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Mesquite pod flour (*Prosopis laevigata*): Physicochemical composition, amino acid profile, water and oil absorption capacity, hemagglutinins, phenols, and flavonoids

Harina de vainas de mezquite (*Prosopis laevigata*): Composición fisicoquímica, perfil de aminoácidos, capacidad de absorción de agua y aceite, hemaglutininas, fenoles y flavonoides

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ABSTRACT

Legumes have been an essential part of the human diet for centuries and are a rich source of protein. However, their nutritional content can vary according to species and varieties. This study aimed to taxonomically identify and physicochemically characterize mesquite (*Prosopis laevigata*) pod flour. Proximate composition (fat: 5.25%. crude fiber: 4.52%. ash: 6.06%. carbohydrates: 71.01%) was analyzed using AOAC methods, while technological properties compacted density (65.0 ± 0.03 g/mL), water absorption capacity (3.5 ± 0.05 mL water/g sample) and oil absorption capacity (3.0 ± 0.07 mL oil/g sample) compared to wheat flour in proximate composition. Non-nutritive factors were quantified using specific assays: trypsin inhibitors (2.1 ± 0.3 TIU/mg), total phenols (1.8 ± 0.2 mg GAE/g) and flavonoids (0.9 ± 0.1 mg QE/g). Saponins (0.1%) and hemagglutinins (0.01 HU/mg) were absent. The amino acid profile analyzed by HPLC revealed higher essential amino acid content than wheat flour, particularly lysine (6.2 and 2.8 g/100 g protein). These results demonstrate that mesquite flour shares key technological properties with wheat flour, such as comparable water absorption capacity (3.5 *vs* 3.2 mL/g) and compacted density (65 *vs* 68 g/mL). Mesquite pod flour is a viable partial substitute in bakery products, enhancing nutritional value while maintaining functional performance.

Keywords: Mesquite pod; flour mesquite; proximal composition; technological properties.

RESUMEN

Las legumbres han sido parte esencial de la dieta humana durante siglos y son una fuente rica de proteínas. Sin embargo, su contenido nutricional puede variar según la especie y la variedad. Este estudio tuvo como objetivo identificar taxonómicamente y caracterizar fisicoquímicamente la harina de vaina de mezquite (*Prosopis laevigata*). La composición proximal (grasa: 5,25%, fibra cruda: 4,52%, cenizas: 6,06%, carbohidratos: 71,01%) se analizó mediante métodos AOAC, mientras que las propiedades tecnológicas (densidad compactada: 65,0 ± 0,03 g/mL), capacidad de absorción de agua (3,5 ± 0,05 mL de agua/g de muestra) y capacidad de absorción de aceite (3,0 ± 0,07 mL de aceite / g de muestra) se compararon con la harina de trigo en composición proximal. Los factores no nutritivos se cuantificaron mediante ensayos específicos: inhibidores de tripsina (2,1 ± 0,3 TIU/mg), fenoles totales (1,8 ± 0,2 mg GAE/g) y flavonoides (0,9 ± 0,1 mg QE/g). No se encontraron saponinas (0,1%) ni hemaglutininas (0,01 HU/mg). El perfil de aminoácidos analizado por HPLC reveló un mayor contenido de aminoácidos esenciales que la harina de trigo, en particular lisina (6,2 y 2,8 g / 100 g de proteína). Estos resultados demuestran que la harina de mezquite comparte propiedades tecnológicas clave con la harina de trigo, como una capacidad de absorción de agua comparable (3,5 vs 3,2 mL/g) y una densidad compactada (65 vs 68 g/mL). La harina de vaina de mezquite es un sustituto parcial viable en productos de panadería, ya que mejora el valor nutricional y mantiene su rendimiento funcional.

Palabras clave: Vaina de mezquite; harina de mezquite; composición proximal; propiedades tecnológicas.

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1. Introduction

Mesquite (*Prosopis* genus. family *Leguminosae*. subfamily Mimosoideae) is a tree which is native to the arid and semi-arid regions of Mexico and other parts of the world. It is historically recognized for its ecological, cultural and nutritional significance. At least eleven species of *Prosopis* have been identified in Mexico, including *P. laevigata* which stands out for its abundance and adaptability to drought conditions. This is a strategic resource in the face of climate change and environmental degradation (Palacios-Romero et al., 2021, Villalón-Mendoza et al., 2023). In recent decades, there has been a growing interest in the use of non-conventional flour, driven by the demand for functional, sustainable, and nutritious foods that meet the needs of health-conscious consumers which address the environmental impact of food production (Bigne et al., 2018; Sciammaro et al., 2018; Zhong et al., 2022). Flours derived from mesquite pods have gained particular attention due to their high content of fiber, proteins, minerals, and antioxidants, as well as their low glycemic index and gluten-free nature, making them suitable for individuals with celiac disease and special dietary requirements (Gonzalez-Macedo et al., 2021, García-Azpeitia et al., 2022, Aguirre-Loredo, 2025). Recent studies have shown that whole mesquite flour can significantly surpass commercial flours in protein and fiber content and can enhance the essential amino acid profile when combined with other ingredients such as oats and amaranth. The use of mesquite in human nutrition is not new; its pods have traditionally been used to prepare beverages, breads, and other products in both Mexico and South America. However, factors such as cultural erosion, migration and land use have led to a decline in the knowledge and appreciation of this resource in many communities, thereby highlighting the need to preserve and promote its use through scientific research and innovative food projects. In the context of the Teotitlán de Flores Magón region in the state of Oaxaca, mesquite represents a valuable alternative for local development, as its utilization can contribute to food security, dietary diversification and the strengthening of the regional economy (Peña-Avelino et al., 2016; Negrete-Sánchez et al., 2017). The decision to study the local mesquite variety is based on its availability in the area specified and the lack of specific information about its properties, which limits its full and sustainable use. Moreover, characterizing the mesquite flour produced in this region will allow for a comparison of its physicochemical and technological properties with those of other non-conventional flours, paving the way for its incorporation into the formulation of high nutritional value and low environmental impact foods (Bigne et al., 2018; Sciammaro et al., 2018; Prokopiuk et al., 2000; De Melo et al., 2022, Korus et al., 2022; Alemán-Huerta et al., 2023; Pérez-Lozano et al., 2025). This study presents the taxonomic identification and characterization of flour obtained from mesquite pods collected in Teotitlán de Flores Magón, Oaxaca. Their physicochemical and techno-functional properties are analyzed with the aim of evaluating their potential as ingredients in the development of healthy and sustainable food products, thereby contributing to the revalorization of ancestral resources and the promotion of innovative dietary practices in the region.

2. Methodology

2.1 Raw material

To obtain unconventional flours, mesquite (*Prosopis* spp.) pods were collected in Teotitlán de Flores Magon, Oaxaca during the months of May and July 2017. Mature pods with similar reddish coloration were selected. Subsequently, the taxonomic identification of *Prosopis* spp. was carried out using the taxonomic keys of Grether et al. (2006) following the guidance given by Dr. Juan Manuel Loeza Corte, a Professor-Researcher at the Universidad de la Cañada (UNCA).

2.2 Obtaining the mesquite pod flour

Mature mesquite pods were manually selected, and any specimens that were damaged or affected by insects were discarded. The selected pods were then subjected to a cleaning and sanitization process. A thermal-chemical treatment was performed by immersing the pods in a 10% NaOH solution at 100 °C for 7 minutes (Corzo, 2008). After treatment, the pods were thoroughly rinsed with potable water and subsequently dried in an oven (ECOSHEL. Model 9053A. USA) at 50 °C for 42 h.

Once dried, the pods were ground using an electric mill (Pulvex 200, Molinos Pulvex, Mexico City, Mexico) and sieved with a #100 mesh size (NMX-F-007-1982). Figure 1 shows the process for obtaining mesquite pod flour. The resulting flour was stored in resealable bags at room temperature (27 °C) until further use and analysis.

Figure 1. Mesquite pod flour process using *Prosopis laevigata*.

2.3 Chemical characterization of mesquite pod flour

The proximate composition of flour mesquite samples includes moisture (method 925.10), crude fat (method 923.05), and ash (method 936.07). Crude protein content was determined with the Kjeldahl method (AOAC 984.13) using a nitrogen-to-protein conversion factor of 5.75 for vegetable proteins (AOAC 2005). Total carbohydrate content was calculated by difference (subtraction method).

2.4 Technological and functional characterization of mesquite pod flour

Amino acid profile

Amino acid profiling was performed following the methodology described by Diaz-Batalla et al. (2018). Briefly, previously defatted flour samples were hydrolyzed for 24 h in 6 N HCl and then evaporated and diluted in citrate buffer pH 2.2. The amino acid content in the hydrolysates was determined with the aid of an amino acid analyzer, using an ion exchange column and post-column detection by reaction with ninhydrin according to the Spackman et al. (1958).

Compacted density

The compacted density was determined following the methodology published by Torres (2015). Two g of powder was poured into a 10 mL capped graduated cylinder which was then placed on an expanded polystyrene plate. It was tapped gently with vertical up-down movements for three minutes until the volume did not present any changes. The occupied volume was then recorded. The compact density was calculated as the ratio between the mass and the volume after the agitation that promoted powder compaction.

$$D = \frac{m}{v} \qquad \text{(Eq. 1)}$$

Where m: mass (g), v: volume (mL) and D: density.

Water/oil absorption capacity

The oil absorption capacity (OAC) and hydration properties such as water absorption capacity (WAC) were determined according to Genevois & de Escalada Pla (2021) and Chandra et al. (2015).

2.5 Determination of non-nutritive compounds in mesquite pod flour

Hemagglutinins and Trypsin inhibitor

The AACC method 22-40 was used which consists of incubating the sample with a known substrate (BAPA) and trypsin. Trypsin activity is indicated by an increase in absorbance at 410 nm. Inhibition of trypsin by the inhibitor present in the sample lowers the absorbance. The internal method (Coscueta et al., 2017; MME-TX-08) was used for hemagglutinin determination.

Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method. A solution of gallic acid was prepared at a concentration of 1 mg/mL and different dilutions were made to generate a calibration curve. Samples were measured at an absorbance at 765 nm using a UV-Vis spectrophotometer (Genesys 10S Thermo Fischer Scientific, Waltham, MA. USA). The absorbances were compared with a calibration curve of standard gallic acid, expressed as mg gallic acid equivalents/mL (Singleton & Rossi. 1965; Cartaya & Reynaldo. 2001).

Determination of Total flavonoid content

Total flavonoid content was determined following the method described by Ming-Yen & Cheng-Chun (2010) using Quercetin as a standard (0 - 2.0 mg/mL). The results were expressed as mg Quercetin equivalent (mg QE/mL of extract).

2.6 Statistical analysis

Experimental data were obtained in triplicate and used to calculate mean values. One-way ANOVA was performed to assess statistical significance ($p \leq 0.05$) and means were compared using Tukey's test ($p \leq 0.05$). Statgraphics Centurion XVI (Statistical Graphics Corp., United Kingdom) was used for all statistical analyses.

3. Results and discussion

3.1 Taxonomic identification of *Prosopis* spp.

The taxonomic identification of Prosopis was carried out to identify the variety of mesquites used in this study. The taxonomic keys from the classification of Grether et al. (2006) were considered for their identification. According to what was described (Figure 2 and 3), it was concluded that the mesquite pods were collected from the genus *Prosopis* and the *laevigata* species.

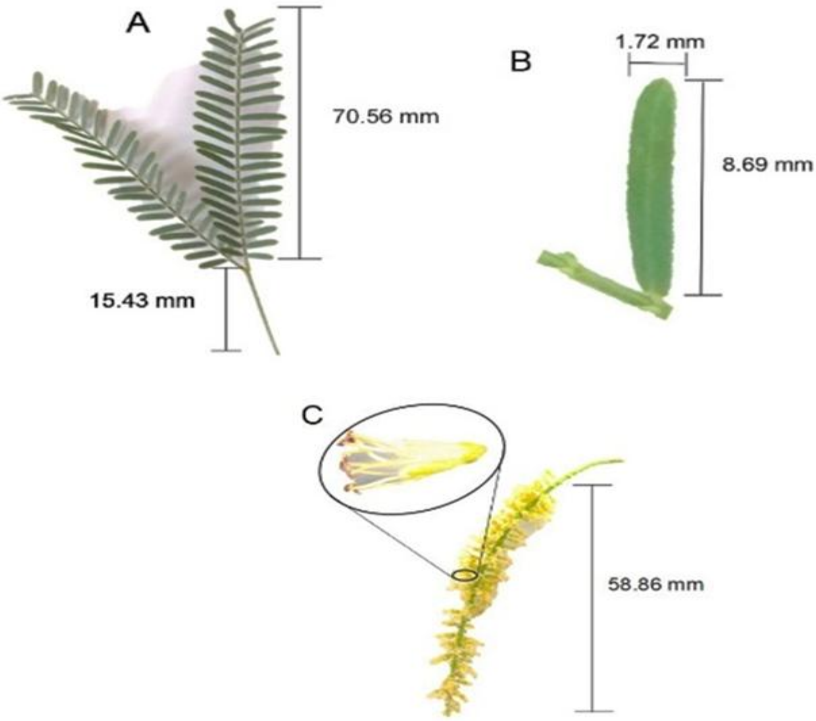

Figure 2. *Prosopis laegivata*. Pinna (A). Leaflet (B). Flower (C).

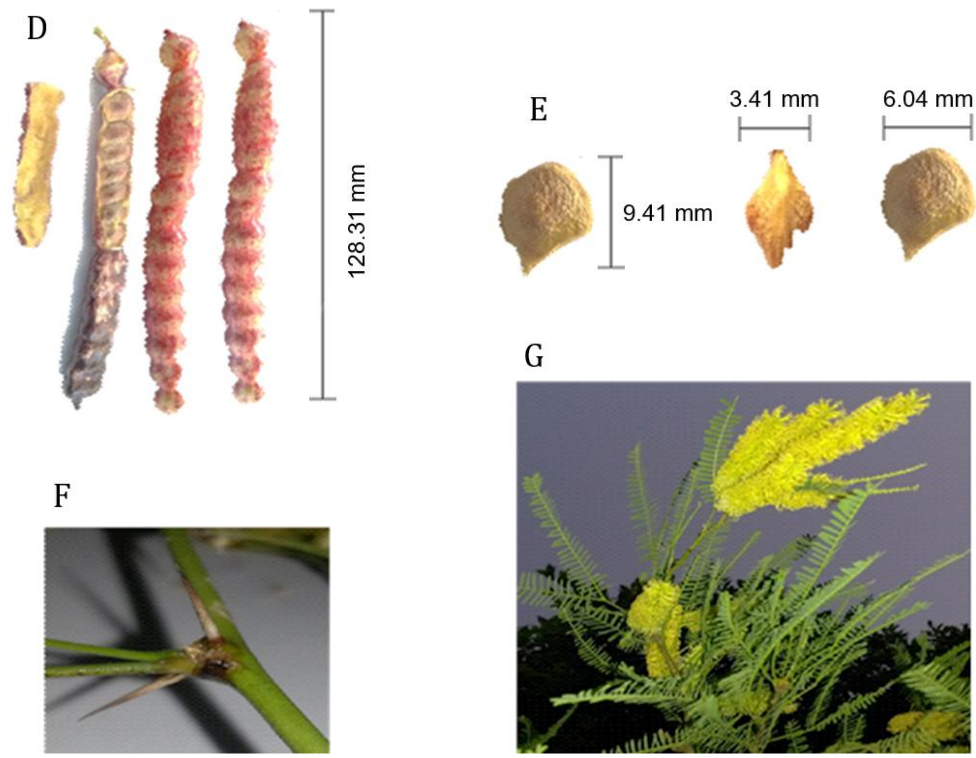

Figure 3. *Prosopis laegivata.* Fruits (D). Seeds (E). Spines (F). Flowering branch (G).

This is based on the work by Grether et al. (2006) who carried out a taxonomic study in the valley of Tehuacán-Cuicatlán, which presents the taxonomic keys of the *Prosopis laevigata* species. The pods used in this study were collected from the trees belonging to this species. Rodríguez Sauceda et al. (2014) conducted a comprehensive monograph on *Prosopis* across Mexico, with particular emphasis on the northern region of the country. The identification keys applied to *Prosopis laevigata* Humb. & Bonpl. ex Willd. in their work are consistent with those employed in the present study. It is worth mentioning that a specimen of the *Prosopis laevigata* species with the voucher UCK 006 was filed in the herbarium "*kixonga naxo*" at the Universidad de la Cañada.

3.2 Characterization of the mesquite (*Prosopis laevigata*) pod flour

Mesquite pod flour exhibited a compact powder appearance with shades of khaki and chamois along with a sweet aroma. This flour was obtained from the mesocarp and pericarp of the mesquite pod belonging to the *Prosopis laevigata* species.

3.3 Physicochemical composition of mesquite (*Prosopis laevigata*) sheath flour

Table 1 shows the comparative physicochemical composition between wheat and mesquite pod flour. As expected, mesquite pod flours presented notably higher levels of moisture, ash, fiber and fat compared to wheat flour, with these differences being statistically significant ($p < 0.05$). In both flours, carbohydrates are the major component, although in wheat flour they were significantly higher ($p < 0.05$). The results obtained in this study are higher than the values reported for moisture content from other studies (Gallegos-Infante et al., 2013; Díaz- Batalla et al., 2018). The results from this study indicated intervals between 9.82% and 8.33%, while for other species even lower values are reported, such as 6.53% for *Prosopis chilensis* (Cerezal Mezquita et al., 2012), 4.22% for *Prosopis juliflora* (Gómez et al., 2008), and intervals of between 2.14% to 7.17% for *Prosopis alba* (Felker et al., 2013; Prokopiuk. 2004; Gómez et al., 2008). Flours are considered powders, and their density gives an indication of their porosity and compacting capacity. Compact density allows the volume to be occupied by a known mass, and not the space between the particles or the mixture of porosity. For this reason, it is necessary to fill all the spaces between the particles with powder by compaction (Alvarado & Aguilera 2001).

Techno-functional properties provide information on how a particular ingredient would behave within a food matrix. These properties play a prominent role during food preparation, processing and

storage, and in turn, correlate with sensory properties. Such properties could be considered as the result of the various conformational changes or interactions that take place between food components, such as protein-protein interactions, protein-polysaccharide interactions, as well as lipid interactions (Patane et al., 2004). Among the techno-functional properties of flour, those related to water stand out, since they play an important role in the main changes that take place during food processing. Therefore, it can be said that hydration properties, among which water absorption capacity and water retention capacity stand out, directly influence the characteristics that make up the food matrix (Miquilena et al., 2016).
Table 1 shows statistically significant differences ($p < 0.05$) in compact density between wheat flour and mesquite pod flour (MF). This result suggests that mesquite pod flour may be grainier with a sandier texture and, consequently, denser (Grossmann et al., 2004).

Table 1
Physicochemical characterization of wheat flour and mesquite pod flour

Component (%)	WF	MF
Moisture	7.03 ± 0.05[a]	15.05 ± 0.05[b]
Protein	7.09 ± 0.04[a]	7.05 ± 0.70[a]
Ash	1.29 ± 0.10[a]	6.06 ± 0.20[b]
Fat	1.46 ± 0.40[a]	5.26 ± 0.40[b]
Fiber	2.49 ± 0.0[a]	4.52 ± 0.48[b]
Carbohydrates	80.64 ± 0.0[a]	62.06 ± 0.0[b]
Compact density (g/mL)	0.45 ± 0.02[a]	0.65 ± 0.03[b]
WAC (mL water/g sample)	4.90 ± 0.08[b]	3.50 ± 0.05[a]
OAC (mL water/g sample)	0.80 ± 0.03[a]	3.00 ± 0.07[b]

WF: Wheat flour. MF: Mesquite pod flour. WAC: water absorption capacity; OAC: oil absorption capacity. Values represent the mean ± standard deviation (n=3). Same letters in the same row indicate no significant difference ($p < 0.05$) by Tukey test.

WHC is the amount of water that remains bound to the hydrated material after the application of an external force. This property is of great importance in pulses and is directly related to the cooking process (Aguilera, 2009). WHC is related to the flour particle size, the amount of free hydroxyl groups capable of interacting with external water, and the amount and size of fiber particles (Katina, 2003). Water absorption values higher than 3.0 g water/g sample are related to improvements in the texture of bakery products (Achouri et al., 2010). The inclusion of mesquite pod flour in bakery products could modify the texture of the crumb in these products (Achouri et al., 2010).

Oil absorption capacity (OAC) is an indirect measure of the hydrophobic protein-fat bonds and their capacity to bind with lipophilic compounds. The oil absorption capacities of the flours were 3.0 ± 0.07 and 0.80 ± 0.03 mL oil/g sample, respectively, meaning that there is a significant difference ($p < 0.05$) between them. This difference in oil absorption capacity between the flour samples could be attributed to the treatment applied to the mesquite pod flour, which probably favored the interaction between the hydrophobic protein-fat bonds. The oil absorption capacity is directly related to the number of non-polar side chains present in proteins, which tend to bind with the hydrocarbon chains of fats (Vegas Niño, 2017). The combined processes of soaking, cooking and drying can cause structural alterations in the protein, which favors the physical retention of fat. This phenomenon appears to be the result of physical trapping of fats by proteins (Onimawo & Akubor, 2012). The WHC of MP meal resembles the value reported for meal from *Prosopis juliflora* which was 3.21 mL oil/g sample (Jaimes-Morales et al., 2015). This suggests similarities in protein-fat interaction properties between these flour varieties.

Amino acid profile of mesquite pod flour

Table 2 shows that mesquite pod flour exceeds the values recommended by FAO, and when compared to wheat flour, it shows a higher level of some essential amino acids.

Table 2
Amino acid profile in wheat flour and mesquite pod flour (g/100 g protein)

Amino acid (Essentials)	MF	WF	*FAO 2013 (g/100 g protein)
Tryptophan	0.804[a]	1.08[a]	0.66
Valine	3.912[b]	2.48[a]	4.0
Isoleucine	2.581[a]	2.56[a]	3.0
Threonine	2.476[a]	2.70[a]	2.5
Phenylalanine	2.853[a]	4.90[b]	4.1
Leucine	7.100[b]	6.07[a]	6.1
Lysine	3.149[b]	1.49[a]	4.8
Methionine	0.741[a]	1.80[b]	2.3
Histidine	2.566[b]	1.27[a]	1.6
Arginine	2.547[a]	2.38[a]	2.3

MF: Mesquite pod flour. WF: Wheat flour. Means are reported ± standard deviation (n = 3). Same letters in the same row indicate no significant difference ($p < 0.05$).
* Amino acid scoring patterns for children aged 3 - 10 years. Adapted from FAO (2013).

Determination of non-nutritive compounds in flour

The non-nutritive compounds are anti-nutritional factors and can be classified into two main groups. The first group is composed of compounds of protein nature, lectins, agglutinins, protease inhibitors (such as trypsin and chymotrypsin inhibitors) and bioactive peptides. The second group consists of non-protein nature, alkaloids, phytic acid, phenolic compounds and saponins (Sánchez-Chino et al., 2015). Table 3 shows the results obtained for the mesquite pod flour. Regarding the trypsin inhibitor, its content was lower compared to that found by Gallegos-Infante et al. (2013), who reported a content of 500 (ITU/g) in flour obtained from the mesocarp and seeds from *Prosopis laevigata* pods. It was also observed that these parameters decrease as the drying temperature and roasting treatment increase.

Table 3
Non-nutritive compounds in the flour obtained from mesquite pods (*Prosopis laevigata*)

Component	Value
Trypsin Inhibitor (ITU/g sample)*	4325.69 ± 0.1
Saponins	Nd
Hemagglutinins	Nd
Total Phenols (mg GAE/g)**	8.91 ± 0.1
Flavonoids (mg EQ/g)***	0.80 ± 0.1

* Units of Trypsin Inhibitors / sample
** Gallic Acid Equivalents / gram
*** Quercetin Equivalents / gram
Nd: not detected.

Much of the trypsin inhibitor activity is eliminated using various treatments such as thermal processes, germination and fermentation, all of which denature the proteins, thereby improving their digestibility. Likewise, the loss of trypsin inhibitors during hydration can be caused by the change in the concentration gradient which changes the diffusion rate. Generally, trypsin inhibitors have low molecular weight proteins, meaning that they pass easily from the seed into the hydration medium (Bishnoi & Khetarpaul, 1994). As for saponins, they are absent on the flour, as are hemagglutinins. Regarding total phenols, the value obtained (Table 3) is within the range reported by Díaz-Batalla et al. (2018), who obtained a value of 8.87 mg GAE/g in flour obtained from the mesocarp of the *Prosopis laevigata* pod. However, in another study conducted by the same authors on flour from the same species but obtained from the seed as well as the extruded seed, the results of total phenols were lower (6.68 - 6.46 mg GAE/g). Similarly, in another study on the same *Prosopis* species, Gallegos-Infante et al. (2013) observed that combining drying and different roasting temperatures on mesquite pods resulted in the decrease of total phenols in the flour. However, when comparing total phenol values reported for other *Prosopis* species, values of 3.3 - 6.6 g GAE/kg were obtained for *Prosopis nigra* and 2.2 – 4.6 g GAE/kg for *Prosopis alba* (Pérez et al., 2014). Therefore, the variability in values may be due to the difference in the sample (meal obtained from mesocarp, mesocarp + pericarp, seed, complete pod) species and treatment applied to the sample. Regarding the result of total flavonoids, a content of 0.80 ± 0.1 mg QE/g is presented, which turns out to be higher than the values reported (0.28 g QE/kg) by Díaz-Batalla et al. (2018) in flour obtained from mesquite mesocarp belonging to the same *Prosopis* species. The same authors also observed that there is a higher content of total flavonoids (0.80) in the flour obtained from heat-treated mesocarp flour. Likewise, studies conducted on flour obtained from the *Prosopis nigra* pod mesocarp reported a total flavonoid content of 0.68 g QE/ 100g flour (Pérez et al., 2014). However, it is worth mentioning that the influence of the treatment or processing carried out in obtaining the flour will probably have an impact on the total flavonoid content. In contrast, studies carried out on six legumes (green and yellow peas, chickpeas, lentils, along with black and red beans) identified higher flavonoid content in seeds (Amarowicz & Pegg, 2008).

4. Conclusions

The pod used in this study was identified as belonging to the *Prosopis laevigata* species. MP flour, from the milling of mesocarp and pericarp of mesquite (*Prosopis laevigata*) pods, was found to have a high nutritional value compared to wheat flour. Regarding the amino acid profile, MP flour had a higher content of essential amino acids than wheat flour, which was mainly lysine. The presence of undesirable factors such as saponins and hemagglutinins was not detected. However, trypsin inhibitor, phenols and flavonoids were present. These results corroborate the potential for the flour to be used in food systems, in particular, baking. According to the results, high content in protein, ash, fiber and amino acid, means that mesquite should be considered as a suitable alternative in the development of foods of high nutritional quality. Several studies have formulated food preparations to totally or partially substitute ingredients such as wheat flour for other types of flour, for example in bread and cookies, since bakery products are suitable matrices for incorporating new ingredients without notoriously modifying their techno-functional and sensory properties. Likewise, there are economic benefits in promoting the use of mesquite, such as the development of sustainable agricultural productivity in Teotitlán de Flores Magón, encouraging the cultivation and further exploitation of a low cost and easily available underutilized source, as well as helping a vulnerable population in a state of malnutrition due to poor access to food.

Finally, as a rich source of macro and micronutrients, mesquite pod flour has great potential for incorporation into various foods, such as baked goods, plans are in progress to use it into varieties of bread, cookies and muffins. However, it is still necessary to study the effect of flour dosage on the physicochemical, rheological, and sensory properties of these products.

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