Chemical and nutritional characterization of raw and thermal-treated flours of Mesquite (Prosopis laevigata) pods and their residual brans

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ABSTRACT

Species of the genus Prosopis were a major staple food in Aridoamerica before the arrival of Europeans. In the present work, chemical and nutritional properties of Prosopis laevigata pods were described. The composition in weight of pods of P. laevigata was 44% mesocarp, 35% endocarp, and 21% exocarp. Sugars, including sucrose, glucose, fructose, and xylose, were important components of pods, reaching a total sugars content of 447 g/kg in mesocarp flour. Considering the FAO-recommended amino-acid scoring patterns for humans older than 3 years, high values of Lys and sulfur-containing amino acids were found in flour. Thermal treatment of flours increased significantly the phenolic compounds content and free-radical scavenging capacity, an effect associated with the generation of Maillard reaction products. Flours of mesquite pods are a good source of phenolic compounds, with significantly higher free-radical scavenging capacity than soybean and common bean.

Caracterización química y nutrimental de harinas crudas y térmicamente tratadas de vainas de Mesquite (Prosopis laevigata) y sus salvados residuales

RESUMEN

Especies del género Prosopis fueron alimento importante en Aridoamérica antes de la llegada de los europeos. En el presente trabajo se describen las propiedades químicas y nutrimentales de vainas de Prosopis laevigata. La composición en peso de las vainas de P. laevigata fue de 21% exocarpio, 44% mesocarpio y 35% endocarpio. Azúcares, incluyendo sacarosa, glucosa, fructosa y xilosa, fueron importantes componentes de la vaina, alcanzando un contenido total de azúcares de 447 g/kg en harina de mesocarpio. Considerando los valores de aminoácidos recomendados por la FAO para mayores de 3 años, altos valores de Lys y aminoácidos azufrados fueron encontrados en las harinas. El tratamiento térmico de las harinas incrementa significativamente el contenido de compuestos fenólicos y la capacidad captadora de radicales libres, un efecto asociado a la generación de productos de la reacción de Maillard. Las harinas de vaina de mezquite son una buena fuente de compuestos fenólicos, con una capacidad captadora de radicales libres más alta que las semillas de soya y frijol.

1. Introduction

Legumes have been an essential part of the human diet for centuries, with a major role in global food security, environmental challenges and healthy diets (FAO, 2016). The genus Prosopis is a group of nitrogen-fixing trees belonging to the Fabaceae family, which involves 44 species distributed mainly in arid and semiarid regions of Asia, Africa, and America (Felker, Takeoka, & Dao, 2013). Prosopis species were a major staple food for indigenous peoples in arid regions of America before the arrival of Europeans. The mature fruit of the genus Prosopis is an indehiscent pod conformed by an exocarp, a developed mesocarp, and a woody endocarp which protects the seed (Felker et al., 2013). Pods and pods fractions of Prosopis species have been described in their nutritional profile: P. africana (Igwe, Ojiako, Anuweje, Nwaogu, & Ujowundu, 2012), P. alba (Cattaneo et al., 2016; Felker, Grados, Cruz, & Prokopiuk, 2003; Sciammarn, Ferrero, & Puppo, 2016), P. chilensis (Astdilullo, Schmeda-Hirschmann, Herrera, & Cortes, 2000), P. glandulosa (Harden & Zolfaghi, 1988), P. juliflora (Marangoni & Alli, 1988), P. laevigata (Barba de la Rosa, Frias-Hernandez, Olalde-Portugal, & Gonzalez-Castañeda, 2006; Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo, & Garcia-Casas, 2013), P. nigra (Felker et al., 2003), P. ruscifolia (Bernardi, Sanchez, Freyre, & Osella, 2010), and P. tamarugo (Astdilullo et al., 2000); active compounds content and in vitro biological activity: P. alba (Cardozo et al., 2010; Cattaneo et al., 2016; Perez et al., 2014; Sciammarr, Ferrero, & Puppo, 2016), P. chilensis (Astdilullo et al., 2000; Briones–Labarca, Munoz, & Maureira, 2011; Schmeda-Hirschmann et al., 2015), P. laevigata (Gallegos-Infante et al., 2013), P. nigra (Cardozo et al., 2010; Perez et al., 2014), P. ruscifolia (Bernardi et al., 2010), and P. tamarugo (Astdilullo et al., 2000); and in vivo biological activity: P. glandulosa (George, Lochner, & Huisamen, 2011; Huisamen, George, Dietrich, & Genade, 2000).
2. Materials and methods

2.1. Plant material

Dry mature pods of 12 P. laevigata trees with the same phenotypic traits and growing conditions were collected during August 2016, at the experimental field of the Universidad Politécnica de Francisco I. Madero in the semi-arid region of the Mezquital Valley in Hidalgo, Mexico. Collected yellow-brown mature pods from different trees were mixed in a single pool of 52 kg. Pods were ground in one and two stages, using a 900 W blender (Nutribullet, Los Angeles, CA, USA). For one-stage milling process, an exhaustive milled pods batch of 15 kg was sieved in 30 and 80 mesh, producing three fractions: mesocarp-seed flour (MSF) and their residual brans, including proximate composition, sugar and phenolic compounds content, amino-acid profile, free-radical scavenging capacity, and Maillard reaction products (MRPs).

2.2. Proximate composition

Proximate composition of the different pods flours and brans was assessed according the methods of the Association of Official Analytical Chemists (AOAC, 2000).

2.3. Free sugars content

Content of sugars in raw flours was determined by HPLC method, according to Akanni, du Preez, Steyn, and Killian (2015) using a Dionex-UltiMate 3000 equipment (Thermo Scientific, MA, USA) supplied with a REZEX RPM-Monosaccharide PB+2(8%) column (300 × 7.8 mm). Sugars separation was developed using water as mobile phase at 80°C column temperature and were detected by refractive index.

2.4. Amino-acid profile

The amino-acid content in raw flours was analyzed after acid hydrolysis, using a cation exchange separation column (LCA K06/Na, 4.6 × 150 mm; Sykam GmbH, Germany) with ninhydrin postcolumn derivatization, in an amino-acid analyzer (Sykam GmbH, Germany) (Li et al., 2012). Same method was used for sulfur-containing amino acids, using performic acid oxidation before hydrolysis (Li et al., 2012). Tryptophan was determined at 620 nm, after enzymatic hydrolysis and reaction with p-dimethylyaminobenzaldehyde (Nielsen, Klein, & Hurrell, 1985).

2.5. Thermal treatment of flours

MF, SF, and MSF were hydrated with 1.5 mL of water per gram of flour and thermal treated, as in a baked process, in a convection oven (Thermo Scientific, MA, USA) at 190°C during 20 min. Mesocarp flour thermal treated (MFT), seed flour thermal treated (SFT), and mesocarpo-seed flour thermal treated (MSFT) were dried and ground.

2.6. Preparation of extracts

Phenolic compounds of raw flours, thermal-treated flours, and brans were extracted with aqueous ethanol, according the method of Kalia, Sharma, Singh, and Singh (2008). Samples of 100 mg were extracted with one milliliter of 40% ethanol in water (v/v) and centrifuged at 12,000 rpm/10 min. The extract was removed and the extraction process was done again with the residual pellet. Both extracts were mixed and diluted with 40% ethanol to obtain a final volume of 25 mL.

2.7. Hydrolysis of extracts

For hydrolysis of phenolic glycosides, a replicate was prepared for each obtained extract. HCI hydrolysis method of Nuutila, Kammiovirta, and Oksman-Caldentey (2002) was used for hydrolysis with an ethyl acetate aglycones recovery. The ethyl acetate was evaporated at 45°C overnight and the residue was diluted with 40% ethanol to obtain a final volume of 25 mL.
2.8. Ultraviolet analysis of Maillard reaction products

Analysis of MRP in extracts of raw and thermal-treated flours was performed using the spectrophotometric method reported by Yu, Zhao, Hu, Zeng, and Bai (2012). Appropriate dilution of extracts were scanned from 240 to 320 nm using an ultraviolet–visible (UV-Vis) spectrophotometer (Genesys 10S, Thermo Scientific, MA, USA). The presence of MRP was evidenced by the increase in the UV absorbance between 270 and 290 nm.

2.9. Total phenolic compounds content

Total phenolic compounds content was determined in raw, thermal-treated and hydrolyzed extracts by the Folin–Ciocalteu method (Pekal & Pyrzynska, 2014). The absorbance was measured at 760 nm and the results were expressed as g of gallic-acid equivalents/kg (mg GAE/kg) of dry weight.

2.10. Total flavonoids content

Total flavonoid content was determined in raw, thermal-treated, and hydrolyzed extracts using two aluminum complexing assays reported by Pekal and Pyrzynska (2014). First assay consisted in the reaction of extracts with an aluminum chloride (AlCl₃) solution and the subsequent absorbance measurement at 425 nm. Results were reported as g of quercetin equivalents/kg (g QE/kg) of dry weight. Second assay consisted in the reaction of extracts, in the presence of sodium nitrite (NaNO₂), with an aluminum chloride solution in alkaline media and the subsequent absorbance measurement at 510 nm. Results were reported as g of catechin equivalents/kg (g CE/kg) of dry weight. Total flavonoids content (g/kg) was reported as the sum of both assays.

2.11. DPPH radical scavenging capacity

The DPPH radical scavenging capacity of raw, thermal-treated, and hydrolyzed extracts was determined according to the Cardozo et al. (2010) method. The absorbance was measured at 515 nm and the results were expressed as g of ascorbic acid equivalent/kg (g AAE/kg) of dry weight.

2.12. ABTS radical scavenging capacity

The ABTS radical scavenging capacity of raw, thermal-treated, and hydrolyzed extracts was determined according to the Pekal and Pyrzynska (2014) method. The absorbance was measured at 734 nm and the results were expressed as mM of trolox equivalent/kg (mM TE/kg) of dry weight.

2.13. Statistical analysis

All assays were performed in triplicate, and expressed as means ± standard deviation. Data were submitted to analysis of variance (ANOVA) and means were compared by Tukey test (p ≤ 0.05) using Statsoft Statistica 8.0. (2007). Correlation analysis (p ≤ 0.05) between absorbance at 290 nm and total phenolic compounds and free-radical scavenging capacity was developed using Statsoft Statistica 8.0. (2007).

3. Results and discussion

3.1. Proximate composition

The proximate composition and pods milling fractions are shown in Table 1. The composition in weight of dry mature pods of P. laevigata was mesocarp, endocarp and exocarp, with 440, 350 and 210 g/kg, respectively, seed in the endocarp represents 160 g/kg. One-stage milling process of the pods produced three fractions; MSF, EnHCB and ExSCB, representing, 353, 215, and 250 g/kg, respectively. Two-stage milling process of the pods produced five fractions; MF, SF, ExB, EnHCB and SCB, representing, 230, 100, 405, 205, and 60 g/kg, respectively. The highest flour yield was obtained by one-stage milling process with 535 g/kg, including the mesocarp rich in sugars and the seed rich in protein. Similar flour yield have been reported previously for P. alba and P. pallida (Felker et al., 2003). The protein MF content reported here for P. laevigata (105 g/kg), is slightly higher than previous reported values for P. alba (Felker et al., 2003), P. glandulosa (Harden & Zolfaghari, 1988) and P. pallida (Felker et al., 2003) with 80, 70, and 100 g/kg, respectively. The protein content reported here for SF of P. laevigata (310 g/kg) is higher than previously reported values for P. africana (Igwe et al., 2012) and similar to P. alba (Sciammaro et al., 2016), P. juliflora (Marangoni & Alli, 1988) and P. glandulosa (Harden & Zolfaghari, 1988). Similar whole pod flours (mesocarp-SF) protein content have been reported for P. chilensis (Astudillo et al., 2000), P. glandulosa (Harden & Zolfaghari, 1988), and P. tamarugo (Astudillo et al., 2000). Using one- or two-stage milling process is possible to obtain

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Table 1. Prosopis laevigata pods milling fractions and proximate composition (g/kg).

| Code | One stage | Two stages | Moisture | Ash | Protein | Fat | Crude fiber |
|------|-----------|------------|----------|-----|---------|-----|------------|
| MF   | 230       |            | 83.3 ± 2.1⁶ | 50.8 ± 1.0⁶ | 105.8 ± 0.5⁶ | 25.0 ± 0.1⁶ | 22.0 ± 0.3⁶ |
| SF   | 100       |            | 65.0 ± 4.1⁶ | 42.0 ± 1.3⁶ | 309.5 ± 3.1⁶ | 40.3 ± 1.5⁶ | 83.5 ± 3.9⁶ |
| MSF  | 535       |            | 77.1 ± 0.7³ | 40.7 ± 0.9⁶ | 119.3 ± 0.5³ | 18.1 ± 0.3³ | 42.0 ± 1.3³ |
| ExB  | 405       |            | 75.0 ± 3.2³ | 41.0 ± 1.3³ | 65.7 ± 3.7³ | 55.5 ± 0.9³ | 254.2 ± 6.9³ |
| EnHCB| 215       | 205        | 67.4 ± 2.6³ | 27.2 ± 1.0³ | 29.7 ± 1.1³ | 15.7 ± 1.8³ | 648.6 ± 8.6³ |
| SCB  | 60        |            | 60.9 ± 1.2³ | 29.5 ± 1.8³ | 5.8 ± 1.4³ | 18.0 ± 1.2³ | 190.8 ± 6.4³ |
| ExSCB| 250       |            | 67.7 ± 1.5³ | 35.1 ± 1.3³ | 60.8 ± 3.0³ | 39.3 ± 6.7³ | 249.7 ± 7.5³ |

MF = mesocarp flour; MSF = mesocarp-seed flour; SF = seed flour; ExB = exocarp bran; EnHCB = endocarp hard coat bran; SCB = seed coat bran; ExSCB = exocarp seed coat bran. *Values were expressed as means ± standard deviation (n = 3). Means accompanied by the same letter in the same column indicate no significant difference between samples (p < 0.05). MF = harina de mesocarpo; MSF = harina de semilla y mesocarpo; SF = harina de semilla; ExB = fibra de exocarpo; EnHCB = fibra de corteza dura del endocarpo; SCB = fibra de corteza de semilla; ExSCB = fibra de corteza de semilla y exocarpo.

*Valores expresados como la media ± la desviación estándar (n = 3). Medias con la misma letra en la misma columna, indica que no existe diferencia significativa entre muestras (p < 0.05).
flour fractions rich in protein (seed), rich in carbohydrates (mesocarp) or a mix of both (mesocarp-seed).

3.2. Free sugars content

The contents of free sugars in raw flours are shown in Table 2. Sucrose and three monosaccharides (glucose, fructose and xylose) were found in MF, MSF, and SF. Sucrose was the main sugar followed by glucose, fructose and xylose. Total sugars in MF, MSF, and SF were 447.81, 304.97 and 95.99 g/kg, respectively. No previous reports were found for sugars content in *P. laevigata*. Felker et al. (2013) found sucrose (382 g/kg) as the main sugar, followed by fructose (96 g/kg), glucose (g/kg), and xylose (2 g/kg) in mesocarp of *P. alba*. Particular importance have monosaccharides in foods as substrates for Maillard reactions and fermentation (Yu et al., 2012).

3.3. Amino-acid profile

The essential amino-acid contents in raw mesocarp, seed and mesocarp-SF are shown in Table 3. Considering the FAO recommended amino-acid scoring patterns for humans older than 3 years (FAO, 2013), limiting amino acids were Ile in MF, Ile, Trp, and Val in SF and mesocarp-SF. Particularly, high values of Lys and sulfur-containing amino acids (Met+Cys) were found in the three raw flours. Previously, Felker and Bandurski (1977) reported Val, Thr, Ile, Lys, Trp, and sulfur-containing amino acids as the limiting amino acids in seeds of *P. chilensis* and *P. juliflora*, meanwhile, whole pods of *P. juliflora* was limited only in sulfur-containing amino acids, suggesting that whole pod has a better amino-acid profile than seed. Marangoni and Alli (1988) and Felker et al. (2013) reported sulfur-containing amino acids as the limiting amino acids in pods of *Prosopis* species, without differenting seed from mesocarp. Thr was the limiting amino acid in seed of *P. Africana* (Igwe et al., 2012). Barba de la Rosa et al. (2006) using a single method for amino-acid analysis, reported a good balance of essential amino acids in *P. laevigata* whole pod flour, with limited content of sulfur-containing amino acids and particularly high values of Trp, an amino-acid profile that contrast with the results presented here for the same species, which could be explained by differences in the used methodology. Particular methodology must be used for amino acids that are unstable during acid hydrolysis (Met, Cys, and Trp) (Li et al., 2012; Nielsen et al., 1985).

3.4. Ultraviolet analysis of Maillard reaction products

The UV–Vis spectra of extracts of raw and thermal-treated flour of *P. laevigata* pods are shown in Figure 1. The absorbance ratios (thermal treated/raw) at 290 nm for seed (0.2/0.13), mesocarp (0.35/0.09), and mesocarp-seed (0.65/0.11) extracts were 1.53, 3.88 and 5.9, respectively (Table 4). The increment in absorbance at 290 nm after thermal treatment was highest in mesocarp-SF, followed by mesocarp and seed. The Maillard reaction between reducing sugars and amines (amino acids, peptides or proteins) during thermal treatments

| Table 2. Contents of free sugars in *Prosopis laevigata* pods flours (g/kg). |
|---------------------------------|----------------|----------------|----------------|----------------|
| Sample | Sucrose | Glucose | Fructose | Xylose |
| MF | 308.6 ± 4.7 | 77.6 ± 0.7 | 47.9 ± 1.1 | 13.5 ± 0.5 |
| MSF | 256.1 ± 4.2 | 26.8 ± 0.3 | 14.3 ± 0.6 | 7.6 ± 0.2 |
| SF | 69.7 ± 0.6 | 13.4 ± 0.6 | 10.8 ± 1.1 | 1.9 ± 0.0 |
| MF | mesocarp flour | MSF | mesocarp-seed flour | SF | seed flour |

**MF = mesocarp flour; MSF = mesocarp-seed flour; SF = seed flour.** Values were expressed as means ± standard deviation (*n* = 3). Means accompanied by the same letter in the same column indicate no significant difference between samples (*p* < 0.05).

| Table 3. Essential amino-acid content in *Prosopis laevigata* pods flours (g/kg of protein). |
|---------------------------------|----------------|----------------|----------------|----------------|
| Amino acid | MF | SF | MSF | FAO* |
| His | 25.7 ± 0.2 | 24.7 ± 0.2 | 29.5 ± 0.2 | 16 |
| Ile | 28.2 ± 0.6 | 28.5 ± 0.1 | 26.6 ± 0.1 | 30 |
| Leu | 75.5 ± 0.2 | 68.9 ± 0.4 | 70.2 ± 0.2 | 61 |
| Lys | 54.0 ± 0.1 | 55.1 ± 0.3 | 52.5 ± 0.1 | 48 |
| Met+Cys | 26.2 ± 0.1 | 34.8 ± 0.3 | 29.7 ± 0.2 | 23 |
| Phe+Tyr | 61.2 ± 0.6 | 58.4 ± 1.1 | 60.8 ± 0.5 | 41 |
| Thr | 34.6 ± 0.5 | 30.2 ± 0.4 | 30.6 ± 0.1 | 25 |
| Trp | 8.7 ± 0.1 | 6.5 ± 0.3 | 6.4 ± 0.1 | 6.6 |
| Val | 40.7 ± 0.4 | 34.1 ± 0.2 | 36.3 ± 0.1 | 40 |

**MF = mesocarp flour; MSF = mesocarp-seed flour; SF = seed flour.** *FAO (2013) recommended amino-acid scoring patterns for humans older than 3 years. Values were expressed as means ± standard deviation (*n* = 3). Means accompanied by the same letter in the same line indicate no significant difference between samples (*p* < 0.05).

**MF = harina de mesocarp; MSF = harina de semilla y mesocarp; SF = harina de semilla.** *FAO (2013) patrón de aminoácidos recomendado para humanos mayores de 3 años. Valores expresados como la media ± la desviación estándar (*n* = 3). Medias con la misma letra en la misma línea, indica que no existe diferencia significativa entre muestras (*p* < 0.02).

**Figure 1. UV–Vis spectra of extract of thermal treated and non-thermal treated samples.** a) mesocarp flour (MF), b) mesocarp-seed flour (MSF), c) seed flour (SF), d) seed flour thermal treated (SFT), e) mesocarp flour thermal treated (MFT), f) mesocarp-seed flour thermal treated (MSFT).

**Figura 1. Espectro UV–Vis de extractos de muestras térmicamente tratadas y no térmicamente tratadas.** a) harina de mesocarp (MF), b) harina de mesocarp y semilla (MSF), c) harina de semilla (SF), d) harina de semilla térmicamente tratada (SFT), e) harina de mesocarp térmicamente tratada (MFT), f) harina de mesocarp y semilla térmicamente tratada (MSFT).
Table 4. Absorbance at 290 nm of raw and thermal-treated Prosopis laevigata pods flours and its correlation with phenolic compounds content and DPPH radical scavenging capacity.

| Sample | Abs 290 nm | Total Phenolics (g GAE/kg) | DPPH (g AAE/kg) |
|--------|------------|----------------------------|-----------------|
| MF     | 0.09 ± 0.01 | 8.87 ± 0.1² | 10.6 ± 0.3³ |
| MFT    | 0.35 ± 0.02 | 12.4 ± 0.1³ | 14.7 ± 0.1² |
| MSF    | 0.31 ± 0.01 | 8.36 ± 0.1³ | 10.1 ± 0.1² |
| MSFT   | 0.65 ± 0.02 | 13.2 ± 0.1³ | 17.9 ± 0.3³ |
| SF     | 0.13 ± 0.01 | 6.59 ± 0.1³ | 8.98 ± 0.1³ |
| SFT    | 0.2 ± 0.02  | 7.74 ± 0.2³ | 10.7 ± 0.3³ |

Correlation (r) with Abs 290 nm

MF = mesocarp flour; MFT = mesocarp flour thermal treated; MSF = mesocarp seed flour; MSFT = mesocarp-seed flour thermal treated; SF = seed flour; SFT = seed flour thermal treated. Values were expressed as means ± standard deviation (n = 3). Means accompanied by the same letter in the same column indicate no significant difference between samples (p < 0.05).

MF = harina de mesocarpo; MFT = harina de mesocarpo térmicamente tratada; MSF = harina de mesocarpo y semilla; MSFT = harina de mesocarpo y semilla térmicamente tratada; SF = harina de semilla; SFT = harina de semilla térmicamente tratada. Valores expresados como la media ± la desviación estándar (n = 3). Medias con la misma letra en la misma columna, indica que no existe diferencia significativa entre muestras (p < 0.05).

3.5. Total phenolic compounds content

Total phenolic compounds contents in raw, thermal-treated, and hydrolyzed samples are shown in Table 5. Total phenolic compounds contents in raw flours were 8.87, 6.57, and 8.36 g GAE/kg for MF, SF, and MSF, respectively. These values are higher than previous reported values of free phenolic compounds for pods of P. nigra (3.3–6.6 g GAE/kg) and P. alba (2.2–4.6 g GAE/kg) (Perez et al., 2014). Thermal treatment of MF, SF, and MSF increases significantly (40%, 17%, and 58%, respectively) the total phenolic compound content when compared with their respective raw flours. This increased values could be attributed to the generation of s, which can be evidenced by the increased absorbance between 270 and 290 nm in thermal-treated samples (Figure 1). Total phenolic compounds content showed high linear correlation (r = 0.76) with absorbance at 290 nm (Table 4), suggesting the reduction of Folin–Ciocalteu reagent by MRP generated during thermal treatment. Folin–Ciocalteu assay is not specific for phenolic compounds and other compounds like MRP can also reduce the Folin–Ciocalteu reagent, a phenomena previously described in carob pods flours thermal-treated (Sahin et al., 2009). Major increases in UV absorbance were found in thermal-treated flours with high levels of monosaccharides (Table 2), a substrate for Maillard reaction. Previous works reported the total phenolic compounds content in flours of Prosopis species using the Folin–Ciocalteu reagent method (Bernardi et al., 2010; Briones-Labarca et al., 2011; Cardozo et al., 2010; Cattaneo et al., 2016; Gallegos-Infante et al., 2013; Perez et al., 2014; Schmeda-Hirschmann et al., 2015; Sciammaro et al., 2016) with variability in the values, which can be due to different samples, species and sample treatment. Sample treatment during drying could enhance Maillard reaction activity, indirectly modifying the total phenolic compound content of Prosopis flours. Acid hydrolysis of extracts of MF, SF, and MSF decreases significantly (11%, 60% and 13%, respectively) the total phenolic compound content when compared with their respective raw flours. During acid hydrolysis free phenolic acids and flavonoids can be partially degraded, while flavonoids as quercetin and kaempferol are considerate acid resistant (Nuutila et al., 2002). Total phenolic compounds content in brans ranged from 3.46 to 7.50 g GAE/kg in raw samples (ExB, EnHCB, SCB, and ExSCB) and from 0.69 to 5.71 g GAE/kg in hydrolyzed samples (ExBH, EnHCBH, SCBH, and ExSCBH). No previous reports were found for total phenolic compounds content in Prosopis brans. Total phenolic compounds contents of SBF and CBF, raw and hydrolyzed are shown in Table 5. For both legume seeds, their values represents between 10% and 20% of the respective mesquite pods flours. Regarding the phenolic compounds content, mesquite pods are more dense foods than soybean and common bean.
3.6. Total flavonoids content

The contents of total flavonoids in raw, thermal-treated, and hydrolyzed extracts are shown in Table 5. Thermal treatment of MF, SF, and MSF flour had a slight effect on total flavonoids contents when compared with their respective raw flours. Acid hydrolysis of extracts of MF, SF, and MSF increases significantly (300%, 32%, and 180%, respectively) the total flavonoids contents when compared with their respective raw flours. Food flavonoids exist mainly in the form of glycosides which are less able to form complex with aluminum. Acid hydrolysis of glycosides produce free aglycones with higher capacity to interact with aluminum, increasing the sensibility of the assays (Denni & Mammen, 2012; Pekal & Pyrzyńska, 2014). The assays conducted in neutral media without NaNO$_2$ must be used only to determine the content of flavonolives and the assay in the presence of NaNO$_2$ in alkaline medium must be used for rutin, catechins, and some phenolic acids, being less specific than the first assay (Pekal & Pyrzyńska, 2014). Together both assays represent a better and complemented measurement of the total flavonoids content in food samples. Major increases after hydrolysis were found in neutral media, suggesting the presence of flavonoid glycosides of the flavonol sub-group. Previous studies reported the presence of glycosides of two flavonoids, apigenin, and quer cetin in mesocarp and seed of $P$. alba (Cattaneo et al., 2016; Perez et al., 2014), $P$. nigra (Perez et al., 2014), and $P$. chilensis (Schmeda-Hirschmann et al., 2015). Total flavonoids content in brans ranged from 1.41 to 3.16 g/kg in raw samples (ExB, EnHCB, SBF, and ExSCB) and from 0.13 to 3.68 g/kg in hydrolyzed samples (ExBH, EnHCBH, SCB and ExSCBH). No previous reports were found for total flavonoids content in Prosopis brans. Total flavonoids content of SBF and CBF, raw, and hydrolyzed are shown in Table 5. For both legume seeds, their values represents between 5% and 15% of the respective mesquite pods flours. Regarding the total flavonoids content, mesquite pods are more dense foods than soybean and common bean.

3.7. Radical scavenging capacity

The radical scavenging capacities of raw, thermal-treated, and hydrolyzed extracts are shown in Table 5. Thermal treatment of MF, SF, and MSF increases significantly (35%, 15%,
and 80%, respectively) the free-radical scavenging capacity when compared with their respective raw flours. These increased values could be attributed to the Maillard reactions involving reducing sugars and amino acids, leading to the formation of a variety of byproducts, intermediates, and brown pigments, which can be evidenced by the increased absorbance between 270 and 290 nm in thermal-treated samples (Figure 1). Mayor increases in absorbance were found in flours samples thermal-treated rich in monosaccharides (Table 2), a necessary substrate for Maillard reaction. Free-radical scavenging capacity (DPPH) showed high linear correlation ($r = 0.93$) with absorbance at 290 nm (Table 4), suggesting the participation of MRP generated during thermal treatment on free-radical scavenging capacity. MRPs have been described as compounds with radical scavenging capacity and its production during thermal treatment can increase the radical scavenging capacity in flours of carob pods (Sahin et al., 2009) and synthetic media (Mondaca-Navarro et al., 2017; Vhangani & Van-Wyk, 2016; Yu et al., 2012). Major food antioxidants are phenolic compounds, but ascorbic acid, MRP and other compounds can reduce DPPH and ABTS radicals (Mondaca-Navarro et al., 2017; Vhangani & Van-Wyk, 2016; Yu et al., 2012). Thermal treatment of Prosopis pods flour have been related to brown color development, due to Maillard reaction (Felker et al., 2013). Acid hydrolysis of extracts of MF had a slightly positive effect in free-radical scavenging capacity, while in SF and MSF acid hydrolysis decreases significantly (40% and 55%, respectively). The reduction of free-radical scavenging capacity in hydrolyzed extracts could be attributed mainly to phenolic acids destruction. In hydrolyzed extracts of thermal-treated seed and MSF radical scavenging capacity increases significantly, an effect attributed to MRP generated during treatment. Hydrolysis of brans extracts decreases the radical scavenging capacity. No previous reports were found for radical scavenging capacity in Prosopis brans. The radical scavenging capacities of SBF and CBF, raw, and hydrolyzed are shown in Table 5. For both legume seeds, their values represents between 20% and 35% of the respective mesquite pod flours. Regarding the radical scavenging capacity, mesquite pods are more dense foods than soybean and common bean.

4. Conclusions

One- or two-stage milling process of P. laevigata pods can produce flours and brans able to be used as ingredient in human food products. These flours are compositionally similar to other Prosopis species. Brans can be considered as a valuable fiber-rich raw material. Raw flours are a good source of Lys and sulfur-containing amino acids. Raw flours and brans are a good source of active phenolic compounds with higher radical scavenging capacity than soybean and common bean. Thermal treatment of P. laevigata flours increases the apparent total phenolic compounds content and radical scavenging capacity, an effect associated with the generation of MRPs. Prosopis laevigata pods flours are very prone to Maillard reaction development, due to the presence of free sugars. Particular importance have MRP due to their recent described properties on health. The identification and quantification of specific antioxidant compounds produced during thermal treatment of flours would be further conducted. Total flavonoid content can be better defined using both aluminum complexation assays after acid hydrolysis, but given the important role in human health of these compounds, analytical definition must be further conducted for specific flavonoids identification. The results presented here highlight the alimentary traits of a currently underutilized biological resource.

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