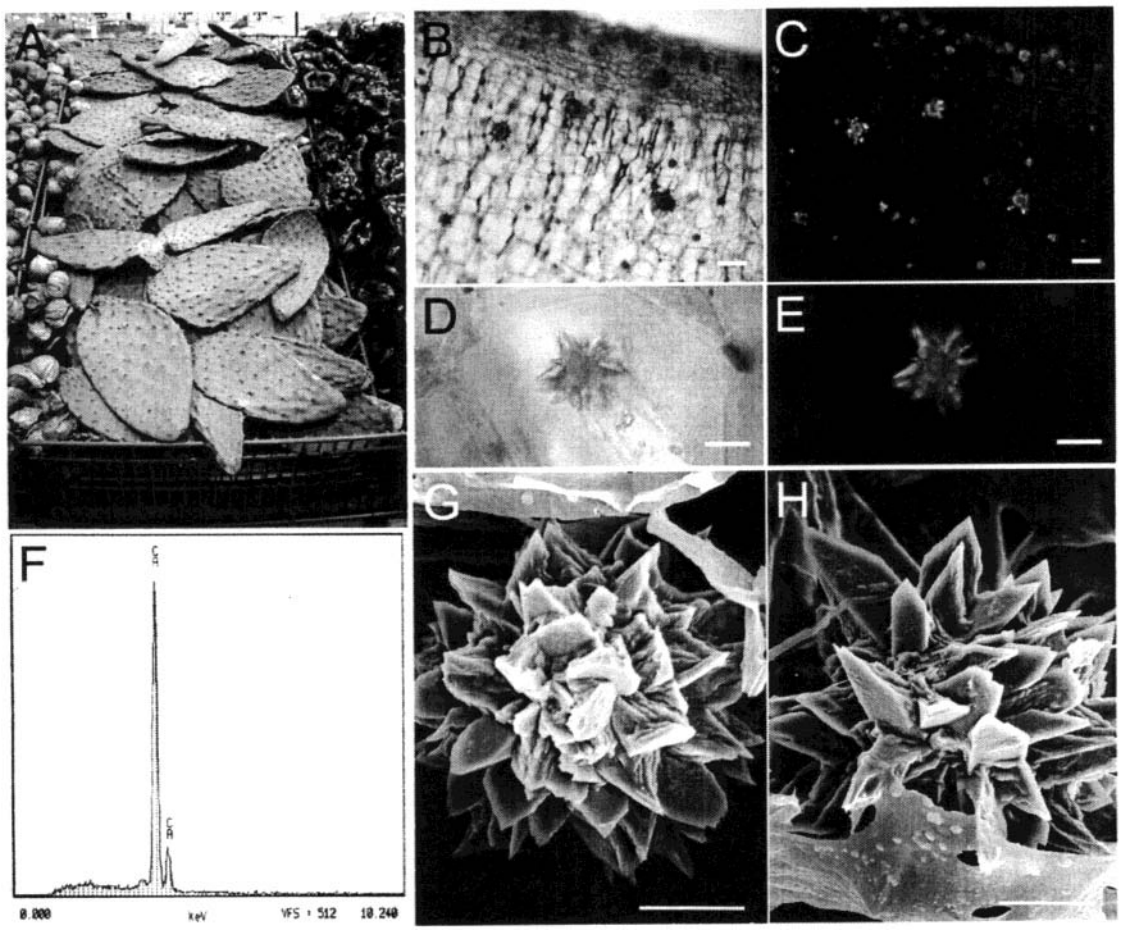

Figure 1. Calcium oxalate crystals in nopales: (**A**) nopales as sold in the local market; (**B**) free-hand section of nopales viewed under bright field illumination (bar = 50 μm); (**C**) same section as in (**B**) viewed between crossed polarizers (bar = 50 μm); (**D**) higher magnification view of crystal within nopales cell viewed under bright field illumination (bar = 20 μm); (**E**) same section as shown in (**D**) viewed between crossed polarizers (bar = 20 μm); (**F**) mineral composition analysis of crystal shown in (**G**); (**G**, **H**) nopales crystal viewed using SEM (bar = 10 μm).

Table 3. Oxalate Content[a] (Milligrams per Gram of Dry Weight)

	soluble	insoluble
spinach	114.4 ± 4.0	22.4 ± 1.2
nopales	0.61 ± 0.08	34.5 ± 7.2
kale	0.33 ± 0.07	1.7 ± 0.7

[a] Values are the average ± SE of three independent samples done in duplicate.

croanalysis of the oxalate crystals confirmed that they are composed of calcium (**Figure 1F**). Thus, the reduction in calcium availability in nopales is attributable, at least in part, to the presence of calcium oxalate crystals.

A number of factors can affect the nutritional quality of edible plant foods. The presence of factors that influence the availability of nutrients is an important consideration in addition to the nutrient composition of the food. In this study we show that such a factor, oxalate, dramatically reduces the calcium availability in nopales. This issue of calcium availability is essential when one considers the number of crop plants that accumulate calcium oxalate (*26*) and the reliance of different populations around the world on plant foods as their main source of calcium and other minerals. Recent studies have indicated that genetic manipulation to reduce the oxalate content in plant foods may be possible soon (*24*, *27*). The removal of oxalates would not only benefit efforts to improve the nutritional quality of plant foods but would also be of benefit from a medical standpoint. New studies have indicated that the absorption of oxalate from plant foods contributes more than previously thought to elevations in urinary oxalate excretion (*28*). High urinary oxalate excretion increases chances of calcium oxalate precipitation and urinary stone formation; >75% of all urinary and kidney stones contain calcium oxalate as their primary component.

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