Vol. 18, No. 1 (2019) 303-312
Revista Mexicana de Ingeniería Química

EFFECT OF EXTRACTION METHOD IN THE CONTENT OF PHYTOESTROGENS AND MAIN PHENOLICS IN MESQUITE POD EXTRACTS (*Prosopis* sp.)

EFECTO DEL MÉTODO DE EXTRACCIÓN EN EL CONTENIDO DE FITOESTRÓGENOS Y PRINCIPALES FENÓLICOS EN LOS EXTRACTOS DE LA VAINA DE MESQUITE (*Prosopis* sp.)

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Received: February 13, 2018. Accepted: June 7, 2018.

Abstract

Different methodologies were tested to extract phytoestrogens and the main phenolic compounds from mesquite pods (*Prosopis* sp.) collected at Tequisquiapan, Queretaro, Mexico. This fruit is used for human consumption as well as feedstuff for ovines, caprines, and other farm animals. Other researchers previously observed changes in hormonal profiles on rats fed with mesquite pod extracts, affecting their reproductive cycle and behavior; those changes were attributed to phytoestrogens such as genistein and daidzein reported in soybeans, possibly present in mesquite due that this plant is also a legume. Given the agronomic and commercial importance of mesquite in this region and other semiarid zones of Mexico, this work was focused on optimization of the extraction conditions for these compounds. The results shown the presence of vanillic acid, vanillin, and that other phenols such as ferulic and caffeic acids which were the most abundant compounds extracted with the studied extraction methods. In addition, the results showed that only genistein was present in mesquite extracts at very low concentrations compared to soy extracts.

Keywords: *Prosopis*, mesquite pods, phytoestrogens, phenols, isoflavones.

Resumen

Se probaron diferentes metodologías para la extracción de fitoestrógenos y principales compuestos fenólicos de las vainas de mezquite (*Prosopis* sp.), recolectada en el municipio de Tequisquiapan, Querétaro, México. Este fruto sirve para consumo humano y de animales como ovinos, caprinos y otros animales de granja. Otros investigadores observaron cambios en el perfil hormonal en ratas al incorporar los extractos de este fruto en la dieta, afectando su comportamiento y ciclos reproductivos. Dichos cambios se atribuyeron a los fitoestrógenos genisteína y daidzeína, que han sido reportados en soya y posiblemente se encuentren en mezquite siendo esta también una leguminosa. Dada la importancia agronómica y comercial del mezquite en esta región y otras zonas semiáridas de México, en este trabajo se establecieron las condiciones óptimas de extracción de estos compuestos. Los resultados mostraron la presencia de ácido vaníllico, vanillina y otros ácidos fenólicos como el ácido ferúlico y el cafeíco que fueron los compuestos mayoritarios en los sistemas de extracción probados. Además, los resultados mostraron que solo estaba presente la genisteína en los extractos de mezquite en muy bajas concentraciones comparado con los extractos de soya.

Palabras clave: *Prosopis*, vaina de mezquite, fitoestrógenos, fenoles, isoflavonas.

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doi: https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n1/Gonzalez ; *issn-e*: 2395-8472

1 Introduction

Mesquite is a legume of genus *Prosopis* native of Central Mexico. Mesquite comes from Nahuatl (the language spoken by the Aztecs) "*mizquitl*" (Bouttier-Figueroa *et al*., 2017), it is abundant in Central and Northern Mexico as well as in Baja California Peninsula (Sauceda *et al*., 2014). It also grows in Texas, California, Utah and Kansas, as well as in Argentina, Chile, Brazil and Bolivia. In these areas, the fruit is used as raw material for flour (Pérez *et al*., 2014; Sauceda *et al*., 2014), gums (Vernon-Carter *et al*., 2000) and honey (Almaraz-Abarca *et al*., 2007). The wood and resin are also utilized, being an important resource in tree-producing areas (Sauceda *et al*., 2014). Mesquite pods are used as feedstuff mainly for sheep and goats as it is a low-cost resource growing in the wild (Andrade-Montemayor *et al*., 2011). Nonetheless, previous studies reported estrogenic activity in mesquite pods extracts, promoting changes in reproductive cycles of rats and abundant epithelial growth, among other effects (Retana-Márquez *et al*., 2012; Retana-Márquez *et al*., 2016); according to these reports, changes were attributed by the presence of phytoestrogens in pod extracts, although there are no reports on the specific chemical family of these compounds.

Phytoestrogens are plant compounds that, due to structural similarity, have similar effects to several mammal hormones (Adeoya-Osiguwa *et al*., 2003; Meza *et al*., 2015). The most abundant phytoestrogens in nature are genistein, daidzein, formonentine and cumestrol. These chemicals can play an important role in mammal reproduction (Lambert & Edwards, 2017; Vernon-Carter *et al*., 2000). The most widely used leguminous as source of hormone replacement is soybean, due to its high isoflavone content, mainly daidzein and genistein (Adjakly *et al*., 2013).

As most phenolic compounds in plants, phytoestrogens are bioactive. Among other properties, they may act as antioxidants, depending on the position and number of substituents in the molecule capable to neutralize free radicals. Phenolic compound derived from hydroxycinnamic acids contain one CH=CH-COOH group, whereas those derived from hydroxybenzoic acids contain one -COOH group (Pandey & Rizvi, 2009). The antioxidant activity of hydroxycinnamic acid derivatives is higher than the activity of hydroxybenzoic acid derivatives. Even though this activity also depends on the concentration in the plant extract. Plants synthetize phytoestrogens as secondary metabolites by the pentose-phosphate, shikimate and phenylpropanoid pathways, induced by stress conditions. In plants, this synthesis is a defense mechanism against insects, microorganisms and other stressors. These compounds contain, as part of their chemical structure, at least one aromatic ring. Isoflavones, like other flavonoids, contain three aromatic rings (Figure 1) (Balasundram *et al*., 2006).

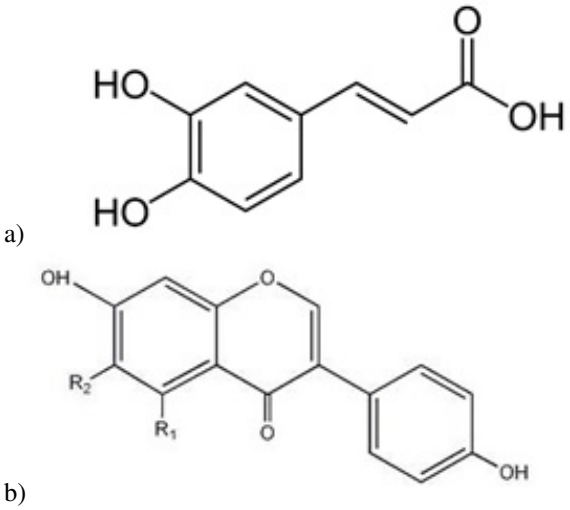


Fig. 1. Chemical structures: a) phenolic acid molecule (caffeic acid, base structure C6-C3); b) base molecule of isoflavone (C6-C3-C6).

Due to their wide diversity, identification of these compounds is a complicated task. However, by techniques including mass spectrometry it is possible to identified molecular ions of interest. The objective of the present study was to identify the most abundant phytoestrogens and other phenolic compounds in mesquite pods, and to analyze their antioxidant capacity and chemical structure. Soybean was used as a reference, due that this material has been extensively studied.

2 Materials and methods

2.1 Raw material and conditioning

Mesquite pods (*Prosopis* sp.) were collected at Tequisquiapan, Queretaro, Mexico (20°31'14"N; 99°53'45"O; 1880 MASL; 18°C average temperature; 514 mm average rainfall), during Summer (July 15 to August 15). The climate corresponds to BSh warm

semi-arid, according to Köppen-Geiger classification. All studies were also followed in soybean (*Glycine max*) as a reference, purchased to a wholesaler in Mexico City. Mesquite pods and soybean were dried at room temperature (approximately 18ºC), ground in a commercial grinder (Sanitary, Mexico City) through a 2-mm mesh to obtain a fine powder, and stored at room temperature in closed containers until used.

2.2 Bromatological analysis

Chemical composition of samples was analyzed according to the following Mexican Official Analytical Methods: fat (NMX-F-089-S-1978), nitrogen (NMX-F-068-S-1980), fiber (NMX-F-090-S-1978), water content (NMX-F-083-1986) and ashes (NMX-F-066-S-1978). Total carbohydrates were reported as difference to 100%.

2.3 Extraction of isoflavones and phenolic compounds

Two previously reported methods, with some modifications, were carried out (Peñalvo *et al*., 2004). For total isoflavone extraction (Extraction Method 1) 2 g sample was used, mixed with 2.5 mL 80% ethanol (EtOH80) and thoroughly stirred for 2 min in a vortex; samples were centrifuged at 5000 x g for 5 min at room temperature (Beckman centrifuge, model J2-M1, Palo Alto, CA). The supernatant was extracted and placed in a 10 mL-volumetric flask covered with aluminum foil to prevent light oxidation. Samples were extracted three times with ethanol; the extracts were mixed, and the total volume taken to 10 mL with EtOH80.

Aglycones were extracted by Extraction Method 2, using 2 g sample mixed with 2.5 mL acid ethanol (AcEtOH; EtOH80 in 1M HCl), incubated at 80°C in a water bath for 1 h, cooled down for 1 min and thoroughly stirred in a vortex for 2 min. Samples were then centrifuged at 5000 x g for 5 min; the supernatant was collected and placed in a 10 mL-volumetric flask, samples were extracted three times, the extracts were mixed and taken to the total volume with AcEtOH. Each extraction was carried out in triplicate. The extracts were placed in amber flasks and injected with nitrogen to remove oxygen from the headspace. All samples were stored at 4oC until analysis.

2.4 Phenolic compounds analysis by Folin-Ciocalteu assay

The concentration of phenolic compounds was analyzed by the Folin-Ciocalteu colorimetric method, previously described (Singleton *et al*., 1999). It is based on phenol reaction with oxidants, forming bright blue phosphomolybdenum and phosphotungsten complexes. Electron transference at basic pH reduces these complexes to oxides; color formation is proportional to the number of hydroxyl groups in the molecule. 100 μL sample was mixed with 750 μL diluted Folin-Ciocalteu reagent (1:10), left standing for 5 min and mixed with 750 μL sodium bicarbonate. The reaction proceeded for 90 min at room temperature in the dark. Absorbance was then read at 725 nm (Beckman spectrophotometer, model DU 650, Palo Alto, CA). Readings were interpolated in a standard curve of gallic acid equivalents to obtain mg GAE/g (dry weight).

2.5 Antioxidant activity analysis by $ABTS^+$ radical assay

It was carried out according to the previously described assay (Charurin *et al*., 2002). The working solution was prepared (absorbance = 0.7±0.2 at 734 nm) from a 2.45 mM $ABTS^+$ stock solution [2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid]. 10 μL sample was mixed with and 990 μL radical solution. The absorbance was read every minute during 5 min total reaction time. Inhibition percentage was obtained from absorbance difference (Equation 1) in a Trolox standard curve. Results were obtained as μM Trolox / g (dry weight).

$$\Delta A_{sample} = \frac{(A_{t0(sample)} - A_{t5(sample)})}{A_{t0(sample)} - \frac{A_{t0(solvent)} - A_{t5(solvent)}}{A_{t0(solvent)}}} \quad (1)$$

2.6 Identification and relative quantification of phenolic compounds by HPLC-MS

Phenolic compound identification was carried out by HPLC-MS analysis using an Ultimate 3000 equipment (Dionexcorp, CA) fitted with a photodiode arrangement and a micrOTOF Q-II analyzer in electrospray ionization system (ESI) mode (BrukerDaltonics, Billerica, MA). External calibration was carried out before analysis, using a 74900-00-05 Cole Palmer pump (Billerica, MA), directly connected to the interphase with sodium formate solution. Analysis were carried out using formic acid 0.01% (A)

and acetonitrile (B) phases, programmed as follows: 0 to 10 min, 10-60%; 10 to 15 min, 60-90%. Other conditions of analysis were: 0.3 mL/min flow rate, 35 °C. The chromatograms were obtained at 280 nm. MS analysis was carried out using nitrogen as drying gas, 4 L/min, 180 °C, 0.4 bar negative mode (-ESI), from 50 m/z to 3000 m/z, 2700 V. Data were analyzed by Data Analysis software 4.0 (Bruker Daltonics, Billerica, MA) to obtain total detected compounds and relative concentrations.

2.7 Statistical analysis

Data were subjected to one-way analysis of variance and reported as means and standard deviations at $\alpha = 0.01$, using Excel 2013 Microsoft Data Analysis. All analysis was carried out in triplicate.

3 Results and discussion

3.1 Bromatological composition

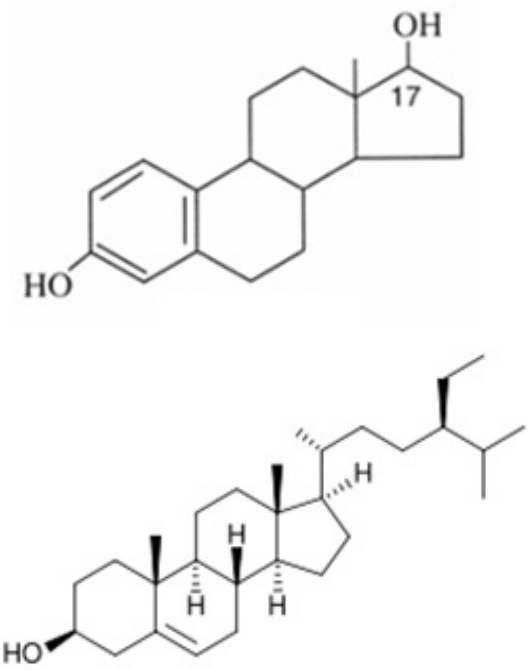
Soybean chemical composition obtained in this study was in agreement to data reported by other authors (Karr-Lilienthal *et al*., 2006). A higher content of carbohydrate, fiber, protein and fat (Table 1) were observed in mesquite.

Other researchers indicated 52.08±0.09 and 46.37±0.08 % (dry weight) carbohydrate content in *Prosopis alba* and *Prosopis nigra* pods, respectively (Cardozo *et al*., 2010). Although carbohydrate concentration in our studied mesquite samples was lower (39.32±2.82 %), there was a non-significant different with respect to *P. nigra* or *P. alba* ($p > 0.01$). According to other authors, soluble carbohydrates, easy to digest, are present in *Prosopis pallida* L., mainly glucose, fructose, galactose and arabinose (Bravo *et al*., 1994), whereas Bouttier-Figueroa *et al*., (2017) reported high galactomannan concentration in mesquite seed extracts, 58.46 % was mannose and 28.5 % galactose.

Mesquite pod fiber content (13.36±0.49 %) was even higher than in soybean (3.93±0.13 %) and other plants for animal and human consumption, such as quintonil (*Amaranthus hybridus*) containing 8.61% fiber (Baeza-Jiménez *et al*., 2017). Andrade-Montemayor *et al*. (2009) reported several anti-nutritional factors in fiber, although heat treatments may inhibit these compounds.

Table 1. Bromatological analysis of soy bean (*Glycine max*) and mesquite pod (*Prosopis* sp.).

Component	Soy (%)	Mesquite (%)
Moisture	7.13±0.07	5.73±0.58
Ashes	4.32±0.11	2.79±0.06
Ether extract	15.49±0.73	23.95±1.97
Protein	44.95±0.81	14.83±0.15
Raw fiber	3.93±0.13	13.36±0.49
Carbohydrates	24.17±0.01	39.32±2.82



a)

b)

Fig. 2. Chemical structures. a) 17 β-estradiol, b) β-sitosterol.

For instance, roasting increases pod digestibility and promotes tannin formation, a highly antioxidant phenol (Hagerman *et al*., 1998). In general, heating induces the formation of simple phenolic dimer and trimers. When these compounds polymerize their antioxidant capacity considerably increases (Arrieta-Baez *et al*., 2012). Tannins formation may be undesirable in foods, due to their astringent and bitter flavor. However, tannins are present in several fruits, vegetables and products such as wine (Vidal *et al*., 2004), making a positive contribution to flavor if present in small quantities. Therefore, roasting can be applied as pre-treatment to mesquite pods before consumption, due to the inhibition of anti-nutritional compounds and development of a desirable flavor.

Table 2. Phenolic compound concentration and antioxidant activity of soy (*Glycine max*) and mesquite (*Prosopis* sp.) extracts.

Phenolic compound concentration (mg GAE g^{-1})	Method 1	Method 2
Soy	0.253±0.017 a	0.230±0.004 a
Mesquite	0.494±0.038 a	0.375±0.011 a
Antioxidant activity (μM Trolox g^{-1})		
Soy	503.19±40.55 b	433.51±62.19 b
Mesquite	771.82±68.41 b	888.58±174.55 b

a no significant difference between methods (P≤0.01)
b significant difference between methods (P≤0.01)

According to our results obtained with ether extracts, mesquite pods contain 1.5 times more fatty acid concentration than soybean (ether extract = 15.49+0.73 and 23.95+1.97% for soybean and mesquite pods, respectively). The presence of linoleic and oleic acids, and β-sitosterol has been reported in considerable amounts in seeds of different *Prosopis* varieties (Lamarque *et al*., 1994). These phytosterols are generally esterified or conjugated with phenolic acids. Among other biological functions, they reduce cholesterol concentration (Andreu Palou *et al*., 2005). Due to the structural similarity with steroid hormones (Figure 2) these compounds can also be responsible of hormonal changes in rats (Retana-Márquez *et al*., 2016). Although isoflavones were present in very small concentrations in the studied mesquite pods, it is necessary to confirm a possible synergistic effect between isoflavones and several sterols present in these legumins.

3.2 Phytochemical composition

Phenolic compound concentration in soybean and mesquite pods was 0.253±0.017 and 0.494±0.038 mg GAE/g (dry weight), respectively, as analyzed by Method 1. As the extraction system included ethanol, we concluded that these figures were mainly free phenolic acids, as well as non-conjugated or intertwined flavonoids. Results obtained by Method 2 gave 0.230±0.004 and 0.375±0.011 mg GAE/g (dry weight) for soybean and mesquite pods, respectively. There was a non-significant difference ($p > 0.01$) between the two extraction methods for estimating total phenolic compounds concentration. Other authors reported methods for phenol extraction in food residues that could be applicable for mesquite. According to their results, the optimal extraction condition for phenols was 60/30/10 water-acetone-ethanol at 35ºC for 2 h (Felix *et al*., 2017). However, extraction with EtOH80 and acidified EtOH was focused on some specific phenols and to extract aglycones. This approach was used to study the effect of soy milk addition to smoothies, where Morales-de la Peña *et al*. (2017) pointed out that ultrasound treatments increased isoflavone content and aglycone formation.

The antioxidant activity was significantly different between extraction methods ($p < 0.01$) (Table 2), due to specifically extracted compounds. When samples were acidified and incubated (Method 2), the glycosylated moiety attached to phenols was removed (Peñalvo *et al*., 2004).

However, the antioxidant capacity of mesquite was higher than soy in both methods. Results showed that the antioxidant activity of mesquite pods, extracted by Method 1, is 1.5 times higher than soybean, and twice by Method 2. Compounds extracted from mesquite pods contain more active structures for neutralizing free radicals than those extracted from soybean. Therefore, the next step was to identify the extracted compounds.

As discussed later, HPLC-MS analysis showed the presence of caffeic and ferulic acids in mesquite pods. These compounds have several pharmaceutical applications. A study in India with *Prosopis cineraria*, demonstrated that the identified compounds are anti-inflammatory and antioxidants, among other healing properties (Liu *et al*., 2012).

Genistein was detected in mesquite pods, although in a very small concentration as compared to soybean (Figure 3) where also daidzein was present. Extraction Method 1 produced a higher concentration of genistein from soybean (7.41±0.44%) than Method 2 (3.16±1.01%) (Table 2).

Table 3. Relative concentration of phytoestrogens and some phenolic compounds detected in soy (*Glycine max*) and mesquite (*Prosopis* sp.) extracts.

Compound	ion m/z [M-H]-	Method 1		Method 2	
		Relative percentage (%)			
		soy	mesquite	soy	mesquite
Vainillin (4-Hydroxy-3-methoxybenzaldehyde)	151.1	nd	0.38±0.06	nd	0.87±0.48
Vanillic acid (4-Hydroxy-3-methoxybenzoic acid)	167.1	nd	0.51±0.1	nd	1.19±0.87
Genistein (4',5,7-Trihydroxyisoflavone)	269.0	7.41±0.44	0.36±0.02	3.16±1.01	0.11±0.8
Daidzein (4',7-Dihydroxyisoflavone)	253.0	5.28±0.16	nd	2.7±0.27	nd
Caffeic acid (3,4-dihydroxyphenyl) acrylic acid)	179.1	nd	0.37±0.1	nd	0.23±0.12
Ferulic acid (4-Hydroxy-3-methoxycinnamic acid)	193.1	nd	nd	nd	0.08±0.01

*nd- not detected

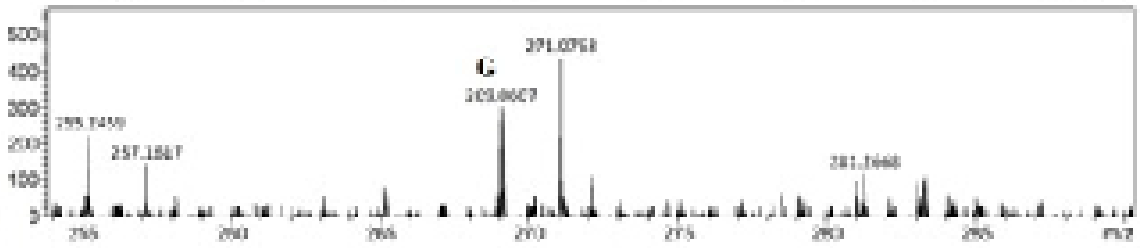


Fig. 3. Genistein (G) detection in mesquite extracts by HPLC-MS, ESI negative mode.

According to several authors, genistein is the most abundant phytoestrogen in soybean. These studies pointed out that this compound has anticancer effects in different cell types. A study in prostate cancer reports that genistein takes part in the epigenetic cell modulation and regulation cycles, inhibiting growth and promoting the apoptotic process (Adjakly *et al*., 2013). Even though, other authors pointed out that the effect of soybean extracts was more efficient on breast cancer tumors than direct genistein supply to rats through the diet (Kim *et al*., 2008). Genistein concentration in mesquite pods extracts was 0.36±0.8% and 0.11±0.8% using Methods 1 and 2, respectively. In both plant extracts, mesquite pods and soybean, there was a reduction in relative concentration by Method 2, involving incubation with acid ethanol at 80°C. At 70-90 °C, genistein tends to form glycosylated compounds with lysine via Maillard reaction, with further degradation. This process also affects bioactive properties, such an antioxidant activity.

Daidzein was also detected in soybean extracts, although in low concentrations (5.28±0.16% and 2.7±0.27% for Methods 1 and 2, respectively). A degradation process was observed for daidzein. However, it has been reported that this compound is stable to high temperatures (Ungar *et al*., 2003). Therefore, the degradation process may proceed through a different mechanism than for genistein, although it has not been fully elucidated. Daidzein was not detected in mesquite pods, although other aglycones (vanillic acid, vanillin, caffeic acid and ferulic acid) were extracted (Table 3).

Vanillin is a hydroxybenzoic acid derivative of high commercial value, widely used in food and other industries as flavoring. It is also a precursor of other compounds used in the pharmaceutical industry and as sanitizer due to its antimicrobial and fungicide properties (Walton *et al*., 2003). As natural vanillin mainly obtained from *Vanilla plan folia*, is an expensive crop (Walton *et al*., 2000), vanillin synthesis is preferred for industrial and commercial

production. In domesticated and wild vanilla tree varieties, vanillin is present in concentrations between 391 and 1117 ppm (Herrera-Cabrera *et al*., 2016). Among other biotechnological processes available for vanillin production, ferulic acid is enzymatically modified by *Pseudomonas* sp. (Walton *et al*., 2003). Vanillic acid provides aromatic properties in fruits and, as a major component on mesquite extracts, its presence could be a source of aroma in processed foods which include mesquite.

Two phenylpropanoids, caffeic and ferulic acids, were also detected in mesquite pods extracts, both compounds were reported as having antimicrobial properties (González-Quijano *et al*., 2014; Kim *et al*., 2017). Caffeic acid concentration in the extracts obtained by Methods 1 and 2 were 0.37±0.1% and 0.23±0.12%, respectively. This compound is normally detected as esterified chlorogenic acid, and can be recovered by enzymatic reactions of pericarp and other food residues, as indicated by Ramírez-Velasco *et al*. (2016). Other compound present on mesquite extracts was ferulic acid; it was extracted only by Method 2 (0.08±0.01%). It is a precursor of other compounds, such as vanillin, with several bioactive effects such as antioxidant, antibacterial, and antifungal, among others. Ferulic acid is present in most vegetables, fruits and cereals as a constituent of the cell wall (Walton *et al*., 2000). Generally, it is present in a free form, but also linked to arabinose and lignin (Šukalović *et al*., 2017).

Conclusion

Mesquite pods most abundant compounds are precursors of vanillin and simple phenolic acids with higher antioxidant activity than soybean. Conversely as reported by other authors, concentration of isoflavones was very low in mesquite. Therefore, it is not likely that the presence of these compounds is responsible of hormonal changes in laboratory animals. Mesquite contain only genistein in trace amounts as compared to soybean. Further studies on the presence of glycosylated phenolic compounds, isoflavone isomers or precursors or steroid-like compounds are necessary to find out possible effects of mesquite pods on reproductive activity of laboratory or farm animals, or its transfer to meat or meat products for human consumption.

Nomenclature

EtOH80	ethanol 80%
AcEtOH	acidified ethanol
GAE	galic acid equivalents
ΔA	absorbance difference
A_{t0}	initial absorbance
A_{t5}	five-minutes absorbance
ESI-	ionization by electrospray in negative mode

Acknowledgements

G.K. González-Quijano thanks the National Council of Science and Technology (CONACYT-Mexico) for a postdoctoral scholarship, and her present position in the Cátedras CONACYT-program. This work was part of the research project “Animal Substrates: biomolecules involved in their functionality" approved and supported by Universidad Autónoma Metropolitana-Iztapalapa. The authors also thank Centro de Nanociencias y Micro y Nanotecnologías (CNMN, National Polytechnic Institute) for the use of research facilities.

References

Adeoya-Osiguwa, S. A., Markoulaki, S., Pocock, V., Milligan, S. R., Fraser, L. R. (2003). 17β-Estradiol and environmental estrogens significantly affect mammalian sperm function. *Human Reproduction 18*, 100-107.

Adjakly, M., Ngollo, M., Boiteux, J.-P., Bignon, Y.-J., Guy, L., Bernard-Gallon, D. (2013). Genistein and daidzein: Different molecular effects on prostate cancer. *Anticancer Research 33*, 39-44.

Almaraz-Abarca, N., da Graça Campos, M., Ávila-Reyes, J. A., Naranjo-Jiménez, N., Herrera Corral, J., González-Valdez, L. S. (2007). Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (*Prosopis* juliflora, Leguminosae). *Journal of Food Composition and Analysis 20*, 119-124.

Andrade-Montemayor, H. M., Alegría-Ríos, F. S., Pacheco-López, M., Aguilar-Borjas, J. H., Villegas-Díaz, J. L. O., Basurto-Gutierrez, R., . . . Vera-Avila, H. R. (2009). Effect of dry roasting

on composition, digestibility and degradability of fiber fractions of mesquite pods (*Prosopis* laevigata) as feed supplement in goats. *Tropical and Subtropical Agroecosystems 11*, Special Issue - IGC, Queretaro, México.

Andrade-Montemayor, H. M., Cordova-Torres, A. V., García-Gasca, T., Kawas, J. R. (2011). Alternative foods for small ruminants in semiarid zones, the case of Mesquite (*Prosopis* laevigata spp.) and Nopal (*Opuntia* spp.). *Small Ruminant Research 98*, 83-92.

Andreu Palou, O., Segura, C. P., Bonet, M.L.P., Oliver, P.V., Serra, F.V., Rodríguez, A.M.G., Ribot, R.J. (2005). *El Libro Blanco de los Esteroles Vegetales* (2 ed.). España: Unilever Foods S.A.

Arrieta-Baez, D., Dorantes-Álvarez, L., Martinez-Torres, R., Zepeda-Vallejo, G., Jaramillo-Flores, M. E., Ortiz-Moreno, A., Aparicio-Ozores, G. (2012). Effect of thermal sterilization on ferulic, coumaric and cinnamic acids: dimerization and antioxidant activity. *Journal of the Science of Food and Agriculture 92*, 2715-2720.

Baeza Jiménez, R., López García, G., López Martínez, L. X., Dublán García, O. (2017). Extraction and characterization of the fatty acid profile of quintonil (*Amaranthus hybridus*). *Revista Mexicana de Ingeniería Química 16*, 835-844.

Balasundram, N., Sundram, K., Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry 99*, 191-203.

Bouttier-Figueroa, D. C., Quevedo-López, M. A., Rosas-Durazo, A., Sotelo-Lerma, M. (2017). Hydrothermal technique for isolation of galactomannan from seeds of sonoran mezquite (*Prosopis* spp.). *Revista Mexicana de Ingeniería Química 16*, 457-465.

Bravo, L., Grades, N., Saura-Calixto, F. (1994). Composition and potential uses of mesquite pods (*Prosopis* pallida L): Comparison with carob pods (*Ceratonia siliqua L*). *Journal of the Science of Food and Agriculture 65*, 303-306.

Cardozo, M. L., Ordoñez, R. M., Zampini, I. C., Cuello, A. S., Dibenedetto, G., Isla, M. I. (2010). Evaluation of antioxidant capacity, genotoxicity and polyphenol content of non conventional foods: *Prosopis* flour. *Food Research International 43*, 1505-1510.

Charurin, P., Ames, J. M., del Castillo, M. D. (2002). Antioxidant activity of coffee model systems. *Journal of Agricultural and Food Chemistry 50*, 3751-3756.

Felix, A. C. S., Alvarez, L. D. G., Santana, R. A., Valasques-Junior, G. L., Bezerra, M. d. A., de Oliveira-Neto, N. M., . . . Nascimento Junior, B. B. d. (2018). Application of experimental designs for evaluate the total phenolics content and antioxidant activity of cashew apple bagasse. *Revista Mexicana de Ingeniería Química 17*, 165-175.

González-Quijano, G. K., Dorantes-Alvarez, L., Hernández-Sánchez, H., Jaramillo-Flores, M. E., Perea-Flores, M. d. J., León, A. V. P. d., Hernández-Rodríguez, C. (2014). Halotolerance and survival kinetics of lactic acid bacteria isolated from Jalapeño pepper (*Capsicum annuum* L.) fermentation. *Journal of Food Science 79*, M1545-M1553.

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., Riechel, T. L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry 46*, 1887-1892.

Herrera-Cabrera, B. E., Hernández-Ruíz, J., Delgado-Alvarado, A. (2016). Variación de aroma en vanilla planifolia Jacks. ex Andrews silvestre y cultivada. *Agroproductividad 9*, 11-17.

Karr-Lilienthal, L. K., Bauer, L. L., Utterback, P. L., Zinn, K. E., Frazier, R. L., Parsons, C. M., Fahey, G. C. (2006). Chemical composition and nutritional quality of soybean meals prepared by extruder/expeller processing for use in poultry diets. *Journal of Agricultural and Food Chemistry 54*, 8108-8114.

Kim, H.-A., Jeong, K.-S., Kim, Y. K. (2008). Soy extract is more potent than genistein on tumor growth inhibition. *Anticancer Research 28*, 2837-2841.

Kim, J.-H., Yu, D., Eom, S.-H., Kim, S.-H., Oh, J., Jung, W.-K., Kim, Y.-M. (2017). Synergistic antibacterial effects of chitosan-caffeic acid conjugate against antibiotic-resistant acne-related bacteria. *Marine Drugs 15*, 167.

Lamarque, A. L., Maestri, D. M., Grosso, N. R., Zygadlo, J. A., Guzmán, C. A. (1994). Proximate composition and seed lipid components of some *Prosopis* (leguminosae) from argentina. *Journal of the Science of Food and Agriculture 66*, 323-326.

Lambert, M. R., Edwards, T. M. (2017). Hormonally active phytochemicals and vertebrate evolution. *Evolutionary Applications 10*, 419-432.

Liu, Y., Singh, D., Nair, M. G. (2012). Pods of Khejri (*Prosopis* cineraria) consumed as a vegetable showed functional food properties. *Journal of Functional Foods 4*, 116-121.

Meza, M., Luz, D., Roa Mercado, C., Ahumada Barraza, C. (2015). The effects of soybean isoflavones over the bone health of adult and children. *Revista Salud Uninorte 31*, 138-152.

Morales-de la Peña, M., Rosas-González, M. C., Martín-Belloso, O., Welti-Chanes, J. (2017). Changes in bioactive compounds concentration and physicochemical properties of mango smoothies treated by ultrasound. *Revista Mexicana de Ingeniería Química 17*, 131-144.

Pandey, K. B., Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity 2*, 270-278.

Peñalvo, J. L., Nurmi, T., Adlercreutz, H. (2004). A simplified HPLC method for total isoflavones in soy products. *Food Chemistry 87*, 297-305.

Pérez, M. J., Cuello, A. S., Zampini, I. C., Ordoñez, R. M., Alberto, M. R., Quispe, C., . . . Isla, M. I. (2014). Polyphenolic compounds and anthocyanin content of *Prosopis* nigra and *Prosopis* alba pods flour and their antioxidant and anti-inflammatory capacities. *Food Research International 64*, 762-771.

Ramírez-Velasco, L., Armendáriz-Ruiz, M., Arrizon, J., Rodríguez-González, J., Mateos-Díaz, J. (2016). Liberación de ácido cafeíco de la pulpa de café empleando un extracto con actividad clorogenato esterasa de *Aspergillus ochraceus* producido por fermentación sólida. *Revista Mexicana de Ingeniería Química 15*, 503-512.

Retana-Márquez, S., Aguirre, F. G., Alcántara, M., García-Díaz, E., Muñoz-Gutiérrez, M., Arteaga-Silva, M., . . . Delgadillo, J. A. (2012). Mesquite pod extract modifies the reproductive physiology and behavior of the female rat. *Hormones and Behavior 61*, 549-558.

Retana-Márquez, S., Juárez-Rojas, L., Hernández, A., Romero, C., López, G., Miranda, L., . . . Delgadillo, J. A. (2016). Comparison of the effects of mesquite pod and Leucaena extracts with phytoestrogens on the reproductive physiology and sexual behavior in the male rat. *Physiology & Behavior 164*, 1-10.

Sauceda, E. N. R., Martínez, G. E. R., Valverde, B. R., Ruiz, R. M., Hermida, M. d. l. C. C., Torres, S. M. M., Ruiz, H. H. P. (2014). Análisis técnico del árbol del mezquite (*Prosopis* laevigata Humb. & Bonpl. ex Willd.) en México. *Ra Ximhai 10*, 173-193.

Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology 299*, 152-178.

Šukalović, V. H.-T., Vuletić, M., Marković, K., Vučinić, Ž., Kravić, N. (2017). Modification of antioxidant systems in cell walls of maize roots by different nitrogen sources. *Spanish Journal of Agricultural Research 14*, 0808.

Ungar, Y., Osundahunsi, O. F., Shimoni, E. (2003). Thermal stability of genistein and daidzein and its effect on their antioxidant activity. *Journal of Agricultural and Food Chemistry 51*, 4394-4399.

Vernon-Carter, E.J., Beristain, C.I., Pedroza-Islas, R. (2000). Mesquite gum (*Prosopis* gum). *Developments in Food Science 41*, 217-238.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Analytica Chimica Acta, 513, 57-65.

Walton, N. J., Mayer, M. J., Narbad, A. (2003). Vanillin. *Phytochemistry 63*, 505-515.

Walton, N. J., Narbad, A., Faulds, C., Williamson, G. (2000). Novel approaches to the biosynthesis of vanillin. *Current Opinion in Biotechnology 11*, 490-496.