SHST
Horticulture and Forestry Society of Transylvania

Chávez-Mendoza C and Sánchez E (2023)
Notulae Scientia Biologicae
Volume 15, Issue 1, Article number 11353
DOI:10.15835/nsb15111353
Research Article

NSB
Notulae Scientia Biologicae

Antioxidant capacity and nutraceutical compounds content of six common bean (*Phaseolus vulgaris* L.) varieties harvested in Morelos, Mexico

Celia CHÁVEZ-MENDOZA, Esteban SÁNCHEZ*

*Centro de Investigación en Alimentación y Desarrollo A. C. Av. Cuarta Sur #3820. Fracc. Vencedores del Desierto, C.P. 33089, Delicias, Chihuahua, México; celia.chavez@ciad.mx; esteban@ciad.mx (*corresponding author)*

Abstract

Common bean is considered one of the most important legumes in the world. It is the main source of protein, calories, B vitamins, minerals, polyphenols and other elements, which collectively give it a high nutraceutical value. In Mexico a great agrobiodiversity exists in the production of this grain, which implies the need to generate information regarding its nutritional quality as a tool to apply future genetic improvement programs. The purpose of this study was to characterize the antioxidant capacity (AC) and nutraceutical content of six bean varieties produced in Morelos State, Mexico. Grain morphometric characteristics, color (L, a*, b* chroma and °hue), nutritional quality, AC (DPPH), nutraceutical compounds content, micro and macro nutrients were determined. A significant effect ($p < 0.05$) of variety on almost all the variables evaluated except for phytic acid, P, K, Ca, C, S and H was observed. Lower lightness was obtained in varieties with darker colors such as 'Negro'/102 (24.96), 'Negro'/104 (26.85) and 'Sangre de Toro' (32.41) and higher lightness in lighter colored varieties such as 'Peruano' bean (69.21), 'Pinto' (65.94) and 'Flor de Mayo' (50.14). Nutritional and nutraceutical quality of the latter genotype stood out, as it had the highest crude fiber content (5.71 %), total phenols (4.24 mg GAE g^{-1}), flavonoids (1.99 mg CE g^{-1}), AC (96.76% Inhibition), and a high protein content (23.29%). Results also exhibited significant correlation ($p < 0.05$) between total phenols and flavonoids with AC. It is concluded that the nutritional characterization carried out on bean varieties from important producing areas in Mexico provide a valuable database for genotype selection with high functional and nutritional character, either to be grown for direct consumption, future biofortification or breeding programs.

Keywords: antioxidant capacity; bioactive compounds; micronutrients; Mexican bean; Morelos

Introduction

Nutraceutical products are defined as chemical or biological substances that can be found as natural components of food or added to it, and that are particularly beneficial, both in disease prevention and in improvements in physiological functions of the organism. The consumer's and the general population's interest in obtaining optimal diets to maintain good health and prolong lifespan has led to an increase in natural food markets in which this type of product has priority (Pérez, 2006). Common bean is considered one of the most important legumes in the world. It is the main source of protein, calories, B vitamins, minerals, polyphenols

Received: 20 Sep 2022. Received in revised form: 08 Mar 2023. Accepted: 15 Mar 2023. Published online: 17 Mar 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae 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.

and other elements, which collectively give it a high nutraceutical value (Herrera-Hernández *et al*., 2018). This legume is considered a functional food and has gained great interest for study due to its high content of bioactive compounds such as enzyme inhibitors, lectins, phytates, oligosaccharides and phenolic compounds that may have metabolic roles in humans and animals that consume them frequently. Its antioxidant capacity, antimutagenic and antiproliferative effect have been associated with the presence of phenolic compounds, and this could explain the numerous scientific studies that have suggested that the consumption of this food is related to several beneficial health effects, such as reduction of coronary heart disease, protective effects against cancer, and decrease of diabetes and obesity risk (Gálvez *et al*., 2007). The antioxidant activity of beans is due to the presence of phenolic acids and flavonoids, mainly tannins, both raw and cooked, gallic, vanillic, p-coumaric, ferulic, sinapic and chlorogenic acid, which are of great importance as precursors in the synthesis of phenolic compounds in plants (Huber *et al*., 2016).

Legumes are also an excellent source of micronutrients. They are a source of Se, thiamine, niacin, folate, riboflavin and pyridoxine. They also contain vitamin E and A as well as Fe and Zn, although the Fe content can vary greatly depending on the variety, for example, white beans contain almost twice as much Fe as black beans. However, most of the Fe contained is tightly bound to phytates, which reduce absorption and may contribute to Fe deficiencies in countries where beans and other legumes are a staple food (Mudryj *et al*., 2014). Nevertheless, beans have the potential to treat Fe deficiency anemia and other diseases associated with micronutrient deficiencies that affect a large number of people around the world. In this regard, biofortification of this crop is a technique that has been launched with the rationale that high mineral content grains will increase the supply and availability of non-heme Fe in various human populations. Such nutritional improvement focuses on both increasing nutrient content and reducing the anti-nutritional factors contained in the plant, such as oxalates, phytates and tannins, which together affect the bioavailability of these nutrients to consumers (Diaz *et al*., 2010).

Common beans are a staple food in many Latin American and African countries. Mexico is considered the center of origin of this legume, which has been consumed since pre-Hispanic era (Espinosa-Alonso *et al*., 2006). This country has the widest variety of beans and is accepted as the center of origin of the common bean, since 47 out of 52 species classified in the *Phaseolus* genus were identified in Mexico (Silva-Cristobal *et al.,* 2010). In Mexico, there is a great diversity of this crop, in which color is one of the attributes that determine consumption preferences in different Mexican regions, such as yellow in the northwest, beige with brown and cream spots in the northeast, black in the south and various specific colors in the central region (Espinosa-Alonso *et al.,* 2006). It is known that the coat color is attributed to the presence and quantity of polyphenols such as flavonol glycosides, condensed tannins and anthocyanins which function to protect the seed against predators and pathogens (Beninger *et al*., 1999; Takeoka *et al.,* 1997).

Since there are several factors that influence the nutritional quality of the bean grain, and due to the wide agrobiodiversity that exists in Mexico, the objective of this study was to characterize the antioxidant capacity and nutraceutical content of six bean varieties from Morelos, Mexico, in order to select varieties that have the potential to be bio-fortified with micronutrients, with a focus on improving bean nutritional quality, thus contributing to the nutrition of the population, especially the most unprotected whose food base is legumes.

Materials and Methods

Sample preparation

Six common bean varieties representative of Morelos State, Mexico, were used for the study were used, which are presented in Table 1. Seeds were harvested in 2018 and collected and analysed in the same year.

Table 1. Bean varieties grown in Morelos State, Mexico used in the study

Variety	Origin	Laboratory work classification number	Color	Photography
'Sangre de Toro'	Tlayacapan Morelos	98	Red	
'Peruano'	Cuautla Morelos	100	Yellow	
'Flor de Mayo'	Cuautla Morelos	101	Spotted	
'Negro'	Cuautla Morelos	102	Black	
'Pinto'	Cuautla Morelos	103	Pinto	
'Negro'	Cuautla Morelos	104	Black	

For the analysis, 100 grains of each variety were used. Samples were ground to a fine powder which was stored in polyethylene bags and kept in a desiccator until analysis. The determinations were made in triplicate.

Morphometric characteristics determination of beans

Seed weight.

One hundred seeds of each bean variety were placed in a Petri dish and weighted using an analytical scale (And Company Limited, Milpitas, CA, U.S.A.). The result was reported in g of 100 seeds.

Length, width and thickness.

These were determined using a digital vernier (Steren®, Azcapotzalco, Mexico City, Mexico). One hundred seeds of each variety studied were used for the test. Results were expressed in mm.

Color determination

For color evaluation, 100 seeds of each variety were taken and placed in a glass petri dish until the container was full, and using a portable colorimeter Konica Minolta DP-400 (Minolta Co. Ltd. Osaka, Japan) the CIELAB system color coordinates (L*, a* and b*) were obtained from the surface of these samples. Where parameter L* represents brightness, which varies from 0 (black) to 100 (white); a* can have either positive (red) or negative (green) values; and b* represents yellow when the value is positive and blue when it is negative.

Color coordinates L*, a* and b* were used to obtain the CIEL*C*h° color space, where C represents chroma or color saturation and h° is the hue angle or hue representing color according to the angle on the 360

° color wheel, with red-purple at 0 °, yellow at 90 °, blue-green at 180 ° and blue at 270°, counter clockwise (McGuire, 1992).

Chroma and hue were calculated using the following formulas (McGuire, 1992):

$$C* = (a*^2 + b*^2)^{1/2} \quad (1)$$

$$°h = 360° + [(\arctan (b*/a*)) / 6.2832]*360 \quad (2)$$

When a*>0 and b*<0

Or

$$°h = [(\arctan (b*/a*)) / 6.2832]*360 \quad (3)$$

When a>0 y b>0

Or

$$°h = 180 + [(\arctan (b*/a*)) / 6.2832]*360 \quad (4)$$

When a<0 y b<0

Nutritional quality analysis of bean

Ash content determination

Ash analysis was performed using the method proposed by Mexican Norm NMX-F-F066-S-1978 (1978). One g of sample was weighed in a crucible and kept at constant weight (100 °C for 2 h) then placed in a desiccator and taken to a muffle (Felisa®) where it was kept at 600 °C to carbonize the sample until calcination was reached. Results were expressed as percentage of ash.

Fat content determination

Fat content in the bean samples was determined using the Golfish method proposed by the Association of Official Analytical Chemist (AOAC) (2000). Goldfish flasks were dried and kept to constant weight in an oven. The Goldfish Labconco® grease extractor was then assembled and the sample was placed inside filter paper and covered with absorbent cotton, to be introduced into the equipment. The solvent (petroleum ether) was added and kept under reflux for 2.5 h. After the extraction time was over, the solvent was recovered by distillation, retaining only the fat. Finally, the flask with fat was weighed and expressed as a percentage.

Moisture content determination

Moisture was obtained by the open-capsule drying method proposed by the AOAC (2000). For the analysis, 1 g of sample was taken from each bean variety, which was weighed in an aluminium capsule that was previously dried at 75 °C until constant weight. Each capsule with the sample was weighed and placed in an oven (Felisa® St. Livonia, Michigan, U.S.A) for 12 h at 75 °C. Then, the capsule was removed from the oven and placed in a desiccator. Afterwards, its weight was taken. Moisture content was expressed as a percentage.

Fiber content determination

Crude fiber was determined using the method proposed by Mexican Norm NMX-F-90-S-1978 (1978). The analysis was performed on the previously defatted sample. First, the sample weight was taken and recorded, then each sample was transferred to a beaker for fiber determination and 200 mL of 1.25% sulfuric acid and 1 mL of isoamyl alcohol as defoamer were added to each beaker. Mixture was kept at boiling point for 30 min. Afterwards, it was rinsed to remove sulfuric acid and isoamyl alcohol residues and at the same time to neutralize the mixture. Subsequently, 200 mL of 1.25% sulfuric acid was added, kept boiling for 30 min and then rinsed in glass fiber until neutralized. Thereafter, fiberglass with the sample in the capsule was placed in an oven (Felisa) and left to dry for 12 h until sample was completely dry. Once the drying process was finished, the capsule with the fiberglass and sample was weighed, and then the fiber percentage of each bean variety was obtained by weight difference.

Carbohydrate content determination

Carbohydrates were obtained by difference with ash, fat and moisture content obtained and reported as a percentage.

Protein content determination

To quantify protein concentration, the Flash 2000 Organic Elemental Analyzer (Thermo Scientific® Corporation, Cambridge, UK) was used, whose procedure is based on the Dumas method proposed by Reussi-Calvo *et al.* (2008). First, a tin capsule was taken and placed on a microbalance (Mettler Toledo®, Columbus, Ohio, USA), then 9 µg of vanadium pentoxide and 3 µg of the bean sample were weighed. Finally, sample was placed in the Flash 2000 autosampler for analysis. Protein concentration was expressed as a percentage.

Energy determination

Energy contained in each sample was measured as the sum of calories contained in carbohydrates, fat and protein as described in the Mexican Official Norm NOM-051-SCFI/SSA1-2010 (2010). Energy expressed in Kcal 100^{-1} g^{-1}.

Mineral analysis

Micronutrient determination

One g of finely ground sample was weighed on an analytical balance (And Company Limited, Milpitas, CA, U.S.A.). Afterwards, acid digestion of the dehydrated plant tissue was performed by adding 25 mL of triacid mixture (1000 mL nitric acid, 100 mL hydrochloric acid and 25 mL sulfuric acid), and placed in a digester (Labconco® Corporation, Kansas City, MO, U.S.A.). After digestion, sample was filtered and volumetrically diluted with tridistilled water in a 50 mL flask. Finally, samples were poured into polypropylene tubes for further analysis. Fe, Zn, Mn, Cu and Ni concentration was determined by atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer iCE 3000 Thermo Scientific® Corporation, Cambridge, UK) and concentration was expressed in ppm.

Macronutrient determination

Magnesium (Mg), potassium (K), calcium (Ca) were quantified by atomic absorption spectrophotometry (atomic absorption spectrophotometer iCE 3000 Thermo Scientific) in the same way as micronutrients were obtained and expressed as a percentage.

Phosphorus (P) was determined by colorimetry using the ammonium metavanadate-molybdate method. 500 µL of the digested sample was taken for minerals, 1 mL of phosphorus reagent (ammonium metavanadate-molybdate) and 3.5 mL of tridistilled water was added; it was stirred in a Vortex (VWR, Thorofare, New Jersey, U.S.A.) and allowed to stand for 1 h. After that time, the samples were read in a UV/Vis spectrophotometer (JENWAY Spectrophotometer, Jenway Limited®, Essex, England) at a wavelength of 430 nm; a calibration curve was performed with a phosphorus standard (Ion Chromatography Standard (IC) AcculonTM Reference Standard, New Haven, Connecticut, U.S.A.) and results were expressed as dry weight percentage (%).

C, H, S and N organic compounds determination

C, H, S and N were determined by the Dumas method proposed by Reussi-Calvo *et al.* (2008). First, a tin capsule was taken and placed on a microbalance (Mettler Toledo®, Columbus, Ohio, USA). Nine µg of vanadium pentoxide and 3 µg of the bean sample were weighed, placed in a capsule and then closed. Finally, sample was placed in the Flash 2000 organic elemental analyzer (Thermo Scientific® Corporation, Cambridge, UK) and the compound concentration was reported as a percentage. Three replicates were performed for the analysis.

Antioxidant capacity determination (2, 2-diphenyl-1-picrylhydrazyl (DPPH))

The analysis was carried out using the method proposed by Hsu *et al*. (2003). Extract was obtained by maceration of 1 g of finely ground seed in 5 mL of 80% methanol, which was centrifuged (Allegra® Refrigerated Centrifuge, Beckman Coulter, Inc.; Fullerton, California, U.S.A.) at 6000 rpm for 10 min at 4 °C. Once extract was centrifuged, 0.5 mL of the supernatant was taken and mixed with 2.5 mL of freshly prepared 0.1 mM DPPH solution. Thereafter, sample was incubated (Boekel Scientific incubator) for one h in dark conditions and at room temperature. After this time, samples were read in a UV/Vis spectrophotometer (Genesys 10S, Thermo Scientific® Corporation, Cambridge, UK) at a wavelength of 517 nm. Resulting antioxidant capacity was reported as percentage inhibition.

Nutraceutical compound determination

Total phenols extraction and quantification

Phenolic compounds were extracted using the colorimetric method proposed by Singlenton and Rosi (1965). For analysis, 0.5 g of ground bean seed was mixed with 2.5 mL of methanol, 2.5 mL of chloroform, and 1.25 mL of 2% NaCl solution (J. T. Baker, State of Mexico, Mexico) and macerated to obtain an extract. Subsequently, mixture was homogenized, then centrifuged (Allegra® Refrigerated Centrifuge, Beckman Coulter, Inc.; Fullerton, California, U.S.A.) at 5000 rpm for 10 min and three phases were obtained, a methanol phase, which contains the phenolic acids, interphase containing the proteins precipitated by NaCl and the chloroform phase constituted by dissolved lipids. For the reaction, 750 µL of 2% Na_2CO_3 was placed in a test tube and mixed with 250 µL of Folin-Ciocalteau reagent (Sigma-Aldrich, St. Louis, MO, USA), 1375 µL of deionized water and 250 µL of enzyme extract. Finally, mixture was incubated at room temperature for 60 min. Quantification was obtained using a standard curve of gallic acid (10-100 µg ml^{-1}) at an absorbance of 725 nm. Results are shown in mg of gallic acid equivalents per g of sample (mg GAE g^{-1}) (dry weight).

Flavonoid content determination

Flavonoid analysis was performed according to the method proposed by Zhishen *et al.* (1999). Extract was obtained by macerating 0.5 g of ground seed with 5 mL of 85% methanol. Subsequently, it was centrifuged (Allegra® Refrigerated Centrifuge, Beckman Coulter, Inc.; Fullerton, California, U.S.A.) at 4000 rpm for 10 min. Then an aliquot of 250 µL was placed in a test tube, and 75 µL of $NaNO_2$ (J. T. Baker, State of Mexico, Mexico) was added, the mixture was homogenized in a vortex (VWR, Thorofare, New Jersey, U.S.A.) and allowed to stand for 5 min. Afterwards, 150 µL of $AlCl_3$ (Sigma-Aldrich, St. Louis MO, USA) and 500 µL of NaOH (J.T. Baker, State of Mexico, Mexico) were added and diluted to a final volume of 2.025 mL with tridistilled water. Absorbance was then measured at 510 nm in a UV/Vis spectrophotometer (Genesys 10S, Thermo Scientific® Corporation, Cambridge, UK).

Results obtained were expressed as mg catechin equivalents (CE) per g of sample (mg CE g^{-1}) based on dry weight.

Anthocyanin determination

Anthocyanin content was determined by pH differential according to the method proposed by Wrolstad *et al.* (2005). 0.5 g of finely ground bean was mixed with 5 mL of methanol (J.T. Baker, Estado de Mexico, Mexico). Mixture was centrifuged (Allegra® Refrigerated Centrifuge, Beckman Coulter, Inc.; Fullerton, California, U.S.A) at 4000 rpm for 10 min. After the centrifugation time, 2 phases of the sample were obtained; 0.5 mL of the first phase were taken and placed in a test tube, then 2 mL of potassium chloride (KCl) (J.T. Baker, Estado de México, México) were added, homogenized in a vortex (VWR, Thorofare, New Jersey, U.S.A.) and its absorbance was obtained in a UV/Vis spectrophotometer (Genesys 10S, Thermo Scientific® Corporation, Cambridge, UK) at 460 nm. Subsequently, 0.5 mL of the second phase was taken and deposited in a test tube, 2 mL of sodium acetate was added, homogenized in the vortex and the absorbance reading was taken in the UV/Vis spectrophotometer. Results were expressed as mg Cyanidin-3-glucoside (C3G) g^{-1} (dry weight$)^{-1}$.

Phytic acid determination

For phytic acid determination, the method proposed by McKie and McCleary (2016) was used, first total phosphorus was obtained by the ammonium metavanadate-molybdate method in an absorption range of 430 nm and a potassium phosphate standard curve. Once the results were obtained, the following formula was applied:

$$\text{Phytic acid} = (\text{Total phosphate}) / 0.282 \quad (5)$$

Where 0.282 is a conversion factor from total phosphorus to phytic acid. Results were expressed in g of phytic acid per 100 g (g 100^{-1} g^{-1}) based on dry weight of sample.

Statistical analysis

Data was subjected to an analysis of variance in a completely randomized one-factor design to evaluate the effect of bean variety on the different variables studied, as well as an analysis of comparison of means using the Tukey test and an analysis of correlation between variables performed with SAS statistical package (SAS Institute, INC; Cary, NC, USA) Means were accepted as significantly different at a 95% confidence interval ($p \leq 0.05$). Results were reported as mean± standard deviation.

Results and Discussion

Morphometric characteristics of bean seeds

Table 2 shows weight, length, width and thickness of bean seeds of the evaluated varieties. Statistical analysis showed a significant difference ($p \leq 0.05$) between varieties for the four variables mentioned above. Seed width ranged from 5.5 to 9.1 mm, with the lowest value for 'Flor de Mayo' and the highest for 'Sangre de Toro', the latter being the only variety with a statistical difference compared to other varieties. Bean thickness ranged from 4.9 to 6.8 mm, with 'Pinto' being the lowest and 'Peruano' the highest, although the latter variety was statistically equal to 'Flor de Mayo', 'Negro'/102 and 'Negro'/104. In addition, kernel length ranged from 10.8 to 17.3 mm, with 'Negro'/102 being the shortest variety and 'Sangre de Toro' the longest. Finally, the weight of 100 seeds ranged from 23.06 to 53.42 g, with 'Negro'/102 being the shortest and 'Sangre de Toro' the heaviest. In summary, 'Sangre de Toro' had the highest values for width, length and weight among all analysed varieties, while 'Negro'/102 was the shortest and lightest.

Results obtained from dimensions of the bean varieties analysed in the present work are lower than those reported by Herrera-Hernández *et al.* (2018) in bean varieties grown in Zacatecas, Mexico. Likewise, the weight of 100 seeds was higher than reported by Mederos and Reynaldo (2007) in 'Cuban bean' varieties with black and red coat. According to the classification of Aguirre and Gómez-Aldapa (2010), 'Sangre de Toro' and 'Peruano' varieties are classified as large (>40 g) and 'Flor de Mayo', 'Negro'/102, 'Pinto' and 'Negro'/104 as medium (26 to 40 g). Other studies have shown great variability in the weight of 100 seeds, for example Pliego-Marín *et al.* (2013) found a large amplitude of this variable in seeds collected in Central Valleys of Oaxaca, Mexico, with intervals ranging from 11.2 to 74.8 g of 100 seeds, being out of the range of what was found in this study, with very small genotypes such as 'Negro Delgado' with a weight of 11.2 g of 100 seeds coming from Zaachila and 'Frijolon' with 74.8 g weight with the same origin.

Table 2. Morphometric characteristics of several common bean varieties from Morelos State, Mexico

Variety/ Classification number*	Width (mm)	Thickness (mm)	Length (mm)	Weight (g of 100 seeds)
'Sangre de Toro'/98	9.1 ± 0.01a	6.4 ± 0.02ab	17.3 ± 0.09a	53.42 ± 3.59a
'Peruano'/100	8.2 ± 0.05b	6.8 ± 0.06a	13.6 ± 0.04b	44.41 ± 1.01b
'Flor de Mayo'/101	5.5 ± 0.02b	5.5 ± 0.02bc	12.6 ± 0.05bc	30.97 ± 0.18c
'Negro'/102	7.3 ± 0.02 b	5.5 ± 0.02bc	10.8 ± 0.03d	23.06 ± 0.33d
'Pinto'/103	7.5 ± 0.07b	4.9 ± 0.03c	13.1 ± 0.09bc	29.07 ± 0.36c
'Negro'/104	7.4 ± 0.02b	5.6 ± 0.05bc	11.46 ± 0.04cd	30.29 ± 0.84c

Data correspond to mean ± SD. Different letters per column indicate significant statistical difference between varieties. Tukey test ($p \leq 0.05$). *Laboratory work classification.

Bean seed color characteristics

Bean color is an important aspect for consumer taste so its evaluation is necessary (Aguirre-Santos *et al*., 2011). Table 3 presents the colour characteristics L*, a*, b*, chroma and hue of common bean seeds from Morelos, Mexico, the statistical analysis showed significant difference ($p \leq 0.05$) between varieties in all five variables analysed. Luminosity ranged from 24.96 to 69.21, with 'Negro'/102, 'Negro'/104 and 'Sangre de Toro' being the bean varieties with the lowest luminosity corresponding to darker bean colors. While 'Peruano bean', 'Pinto' and 'Flor de Mayo' had the highest L* value corresponding to lighter colors of all varieties analysed. These values correspond to those reported by Chávez-Mendoza *et al*. (2019) in bean varieties from different regions of Mexico. They are also similar to those reported by Aguirre and Gómez-Aldapa (2010) for 'Pinto Saltillo', 'Bayo Victoria' and 'Negro San Luis' bean varieties with 7.125, 57.1 and 21.85, respectively. Regarding the a* value, it ranged from 0.71 to 26.043. Where 'Peruano' and 'Negro'/104 beans had the lowest values for this color characteristic, with no statistical difference between them, indicating a lower tendency to red and a higher tendency to green. In addition, 'Sangre de Toro' had the highest a* value of all varieties analysed, which coincides with its strong red color. On the other hand, b* value was within a range of -1.42 to 33.44. 'Negro'/102 and 'Negro'/104 had negative values indicating a greater tendency to blue color, while 'Peruano' bean had the highest b* value with a more yellow tendency. Other studies reported negative values of b* for dark-skinned beans such as 'Negro Puebla', 'Negro 151', 'Negro 152', 'Negro Querétaro', 'Negro San Luis', 'Negro Sinaloa', 'Negro Veracruz', 'Medellín', 'Nayarit 80', 'Jamapa', 'Negro Perla', 'Merentral', 'Altiplano' and 'Negro Puebla 152', which places them in the third quadrant of the tri-stimulus hunter L scale, a* b* corresponding to blue-green coloration (Salinas-Moreno *et al*., 2005).

Regarding chromaticity, it showed a range from 1.77 to 33.45, with 'Negro'/102 and 'Negro'/104 having the lowest values or lowest color saturation, whereas the 'Peruano bean' had the highest color clarity or chromaticity while 'Flor de Mayo' and 'Negro'/104 were in the intermediate range for this variable. This range was higher than that reported by Aguirre and Gómez-Aldapa (2010) in 'Negro San Luis', 'Pinto Saltillo' and 'Bayo Victoria' varieties.

Hue angle was within the range of 11.75 ° and 126.64 °, with 'Sangre de Toro' variety having the lowest value, which is coincidentally in the red tone of the color wheel (McGuire, 1992), while 'Negro'/102 variety had the highest angle with a bluish-green tone, equal to the coloration reported by Aguirre and Gómez-Aldapa (2010) for 'Negro San Luis' bean, which corresponds to the third quadrant of the tristimulus scale. Likewise, 'Peruano' and 'Pinto' varieties showed a shade closer to yellow, since their hue angle was close to 90 °, as reported by McGuire (1992).

Table 3. Seed color characteristics of several common bean varieties from Morelos State, Mexico

Variety/ Classification number*	L	a*	b*	Chroma	° Hue
‘Sangre de Toro’/98	32.41 ± 0.09d	26.04 ± 1.03a	5.42 ± 0.20d	26.60 ± 1.04b	11.75 ± 0.25f
‘Peruano’/100	69.21 ± 1.1a	0.71 ± 0.68d	33.44 ± 1.07a	33.45 ± 1.07a	88.78 ± 1.75c
‘Flor de Mayo’/101	50.14 ± 0.71c	14.81 ± 0.51b	11.46 ± 0.59c	18.73 ± 0.58c	37.74 ± 1.62e
‘Negro’/102	24.96 ± 0.40f	1.05 ± 0.11d	-1.42 ± 0.09e	1.77 ± 0.05d	126.64 ± 4.49a
‘Pinto’/103	65.94 ± 0.67b	6.21 ± 0.24c	16.46 ± 0.06b	17.59 ± 0.05c	69.32 ± 0.08d
‘Negro’/104	26.85 ± 0.60e	0.91 ± 0.07d	-1.80 ± 0.12e	2.03 ± 0.07d	116.99 ± 3.55b

Data correspond to mean ± SD. Different letters per column indicate significant statistical difference between varieties. Tukey ($p \leq 0.05$). *Laboratory work classification.

Nutritional analysis

Table 4 reports the nutritional composition of the bean varieties analysed in the present study. Statistical analysis showed significant differences ($p \leq 0.05$) between varieties in protein, ash, fat, moisture, carbohydrate, fiber and energy content.

Protein concentration ranged from 18.03 to 26.92%. ‘Sangre de Toro’ and ‘Pinto’ varieties had the lowest content, ‘Flor de Mayo’, ‘Negro’/102 and ‘Negro’/104 had intermediate values and ‘Peruano’ bean showed the highest concentration. The results obtained in most of the varieties analysed in this study are similar to those reported by Armendáriz-Fernández *et al*. (2019) in bean varieties harvested in Oaxaca, Mexico. As well as to those found by Herrera-Hernández *et al.* (2018) in varieties harvested in Zacatecas, Mexico. They also coincide with the results obtained by Peña-Betancourt and Conde-Martínez (2012) on wild bean varieties (‘Durango Atypical’ and ‘Typical’, ‘Oaxaca Chico’ and ‘Tlaxcala Atypical’ and ‘Typical’). Also, commercial beans such as ‘Flor de Mayo’, ‘Peruano’, ‘Garbancillo’ and ‘Flor de Junio’. Mederos (2006), on the other hand, indicates that protein in beans ranges between 16 and 30% and that varieties most consumed in Latin America have an average concentration of 20%, which is in agreement with results obtained in the present study. According to the same author, bean protein has a high lysine and phenylalanine plus tyrosine content, so it fulfils all the minimum requirements recommended by the Food and Agriculture Organization (FAO) or the World Health Organization (WHO).

Ash content ranged from 4.0 to 4.96%. ‘Flor de Mayo’ and ‘Negro’/104 varieties had the lowest concentration with no statistical difference between them, followed by ‘Pinto’, ‘Negro’/102, and ‘Peruano’ with intermediate values, and finally ‘Sangre de Toro’ with the highest concentration among all six varieties analysed. The obtained range was higher than reported by Herrera-Hernández *et al.* (2018) in varieties harvested in Zacatecas, Mexico and slightly lower than results obtained by Armendariz-Fernández *et a*l. (2019) in bean varieties harvested in the state of Oaxaca, Mexico. Aguirre and Gómez-Aldapa (2010) reported slightly lower results in ‘Negro San Luis’, ‘Pinto Saltillo’ and ‘Bayo Victoria’ varieties, noting that ash content may vary depending on cultivar genetics and soil characteristics.

Fat concentration was within the range of 0.96 to 1.64%. ‘Negro’/104 had the lowest value for this variable while ‘Peruano’ had the highest content of all evaluated variables, with no statistical difference between ‘Sangre de Toro’ and ‘Negro’/102. These results are similar to those reported by Aguirre and Gómez-Aldapa (2010) on ‘Negro San Luis’, ‘Pinto Saltillo’ and ‘Bayo Victoria’ varieties corresponding to a range of 0.92 to 1.71%. In addition, they are higher than those reported by Fernandez and Sanchez (2017). Lipid fraction of bean is the smallest, and is constituted by a mixture of acylglycerides whose predominant fatty acids are monounsaturated and polyunsaturated (Ulloa *et al*., 2011).

Moisture content of common bean was within the range of 10.29 and 14.58%, being ‘Negro’/104 the variety with the lowest moisture content and ‘Negro’/102 with the highest value. Results obtained in this last variety were much higher than those reported in other black beans such as ‘Negro San Luis’, which had a moisture content of 11.95 % (Aguirre and Gómez-Aldapa, 2010), but were similar to those found in some Cuban varieties (Mederos and Reynaldo, 2007); however, the remaining varieties analysed in the present work were much lower than the values reported by the latter authors. Other studies have shown results different from those found in this work such as the one conducted by Armendariz-Fernández *et al*. (2019) who reported that 'Sangre de Toro' and 'Peruano' varieties had a lower moisture content, while the 'Flor de Mayo' variety had a higher amount (12.8%) than that observed in this study. Meanwhile, Herrera-Hernández *et al*. (2018) reported much lower moisture values in bean varieties produced in Zacatecas, Mexico, ranging from 6.14 to 7.42%. Peña-Betancourt and Conde-Martínez (2012) found much lower results in wild and improved bean varieties, suggesting that the lower moisture content of wild varieties was due to increased temperature and lack of irrigation at the production site, in addition to prolonged storage. Aguirre and Gómez-Aldapa (2010) reported that moisture content is related to seed age and postharvest handling, as well as to processing methods and conditions.

Carbohydrates constitute the main fraction in legume beans (Mederos, 2006), 100 g of raw beans provide 52 to 76 g depending on variety (Ulloa *et al.,* 2011). Carbohydrate concentration obtained in the present study was between 51.21 and 61.53%, where 'Pinto' variety had the highest content, while 'Peruano' had the lowest. No statistical difference was obtained between the concentration of 'Negro'/104 (58.28%) and 'Sangre de Toro' (58.01%), nor between 'Flor de Mayo' (53.58%) and 'Negro'/102 (53.67%), which had intermediate values for this variable. Other studies have shown values different from these results; thus, Armendariz-Fernández *et al*., (2019), reported a higher carbohydrate content in 'Flor de Mayo' (55 %) and 'Peruano' (56.6%) varieties, while in 'Sangre de Toro' beans the found value was lower (55.2%). For their part, Herrera-Hernández *et al*. (2018) reported a higher range (57.16% to 65.79%) of carbohydrate content in common bean varieties grown in Zacatecas, Mexico, including 'Flor de Mayo', 'Negro', 'Pinto Saltillo' bean, among others. While Fernandez and Sanchez (2017) found much lower values than those obtained in the present study in different varieties of beans produced and consumed in Mexico purchased in a local market in Delicias, Chihuahua, Mexico, such as 'Bayo', 'Pinto', 'Negro', 'Alubia', 'Flor de Mayo' and 'Peruano', where the latter was the exception as it showed a higher concentration than that obtained in this study. Bean carbohydrates consist mainly of starch and other polysaccharides (dietary fiber) with small but significant amounts of oligosaccharides; starch represents more than 50% of seed weight and is the dominant carbohydrate in the human diet, hence the importance of this legume (Mederos, 2006).

Bean is also a good source of fiber which ranges in value from 14-19 g 100^{-1} g^{-1} of the raw food, from which up to half may be of the soluble form. The main chemical components of fiber in beans are pectins, pentosans, hemicellulose, cellulose and lignin (Ulloa *et al*., 2011). Half a cup of beans provides between 5.2 and 7.8 g of total fiber (Messina, 2014). Fiber content in the present study ranged from 3.21 to 5.71%, 'Flor de Mayo' variety had the highest value while 'Negro'/104 bean had the lowest. These results were far higher than those reported by Herrera-Hernández *et al*. (2018) in genotypes grown in Zacatecas, Mexico and to those reported by Armendariz-Fernández *et al.* (2019) on varieties produced in Oaxaca, Mexico.

Finally, the energy content of the analysed beans was between 318.39 and 334.6 Kcal, with 'Negro'/104 being the variety with the highest energy content and 'Negro'/102 the lowest. Similar results were reported by Armendariz-Fernández *et al*. (2019) on several bean varieties produced in Oaxaca, Mexico, including 'Sangre de Toro', 'Bayo', 'Peruano' among others. Likewise, results were lower than those reported by Herrera-Hernández *et al*. (2018) in bean varieties produced in Zacatecas, Mexico.

Table 4. Nutritional composition of several common bean varieties from Morelos State, Mexico

Variety/ Classification number*	Protein (%)	Ash (%)	Fat (%)	Moisture (%)	Carbohydrates (%)	Crude fiber (%)	Energy (Kcal $100^{-1}g^{-1}$)
'Sangre de Toro'/98	18.03 ± 0.13d	4.96 ± 0.03a	1.53 ± 0.04a	13.45 ± 0.04b	58.01 ± 0.15b	3.40 ± 0.02c	320.41 ± 0.23d
'Peruano'/100	26.92 ± 1.87a	4.22 ± 0.02c	1.64 ± 0.05a	11.93 ± 0.02c	51.21 ± 0.10d	3.81 ± 0.02b	328.36 ± 0.13c
'Flor de Mayo'/101	23.29 ± 1.40b	4.0 ± 0.02d	1.11 ± 0.07cd	11.53 ± 0.02d	53.58 ± 0.12c	5.71 ± 0.02a	320.59 ± 0.39d
'Negro'/102	21.72 ± 1.15bc	4.26 ± 0.01c	1.47 ± 0.07ab	14.58 ± 0.03a	53.67 ± 0.12c	3.4 ± 0.02c	318.39 ± 0.55e
'Pinto'/103	19.66 ± 1.33cd	4.45 ± 0.05 b	1.26 ± 0.13bc	10.63 ± 0.02e	61.53 ± 0.15a	3.31 ± 0.04d	332.74 ± 0.80b
'Negro'/104	22.71 ± 0.66bc	4.05 ± 0.05d	0.96 ± 0.05d	10.29 ± 0.01f	58.28 ± 0.13b	3.21 ± 0.03e	334.6 ± 0.14a

Data correspond to mean ± SD. Means with the same letter between rows indicate that there is no statistical difference between varieties. Tukey test ($P \leq 0.05$). *Laboratory work classification.

Mineral content analysis

Micronutrient content

Table 5 shows the micronutrients present in the common bean varieties produced in Morelos State, Mexico. Statistical analysis showed a significant difference ($p < 0.05$) between varieties in the content of all the microelements analyzed.

Iron (Fe) concentration ranged from 89.225 to 136.416 ppm, with 'Flor de Mayo' and 'Sangre de Toro' varieties having the lowest Fe content and no significant difference between them, while 'Pinto beans' had the highest concentration. These results are higher than those reported by Akond *et al.* (2011a) on 14 genotypes from the International Center for Tropical Agriculture and the United States of America. As well as those obtained by Armendáriz-Fernández *et al.* (2019) on bean varieties produced and consumed in Oaxaca, Mexico. On the other hand, they are lower than those obtained in most of the bean varieties analyzed by Chávez-Mendoza *et al*. (2019), who found that this micronutrient is present in greater proportion in the coat than in the bean cotyledon. Fe deficiency has been mainly related to anemia. This micronutrient is also part of a large number of enzymes involved in energy production and in the proper functioning of the immune response in humans. Beans contribute approximately 40% of Fe to the diet of people who base their diet on this legume and corn (Mederos, 2006), hence the importance of studying this micronutrient in the different genotypes produced in Mexico, which serves as a basis for biofortification and genetic improvement studies.

Zinc (Zn) content ranged from 24.82 to 35.78 ppm, 'Flor de Mayo' variety had the lowest value, while 'Peruano' had the highest. These results were similar to those reported by Armendáriz-Fernández *et al*. (2019) in bean varieties produced and consumed in the state of Oaxaca Mexico. Some studies report that Zn content in beans is one of the highest among vegetables, almost equal to that found in dairy products, although lower than that found in meat. Evaluations carried out on bean collections reveal ranges in the content of this micronutrient from 21 to 54 ppm with an average of 35 ppm (Mederos, 2006), which coincides with the results found in this work. Importantly, this microelement is associated with decreased oxidative stress in cells and improved immune cell function. In addition, its deficiencies have been shown to cause DNA damage in peripheral blood cells in rats (Mudryj *et al*., 2014).

Nickel (Ni) concentration in analyzed varieties ranged from 2,113 to 6,783 ppm with 'Negro'/102 bean having the lowest value and 'Sangre de Toro' having the highest. These data are similar to those reported by Chávez-Mendoza *et al.* (2019) in different bean varieties, where a higher presence of this micronutrient was observed in the cotyledon than in the coat, with 'Bayo' having the highest value at 8.62 ppm, while 'Negro' and 'Flor de Mayo' beans had the lowest, coinciding with what was reported in the present study.

Manganese (Mn) content ranged from 9.036 to 18.82 ppm, with 'Negro'/102 having the lowest concentration of this micronutrient, while 'Pinto' had the highest value. These results were similar to those reported by Chávez-Mendoza *et al*. (2019) in several bean varieties, including 'Flor de Mayo', 'Pinto Saltillo', 'Negro San Luis', 'Negro 8025', 'Negro Jamapa', 'Higuera Azufrado' among others. However, they are lower than those obtained by Herrera-Hernández *et al*. (2018) in some varieties grown and consumed in Zacatecas, Mexico, including the 'Flor de Mayo' variety in which a higher content of this microelement was obtained (25.45 pm) than that obtained in the present work.

Lastly, Cu concentrations of the varieties studied ranged from 3.48 to 6.69 ppm. 'Sangre de Toro' had the lowest content of this micronutrient while 'Pinto' had the highest. These results are much lower than those recorded by Herrera-Hernández *et al*. (2018) in bean varieties grown in Zacatecas state, Mexico.

Summarizing, 'Pinto' had the highest concentration of Fe and Mn, 'Peruano' had the highest concentration of Cu and Zn, while 'Sangre de Toro' had the highest Ni content and the lowest amount of Fe and Cu. The average concentration of these micronutrients in the analysed seed in descending order was as follows: Fe > Mn > Zn> Cu > Ni. This behaviour is similar to that reported by Chávez-Mendoza *et al.* (2019) in several bean varieties, with the exception of manganese, which, unlike the present study, was in lower concentration than zinc.

Observed differences in mineral content among the varieties analysed in this study can be attributed to the genotype and the environment in which they are produced. Previous studies have shown that the concentration of these compounds in beans has varied as a function of genetic material, crop management and storage conditions (Espinoza-García *et al*., 2016).

Table 5. Micronutrient concentration in common bean varieties produced in Morelos State, Mexico

Variety/ Classification number*	Fe (ppm)	Zn (ppm)	Ni (ppm)	Mn (ppm)	Cu (ppm)
'Sangre de Toro'/ 98	89.22 ± 1.65c	32.97 ± 3.70ab	6.78 ± 0.21a	13.27 ± 0.64bc	3.48 ± 0.42b
'Peruano'/ 100	101.40 ± 3.24bc	35.78 ± 5.10a	3.28 ± 0.46c	11.05 ± 0.41cd	6.69 ± 0.50a
'Flor de Mayo'/ 101	91.06 ± 6.49c	24.82 ± 0.66b	2.45 ± 0.33cd	10.54 ± 0.65cd	3.95 ± 0.68b
'Negro'/ 102	99.76 ± 5.83bc	31.85 ± 1.133ab	2.11 ± 0.30d	9.036 ± 1.43d	6.09 ± 0.56a
'Pinto'/ 103	136.41 ± 10.01a	31.81 ± 3.48ab	5.23 ± 0.46b	18.82 ± 1.83a	4.21 ± 0.68b
'Negro'/ 104	113.43 ± 6.09b	34.38 ± 0.90a	2.49 ± 0.28cd	14.491 ± 0.73b	4.39 ± 0.38b

Data correspond to mean ± SD. Different letters per column indicate significant statistical difference between varieties. Tukey ($p \leq 0.05$). *Laboratory work classification.

Macronutrient determination

Ca, Mg and K are the main cations in common bean. There is greater availability of Ca than Mg or K (Suárez-Martínez *et al.,* 2016).

Table 6 shows the P, K, Mg and Ca contents obtained in the bean samples of the different varieties analysed. Statistical analysis showed a significant difference ($p < 0.05$) between varieties in Mg concentration but not in P, K and Ca content.

Phosphorus, plays a relevant role in various metabolic processes vital to all living organisms such as being part of macromolecular structures, in energy generation and metabolic regulation, hence the importance of its availability in free form as inorganic P, which can be accessed by organisms' cells, as well as other nutrients that are chelated (Rodriguez-Blanco *et al*., 2018). P concentration in bean genotypes evaluated was within a range of 0.149 to 0.194%, although no statistical difference was observed between varieties for this variable, 'Negro'/104 bean had the lowest concentration of this macronutrient while 'Negro'/102 had the highest.

Results obtained in the present study were higher than those described by Armendáriz-Fernández *et al*. (2019) in common beans produced in Oaxaca, Mexico, where 'Peruano' and 'Sangre de Toro' had the highest concentrations with 0.17% for both, and varieties such as 'Negro Michigan' and 'Bayo Bola' had concentrations as low as 0.01%. Fernandez and Sanchez (2017) meanwhile reported higher P values than those obtained here in 'Pinto', 'Peruano' and kidney bean.

As for K concentration, it ranged from 1.464 to 1.815%. These results are higher than those recorded by Armendáriz-Fernández *et al.* (2019) in common bean produced in the State of Oaxaca, Mexico. They also exceed the concentrations of this macronutrient obtained by Fernandez and Sanchez (2017) in beans commonly consumed in Mexico such as 'Bayo', 'Pinto', 'Peruano', 'Negro', kidney bean, 'Flor de Mayo' and green bean.

Ca, Mg and Cu are deficient in developed and developing countries which is attributable to the low availability of mineral elements in the soil and/or the low ability of plants to store them in their tissues, associated at the same time with the scarce availability of food of animal origin (Araméndiz-Tatis *et al.,* 2016). For the present study, the Mg content in the analysed beans ranged from 0.169 to 0.201%, with the 'Negro'/104 bean having the lowest concentration, while the 'Negro'/102 bean had the highest amount. Results were much higher than those reported by Herrera-Hernández *et al*. (2018) in common bean varieties produced in Zacatecas, Mexico, in which 'Patola' and 'Japanese' beans had the highest concentrations of this macronutrient with a concentration of 0.16% in both genotypes. Fernandez and Sanchez (2017) also found lower concentrations than those obtained here, in bean varieties produced and consumed in Mexico.

Ca belongs to the group of minerals that should always be part of our diet. It is the most abundant mineral element in our body, as it is an important part of the skeleton and teeth. It accounts for about 2% of body weight and is an essential cellular component for maintaining and/or performing the various specialized functions of virtually all cells in the body. These functions, non-skeletal, we can divide them into structural and properly regulatory (Martinez, 2016). In this study, Ca content ranged from 0.154 to 0.273%. These results are similar to those covered by Herrera-Hernández *et al.* (2018) on common bean varieties produced in Zacatecas, Mexico. Other studies have disclosed lower concentrations than those obtained in the present investigation in 'Pinto', 'Flor de Mayo' and 'Peruano' beans (Fernandez and Sanchez, 2017).

In summary, 'Negro'/102 had the highest Mg concentration while 'Negro'/104 had the lowest. Moreover, all the varieties analysed had the same P, K and Ca contents according to the statistical analysis.

Table 6. Macronutrient concentration (%) in common bean varieties produced in Morelos State, Mexico

Variety/ Classification number*	P	K	Mg	Ca
'Sangre de Toro'/ 98	0.180 ± 0.009a	1.815 ± 0.04a	0.192 ± 0.002ab	0.228 ± 0.073a
'Peruano'/ 100	0.182 ± 0.026a	1.655 ± 0.048a	0.188 ± 0.006bc	0.1545 ± 0.022a
'Flor de Mayo'/ 101	0.170±0.015a	1.659 ± 0.033a	0.173 ± 0.003d	0.218 ± 0.0651a
'Negro'/ 102	0.194 ± 0.084a	1.605 ± 0.315a	0.201 ± 0.002a	0.273 ± 0.025a
'Pinto'/ 103	0.175 ± 0.059a	1.464 ± 0.080a	0.179 ± 0.001dc	0.245 ± 0.034a
'Negro'/ 104	0.149 ± 0.012a	1.687 ± 0.062a	0.169 ± 0.001d	0.239 ± 0.058a

Data correspond to mean ± SD. Different letters per column indicate significant statistical difference between varieties. Tukey test ($p \leq 0.05$). *Laboratory work classification.

Organic compound determination C, S, N, H

Table 7 presents the C, S, N and H contents obtained in the common bean samples of the different varieties analysed. Statistical analysis showed significant difference ($p < 0.05$) between varieties in N concentration, but not in C, S, and H content ($p > 0.05$).

Carbon concentration in the analysed samples ranged from 40.441 to 42.349%, S from 0.040 to 0.082% and H from 6.153 to 6.391% with no significant statistical difference between varieties for the three elements. These results are lower than those shown by Chávez-Mendoza *et al.* (2019) in several bean varieties, in which no significant difference was observed between varieties in the cotyledon, as occurred in the present investigation; however, these authors did observe significant differences in the content of these elements in the seed coat. On the other hand, Paredes *et al.* (2009) found C and S concentrations higher than those found in the present study in Chilean, Nuevo Granada, Durango and Mesoamerican breeds.

Nitrogen content ranged from 2.926 to 4.0%, with 'Sangre de Toro' variety having the lowest concentration and 'Peruano' the highest. These results are lower than those obtained by Chávez-Mendoza *et al.* (2019) in several bean varieties, both in coat and cotyledon, including 'Flor de Mayo', 'Pinto Saltillo', 'Negro Jamapa', 'Negro San Luis', 'Negro 8025', among others.

Summarizing, it was observed that 'Sangre de Toro' variety presented the lowest values in N concentration, while 'Peruano' bean and 'Flor de Mayo' had the highest amount of this compound. Furthermore, all varieties analysed had statistically the same C, S and H concentration.

Table 7. C, S and N concentration (%) in common bean varieties produced in Morelos State, Mexico

Variety/ Classification number*	C	S	N	H
'Sangre de Toro'/98	40.441 ± 0.53a	0.087 ± 0.018a	2.926 ± 0.12c	6.153 ± 0.203a
'Peruano'/100	41.150 ± 0.11a	0.097 ± 0.010a	4.000 ± 0.21a	6.332 ± 0.064a
'Flor de Mayo'/101	41.418 ± 0.69a	0.094 ± 0.023a	3.660 ± 0.28ab	6.315 ± 0.166a
'Negro'/102	41.625 ± 1.00a	0.086 ± 0.019a	3.542 ± 0.232abc	6.391 ± 0.176a
'Pinto'/103	42.349 ± 2.74a	0.040 ± 0.036a	3.068 ± 0.43bc	6.513 ± 0.49a
'Negro'/104	41.598 ± 0.52a	0.082 ± 0.015a	3.542 ± 0.13abc	6.347 ± 0.098a

Data correspond to mean ± SD. Different letters per column indicate significant statistical difference between varieties. Tukey test ($p \leq 0.05$). *Laboratory work classification.

Antioxidant capacity and nutraceutical compounds

<u>Antioxidant capacity</u>

The antioxidant capacity obtained in the analysed bean varieties is presented in Figure 1. Statistical analysis showed significant difference ($p \leq 0.05$) between varieties in this variable, which ranged from 73.59 to 96.76% inhibition. 'Peruano' bean had the lowest antioxidant capacity, with no statistical difference with 'Pinto' and 'Negro'/104, while the highest value was for the 'Flor de Mayo' variety, which was statistically equal to 'Sangre de Toro' and 'Negro'/102. Study by Chávez-Mendoza *et al.* (2019) also revealed 'Flor de Mayo', 'Negro 8025' and 'Negro San Luis' as the varieties with the highest antioxidant capacity, finding differences in this variable between cotyledon and coat, with the latter having the highest percentage of inhibition. These authors noted that these varieties had the highest phenolic content, which could explain their higher antioxidant activity, as was the case in the present study, where 'Flor de Mayo', 'Sangre de Toro' and 'Negro'/102 had the highest concentration of total phenols, flavonoids and anthocyanins. Silva-Cristobal *et al.* (2010) also disclosed that the antioxidant capacity of legumes depends on the total polyphenol content. Aguilera *et al.* (2011) point out that the antioxidant activity is directly related to the polyphenol structure, such as the number of hydroxyl groups, degree of glycosylation etc. González de Mejía *et al.* (1999) found that phenolic compounds extracted from the coat of common bean inhibit the mutagenicity induced by benzopyrene and 1-nitropyrene (premutagenic and mutagenic agents, respectively), hence the importance of studying these compounds present in common bean. On the other hand, Armendariz-Fernández *et al.* (2018) reported lower results in bean varieties grown in Oaxaca, Mexico also finding that 'Sangre de Toro' and 'Flor de Mayo' variety along with two other varieties, had the highest values of antioxidant ability with 82.1% and 79.1% respectively. Some authors have stated that 'Flor de Mayo' is the preferred variety for consumption in Central Mexico (Cardador-Martínez *et al.*, 2002), which confirms the importance of this variety for Mexican consumers.

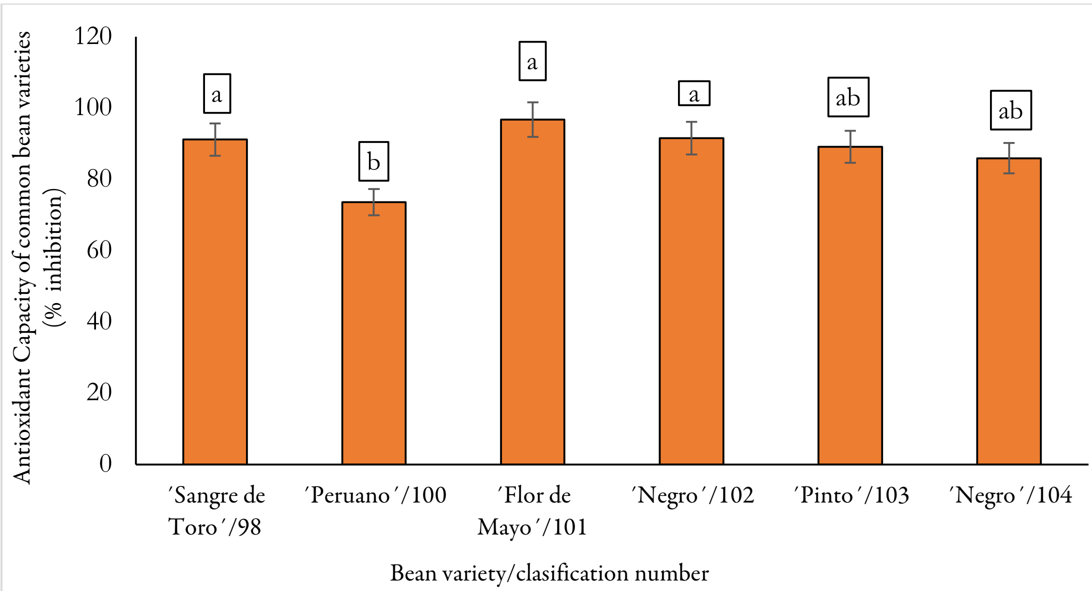

Figure 1. Antioxidant capacity of common bean varieties grown in Morelos State, Mexico. Means with the same letter are not significantly different, Tukey test ($p \leq 0.05$). *Laboratory work classification.

Nutraceutical compounds

Table 8 shows the nutraceutical compounds evaluated in common bean varieties produced in Morelos State, Mexico. Statistical analysis showed significant difference ($p \leq 0.05$) between varieties in the content of total phenols, flavonoids and anthocyanins, but not in the phytic acid concentration ($p > 0.05$).

Total phenol content ranged from 1.76 to 4.24 (mg GAE g^{-1}). Statistical analysis showed significant differences between varieties in the content of this compound. 'Negro'/104 was the variety with the lowest phenol concentration along with 'Negro'/102, whereas 'Flor de Mayo' had the highest content, followed by 'Sangre de Toro', while 'Pinto' and 'Peruano' beans had intermediate concentrations. Other studies have found that these compounds are found in greater amounts in the bean coat than in the cotyledon; and have reported results similar to those obtained in the present work in some common Mexican bean varieties, such as 'Flor de Mayo', 'Negro 8025', 'Negro San Luis', 'Pinto Saltillo' among others (Chávez-Mendoza *et al.*, 2019). Likewise, the results obtained were superior to those found by Espinosa-Alonso *et al.* (2006) in wild and weedy Mexican bean germplasm materials from Chiapas, Chihuahua, Durango, Guerrero and Jalisco, as well as in 'Pinto' and 'Negro Jamapa' beans. The total phenol level obtained in the bean of the present study exceeds that observed in wild berry species of the genus *Vaccinium*, which are among the most important sources of these bioactive compounds in fruits with a content of 0.81 to 1.70 mg GAE g^{-1} (Taruscio *et al.* 2004), which denotes the importance of consumption of the common bean studied in this work. According to Rodriguez *et al.* (2021) the differences in phenolic composition between varieties may be related to the color of the seed coat, however, other studies have suggested that this variability is due more to genotype than to color, as well as to environmental conditions under which it is produced.

Phytic acid is bound to minerals and does not allow mineral availability due to its chelating property, it has been reported to inhibit the absorption of Fe, Zn, Ca, Mg and Mn (Gupta *et al*., 2015). However, it is also considered an antioxidant and anti-carcinogen with potential human health benefits. This accounts for 60 to 85% of the total P in the seed (Akond *et al.,* 2011b). In the present study, phytic acid concentration ranged from 0.149 to 0.194 g 100^{-1} g^{-1}, with no significant difference between varieties. Similar results were disclosed by Iniestra-González *et al*. (2005) on a group of 16 Mexican common bean varieties with different bean types, 'Negro', 'Pinto', 'Crema' or 'Bayo', 'Azufrado', 'Flor de Mayo' and 'Blanco'. While they were much lower than those obtained by Díaz-Batalla *et al.* (2006) in wild and cultivated varieties of Mexican common raw and cooked beans and by De Paula *et al*. (2018) in different genotypes of Colombian cowpea, in which the control 'Criollo Córdova' showed the maximum value with 12.27 mg g^{-1}. On the other hand, the results obtained were superior to those shown by Akond *et al*. (2011a) in several common bean genotypes from USA, Brazil and the International Center for Tropical Agriculture. Although phytic acid is considered an anti-nutrient, it does not represent a problem and can positively affect human health if a diverse diet is consumed in which micronutrient intake and bioavailability are high (Diaz-Batalla *et al*., 2006).

Flavonols quercetin and kaemferol are the most important flavonoids in foods and their consumption has been linked to an inverse association between lung cancer and risk of cardiovascular disease (Díaz-Batalla *et al.,* 2006). Their presence affects the flavour and color of common bean (Yang *et al.,* 2018). In the present research, flavonoids were present in a concentration ranging from 0.275 to 1.991 mg CE g^{-1}. 'Peruano' bean had the lowest concentration, which showed no significant difference with 'Negro'/104, 'Negro'/102 and 'Pinto' bean. Whereas 'Flor de Mayo' had the highest content of these nutraceutical compounds, whose concentration was statistically equal to that obtained in the 'Sangre de Toro' variety. Result obtained in 'Flor de Mayo' was slightly higher than that reported by Herrera-Hernández *et al.* (2018) in this same variety but grown in Zacatecas, Mexico, in general the analysed varieties in this study had a higher content of flavonoids than those evaluated by those authors such as 'Bayo' bean, 'Flor de Junio', 'Reata', 'Canario', 'Pinto Saltillo', 'Negro' among others. Other studies have shown that there is a difference in the content of these nutraceutical compounds between the seed coat and the whole grain (Aquino-Bolaños *et al.,* 2016). These authors disclosed results lower than those found in the present work in seed samples from 26 common bean populations collected in several rural communities in the states of Oaxaca, Guerrero, Puebla, Tlaxcala and Estado de México, presenting a range in whole seed from 0.10 to 0.78 mg CE g^{-1}. Rodriguez *et al.* (2021) observed that color influences seed flavonoid content in genotypes of Spanish origin, with those of white coat having the lowest amount and those of red color the highest, which coincides with the results obtained in this study with the 'Sangre de Toro' variety. Other lines that showed high concentrations of these nutraceutical compounds were pink, brown and black, which does not coincide with the results found in the present work.

Anthocyanins constitute one of the most important groups of natural pigments and are responsible for many of the colors of fruits and vegetables, as well as flowers; in beans they are present in higher amounts in black or blue-violet seeds (Guevara-Lara *et al*., 2006). Anthocyanins in the samples analysed ranged from 0.763 to 2.400 mg C3G g^{-1}. No significant difference was found between 'Pinto' and 'Negro'/104 beans, which had the lowest concentration of these compounds, while 'Sangre de Toro', 'Peruano' and 'Flor de Mayo' showed the highest values with no statistical difference between them. Results were higher than those shown by Guevara-Lara *et al.* (2006) in wild bean and weed samples from Chiapas, Chihuahua, Durango, Guerrero, Jalisco, Michoacán, Morelos, Nayarit, Oaxaca, Sinaloa and Zacatecas. Likewise, values obtained also exceeded the results presented by Reynoso-Camacho *et al*. (2007) in 'Pinto Zapata', 'Flor de Mayo', 'Anita' and 'White Tlaxcala' beans, but were lower than those obtained in the 'Flor de Junio Marcela' variety. They were also higher than the values reported by Herrera-Hernández *et al.* (2018) in several common bean varieties grown in the state of Zacatecas Mexico. Differences in the content of these bioactive compounds are due to factors such as genotype and place of origin. In contrast to the present study, Rodriguez *et al*. (2021) found the highest concentration of monomeric anthocyanins in black coat bean samples with an average of 4.40 mg C3G g^{-1}. This same result was obtained by Salinas-Moreno *et al*. (2005) in 15 Mexican black bean varieties. Whereas Aquino-Bolaños *et al*. (2016) stated that the highest concentration of this bioactive compound was present in the cream-pinkish varieties, with similarity to that obtained in 'Flor de Mayo' variety of the current study. Meaning that the color may be independent of the anthocyanin content in the common bean.

In summary, of all the varieties analysed, 'Negro'/104 beans had the lowest concentration of total phenols, flavonoids and anthocyanins, while 'Flor de Mayo' and 'Sangre de Toro' had the highest values.

Table 8. Nutraceutical compounds of common bean varieties produced in Morelos State, Mexico

Variety/ Classification number*	Total phenols (mg EAG g^{-1})	Phytic acid (g100^{-1} g^{-1})	Flavonoids (mg CE g^{-1})	Anthocyanins (mg C3G g^{-1})
'Sangre de Toro'/98	2.89 ± 0.40ab	0.18 ± 0.009a	1.579 ± 0.10a	2.400 ± 0.11a
'Peruano'/100	2.24 ± 0.83b	0.182 ± 0.02a	0.275 ± 0.07b	2.147 ± 0.22a
'Flor de Mayo'/101	4.24 ± 0.57a	0.170 ± 0.01a	1.991 ± 0.09a	2.134 ± 0.18a
'Negro'/102	1.97 ± 0.57b	0.194 ± 0.08a	0.518 ± 0.12b	1.599 ± 0.07b
'Pinto'/103	2.19 ± 0.28b	0.175 ± 0.05a	0.638 ± 0.27b	0.763 ± 0.15c
'Negro'/104	1.76 ± 0.26b	0.149 ± 0.01a	0.416 ± 0.16b	1.133 ± 0.20c

Data correspond to mean ± SD. Different letters per column indicate significant statistical difference between varieties. Tukey test ($p \leq 0.05$). *Laboratory work classification

Correlation analysis

Table 9 shows the Pearson correlation coefficients obtained between color variables, nutraceutical compounds and antioxidant ability.

Table 9. Pearson correlation coefficients between color variables, nutraceutical compounds and antioxidant capacity of different varieties of common bean grown in Morelos State, Mexico.

	L	a*	b*	Chroma	°Hue	AF	AC	TF	Flavonoids	Anthocyanins
L	1									
a*	-0.13	1								
b*	0.92**	-0.13	1							
Chroma	0.69*	0.42	0.82**	1						
°Hue	0.70*	0.29	0.58*	0.60*	1					
AF	0.05	0.03	0.08	0.10	0.15	1				
AC	-0.38	0.44	-0.56*	-0.36	-0.01	0.11	1			
TF	0.14	0.64*	0.06	0.31	0.34	-0.21	0.50*	1		
Flavonoids	-0.10	0.83**	-0.16	0.23	0.28	0.04	0.61*	0.86**	1	
Anthocyanins	-0.04	0.54*	0.22	0.57*	0.08	0.10	-0.08	0.429	0.49*	1

*Significant linear correlation ($p < 0.05$); **highly significant linear correlation ($p < 0.0001$). °Hue = Hue angle. FA = phytic acid. AC = antioxidant capacity. TF = total phenols.

Brightness showed a highly significant positive correlation with b* value (r=0.92), as well as significant with chroma (r=0.69) and hue angle (r=0.70). Likewise, a* value was highly positively correlated with flavonoids (r=0.83) and significantly with total phenols (r=0.64) as well as anthocyanins (r=0.54). As for b* value, it showed a highly significant positive linear correlation with chroma (r=0.82) and significant with hue angle (r=0.58), it also had a significant negative correlation (r=-0.56) with antioxidant capacity. On the other hand, chroma correlated positively and significantly with hue angle (r=0.60) and anthocyanins (r=0.60). While phytic acid showed no correlation with any of the color variables and nutraceutical compounds evaluated; total phenols were highly positively correlated with flavonoids (r=0.86) and significantly correlated with antioxidant capacity (r=0.50) whereas flavonoids were significantly and positively correlated with anthocyanins (r=0.49).

The results of the present investigation revealed an important contribution of nutraceutical compounds such as total phenols, flavonoids and anthocyanins in the a* value of common bean grown in the producing region of Morelos State, Mexico. Some authors have suggested that the color of the coat of this crop is closely related to the content of several phenolic compounds, prominent among them being flavonoids (Yang *et al*., 2018) Likewise, in the current work a good correlation was observed between total phenols and flavonoids with antioxidant capacity, this result has been obtained by other authors (Rodriguez *et al*., 2021; Mastura *et al*., 2017). However, contrary to what was obtained by other authors (Aquino-Bolaños *et al*., 2016), in this study no linear correlation ($p > 0.05$) was observed between anthocyanins and antioxidant capacity.

Conclusions

The current research showed that genotype has a significant effect on antioxidant capacity, and nutraceutical compound content in bean seed. Obtained colour values coincided with the coloration perceived in the different varieties evaluated. Thus, lower lightness was observed in varieties with darker colours such as 'Negro'/102, 'Negro'/104 and 'Sangre de Toro' as well as higher lightness in lighter coloured varieties such as 'Peruano' bean, 'Pinto' and 'Flor de Mayo'. Furthermore, the highest a* value corresponded to the varieties with a greater tendency to red coloration, such as 'Sangre de Toro', and the highest b* value corresponded to those with a greater tendency to yellow coloration, such as 'Peruano'. On the other hand, the relationship between color and nutraceutical compound content was not very clear in the present study, since the only component that had a significant positive correlation with these was the a* value, which may indicate that the content of these compounds in the seed depends more on the genotype than on the seed coat color. In terms of nutritional and nutraceutical quality, of all the varieties studied, 'Flor de Mayo' beans had the highest crude fiber content, total phenols, flavonoids, anthocyanins and antioxidant ability, and, after 'Peruano' beans, the highest protein content. The correlation found between flavonoids and total phenols with antioxidant capacity shows these nutraceutical compounds as important indicators of antioxidant capacity of the seeds evaluated. Finally, the study showed that the bean varieties produced in Morelos State, Mexico, may be of interest from a functional and nutritional point of view. Likewise, these varieties are favourable lines to be biofortified or used in genetic improvement programs in the future, for benefiting the population whose basic source of protein is this legume or those with scarce resources.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

We thank to Consejo Nacional de Ciencia y Tecnología (CONACyT)-México (National Council of Science and Technology, Mexico) for the support granted in the "Convocatoria Proyectos de Desarrollo Científico para Atender Problemas Nacionales 2015" (Call for Scientific Development Projects to Address National Problems 2015), to Project 1529 "Biofortificación de cultivos agrícolas básicos, clave para combatir la desnutrición" (Biofortification of basic agricultural crops, key to fight malnutrition).

Conflict of Interests

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

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