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Changes in the fruits and seeds morphometric, germination, phytochemicals content, and antioxidant capacity in seed ripening of *Echinocereus stramineus*

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Abstract. Within the cacti family, most studies have focused on the physicochemical characterization of the stems, fruits, and seeds from *Mammillaria*, *Opuntia*, *Hylocereus*, and *Stenocereus* genera. However, few information is focused on the morphological and physicochemical characterization of the stems, fruits, and seeds of other genera widely distributed in the arid and semiarid zones of the Chihuahuan desert, such as the genus *Echinocereus*. The objective of this study was to analyze the fruit morphology, morphometry, germination process, and phytochemical content in four stages of seed in fruit ripening from *E. stramineus*. Morphometric parameters were measured by picking ten fruits and sixty seeds for each ripening stage. The characterization of the germination process included the germination percentage, mean germination time, germination speed, and mean germination speed which were computed 21 days after germination. The quantification of total phenols, flavonoids, tannins, reducing sugars, protein, and antioxidant activity of seeds was determined using colorimetric approaches under basal conditions. The morphometric results revealed a negative correlation between the fruit ripening stage and total mass (r= -0.980, p = 0.020), shell mass (r= -0.986, p = 0.014), pulp mass (r = -0.979, p = 0.021), fruit length (r = -0.978, p = 0.022), fruit width (r = -0.968, p = 0.032) and fruit area (r = -0.960, p = 0.04). The germination characterization process showed a negative association between the fruit ripening stage and the seed germination percentage (r = -0.979, p = 0.021) and between the seed mean germination time and the mean germination speed (r= -0.986, p = 0.014). The content of flavonoids, reducing sugars, proteins, and antioxidant capacity showed significant differences among the four stages of fruit ripening; however, no association was found between seed phytochemical content and the ripening stage. This study provides the first data on seed phytochemicals and information on the germination process of *E. stramineus* seeds.

Key words: Fruits variation, germination indexes, seed phytochemical.

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Introduction

In México, the most important families of plants that grow in arid and semiarid areas are the Agavaceae, Euphorbiaceae, Crassulaceae, and Cactaceae (Anderson, 2001). Some authors consider that the Cactaceae family integrates 1,500 to 1,600 species distributed between 110 to 124 genera (Lebgue *et al*., 2011). In Mexico, about 669 species have been identified, catalogued in 63 genera (Lebgue *et al*., 2011), of which Opuntia, Ferocactus, Mammillaria, Echinocereus, Coryphantha, and Cylindropuntia are the most important in abundance (Hunt, 2016).

In the cactus family, most studies have focused on the physicochemical characterization of the stems, fruits, and seeds of the genera *Mammillaria* (Aparicio-Fernández *et al*., 2013; Loza-Cornejo *et al*., 2017), *Opuntia* (Pichereaux *et al.,* 2016; Mena *et al.,* 2018; Núñez-Gastélum *et al.,* 2018; Valero-Galván *et al*., 2021), *Hylocereus* (Ibrahim *et al*., 2018; Magalhães *et al*., 2019), and *Stenocereus* (García-Cruz *et al*., 2017; Castro-Enríquez *et al*., 2020). However, scarce studies have been reported on the genus *Echinocereus*, which is also widely distributed in the arid zones of the Chihuahuan desert.

The *Echinocereus* genus has a great diversity of species, being distributed from the central region of northern of Mexico to the central-southern part of the United States of America, predominantly in the arid and semiarid regions (Bravo-Hollis and Sánchez, 1978). In Mexico, about 64 species have been recorded (Bravo-Hollis and Sánchez, 1978). This genus is classified into eight sections according to the morphometric characteristics of the plants (Sánchez *et al*., 2018), which include perennial plants with short, single or branched stems showing few to several ribs, mostly smooth, globose to cylindrical, and sometimes very long, erect or prostrate; sometimes pendulous, with vegetative and floriferous areoles. The flowers are diurnal, grown laterally, and show a medium-length receptacle tube with trichomes and spines (Bravo-Hollis and Sánchez, 1978). The fruit is fleshy and colorful, with a thin pericarp, thorny areolas, deciduous when ripe, and black and with tuberculate seeds. The *Echinocereus* species have various uses depending on the region where these plants are grown; for example, plants can serve as hedges due to their prickly nature. Besides, these species produce fleshy fruits called ‘pitayas’, which have a pleasant flavor, and different shades of color (white, pink, orange, red, purple), that contain small, soft, and edible seeds (Manzano, 2014). Local people from the arid and semiarid regions collect the ‘pitayas’, from June to September, to consume them fresh or processed to fresh water and jam. Also, the farmers use the stems to help expel or disintegrate the thorns; for example, when the cattle get thorns and cannot remove them, they place a piece of *E. stramineus* stems on the affected part and splint it.

Echinocereus stramineus (known as ‘alicoche’) has a wide distribution in Mexico, including the states of Chihuahua, Coahuila, Durango, Nuevo León, San Luis Potosí, Tamaulipas, and Zacatecas, but is also found in the states of New Mexico and Texas in the United States of America. It thrives in xerophilous thickets but can also be spotted in the pine-oak forests located within the transition zones, growing on rocky hills, on limestone or volcanic soils at heights of about 1,200 to 2,100 m.a.s.l. (Miller, 1988). The plants develop in a group of mono articulated cacti, not shrubby growth, that present small stems of globose to cylindrical shape reduced at the apex of 45 cm high and 8 cm wide, with 10 to 17 slightly tuberculate green ribs that form hemispherical conglomerates of more than 1 m wide and with up to 10 stems. Each plant is armed with 1 to 4 stout central spines, straight or curved, straw yellow to whitish, 4 to 9 cm long, with 7 to 14 pinkish to yellowish radial spines of 3 cm long. The flower does not appear at the apex of the stem, and it is funnelform, bright magenta, from 6 to 12 cm in diameter (Taylor, 1988). The fruit is globose with spines, red in color, fleshy, and has a strawberry aroma and flavor.

The recent studies reported a high content of alkaloids in stems of *E. merkeri*, *E. enneacanthus*, *E. chloranthusse,* and *E. triglochidiatus* species (Agurell *et al*., 1969). Similarly, flavonoids and sugars were quantified in flowers of *E. rigidissimus*, *E. stoloniferus*, *E. engelmannii*, *E. scopulorum,* and *E. triglochidiatus* (Miller, 1988). Furthermore, phenolics, terpenes, and alkaloids were measured in *E. stramineus* stems (Treviño *et al*., 2012). However, aspects of the ecology and bromatological composition of *E. stramineus* seeds remain uncharacterized. Therefore, the objective of this study was

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to determine the natural variation of *E. stramineus* seeds from the Samalayuca region (Northern Chihuahua, Mexico) by analyzing their morphometry, germination, and phytochemical composition in four fruit ripening stages.

Materials and Methods

Plant material

Samples of *E. stramineus* fruits were collected at the Sierra del Presidio, Samalayuca, Ciudad Juárez, Chihuahua, Mexico (latitude 31º 20' 33'' N; longitude 106º 24' 35'' W) at an altitude of 1300 m.a.s.l. This area has a hot and dry climate, with an average annual rainfall of 253.5 mm (the rainy season is from June to September and the dry season from October to May), and a mean monthly minimum temperature of 8.2 °C and a mean monthly maximum temperature of 26.4 °C. The predominant varieties of vegetation in this zone are crasicaule, microphyllous, and thorny scrub. On the slopes of the Sierra de Presidio, an area of 2 ha was selected where *E. stramineus* develops abundantly (Figure 1A). At this site, fifty plants (without apparent damage caused by insects and animals) of *E. stramineus* were randomly selected and observed every two weeks from April 1st to June 30th, 2023. In this period, after two weeks of observation, the flowering of the plants emerged (Figure 1B), and the first fruits appeared on June 1 (Figure 1C). For assessing the impact of fruit ripening on the morphometric and biochemical characteristics and germination process of seeds, twenty fruits were selected at four different maturation stages based on fruit coloration [green (EI) 60 days after flowering, green red (EII) 70 days after flowering, red (EIII) 80 days after flowering, and dry state (EIV) 90 days after flowering] (Figure 1C). Once collected, they were placed in hermetically sealed plastic bags and immediately transported to the laboratory.

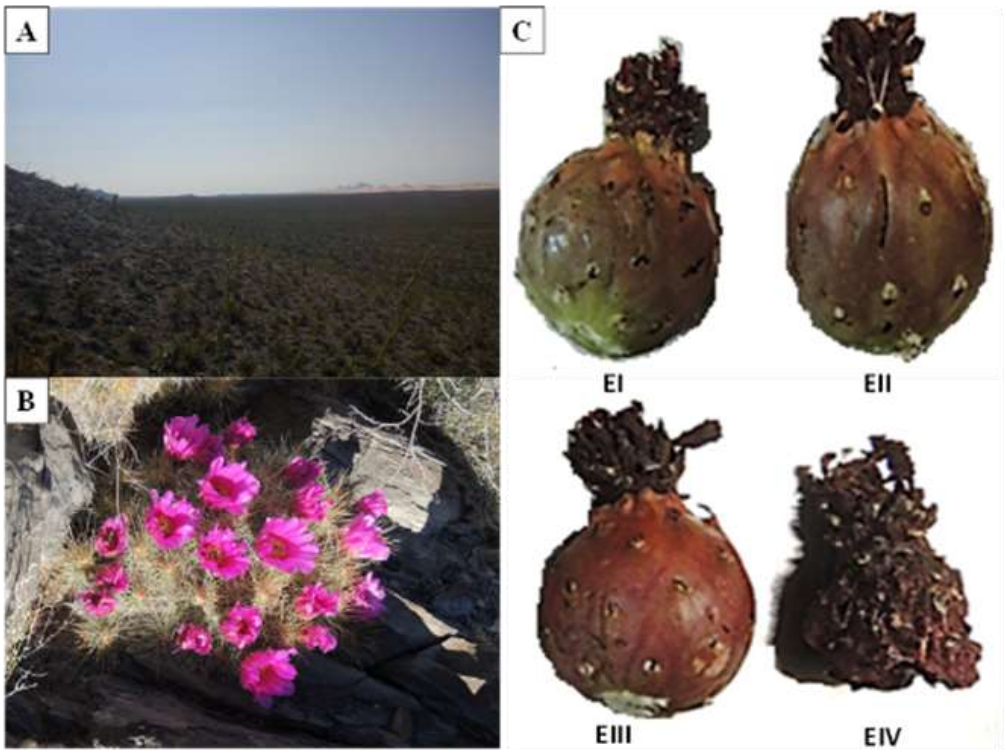

Figure 1. Panoramic view of the harvest location of *E. stramineus* fruits at Sierra del Presidio, Samalayuca, Ciudad Juárez, Chihuahua, Mexico (A); *E. stramineus* plant flowering (B); four ripening stages (EI to EIV) of *E. stramineus* fruits (C).

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Morphometric analysis of fruits and seed extraction

The collected fruits were washed with 500 mL of soapy water by shaking in an orbital shaker (Labnet®, USA) for 10 min. Then, the soap residues were removed with two washes of distilled water under the same conditions as the previous step, and finally, fruits were dried with blotting paper. After that, 10 fruits were randomly selected, and the mass was weighed an analytical balance (Velab®, USA) for each of the ten fruits from each ripening stage. Next, a digital photograph of the ten fruits from each ripening stage was taken to measure the area and perimeter (the surface observed in the image recorded in the photograph), length, and width by the ImageJ digital image processing software (National Institute of Health, USA). Then, fruits were cut longitudinally to separate the peel from the pulp. After that, the pulp and peel were weighed individually for each fruit and ripening stage using the analytical balance. Subsequently, seeds were recovered from fruit pulp by placing it in a sieve and washing it with distilled water until pulp was removed. Finally, seeds were disinfected with 5 % (v/v) sodium hypochlorite for 5 min, washed with distilled water twice and left to dry at 25 ºC for five days.

Morphometric analysis of seeds

Once dried, 60 seeds from each ripening stage, were randomly selected and the individual mass was measured on the analytical balance. Subsequently, the 60 seeds for each ripening stage were then placed evenly on a millimeter sheet and a digital photograph was taken per each ripening stage. The digital images were used to measure the seed area and perimeter (the surface observed in the image recorded in the photograph), length, and width using the ImageJ digital image processing software (National Institute of Health, USA). In addition, seeds were observed under an optical microscope (VanGuard®, China) to define the testa characteristics such as shape, color, and texture.

Seed germination

Before the germination process, seeds were disinfected using 70% (v/v) sodium hypochlorite solution for 3 min, rinsed with abundant distilled water for 1 min, and immersed in fungicide solution (Captan 1 g 20 mL^{-1}) for 2 min, to be subsequently sown. Next, 30 seeds were placed, in groups of 10, in sterilized Petri dishes, to which 20 g of pre-sterilized sandy-type soil followed by 13 mL of distilled water were added (Reyes-Corral *et al*., 2022). Finally, plates were placed in a bioclimatic chamber at 25 °C, applying a photoperiod of 12 h light/darkness. The germination progress of each seed was examined every three days for 25 days, counting the number of germinated seeds. Those not germinated after this period were considered non-viable or latent, and those with a root protrusion of 1 mm or more were marked as germinated (Baskin and Baskin, 2014). Finally, to find out the differences of seed germination characteristics among the fruit ripening stages, different germination indexes were calculated, including the average germination rate, average germination time, germination speed index, and percentage of germination, according to the methodology proposed by Souza *et al*. (2016).

Quantification of seed phytochemicals

A standard extract was obtained according to the method proposed by Reyes-Corral *et al.* (2022) and Álvarez-Parrilla *et al*. (2011). From each ripening stage, 0.1 g of seeds were manually ground in a mortar using a pestle until a fine powder. Then, 500 mL of 80% (v/v) methanol (JT Baker®, USA) solution was added and blended with the help of the same pestle. Next, the homogenate was transferred to a tube to be stirred at 500 rpm for 10 min in darkness and sonicated for 30 min at 4 °C in darkness. Afterward, the extract was centrifuged (Eppendorf, USA) at 3,500 rpm for 15 min at 4 °C, and the supernatant was collected into a new tube. This methodology was repeated twice, and the two

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supernatants were mixed and brought to a final volume of 2 mL. The samples were stored at -20 °C until further analyses.

The content of reducing sugar was measured by the spectrophotometric approach described by Ávila-Núñez *et al*. (2012). Briefly, 100 µL of each standard extract was mixed individually with 300 µL of the DNS (3,5-dinitrosalicylic acid) reagent in an assay tube. Then, samples were incubated at 95 °C in a dry bath for 5 min, and immediately later, the mixture was cooled in an ice bath for 5 min. Next, 250 µL of each sample was taken and individually placed in a 96-well microplate, and the absorbance was measured at 540 nm. The calibration curve was performed using glucose as the standard, and results were expressed as mg of glucose equivalents (GE) per g of seed dry weight (DW) (mg GE·g^{-1} DW).

The total phenolic content was determined by a spectrophotometric approach, according to Georgé *et al.* (2005). Briefly, 25 µL of standard extract, 125 µL of the Folin–Ciocalteu reagent (10% v/v), and 100 µL of Na_2CO_3 were placed into a well of 96-well microplate, and the mixture was incubated at 25 ºC for 15 min in darkness. Finally, the absorbance was measured at 740 nm. The calibration curve was performed using gallic acid as standard, and data were expressed as mg gallic acid equivalents (GAE) per g of seed (mg GAE·g^{-1} DW).

The total flavonoids were determined by a spectrophotometric approach, according to the methodology defined by Georgé *et al.* (2005). Briefly, 62.5 µL of standard extract, 46.5 µL of $NaNO_2$ (5%), 46.5 µL of $AlCl_3$ (10%), and 62.5 µL of 0.5 M NaOH were placed into a well of 96-well microplate, and the mixture was incubated for 30 min at 25 ºC in darkness. Finally, the absorbance was measured at 510 nm. The calibration curve was performed using catechin reactive as standard, and data were expressed as mg catechin equivalents (CE) per g of seed (mg CE·g^{-1} DW).

The total tannins were determined by the colorimetric method using the 4-(dimethylamino) cinnamaldehyde (DMAC) assay (Reyes-Corral *et al*., 2022). In brief, 50 µL of each standard extract was mixed individually with 200 µL of 0.1% DMAC reactive in a 96-well microplate, and the reaction was allowed to proceed for 5 min at 25 ºC in darkness. The absorbance was measured at 640 nm. The calibration curve was performed using catechin reactive as the standard, and data were presented as mg catechin equivalents (CE) per g of seed (mg CE·g^{-1} DW).

All absorbances were measured in a BioRad xMarkTM Plus Microplate Absorbance Spectrophotometer (Hercules®, USA), and data were acquired using the Microplate Manager 6.0 (Tokyo, Japan) computer software. All quantifications were carried out in triplicates (biological replicates) per each fruit ripening stage.

Quantification of seed antioxidant activity

The antioxidant activity was analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) methods, according to the methodology proposed by Moreno-Escamilla *et al*. (2017).

For the quantification by DPPH assay, 25 µL of the standard extract was placed in a well of a 96-well microplate and blended with 200 µL of DPPH reagent (190 mM DPPH (Sigma-Aldrich, México) in 100% (v/v) methanol). Then, the mixture was incubated for 30 min at room temperature in darkness, and absorbance was read at 517 nm every minute for 1 h.

For the quantification by FRAP assay, 24 µL of the standard extract was placed in a well of 96-well and mixed with 180 µL of FRAP reagent (10 mM 2,4,6-tri[pyridil]-s-triazine (TPTZ) (Thermo-Fisher®, México); 300 mM $C_2H_3O_2Na$ (Thermo-Fisher®, México); 20 mM $FeCl_3$ (Thermo-Fisher®, México). Then, the mixture was incubated for 30 min at 37 °C, and the absorbance was read at 595 nm every minute for 30 min.

All absorbances were measured in a BioRad xMarkTM Plus Microplate Absorbance Spectrophotometer (Hercules®, USA), and data were acquired using the Microplate Manager® 6.0 (Tokyo, Japan) computer software. For both methods, the calibration curve was performed using TROLOX (Sigma-Aldrich®, México) as standard, and results were expressed as µM of TROLOX equivalents per g^{-1} of dry weight (mg TE·g^{-1} DW). All determinations were carried out in triplicates (biological replicates) per each fruit ripening stage.

Extraction and quantification of seed proteins

The seed proteins were extracted from 0.1 g of seeds by the TCA/acetone-phenol method (Valero-Galvan *et al*., 2014). The final pellet of proteins was solubilized in 50 µL of a solution of 7 M urea (Jalmek-Scientific®, México). The insoluble material was removed by centrifugation. Finally, solubilized proteins in the supernatant were quantified, according to the Bradford method using BSA as the standard (Merck®, México) (Ramagli and Rodriguez, 1985). All seed protein extractions were carried out in triplicates per each ripening stage.

Statistical analysis

Before the statistical analysis, data were analyzed using the Shapiro-Wilk normality test. The fruit and seed morphometry data, seed germination indexes, and seed phytochemical results of the four ripening stages were analyzed by one-way ANOVA, followed by multiple comparisons of the Duncan test. In addition, a Pearson correlation was performed to establish the relationships between fruit and seed morphometry, germination indexes, and the seed phytochemical content with the fruit ripening stage. The significant differences were established with a 95% confidence level among the groups. The data matrix was processed using the IBM SPSS® Statistics Base 22.0 software.

Results and discussion

Fruits and seeds morphometry

In the current study, a variation in the *E. stramineus* fruit shell color occurred, classifying them into four stages (Figure 1). The first ripening stage (EI) was categorized as an immature fruit phase, in which fruits showed a greenish-brown color (Figure 1C - EI). The second one (EII) was classified as an intermediate ripe fruit phase, and the fruits exhibited a brown color (Figure 1C - EII). The third one (EIII) was categorized as a soft ripe stage, in which fruits displayed a burgundy color (Figure 1C - EIII). The fourth stage was classified as a dry ripe stage, with dry fruits of a dark brown color (Figure 1C - EIV). Similar patterns of color have been identified in the fruit ripening of other cactus species. In *Hylocereus undatus*, fruits started with a light green skin mixed with a hint of red 25 days after flowering; over the next four days, the color intensified to 70% bright red before turning into a purple-red color after an additional two days (Centurion *et al*., 2008). This change has been associated with a decrease in the firmness of the fruit peel and pulp, which could be due to the increase in the activity of the pectin methyl esterase enzyme (Centurion *et al*., 1999). Likewise, in fruits of pitahaya (*H. undatus*) harvested in different stages of maturation and stored for 12 days at 20 ºC, the skin color changed from green, pinkish-yellow, and red to reddish-orange, red, and red, respectively, after the

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storage period (Osuna *et al*., 2011). Similarly, changes in the fruit color in different ripening stages were observed in *Cylindropuntia spinosior* (González-Fernández *et al*., 2023).

The values of the fruit morphometric variables showed a progressive decline during ripening (Table 1). The EI ripening stage showed the highest values in total mass, shell mass, pulp mass, length of fruit, width, area, and perimeter, while the EIV stage showed the lowest ones in total mass, shell mass, flesh mass, length, width, area, and the perimeter. The decrease in the total weight, shell weight, and pulp weight observed was about nine-, five-, and eleven-fold, respectively, from the hard-mature green stage (EI) to the final dry ripe stage (EIV) (Table 1). In addition, the fruit ripening stage was negatively correlated with total mass (r = -0.980, p = 0.020), shell mass (r = -0.986, p = 0.014), pulp mass (r = -0.979, p = 0.021), fruit length (r = -0.978, p = 0.022), fruit width (r = -0.968, p = 0.032) and fruit area (r = -0.960, p = 0.040), suggesting that these characteristics decrease as the fruit ripening stage increases (Table 1).

Table 1. Morphometric variables of *E. stramineus* fruits from the four fruit ripening stages.

Variables	Fruit stages				ANOVA
	EI	EII	EIII	EIV	
Total weight (g)	38.2±3.9^{d}*	29.0±6.5^{c}	9.9±3.4^{b}	4.2±1.7^{a}	0.001
Shell weight (g)	7.8±2.6^{d}	6.6±1.0^{c}	3.3±1.4^{b}	1.4±0.9^{a}	0.001
Pulp weight (g)	31.5±3.1^{d}	21.2±6.3^{c}	6.6±2.5^{b}	2.8±0.8^{a}	0.001
Length (cm)	4.6±0.1^{c}	3.9±0.1^{c}	3.7±0.1^{b}	2.9±0.1^{a}	0.001
Width (cm)	3.3±0.0^{c}	3.2±0.1^{c}	2.7±0.1^{b}	2.2±0.1^{a}	0.001
Length/width ratio	1.39±0.05^{d}	1.21±0.1^{a}	1.37±0.1^{c}	1.31±0.1^{b}	0.001
Area (cm^2)	10.1±0.2^{d}	9.5±1.0^{c}	8.2±0.3^{b}	4.9±1.1^{a}	0.001
Perimeter (cm)	11.3±0.2^{c}	11.2±0.1^{c}	10.2±0.2^{b}	7.9±0.2^{a}	0.001

Data are expressed as the mean ± standard deviation (n= 10) and were analyzed using a one-way ANOVA ($p \leq 0.05$).
*Values in rows with different letters differ significantly (Duncan $p \leq 0.05$).

The decrease in the values of morphometric variables of the fruits evaluated in the present study showed a similar tendency to those observed in the fruit ripening stages of the pitayas of *Stenocereus pruinosus*, *S. stellatus*, and *C. spinosior* (Luna-Morales, 2004; González-Fernández *et al*., 2023). However, those results differed from those found in the fruit ripening stages of *Selenicereus megalanthus*, *Hylocereus undatus* and *Escontria chiotilla*, in which the morphological variables increased as the ripening process advanced (Centurion *et al*., 2008; Ruiz-Huerta *et al*., 2015, Sotomayor *et al*., 2019).

In the current study, significant differences ($p \leq 0.05$) were also observed in morphometric variables of mass, length, width, area, and perimeter of the seeds during the ripening stages (Table 2). The EIII maturation stage showed seeds with the highest weight, while the EI, EII, and EIV stage seeds exhibited similar values in this characteristic. Moreover, seeds of the EI stage exhibited the highest values in width and length, while the EIII and EV stage seeds presented the lowest ones. The area and perimeter of the seed were higher in the EI stage, while the EII, EIII, and EIV stages exhibited the lowest values in both characteristics. Finally, no significant differences were observed between the length/width ratio of the seeds of the different stages of fruit ripening (Table 2). Further, no correlations were observed between seed morphometric variables and the fruit ripening stage.

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Table 2. Morphometric variables of *E. stramineus* seeds from the four fruit ripening stages.

Variables	Fruit ripening stages				ANOVA
	EI	EII	EIII	EIV	
Weight (mg)	0.40±0.0[a]*	0.40±0.0[a]	0.60±0.0[b]	0.40±0.0[a]	0.001
Length (mm)	1.26±0.08[c]	0.32±0.03[b]	0.23±0.02[a]	0.25±0.02[a]	0.001
Width (mm)	0.89±0.08[c]	0.24±0.02[b]	0.17±0.02[a]	0.17±0.02[a]	0.001
Length/width ratio (mm)	1.42±0.11[a]	1.36±0.21[a]	1.40±0.16[a]	1.45±0.16[a]	0.380
Area (mm^2)	0.87±0.11[b]	0.06±0.01[a]	0.03±0.01[a]	0.04±0.00[a]	0.001
Perimeter (mm)	3.53±0.21[c]	0.94±0.06[b]	0.68±0.07[a]	0.72±0.04[a]	0.001

Data are expressed as the mean ± standard deviation (n= 60) and were analyzed using a one-way ANOVA ($p \leq 0.05$). *Values in rows with different letters differ significantly (Duncan p ≤ 0.05).

In the cactus family, the knowledge of the seed morphometric characteristics (mass, width, length, and thickness) could be associated with the germination rates and the subsequent establishment of the seedlings (Rojas-Aréchiga *et al.*, 2001; González-Cortés *et al*., 2018;). Likewise, the seed mass has been related to a better dispersion, viability, emergence, survival, and competitive ability of seedlings (Harper, 1970). In addition, knowledge of the morphometric variables of the seeds could be the basis for the identification, *ex-situ* conservation, better seed collection, and storage process of these species (González-Cortés *et al*., 2019). Also, physiological seed maturity is attained at maximum dry matter accumulation and is related to maximum yield and physiological seed quality (Zavala-Hernández *et al*., 2015). Although no data on the kinetics of variables associated with the seed morphometric variation have been reported in seeds from different fruit ripening stages of *E. stramineus*, also there are few studies on other species of plants. These results were like that observed in the kinetics of variables related to seed morphometric variation at four fruit ripening stages of *Cylindropuntia spinosior*, where no correlations were observed between seed morphometric variables and the fruit ripening stages (González-Fernández *et al.*, 2023). However, the results differed in the dry weight, fresh weight, length, diameter, and length/diameter ratio in *Jatropha curcas* seeds that increased as the days after anthesis of the seeds grew; however, 75 days after anthesis showed a trend to decrease as the day of this process increased (Zavala-Hernández *et al*., 2015).

Although no information on the morphometric variables in seeds of *E. stramineus* has been reported, these variables were analyzed recently in other species of the cactus family. The seeds of *Mammillaria parkinsonii* showed values of 0.4 mg in mass, 0.98 mm in length, and 0.62 mm in width, as well as a length/width ratio of 1.58, considering them as oval seeds (Uribe-Salazar *et al*., 2022). These results were like those showed in the current study by seeds from the EI ripening stage (Table 2). However, the mass, length, and width values of these seeds were lower than those found in *C. leptocaulis* (0.007 g, 4.1 mm, and 3.40 mm, respectively), *C. imbricata* (0.020 g, 4.1 mm, and 3.87 mm, respectively), and *C. spinosior* (0.018 g, 4.2 mm, and 3.80 mm, respectively) (Reyes-Corral *et al.,* 2022), and in *Opuntia polyacantha* (0.026 g, 5.4 mm, and 4.58 mm, respectively), *O. phaeacantha* (0.028 g, 4.5 mm, and 3.77 mm, respectively), *O. macrocentra* (0.017 g, 4.3 mm, and 3.72 mm, respectively), *O. engelmannii* (0.009 g, 3.5 mm, and 3.07 mm, respectively) (Núñez-Gastélum *et al*., 2018), being all these samples collected in the same area where *E. stramineus* plants evolve. In the same way, the length and width values of *E. stramineus* seeds were lower than those found in fruits of *O. ficus-indica* (4.8 mm and 4.5 mm), *O. creeper* (4.4 mm and 4.1 mm), *O. megacantha* (4.2 mm and 3.8 mm), *O. stenopetala* (3.4 mm and 3.1 mm), *O. durangensis* (3.3 mm and 2.9 mm), *O. engelmannii* (2.7 mm and 2.5 mm), and *O. microdasys* (2.5 mm and 2.3 mm) collected in Coahuila State (Mexico) (González-

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Cortés *et al*., 2019). In another study, the mass, length, and width values of the seeds from 24 genotypes of *Opuntia* spp. were higher than those found in the present study (Guerrero-Muñoz *et al*., 2006). Likewise, *E. stramineus* seeds revealed lower values in mass, length, and width than those found in five species of Cactaceae located in Jalisco State, Mexico, which were *Coryphantha clavata* (1.8 mg, 1.4 mm, and 0.9 mm, respectively), *C. bumamma* (1.9 mg, 2.3 mm, and 0.7 mm, respectively), *Mammillaria uncinata* (1.1 mg, 1.0 mm, and 0.7 mm, respectively), and *Ferocactus histrix* (0.6 mg, 1.4 mm, and 0.9 mm, respectively); however, the length and width values for the EI ripening stage seeds were similar to those observed in *C. cornifera* seeds (0.9 mm and 0.6 mm) (Loza-Cornejo *et al*., 2012).

Germination process

The germination percentage (G), mean germination time (MGT), germination rate index (GRI), and mean germination rate (MGR) showed significant differences among the four ripening stages (Table 3). The EIV stage presented the lowest values of G, followed by the EIII, the EII, and the EI. In addition, the Pearson correlation showed negative associations between the fruit ripening stage and the germination percentage (r = -0.979, p = 0.021). The EIII stage presented the lowest values of MGT, followed by the EIV, the EII, and the EI. These results showed that EIII stage seeds germinate faster than seeds from the other three stages, while EI stage seeds were the slowest to sprout, supporting that the EIII stage fruits have an optimal level of ripening. The GRI of the EII stage seeds showed the highest values, while the EIV stage showed the lowest ones. The MGR of the EIII stage seeds showed the highest values, while the values of the EI stage presented the lowest.

Table 3. Germination indexes from of *E. stramineus* seeds from the four fruit ripening stages.

Index	Fruit ripening stages				ANOVA
	EI	EII	EIII	EIV	
G (%)	13.3±1.0^{d}*	11.7±1.1^{c}	7.5±1.0^{b}	2.5±1.1^{a}	0.001
MGT (Days)	15.87±0.31^{d}	14.26±0.20^{c}	8.08±0.40^{a}	10.68±0.10^{b}	0.001
GRI (%Days^{-1})	1.24±0.10^{c}	1.54±0.20^{d}	1.02±0.13^{b}	0.22±0.15^{a}	0.001
MGR (Days^{-1})	0.06±0.01^{a}	0.07±0.02^{a}	0.12±0.04^{c}	0.09±0.01^{b}	0.001

Data are expressed as the mean ± standard deviation (n= 60) and were analyzed using a one-way ANOVA ($p \leq 0.05$.
*Values in rows with different letters differ significantly (Duncan p ≤ 0.05). MGT, mean germination time; GRI, germination rate index; MGR, mean germination rate; G, germination percentage.

In the Cactaceae family, a high germination percentage has been associated with seed coat size by functioning as a regulator of seed imbibition (González-Cortés *et al*., 2019). However, the physical and morphometric dormancy of the seed is the most significant factor for seed germination in species such as *Opuntia* and *Cylindropuntia* genera (Mandujano *et al*., 2005; Orozco-Segovia *et al*., 2007). Similarly, studies have found that *Opuntia* seeds present an innate and forced dormancy regulated by environmental factors such as temperature and light (Souza *et al*., 2016). Furthermore, knowledge of seed germination parameters could provide information about species' capacity for inhabiting different environments. Unfortunately, only a few studies have determined seed germination parameters in the cactus family, and to date, no research has addressed the germination of *E. stramineus* species in depth.

The G determined in this species was relatively low (Table 3). These results were lower than those calculated for *E. stramineus* seeds sown in Murashige and Skoog medium, where seeds presented a

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G of 80% (Garza-Padrón *et al*., 2010). However, the results determined in the present study were like those found in seeds of same genus as *E. pectinatus* (2.5-16.5%) (Díaz-Baca *et al*., 2020) and from the genus *Cylindropuntia* as *C. leptocaulis* (13.2 %), *C. imbricata* (10.0 %), and *C. spinosior* (3.2 %) (Reyes-Corral *et al*., 2022). Contrariwise, although most seeds from the EI stage required more time to sprout, they exhibited the highest G. Similarly, the results determined in the present study were lower than those estimated for seeds of other cactus species like *Mammillaria albilanata* (92.6 %), *M. bocasana* (93.3 %), *M. rhodantha* (99 %)*, M. columbiana* (93.9 %) and *M. spinosissima* (55.1 %) (Ramírez-González *et al*., 2019); *C. hankeanus* (28.8 %), *C. baumannii* (85.5 %), *E. aurea* (32.2 %), *E. leucantha* (68.6 %), *E. candicans* (45 %), *E. spiniflora* (64.4 %), *G. bruchii* (38 %), *G. capillense* (87.5 %), *G. castellanosii* (93.3 %), *G. monvillei* (20.6 %), *G. mostii* (60 %), *G. quehlianum* (83.3 %), *G. schickendantzii* (51.1 %), *G. stellatum* (63.3 %), *H. pomanensis* (53.3 %), *P. mammulosa* (48.8 %), and *S. coryne* (88.3 %) (Sosa-Pivatto *et al*., 2014).

The mean germination time (MGT) is the average length of time needed by seeds for maximum seed germination, where the number of seeds germinated in a predetermined time interval was used as the basis for calculating the germination time (Table 3). Compared with other cactus species, values determined in this study were higher than those found in germination of seeds from *Pereskia aculeata* (5.3 days), *Astrophytum capricorne* (6 days), *A. myriostigma* (6 days), and *A. ornatum* (5 days) (Muro-Pérez *et al*., 2013; Souza *et al*., 2016). However, the results of this study were similar to those determined for germinated seeds of *P. grandifolia* (11.57 days), *Cereus hankeanus (*13.15 days), *Cleistocactus baumannii* (9.94 days), *Echinopsis aurea* (12.75 days), *E. leucantha* (15.51 days), *E. candicans* (13 days), *E. spiniflora* (11.53 days), *Gymnocalycium bruchii* (10.18 days), *G. capillense* (9.78 days), *G. castellanosii* (11.54 days), *G. monvillei* (12.94 days), *G. mostii* (9.69 days), *G. quehlianum* (12.58 day), *G. schickendantzii* (18.44 days), *G. stellates* (10.79 days), *Harrisia pomanensis* (15.38 days), *Parodia mammulosa* (8.45 days), and *Stetsonia coryne* (13.09 days) (Sosa-Pivatto *et al*., 2014; Souza *et al*., 2016).

The GRI indicates the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination (Table 3). These results were lower than those calculated for seeds of *Pilosocereus gounellei* (2.1 %·days^{-1}) (Fernandes *et al*., 2012); however, they were highest than those estimated for seeds from *C. leptocaulis* (0.05 %·days^{-1}), *C. imbricata* (0.07 %·days^{-1}), and *C. spinosior* (0.02 %·days^{-1}) (Reyes-Corral *et al*., 2022).

The MGR is defined as the reciprocal of the mean germination time (Table 3). The results of this study were highest than those determined in seeds of *C. leptocaulis* (0.04 days), *C. imbricata* (0.07 days), and *C. spinosior* (0.02 days) (Reyes-Corral *et al*., 2022).

An important factor for obtaining good quality seed in plants is the right time to harvest, which could be associated for the ripening stages of the fruit and seed, which in turn could impact the seed germination process. In various meaty fruits, the physiologic maturity of the seeds, usually, coincides with the begin of the alteration in the coloration of the epidermis of the fruits (Firmino de Azevedo *et al*., 2009). However, in the present study, a negative correlation was observed between the stage of fruit ripening and the percentage of seed germination. The seed harvesting and extraction before the physiological maturity attainment was also detrimental to germination in the genus *Schlumbergera* (Boyle *et al*., 1995). However, intermediate and mature fruit stages of *Opuntia dillenii* provided the seeds with the highest germination percentage (Firmino de Azevedo *et al*., 2009). Furthermore, other

studies have observed that the germination percentage of seeds collected in the early and late stages of fruit ripening tends to be low in the intermediate stages of ripening (Buitrago-Guacaneme *et al*., 2015), this low seed germination in early stages of maturity can be explained because of in some species, the newly ripened seeds have embryos smaller compared to the size of the seed, having a large amount of endosperm, and when these embryos have distinguishable cotyledons and radicle, they must grow to a critical length before the radicle emerges from the seed (Walck *et al*., 2002; Cárdenas *et al*., 2004).

Determination of phytochemical content and antioxidant capacity from seeds

The phytochemical composites are extensively spread in different plant tissues, gaining much attention due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health. In the present study, the seed content of flavonoids, reducing sugars, proteins, and antioxidant capacity determined by DDPH and FRAP showed statistically significant differences. However, no differences were observed between the content of phenolics and tannins (Table 4).

Table 4. Phytochemical content and antioxidant activity of *E. stramineus* seeds from four fruit ripening stages.

Bioactive compounds	Fruit ripening stages				ANOVA
	EI	EII	EIII	EIV	
Phenolics (mg GAE·g^{-1})*	10.7±1.4^{a}¥	10.5±2.6^{a}	10.8±1.2^{a}	9.2±1.4^{a}	0.59
Flavonoids (mg CE·g^{-1})**	20.1±1.5^{c}	18.3±0.6^{b}	20.1±1.0^{c}	16.7±0.4^{a}	0.04
Tannins (mg CE·g^{-1})**	0.59±0.1^{a}	0.69±0.09^{a}	0.69±0.0^{a}	0.73±0.1^{a}	0.49
Reducing sugars (mg GE·g^{-1})***	100.6±1.8^{b}	106.3±6.0^{b}	85.4±1.7^{a}	80.8±2.7^{a}	0.03
Proteins (mg·g^{-1})	1.58±0.5^{a}	1.46±0.2^{a}	1.46±0.3^{a}	5.2±1.4^{b}	0.00
DPPH (mM TE·g^{-1})****	27.7±1.9^{b}	14.3±3.3^{a}	22.5±3.1^{b}	23.2±3.3^{b}	0.00
FRAP (mM TE·g^{-1})****	69.3±2.6^{a}	97.3±11.1^{c}	85.2±5.0^{b}	108.0±7.3^{c}	0.05

Data are expressed as the mean ± standard deviation (n = 5) and were analyzed using a one-way ANOVA ($p \leq 0.05$). ¥Values in rows with different letters differ significantly (Duncan p ≤ 0.05). *GAE: gallic acid equivalents, **CE: catechin equivalents, ***GE: glucose equivalents, and ****TE: Trolox equivalents.

In this study, the analysis of phenolic content demonstrated that seeds of *E. stramineus* could be a good source of phenolic compounds, although seeds from fruit in the four ripening stages showed no differences. No data on the total phenolic content have been reported in seeds from different fruit ripening stages of *E. stramineus*; however, there are several studies on other species of the Cactaceae family. The results of the present study contrast with those shown when comparing two ripening stages of *Opuntia ficus-indica* fruits, where seed total phenolics contents were higher in unripe than in mature fruits (Cardador-Martínez *et al*., 2011), as well as in ripe than in overripe ones (Tounsi *et al*., 2011). Furthermore, the total phenolic content increased throughout the different fruit ripening stages in twelve accessions of *Opuntia* spp. (Pinedo-Espinosa *et al*., 2017). The phenolic content determined in this study was higher than that quantified in seeds from other cactus species as *O. microdasys* (0.36 mg GAE·g^{-1}), *O. macrorhiza* (0.95 mg GAE·g^{-1}), *C. imbricata* (1.0 mg GAE·g^{-1}), *O. ficus indica* (0.95-1.72 mg GAE·g^{-1}), *C. leptocaulis* (2.6 mg GAE·g^{-1}), *C. spinosior* (3.1 mg GAE·g^{-1}) (Amrane-Abider *et al*., 2018; Chahdoura *et al*., 2015: Reyes-Corral *et al*., 2022; Tounsi *et al*., 2011), and similar to those quantified in seeds of *O. polyacantha* (10.78 mg GAE·g^{-1}) (Núñez-Gastélum *et al*., 2018), but lower

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than those quantified in seeds of *O. engelmannii* (12.55 mg GAE·g^{-1}), *O. phaeacantha* (12.87 mg GAE·g^{-1}), *O. macrocentra* (12.89 mg GAE·g^{-1}), *O. joconostle* (50.43 mg GAE·g^{-1}), and *O. matudae* (59.48 mg GAE·g^{-1}) (Morales *et al*., 2012; Núñez-Gastélum *et al*., 2018). Also, the phenolic content of *E. stramineus* was higher than that quantified in chia (*Salvia hispanica*) seed (0.88-0.97 mg GAE·g^{-1}) (Reyes-Caudillo *et al*., 2008; Beltrán-Orozco *et al*., 2020).

The seed flavonoid content showed significant differences among the four ripening stages (Table 4). The seeds from the EI and EIII stages presented the highest values, while the EIV the lowest ones. These results were like those previous studies carried out in *O. ficus-indica*, in which total flavonoids were higher in the seeds from ripe than from overripe fruits (Tounsi *et al*., 2011), and no correlation was found between the seed flavonoid content and the different fruit ripening stages (Cardador-Martínez *et al*., 2011). Similarly, a positive correlation occurred between the seed flavonoid range and the fruit ripening stage when analyzing the pulp juice of different ripening fruits of *Opuntia* spp. (Pinedo-Espinosa *et al*., 2017). Likewise, the flavonoid content determined in seeds from *E. stramineus* was higher than those quantified in seeds from *O. ficus-indica* collected at the center of Tunisia (Morocco) (0.6-1.25 mg CE·g^{-1} DW) (Tounsi *et al*., 2011) and collected at La Soledad, Villa de Graciano, San Luis Potosí, Mexico (3.3-4.6 CE·g^{-1} DW) (Cardador-Martínez *et al*., 2011). Also, the flavonoid content of *E. stramineus* was higher than that quantified in chia (*S. hispanica*) seed (0.35 mg CE·g^{-1}) (Beltrán-Orozco *et al*., 2020).

The tannin content in seeds from the four ripening stages of *E. stramineus* fruits did not shows significant differences, showing a range between 0.73 to 0.59 mg CE·g^{-1} (Table 4). These results were like those observed in seeds from *O. ficus-indica* fruits collected at the unripe and ripe stages (1.3-2.0 mg CE·g^{-1} DW), in which no significant change was found between the two stages (Cardador-Martínez *et al*., 2011). However, the total tannins were higher in seeds collected from *O. ficus-indica* fruits at the ripe than overripe stage (0.36 and 0.27 mg CE·g^{-1} DW, respectively) (Tounsi *et al*., 2011). The tannin content determined in our study was higher than those presented by Tounsi *et al*. (2011), but lower than those shown by Cardador-Martínez *et al*. (2011).

The reducing sugar content showed significant differences in seeds from the four ripening stages of *E. stramineus* fruits. The EI and EII stage presented the highest values, while EIII and EIV the lowest ones (Table 4). Although no study about reducing sugars has been conducted in seeds from fruits in different ripening stages, data are found in other tissues of other species of the Cactaceae family. The results of the present study, contrast with those observed during the ripeness stage of *O. ficus indica* fruits, where ripening fruit was influenced by both cultivar and maturity (Kyriacou *et al*., 2016). Furthermore, fruit ripening increased the content of soluble solids, fructose, glucose, and total sugars (Kyriacou *et al*., 2016). Also, the reducing sugar content of *E. stramineus* seeds was higher than the quantified in seeds from other cactus species such as *C. imbricata* (8.1 mg GE·g^{-1}), *C. spinosior* (12.4 mg GE·g^{-1}), and *C. leptocaulis* (31.8 mg GE·g^{-1}) (Reyes-Corral *et al*., 2022).

The protein content showed significant differences in seeds from the four ripening stages of *E. stramineus* fruits. The EIV stage presented the highest values, while the EI, EII, and EIII stages the lowest ones (Table 4). Compared with studies carried out in other species of the Cactaceae family, the results of the present study were higher than those quantified in seeds from *O. joconostle* (0.02 mg·g^{-1}), *O. matudae* (0.03 mg·g^{-1}), and *C. leptocaulis* (0.25 mg·g^{-1}), similar than those observed in seed from *C. spinosior* (4.2 mg·g^{-1}), although lower than those detected in seeds from *C. imbricata*

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(5.6 mg·g^{-1}), *O. phaeacantha* (10.45 mg·g^{-1}), *O. macrocentra* (11.45 mg·g^{-1}), *O. polyacantha* (11.47 mg·g^{-1}), and *O. engelmannii* (14.75 mg·g^{-1}) (Morales et al., 2012; Núñez-Gastélum *et al.*, 2018; Reyes-Corral *et al*., 2022).

The antioxidant activity determined by the DPPH assay, showed significant differences for the seeds in the four maturation stages of *E. stramineus*. The EI stage presented the highest values, while EII presented the lowest values (Table 4). Although the antioxidant activity has not been analyzed in seeds from different ripening stages of *E. stramineus* fruits, several studies were carried out in other tissues of cactus family species. The seed antioxidant activity determined in this study was similar to those quantified from cacti seeds of *C. leptocaulis* (26.37 mmol TE·g^{-1}), although higher than those detected in seeds of *O. polyacantha* (2.25 µmol TE·g^{-1}), *O. engelmannii* (3.41 µmol TE·g^{-1}), *O. macrocentra* (3.81 µmol TE·g^{-1}), *O. phaeacantha* (4.30 µmol TE·g^{-1}), *C. spinosior* (12.02 mmol TE·g^{-1}), *C. imbricata* (13.08 mmol TE·g^{-1}) (Núñez-Gastélum *et al.*, 2018; Reyes-Corral *et al*., 2022), and in chia (*S. hispanica*) seed (41.1 µmol TE·g^{-1}) (Beltrán-Orozco *et al*., 2020).

The antioxidant activity determined by FRAP assay showed significant differences for the EIV stage with highest values, while the EI stage showed the lowest one (Table 4). These results were higher than those quantified in seeds from *C. imbricata* (5.6 mmol TE·g^{-1}), *C. leptocaulis* (10.7 mmol TE·g^{-1}), and *C. spinosior* (11.7 mmol TE·g^{-1}) (Reyes-Corral *et al*., 2022). Also, this value was higher than that quantified in chia (*S. hispanica*) seed (72.3 µmol TE·g^{-1}) (Beltrán-Orozco *et al*., 2020).

Conclusions

The results showed significant differences in the fruit and seed morphometry of *E. stramineus* throughout the four ripening stages. In fruits, the total mass, shell mass, pulp mass, length, width, and area were negatively correlated with the ripening stage, decreasing these variables as the fruit ripened. However, no correlations were observed in seeds. Moreover, the germination indexes calculated for seeds collected from the four fruit ripening stages also showed statistically significant differences, correlating negatively the fruit maturation state with the germination percentage and the mean germination time with the mean germination speed. The seeds of *Echinocereus stramineus* could be in the future industrially exploited as a good and cheap source of natural phytochemicals and antioxidant capacity. However, before considering incorporation as a dietary complement or as a natural food antioxidant, is necessary to carry out further studies to test their *in vivo* activity, bioavailability, and toxicity.

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CONSENT FOR PUBLICATION

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