Chemical characteristics of non-starch polysaccharides of *Opuntia* cladodes as evidence of changes through domestication

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\textbf{A B S T R A C T}

Our knowledge of the complex domestication process originates from a small subset of crops. Non-starch polysaccharides are abundant in cladodes ("nopales") of the *Opuntia* genus, which people consume as vegetables. Chemical characteristics of these polysaccharides often respond to the species domestication. In this study, we assess the partial chemical variation of cladode non-starch polysaccharides in *Opuntia*, in a domestication gradient. For it, we isolated mucilage, pectins, and hemicelluloses. The degree of esterification, methylation, and total galacturonic acid for mucilage and pectins were determined. Also, to calculate structural protein content in those polysaccharides the total nitrogen was measured. A completely randomized experimental design was used, including 14 *Opuntia* variants of five species and six replicates. The degree of mucilage esterification (58.26–69.95%), and pectins (59.43–69.68%), and the degree of methylation of mucilage (25.24–28.66%) were independent of the domestication level. In contrast, the degree of pectins methylation (24.48–27.16%) was higher in wild species than in the domesticated. Total galacturonic acid content in mucilage (1.83–4.10 mMol 100 mg dry biomass) and pectins (6.46–10.33 mMol 100 mg dry biomass) was higher in domesticated species than in the wild ones. However, the content of structural protein in pectins (2.45–2.9%), loosely bound hemicelluloses (0.21–0.28%) and tightly bound hemicelluloses (0.45–0.79%) was higher in wild species. Chemical characteristics of structural polysaccharides significantly vary with domestication, probably a consequence of selection pressure for wide agricultural environments.

\section{1. Introduction}

The morphological, physiological, biochemical and genetic differences between domesticated species and their wild relatives constitute the domestication syndrome (Zohary, 2004). Domestication includes a decrease in seed dispersal capacity, viability, latency and chemical or mechanical protection against herbivores. It may also induce an enlargement of organs which are of interest to humans and often require plants to successfully live in human-made environments (Peña-Valdivia, Trejo, Arroyo-Peña, Sánchez-Urdeneta, & Baloi, 2012). Nonetheless, domesticated plants often become more vulnerable to herbivores, pathogens, and competitors than their wild congeners; this implies a gradual loss of adaptation to the natural environment as domestication takes place (Rosenthal & Dirzo, 1997). Our understanding of the complex domestication processes originates from a relatively small subset of crops, mainly of major economic importance. In order to explore global trends and historical patterns in domestication, large datasets are required to be analyzed which include a broad species range; the information has been critical to gain knowledge of crop breeding traits and fundamental biological processes, such as the tolerance to temperature increase and low water availability, as a result of global climate change (Meyer, DuVal, & Jensen, 2012).

The genus *Opuntia* has recently been used to study the domestication process, as it has a high number of species (188), of which 78 are native to Mexico (Anderson, 2001). Among them, there is a diversity of wild (i.e., *O. streptacantha* Lem. and *O. hyptiancanta* F.A.C. Weber), semi-domesticated (*O. megacantha* Salm-Dyck,) and domesticated (*O. albicarpa* Scheinvar and *O. ficus-indica* (L.) Mill) species (Reyes-Agüero, Aguirre-Rivera, & Flores-Flores, 2005) and they include many variants and cultivars. They are perennial plants that, although grown in different environments, due to their CAM-like metabolism are adapted to semi-arid climate conditions. In addition, fruits and tender and ripe cladodes are edible food for humans and livestock and contribute to food security of population in marginalized areas (Maki-Díaz, Peña...
Valdívia, García-Nava, Arévalo-Galarza, Calderón, & Anaya, 2015). Cladodes also are used in herbal medicine, and as a host of Dactylocyporum coccus used to obtain the carmine dye grana (cochineal extract), which is added to food products (López-Palacios et al., 2015). Changes in fruit during Opuntia domestication include: pulp sweetness, enhanced flavor and size, and seed hardness and seed quantity decrease (López-Palacios et al., 2015; Reyes-Agüiero et al., 2005). Moreover, changes in the young-tender cladodes (“nopalitos”) occurred in shape, color, flavor (Reyes-Agüiero et al., 2005), tissue firmness, turgor control, osmotic potential, membrane permeability, tissue water potential (García-Nava et al., 2015), and in soluble fiber (mucilage, pectins and loosely bound hemicellulose) content and proportion (López-Palacios, Peña-Valdivia, Reyes-Agüiero, & Rodríguez-Hernández, 2012). Spite of current advances in the understanding of the domestication process, humanity in the modern era has not domesticated any plant species, and researchers have to continue exploring how, when, where and why domestication happens (Peña-Valdivia, Aguirre-Rivera, & Arroyo-Peña, 2013). So by comparing the domesticated species with their wild relatives scientists could answer some of these current problems.

In plants, long-chain non-starch polysaccharides located in the cell wall, are major components of dietary fiber (Peña-Valdivia et al., 2012). Besides cellulose, which is considered a homopolysaccharide, they include pectins, mucilage, and hemicelluloses which are an extremely diverse set of biopolymers. These constantly change during plant development and in response to environmental stress or pathogen attacks (Gorshkova, Kozlova, & Mikshina, 2013). Cell wall proteins represent only 5–10% of the cell wall mass, are embedded in the complex matrix of polysaccharides and are relevant during plant development and adaptation to the environment (Albenne, Canut, & Jamet, 2013). Wide variations in the composition and organization of the cell wall between plant species and within the same species hinder cell wall studies. Non-starch polysaccharide hydrocolloids are abundant in tender cladodes and fruits of Opuntia, mainly of *F. ficus-indica*, have been documented (Forni, Penci, & Polesello, 1994; Lim-Vargas, Corrales-García, Valle-Guadarrama, Peña-Valdivia, & Trejo-Marquez, 2014; Majdoub et al., 2001; Sepúlveda, Sáenz, Aliage, & Aceituno, 2007). However, information is not available on the possible changes of chemical characteristics in polysaccharides of *Opuntia* resulting from domestication process.

This study aimed to assess partial chemical characteristics of non-starch polysaccharides from young cladodes of 14 variants of five species of *Opuntia*, Cactaceae in a domestication gradient. We hypothesise that non-starch polysaccharides chemical characteristics of *Opuntia* cladodes change through domestication.

2. Materials and methods

2.1. Plant material

Fourteen variants of five species of *Opuntia* were evaluated. The variants and species were arranged according to the domestication gradient as follows: Cardona de Castilla, Coloradita, and Tuna Loca of wild *O. streptacantha* Lem.; Memelo 1 and San Pedrera of wild *O. hyptiananca* F.A.C. Weber; Amarilla Montesa, Chapeada and Rubí Reyna of semi-domesticated *O. megacantha* Salm-Dyck; Copena Z1, Naranjón Legítimo and Villanueva of domesticated *O. albicarpa* Sheinvar; and Atlixco, Copena V1 and Rojo Vigor of domesticated *O. ficus-indica* (L.) Mill.

Edible young cladodes, 15–20 cm long, were collected from the Germplasm Bank plantation at the Centro Regional Universitario Centro-Norte, Universidad Autónoma Chapingo in the “El Orito” located 4 km southwest Zacatecas city, Mexico (22° 44.7′ N and 102° 36.4′ W, climate BSJKw (w), with a summer rainy season and cold winters) (García, 2004). Cladodes were, segmented, freeze-dried, and grounded in a mortar. The flour obtained was kept in a desiccator at laboratory temperature until used. The spines were previously removed from cladodes.

2.2. Experimental design

We used a completely randomized experimental design, including the 14 *Opuntia* variants of the five species with six replicates. Each repetition was a cladode from different plants.

2.3. Extraction of non-starch polysaccharides

Extraction and purification of non-starch polysaccharides included the sequential extraction of polysaccharides with aqueous solvents (Alvarez & Peña-Valdivia, 2009; Peña-Valdivia et al., 2012). The mucilages were solubilized in water. Ten ml of distilled water were then added to 500 mg of each sample and kept 30 min in a boiling water bath. The samples were then centrifuged 10 min at 1400 × g to separate the supernatant from the solid residue. The supernatant containing the mucilages was kept refrigerated (5 ± 1 °C). Pectins were then solubilized from the pellet by adding 3 ml of ammonium oxalate (0.5% in water w: v). These samples were maintained under agitation for 60 min at environmental temperature. The samples were then centrifuged 10 min at 1400 × g to separate the supernatant from the solid residue. The supernatant containing the pectins was kept refrigerated (5 ± 1 °C).

The interactions between polysaccharides in the cell wall involved several levels. All of them result in integrity of cell wall architecture, wall pore size, and polymers modification, like side chains addition and removal. Based on experimental evidence, the cell wall models include hemicelluloses attached to cellulose microfibrils in several complex levels by hydrogen bound and cross-links between these microfibrils. Those hemicellulosic polysaccharides loosely bound to cellulose microfibrils can be solubilized with strong alkali (5% KOH). On the other hand, the hemicelluloses tightly bound to cellulosic microfibrils can be extracted using strong alkali (24% KOH or 4 M NaOH) (Wakabayashi, 2000).

In the present study, loosely bound hemicelluloses were solubilized as follows. Three ml of 5% KOH in water (w: v) were added to the mucilage-pectin free pellet. Nitrogen gas was then bubbled through the liquid and kept stirring at 5 ± 1 °C, for 24 h. The samples were then centrifuged at 1400 × g for 10 min. The supernatant containing the loosely bound hemicelluloses was kept at 5 ± 1 °C. Then, 3 ml of 24% KOH in water (w:v) were added to the pellet (mucilages, pectins and loosely bound hemicelluloses free residue) to solubilize the tightly bound hemicelluloses. Also, nitrogen gas was bubbled through the liquid and kept under stirring at 5 ± 1 °C, for 24 h. The samples were then centrifuged 10 min at 1400 × g to separate the supernatant, containing tightly bound hemicelluloses, from the solid residue, which was eliminated.

Each polysaccharide type was consecutively three times extracted to effectively carbohydrates removal before to proceed with the next type of polysaccharide. The three supernatants with each type of polysaccharide were pooled. To flocculate the polysaccharides, five volumes of pre-cooled (6–12 h, −20 ± 1 °C) concentrate ethanol (96%) were added to the supernatants and kept in refrigeration for 12 h to allow complete flocculation. The hemicelluloses precipitation was complemented with the addition of four drops of concentrated HCl.

Each class of polysaccharide was recovered, as precipitated, by centrifugation (1400 × g, during 5 min), and decanting the supernatant. Polysaccharides were purified by dialysis against water (renewed every 4 h), during 72 h, with constant agitation (826 × g in orbital agitator
PRO VSOS-4 P, U.S.A.), using tubular dialysis membrane (MWCO 12-14 kDa, ZelluTrans, Roth, Karlsruhe, Germany). Purified polysaccharides were dehydrated by freeze drying.

2.4. Chemical characterization

The degree of esterification (DE) was determined by the titrimetric method, in a dry sample (5 mg) of isolated mucilage and pectins, by hydrochloric acid: sodium hydroxide double titration (Food Chemical Codex, 1988). Five mg of polysaccharide were moisture with 0.01 ml of 65% propan-2-ol, 10 ml of distilled water was added and stirred until polysaccharide had dissolved. The solution was titrated with 0.01 N NaOH (pH 7.5), the volume of titrant represented “de1 volume”. Then 3 ml of 0.01 N NaOH were added, mixture was covered and left for exactly 30 min. An amount of diluted H2SO4 equivalent to 3 ml of 0.01 N NaOH solution was added, while stirring was continued, the mixture was titrated with 0.01 N NaOH solution to pH 7.5. The volume of titrant consumed again represented “de2 volume”. The DE was calculated as: $\text{DE} (%) = 100 \times \frac{\text{de2} - \text{de1}}{\text{de1}}$.

3.1. Degree of esterification of mucilage and pectins

The DE of mucilage was determined by the titrimetric method, in a dry sample (5 mg) of isolated mucilage and pectins, by hydrochloric acid: sodium hydroxide double titration (Food Chemical Codex, 1988). Five mg of polysaccharide were moisture with 0.01 ml of 65% propan-2-ol, 10 ml of distilled water was added and stirred until polysaccharide had dissolved. The solution was titrated with 0.01 N NaOH (pH 7.5), the volume of titrant represented “de1 volume”. Then 3 ml of 0.01 N NaOH were added, mixture was covered and left for exactly 30 min. An amount of diluted H2SO4 equivalent to 3 ml of 0.01 N NaOH solution was added, while stirring was continued, the mixture was titrated with 0.01 N NaOH solution to pH 7.5. The volume of titrant consumed again represented “de2 volume”. The DE was calculated as: $\text{DE} (%) = 100 \times \frac{\text{de2} - \text{de1}}{\text{de1}}$.

The degree of methylation (DM) was quantified in dry mucilage and pectin samples with the FT-IR spectra (Manrique & Lajolo, 2002) in a FT-IR spectrophotometer (Spectrum GX system, Perkin Elmer 1600, USA) at 4 cm⁻¹ resolution and over the 4000–400 cm⁻¹ range with 100 scans. Base line and the FT-IR spectral parameters and area under the curve of each sample were calculated with the software package Spectrum FT-IR. The degree of methylation was obtained at the inferred band at 1730 cm⁻¹ (corresponding to the number of esterified carboxylic groups) and using the sum of the areas of the bands between 1730 and 1640 cm⁻¹ (number of total carboxylic groups); this is proportional to the DM (Manrique & Lajolo, 2002). The DM of samples was calculated according to following equation:

$$\text{DM} = \frac{A_{1730} (A_{1640} + A_{1730})}{A_{1640}} \times 100$$

where $A_{1730}$ and $A_{1640}$ are the peak area at 1730 and 1640 cm⁻¹.

Total galacturonic acid content was quantified by a colorimetric method with some modifications (Liu, Cao, Huang, Cai, & Yao, 2010). Samples (1 mg) were hydrolysed with sodium tetraborate in concentrated H2SO4 at 90 °C for 10 min. Color was developed using a carbazole solution (0.125% carbazole in absolute ethanol) and concentration was determined at $\lambda = 530$ nm with a pure D-galacturonic acid calibration curve.

Protein content was determined by the Kjeldahl method (AOAC, 1990), after mineralization in a Digestion System 20 and distillation by a Kjeltec Auto 1030 Analyser. Protein content was calculated using 6.25 to convert N into protein.

2.5. Data analysis

To determine significant differences among variants and species the data were analyzed with ANOVA, Tukey’s multiple comparison tests and multivariate principal component analysis (PCA).

3. Results

3.1. Degree of esterification of mucilage and pectins

The DE of mucilage was different ($P \leq 0.05$) among 14 variants, ranging from 56.95% in Amarillo Montesa to 73.55% in Coloradita. Differences between variants within species were significant for wild O. streptacantha (between Tuna Loca and Coloradita the difference was 15.23%) and for O. albicarpa (between Villanueva and Coloradita Z1 the difference was 6%). In contrast, no significant differences ($P > 0.05$) within O. hyptiacantha, O. megacantha, and O. ficus-indica were found (Fig. 1a).

The mean DE of mucilage among species ranged from 58% in O. megacantha to 70% in O. streptacantha and O. hyptiacantha. The lowest DE corresponded to a species with an intermediate degree of domestication. However, the mucilage of both wild species (O. streptacantha and O. hyptiacantha) was significantly ($P \leq 0.05$) higher DE (4%) than either of the highly domesticated species (O. albicarpa and O. ficus-indica) (Fig. 2a).

The DE in the pectins significantly varied ($P \leq 0.05$) among the variants of Opuntia, from 49.95% in Coloradita and Copena Z1 to 73.41% in the Rubi Reyna and Rojo Vigor. Significant differences ($P \leq 0.05$) of DE in pectins within species were also observed, the exception was O. hyptiacantha (Fig. 1b).

Differences in DE of pectins between species were significant ($P \leq 0.05$), the concentration in wild species was the lowest (57.7%; $P \leq 0.05$), compared to the high value (66.7%) for the semi-domesticated and domesticated species. The DE of the pectins increased in species along the domestication gradient, but O. albicarpa was an exception and presented a low DE (Fig. 2b).

3.2. Degree of methylation of mucilage and pectins

The degree of methylation (DM) of mucilage differed significantly ($P \leq 0.05$) among variants. The highest differences 23–31% were observed among the variants Copena Z1 and Atlixco ($P \leq 0.05$) of domesticated species; in contrast, there were no significant differences observed between the wild variants of O. streptacantha and O. hyptiacantha (Fig. 1c). In average the DM of mucilage did not show regular changes among species along the gradient of domestication (Fig. 2c).

The DM of pectins was significantly different among the variants (Fig. 1d) and between the species (Fig. 2d). Among the pectins of variants, DM fluctuated between 22% of the semi-domesticated Amarillo Montesa and 29% of the wild Coloradita. Variants of O. megacantha showed the highest difference, up to 17%, between the Amarilla Montesa and Rubi Reyna. In contrast, DM of pectins was similar (averaging 27%) among the domesticated variants, Rojo Vigor, Atlixco and Copena V1.

The pectin’s DM showed a gradient from the wild species O. streptacantha (27%) to the domesticated O. albicarpa (24%). Still, pectins of O. ficus-indica were an exception to the trend (Fig. 2d).

3.3. Galacturonic acid content in mucilage and pectins

Total galacturonic acid content in mucilage among variants was significantly different ($P \leq 0.05$), ranging from 1.45 mM 100 mg⁻¹ dry biomass in Chapeada to 5.05 mM 100 mg⁻¹ dry biomass in Copena V1 (Fig. 3a). Besides, the total galacturonic acid in mucilage was significantly different ($P \leq 0.05$) between variants within a species; a two-fold difference was observed between variants in a higher degree of domestication, such as O. albicarpa and O. ficus-indica (Fig. 4a).

The total galacturonic acid content of pectins in the nopalitos variants (averaged 8.6 mM 100 mg⁻¹ dry biomass) was three times higher than in the mucilage (2.81 mM 100 mg⁻¹ dry biomass on average). The total galacturonic acid content was different ($P \leq 0.05$) among the 14 variants, ranging between 4.67 and 11.91 mM 100 mg⁻¹ dry biomass, in Tuna Loca and Copena V1, each. Within species, there was a large difference in the galacturonic acid content of pectins. For example, between the Tuna Loca and Coloradita (a two-fold difference) variants in the wild species O. streptacantha, and between Amarilla Montesa and Rubi Reyna variants (almost a two-fold difference) in O. megacantha, a species with intermediate degree of domestication, and between Rojo Vigor and Copena V1 (a lesser, but still significant difference) in O. ficus-indica, the species with highest domestication degree (Fig. 3b).

Galacturonic acid content in pectins showed significant differences between species. On average, galacturonic acid concentrations in pectins of the domesticated species O. ficus-indica were 38% higher than those in the wild O. streptacantha (Fig. 4b).
3.4. Protein in pectins, loosely and tightly bound hemicelluloses

The pectin protein content was significantly different ($P \leq 0.05$) among the 14 variants, and within and between species (Figs. 5a, 6a). Among the variants, pectin protein content fluctuated between 2.27% and 3.11% in Copena Z1 and Cardona de Castilla; and within species, variants differed by up to 14% in *O. hyptiacantha* and 18% in *O. megacantha*.

The pectin protein content between variants of wild species and less domesticated variants showed significant differences ($P \leq 0.05$), but among domesticated variants species the differences between variants were not significant ($P > 0.05$) (Fig. 5a). The pectin protein content of the wild *O. streptacantha* was greater by 16% than the domesticated *O. ficus-indica* (Fig. 6a).

Protein content in loosely cellulose-bound hemicelluloses was significantly different ($P \leq 0.05$) among the variants, both, within and among species (Figs. 5b, 6b). Among variants, protein in these polysaccharides ranged between 0.19% and 0.31% in Rojo Vigor and Tuna Loca variants. Within species, significant difference was observed between variants in both wild and domesticated species; the greatest differences were evident within *O. streptacantha* variants. Differences between variants within domesticated species were not significant. On average the protein content in loosely bound hemicelluloses was 19–21% greater in the wild *O. streptacantha* and *O. hyptiacantha* than in the domesticated *O. megacantha*, *O. albicarpa* and *O. ficus-indica* ($P \leq 0.05$) (Fig. 6b).

The variants within the domesticated *O. ficus-indica* showed greater differences in the protein content of cellulose-tightly bound hemicelluloses (Rojo Vigor, 0.48%, and Atlixco, 0.69%) ($P \leq 0.05$). In contrast, the minimum difference between the variants within a species was between Memelo 1 (0.52%) and San Pedroña (0.58%) of *O. hyptiacantha* (Fig. 5c). The protein in tightly bound hemicelluloses was more abundant in wild species *O. streptacantha* (0.79%) than in domesticated species, *O. albicarpa* and *O. ficus-indica* (0.54% average; Fig. 6c). The results showed that on average, and similarly to pectins and loosely bound hemicelluloses, tightly bound hemicelluloses of wild species *O. streptacantha* contained more protein (32%) than domesticated species (Fig. 6c).
4. Discussion

4.1. Degree of esterification of mucilage and pectins

The DE of mucilage was a relatively constant characteristic between variants within a species and no significant differences within variants of three species were found. The DE in mucilage of nopalitos (genus *Opuntia*) does not gradually decrease along the domestication gradient. However, the two wild species presented a higher DE compared with the domesticated species. In contrast to DE, total mucilage content seemed to increase in nopalitos with domestication. Mucilage content in cladodes of an *Opuntia* species group, including wild and semi-domesticated species was up to 63% lower than in that highly domesticated, *i.e.*, *O. ficus-indica* (López-Palacios et al., 2012). These results indicate that domestication affects the content and some chemical characteristics of mucilage in cladodes.

The genus *Opuntia* is characterized by the presence of high concentration of mucilage (4–12% DM) in young (López-Palacios et al., 2012; Peña-Valdivia et al., 2012) and (15–17%) old cladodes (Contreras-Padilla et al., 2016), which form molecular networks that retain high water content (Saag, Sanderson, Moyna, & Ramos, 1975). Mucilages in *Opuntia* are complex non-starch polysaccharides, with a highly branched structure (Goycoolea & Cárdenas, 2004; Matsuhiro, Lillo, Sáenz, Urzúa, & Zárate, 2010) containing arabinose, galactose, rhamnose, xylose, and galacturonic acid in different proportions (Matsuhiro et al., 2010); including one gelling with water-soluble Ca$^{2+}$ fraction and another with non-gelling properties (Goycoolea & Cárdenas, 2004). The water-soluble polysaccharide fraction with thickening properties represented less than 10% of the water-soluble material in *O. ficus-indica* (Majdoub et al., 2001). Mucilage of *O. ficus-indica* peeled fruits contained less than 50% of pectin-like polysaccharide (Matsuhiro et al., 2010). This indicates that mucilage of *Opuntia* is a complex polysaccharide mixture which could be heterogeneous in both chemical composition and polysaccharide proportion. In our research, the DE heterogeneity was observed among wild species as well as in domesticated *Opuntia*.

The DE is one of the characteristics that define polysaccharide functionality. Higher values of DE in polysaccharides are related to a greater solubility in water and a faster gelation rate (Calvo-Arriaga, Hernández-Montes, Peña-Valdivia, Corrales-García, & Aguirre-Mandujano, 2010). Mucilage is responsible for the unpleasant sensation of sliminess when eating raw, boiled or cooked nopalitos. Domestication could involve some changes associated with a lower rate of solubility in broth and gelation of hydrocolloids along with low mucilage concentration in cladodes tissues. Calvo-Arriaga et al. (2010) determined that mucilage aqueous dispersion of *O. ficus-indica* nopalitos presented lower viscosity than citric pectin with low methoxyl content, this is a result of fewer interaction zones among molecules, weaker intramolecular cross-links, shorter polymer chains (Vliet, 1999) or greater DE with respect to low methoxyl citric pectin. Those authors...
also stated that high DE of mucilage of nopalito could form less calcium binding and dispersions of these mucilages had a lower viscosity (Walter, 1991). The later contributes to increasing the polysaccharide solubility whereas the lower or higher polysaccharide hydration might contribute to the process of adapting species to environments with different levels of humanization.

In our research, the DE heterogeneity of pectins among variants and species of Opuntia was quite broad. Species and domestication level showed a strong effect on DE of pectins and in general DE increased in species along the domestication gradient. This contrasted with the pectin content in cladodes of the same Opuntia species studied by López-Palacios et al. (2012). Their experimental evidence showed that pectin content in cladodes of domesticated species, i.e., O. albicarpa and O. ficus-indica, had up to 40% less content of these polysaccharides than wild species. These results indicated that domestication affects pectin content and chemical characteristics in cladodes.

Pectin structure and composition are species dependent and are affected by variant, cladode age, harvest season, rain distribution, environmental temperature, topography, soil type and by the interaction of all these factors (López-Palacios et al., 2012). The DE of pectins might be an indirect result of artificial selection to increase certain physicochemical traits because it is one of the most important features of pectin solutions are mainly dependent on DE. On average the DE of pectins of Opuntia species was as higher (59–70%) as high methoxyl pectins (DE ≥ 50%) (Willats, Know, & Mikkelsen, 2006). However, pectins of variants like Coloradita and Copaena Z1 can be defined as low methoxyl pectins.

On average, the DM of mucilage did not show regular changes among species along the domestication gradient. Similarly, no significant differences in DM of mucilage were observed in nopalitos of both wild species; but, among variants of domesticated species, DM heterogeneity was quite broad. The lower DM in O. albicarpa could be due to an adaptation mechanism respect to O. ficus-indica, since the first one is abundant in the northern zone of Mexican Southern Plateau; according to Reyes-Agüero et al. (2005), it is more likely that O. albicarpa resulted of a long domestication process from wild populations that were preserved and modified in home gardens, from where they were chosen to establish commercial plantations. The above indicates that the agricultural environment seems to be more effective on the DM of mucilages than in wild environments. Besides, the wider contrast in content and chemical characteristics of non-starch polysaccharides, such as DM of mucilage, in nopalitos of domesticated species seems to be related to the wide environmental conditions in which they can grow (López-Palacios et al., 2012).

4.2. Degree of methylation of mucilage and pectins

The DM of pectins was significantly different among the variants and showed a gradient ranging from the wild O. streptacantha species to the domesticated O. albicarpa. The DM is one of the most important properties for characterizing pectins. Values depend on several factors, such as species, tissue type, and tissue maturity; differences observed, depended on the method of polysaccharide extraction which can modify structural characteristics and properties of pectins (De Vries, Hansen, Soderberg, Glanhn, & Pedersen, 1986; Yapo, 2011).

The DM of nopalito pectins of 14 variants was lower than 30%; so pectins of nopalitos of the Opuntia genus included in this study were classified as of low-methoxyl. According to Liu et al. (2010) this type of pectin shows DM lower than 50%. The low degree of methyl-esterification of pectins of nopalito was similar to that measured in peel and pulp fruit of O. ficus-indica (Forni et al., 1994; Majdoub, Picton, Le, & Roudesli, 2010).

Pectin physicochemical properties depend directly on the DM and
contribute to cell wall elasticity (Peaucelle et al., 2011). Thus, modifications of the structure and composition of polysaccharides in cell walls, mainly pectins and loosely bound hemicelluloses, are related to texture changes in plant tissues during postharvest and DM, and play an important role in plant tissue firmness and cohesion (De Vries et al., 1986). DM reduction results in cohesion increase which is particularly evident in heated tissues. Manrique and Lajolo (2002) found that firmness of green, intermediate and ripened papaya (Carica papaya) fruit seemed to show an inverse relationship with the DM associated with middle lamella and primary cell wall during ripening. Higher DM present in the wild Opuntia streptacantha compared with the domesticated O. albicarpa, could be a result of selection for tissue with less firmness.

4.3. Galacturonic acid content in mucilage and pectins

On average, both domesticated species showed twice the galacturonic acid levels in mucilage than the intermediate and wild species. This result suggests a relationship between mucilage chemical modifications and domestication. Likewise, galacturonic acid content was homogeneous for the wild and intermediate species and also for the domesticated group.

Information on the content of galacturonic acid in mucilage of cladodes of the genus Opuntia is scarce; it has only been documented in cladodes mucilage of O. ficus-indica, which contained between 8% and 12.7% of galacturonic acid and various neutral sugars, such as L-arabinose, D-galactose, L-rhamnose, and D-xylose. The presence of D-galacturonic acid in mucilage has been wrongly named as pectin or pectinoid (McGarvie & Parolis, 1981; Nobel et al., 1992). López-Palacios et al. (2012) evaluated the pectin concentration in nopalitos of the same variants and species of Opuntia which were included in our study. Their results showed a relative decreased of 27.7% in pectins with domestication (from 2.53% in the wild species O. streptacantha to 1.83% in the domesticated species O. ficus-indica). This is in opposition to modifications in the composition of the pectins; as is the galacturonic acid content, which increased by 38% with domestication.

4.4. Protein in pectins, loosely and tightly bound hemicelluloses

Proteins can be covalently linked to the cell wall polysaccharides, such as pectins and hemicelluloses, to form structural proteins networks (Albenne et al., 2013). The protein values of pectins in the present study are within the range obtained by Pérez-Martínez et al. (2013) in cladode pectin for O. ficus-indica (2.81–4.5%). In contrast, protein content was not detected by them in the pectin of O. ficus-indica prickly pear peel extracted with alkali and acid. Ramos-Aguilar et al. (2015) reported that alkali-soluble pectin of green and red pepper showed a high protein content (1.9–2.6%). They suggested that the protein-pectin complex may be a distinctive characteristic of pectins with a high content of galacturonic acid and arabinan chains. The results of the present study showed that pectins protein content decreased along the domestication gradient and had an inverse relationship between galacturonic acid and protein in pectins. This probably indicates modifications in cell wall composition and function depending on the wild or agronomic environment where Opuntia variants grow. Similarly, low but significant differences of protein content in loosely and tightly cellulose-bound hemicelluloses among species seem to indicate that wild species, with higher structural protein content, are best adapted to...
exposure of abiotic and biotic stresses. Thus, protein may also take part in wound healing, plant defense impeding pathogen infection and this can be regulated by drought stress (Showalter, 1993).

The results showed that on average, and similarly to pectins, loosely and tightly bound hemicelluloses of wild species O. streptacantha contained more protein (32% more) than domesticated species. Comparable gradients of non-starch polysaccharides, pectins, and loosely and tightly bound hemicelluloses were observed in young cladodes of the same species of Opuntia (López-Palacios et al., 2012). However, pectins of the five species of Opuntia contained more protein (2.62% on average) than loosely and tightly bound hemicelluloses (0.24% and 0.58% on average). Although these hemicelluloses are considered to be an abundant source of xylose and xylo-oligosaccharides, their structural and physicochemical features have been poorly characterized. In this study, the difference in protein content of the pectins and loosely bound hemicelluloses may be due to the cell wall architecture and the polymers that are composed, although composition of the hemicelluloses isolated from different tissues varies considerably (Kenneth, Keegstra, Bauer, & Albersheim, 1973). The multi-layer model proposes that the cellulose microbrils are coated with a layer of xyloglucan and embedded in successive layers of hemicelluloses, each of them more loosely bound than the previous one, forming the cellulose-hemicellulose network. Pectins finally fill the spaces between the networks (Cosgrove, 2000), with different types of links that keep them connected and attached to the cell wall components. Covalent bonds between xyloglucan and laterals chains have been reported, and between rhamnogalacturonan type I and extensins structural proteins (Popper & Fry, 2005), which play a fundamental role in disassembly of the cell wall (Qi, Behrens, West, & Ma, 1995). Moreover, depending on the cell type, the structural proteins express great variations in their abundance development stage and the previous stimulation of the plant (e.g., wounds, pathogen attack) (Cosgrove, Bedinger, & Durachko, 1997). The proportion and type of protein-linking cell wall polysaccharides play a central function in nopal tissues of plants growing in the natural wild environment, but probably differs to those in plants under cropping environment.

4.5. PCA

The multivariate PCA data analysis was carried out to identify the characteristics of polysaccharides that could be involved in the domestication of Opuntia. The first three principal components (PC) explained 63.1% of the variability (Table 1). The PC1 had a higher and more positive correlation with the protein content in each group of polysaccharide and one chemical characteristic of mucilages. This indicated that PC1 was related to the cell wall recognition and signaling events (Albenne et al., 2013). The PC2 had a positive correlation with different polysaccharides chemical properties and to form stable gels with other molecules. The chemical characteristics of the cell wall polysaccharides govern biological roles of these molecules within the cell structure. PC3 appears to represent the relevant influence of domestication on pectins in order to maintain them poor or high negatively charged and also may ionically interact with Ca to form a stable gel with other pectin molecules only if more than ten consecutive unmethyl-esterified galacturonic acid residues are coordinated (Peaucelle et al., 2011). When PC1 was plotted against PC2 (Fig. 7a) and PC3 (Fig. 7b) and this against PC2 (Fig. 7c), no overlap was observed between O. streptacantha, the wild species, and O. ficus-indica and O. albicarpa, two of some long domesticated Opuntia species. Neither overlap was observed between O. streptacantha and O. megacantha, a species with intermediate degree of domestication. The domesticated O. ficus-indica and O. albicarpa are clearly independent biological units compared to the wild O. streptacantha (Fig. 7). Among the Opuntia variants selected for this study, the chemical characteristics that promoted this independence were mainly related to wall structural integrity, cell adhesion, signal transduction, environmental reactions and other important cell wall biological functions.

Non-starch polysaccharides extraction involves some alteration of the structure and composition of each type of polysaccharide. Even so, this methodology has been widely used in many species with several objectives (Alvarez & Peña-Valdivia, 2009; Calvo-Arriaga et al., 2010; López-Palacios et al., 2012; Peña-Valdivia et al., 2012). The present study is an advance in the knowledge of the changes in the chemical composition of cell walls during domestication. This showed that those

<table>
<thead>
<tr>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance</td>
<td>0.2692</td>
<td>0.4760</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE ̈ mucilage</td>
<td>0.45440</td>
<td>−0.03213</td>
</tr>
<tr>
<td>DE pectins</td>
<td>0.01223</td>
<td>0.44968</td>
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<tr>
<td>DM ̈ mucilage</td>
<td>0.15844</td>
<td>0.56866</td>
</tr>
<tr>
<td>DM pectins</td>
<td>0.31366</td>
<td>0.16468</td>
</tr>
<tr>
<td>GA ̈ mucilage</td>
<td>−0.07662</td>
<td>0.30114</td>
</tr>
<tr>
<td>GA pectins</td>
<td>0.02269</td>
<td>0.57313</td>
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<tr>
<td>Protein pectins</td>
<td>0.48100</td>
<td>−0.04990</td>
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<tr>
<td>Protein loosely bound hemicelluloses</td>
<td>0.48907</td>
<td>−0.08074</td>
</tr>
<tr>
<td>Protein tightly bound hemicelluloses</td>
<td>0.43920</td>
<td>−0.13453</td>
</tr>
</tbody>
</table>

* DE: degree of esterification.
* DM: degree of methylation.
* GA: galacturonic acid content.
alterations, and several modifications result from complex interaction such as climate changes, human selection, changes in growth environments and the uses of a complex genus with near to 200 species. Also, the complex CAM-like metabolism adaptation to semi-arid climate conditions of these perennial plants. Taking into account that these species have been used since ancestral times for various human activities, such as food and livestock, herbal medicine, and as hosts for Dactylopius coccus insect for non-synthetic pigment production.

5. Conclusions

The total galacturonic acid content in mucilage and pectins of nopalitos increased during domestication of the genus Opuntia, while DM and protein content in pectins and hemicelluloses decreased. The DE varied between species without a defined trend according with the nopal domestication. In general, the galacturonic acid content in mucilage and pectins of young cladodes was heterogeneous within species. Chemical characteristics of non-starch polysaccharides in young cladodes of Opuntia allowed separation between wild and domesticated species.

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References

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