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Bromatological analysis and quantification of calcium oxalates in tender prickly pear flour from different Opuntia cultivars

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Abstract. The average consumption of *Opuntia* spp. (tender prickly pear) per person in Mexico is 6.3 kg per year due to the convenience of its valuable nutraceutical properties and medicinal benefits and their high concentrations of calcium, fiber, minerals, calcium oxalates, and antioxidants. The *Opuntia* spp. is used in a wide range of applications, and the food industry has developed flours derived from it as a substitute. The objective of this study was to know the chemical and nutritional qualities of Opuntia (tender prickly pear) prickly pear flour using three Opuntia types, Jalpa, Villanueva and Oreja de Elefante. The tender prickly pear was classified into four ranges of fresh weight, No. 1 (<150 g), No. 2 (150-300 g), No. 3 (300-600 g), and No. 4 (>600 g). The results were subjected to statistical analysis (ANOVA) and mean comparisons using the Tukey method. The chemical and nutrient composition varied according to the variety of prickly pear and tender prickly pear and their stage of ripeness, which correlates with the mass of the cladodes. Results showed that as the weight and degree of ripeness increased, the fiber concentration also increased in the Oreja de Elefante cultivar, having a higher concentration of 9.06% compared to the other cultivars. The calcium oxalates had a negative relationship with age in all Opuntia cultivars. The cultivars showed a higher density of crystals in the upper part and a reduction in crystal density in the basal part, with the Villanueva cultivar having the highest in the upper part with 58.50 calcium oxalate crystals and the middle part with 45.69 calcium oxalates; however, for the basal part the Oreja de Elefante cultivar had the highest concentration with 36.00 calcium oxalate crystals.

Keywords: *nutraceutical; crude fiber; calcium; characterization.*

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Introduction

In Mexico, *Opuntia* spp. encompasses a diverse array of species, with its distribution shaped by physiographic, ecological, and management factors to maximize production (Aparicio-Fernández *et al.*, 2017). The optimal climatic conditions for prickly pear cultivation include temperatures from 16°C to 28°C, annual precipitation between 150 and 1800 mm, and elevations ranging from 800 to 1800 MSL. Nevertheless, tender prickly pear cultivation is predominantly concentrated in Mexico's arid and semi-arid regions (Diego-Zarate *et al*., 2021). The genus *Opuntia ficus-indica* is significant due to its various uses, including human consumption (as tender prickly pear and fruit), animal feed, and cochineal dye production. In Mexico, 258 species of Opuntia (tender prickly pear) are found (Vázquez-Alvarado *et al*., 2015). According to the information provided by the Servicio de Información Agroalimentaria y Pesquera (SIAP), in México, the States with the highest *Opuntia* spp. production are Estado de México, Zacatecas, Puebla, Michoacán, Jalisco, Sinaloa, Chihuahua, and Tamaulipas. The per capita consumption of tender prickly pear in Mexico is 6.3 kg per year in daily human diets (SIAP, 2022). The cladodes are highly nutritious, containing calcium, antioxidants, and dietary fiber, making them an integral part of human nutrition. Additionally, tender prickly pear is valued for its nutraceutical properties, as it helps reduce bone-related diseases like osteoporosis due to its calcium content (Rodríguez-García *et al*., 2007); while tender prickly pear or nopalitos have high levels of calcium oxalates, which can limit the absorption of bioavailable calcium (Contreras-Padilla *et al*., 2011). The bacteria from the genus *Oxalobacter formigenes* present in the gastrointestinal tract act as enzymes to degrade a significant proportion of the oxalates (Vázquez-Alvarado *et al*., 2015). The conversion of fresh tender prickly to its dried form is primarily used to produce powders or flours for the food industry (Torres-Ponce *et al*., 2015). This allows for the creation of products such as bread, tortillas, cookies, juices, sweets, and alcoholic beverages, taking advantage of its nutritional properties like vitamins, dietary fiber, calcium, and antioxidants (Solís-García *et al*., 2021).

One of the options to improve the nutritional quality of corn grain silage, is the moistening process, which consists of moistening the grain, aiming to reach the desired moisture content for the fermentation process in the silo, enabling adequate storage conditions. This is an effective procedure, as in addition to improving digestibility, it conserves corn by reducing material losses and deterioration because of pest action, temperature, and humidity changes, reduces transportation costs and presents minimal effects of market price fluctuations (Arcari *et al.,* 2016). Therefore, the moist corn silage technique is an alternative to maximize its use by the animals (Junges *et al.,* 2017). Thus, to improve economic and storage conditions, the farmers can choose to buy corn grains at times when the price is affordable, then adopt the ensiling process with grain moistening and use this silage strategically in periods of forage scarcity and elevated prices to avoid excessive expenses. Another option has been proposed is the use of a wide variety of additives aiming to improve the fermentation process and aerobic stability, through the inhibition of undesirable microorganisms after opening the silo (Morais *et al.,* 2021). The moistening additive is used in grains to improve digestibility (Kung Jr. *et al.*, 2018) and silage quality (Saylor *et al.,* 2020). However, for silages rich in energy such as cereal grains, the control over the population of unwanted microorganisms is also important to ensure feed safety (Sadhasivam *et al.,* 2017). This study seeks to determine the chemical and nutritional composition of prickly pear flour based on its content of calcium oxalates, protein, fat, carbohydrates, and fiber across four maturity stages of cladodes in three cultivars.

Material and Methods

Location of the cactus plantation and collection of cladodes

The cladodes of Villanueva and Jalpa cultivars were collected from a property named Vertia, located on Zuazua-Marin Road in the neighbourhood of General Zuazua, Nuevo León (25°88'N, 100°08'W). The cultivar Oreja de Elefante (O. Elefante) was collected from the Marín campus of the Agronomy Faculty of the Universidad Autonóma de Nuevo León at 375 MSL (25°53'N, 100°03'W).

The tender cladodes from different cultivars, Villanueva, Jalpa, and O. Elefante were cut in various sizes and stages of development, as shown in Figure 1. Four ranges of fresh weight of tender cladodes were established, No. 1 = <150 g; No. 2 = 150-300 g; No. 3 = 300-600 g; and No. 4 = >600 g. The prickly pears were collected in the months of August and September 2021. Each cladode was then

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weighed using a scale (ETEKCITY® model EK8060) and their length, width, and thickness were measured with a digital fractional caliper.

Figure 1. (A) weight, (B) length, (C) width, and (D) thickness measurements.

Site of analysis

The analyses were conducted at the Agronomy Faculty of UANL, Campus Escobedo, located at 25°47'47.3"N, 100°17'8"W at 478 MSL, and at the Marin Campus, located at 25°53'N and 100°03'W with 375 MSL. These analytical procedures were conducted within the respective laboratories, the microbial biotechnology laboratory and the laboratory for soils, water, and plant tissue.

Setting and drying of prickly pear

The cladodes were cut into strips of 2 cm thickness and then dried in an oven (1600 HAFO® Series) at a temperature of 65 °C. Subsequently, they were processed in a high-speed multifunctional electric mill (Grinder® model 700), which possesses a maximum crushing capacity of 700 g and a fineness range of 50 to 300 µm akin to that of cornstarch.

Bromatological analysis

The moisture determination was performed using a reference method and subsequent calculations proposed by Bianco *et al*. (2014). The ash was determined when the cladode samples were incinerated in a muffle furnace at 550 °C for 4 h (Keshun, 2019) and calculated using the Enríquez and Ojeda (2020) equation. The protein content was determined using the micro-Kjeldahl method, multiplied by the protein conversion factor, according to Bermúdez (2011). The crude fiber was assessed using the method of Maldonado-Santoyo and Morales-López (2022). The sum of the values for moisture, total ash, proteins, fats, and crude fiber was used to calculate the total carbohydrates of the nitrogen-free extract (NFE).

Calcium oxalates

The calcium oxalates were determined using the methodology described by Tovar-Puente *et al.* (2007), which is detailed in the flowchart of Figure 2. The original method was followed, except for cross-sectional cutting of thin slices of the tender prickly pear (1 mm) from the upper, middle, and basal parts, as illustrated in Figure 3. Each section provided three samples and a replication of those. The samples were immersed in 70% ethyl alcohol and 10% potassium hydroxide (KOH). Subsequently, one of the samples was placed on a glass slide and examined using the VE-BC3 PLUS Microscope with a digital camera at 10X magnification. The calcium oxalates were counted using the ImageJ image processing software, version IJ1.46r.

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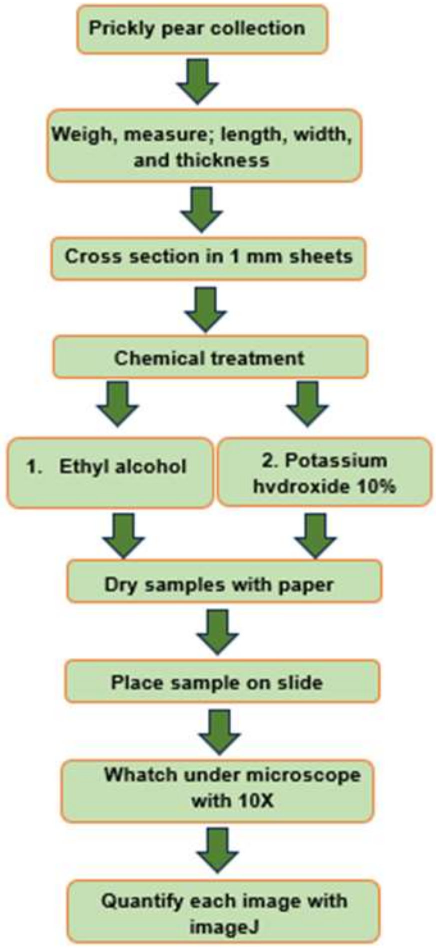

Figure 2. Calcium oxalate determination flowchart.

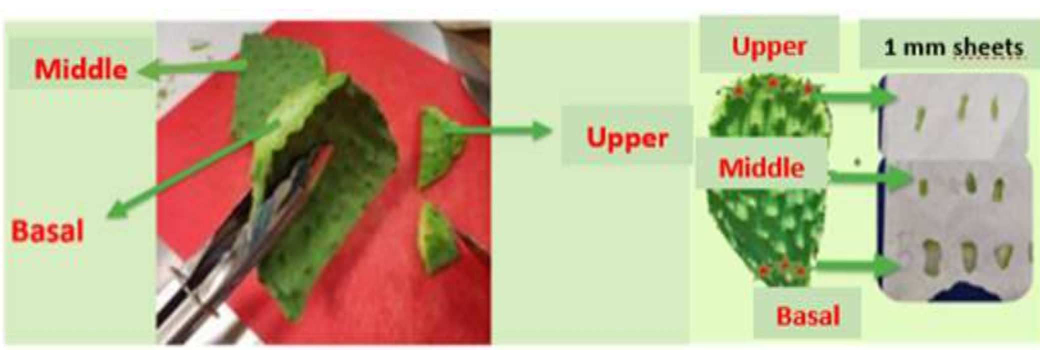

Figure 3. Cut of each tender, prickly pear slice from the upper, middle, and basal parts.

Experimental design and statistical analysis

The experimental design used was a completely random with two factors, factor one the cultivars with three levels, Villanueva, Jalpa, and O. Elefante and factor two with four ranges of fresh weight of tender cladodes, No. 1 = <150 g; No. 2 = 150-300 g; No. 3 = 300-600 g; and No. 4 = >600 g with three replications. An analysis of variance (ANOVA) and mean comparisons using the Tukey method were done. The collected data underwent statistical analysis using SPSS software and the experimental designs software of the FA-UANL.

Results and discussion

Bromatological analysis

The mean comparisons of bromatological variables are listed in Table 1, corresponding to each cultivar within their respective weight ranges.

Nitrogen content

The means of nitrogen content within each cultivar for different weight ranges are showed in Table 1. Jalpa showed the highest nitrogen content in the weight ranges of 1, 3, and 4. In contrast, no significant differences were found between the various weight ranges in Villanueva. Regarding the O. Elefante, the highest nitrogen content was observed in the weight range of 1, being the cultivar with the highest nitrogen content with 0.45%. The result of the total nitrogen is crucial for determining the protein content, followed by the multiplication of a factor suggested by Rössel-Kipping *et al*. (2021).

Protein content

The protein content of cultivars within each weight range are showed in Table 1. Jalpa showed the highest protein content in weight ranges of 1 and 4. There were no significant differences in Villanueva. Conversely, the O. Elefante, intended for forage and vegetable use, exhibited the highest protein percentage in range 1, with a value of 2.84%; however, this value is lower compared to the protein content reported for forage cactus by Torres-Ponce *et al*. (2015) which ranged from 4 to 6.4%.

Total ashes content

The total ash content showed no significant differences for Jalpa, as well as for O. Elefante across its weight ranges. However, Villanueva exhibited significant differences across with highest total (29.08%) ash content in the weight range of 4. The ash content of Villanueva is consistent with the findings reported by Rives-Castillo *et al*. (2021) for cladodes harvested in August and September, the same months that prickly pear in this study were collected.

Table 1. Comparison of means of bromatological analyses of prickly pear flour weight ranges within each cultivar.

Cultivars	Weight range	Nitrogen (%)	Protein (%)	Total ashes (%)	Crude fiber (%)	Organic matter (%)	Fat (%)	Carbohydrates (%)
Jalpa	1	0.44 [a*]	2.78 [a]	19.74 [a]	5.25 [c]	71.59 [a]	2.25 [a]	61.29 [a]
	2	0.41 [b]	2.62 [b]	18.08 [a]	5.09 [c]	70.70 [a]	2.10 [a]	60.88 [a]
	3	0.43 [a]	2.73 [a]	19.49 [a]	5.98 [b]	67.71 [ab]	2.14 [a]	56.85 [a]
	4	0.44 [a]	2.78 [a]	16.88 [a]	8.37 [a]	64.02 [b]	2.30 [a]	50.55 [b]
Villanueva	1	0.43 [a]	2.73 [a]	18.29 [c]	7.95 [c]	70.41 [a]	1.82 [a]	57.89 [a]
	2	0.42 [a]	2.67 [a]	22.63 [b]	8.30 [bc]	67.89 [a]	1.61 [a]	55.28 [a]
	3	0.44 [a]	2.76 [a]	23.41 [b]	8.42 [b]	65.60 [a]	1.68 [a]	52.73 [a]
	4	0.43 [a]	2.70 [a]	29.08 [a]	8.90 [a]	59.40 [b]	1.67 [a]	46.12 [b]
O. Elefante	1	0.45 [a]	2.84 [a]	13.35 [a]	7.89 [b]	76.93 [a]	1.98 [a]	64.21 [a]
	2	0.42 [b]	2.64 [b]	14.43 [a]	9.04 [a]	75.39 [a]	2.05 [a]	61.65 [a]
	3	0.40 [c]	2.53 [c]	14.79 [a]	8.01 [b]	75.17 [a]	2.04 [a]	62.57 [a]
	4	0.43 [b]	2.70 [b]	16.82 [a]	9.06 [a]	73.06 [a]	2.17 [a]	59.12 [a]

* Means followed by the same letter in the same column are not significantly different. The significance level was set at p≤0.05. (n=4).

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Crude fiber content

The crude fiber content is exhibited in Table 1, indicating that Jalpa and Villanueva showed significant differences between weight ranges. Both cultivars exhibited the highest contents in range 4, with 8.37 and 8.90%, respectively. For the O. Elefante ranges 2 and 4 had the highest crude fiber content with 9.04 and 9.06%. These values are within the range estimated by Ponce-Luna *et al*. (2023), which indicates crude fiber content of 8-17%, and align with Guzmán and Chávez (2007), who noted that increasing weight ranges lead to higher fiber concentrations in prickly pear.

Fat content

The means of fat content is displayed in Table 1, indicating no significant differences. The values ranged from 1.61 to 2.25%, which are consistent with the 1.50% fat content reported by Rodiles-López *et al*. (2019).

Carbohydrates content

The means of carbohydrates content for the cultivars across their respective weight ranges is illustrated in Table 1, indicating no significant differences among the three cultivars. However, O. Elefante had the highest content in weight range 1. Albergamo *et al*. (2022) reported similar values, with carbohydrate content in prickly pear ranging from 55.1 to 66.5%. It is noteworthy that the concentration of carbohydrates decreases as the weight range, and consequently the age, of the prickly pear fruit plant increases, as stated by Guzmán and Chávez (2007). Castillo *et al*. (2013) reported protein content of 12.66%, fat of 3.03%, fiber of 47.65%, and ash of 20.28% in prickly pear flour. These values differ from those obtained in this study, where lower percentages were recorded, comprising 2.84% protein, 2.20% fat, 9.06% fiber, and 23.35% ash. The disparities in the results can be attributed to several reasons, such as the use of different cultivars, different weight ranges, and variations in soil type, water, and climatic conditions compared to Castillo's study. Furthermore, the chemical composition of prickly pear flour is influenced by the maturity of the cactus and the cultivar used.

Calcium oxalates content

Calcium oxalates change shape, size, and amount depending on where they are in the top, middle, and bottom parts of a plant called Jalpa. According to the findings of Zuñiga *et al*. (2018) who conducted a count of calcium oxalates in prickly pear subjected to water stress at various concentrations; calcium oxalates generally possess a diameter of 8.50 µm. The increased presence of calcium oxalate in prickly pear is linked to the plant's management and conditions.

Calcium oxalate crystals in the tender prickly pear are depicted in Figure 6, revealing distinctive features between the basal part (A) and the upper part (B). In the basal part, the calcium oxalate crystals revealed notable and substantial drusen, while in the upper part these drusen are less defined and of lesser importance. This variation is attributed to the distribution and concentration of calcium oxalate crystals, which tend to decrease in the basal part, resulting in more uniform crystals in the upper part. To have a better identification of the structures of calcium oxalate crystals, X-ray diffraction is suggested as mentioned by Contreras-Padilla *et al*. (2011) where they used scanning electron microscopy images to study the morphology of calcium oxalates.

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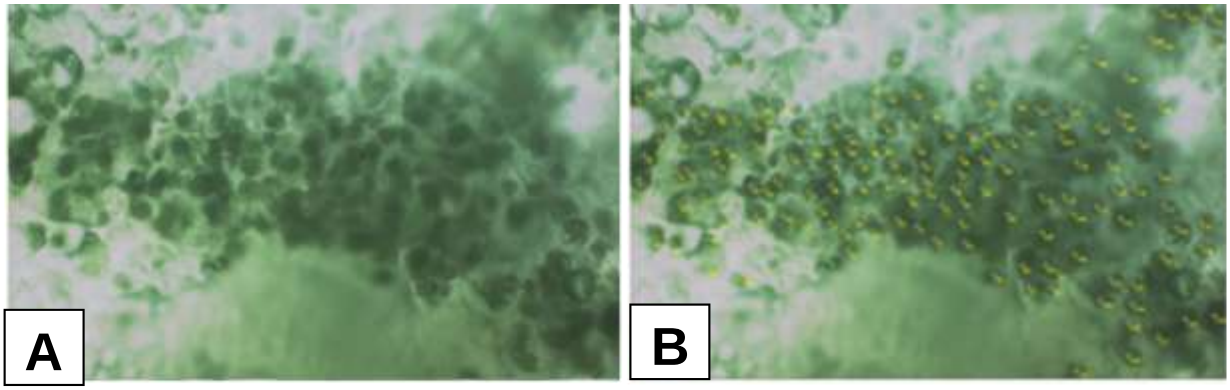

Figure 4. llustrates quantifying calcium oxalates using the ImageJ image processing software. (A) Image of oxalates without ImageJ software, and (B) Image of oxalates with ImageJ software.

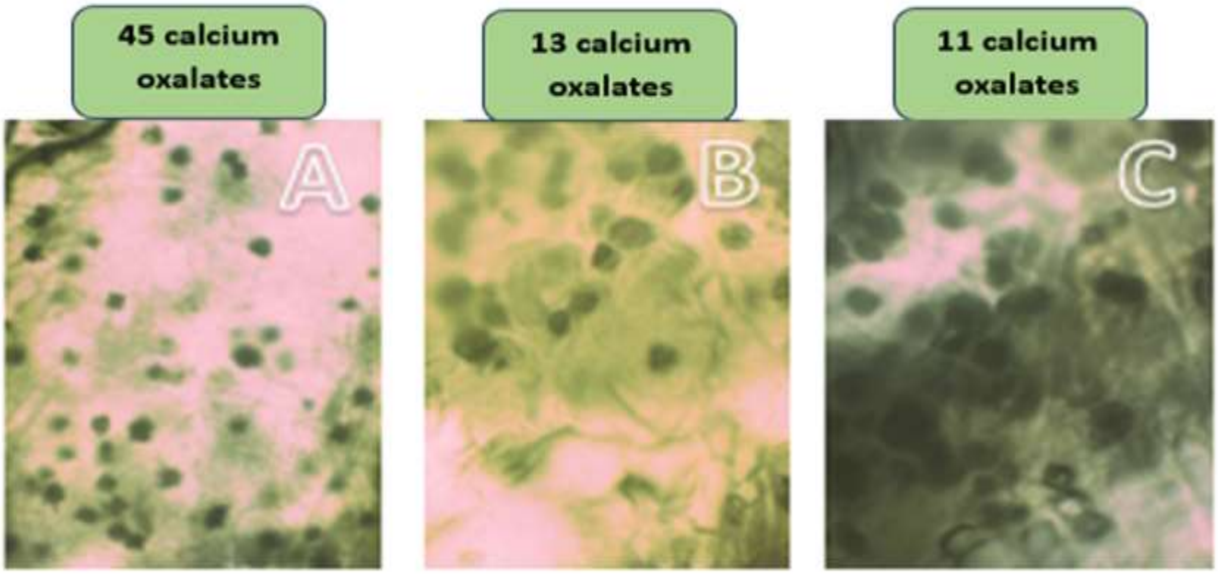

Figure 5. Characteristics of the oxalate crystals according to their location, (A) upper, (B) middle, and (C) basal of the Jalpa cultivar.

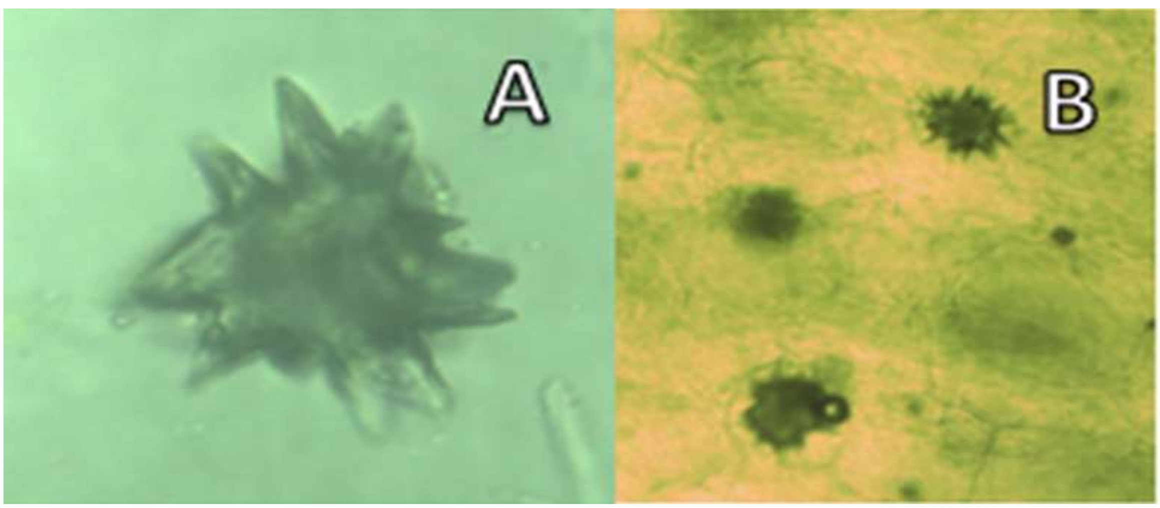

Figure 6. (A) Oxalate crystal basal and (B) Oxalate crystals upper part of the cladode in 10 X objective.

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The evaluation of the average comparison of the number of calcium oxalate crystals in the upper, middle and basal parts of the tender prickly pear did not reveal interactions between the cultivars. To this end, a comparison of general means was carried out for the analysis of cultivars, as described in Table 2.

The levels of calcium oxalates in the cultivars, both in the upper and middle parts of the tender prickly pear (Table 2), indicated that Villanueva exhibits the highest concentration, recording 58.50 and 45.69, respectively. In the basal part, the highest concentration was observed in O. Elefante, with 36.00 calcium oxalate crystals. The total amount of calcium oxalates covering the upper, middle, and basal parts was determined to be 131.87 for Villanueva and 102.82 for O. Elefante. Berhe *et al*. (2023), in their study on calcium oxalate and other cladode features in *Opuntia ficus-indica* resistant cultivars to *Dactylopius coccus* Costa reported a lower concentration of calcium oxalate crystals than those found in this study, with 20.7 ± 2.08 in the *O. cochenillifera* cultivar. However, they note that calcium oxalate crystals function as defense mechanisms against *D. coccus* in *O. ficus-indica* cultivars. The number of oxalates varies depending on the cultivar, growth stage, and other agronomic conditions.

Table 2. Comparison of general means number of calcium oxalates of tender prickly pear among cultivars.

Cultivars	Upper	Middle	Basal	Total oxalates
Jalpa	34.01 [b]	24.12 [b]	26.70 [a]	84.84 [b]
Villanueva	58.50 [a]	45.69 [a]	27.68 [a]	131.87 [a]
O. Elefante	30.99 [b]	35.83 [ab]	36.00 [a]	102.82 [ab]

The mean comparison of calcium oxalate crystals in the upper, middle, and basal parts of the tender prickly pear indicated a reduction in the number of oxalates as the weight range increased. However, no significant differences were detected in the basal part. The trend for the total calcium oxalate count was linear, indicating a decrease in oxalate content with increasing weight range (Table 3). This finding aligns with Rodríguez-Garcia *et al*. (2007), demonstrating that greater weight and maturation of the tender prickly pear correspond to lower concentrations of calcium oxalates.

Table 3. Comparison of general means number of calcium oxalates if tender prickly pear between weight ranges.

Ranges	Weight	Upper	Middle	Basal	Total Oxalates
1	(<150 g)	58.42 [a]	49.63 [a]	30.55 [a]	138.61 [a]
2	(150-300 g)	44.66 [ab]	45.46 [a]	33.14 [a]	123.27 [ab]
3	(300-600 g)	36.93 [bc]	24.07 [b]	27.88 [a]	88.90 [bc]
4	(600 g >)	24.64 [c]	21.70 [b]	28.92 [a]	75.27 [c]

The average concentration of calcium oxalates in tender prickly pear across different weight ranges was 58.42, according to the overall mean comparison of the calcium oxalates in tender prickly pear. However, range 4 (weighing less than 600 g) had the lowest amount of calcium oxalate crystals. This is because calcium oxalate crystals are more abundant when the prickly pear is young and decrease as it gets heavier.

In the middle region of the delicate prickly pear (Table 3), 49.63 crystals of calcium oxalates were observed for rank 1 (150 g), indicating the highest concentration. The opposite situation occurred when

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range 4 (600 g) contained 21.70 crystals of calcium oxalates, demonstrating the same correlation between the lighter weight of the nopal and the higher concentration of calcium oxalates. The concentration of calcium oxalates in the basal part (Table 3) suggests that the weight range 2 (150-300 g) exhibits a higher quantity compared to the other weight ranges. It is important to note that the count of calcium oxalates in the basal part does not follow the sequence observed in other sections where the count of these crystals was conducted. Villanueva had the highest concentration of calcium oxalates, registering 131.87, followed by Jalpa with 84.84 calcium oxalates. While assessing the oxalate concentration in Villanueva, Tovar-Puente *et al.* (2007) observed that this cultivar exhibited the lowest amount of calcium oxalate crystals compared to other cultivars analyzed in this study. This was attributed to the location where the tender prickly pear was cultivated, as well as its management and irrigation availability.

Conclusions

The chemical and nutrient content of prickly pear flour varies according to the variety and maturity stage, which is directly related to the weight. The cultivars Jalpa, Villanueva, and O. de Elefante exhibited significant differences in their characteristics, likely influenced by the maturity stage of the cladode. The amount of fiber present in the tender prickly pear flour from the three cultivars indicated a linear relationship, where it is observed that the fiber concentration increases as the nopal reaches a higher degree of maturity. Calcium oxalates were found in higher quantities in the upper region compared to the middle and basal regions. This response is consistent among all three cultivars. The amount of calcium oxalates showed an inversely proportional variation with age ranges; in other words, as you get younger, the presence of calcium oxalates is higher. This pattern remained consistent across all three cultivars.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Not applicable.

AUTHOR CONTRIBUTIONS

Conceptualization, M.G.A and R.E.V.A; methodology, M.G.A and R.E.V.A; software, M.G.A, R.E.V.A and E.O.S; validation, M.G.A, R.E.V.A and E.O.S; formal analysis, M.G.A, R.E.V.A and E.O.S; investigation, M.G.A, R.E.V.A and E.O.S; resources, M.G.A and R.E.V.A; data curation, M.G.A and E.O.S; writing—original draft preparation, M.G.A, R.E.V.A and E.O.S; writing—review and editing, M.G.A, R.E.V.A, E.O.S, E.V.G.C and M.C.O.Z; visualization, MGA; supervision, M.G.A, R.E.V.A and

E.O.S; project administration, M.G.A and R.E.V.A. All authors have read and agreed to the published version.

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REFERENCES

Aparicio-Fernández, X., Loza-Cornejo, S., Torres-Bernal, M.G., Velázquez-Placencia, N.J. and Arreola-Nava, H.J. 2017. Características fisicoquímicas de frutos de variedades silvestres de Opuntia de dos regiones semiáridas de Jalisco, México. Polibotánica. 43:1-26

Albergamo, A., Potosí, A.G., Di Bella, G., Amor, N., Lo Vecchio, G., Nava, V., Rando, R., Mansour, H. and Turco, V. 2022. Chemical characterization of different products from the Tunisian *Opuntia ficus-indica* (L.) Mill. *Foods, 11*, 155. https://doi.org/10.3390/foods11020155

Arcari, M.A., Martins, C.M.M.R., Tomazi, T., Gonçalves, J.L. and Santos, M.V. 2016. Effect of substituting dry corn with rehydrated ensiled corn on dairy cow milk yield and nutrient digestibility. *Animal Feed Science and Technology*. 221:167-173. https://doi.org/10.1016/j.anifeedsci.2016.08.005

Berhe, Y., Portillo, L., Delgado-Aceves, L., Palacios-Juárez, H. and Vigueras, A. 2023. Calcium oxalate and other cladode features in *Opuntia ficus-indica* resistant cultivars to *Dactylopius coccus* Costa. Journal of Agricultural Chemistry and Environment. 12:112-123. https://doi.org/10.4236/jacen.2023.122009

Bermúdez J.E. 2011. Obtención de harinas de cereales y leguminosas precocidas y su aplicación en alimentos para el adulto mayor. Instituto Politécnico Nacional. 168 p.

Bianco, H.W., Capote, T. and Garmendia, C. 2014. Determinación de humedad em harina precocida de maíz blanco utilizando un horno de microondas doméstico. Revista del Instituto Nacional de Higiene "Rafael Rangel". 45(2):29-36.

Castillo, S. F., Estrada, L., Margalef, M. I. and Tóffoli, S. L. 2013. Obtención de harina de nopal y formulación de alfajores de alto contenido en fibra. *Diaeta*. 31(142):20-26.

Contreras-Padilla, M., Pérez-Torrero, E., Hernández-Urbiola, M., Hernández-Quevedo, G., Real, A., Rivera-Muñoz, E.M. and Rodríguez-García, M.E. 2011. Evaluation of oxalates and calcium in nopal pads (*Opuntia ficus-indica* var. redonda) at different maturity stages. *Journal of Food Composition and Analysis.* 4(1):38-43. https://doi.org/10.1016/j.jfca.2010.03.028

Guzmán, L.D. and Chávez. J. 2007. Estudio bromatológico del cladodio del nopal (*Opuntia ficus-indica*) para el consumo humano. *Revista de la Sociedad Química del Perú*. 73(1):41-45.

Diego-Zarate, L.M., Méndez-Zamora, G., Rivera-De Alba, J.A. and Flores-Girón, E. 2021. Efecto del nopal (*Opuntia* spp) deshidratado en polvo sobre las propiedades fisicoquímicas y sensoriales de salchichas Viena. *Biotecnia. Revista de Ciencias Biológicas y de la Salud.* 23(2):89-95.

Enríquez, M.A.E. and Ojeda, G.L. 2020. Evaluación bromatológica de dietas alimenticias, con la inclusión de harina de plátano de rechazo. *Revista Espamciencia*. 11(1):12-18.

Junges, D., Morais, G., Spoto, M.H.F., Santos, P.S., Adesogan, A.T., Nussio, L.G. and Daniel, J.L.P. 2017. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. Journal of Dairy Science. 100(11):9048-9051. https://doi.org/10.3168/jds.2017-12943

Keshun, L. 2019. Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. Algal Research. Volume 40. 1-5 p. https://doi.org/10.1016/j.algal.2019.101486

Kung Jr. L., Shaver, R.D., Grant, R.J. and Schmidt, R.J. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*. 101(5):4020-4033. https://doi.org/10.3168/jds.2017-13909

Maldonado-Santoyo, M. and Morales-López G. 2022. Análisis químico y nutricional en hojas de *Ricinus communis*. *Revista Cubana de Química*. 34(1):3-18.

Morais, J.P.G. de, Cantoia Júnior, R., Garcia, T.M., Capucho, E., Campana, M., Gandra, J.R., Lucas G. Ghizzi, and Del Valle, T.A. 2021. Chitosan and microbial inoculants in whole-plant soybean silage. *The Journal of Agricultural Science*. 159 (3-4):227-235. https://doi.org/10.1017/S0021859621000447

Ponce-Luna, A., Pérez-Escalante, E., García-Curiel, L., Hernández-Hernández, A.A., Contreras-López, E. and Pérez-Flores, J. G. 2023. Potencial del nopal (*Opuntia ficus-indica*) para su incursión en la industria de la confitería: Una breve revisión. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos*. 8:491-500.

Rives-Castillo, S.C.H., Correa-Pacheco, Z.N., Bautista-Baños, S., Ventura-Aguilar, R. I., Schettino-Bermúdez, B.S.S., Ortega-Gudiño, P. and Barajas-Cervantes, A. 2021. Análisis químico proximal y térmico en harinas de productos residuales de nopal (*Opuntia ficus-indica*) para obtención de compuestos poliméricos biobasados. *Agrociencia* 55(5): 403-416. DOI: https://doi.org/10.47163/agrociencia.v55i5.2516

Rodiles-López, J.O., Arriaga-Martínez, L. P., Martínez-Flores, H.E., Zamora-Vega, R. and García, Martínez, R.M. 2019. Desarrollo de una tortilla adicionada con harinas de aguacate y nopal y su efecto en la reducción de colesterol, triglicéridos y glucosa en ratas. *Biotecnia*. 21:71-77. https://doi.org/10.18633/biotecnia.v21i2.909

Rodríguez-García, M.E., Lira, C., Hernández-Becerra, E., Cornejo-Villegas, M.A., Palacios-Fonseca, A.J., Rojas-Molina, I., Reynoso, R., Quintero, L.C., Del Real, A., Zepeda, T.A. and Muñoz-Torres, C. 2007. Physicochemical characterization of nopal pads (*Opuntia ficus indica*) and dry vacuum nopal powders as a function of the maturation. Plant Foods for Human Nutrition. 62:107-112. https://doi.org/10.1007/s11130-007-0049-5

Rössel-Kipping, E.D., Ortiz-Laurel, H., Moreno-Ovalle, F.M., López-Martinez, L.A. and Amante-Orozco, A. 2021. Physical and chemical attributes of prickly pear cactus (*Opuntia ficus-indica*) varieties Copena, Pelon Blanco and Pelon Rojo. *Agroproductividad*. 14(4):127-134. https://doi.org/10.32854/agrop.v14i4.1843

Sadhasivam, S., Britzi, M., Zakin, V., Kostyukovsky, M., Trostanetsky, A., Quinn, E. and Sionov, E. 2017. Rapid detection and identification of mycotoxigenic fungi and mycotoxins in stored wheat grain. *Toxins.* 9(10):302. https://doi.org/10.3390/toxins9100302

Saylor, B.A., Fernandes, T., Sultana, H., Gallo, A., Ferraretto, L.F. 2020. Influence of microbial inoculation and length of storage on fermentation profile, N fractions, and ruminal in situ starch disappearance of whole-plant corn silage. Animal Feed Science and Technology. 267, 114557. https://doi.org/10.1016/j.anifeedsci.2020.114557

Servicio de Información Agroalimentaria y Pesquera SIAP.2022. Producción Anual Agrícola de nopalitos.

Solís-García, S.M., Mendoza-Diaz, S.M., Juárez-García, M., Mancillas-Medina, J., Ávila-Ontiveros, M., Leyva-Maldonado, A. and Ramírez-García, J.F. 2021. Evaluación nutracéutica de un pan tipo coyota a partir de harinas de trigo, frijol y nopal con mermelada de higo. *Investigación y Ciencia*, 29(82):24-32. https://doi.org/10.33064/iycuaa2021823539

Torres-Ponce, R.L., Morales-Corral, D., Ballinas-Casarrubias, M.L. and Nevárez-Moorillón, G.V. 2015. El nopal: planta del semidesierto con aplicaciones en farmacia, alimentos y nutrición animal. *Revista Mexicana de Ciencias Agríc*olas. 5:1129-1142.

Tovar-Puente, A., Pando-Moreno, M., González-Rodríguez, H., Scott-Morales, L. and Méndez-Gallegos, S.J. 2007. Density of Calcium Oxalate Crystals in 15 Tender prickly pear Cultivated Species. *Journal of the Professional Association for Cactus Development*. 9:91-98.

Vázquez-Alvarado R.E., Valdez-Cepeda, R.D., Blanco-Macías, F., Ojeda-Zacarías, M.C. Méndez-Gallegos, S.J., Santos-Haliscak, J.A. and Olivares-Sáenz, E. 2015. Manejo de condiciones de estrés hídrico y nutricional en la producción de oxalatos de calcio en nopal verdura. XIV Simposium-Taller Nacional y VII Internacional "Producción y Aprovechamiento del Nopal y Maguey XIV Simposium-Taller Nacional y VII Internacional "Producción y Aprovechamiento del Nopal y Maguey".

Zúñiga, R., Gutiérrez, E.V., Gutiérrez, M.C., Olivares, E., Méndez, S.J., Carranza, R. and Vázquez, E.R. 2018. Caracterización $CaCO_3$ y CaC_2O_4 con análisis microfotográfico en *Opuntia ficus-indica* (L.) Miller. *Revista Mexicana de Ciencias Agrícolas.* 9(7):1524-1531.