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Comparative Analyses of Total Phenols, Antioxidant Activity, and Flavonol Glycoside Profile of Cladode Flours from Different Varieties of *Opuntia* spp.

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ABSTRACT: The phenolic, flavonoid, and antioxidant contents of methanol extracts of nine samples of Mexican cactus (*Opuntia* spp.) cladodes processed into flours were studied. *Opuntia undulata* contained the highest amount of phenols [905.08 ± 64.51 μg of gallic acid equivalents (GAE)/g]. The oxygen radical absorbance capacity (ORAC) of the cladode flour extracts indicated that *Opuntia robusta* var. Gavia [738.8 ± 89.9 μmol of Trolox equivalents (TE)/g] contained the highest antioxidant capacity. ORAC values significantly correlated to total phenols but not to flavonoid contents and were comparable to cranberries and blackberries. Glycosidic forms of isorhamnetin and kaempferol were identified via high-performance liquid chromatography−photodiode array (HPLC−PDA) and HPLC−mass spectrometry (MS), with isorhamnetin being the most abundant flavonol in all samples, except for *Opuntia lindheimeri*. The effectiveness of acid hydrolysis varied among species because of the different flavonol profiles. For some varieties, the triglycosidic forms were partially acid-hydrolyzed, giving an increase in the content of diglycosides. Optimization of hydrolysis for each species is required to estimate the total amount of each flavonol.

KEYWORDS: *Opuntia* flour, flavonoids, flavonol glycosides, phenols, antioxidant, ORAC

INTRODUCTION

Cacti from the genus *Opuntia* are endemic to the American continent and are particularly well-adapted to arid lands. Prickly pear cacti are highly adaptable to a diversity of climates and can be found all over the world, with more than 258 species, including countries in the Mediterranean, southern U.S.A., South America, Middle East, South Africa, and India.[1] It is used mainly for fruit production, but the vegetative parts (cladodes) are also eaten as a vegetable in some countries.[2] Over 10 000 ha are cultivated in Mexico for human consumption, with a total yearly production of 600 000 tons. The most common types are species of *Opuntia robusta*, *Opuntia streptacantha*, *Opuntia leucotricha*, *Opuntia hyptiacantha*, and *Opuntia chavena*.[1] Since ancient times, the cactus has been one of the main traditional crops in Mexico, where it is considered together with maize (*Zea mays*) and agave (*Agave* spp.) the base of subsistence agriculture.[3]

The fruits and cladodes or pads of *Opuntia* are edible. The immature pads are consumed as vegetables called "nopalitos" in a variety of typical Mexican dishes. Besides being a food source, the cladodes have been traditionally used as a medicinal plant to treat a variety of diseases, particularly diabetes and hypercholesterolemia.[4] Furthermore, the consumption of cladodes has also been related to other health effects, including anti-inflammatory and wound-healing,[5] antigenotoxic,[6] neuroprotective,[7] hypotensive,[8] immunomodulatory,[9] antiviral,[10] and antioxidant to combat oxidative stress.[11−13] More details about medicinal uses of *Opuntia* cladodes have been reviewed elsewhere.[1,2]

Some of the beneficial effects of cactus cladodes on health have been attributed to its dietary fiber.[14] In addition, the plant contains other phytochemicals, such as phenolic acids, flavonoids, carotenoids, and vitamins.[1,2] In recent years, an interest has raised the study of the phytochemicals associated with *Opuntia* cladodes, as well as their mechanism of action. Research has focused on the antioxidant capacity of the cladode hydrophilic extracts mainly exerted by phenols and flavonoids.[11,12] Flavonoid compounds with a well-known antioxidant capacity have been isolated from several species and varieties. They are mainly quercetin, isorhamnetin, and kaempferol, as both aglycones and glycosides.[7,15,16] Flavonol derivatives isolated from *Opuntia* stems have been shown to effectively inhibit *in vitro* free radicals.[7] Several glycosylated flavonols have also demonstrated diphenylpicrylhydrazyl (DPPH) radical scavenging activity with an IC_{50} value of 45.58 μg/mL for glycosylated and feruylated derivatives of isorhamnetin.[17] Likewise, the IC_{50} values for kaempferol and quercetin glycosides were 12−13 and 4.3−6.1 μM, respectively.[15] Consequently, there has been growing interest in the use of cactus flour as a nutraceutical supplement in various processed foods, such as tortillas and bread.[18,19]

The purpose of this study was to evaluate and compare the flavonol profiles, phenol content, and antioxidant capacity of methanol extracts of nine different varieties of Mexican cactus previously processed into flours. The glycosides of three flavonols were acid-hydrolyzed to identify their aglycones. Compound identification was centered on flavonols because they possess a high antioxidant activity[20] and have previously been reported in significant amounts in *Opuntia* species.[1,2] Correlation analyses between the parameters analyzed were carried out to identify the main components responsible for the observed antioxidant capacity.

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Table 1. Nomenclature and Scientific Names of the Nine Varieties of *Opuntia* spp. Analyzed[a]

entry	scientific name
OFI-J	*Opuntia ficus-indica* (L.) Mill. var. Jalpa
OL	*Opuntia lindheimeri* Englem.
OR-G	*Opuntia robusta* H.L. Wendl var. Gavia
OS	*Opuntia streptacantha* Lem.
OU	*Opuntia undulata* Griffiths.
OR-R	*Opuntia rastrera* FAC Weber
OR-T	*Opuntia robusta* H.L. Wendl var. Tapón
OL-D	*Opuntia leucotricha* DC.
OFI-V	*Opuntia ficus-indica* (L.) Mill var. Villanueva

[a] *Opuntia* species were identified by Rigoberto E. Vázquez-Alvarado (School of Agronomy of Universidad Autónoma de Nuevo León, Monterrey, Mexico).

MATERIALS AND METHODS

Preparation of Methanol Extracts from *Opuntia* spp. Cladode Flour. Nine different varieties of *Opuntia* cladodes (Table 1) collected in the spring of 2008 in the northeastern region of Mexico were obtained as a dried powder. To produce the nopal powders, fresh cladodes with spines were sanitized in water containing sodium hypochlorite (200−400 ppm) for 10 min. The samples were cut and dehydrated in a convection oven at 60 °C for 8−9 h. The dehydrated cladodes were ground in a hammer mill fitted with a 2 mm round orifice screen.

A total of 2 g from each powder was extracted using a mixture of 80% methanol (DEQ, Monterrey, Mexico)/water in a 1:10 mass/volume ratio for 2 h at room temperature and 150 rpm. Samples were centrifuged 10 min at 3000 rpm. The resulting supernatant was collected, concentrated at 65 °C under vacuum (Savant SC25EXP SpeedVac concentrator, Thermo Electron Corporation, Waltham, MA), and stored at −80 °C until use.

Determination of Total Phenols. The total phenol content of raw extracts was measured using the methodology proposed by Vinson et al.[21] using gallic acid as a standard (Sigma, St. Louis, MO). Folin−Ciocalteu reagent (2 N, Sigma, St. Louis, MO) was added to oxidize an aliquot of the extract in a 10:1 volume/volume ratio. Samples were incubated for 20 min in 96-well microplates at room temperature, and absorbance was measured at 750 nm in a microplate reader (Synergy HT, Bio-Tek, Winooski, VT). Results were expressed as micrograms of gallic acid equivalents per gram of dry sample (μg of GAE/g).

Determination of Hydrophilic Antioxidant Capacity. Hydrophilic antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) assay according to the methodology established previously.[22] 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) (Sigma, St. Louis, MO) was used to produce peroxide radicals, using fluorescein (Sigma, St. Louis, MO) as a substrate and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Sigma, St. Louis, MO) as a standard. The assay was carried out in a 96-well black microplate at 37 °C. All samples and reagents were dissolved in a pH 7.4 phosphate buffer (Sigma, St. Louis, MO), which was also used as a blank. A total of 25 μL of sample was incubated for 30 min with 150 μL of 0.1 μM fluorescein. After incubation, 25 μL of 153 mM AAPH was added. Fluorescence was measured using a microplate reader (Synergy HT, Bio-Tek, Winooski, VT), with excitation and emission wavelengths of 485 ± 20 and 530 ± 25 nm, respectively. The flourescence was monitored for 1 h at 2 min intervals. Results were expressed as micromoles of Trolox equivalents per gram of dry sample (μmol of TE/g).

Hydrolysis of Methanolic Extracts. Acid hydrolysis of raw extracts containing the flavonols was performed using the methodology previously described by Nuutila et al.,[23] with slight modifications. Briefly, samples were mixed with 3.2 M hydrochloric acid (DEQ, Monterrey, Mexico) at a 5:3 ratio and hydrolyzed at 85 °C for 20 min. The optimal hydrolysis time was determined by a kinetics analysis (data not shown).

Samples (5 mL) of raw and hydrolyzed extracts were fractionated using a solid-phase extraction (SPE) C_{18}-Aq column (Grace, Deerfield, IL). The first fraction was eluted with a 25% high-performance liquid chromatography (HPLC)-grade methanol (Honeywell, Morristown, NJ) and water (Honeywell, Morristown, NJ) mixture and discarded. The fraction of interest was eluted with 100% HPLC-grade methanol and concentrated to a volume of 250 μL under vacuum.

Identification of Flavonol Glycosides by HPLC−Photodiode Array (PDA) and HPLC−Mass Spectrometry−Time of Flight (MS−TOF). Raw methanol extracts and their acid hydrolyzates fractionated by SPE were analyzed by HPLC−PDA (1200 Series, Agilent Technologies, Santa Clara, CA). Chromatograms were obtained at 365 nm after injection of 2 μL of sample. Separation was performed in a Zorbax-SB C_{18}, 3.0 × 100 mm, 3.5 μm column (Agilent Technologies, Santa Clara, CA) with a flow of 0.5 mL/min. The mobile phase used was (A) HPLC-grade water with 0.1% formic acid (Sigma, St. Louis, MO) and (B) HPLC-grade methanol. Separation was achieved starting with 30% B for the first 5 min, increasing the B concentration to 60% until 20 min, and then increasing the B concentration to 90% for the next 5 min. Pure flavonoid aglycones (Sigma, St. Louis, MO) were used as standards. Ultraviolet−visible (UV−vis) absorption spectra were recorded for the predominant peaks.

Glycosides were not quantified because of the lack of pure standards; instead, percentages of glycosides remaining in the extract after 20 min of acid hydrolysis were recorded. These calculations were performed integrating the area under the curve (AUC) using the following equation:

$$\text{percentage of glycosides remaining} = \frac{\text{AUC}_{\text{after hydrolysis}}}{\text{AUC}_{\text{raw extract}}} \times 100$$

The identification of flavonol glycosides in raw extracts was confirmed by HPLC−MS−TOF. Chromatographic conditions used were the same as those described for HPLC−PDA analysis, with an injection volume of 1 μL. Mass spectra were collected using an electrospray source in positive mode (ESI+) under the following conditions: m/z range, 100−1500; nitrogen gas; gas temperature, 350 °C; drying gas flow rate, 13 L/min; nebulizer pressure, 50 psig; capillary voltage, 4000 V; and fragmentor voltage, 70 V.[16] Extracted ion chromatograms were obtained considering the accurate mass obtained for the aglycone standards and an error range of 0.01 units using the Analyst QS 1.1 software (Applied Biosystems, Carlsbad, CA). The mass spectrum of each peak was obtained to confirm the presence of the ion representing the aglycone and the glycosidic form of the flavonol previously reported for this plant.

Statistical Analysis. All measurements were performed in triplicate for each assay, and results were expressed as the mean ± standard deviation. Data were analyzed by analysis of variation (ANOVA) procedures, and differences among means were compared using Turkey tests with a level of significance of $p < 0.05$. Correlation analysis was performed by the Pearson correlation method with a level of significance of $p < 0.05$. Minitab 14.1 was used for all statistical analyses.

RESULTS AND DISCUSSION

Total Phenol Content. Table 2 depicts the total phenol concentration of the nine varieties analyzed herein. Five of the varieties analyzed contained similar total phenols (OR-G, OS, OR-R, OR-T, and OFI-V). The most outstanding sample was *O. undulata* (OU), which contained 905.1 ± 64.5 μg of GAE/g of

Table 2. Free Phenolic Content and Antioxidant Capacity of the Cladode Flours of Nine Varieties of *Opuntia* spp.[a]

sample	free phenols (μg of GAE/g)	ORAC (μmol of TE/g)
OFI-J	318.11 ± 34.18 d	382.52 ± 47.94 d
OL	754.26 ± 115.11 b	466.04 ± 50.57 c,d
OR-G	561.89 ± 37.79 c	738.83 ± 89.82 a
OS	663.56 ± 57.52 c	471.69 ± 60.53 c
OU	905.08 ± 64.51 a	585.20 ± 67.18 b
OR-R	689.46 ± 46.71 b,c	396.22 ± 58.77 d
OR-T	643.53 ± 59.69 c	496.83 ± 54.70 c
OL-D	802.01 ± 57.87 b	431.57 ± 50.80 c,d
OFI-V	593.1 ± 36.4 c	264.56 ± 32.20 e

[a] Abbreviations: GAE, gallic acid equivalent; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents. Values are expressed on a dry basis. Data with different letters in the same column are statistically different.

dry sample. On the other hand, the variety with the lowest amount of phenols was OFI-J (318.1 ± 34.2 μg of GAE/g). This flour contained approximately one-third of the total phenols assayed in the OU counterpart. As reviewed before,[24] polyphenols in plants have a highly variable composition and their total content as well as their profile variations may occur even within a single species. Factors that contribute to this variability in the genus *Opuntia* include genetic factors, environmental stress and conditions, and age.[1]

Other research studies have reported a higher amount of total phenols for *Opuntia* varieties. Lee and collaborators[11] reported 4140 μg of GAE/g of dry weight in cladode samples of *O. ficus-indica* var. Saboten. The analysis of *O. ficus-indica* var. Milpa Alta cladodes yielded 3081 μg of GAE/g of dry sample of total phenols.[12] These comparative values are 2−15 times higher compared to the values observed in this study. During flour preparation, the fresh cladodes were first soaked in chlorinated water (200−400 ppm) for 10 min and dehydrated at 60 °C for 8−9 h. Thus, part of the phenolic compounds might have leached into the sanitizing solution during disinfection and/or been thermally damaged during drying.

The *Opuntia* flours analyzed contained total phenols comparable to fruits, such as melon (259.2 μg of GAE/g), orange (358.9 μg of GAE/g), pineapple (697.9 μg of GAE/g), and white grapes (797.6 μg of GAE/g).[21]

Identification of Flavonol Glycosides by HPLC−PDA and HPLC−MS−TOF. The PDA chromatograms at 365 nm and ion chromatograms (m/z 317 and 287) for raw (Figure 1) and acid-hydrolyzed (Figure 2) extracts of OFI-J were used to characterize the flavonol profile of all of the *Opuntia* spp. flours. Peaks were identified (Table 3) as kaempferol (K) and isorhamnetin (I), as well as their respective glycosides (IG1, IG2, IG3, IG4, KG1, IG5, and IG6). Identification was corroborated by the UV−vis and MS spectra for each flavonoid in comparison to a previous research paper.[16]

Quercetin and its derivatives have been previously reported in *Opuntia* species[2,7,15,25,26] but were not detected in any of the samples studied herein. A peak eluting at the same retention time of a pure quercetin standard was observed, but the HPLC−MS−TOF m/z ion detected did not correspond to this particular compound. This peak was detected in extracts before hydrolysis, and according to the mass spectra, it corresponded to a diglycosidic form of isorhamnetin (IG6) with a m/z of 633 [(I + Hex + Pen) + Na^+]. Samples analyzed in this study were all Mexican species and varieties of *Opuntia*, whereas other research reports have studied Asian[7,15,25] and Italian[16] samples. Thus, the different genotypes and environmental conditions could be the possible reason of the observed absence of quercetin.[24]

The concentration of kaempferol as aglycone increased at least 10 times after hydrolysis. In fact, the aglycone was only detected for OFI-J, OFI-V, and OR-G raw extracts. Particularly, the amount of kaempferol went from 2.4 to 119.8 μg/g for OFI-J and from 4.9 to 54.3 μg/g for OR-G (Table 4). Only one glycosidic form of kaempferol was identified (Table 3), kaempferol-glucosyl-rhamnoside (KG1). The kaempferol concentration after acid hydrolysis ranged from 1.8 ± 0.3 to 474.6 ± 23.1 μg/g (Table 4). Previous reports for enzymatically hydrolyzed fresh cladode extracts found 60 μg/g of kaempferol when treatment with pectinase managed to hydrolyze up to 30.8% of the glycosides.[16] In the present study, 100% of hydrolysis of KG1 extracts was achieved for most samples. Results indicate that the efficiency of the hydrolysis was not related to the concentration of the flavonol. The sample extract with the highest concentration of kaempferol was OFI-V, and only 8.1 ± 4.2% of KG1 remained after acid hydrolysis. In contrast, the OU sample extract, which contained the second lowest concentration, had 74.6 ± 32.9% KG1 remaining after the acid hydrolysis treatment.

Isorhamnetin was the most abundant flavonoid in all samples, except OL, which did not contain this flavonol. Previous investigations indicate that isorhamnetin is indeed the predominant flavonoid in cladodes of *Opuntia* spp.,[16] and the one that has the widest variety of glycosidic derivatives.[16,25] As observed for kaempferol, isorhamnetin was detected as aglycone only in OFI-J, OFI-V, and OR-G extracts in a range of 4.9−10.7 μg/g. Table 3 shows details of the six glycosidic forms detected, which were previously reported in *O. ficus-indica*.[16] Three triglycosides, isorhamnetin-glucosyl-rhamnosyl-rhamnoside (IG1), isorhamnetin-glucosyl-rhamnosyl-pentoside (IG2), and isorhamnetin-hexosyl-hexosyl-pentoside (IG3), and three diglycosides, isorhamnetin-glucosyl-pentoside (IG4), isorhamnetin-glucosyl-rhamnoside (IG5), and isorhamnetin hexosyl-pentoside (IG6) were detected in four of the nine samples analyzed (Table 4). The minimum and maximum concentrations of isorhamnetin after acid hydrolysis ranged from 58.9 ± 14.8 μg/g (OS) to 726.2 ± 106.2 μg/g (OFI-J).

Some glycosidic forms of isorhamnetin were not totally acid-hydrolyzed. The most labile form was IG1, from which only 1.7% remained in OU. The other triglycosides IG2 and IG3 were totally hydrolyzed in three of five samples. Once more, the amount left of the nonhydrolyzed triglycosides was not related to the concentration of the aglycone. For OFI-J, which contained the highest concentration of isorhamnetin, the hydrolysis of triglycosides was totally effective. In contrast, OFI-V had similar amounts of isorhamnetin, but 13.41% IG2 and 44.5% IG3 remained after acid hydrolysis. The type of triglycoside was not related to the hydrolysis efficiency. For example, in the OL-D sample, 60.8% IG2 and 12.4% IG3 remained after acid treatment, contrary to what was observed in OFI-V, where 13.4 and 44.5% of these diglycosides were not hydrolyzed, respectively.

Hydrolysis of triglycosidic forms produced an accumulation of diglycosides of isorhamnetin. For the specific case of OL-D, 118% IG4 remained after acid hydrolysis. The remnants of IG5 observed in OFI-J, OR-G, OR-R, OR-T, OL-D, and OFI-V varied from 10.4 to 1016.5%. The dramatic increase observed in the OU

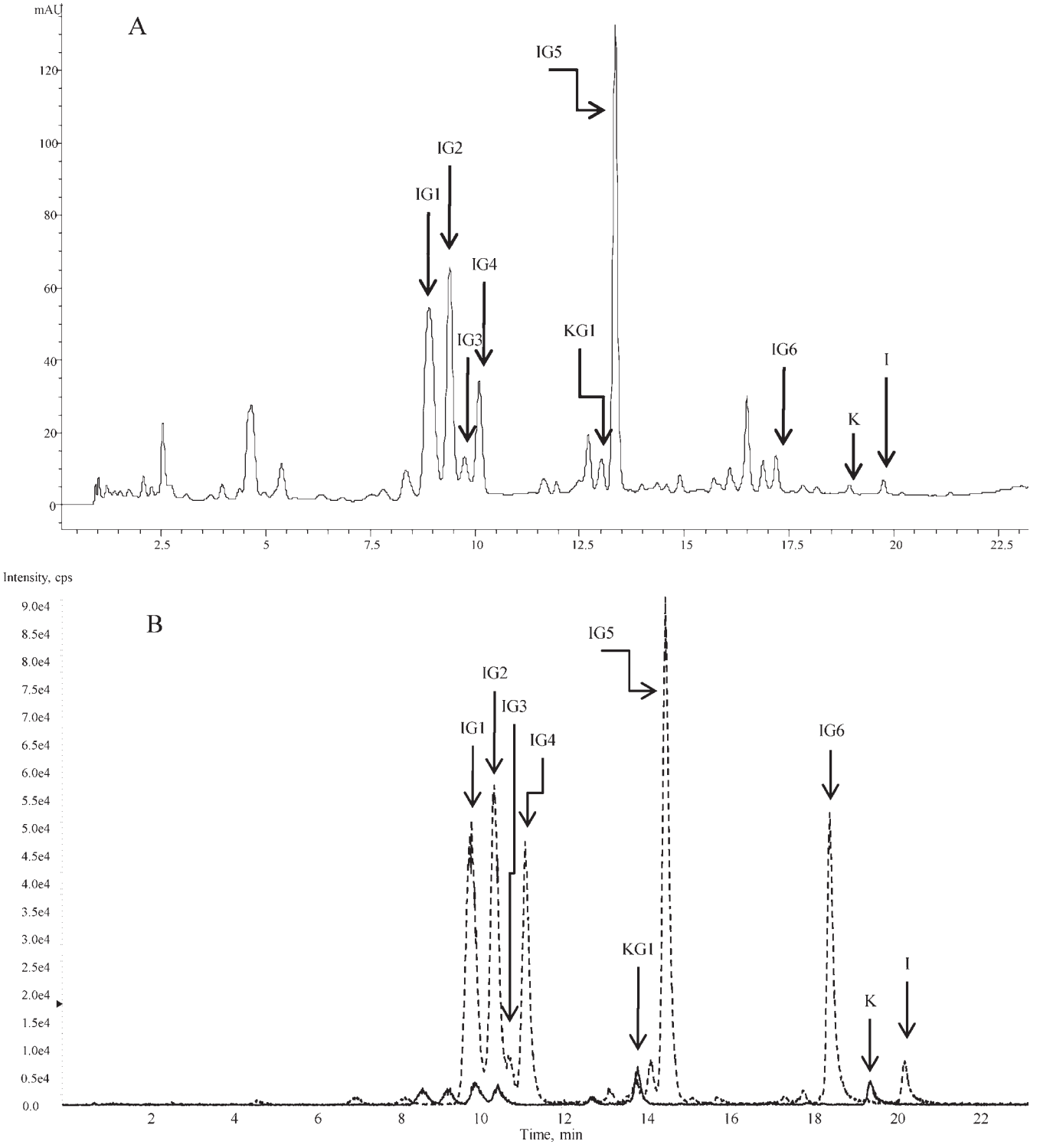

Figure 1. (A) UV at 365 nm and (B) extracted ion at *m*/*z* 287 and 317 chromatograms for the raw extract of *O. ficus-indica*. Identified peaks correspond to glycosides and aglycones of the flavonols, kaempferol (K) and isorhamnetin (I), as given in Table 3. In panel B, the dashed line corresponds to peaks containing the isorhametin aglycone ion (317) and the solid line represents peaks containing the kaempferol ion (287).

extract could be related to the high amount of IG3 because it was the sample with the highest AUC of this triglycoside (data not shown). Interestingly, for OS, none of the triglycosides was detected before or after hydrolysis, and therefore, diglycosides could have been liberated from other flavonol conjugates. IG6 also showed a marked increase in the OS extract. The rest of the sample extracts had less than 50% left of this particular glycoside. The concentration of isorhamnetin reported could be much higher than those reported in Table 4, considering that hydrolysis of glycosides was not complete in most samples.

It should be mentioned that each species showed a different profile of glycosides, and thus, hydrolysis of each sample should

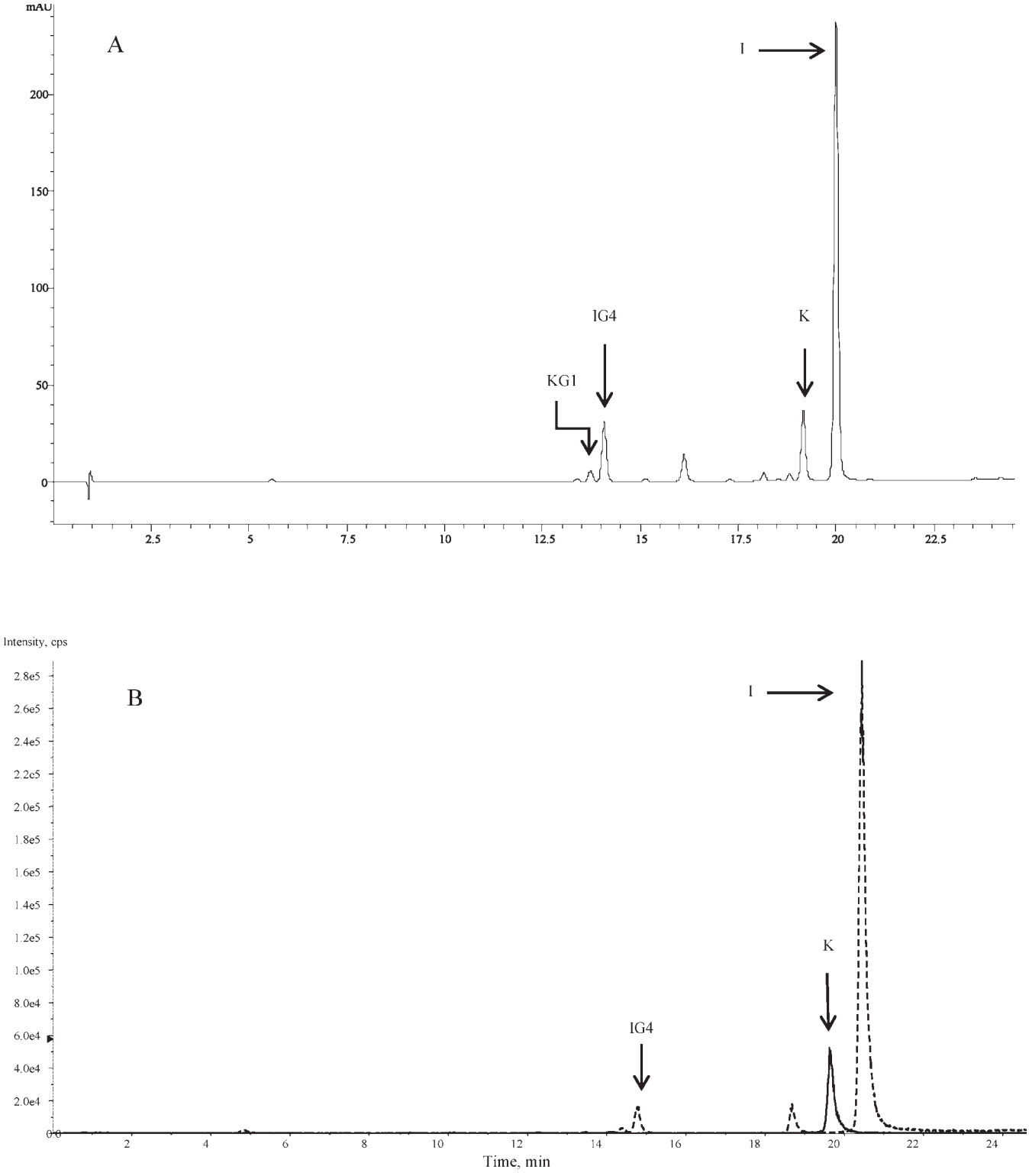

Figure 2. (A) UV at 365 nm and (B) extracted ion at *m*/*z* 287 and 317 chromatograms for the hydrolyzed extract of *O. ficus-indica*. Identified peaks correspond to glycosides and aglycones of the flavonols, kaempferol (K) and isorhamnetin (I), as given in Table 3. In panel B, the dashed line corresponds to peaks containing the isorhametin aglycone ion (317) and the solid line represents peaks containing the kaempferol ion (287).

be optimized to determine the total amount of flavonols present. Only the *O. robusta* varieties (OR-G and OR-T) were efficiently hydrolyzed after 20 min. Previous experiments with OFI-J (data not shown) indicated that longer hydrolysis times degraded the free kaempferol. Other samples, such as OS, OU, and OL-D, had a significant accumulation of some diglycosides, indicating that the process must be optimized.

Antioxidant Capacity (ORAC). In general, a higher level of phenols resulted in a higher antioxidant capacity (Table 2), with the exceptions of OR-G and OFI-V. A positive correlation

Table 3. Identification of Flavonoids in *Opuntia* spp. Extracts by UV−vis and MS Spectra[a]

peak	compound name	UV−vis λ_{max}	m/z fragments
IG1	isorhamnetin-glucosyl-rhamnosyl-rhamnoside	254, 354	317 $[I + H^+]$ 479 $[(I + Glu) + H^+]$ 625 $[(I + Glu + Rha) + H^+]$ 771 $[(I + Glu + Rha + Rha) + H^+]$ 793 $[(I + Glu + Rha + Rha) + Na^+]$
IG2	isorhamnetin-glucosyl-rhamnosyl-pentoside	253, 354	317 $[I + H^+]$ 479 $[(I + Glu) + H^+]$ 625 $[(I + Glu + Rha) + H^+]$ 757 $[(I + Glu + Rha + Pen) + H^+]$ 779 $[(I + Glu + Rha + Pen) + Na^+]$
IG3	isorhamnetin + 1 hexose + 1 methylpentose + pentose	253, 354	317 $[I + H^+]$ 779 $[(I + Hex + MP + Pen) + Na^+]$
IG4	isorhamnetin-glucosyl-pentoside	254, 354	317 $[I + H^+]$ 479 $[(I + Glu) + H^+]$ 633 $[(I + Glu + Pen) + Na^+]$
KG1	kaempferol-glucosyl-rhamnoside	262, 351	287 $[K + H^+]$ 449 $[(K + Glu) + H^+]$ 595 $[(K + Glu + Rha) + H^+]$ 617 $[(K + Glu + Rha) + Na^+]$
IG5	isorhamnetin-glucosyl-rhamnoside	253, 354	317 $[I + H^+]$ 479 $[(I + Glu) + H^+]$ 625 $[(I + Glu + Rha) + H^+]$ 647 $[(I + Glu + Rha) + Na^+]$
IG6	isorhamnetin + 1 hexose + 1 pentose	253, 354	317 $[I + H^+]$ 339 $[I + Na^+]$ 633 $[(I + Hex + Pen) + Na^+]$
K	kaempferol	266, 363	287 $[K + H^+]$ 309 $[K + Na^+]$
I	isorhamnetin	253, 370	317 $[I + H^+]$ 339 $[I + Na^+]$

[a] Flavonol glycoside UV−vis and mass spectra were compared to those reported by Ginestra et al.[16]

Table 4. Concentration of Flavonol Aglycones and Percentage of Remaining Glycosides after 20 min of Acid Hydrolysis[a]

	aglycone (μg/g)		glycosides (% remaining after hydrolysis)						
ID	kaempferol	isorhamnetin	IG1	IG2	IG3	IG4	KG1	IG5	IG6
OFI-J	119.8 ± 1.7 b	726.2 ± 106.2 a	0.0 b	0.0 c	0.0 c	0.0 b	25.3 ± 28.5 b	13.4 ± 2.5 c	32.4 ± 23.2 b
OL	1.8 ± 0.3 e	0.0 ± 0.0 d	NF	NF	NF	NF	NF	NF	NF
OR-G	54.3 ± 5.8 c	582.9 ± 122.4 a,b	0.0 b	0.0 c	0.0 c	0.0 b	0.0 d	0.0 d	11.6 ± 2.5 b
OS	42.2 ± 4.2 c	58.9 ± 14.8 c,d	NF	NF	NF	NF	NF	311.6 ± 31.7 b	380.4 ± 75.5 a
OU	12.9 ± 2.2 d,e	326.9 ± 74.4 b,c	1.7 ± 0.4 a	NF	8.5 ± 0.6 b	NF	74.6 ± 32.9 a	1016.5 ± 183.6 a	NF
OR-R	28.9 ± 4.7 d	199.8 ± 24.2 c	0.0 b	NF	NF	NF	0.0 d	0.4 ± 0.8 d	56.1 ± 20.9 b
OR-T	45.6 ± 1.2 c,d	99.58 ± 47.6 c,d	0.0 b	0.0 c	NF	NF	0.0 d	0.0 d	0.0 c
OL-D	39.6 ± 4.3 c,d	418.7 ± 9.4 b	0.0 b	60.8 ± 8.8 a	12.4 ± 6.7 b	118.6 ± 55.9 a	0.0 d	27.0 ± 24.9 c,d	38.0 ± 9.3 b
OFI-V	474.6 ± 23.1 a	654.0 ± 0.9 a	0.0 b	13.4 ± 1.9 b	44.5 ± 2.2 a	0.0 b	8.1 ± 4.2 c	10.4 ± 1.4 c	51.9 ± 1.9 b

[a] Values are expressed on a dry basis. NF = glycoside not found in the sample. Data with different letters in the same column are statistically different.

($p < 0.05$) was found between total phenols and ORAC (Figure 3), and interestingly, the OR-G flour (738.8 ± 89.9 μmol of TE/g) that contained the highest antioxidant capacity did not contain the highest total phenol concentration. Comparatively, the variety OFI-V (264.6 ± 32.2 μmol of TE/g) exerted approximately only one-third of the ORAC capacity. Corral-Aguayo et al. analyzed the antioxidant capacity of *O. ficus-indica* var. Milpa Alta and found values of 770.4 μmol of TE/g on a dry basis for fresh cladode samples.[12] This value is similar to the OR-G flour.

The OR-G flour ORAC value is comparable to cranberries (717.5 μmol of TE/g). The rest of the ORAC values were comparable to blackberries (400.4 μmol of TE/g) and raspberries

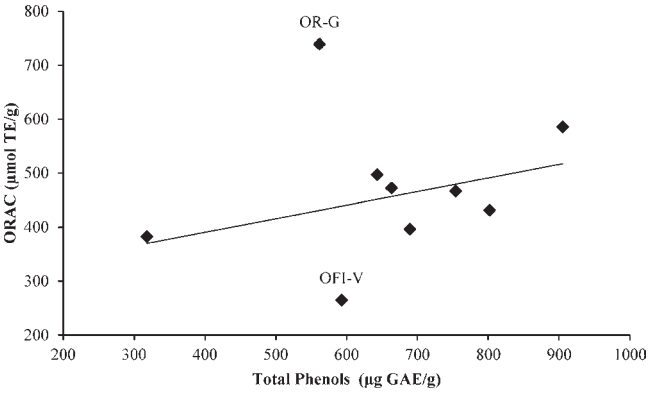

Figure 3. Linear correlation between total phenol (μg of GAE/g) content and antioxidant capacity ORAC (μmol of TE/g) of different cladode flours. Values represent the means of each assay. OR-G and OR-V correspond to samples that did not fit into the general observed behavior.

(335.6 μmol of TE/g).[22] Interestingly, the antioxidant activities were similar to previous reports,[12] although some phenols were lost during flour preparation.

There were not significant correlations ($p > 0.05$) between either individual and total flavonols and ORAC values. One possible explanation for the lack of correlation is that most of the flavonoids present in the extract were glycosidic forms and some contained sugar moieties attached to C3. In a previous investigation that researched a variety of flavonoids, it was established that these compounds lose assayable antioxidant capacity when glycosylated especially in C3.[20] Other compounds with known antioxidant activity have also been reported for *Opuntia* species, such as carotenoids[1,12,27] and tocopherols.[12] These lipophilic compounds probably contributed to the antioxidant activity observed in the extracts.

The current study shows that the flavonoid profile of the *Opuntia* genus was significantly different. The identified flavonols, isorhamnetin and kaempferol, were found mostly in their glycosidic forms in the raw extracts. The acid treatment greatly increased the concentration of aglycones. The most abundant flavonol was isorhamnetin, which was found in six glycosidic forms. Some of these glycosides were not fully hydrolyzed after 20 min; therefore, hydrolysis must be optimized for each species. The ORAC values correlated to total phenols but not to individual or total flavonoid contents.

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ABBREVIATIONS USED

GAE, gallic acid equivalents; ORAC, oxygen radical absorbance capacity; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; Trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; TE, Trolox equivalents; SPE, solid-phase extraction; OFI-J, *Opuntia ficus-indica* var. Jalpa; OL, *Opuntia lindheimeri*; OR-G, *Opuntia robusta* var. Gavia; OS, *Opuntia streptacantha*; OU, *Opuntia undulata*; OR-R, *Opuntia rastrera*; OR-T, *Opuntia robusta* var. Tapón; OL-D, *Opuntia leuchotricha* var. Duraznillo; OFI-V, *Opuntia ficus-indica* var. Villanueva; K, kaempferol; I, isorhamnetin; KG1, kaempferol-glucosyl-pentoside; IG1, isorhamnetin-glucosyl-rhamnosyl-rhamnoside; IG2, isorhamnetin-glucosyl-rhamnosyl-pentoside; IG3, isorhamnetin-hexose-hexose-pentose; IG4, isorhamnetin-glucosyl-pentoside; IG5, isorhamnetin-glucosyl-rhamnoside; IG6, isorhamnetin-hexose-pentose; Glu, glucose; Rha, rhamnose; Pen, pentose; Hex, hexose; MP, methyl-pentose; NF, not found

REFERENCES

(1) Stintzing, F.; Carle, R. Cactus stems (*Opuntia* spp.): A review on their chemistry, technology, and uses. *Mol. Nutr. Food Res.* **2005**, *49*, 175–194.

(2) Feugang, J. M.; Konarski, P.; Zou, D.; Stintzing, F. C.; Zou, C. Nutritional and medicinal use of cactus pear (*Opuntia* spp.) cladodes and fruits. *Front. Biosci.* **2006**, *11*, 2574–2589.

(3) Reynolds, S. G.; Arias, E. Introduction. In *Cactus (Opuntia spp.) as Forage*; Mondragón-Jacobo, C., Ed.; Food and Agriculture Organization (FAO): Rome, Italy, 2001; pp 1−4.

(4) Gutierrez, M. A. Medicinal use of the latin food staple nopales: The prickly pear cactus. *Nutr. Bytes* **1998**, *4* (2), 1–3.

(5) Galati, E. M.; Mondello, M. R.; Monforte, M. T.; Galluzo, M.; Miceli, N.; Tripodo, M. M. Effect of *Opuntia ficus-indica* (L.) Mill. cladodes in wound-healing process. *J. Prof. Assoc. Cactus Dev.* **2003**, *5*, 1–16.

(6) Zorgui, L.; Ayed-Boussema, I.; Ayed, Y.; Bacha, H.; Hassen, W. The antigenotoxic activities of cactus (*Opuntia ficus-indica*) cladodes against mycotoxin zearalenone in Balb/c mice: Prevention of micronuclei, chromosome aberrations and DNA fragmentation. *Food Chem. Toxicol.* **2009**, *47*, 662–667.

(7) Dok-Go, H.; Lee, K. H.; Kim, H. J.; Lee, E. H.; Lee, J.; Song, Y. S.; Lee, Y. H.; Jin, C.; Lee, Y. S.; Cho, J. Neuroprotective effects of antioxidative flavonoids quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica* var. Saboten. *Brain Res.* **2003**, *965*, 130–136.

(8) Saleem, R.; Ahmad, M.; Azmat, A.; Ahmad, S. I.; Faizi, Z.; Abidi, L.; Faizi, S. Hypotensive activity, toxicology and histopathology of opuntioside-I and methanolic extract of *Opuntia dillenii*. *Biol. Pharm. Bull.* **2005**, *28*, 1844–1852.

(9) Schepetkin, I. A.; Xie, G.; Kirpotina, L. N.; Klein, R. A.; Jutila, M. A.; Quinn, M. T. Macrophage immunomodulatory activity of polysaccharides isolated from *Opuntia polycantha*. *Int. Immunopharmacol.* **2008**, *8*, 1455–1466.

(10) Ahmad, A.; Davies, J.; Randall, S.; Skinner, G. R. Antiviral properties of extract of *Opuntia streptacantha*. *Antiviral Res.* **1996**, *30*, 75–85.

(11) Lee, J. C.; Kim, H. R.; Kim, J.; Jang, Y. S. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. *J. Agric. Food Chem.* **2002**, *50*, 6490–6496.

(12) Corral-Aguayo, R. D.; Yahia, E. M.; Carrillo-Lopez, A.; González-Aguilar, G. Correlation between some nutritional components and the total antioxidant capacity measusured with six different assays in eight horticultural crops. *J. Agric. Food Chem.* **2008**, *56*, 10498–10504.

(13) Budinsky, A.; Wolfram, R.; Oguogho, A.; Efthimiou, Y.; Stamatopoulos, Y.; Sinzinger, H. Regular ingestion of *Opuntia robusta* lowers oxidation injury. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2001**, *65*, 45–50.

(14) García-Verdi, G.; Olguín-Rubio, A.; Ramos-Gómez, M.; Rodríguez-García, M. E.; Reynoso-Camacho, R. Efecto antidiabético

del cladodio del nopal comercial en ratas sanas y diabéticas. *Rev. Salud Publica Nutr.* **2006**, *7* (2), 1–5.

(15) Qiu, Y.; Chen, Y.; Pei, Y.; Matsuda, H.; Yoshikawa, M. Constituents with radical scavenging effect from *Opuntia dillenii*: Structures of new α-pyrones and flavonol glycoside. *Chem. Pharm. Bull.* **2002**, *50*, 1507–1510.

(16) Ginestra, G.; Parker, M.; Bennett, R.; Robertson, J.; Mandalari, G.; Narbad, A.; Lo-Curto, R.; Bisignano, G.; Faulds, C.; Waldron, K. Anatomical, chemical, and biochemical characterization of cladodes from prickly pear [*Opuntia ficus-indica* (L.) Mill.]. *J. Agric. Food Chem.* **2009**, *57*, 10323–10330.

(17) Saleem, M.; Kim, H. J.; Han, C. K.; Jin, C.; Lee, Y. S. Secondary metabolites from *Opuntia ficus-indica* var. Saboten. *Phytochemistry* **2006**, *67*, 1390–1394.

(18) Sáenz, C.; Sepúlveda, E.; Pak, N.; Vallejos, X. Use of nopal dietary fiber in a powder dessert formulation. *Arch. Latinoam. Nutr.* **2002**, *52*, 387–392.

(19) Sandoval-Iturbide, A.; Navarro-Cruz, A. R.; Avila-Sosa, S. R.; Lazcano-Hernández, M.; Dávila-Márquez, R. M. Elaboración de un producto de panificación utilizando harina de nopal viejo o pie de cría (*Opuntia ficus-indica*). *Rev. Salud Publica Nutr.* **2008**, *8*, 1–8.

(20) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774–2779.

(21) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: Fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315–5321.

(22) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Lipophylic and hydrophylic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **2004**, *52*, 4026–4037.

(23) Nuutila, A. M.; Kammiovitra, K.; Oksman-Caldentey, M. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. *Food Chem.* **2002**, *76*, 519–525.

(24) Cheynier, V. Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr.* **2005**, *81* (Supplement), 223S–229S.

(25) Kim, H.; Park, S. H. Metabolic profiling and discrimination of two cacti cultivated in Korea using HPLC−ESI−MS and multivariate statistical analysis. *J. Korean Soc. Appl. Biol. Chem.* **2009**, *52*, 346–352.

(26) Lee, E. H.; Kim, H. J.; Song, Y. S.; Jin, C.; Lee, K. T.; Cho, J.; Lee, Y. S. Constituents of the stems and fruits of *Opuntia ficus-indica* var. Saboten. *Arch. Pharmacal. Res.* **2003**, No. 26, 1018–1023.

(27) Bensadon, S.; Hervert-Hernández, D.; Sáyago-Ayerdi, S. G.; Goñi, I. By-products of *Opuntia ficus-indica* as a source of antioxidant dietary fiber. *Plant Foods Hum. Nutr.* **2010**, *65*, 210–216.