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Classification of Seven Species of Cactaceae Based on Their Chemical and Biochemical Properties

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Fast protein liquid and ion-exchange chromatography, fluorescence, FT-IR spectra, and elemental and electrophoretic analyses were used to characterize proteins from seven species of Cactaceae, which can be divided into three groups based on their chemical and biochemical properties. Some cactus juice proteins precipitated by ammonium sulfate yielded complex electrophoretic patterns where the major bands correspond to approximately 24,000 Da and 32,000 Da in the presence of sodium dodecyl sulfate (SDS). The SDS–polyacrylamide gel electrophoretic (PAGE) patterns did not differ by the year of sample collection (1986 and 1992). Chromatographic analysis showed that protein characterization of cactus juices may be useful in cactus taxonomy at the family level. Fluorescence emission and FT-IR spectra of studied cacti species were measured to compare protein structure. Differences in the emission peak response and fluorescence intensity, as well as the changes in amide band content were found.

Several species of Mexican Cactaceae have been used as food and in traditional medicine as hallucinogenic, anticancer, and anti-inflammatory agents.[1–5] Researchers have shown that the *Opuntia ficus indica* and other species can be used for ethanol production[6] and as a component of sorghum feed.[7–11] The nutritional value of Cactaceae has been based on crude protein and fiber.[12–15] Some chemical components have been identified in the Cactaceae of Baja California[4,16,17] and other regions.[12,18–20]

Until now, Cactaceae taxonomy has been based on alkaloids, sterols, and fatty acids,[17,21–23] thus the protein composition has not been analyzed except to monitor stress in cactus roots[24] and for the purification and characterization of lectins and isolectins.[25,26]

In this work, we have analyzed and characterized the chemical and protein composition of cactus juice from seven species. The observed chromatographic and protein patterns may be used as an indicator for taxonomic purposes in the future.

Materials and Methods

Plant material. Seven common species of wild Cactaceae were collected on 1986 and 1992 in different areas of the Baja California desert near Comitan, Puerto San Carlos and Los Divisaderos, Mexico.[27] These species included *Pachycereus pringlei* (*Cardon*), *Machaerocereus eruca* (*Chirinola*), *Cholla opuntia* (*Cholla*), *Echinocereus engelmannii* (*Viejito*), *Lophocereus schottii* (*Garambullo*), *Stenocereus thurberi* (*Pitahaya dulce*), and *Machaerocereus gummosus* (*Pitahaya agria*).

Elemental organic composition (C, H, N, S, P, and O), carbohydrates (total and reduced sugars), and polyphenols were analyzed as described in ref. 28. Sodium, calcium, and iron were measured by atomic absorption spectrophotometry. All data were calculated as averages of triplicate measurements.

Protein extraction. About 1 kg of each sample of seven cacti species was cut into small pieces, homogenized in a Retsch ultra centrifugal mill, type ZM 1 (5 min at 15,000 × *g*) and filtered through cloth. The supernatant, about 200 ml, was then clarified by centrifugation (20 min at 7000 × *g*) and filtered through a Whatman No. 1 filter. This fraction is the crude extract. Solid ammonium sulfate was added to the supernatant to obtain 80% relative saturation, and the precipitate formed during 48 h at room temperature was collected by centrifugation (20 min at 7000 × *g*). After the pellet was resuspended in 40 ml of 20 mM Tris buffer, pH 7.4, and the insoluble material was removed by further centrifugation (20 min at 7000 × *g*), the clear supernatant was dialyzed extensively against 20 mM Tris buffer, pH 7.4, using cellulose tubing (Sigma) with a M_r cutoff of 2000 and lyophilized before storage at 4°C until use. This fraction was designated as Ammonium Sulfate Protein Precipitate (ASPP).

Protein content. The protein content was measured by the procedure of Bradford[29] and bovine serum albumin (BSA) was used as a standard.

Fast protein liquid chromatography. Ten mg of ASPP were dissolved in one ml of distilled water. The sample was put on a G-10 column (Pharmacia) equilibrated with 100 mM phosphate buffer, pH 8, for desalting. The column was washed with the same buffer, and the sample recovered in the exclusion volume of the column (3 ml). The filtered samples (0.5 ml) were put on a 2-ml Mono-Q column (Pharmacia) equilibrated with phosphate buffer. The FPLC worked with two buffer reservoirs. Reservoir A was filled with phosphate buffer as was mentioned above and reservoir B with 100 mM Gly-HCl, pH 2.5, containing 100 mM NaCl. A non-linear pH gradient was made by mixing the two buffers in different proportions. When the sample was put onto the column, only buffer A was applied (100% A, 0% B) and then the first peak was obtained (this is considered as non-retained material). The first eluted fraction (second peak) was obtained by varying the pH to 5.5 (50% A, 50% B). The next peaks were eluted at pH 5.0; 3.5; 3.0, and the last one at pH 2.5 (0% A; 100% B). The proportions of two buffers are indicated in the chromatograms on vertical lines in Fig. 1. The protein content was monitored at 280 nm.

Ion exchange chromatography. One and a half g of ASPP were dissolved in 5 ml of THA (10 mM Tris–HCl + sodium azide 0.02%; pH 8.0) and dialyzed against 500 ml of the same buffer (3 times, 12–16 h, 10°C). The sample was put on a the DEAE-Sepharose (Pharmacia Fine Chem.) column (53 ml) equilibrated with THA, and washed with the same buffer until the absorbance (280 nm) was near zero. The absorbed proteins were eluted with NaCl–THA (0.5 M NaCl dissolved in THA) by washing the column until the absorbance was near zero. The 2-ml fractions were collected and the absorbance at 280 nm was measured.

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Electrophoretic separation. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was done by the procedure of Laemmli[30] on acrylamide gradients of 10–15% on precast commercial gels (Phast Gel high density from Pharmacia) using the Phast System (Pharmacia) electrophoresis apparatus.

M_r markers (Sigma) were from 14,000 to 70,000 Da. The sample buffer contained 10 mM Tris–HCl, pH 8.0, 2.5% (w/v) SDS, 1 mM EDTA, 0.01% bromophenol blue, and in the case of reduced peptides, 5% (w/v) 2-mercaptoethanol (2-ME). Native-PAGE was done on acrylamide gradients of 8–25%, using the same sample buffer, but without SDS and ME. M_r protein markers were from 14,200 to 545,000 Da.

One part of dialyzed and lyophilized crude extract of cactus juices was dissolved in sample buffer, and another part was treated with polyvinyl-polypirollidon (PVPP). Three-tenths of a g of crude cactus juice was dissolved in 0.75 ml of 10% PVPP, then centrifuged. The supernatant was mixed with the sample buffer. Then all prepared protein samples were the put on gels. Proteins were fixed after electrophoresis in 6% glutaraldehyde and Coomassie Blue R, or silver stained.

Also mini-gel SDS–PAGE was done using a 10–18% acrylamide gradient. The samples (400 ng) in 10 μl of sample buffer were put on the gels. The run was done for 4 h, and the gel was fixed, stained with Coomassie Brilliant Blue R-250, and destained, as reported by Van-Seuningen and Davril.[31]

Isoelectric focusing (IEF) was done on precast (Pharmacia) polymerized carrier ampholyte polyacrylamide gels rehydrated in 8 M urea using marker proteins in the p*I* range from 3.5 to 10.6 (Pharmacia) according to instructions in the booklet.[32]

Fluorescence and FT-IR spectra. Fluorescence measurements were done using a Model FP-770 Jasco spectrofluorometer. The temperature of the samples was maintained at 30°C with a thermostatically controlled circulating water bath. Fluorescence emission spectra for all samples were taken at excitation wavelengths (nm) of 274 and 295 and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm.

A Perkins Elmer 2000 FT-IR spectrometer was used to record IR spectra. Lyophilized material was mixed with KBr and the pellet was pressed at 10,000 kg/cm^2 for 15 s.

Results

The chemical analysis of the juice of seven Cactaceae species is given in Tables IA and IB. Elemental analysis (Table IA) showed that the carbon content was the highest for *Pitahaya agria* (41.55%) and the lowest for *Viejito* (24.97%). Interestingly, the percentage of hydrogen varied slightly between the 5.87% in the case of *Pitahaya agria* and 4.11% for *Viejito* among the cactus species studied. The amount of elemental nitrogen, sulfur, and phosphorus on the other hand was the highest for *Viejito.* Sodium content (Table IB) varied between the highest value found for *Pitahaya dulce* (2.1%), and the lowest of *Cholla* (0.1%). Calcium content was low in most samples (0.05%–0.08%) compared to *Chirinola* (2.30%) and *Pitahaya dulce* (0.21%) content. The iron content was nearly the same for all samples (0.11–0.15%). Total polyphenols in all samples varied from 0.93% for *Garambullo* to 0.04% for *Chirinola.* The amount of protein was the highest for *Chirinola* (1.80%) and the lowest for *Cardon* (0.38%).

Ammonium sulfate precipitable proteins from the crude extract of the cactus stem juices were analyzed by electrophoretic and chromatographic techniques.

When put into a FPLC equipped with a Mono-Q column, part of the proteins were not retained under our experimental conditions. When a pH linear gradient was made to detect the elution profile, proteins were not detected at pH from 7.9 to 5.5 (data not shown). These results permitted us to discover the optimum scale of pH. Since the proteins were not detected in the range of pH from 7.9

Table IA. Chemical Analysis of Cactus Juices[a]

Species	Dry weight (%)	Elemental analysis (%)					
		C	H	N	S	P	O
Cardon *Pachycereus pringlei*	7.27	34.17	5.45	2.54	0.0	0.83	57.01
Chirinola *Machaerocereus eruca*	5.63	39.45	5.44	1.37	0.5	0.83	52.41
Cholla *Cholla opuntia*	6.92	29.96	4.21	1.37	0.6	0.78	63.08
Vlejito *Echinocereus engelmannii*	3.54	24.97	4.11	4.04	1.0	1.65	64.23
Garambullo *Lophocereus schottii*	6.83	36.13	4.92	3.25	0.5	0.65	54.55
Pitahaya dulce *Stenocereus thurberi*	5.26	26.70	4.23	3.47	0.0	0.56	65.04
Pitahaya agria *Machaerocereus gummosus*	5.87	41.55	5.87	1.28	0.5	0.52	50.28

[a] All values are a mean of three samples. The standard deviation was 10% of the values.

Table IB. Chemical Analysis of Cactus Juices[a]

Species	Metals (%)[b]			Polyphenols (%)	Proteins (%)	Sugars (%)	
	Na	Ca	Fe			Total	Reduced
Cardon *Pachycereus pringlei*	0.3	0.08	0.12	0.88	0.38	19.6	18.8
Chirinola *Machaerocereus eruca*	0.7	2.30	0.13	0.04	1.80	12.8	5.6
Cholla *Cholla opuntia*	0.1	0.06	0.14	0.32	1.43	n.d.[c]	4.9
Vlejito *Echinocereus engelmannii*	0.9	0.06	0.12	0.06	1.11	82.0	22.0
Garambullo *Lophocereus schottii*	0.6	0.05	0.11	0.93	1.23	n.d.	9.5
Pitahaya dulce *Stenocereus thurberi*	2.1	0.21	0.15	0.08	1.14	70.0	13.0
Pitahaya agria *Machaerocereus gummosus*	0.9	0.04	0.11	0.06	1.65	82.00	22.0

[a] All values are a mean of three samples. The standard deviation was 10% of the values.

[b] Metals (%), polyphenols (%), proteins (%), and sugars (%) were measured separately using cactus juices dry matter. Dry weight (%) is shown in Table IA.

[c] n.d. = not determined.

to 5.5, it was unnecessary to use this range of pH. Therefore a decreasing pH linear gradient (from 5.5 to 2.5) was made in steps. Elution patterns obtained at pH 5.0, 3.5, 3.0, and 2.5 for *Pitahaya agria*, *Chirinola*, *Pitahaya dulce*, and *Viejito* and shown in Fig. 1, profiles 1, 2, 5, and 8, were similar. *Cardon* (Fig. 1, profiles 3 and 4) and *Garambullo* (Fig. 1, profile 6) shows different patterns in number, size, and form of peaks in comparison with *Pitahaya agria*, *Chirinola*, *Pitahaya dulce*, and *Viejito*. It was difficult to obtain a sharp separation in *Cardon*, therefore this sample was applied in dialyzed (Fig. 1, profile 3) and in non-dialyzed (Fig. 1, profile 4) forms. Dialysis against distilled water did not improve resolution of the obtained patterns. *Cholla* (Fig. 1, profile 7) showed additional peaks and differed from other samples. The cacti ASPP fractions demonstrated very similar isoelectric patterns. Only *Garambullo*, *Cardon*, and *Cholla* extracts showed in IEF one additional band with an isoelectric point (p*I*) of 7.40 (data are not shown). *Garambullo*, *Cardon*, and *Cholla* differed from other Cactaceae. There was observed a correlation between Q-Sepharose and isoelectric patterns, and polyphenol content. Native gel electrophoresis (Fig. 2) confirmed previous data: all seven species had some bands between 29,000 Da and 132,000 Da. The *Pitahaya agria* (1), *Pitahaya dulce* (2), *Garambullo* (3), *Viejito* (4), and *Chirinola* (6) demonstrated very similar bands, but *Cholla* (5) and *Cardon* (7) differed from them and from one another. Some of the patterns were diffused. SDS–PAGE was done to get a better resolution. There was a similarity between ASPP patterns seen by SDS–PAGE in all seven species (Fig. 3). *Chirinola* (lane 6), *Cholla* (lane 5), *Viejito* (lane 4), *Pitahaya dulce* (lane 2), and *Pitahaya agria* (lane 1), on the other hand showed very sharply defined major and minor bands (Fig. 3). It is noteworthy that *Pitahaya agria* (lane 1), *Pitahaya dulce* (lane 2), *Viejito* (lane 4), and *Chirinola* (lane 6) had two bands in common of 24,000 Da and 32,000 Da (positions A and B on Fig. 3). Electrophoretic patterns of *Cholla* proteins (lane 5) were different in their molecular mass range in comparison with the previous group. *Garambullo* (lane 3) and *Cardon* (lane 7) also differed from *Pitahaya agria*, *Pitahaya dulce*, *Viejito*, *Chirinola*, and *Cholla*.

The electrophoretic patterns of cacti proteins from species collected at different times (1986 and 1992) showed similar protein composition and protein content for the same species from the same area, collected 6 years apart. Only one cactus, *Pitahaya agria*, showed slightly diffused electrophoretic bands, which can be explained or by the proteolytic digestion of the crude sample caused by storage or by experimental error (Fig. 4, lanes 4 and 3). A protein band seen at 32,000 Da may correspond to a lectin,[26]

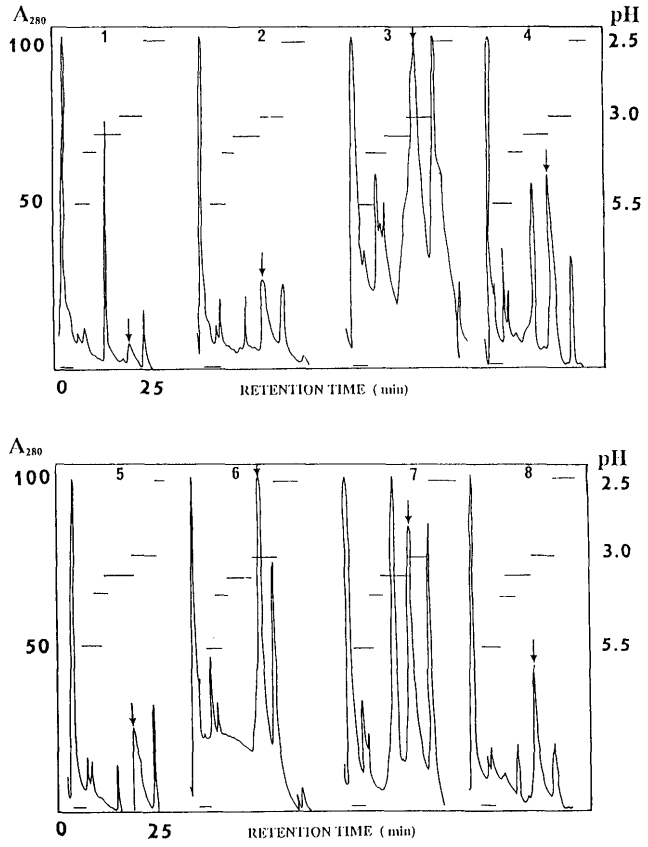

Fig. 1. FPLC Profiles of Ammonium Sulfate-precipitable Proteins from Cactaceae Samples.

1, 2, 3, 4, 5, 6, 7, and 8-respectively profiles of *Pitahaya agria*, *Chirinola*, *Cardon* (dialyzed), *Cardon*, *Pitahaya dulce*, *Garambullo*, *Cholla*, and *Viejito*. Experimental conditions are presented in the text. Vertical lines show the pH step gradient from pH 2.5 to pH 5.5.

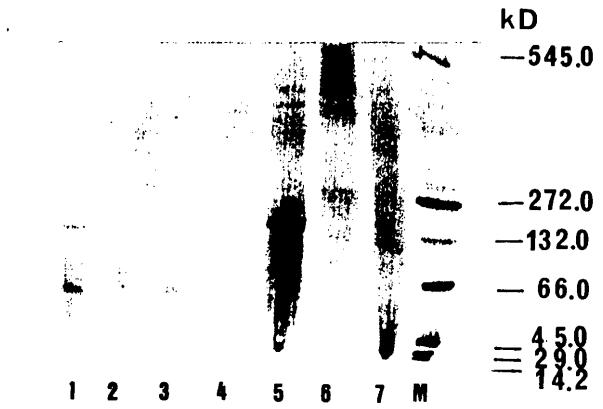

Fig. 2. Native–PAGE Analysis of Proteins Extracted from Cactus.

M_r markers (lane M) had the following sizes: 14,200; 29,000; 45,000; 66,000; 132,000; 272,000; and 545,000 Da. Lane 7, ASPP from *Cardon*; lane 6, ASPP from *Chirinola*; lane 5, ASPP from *Cholla*; lane 4, ASPP from *Viejito*; lane 3, ASPP from *Garambullo*; lane 2, ASPP from *Pitahaya dulce*; lane 1, ASPP from *Pitahaya agria*. The proteins (30 ng/μl) were separated on precast phast Gel 8–25% gradient gels.

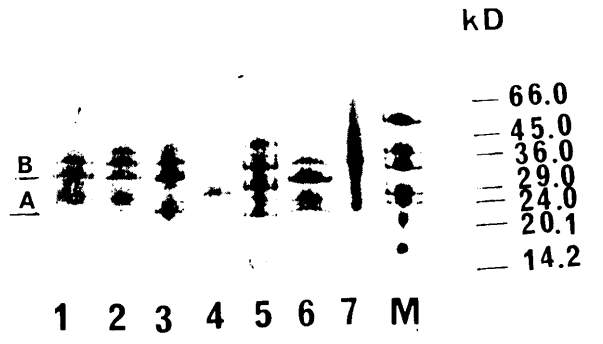

Fig. 3. SDS–PAGE Analysis of Proteins from Different Species of Cactuses.

M_r markers (lane M) had the following sizes: 14,200; 20,100; 24,000; 29,000; 36,000; 45,000 and 66,000 Da. Lane 1, reduced ASPP from *Pitahaya agria*; lane 2, reduced ASPP from *Pitahaya dulce*; lane 3, reduced ASPP from *Garambullo*; lane 4, reduced ASPP from *Viejito*; lane 5, reduced ASPP from *Cholla*; lane 6, reduced ASPP from *Chirinola*; lane 7, reduced ASPP from *Cardon*. The proteins (40 ng/nl) were separated on 10–18% mini gradient gels.

present in the cactus juice already reported, but we did not find the heat shock protein families that have appeared at 74,000 Da and 62,000 Da and corresponded to the data in literature.[24]

The results of chromatography on DEAE-Sepharose (Table II) showed that part of the *Pitahaya agria* crude extract was not absorbed to the column (Peak A), while the main protein content is retained and could be eluted with NaCl (Peak B), and the yield was about 91.3%. The peaks A and B contain the color of the crude extract, but peak A had a more intense in color than peak B. Peak C, which was eluted from the column by 0.1 M HCl, was colorless. All ion-exchange chromatography patterns of Cactaceae from the seven crude extracts were different from those, which are shown in Table II but with slightly different intensities of colors in peaks A and B (data not shown).

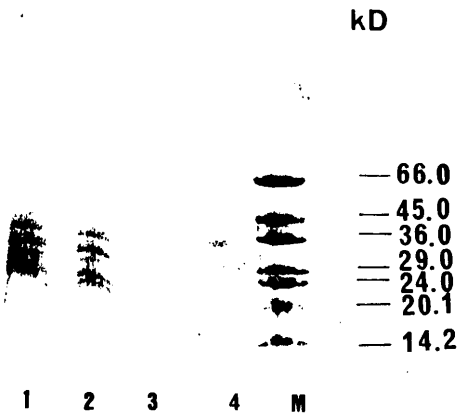

Fig. 4. SDS–PAGE Analysis of Proteins Extracted from *Pitahaya Agria*.
The same M_r marker (lane M) and the same conditions of separation as in Fig. 2. Lane 4, reduced proteins extracted from crude extract of *Pitahaya agria*, collected in 1986; Lane 3, reduced protein extracted from crude extract of *Pitahaya agria*, collected in 1992; lane 2, reduced ASPP; lane 1, unreduced ASPP.

Table II. Protein Content and Percentage of Recovery by DEAE-Sepharose Chromatography

Step	Volume (ml)	Total protein (mg)	Yield (%)
1. Crude extract	5	26.00	100
2. DEAE-Sepharose			
Peak A	19	1.69	6.5
Peak B	25	23.75	91.3
Peak C	17	<0.005[a]	0.0

[a] According to sensitivity of the Bradford method.

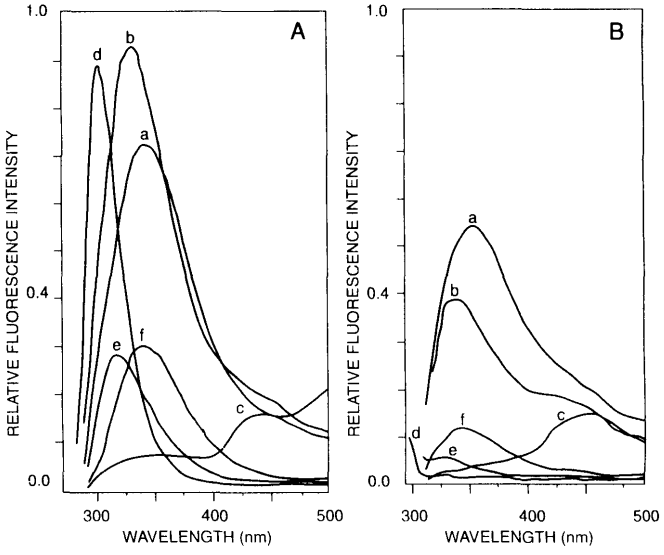

Fig. 5. Fluorescence Emission Spectra of a, *Garambullo* protein; b, *Pitahaya agria* protein; c, *Cardon protein*; d, *Cholla crude*; e, *Cardon crude*; and f, *Cholla protein*. Excitations (nm) at 274 and 295 on the A and B, respectively.

Lyophilized material of ASPP was used for fluorescence studies. Fluorescence spectra of the extracts obtained from the seven cacti species studied here are shown on Fig. 5. *Garambullo* (a) and *Pitahaya agria* (b) proteins demonstrate spectra typical for proteins containing tryptophan. However, the emission peak in *Garambullo* (a) is displaced to the right side, *i.e.*, the tryptophan residue in this protein is situated in a more polar environment; maybe the structure is less compact. At excitation of 274 nm can be seen a very slight shoulder in *Garambullo* protein, connected with the presence of tyrosine. *Cardon* protein (c) demonstrated very slight peak, apparently, it has a low tryptophan content. *Cholla* crude extract (d) showed a spectrum different from the *Cholla* protein (f). At excitation the 274 nm peak of *Cholla* crude extract (d) had a considerable shift to the left side, and at 295 nm was fully absent. It is the evidence of tyrosine presence and absence of tryptophan. The spctrum of *Cholla* protein (f) is typical for proteins containing tryptophan. Perhaps, *Cholla* crude extract (d) contains a mixture of proteins, and the content of this protein is low.

Infrared spectra of Cactaceae proteins are shown in Fig. 6. All proteins show similar bands at 3300 cm^{-1} (amino

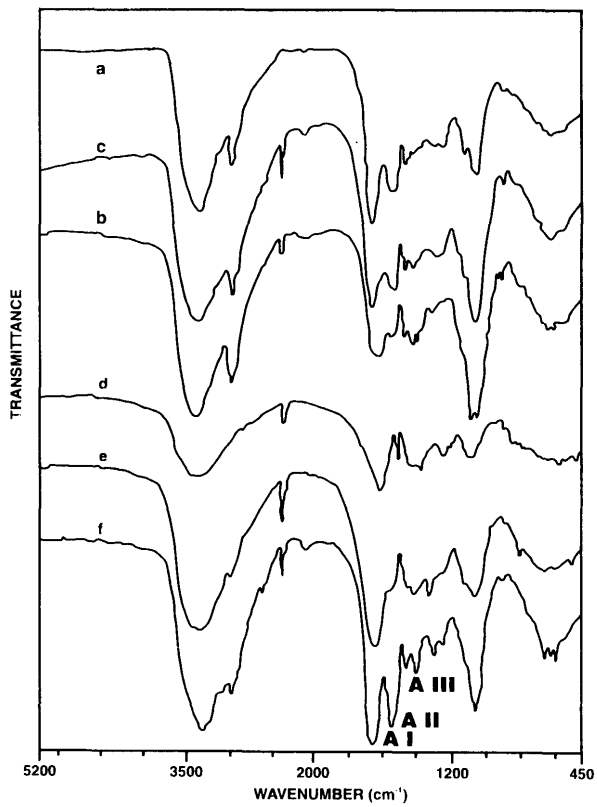

Fig. 6. FT-IR Spectra of *Pitahaya agria* protein (a), *Cardon* protein (b), *Garambullo* protein (c), *Cholla* crude (d), *Cardon* crude (e), and *Cholla* protein (f).

acid peak) and at 2900 cm^{-1} (–CH_2–stretching). Amide I (AI), Amide II (AII), and Amide III (AIII) (Fig. 6, position f) bands (in the range of 1650 cm^{-1}, 1530 cm^{-1}, and 1300–1250 cm^{-1}) differ for various species of cactuses (Fig. 6, positions a, b, c, d, e, and f). Displacement of band positions and absence of the Amide II band can be seen in some samples such as *Cardon* crude (e).

Discussion

Studies on the chemical composition of the stem juice from cactus plants are practically non-existent. This is, to our knowledge, the first report attempting to characterize the chemical composition of the juice of some cacti collected in the Baja California Peninsula of Mexico with the idea of establishing their relationships, similarities, and/or differences for taxonomical purposes.

First, it is important to mention that all seven cactus species selected for this study are quite different in morphology and distribution throughout the Peninsula, yet they are among the best represented species of the 700 different species found in the region. Thus, there are no other special criteria used in their selection.

In regard to the dry matter obtained after lyophilization of whole juice, the seven cactus plant studied had the large proportion of water that is characteristically retained by succulent plants, which in these cases was between 93% and 96% (Tables IA and IB). However, a note of caution should be sounded since the plants were collected from different environments, at different dates, and this may influence the extent of water and its variation. Another important finding is that the extracts that suffered oxidation more rapidly were those with the highest amounts of polyphenol content, such as *Garambullo*, *Cardon*, and *Cholla*, while the extracts containing low amounts of polyphenols remained transparent and clear throughout the study. The elemental composition of the different extracts does not show a clear correlation with either the amount of protein or sugar present in the sample, and among the metals measured, only sodium and calcium showed important variations, otherwise they are practically the same in iron content in all cases. In sum, from the standpoint of either elemental analysis, metals, or protein content, there are no sufficient deviations from a standard or normal value in the stem juices of cactus plants, as to assign them any meaning for taxonomic purposes.[33–36] The most striking differences among the cactus juices were their carbohydrate and polyphenol contents. In this case, it would be interesting to study in deeper detail the actual chemical nature of these fractions to gain more information about their physiological or ecological roles.

Since, as discussed above, the protein content was not considered a useful parameter for taxonomic purposes, we thought it convenient to analyze its composition to discover if some difference could arise to serve this objective. FPLC showed some differences in the elution patterns of the seven different cactus extracts put into a Mono-Q column. For all cacti, two peaks were obtained at pH 5.5, except *Cardon* (3) and *Garambullo* (6). Although the number of protein peaks is about the same, their relative position, proportion, and shape varies in each cactus species. Take for example the fraction eluted at pH 3.0; this protain band is present in all pattens (the peaks are marked with arrows on Fig. 1). This peak is highest for *Garambullo* (6), *Cardon* (3), and *Cholla* (7), followed by *Viejito* (8), *Chirinola* (2), *Pitahaya dulce* (5), and *Pitahaya agria* (1). The fractions obtained at pH 5.0, 3.5, 3.0, and 2.5 were similar for *Pitahaya agria*, *Chirinola*, *Pitahaya dulce*, and *Viejito*, with slight differences in position and quantity variations. Again, at this stage it would be difficult to support the idea of using a given protein found in the crude extract of a cactus stem as a marker. Therefore electrophoretic techniques such as IEF and native– and SDS–PAGE were used to examine the data found in FPLC profiles.

The electrophoretic patterns of cactus juice proteins, obtained from either SDS– or native–PAGE analysis, showed differences between the investigated cactus species. These cactus species can be classified on the basis of polyphenol content and electrophoretic and chromatographic patterns into three groups: I, *Pitahaya agria*, *Pitahaya dulce*, *Chirinola*, *Viejito*; II, *Garambullo*, *Cardon*; and III, *Cholla*. It is interesting to note that there were no differences between patterns obtained from plants collected 6 years apart.

In fluorescence emission spectra of the crude extract and of ASPP from *Cardon* and *Cholla*, as well as of ASPP from *Pitahaya Agria* (b), and *Garambullo* (a), we do find important differences that give some useful information with regard to the structure of cacti proteins (Fig. 5). For example, for the Cardon crude extract (e), Fig. 5 shows the presence of tyrosine and tryptophan residues, as commonly observed in emission spectra at 274 and 295 nm, respectively. But in ASPP from *Cardon* the change in the protein emission spectra causes appearance of such residues in a more polar environment. It means that the protein is in a more opened or relaxed state, which in turn reflects its instability or tendency to denaturation. This is not the case with the crude extract (d) or precipitated protein fraction of *Cholla* (Fig. 5, f), where the shifts are less noticeable, thus suggesting a more compact and stable protein fraction than that of *Cardon* (c). Small shoulder in the spectrum of the *Garambullo* sample (a) points out on the presence of tyrosine residues in this protein. However spectra of *Pitahaya dulce* and *Chirinola* (data not shown) were almost consistent with the *Pitahaya agria* (b) spectrum. Also, the spectra of the precipitated proteins suggest different amino acid content and composition in the number of tryptophan and tyrosine residues in all cases. The proteins from *Cardon* (c) and *Cholla* (f) contain very small quantities of chromophors, as compared to *Pitahya agria* (b) and *Garambullo* (a).

Fluorescence measurements do not allow us to classify proteins because of their low specificity. But florescence data showed a correlation with Q-Sepharose patterns and electrophoretic results which attribute *Pitahaya agria*, *Pitahaya dulce*, *Chirinola*, and *Viejito* to separate group of proteins based on their similar behavior in all analyses. In the same time *Cardon*, *Cholla*, and *Garambullo* demonstrate evident differences from the first protein group.

FT-IR spectra of *Cholla* (f), *Cardon* (b), *Pitahaya agria* (a), and *Garambullo* (c) show also some interesting differences among the extracts or precipitated proteins. Most notable is the absence of the 2600 cm^{-1} band in *Garambullo* proteins (c), and of the band at 1530 cm^{-1} corresponding to an Amide II type bond in *Cardon* extract (e) in Fig. 6.

Seven species of Cactaceae based on their chemical

(elemental analysis) and biochemical (Q-Sepharose patterns, fluorescence, FT-IR, isoelectric and SDS–PAGE electrophoretic patterns) properties were classified into three groups.

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