

Influence of fertilization schemes on bioactive compounds, antioxidant activity and volatile profile in Pinto Centauro bean (*Phaseolus vulgaris* L.)

Nora A. SALAS-SALAZAR¹, Esteban SÁNCHEZ-CHÁVEZ², Dayany E. SÁNCHEZ¹, América CHÁVEZ-MARTÍNEZ³, Mayra C. SOTO-CABALLERO¹, María A. FLORES-CORDOVA^{1*}

¹Universidad Autónoma de Chihuahua, Facultad de Ciencias Agrotecnológicas, Departamento de Poscosecha, Avenida Pascual Orozco, Campus 1, Santo Niño, CP 31350, Chihuahua, México; nsalas@uach.mx; desc180196@hotmail.com; masotoc@uach.mx; mafloresc@uach.mx (*corresponding author)

²Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD), Departamento de Fisiología Vegetal, Av. Cuarta Sur No. 3820 Fraccionamiento Vencedores del Desierto, Delicias 33089, Chihuahua, México; esteban@ciad.mx

³Universidad Autónoma de Chihuahua, Facultad de Zootecnia y Ecología, Departamento de Ecología, Periférico Francisco R Almada, Km 1, CP 31453, Chihuahua, México; amchavez@uach.mx

Abstract

Beans are a nutritional staple food in many countries. Its high content of bioactive compounds provides antioxidant activity. Additionally, it contains volatile compounds that affect their sensory quality. This work aimed to study the effect of fertilization on the bioactive compounds, antioxidant activity, and volatiles in Pinto Centauro beans. An experimental design was used in randomized blocks with four fertilization treatments and four replications. The fertilization treatments were: Control (T0): N 41, P 46, and K 22 kg ha⁻¹; Treatment 1 (T1): N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹; Treatment 2 (T2): N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹; Treatment 3 (T3): N 50, P 66, K 25, S 25, and Zn 2 kg ha⁻¹. The concentration of bioactive compounds (total phenols, flavonoids, anthocyanins, and tannins) and antioxidant activity were measured in the testa, cotyledon, and the seed. The abundance of volatile compounds was determined in the seed. Results indicated that the testa showed a higher ($p > 0.05$) concentration of bioactive compounds (total phenols 78.40 (T2), flavonoids 6.22 (T2), anthocyanins 6.95 (T1), and tannins 3.12 (T1) mg g⁻¹) compared to the cotyledon and seed. This was reflected in the higher antioxidant activity that presented the testa ($p > 0.05$). About fertilization, T1 and T2 presented the highest values of bioactive compounds and antioxidant activity. Additionally, a positive and significant correlation was found between phenolic content and antioxidant activity. In addition, T2 favors the profile and abundance of volatile compounds, respectively. Finally, it is concluded that fertilization with T2 and the lowest concentrations of N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹ is the most suitable, as it favored the accumulation of bioactive compounds in the Pinto Centauro bean.

Keywords: anthocyanins; fertilization; Pinto bean; reducing power

Received: 25 Mar 2025. Received in revised form: 24 Jun 2025. Accepted: 25 Jun 2025. Published online: 26 Jun 2025.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Beans are the most important legume in world production, accounting for 85% of total legume production. India, Brazil, Myanmar, and China contribute 55% of this production, and Mexico 7% (CEDRSSA, 2020). Per capita consumption of beans worldwide is 2.46 kg per year (FIRA, 2022). Due to its source of carbohydrates (35-54%), low-cost proteins (16-33%), lipids (1-2%), vitamin B, minerals (3%), iron (Kan *et al.*, 2018), and fiber (5-10%), which represents 9% of the total seed content, it contributes to reducing cholesterol and promoting intestinal transit (Iniestra *et al.*, 2005).

Additionally, due to its content of functional ingredients such as phenolic compounds, flavonoids, anthocyanins, polyphenols, and tannins, it presents a high antioxidant activity (Rochin-Medina, 2022). Because of this, it is considered to have therapeutic properties (anti-mutagenic, vasodilatory, anti-inflammatory, and anticancer properties) (Abdulrahman *et al.*, 2020). The same ones that are associated with the prevention or reduction of chronic degenerative diseases such as diabetes, obesity, cardiovascular diseases, and colon, breast, intestine, and ovarian cancer (Bedoya and Maldonado, 2022). Such diseases lead to cellular degeneration through oxidative mechanisms, starting from the formation of free radicals in their initiation, propagation, and termination stages, which are formed by environmental pollution, stress, or poor nutrition. Although the free radicals are indispensable in various enzymatic reactions, high concentrations cause damage to cellular structures, generating diseases (Guija-Guerra and Guija-Poma, 2023).

Beans, with their antioxidant effect, can inhibit reactive oxygen species by preventing their oxidation and expansion, achieving a balance (Pérez-Pérez *et al.*, 2020). Beans also present the highest number of polyphenols compared to soybeans and chickpeas (García-Lafuente *et al.*, 2014) and exhibit antioxidant activity in the CaCo-2 cell line (Chen *et al.*, 2019).

However, region, type of cultivation, climatic conditions, soil, and management can affect their properties (Dong *et al.*, 2004). The above is correlated with modern agriculture, which promotes high yields at lower cost (Arreola and Sánchez, 2016). Therefore, balanced fertilization can increase the nutrient concentration in bean crops.

In addition, the inclusion of micronutrients such as zinc (Zn) and secondary macronutrients such as sulfur (S) in fertilization regimes represents a key agronomic strategy for improving the functional and nutraceutical quality of beans (*Phaseolus vulgaris* L.). Both elements participate in essential metabolic pathways, directly influencing the biosynthesis of bioactive compounds and the regulation of the plant's antioxidant system (Akhtar *et al.*, 2019).

Zinc is essential for the activity of more than 300 enzymes, including those related to antioxidant defense, such as superoxide dismutase (SOD), which protects cells from oxidative damage (Chasapis *et al.*, 2020). Several studies have reported that its application in beans contributes to a greater accumulation of phenolic compounds and flavonoids, significantly improving the antioxidant capacity of grains (Rengel, 2015; Rosa-Valdés *et al.*, 2020). Furthermore, zinc is linked to the regulation of genes involved in the synthesis of secondary metabolites, positioning it as a key element in improving the nutraceutical quality of seeds (Liu *et al.*, 2025).

Furthermore, sulfur is essential for the synthesis of amino acids such as cysteine and methionine, as well as glutathione, a broad-spectrum intracellular antioxidant. In beans, adequate sulfur intake has been shown to increase levels of anthocyanins, tannins, and other phenolic compounds, especially in the testa and cotyledons, resulting in greater antioxidant activity (Koprivova and Kopriva, 2014). This action is due, in part, to the participation of zinc in assimilation pathways that promote the synthesis of thiols and other bioactive metabolites of high functional value (Burow *et al.*, 2008).

Overall, fertilization with zinc and sulfur not only favors crop growth and development but also enhances its nutritional and functional value, positioning beans as a strategic food in healthy diets and biofortification programs (Gío-Trujillo *et al.*, 2022).

However, the acceptance or rejection of a food is related to the consumer's subjective perception, the same that are related to color, flavor, texture, consistency, and presentation of the product (Sharif *et al.*, 2017). Among these, flavor plays a central role, as it determines consumer acceptance. When flavor expectations are exceeded, the experience is communicated, and the purchase is repeated (Brückner and Wyllie, 2008).

Volatile compounds (VC) are responsible for the aroma and contribute to the flavor of foods. In beans, VC can characterize bad odors, affecting their properties. These are produced as a defense mechanism to adversities, signaling a response to activate their protection in the affected part (Brosset and Blande, 2022). These compounds are intensified by water stress, frost, attacks by microorganisms, or storage conditions such as heat, light exposure, presence of O₂, harvesting, and shelling, generating undesirable volatile compounds (Karolkowski *et al.*, 2023). A better understanding of the volatile compounds involved in unpleasant flavors and their origins should allow for the design of efficient strategies to limit their impact on overall perception for a more acceptable design of healthy foods. Regarding the influence of these minerals on the aromatic profile, several studies have shown that Zn fertilization not only contributes to improving the nutritional status of crops but can also indirectly modulate the formation of volatile compounds through its influence on metabolic pathways related to oxidative stress. In cereals such as corn (*Zea mays*) and wheat (*Triticum aestivum*), it has been reported that the application of Zn increases the activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, thereby promoting better management of oxidative stress (Cakmak, 2008; Wang *et al.*, 2021). This improvement in antioxidant activity can impact the reduction of aldehydes such as hexanal and nonanal, commonly associated with lipid peroxidation and, therefore, with sensory defects. In turn, this more balanced metabolic state may favor the synthesis of other compounds with greater aromatic value, such as terpenes derived from carotenoid degradation. Although this relationship has not yet been explored in depth in beans (*Phaseolus vulgaris* L.), the extrapolation of these findings to legumes raises the hypothesis that nutritional management with Zn could significantly influence the volatile profile of the grain, with potential implications for its sensory, technological, and commercial quality.

Therefore, the objective of this study was to determine the content of bioactive and volatile compounds and antioxidant activity of the seed, seed coat, and cotyledon from the Pinto Centauro bean cultivar with different fertilization sources.

Materials and Methods

Plant material

Beans from the Pinto Centauro cultivar were used because they are the most tolerant race of anthracnose, rust, and blight, with an early cycle and a seed coat tolerant to darkening, which favors its shelf life (Rico-Alderete *et al.*, 2020). The experiment was established at the "San Pedro" Ranch in the municipality of Cusihuirachi, Chihuahua (latitude: 28° 14' 22.075" N; longitude: 106° 50' 1.907" W; 1997 meters above sea level). During the evaluation period, average maximum temperatures ranged between 23 °C and 34 °C, and minimum temperatures between 2 °C and 16 °C, with an average precipitation of 108.44 mm (Quinta Lupita UNIFRUT meteorological station).

Experimental design

A completely randomized block design was used with four fertilization treatments and four replications. The fertilization treatments were: Control (T0): N 41, P 46, and K 22 kg·ha⁻¹; Treatment 1 (T1): N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹; Treatment 2 (T2): N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹; Treatment 3 (T3): N 50, P 66, K 25, S 25, and Zn 2 kg ha⁻¹. The planting was established in 1 ha (10,000 m²) with 20,833 plants for Blok in furrows 0.30 m high, 0.80 m wide, and 0.50 m long, with 0.15 m between plants and 0.80 m between furrows. A base fertilization was applied at the time of planting.

Samples of bean seeds from each treatment were taken under physiological maturity conditions and stored in refrigeration at 4 °C for subsequent analysis. For the experiment, 60 g of Pinto Centauro beans were used, which were soaked in 70 ml of distilled water for 12 hours. Subsequently, a manual separation was carried out, removing the seed coat from the seed and separating the cotyledons for each bean treatment. Once the testa and cotyledon were separated, they were left to dry at room temperature and ground in a coffee grinder (Protor Silex, China) until some flour was obtained and weighed with an electronic scale (Velab, New York, USA).

Weight

For the seed weight, 100 seeds were randomly taken, and the weight was recorded as g/100 seeds using an electronic scale (Velab, New York, USA), and this was done in triplicate (Aguirre-Santos and Gómez-Aldapa, 2010).

Seed dimension

The dimensions of beans (length, width, and thickness) were determined using a Vernier caliper Mettrod made in China. For this, 100 seeds per treatment were evaluated (Espinosa-Pérez *et al.*, 2015).

Extraction of bioactive compounds

Three grams of the flour (seed, testa, and cotyledon) were taken separately and homogenized with 25 ml of 70% acidified acetone (acetone/water/acetic acid, 70:29.5:0.5, v/v/v). Subsequently, the extracts were centrifuged (Thermo Scientific, ST 16R, USA) at 4000 rpm for 20 minutes at 10 °C. Then, the supernatant was separated, and the residue was extracted once more.

The determination was carried out according to the methodology described by Singleton and Ross (1965). In a test tube, 1.5 ml of 2% Na₂CO₃, 0.5 ml of 50% Folin-Ciocalteu reagent, 2.75 ml of deionized water, and 0.5 ml of the extract were added. The mixture was incubated at room temperature and in the dark for 60 minutes. Then, the absorbance was measured at 725 nm in a spectrophotometer (Thermo Scientific, G 10S UV Vis, USA). The results were expressed as milligrams of gallic acid per gram of dry weight (mg GAE g⁻¹).

The quantification of flavonoids was done according to the method of Zhishen *et al.* (1999). First, 250 µL of extract was placed and mixed with 75 µL of 5% sodium nitrite (NaNO₂) and stirred using a vortex (Genie2, Germany). The reaction was allowed to rest for 5 minutes, then 150 µL of 10% aluminum chloride (AlCl₃) and 500 µL of 1 M NaOH were added. The mixture was then diluted to a final volume of 3 mL with distilled water. The absorbance was measured at 510 nm in a spectrophotometer (Thermo Scientific, G 10S UV Vis, USA). Flavonoids were quantified based on a catechin standard curve (0.2-0.12 mg/mL). The resulting values were expressed in mg of catechin equivalents per gram of sample (mg CE g⁻¹).

Anthocyanins were determined according to the differential pH method described by Wrolstad (1976). The extracts were diluted 1:5 with 0.025 M potassium chloride (pH 1.0) and 0.4 M sodium acetate (pH 4.5) as buffers. Then, the absorbance was measured at 460 nm and 710 nm in a spectrophotometer (Thermo Scientific, G 10S UV Vis, USA). It was calculated using the formula $AM = (A \times MW \times DF \times 1000) / (\epsilon \times l)$, where the absorbance of the sample $A = (A_{\lambda \text{ vis-max}} - A_{700})_{\text{pH}1.0} - (A_{\lambda \text{ vis-max}} - A_{700})_{\text{pH}4.5}$, MW is the molecular weight (449.2), ϵ is the molar absorptivity of cyanidin-3-glucoside (26,900), DF is the dilution factor used, and l is the path length (1 cm). The results are reported as cyanidin-3-glucoside (mg C3G) g⁻¹ of sample (dry weight).

Condensed tannins were evaluated using the vanillin assay according to Flores-Córdova *et al.* (2017) and Price *et al.* (1978). First, 1 ml of the extract was taken, and 4 ml of HCl solution in MeOH (8% v/v) and a vanillin solution in MeOH (4% v/v) were added in a 1:1 ratio. The resulting mixture was maintained in a water bath at 30 °C for 20 minutes. Finally, the absorbance was measured at 500 nm in a spectrophotometer

(Thermo Scientific, G 10S UV Vis, USA). Tannins were quantified using a standard curve based on catechin and expressed as mg equivalents of catechin per fresh weight of the sample (mg EC g⁻¹).

Antioxidant activity

The antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) methodology was carried out according to the methodology of Meir *et al.* (1995). First, 5 g of the fruit was macerated in 5 mL of methanol (80%) and centrifuged at 6000 rpm for 10 min (Thermo Scientific, ST 16R, USA). From the resulting supernatant, 0.5 mL of the extract was taken and mixed with 2.5 mL of a freshly prepared DPPH solution (0.1 mM). The mixture was then incubated for 60 minutes in the dark, and the absorbance was measured at 517 nm in a spectrophotometer (Thermo Scientific, G 10S UV Vis, USA). The analysis was made in triplicate, and the results were expressed as mg TEAC g⁻¹ (Trolox equivalent antioxidant activity) of dry weight based on a calibration curve of Trolox.

Reducing power

The evaluation of the reducing power of the ferric ion (Fe, III) was carried out according to Flores-Córdova *et al.* (2018) and Oyaizu (1986). For this, 1 mL of the extract was mixed with 2.5 mL of phosphate buffer (5 mL, 0.2 M, pH 6.6) and 5 mL of 1% potassium ferricyanide K₃Fe (CN)₆. After 20 minutes of incubation (50 °C), 5 mL of 10% trichloroacetic acid were added to the reacting mixture and then centrifuged at 4500 rpm for 15 minutes (Thermo Scientific, ST 16R, USA). The supernatant of the solution (5 mL) was mixed with distilled water (5 mL) and ferric chloride (1 mL at 1%). Then, the absorbance was measured at 700 nm in a spectrophotometer (Thermo Scientific, G 10S UV Vis, USA). The results are expressed in absorbance units. Higher absorbance values indicate greater reducing power.

Volatile compounds

The preparation of the samples, the grinding process of the Pinto Centauro bean, was carried out in an Oster blender, 2006, USA, for each treatment. First, 5.0 g of ground sample was placed in a 40 ml amber vial sealed with PTFE/silicone septa (Supelco). Subsequently, the sample was heated at 50 °C for one hour and then exposed to the fiber for one hour. After this, the fiber was desorbed in the injection port for 5 minutes at 200 °C within the GC-MS system (Agilent USA), equipped with a DB-WAX column (60m × 0.25 mm, 0.25 μm, Agilent USA). The chromatographic conditions were as follows: an injector temperature of 200 °C, an initial oven temperature of 33 °C held for 5 min, increasing to 50 °C at a rate of 2 °C min, then the temperature was increased to 250 °C at a rate of 5 °C per minute, holding it for 6.5 min. Helium was used as the carrier gas with a linear velocity of 30 cm/s. The mass spectra were obtained using electron ionization at 70 eV. The temperatures of the transfer line and the ion trap were 250 and 180 °C, respectively. The identification of the volatile compounds was carried out by comparing the mass spectra obtained from the samples against those reported in the NIST MS 2014 library.

Statistical analysis

Treatments were developed under a completely randomized design. Data was analyzed using an analysis of variance (ANOVA), and a Tukey's test was conducted when treatments showed significant differences ($p < 0.05$). Each treatment was carried out on triplicate batches ($n = 3$).

Results and Discussion

Physical properties

Physical properties are synonymous with quality; weight, width, and length parameters showed significant differences ($p < 0.05$) between treatments. T3 had the highest ($p < 0.05$) weight (32.25 g), and T0

had the lowest ($p < 0.05$) (30.46 g). The largest width ($p < 0.05$) was found in T2 (8.46 mm) and T1 (8.36 mm), without showing significant differences between them ($p > 0.05$). Regarding length, T2 and T3 had the highest values, 13.46 and 13.24 mm, respectively, without showing significant differences between them ($p > 0.05$). The largest length ($p < 0.05$) was found in T2 (13.46 mm). The highest thicknesses ($p < 0.05$) were presented in T2 (5.28 mm) and T3 (5.20 mm) without presenting differences among them ($p > 0.05$).

Therefore, it could be said that the application of fertilizers benefits the quality of the bean. In a work carried out by Márquez-Quiroz *et al.* (2018) in cowpea beans, with an application of ZnSO_4 , they obtained a weight of 20.5 g in 100 seeds of cowpea beans, a weight slightly lower than that obtained in this work, probably due to the different variety worked.

The results obtained showed significant differences ($p < 0.05$) in yield (Table 1). Furthermore, an increase in yield was observed with increasing doses of N, P, S, and Zn. T3 recorded the highest yield ($p < 0.05$), indicating that the highest combination of nutrients evaluated (N 50, P 66, K 25, S 25, Zn 2 kg ha^{-1}) favored crop productivity under the experimental conditions. However, it is important to highlight that T2, with a lower concentration of inputs, achieved a competitive yield (2,050 kg ha^{-1}). Togay *et al.* (2024) conducted a study on beans grown under rainfed conditions, with six doses of Zn (0, 15, 20, 25, 30, 35 kg ha^{-1}) applied to the soil, and obtained a yield of 2766 kg ha^{-1} with the application of 25 kg ha^{-1} . Likewise, Kachinski *et al.* (2020), using two bean varieties (BRS Esteio and IPR Campos Gerais) in a direct sowing system without irrigation, with foliar applications of Zn (600 g ha^{-1} of Zn), reported that the BRS Esteio cultivar reduced the yield from 3399 to 2643 kg ha^{-1} , while in the IPR Campos Gerais cultivar, it increased from 3212 to 3744 kg ha^{-1} . Therefore, the effect of Zn application on yield is dependent on the bean genotype and the application method (Rico *et al.*, 2020; Togay *et al.*, 2024).

Table 1. Physical characteristics of Pinto Centauro beans

| Treatments | Yield (kg h^{-1}) | Weight 100-seed (g) | Length (mm) | Width (mm) | Thickness (mm) |
|------------|---------------------------------|------------------------|---------------------|--------------------|--------------------|
| T0 | 1684 \pm 2.04 a | 30.46 \pm 0.044 c | 11.98 \pm 0.05 c | 8.12 \pm 0.013 b | 4.74 \pm 0.008 b |
| T1 | 1757 \pm 1.25 a | 31.42 \pm 0.012 b | 12.80 \pm 0.01 b | 8.36 \pm 0.011 a | 4.92 \pm 0.015 b |
| T2 | 2050 \pm 0.87 b | 31.60 \pm 0.011 b | 13.46 \pm 0.01 a | 8.46 \pm 0.011 a | 5.28 \pm 0.013 a |
| T3 | 2233 \pm 0.64 c | 32.25 \pm 0.018 a | 13.24 \pm 0.01 ab | 8.12 \pm 0.010 b | 5.20 \pm 0.029 a |

T0, beans fertilized with N 41, P 46, and K 22 kg ha^{-1} ; T1, beans fertilized with N 41, P 46, K 22, S 12, and Zn 1 kg ha^{-1} ; T2, beans fertilized with N 45, P 60, K 22, S 22, and Zn 1.5 kg ha^{-1} ; T3, beans fertilized with N 50, P 66, K 25, S 25, and Zn 2 kg ha^{-1} . a,b,c Different literals between columns denote significant differences ($p < 0.05$) between treatments. Values are expressed as mean \pm standard deviation ($n = 3$)

Phenols and flavonoids

The phenolic and flavonoid content obtained in the testa and cotyledon of Pinto Centauro beans is shown in Table 2. The seed coat of T2 and T3 presented the highest content ($p < 0.05$) of phenolic compounds, 75.10 and 78.40 mg GAE g^{-1} , respectively. And the highest flavonoid content ($p < 0.05$) was observed in the seed coat of T2 (78.40 mg g^{-1}) and T3 (75.1 mg g^{-1}), without showing significant differences between them ($p > 0.05$).

In the seed, T1 obtained the higher phenolic content ($p < 0.05$) with 28.10 mg GAE g^{-1} . Regarding the flavonoid content, T1 had the highest concentration ($p < 0.05$) (59.80 mg GAE g^{-1}). In the cotyledon, T1 showed the highest values ($p < 0.05$) of phenols (23.10 mg GAE g^{-1}) and flavonoids (1.27 mg GAE g^{-1}).

In a study conducted by Chávez-Mendoza *et al.* (2019) on different bean varieties, it was shown that the seed coat obtained the highest values compared to the cotyledon, ranging from 0.69 mg GAE g^{-1} to 3.32 mg GAE g^{-1} , while in the cotyledon, these compounds were within the range of 0.44 mg GAE g^{-1} to 0.99 mg GAE g^{-1} . Meanwhile, García-Díaz *et al.* (2018) conducted a study on pinto beans and mentioned that the testa shows the highest phenolic values compared to the seed, obtaining a value of 34.1 mg GAE g^{-1} in the testa and 1.69

mg GAE g⁻¹ in the cotyledon. Regarding flavonoids, they obtained 2.8 mg ECat g⁻¹ in the testa and 0.26 mg ECat g⁻¹ in the cotyledon. These values are lower than those obtained in this study. It is worth noting that the study they conducted was merely a characterization and not an application of minerals, which probably influenced the result obtained, unlike the one obtained in this study with higher values. Likewise, Zárte-Martínez *et al.* (2021) infer the importance of phenolic acids in the physiology and morphology of the plant with antioxidant properties, which correlates with the phenolic content. According to Bedoya *et al.* (2022), it explains that the number of phenolic compounds stored in the seed is likely due to its ability to inhibit early germination.

Physiologically, the accumulation of phenolic compounds in the seed coat, particularly in T2 and T3, could be associated with defense mechanisms activated by the availability of micronutrients such as sulfur and zinc, which participate in metabolic pathways related to phenylpropanoid biosynthesis (Kopriva and Koprivova, 2014). These secondary metabolites contribute to strengthening cell walls, inhibiting pathogens, and protecting against oxidative stress, which can be reflected in improved physiological quality, viability, and tolerance to adverse conditions of the seed during germination and storage.

From a nutritional perspective, the increase in the content of phenolic compounds and flavonoids is particularly relevant due to their beneficial effects on human health. These compounds have been widely recognized for their antioxidant capacity, which is associated with the prevention of chronic non-communicable diseases, such as type 2 diabetes, cardiovascular disease, and some types of cancer (Luthria and Pastor-Corrales, 2006; Rocha-Guzmán *et al.*, 2007). The phenol and flavonoid levels obtained in T2 and T3 exceed those reported in other studies (Chávez-Mendoza *et al.*, 2019; García-Díaz *et al.*, 2018), suggesting that the targeted application of nutrients can substantially improve the nutraceutical value of beans.

Furthermore, considering that a large portion of phenolic compounds are in the testa, the use of whole or minimally processed beans could be more beneficial to human health, implying an important change in the approach to bean consumption and processing in the food industry (Ganesan and Xu, 2017). In this sense, T2 stands out not only for achieving good agricultural yields but also for promoting the natural biofortification of the grain, consolidating its potential as a functional food within public nutrition or sustainable rural development programs.

Table 2. Determination of phenolic and flavonoid content in the seed, seed coat, and cotyledon of Pinto Centauro beans

| | T0 | T1 | T2 | T3 |
|--|---------------|---------------|----------------|----------------|
| Phenols mg GAE g⁻¹ | | | | |
| Seed | 19.6 ± 0.94 c | 26.1 ± 2.03 a | 28.1 ± 0.02b a | 22.1 ± 0.02 bc |
| Seed coat | 64.5 ± 0.15 b | 59.8 ± 1.54 c | 78.40 ± 1.53 a | 75.1 ± 0.48 a |
| Cotyledon | 17.9 ± 0.37 b | 23.1 ± 0.45 a | 19.4 ± 1.91 b | 19.7 ± 3.61 b |
| Flavonoids mg EQ g⁻¹ | | | | |
| Seed | 0.68 ± 0.13 b | 0.96 ± 0.09 a | 0.56 ± 2.53 c | 0.60 ± 1.73 cb |
| Seed coat | 3.48 ± 0.41 c | 4.19 ± 0.72 b | 6.22 ± 2.62 a | 6.28 ± 0.57 a |
| Cotyledon | 1.04 ± 0.08 c | 1.27 ± 0.56 a | 1.15 ± 0.04 b | 1.11 ± 2.28 cb |

T0, beans fertilized with N 41, P 46, and K 22 kg ha⁻¹; T1, beans fertilized with N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹; T2, beans fertilized with N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹; T3, beans fertilized with N 50, P 66, K 25, S 25, and Zn 2 kg ha⁻¹. a,b,c Different literals between columns denote significant differences (p<0.05) between treatments. GAE = Gallic Acid Equivalents; EQ = Quercetin Equivalents Values are expressed as mean ± standard deviation (n = 3)

Anthocyanins and tannins

The colors present in the bean testa are due to anthocyanins, which provide them with various colors ranging from red to black. The content of anthocyanins and tannins evaluated in the testa and cotyledon of Centauro pinto bean seeds shows significant differences among treatments (Table 3). It was found that T1, in the seed coat, presented the highest (p < 0.05) anthocyanin content (6.95 mg C3G g⁻¹), and T0 the lowest (p

< 0.05) (1.77 mg C3G g⁻¹). In the seed, T1 had the highest ($p < 0.05$) concentration (6.53 mg C3G g⁻¹), and T0 had the lowest ($p < 0.05$) (1.09 mg C3G g⁻¹). In the cotyledon, T2 had the highest ($p < 0.05$) value (6.12 mg C3G g⁻¹) and T0 the lowest ($p < 0.05$) (0.78 mg C3G g⁻¹).

Rochin-Medina *et al.* (2022) evaluated anthocyanins in the seed of Saltillo pinto beans and obtained 0.23 mg C3G 100 g⁻¹. Salinas-Moreno *et al.* (2005) mentioned that the pigmentation that appears in the epidermis, which is due to the synthesis of anthocyanins, occurs in the subepidermal layer, within the cellular body called anthocyanoplast. For this reason, in most studies, the seed coat shows the highest results.

Regarding the tannin content, T1 presented the highest values ($p < 0.05$) in the seed (3.19 mg EC g⁻¹), the seed coat (3.12 mg EC g⁻¹), and the cotyledon (0.79 mg EC g⁻¹). Compared to the control with 0.89 mg EC g⁻¹. The seed coat showed the highest content with 1.90 mg EC g⁻¹, and the lowest ($p < 0.05$) content was obtained by the control with 0.59 mg EC g⁻¹. Regarding the cotyledon, the highest was for T2 with 3.12 mg g⁻¹, and the lowest value corresponded to T3 with 0.12 mg g⁻¹.

Rochin-Medina *et al.* (2022) obtained 504.70 mg EC 100g⁻¹ in the seed of Saltillo pinto beans. Melo *et al.* (2010) determined a condensed tannin content of 0.72 mg g⁻¹ in the Nariño bean variety. Meanwhile, Sharma (2019) mentions that tannins are present in the seed coat and, through their astringency, protect the seed from microorganisms and predators due to their defense system. It is worth mentioning that the lowest dose of N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹ (T1) had the highest concentration of these compounds, surpassing the control.

Table 3. Anthocyanin and tannin content in the seed, seed coat, and cotyledon of Pinto Centauro beans.

| | T0 | T1 | T2 | T3 |
|---|---------------|---------------|---------------|---------------|
| Anthocyanins mg C3G g⁻¹ | | | | |
| Seed | 1.09 ± 0.14 d | 6.53 ± 0.11 a | 5.08 ± 0.94 b | 2.88 ± 0.24 c |
| Seed coat | 1.77 ± 0.18 c | 6.95 ± 0.14 a | 2.45 ± 0.24 c | 5.41 ± 0.14 b |
| Cotyledon | 0.78 ± 0.13 d | 3.72 ± 0.19 c | 6.12 ± 0.52 a | 2.38 ± 0.27 b |
| Tannins mg EC g⁻¹ | | | | |
| Seed | 0.89 ± 1.14 a | 3.19 ± 1.25 a | 1.01 ± 1.42 c | 2.50 ± 1.74 b |
| Seed coat | 0.52 ± 1.29 b | 3.12 ± 1.13 b | 1.90 ± 1.04 a | 0.10 ± 0.83 c |
| Cotyledon | 0.47 ± 1.67 b | 0.79 ± 1.46 a | 0.48 ± 1.17 b | 0.12 ± 0.91 b |

T0, beans fertilized with N 41, P 46, and K 22 kg ha⁻¹; T1, beans fertilized with N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹; T2, beans fertilized with N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹; T3, beans fertilized with N 50, P 66, K 25, S 25, and Zn 2 kg ha⁻¹. a,b,c Different literals between columns denote significant differences ($p < 0.05$) between treatments. C3G = cyanidin-3-glucoside equivalents; EC = epicatechin equivalents. Values are expressed as mean ± standard deviation (n = 3)

The antioxidant activity, reducing power, and inhibition percentage of the seed, testa, and cotyledon are shown in figure 1. Significant differences ($p < 0.05$) were observed among treatments. The highest percentage of inhibition ($p < 0.05$) was presented in the testa of T2 (86.89%). In seed, T3 obtained the highest percentage ($p < 0.05$) (81.02). Concerning the tested DPPH method, the testa shows the highest content with T2 at 17.08 mg ET g⁻¹, and the lowest value corresponds to the control with a total of 15.03 mg ET g⁻¹. For the seed, the highest value was obtained by T2, with 4.40 mg g⁻¹, and the lowest corresponds to the control, with a value of 1.86 mg g⁻¹. Finally, the cotyledon in T2 presents the highest value with 13.45 mg ET g⁻¹, and the lowest is from the control with 3.12 mg ET g⁻¹.

The reducing power activity shows that T1 in the seed coat presented the highest value with an absorbance of 3.52; in the seed, T1 presented an absorbance of 1,378, and in the cotyledon, all treatments were equal. Therefore, both methodologies can capture free radicals; however, in the reducing power, the Fe³⁺ ion is reduced to Fe²⁺, which could be due to the reducing flavonoids and anthocyanins, as it has also been observed

in T1 that they contain these compounds (Bedoya Maldonado, 2022). Therefore, the technique used for reducing power is a good indicator of its antioxidant potential. This aligns with Flores-Cordova *et al.* (2018), who mention that the reducing power measures the ability of an antioxidant to donate an electron; therefore, an increase in absorbance is indicative of a high reducing power. And the T1 in testa was favored by the lower applications made.

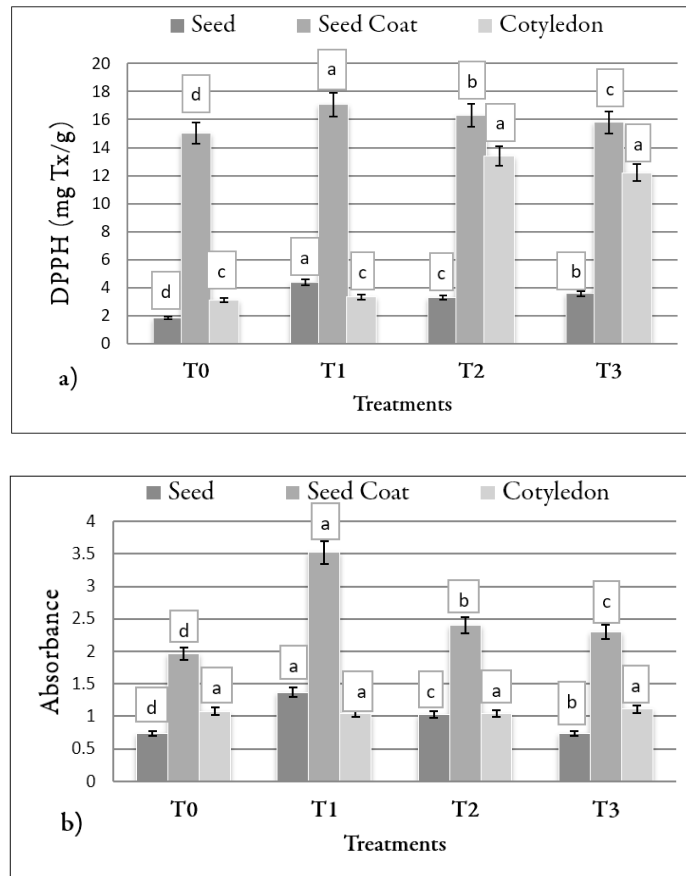


Figure 1. a) Antioxidant activity of Pinto Centauro beans, DPPH method, and b) reducing power (mean \pm standard deviation). T0, beans fertilized with N 41, P 46, and K 22 kg ha⁻¹; T1, beans fertilized with N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹; T2, beans fertilized with N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹; T3, beans fertilized with N 50, P 66, K 25, S 25, and Zn 2 kg ha⁻¹. a,b,c Different letters between treatments denote significant differences ($p < 0.05$) between treatments

Correlation between variables

Table 4 shows the Pearson correlation coefficients between variables. There was a significant and positive correlation between antioxidant activity and phenols and flavonoids; phenols with flavonoids and RP; flavonoids with antioxidant activity and phenols; and reducing power with antioxidant activity, phenols, and flavonoids.

Flavonoids are the main components of phenolic compounds since, in each treatment, the correlation between phenols and flavonoids was significant. These compounds seem to be responsible for conferring the antioxidant activity and reducing power of Centauro pinto beans. Aquino-Bolaños *et al.* (2016) reported a positive correlation between antioxidant activity and polyphenols in seed coats. In addition, Mastura *et al.* (2017) and Chávez-Mendoza *et al.* (2019) evaluated different varieties of beans and established that the antioxidant activity is dependent on phenolic content.

Table 4. Pearson correlations between bioactive compounds of Pinto Centauro beans

| | AA | TF | F1 | AN | TA | RP |
|---------|----------|----------|----------|---------|-----------|----------|
| AA | 1.0000 | 0.7514** | 0.7844** | 0.2503 | -0.4655** | 0.7392* |
| p-value | | <0.0001 | <0.0001 | 0.1409 | 0.0042 | <0.0001 |
| TF | 0.7514** | 1.0000 | 0.9578** | 0.2508 | -0.2055 | 0.8033** |
| p-value | <0.0001 | | <0.0001 | 0.1400 | 0.2292 | <0.0001 |
| F1 | 0.7844** | 0.9578** | 1.0000 | 0.3191 | -0.2259 | 0.8048** |
| p-value | <0.0001 | <0.0001 | | 0.0578 | 0.1852 | <0.0001 |
| AN | 0.2503 | 0.2508 | 0.3191 | 1.0000 | 0.33260* | -0.0183 |
| p-value | 0.1409 | 0.1400 | 0.0578 | | 0.0475 | 0.9152 |
| TA | -0.4654 | 0.2055 | -0.2259 | 1.000 | 1.000 | 0.3417* |
| p-value | 0.0042 | 0.2292 | 0.1852 | 0.0475 | | 0.0413 |
| RP | 0.7392** | 0.8033** | 0.8048** | -0.0183 | -0.3417* | 1.0000 |
| p-value | <0.0001 | <0.0001 | <0.0001 | 0.9152 | 0.0413 | |

AC = Antioxidant activity, TP = Phenols, Fl = Flavonoids, AN = Anthocyanins, TN = Tannins, RP RP= Reducing power. * = The correlation is significant at the 0.05 level (two-tailed). ** = The correlation is significant at the 0.01 level (two-tailed)

Volatile compounds

Although a total of 30 volatile compounds were identified (Table 5), it is important to highlight that not all these compounds have the same sensory or technological relevance. Therefore, we prioritize the discussion of those volatiles with proven aromatic impact, greater relative abundance, and possible involvement in the quality or processing of dried beans.

Among the aldehydes, benzaldehyde was one of the most consistent and abundant compounds across all treatments. This compound is widely recognized for its bitter, almond-like aroma and is associated with pleasant aromatic notes in legume products (Oomah *et al.*, 2007). Its constant presence suggests a possible positive contribution to the typical aromatic profile of dried beans, in addition to acting as a stable sensory marker under moderate storage or heat processing conditions.

Hexanal and nonanal, also detected, are associated with green, herbaceous, and fatty aromas (Burdock, 2010) derived from the oxidation of unsaturated fatty acids (MacLeod and Ames, 1988). While at low levels they can contribute aromatic complexity, high concentrations of hexanal can indicate unwanted lipid oxidation, especially in beans stored for long periods or poorly protected from light.

Regarding terpenes, D-limonene was one of the dominant compounds, especially in the control treatment. This compound is recognized for its fresh citrus aroma and has been reported as one of the main terpenes in legumes (Karolkowski *et al.*, 2021). The presence of D-limonene can be associated with a brighter, cleaner aroma, especially valued in bean-based processed food applications (such as snacks or flours). Its reduction in improved treatments suggests a possible chemical transformation by interaction with fertilizer nutrients or soil microbiota.

β -Pinene (T1) and γ -terpinene (T2), although not abundant, contribute fresh, resinous, or herbal notes that could enrich the sensory profile of the beans. Their appearance in specific treatments suggests that they could be modulated by specific nutritional or microbiological conditions.

Regarding alcohols, 2-ethyl-1-hexanol, identified in all treatments, is associated with herbaceous and green notes. Although these compounds contribute less to the aroma due to their higher sensory threshold, their presence may indicate the oxidation state of lipids and their interaction with phenolic precursors, affecting product stability during thermal processing.

Table 5. Volatile compounds identified in Pinto Centauro beans

| Tr | Volatile compounds | Treatment | | | |
|-------|------------------------------|----------------|----------------|----------------|---------------|
| | | T0 | T1 | T2 | T3 |
| | Aldehydes | | | | |
| 18.11 | Hexanal | 1.08 ± 0.20 a | 0.24 ± 0.01 b | N.D. | 0.32 ± 0.09 b |
| 26.4 | Octanal | 0.31 ± 0.06 a | 0.28 ± 0.01 a | 0.43 ± 0.04 a | 0.18 ± 0.05 a |
| 29.68 | Nonanal | 1.88 ± 0.22 a | 2.60 ± 0.12 a | 1.63 ± 0.35 a | 1.29 ± 0.50 a |
| 32.63 | Decanal | 0.29 ± 0.07 a | 0.20 ± 0.02 a | 0.55 ± 0.03 a | 0.17 ± 0.04 a |
| 33.34 | Benzaldehyde | 19.63 ± 3.73 a | 2.09 ± 0.12 b | 2.03 ± 0.25 b | 1.06 ± 0.07 b |
| | Alcohols | | | | |
| 26.75 | 2-Methyl, 1-pentanol | 0.15 ± 0.03 | N.D. | N.D. | N.D. |
| 28.4 | 1-Hexanol | 0.62 ± 0.04 a | 0.23 ± 0.01 b | N.D. | 0.27 ± 0.01 b |
| 32.26 | 2 Ethyl,1-hexanol | 0.44 ± 0.06 a | 0.36 ± 0.00 a | 0.30 ± 0.05 a | 0.92 ± 0.03 a |
| 34.05 | 1-Octanol | N.D. | 0.14 ± 0.00 | N.D. | N.D. |
| 41.55 | Benzyl alcohol | 0.22 ± 0.07 | N.D. | N.D. | N.D. |
| | Ketones | | | | |
| 28.4 | 6 Methyl 5-hepten-2-one | N.D. | N.D. | 0.14 ± 0.03 a | 0.08 ± 0.02 a |
| 33.18 | 3, 5-Octadien-2-one | N.D. | 0.15 ± 0.01 a | 0.21 ± 0.06 a | 0.17 ± 0.06 a |
| 36.61 | Acetophenone | 1.63 ± 0.06 a | 1.01 ± 0.08 ab | 1.08 ± 0.24 ab | 0.70 ± 0.03 b |
| | Esters | | | | |
| 17.76 | Butyl acetate | 0.23 ± 0.03 | N.D. | N.D. | N.D. |
| 23.96 | Butyl butanoate | 0.38 ± 0.04 a | 0.12 ± 0.01 b | 0.13 ± 0.02 b | N.D. |
| 24.51 | Ethyl hexanoate | 0.42 ± 0.01 a | 0.18 ± 0.01 a | 0.31 ± 0.02 a | 0.07 ± 0.00 a |
| 25.85 | Hexyl acetate | 1.56 ± 0.01 a | 0.70 ± 0.03 bc | 0.82 ± 0.02 b | 0.31 ± 0.00 c |
| 30.24 | Butyl hexanoate | 0.50 ± 0.06 a | N.D. | 0.33 ± 0.08 ab | 0.19 ± 0.07 b |
| 30.32 | Hexyl butanoate | N.D. | 0.26 ± 0.00 | N.D. | N.D. |
| 30.67 | Hexyl 2-methyl butanoate | 0.84 ± 0.13 a | 0.65 ± 0.01 a | 0.74 ± 0.03 a | 0.49 ± 0.02 a |
| | Terpenes | | | | |
| 19.27 | β-Pinene | N.D. | 6.10 ± 0.47 | N.D. | N.D. |
| 23.16 | D-limonene | 18.41 ± 3.83 a | 4.24 ± 0.41 b | 5.40 ± 1.86 b | 3.29 ± 1.02 b |
| 24.92 | γ-Terpinene | N.D. | N.D. | 5.36 ± 1.67 | N.D. |
| | Alkanes | | | | |
| 16.22 | Decane 2,4,6-trimethyl | N.D. | 0.19 ± 0.01 | N.D. | N.D. |
| 18.71 | Decane | N.D. | 0.28 ± 0.04 | N.D. | N.D. |
| | Aromatic hydrocarbons | | | | |
| 25.23 | Styrene | 6.79 ± 2.08 a | 2.22 ± 0.17 a | 1.93 ± 0.47 a | 2.04 ± 0.22 a |
| 25.76 | o-cymene | N.D. | N.D. | 7.90 ± 0.63 | N.D. |

Tr, retention time; N.D., not detected. T0, beans fertilized with N 41, P 46, and K 22 kg ha⁻¹; T1, beans fertilized with N 41, P 46, K 22, S 12, and Zn 1 kg ha⁻¹; T2, beans fertilized with N 45, P 60, K 22, S 22, and Zn 1.5 kg ha⁻¹; T3, beans fertilized with N 50, P 66, K 25, S 25, and Zn 2 kg ha⁻¹. a,b,c Different literals between columns denote significant differences (p<0.05) between treatments. Values are expressed as mean ± standard deviation (n = 3).

1-Octanol, which contributes a fatty or cheesy aroma, could negatively affect perception at high concentrations, as these notes are often associated with sensory deterioration in stored beans (Oomah *et al.*, 2007). The fact that it appears only in T1 may have implications for the selection of treatments with better postharvest performance.

As for ketones, such as 6-methyl-5-hepten-2-one, these contribute notes of fresh fruit or vegetables and are generated by lipid degradation. In particular, the presence of 3,5-octadiene-2-one (a mushroom aroma) in certain treatments can enrich sensory complexity, although it can also be associated with undesirable earthy notes if it exceeds certain thresholds.

The detection of alkanes (2,4,6-trimethyl decane and decane) in the T1 treatment is technologically relevant, as these compounds are byproducts of lipid oxidation and are considered chemical markers of the onset of aroma deterioration. Their absence in the improved treatments could suggest improved oxidative stability of the grain, which is desirable in products that will be stored or cooked.

Finally, the appearance of compounds such as p-cymene, which was present only in the improved treatment, suggests possible biosynthetic pathways induced by nutritional management, opening a line of interest for the study of functional aroma precursors.

The review of the volatile profile has allowed us to identify key compounds with a positive sensory impact (e.g., benzaldehyde, D-limonene, β -pinene), as well as others related to lipid oxidation or potential deterioration (e.g., hexanal, alkanes). This information is relevant not only for aroma characterization but also for understanding the technological implications of fertilization treatments on postharvest quality and processing potential of beans.

Conclusion

This study demonstrated that the fertilization regime significantly influences the accumulation of bioactive compounds and the antioxidant activity of different anatomical parts of the Centaury pinto bean (*Phaseolus vulgaris L.*), specifically the seed, the tegument (testa), and the cotyledon. Among these, the testa consistently presented the highest concentrations of total phenols, flavonoids, anthocyanins, and tannins, which was associated with greater antioxidant activity, confirming that it is the main site of phytochemical accumulation.

Based on the results, T2, which included moderate fertilization (N 45, P 60, K 22, S 22, Zn 1.5 kg ha⁻¹), is recommended as the optimal strategy to maximize the functional quality of the Centauro pinto bean. This approach not only promoted the accumulation of health-promoting bioactive compounds but also improved the antioxidant and volatile profile of the bean without requiring excessive nutrient application.

Regarding the volatile component profile results, key compounds with positive sensory impact were identified (e.g., benzaldehyde, D-limonene, and β -pinene), as well as others related to lipid oxidation or potential deterioration (e.g., hexanal, alkanes). This information is relevant not only for aroma characterization but also for understanding the technological implications of fertilization treatments on postharvest quality and bean processing potential.

These findings underscore the importance of implementing tailored fertilization strategies to enhance the nutraceutical value of the bean. Furthermore, the results open new lines of research aimed at evaluating the stability of these effects across different bean varieties and environmental conditions, with the goal of strengthening their agronomic and functional impact

Authors' Contributions

M.A.F.C. and E.S.CH. designed the study. M.C.S.C. analyzed the data. A.CH.M., N.A.S.S. and M.A.F.C. prepared the manuscript, while D.E.S., M.A.F.C., and E.S.CH. conducted the experiments. M.C., S.C. and D.E.S. organized the data and performed the statistical analysis. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abdulrahman BO, Bala M, Bello OM (2020). Bioactive compounds of black bean (*Phaseolus vulgaris* L.). In: Murthy HN, Paek KY (Eds). Bioactive compounds in underutilized vegetables and legumes. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-44578-2_38-1
- Aguirre-Santos EA, Gómez-Aldapa CA (2010). Evaluación de las características fisicoquímicas en la especie de frijol *Phaseolus vulgaris* de las variedades; Pinto Saltillo, Bayo Victoria y Negro San Luis. XII Congreso Nacional de Ciencia y Tecnología de Alimentos pp 1101-1108. Retrieved 2024 November 14 from <https://repository.uach.edu.mx/bitstream/items/4e712055-5fa5-400a-b950-b29f9a9892d8>
- Akhtar M, Yousaf S, Sarwar N, Hussain S (2019). Zinc biofortification of cereals—role of phosphorus and other impediments in alkaline calcareous soils. *Environmental Geochemistry and Health* 41(5):2365-2379. <https://doi.org/10.1007/s10653-019-00279-6>
- Aquino-Bolaños EN, García-Díaz YD, Chavez-Servia JL, Carrillo-Rodríguez JC, Vera-Guzmán AM, Heredia-García E (2016). Anthocyanin, polyphenol, and flavonoid contents and antioxidant activity in Mexican common bean (*Phaseolus vulgaris* L.) landraces. *Emirates Journal Food Agriculture* 28:581-588. <https://doi.org/10.9755/ejfa.2016-02-147>
- Arreola SJ, Sánchez ChE (2016). Biofortificación del frijol estrategia potencial para combatir la desnutrición y mejorar la salud humana. *Ciencia y Desarrollo* 41(286):37-43.
- Bedoya RA, Maldonado ME (2022). Características nutricionales y antioxidantes de la especie de frijol petaco (*Phaseolus coccineus*). *Revista Chilena de Nutrición* 49(1):34-42. <http://dx.doi.org/10.4067/S0717-75182022000100034>
- Brosset A, Blande JD (2022). Volatile-mediated plant–plant interactions: Volatile 561 organic compounds as modulators of receiver plant defence, growth, and 562 reproductions. *Journal of Experimental Botany* 73(2):511-528. <https://doi.org/10.1093/jxb/erab487>
- Brückner B (2008). Consumer acceptance of fruit and vegetables: the role of flavour and other quality attributes. Brückner B, Wyllie SG (Eds). *Fruit and vegetable flavour*. Woodhead Publishing. <https://doi.org/10.1533/9781845694296.1.3>
- Burdock GA (2010). *Handbook of Flavor Ingredients*, 6th ed.; CRC Press/Taylor and Francis Group: Boca Raton, FL, USA, ISBN 9780429150838. <https://doi.org/10.1201/9781439847503>
- Burow M, Wittstock U, Gershenzon J (2008). Sulfur-Containing Secondary Metabolites and their Role in Plant Defense. In *Sulfur Metabolism in Phototrophic Organisms*. In: Hell R, Dahl C, Knaff D, Leustek T (Eds). *Sulfur metabolism in phototrophic organisms*. *Advances in Photosynthesis and Respiration*. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6863-8_11
- Cakmak I (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil*, 302(1-2):1-17. <https://doi.org/10.1007/s11104-007-9466-3>
- CEDRSSA (2020). Mercado del frijol, situación y prospectiva. Retrieved 2024 November 14 from <http://www.cedrssa.gob.mx/files/b/13/53Mercado%20del%20frijol.pdf>
- Chasapis ChN, Panagoula-Stamatina AN, Chara AS, Stefanidou M.E (2020). Recent aspects of the effects of zinc on human health. *Archives of Toxicology* 94:1443-1460. <https://doi.org/10.1007/s00204-020-02702-9>

- Chávez-Mendoza C, Hernández-Figueroa KI, Sánchez E (2019). Antioxidant capacity and phytonutrient content in the seed coat and cotyledon of common beans (*Phaseolus vulgaris* L.) from various regions in Mexico. *Antioxidants* 8(5):2-19. <https://doi.org/10.3390/antiox8010005>
- Chen Y, Zhang H, Liu R, Mats L, Zhu H, Pauls K, ... Deng ZT (2019). Antioxidant and anti-inflammatory polyphenols and peptides of common bean (*Phaseolus vulgaris* L.) milk and yogurt in Caco-2 and HT-29 cell models. *Journal of Functional Foods* 53:125-135. <https://doi.org/10.1016/j.jff.2018.12.013>
- Dong Y, Zhao L, Liu B, Wang Z, Jin Z, Sun H (2004). The genetic diversity of cultivated soybean grown in China. *Theoretical and Applied Genetics* 108(5):931-936. <https://doi.org/10.1007/s00122-003-1503-x>
- Espinosa-Pérez EN, Ramírez-Vallejo P, Crosby-Galván M, Estrada-Gómez JA, Lucas-Florentino B, Chávez-Servia JL (2015). Clasificación de poblaciones nativas de frijol común del centro-sur de México por morfología de semilla. *Revista Fitotecnia Mexicana* 38(1):29-38.
- FIRA Fideicomiso instituidos en relación con la agricultura (2022). Panorama Agroalimentario. Dirección de Investigación y Evaluación Económica y Sectorial Subdirección de Análisis del Sector. Retrieved 2024 November 14 from <https://www.gob.mx/fira/documentos/panorama-agroalimentario>
- Flores-Córdova MA, Sánchez ChE, Muñoz-Márquez E, Ojeda-Barrios L, Soto PJM, Preciado-Rangel P (2017). Phytochemical composition and antioxidant capacity in Mexican pecan nut. *Emirates Journal of Food and Agriculture* 29(5):346-350. <https://doi.org/10.9755/ejfa.EJFA-2016-08-1075>
- Flores-Córdova MA, Soto PJM, Salas Salazar NA, Sánchez ChE, Piña RFJ (2018). Efecto del subproducto industrial CaCO₃ en los atributos de calidad, contenido fenólico y capacidad antioxidante de manzana cvs Golden Delicious y Top Red. *Nova Scientia* 10(20):64-82. <https://doi.org/10.21640/ns.v10i20.1190>
- Ganesan K, Xu B (2017). Polyphenol-rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *International Journal of Molecular Sciences* 18(11):2331. <https://doi.org/10.3390/ijms18112331>
- García-Díaz YD, Aquino-Bolaños EN, Chávez-Servia JL, Vera-Guzmán, AM, Carrillo-Rodríguez JC (2018). Bioactive compounds and antioxidant activity in the common bean are influenced by cropping season and genotype. *Chilean Journal of Agricultural Research* 78(2):255-265. <https://dx.doi.org/10.4067/S0718-58392018000200255>
- García-Lafuente A, Moro C, Manchón N, Gonzalo-Ruiz A, Villares A, Guillamón E, Rostagno M, Mateo-Vivaracho L (2014). *In vitro* anti-inflammatory activity of phenolic rich extracts from white and red common beans. *Food Chemistry* 161:216-223. <https://doi.org/10.1016/j.foodchem.2014.04.00>
- Gío-Trujillo JA, Alvarado-López CJ, Pacheco-López NA, Cristóbal-Alejo J, Reyes-Ramírez A (2022). Perspectivas futuras de la biofortificación de alimentos: la asociación con microorganismos del suelo. *Revista Ra Ximhai* 18(4):175-199. <https://doi.org/10.35197/rx.18.04.2022.08.jg>
- Guija-Guerra H, Guija-Poma E (2023). Radicales libres y sistema antioxidante. *Horizonte Medico* 23(2):e2158. <http://dx.doi.org/10.24265/horizmed.2023.v23n2.12>
- Iniestra GJJ, Ibarra PFJ, Gallegos IJA, Rocha GNE, González LRF (2005). Factores antinutricios y actividad antioxidante en variedades mejoradas de frijol común (*Phaseolus vulgaris*). *Agrociencia* 39(6):603-610. <https://www.agrociencia-colpos.org/index.php/agrociencia/article/view/424>
- Kan L, Nie S, Hu J, Wang S, Bai Z, Wang J, Song K (2018). Comparative study on the chemical composition, anthocyanins, tocopherols and carotenoids of selected legumes. *Food Chemistry* 260:317-326. <https://doi.org/10.1016/j.foodchem.2018.03.148>
- Karolkowski A, Gourrat K, Bouzidi E, Albouy JF, Levavasseur L, Briand L, Guichard E, Salles C (2023). Origins of volatile compounds and identification of odour-active compounds in air-classified fractions of faba bean (*Vicia faba* L. *minor*). *Food Research International* 163:112260. <https://doi.org/10.1016/j.foodres.2022.112260>
- Karolkowski A, Guichard E, Briand L, Salles C (2021). Volatile compounds in pulses: A review. *Foods* 10(12):3140. <https://doi.org/10.3390/foods10123140>
- Koprivova A, Kopriva S (2014). Molecular mechanisms of regulation of sulfate assimilation. *Frontiers in Plant Science* 5(589):1-11. <https://doi.org/10.3389/fpls.2014.00589>
- Liu C, Xu H, Li Z, Wang Y, Qiao S, Zhang H (2025). Aplicación y avances de la genómica para descifrar los mecanismos de regulación genética de los metabolitos secundarios de las plantas. *Plants* 14(9):1316. <https://doi.org/10.3390/plants14091316>
- Luthria DL, Pastor-Corrales MA (2006). Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. *Journal of Food Composition and Analysis* 19(2-3):205-211. <https://doi.org/10.1016/j.jfca.2005.06.006>
- MacLeod G, Ames J (1988). Soy flavour and its improvements. *Critical Reviews in Food Science and Nutrition* 27(4):219-400. <https://doi.org/10.1080/10408398809527487>

- Márquez-Quiroz C, de la Cruz-Lázaro E, Osorio-Osorio R, Sánchez-Chávez, E, Huijara-Vasconcelos JJ, Sida-Arreola JP (2018). Contenido de zinc y rendimiento del frijol caupí biofortificado. *Revista Mexicana de Ciencias Agrícolas* 9(20):4175-4185. <https://doi.org/10.29312/remexca.v0i20.988>
- Mastura HY, Hasnah H, Dang TN (2017). Total phenolic content and antioxidant capacity of beans: Organic vs. inorganic. *International Food Research Journal* 24(2):510-517.
- Meir S, Kanner J, Akiri B, Hadas SP (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal Agricultural Food Chemistry* 43(7):1813-181. <https://doi.org/10.1021/jf00055a012>
- Melo I, Ligarreto G (2010). Contenido de taninos en el grano y características agronómicas en cultivares de frijol común "tipo reventón". *Agronomía Colombiana* 28(2):147-154.
- Oomah BD, Liang LS, Balasubramanian P (2007). Volatile compounds of dry beans (*Phaseolus vulgaris* L.). *Plant Foods for Human Nutrition* 62:177-183. <https://doi.org/10.1007/s11130-007-0059-3>
- Oyaizu M. (1986). Studies on the products of browning reaction prepared from glucose amine. *Japan Journal of Nutrition* 44:307-315. <http://dx.doi.org/10.5264/eiyogakuzashi.44.307>
- Pérez-Pérez LM, Del Toro SCL, Sánchez ChE, González VRI, Reyes DA, Borboa FJ, Soto PJM, ... Flores-Córdova MA (2020). Bioaccesibilidad de compuestos antioxidantes de diferentes variedades de frijol (*Phaseolus vulgaris* L.) en México, mediante un sistema gastrointestinal in vitro. *Biotecnia* 22(1):117-125. <https://doi.org/10.18633/biotecnia.v22i1.1159>
- Price ML, Van Scoyoc S and Butler LG (1978). A critical evaluation of the vanillin reaction as an assay for tannin in Sorghum. *Journal of Agricultural and Food Chemistry* 26(5):1214-1218. <https://doi.org/10.1021/jf60219a031>
- Rengel Z (2015). Availability of Mn, Zn and Fe in the rhizosphere. *Journal of Soil Science and Plant Nutrition*, 15(2):397-409. <https://doi.org/10.4067/S0718-95162015005000031>
- Rico-Alderete IA, Sánchez-Chávez E, Soto-Parra JM, Antillón-Leyva R, Salas-Salazar, NA, Ojeda-Barrios DL, Flores-Córdova MA (2020). Manejo de fertilización en frijol 'Pinto Centauro' y su impacto en el rendimiento, calidad nutricional e índice de rentabilidad. *Revista Chapingo Serie Horticultura* 26(3):207-222. <https://doi.org/10.5154/r.rchsh.2020.03.005>
- Rocha-Guzmán NE (2007). Antioxidant activity and phenolic content of dry beans of different color. *Plant Foods for Human Nutrition* 62(1):31-35. <https://doi.org/10.1016/j.lwt.2007.11.025>
- Rochin-Medina JJ, Mora-Rochín S, Navarro-Cortez RO, Tovar-Jimenez X, Quiñones-Reyes G, Ayala-Luján JL, Aguayo-Rojas J (2022). Contenido de compuestos fenólicos y capacidad antioxidante de variedades de frijol sembradas en el estado de Zacatecas. *Acta Universitaria* 31:1-13. <https://doi.org/10.15174/au.2021.3059>
- Rosa-Valdés ML (2020). Effect of zinc fertilization on the bioactive compounds and antioxidant activity in black bean (*Phaseolus vulgaris*). *Journal of Food Composition and Analysis* 88:103427. <https://doi.org/10.1016/j.jfca.2020.103427>
- Salinas-Moreno Y, Rojas-Herrera L, Sosa-Montes E, Pérez-Herrera P (2005). Composición de antocianinas en variedades de frijol negro (*Phaseolus vulgaris* L.) Cultivadas En México. *Agrociencia* 39(4):385-394. <https://dialnet.unirioja.es/servlet/articulo?codigo=1215922>
- Sharif MK, Butt MS, Sharif HR, Nasir M (2017). Sensory evaluation and consumer acceptability. In: Jeantet R, Croguennec T, Schuck PM, Brulé G (Eds). *Handbook of Food Science and Technology*, Wiley Online Library.
- Sharma KP (2019). Tannin degradation by phytopathogen's tannase: A plant's defense perspective. *Biocatalysis and Agricultural Biotechnology* 21:101342. <https://doi.org/10.1016/j.bcab.2019.101342>
- Singleton FB, Ross CW (1965). Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal Enology Viticulture* 16:144-158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Wang X, Chen T, Zhang W, Fan X, Song W (2021). Zinc application improves the antioxidant defense system in maize leaves under drought stress. *Agronomy* 11(4):740. <https://doi.org/10.3390/agronomy11040740>
- Wrolstad, RE (1976). Color and pigment analyses in fruit products. *Station Bulletin* 624, Agricultural Experiment Station Oregon State University: Corvallis, 1976.
- Zárate-Martínez W, González-Morales S, Ramírez GF, Robledo-Olivo A, Juárez-Maldonado A (2021). Efecto de los ácidos fenólicos en el sistema antioxidante de plantas de tomate (*Solanum lycopersicum* Mill.) *Agronomía Mesoamericana* 32(3):854-868. <https://doi.org/10.15517/am.v32i3.45101>
- Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64(4):555-559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Norulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.