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# Supply of essential and nonessential amino acids, proteins, antioxidants, iron and zinc from the main varieties of beans consumed in Mexico and their potential for biofortification

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## Abstract

The objective of the present study was to characterize the contribution of essential and non-essential amino acids, protein, iron (Fe), and zinc (Zn) concentration, and antioxidant activity of the main bean varieties produced and consumed in Mexico. 23 varieties of beans were selected, and their amino acid profile, Fe and Zn concentration, protein and antioxidant activity were evaluated. The data obtained were subjected to an analysis of variance, test of separation of means and a correlation analysis. The results obtained indicate that the beans analyzed are an excellent source of essential amino acids (Histidine, threonine, valine, methionine, lysine, isoleucine, leucine and phenylalanine) and non-essential amino acids (Aspartic acid, serine, glutamic acid, glycine, alanine, proline, cysteine, tyrosine and arginine). The most prominent amino acids in Mexican bean varieties were: Glutamic acid, proline, aspartic acid, serine, lysine, and leucine. Regarding antioxidant activity, the most outstanding concentrations ranged from 91.18 to 96.76% inhibition. The bean varieties with the highest accumulation of amino acids, proteins, Fe, Zn and antioxidant activity were: 1) Black-eyed (23); 2) Peruvian (154); 3) Flor de Junio (150); 4) Pinto Saltillo (155). Finally, it was found that the common bean (*Phaseolus vulgaris* L.) is an excellent vehicle for the biofortification of Fe and Zn, which can contribute significantly to combat malnutrition problems and health of vulnerable communities in the urban and rural sectors of Mexico, as well as in the developing countries of the world.

***Keywords:*** biofortification; glutamic acid; green bean; *Phaseolus vulgaris* L.; proline

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## Introduction

In recent years, consumer tastes have changed towards foods that encourage a higher quality lifestyle. A new social and cultural approach to food has contributed to the emergence of new eating-habit trends. On the other hand, for some populations of the world, the inclusion of high-quality proteins in their diets is a problem, especially for those who rarely consume animal products. Even when energy intake from other foods is adequate, insufficient concentrations of essential amino acids can contribute to the prevalence of undernutrition (Ganesan and Xu, 2018).

One way to deal with the problem of amino acid deficiency is to identify grains with high biological value proteins. There are food plants that have not been fully exploited; among them is the common bean (*Phaseolus vulgaris* L.), the most important legume for human consumption in the world (Espinosa-Alonso *et al.,* 2006). It has nutritional properties, among which its high protein content stands out. Proteins are by far the most important nutrients; their functional diversity is enormous. They have the potential to avoid the risk of diseases and to promote optimal health conditions; furthermore, their nutritional value makes them capable of exerting specific biological effects. They are formed by macromolecules made up of chains of building blocks, known as amino acids, which are linked by peptide bonds between the carboxyl and a-amino groups, resulting in the loss of a $H_2O$ molecule (Gupta *et al*., 2021).

The proteins ingested in the diet are the fundamental source of amino acids for building the body's own proteins. They carry out most of functions in the cells of living beings. They are part of the basic structure of tissues (muscles, tendons, skin, nails, etc.); they regulate the assimilation of nutrients, fat-soluble vitamins and minerals and are responsible for the transport of oxygen and fats in the blood, as well as for the elimination of toxic materials. However, the anabolic pathways of the human body do not allow for the synthesis of the full range of compounds needed for normal cell metabolism, and a significant amount of them must be provided by the diet (Hou *et al.,* 2015).

The biological value of a protein depends primarily on its amino acid composition and the ratio among them, and its maximum value is considered to be when these ratios are necessary to meet nitrogen demands that are required for growth, synthesis, and tissue repair (Marrugo-Ligardo *et al*., 2016). Human beings need a total of twenty amino acids, of which eleven are synthesized by themselves, these are called nonessential. The remaining nine are not synthesized by the body and must be provided by the diet. These are called essential amino acids. If any of them is absent, it will be impossible to synthesize the proteins that require such amino acids. This can lead to different types of undernutrition, depending on the amino acid deficiency, since the missing amino acid that is not present in the dietary protein cannot contribute to the synthesis of new proteins (Gupta *et al*., 2021).

Tryptophan, Lysine and Methionine are the essential amino acids that cause the main problems related to human nutrition, since their deficiency is typical in populations that have difficult access to products of animal origin, and therefore, cereals or tubers become the basis of their diet (Galili and Amir, 2013).

Lysine is needed in the body for the development of carnitine, which is used in lipid metabolism. This amino acid stimulates cholesterol synthesis in the liver. In addition, it takes part in the production of collagen and elastin (Huang *et al*., 2021).

Methionine is used in the manufacture of Taurine, which is an important amino acid for heart function. It is also a neurotransmitter in the brain. Methionine deficiency has been found to be associated with low-quality protein intake. Its deficiency may also result in inadequate phosphatidylcholine synthesis and other phospholipids. These substances are essential for the functioning of the nervous system, as well as for preventing the agglutination of blood cells. Methionine is also transformed into Homocysteine, which is again turned into Methionine by the trans-sulfuration pathway (Wu, 2020).

Homocysteine should not accumulate in the body. If it happens, it is associated with an increased risk of heart disease and atherosclerosis (disease that occurs in the coronary arteries). A deficient conversion of

Homocysteine to Methionine is caused by vitamin B6 deficiency in genetically susceptible people. This genetic defect does not allow proper conversion of Homocysteine to Methionine. This is associated with an increased risk of atherosclerosis (Moretti and Caruso, 2019).

FAO has stated that the protein in a food is biologically complete when it contains all the amino acids in an amount equal to or greater than that established for each amino acid needed for a reference or standard protein. The standard requirement of amino acids for preschoolers and adults corresponds to the recommendations given by FAO/WHO/ONU for preschoolers 1.10 g/kg/d and for adults 0.75 g/kg/d (FAO, 2013).

The importance of antioxidant components in maintaining health and protecting against coronary heart disease and cancer is generating interest among scientists, food manufacturers and consumers, as it has been shown to improve health, delay aging, they prevent chronic diseases, prolong life expectancy and support body structure (Khan *et al*., 2021). In addition to this, the future trend is moving towards functional foods with specific health effects. Synthetic antioxidants can present a certain degree of toxicity and require high manufacturing costs but with less efficiency than natural antioxidants. Therefore, it is necessary to identify natural, inexpensive foods with outstanding antioxidant effects and with the potential to be incorporated into processed foods. Beans have beneficial levels of antioxidant capacity, substances capable of retarding oxidation by protecting the body from the action of free radicals (Gan *et al*., 2016). Therefore, it is of great interest to evaluate the antioxidant capacity in varieties of Mexican beans.

Mexico has a large agrobiodiversity of beans throughout the national territory. Currently, it has reported the presence of 70 species of the 150 existing worldwide within the genus *Phaseolus*. In 2017, its production amounted to 1,183,000 tons, making it the seventh-largest producer in the world and the second largest in terms of total area planted nationally, only after corn (SIAP, 2021). The protein concentration of the bean ranges from 15 to 30%; moreover, it has enough content to meet the basic daily needs of amino acids: Lysine (6.4-7.6 g100 $g^{-1}$ of protein) and phenylalanine + tyrosine (5.3-8.2 g 100 g-1 of protein) (Petry *et al.,* 2015).

Hence, it is considered as a strategic crop which satisfies the recommended intake values that avoids nutritional deficiencies. This has not only a nutritional implication, but from the economic point of view it is considered feasible to use beans in diets and social programs as a strategy to prevent undernutrition (SIAP, 2021).

In addition, since there is little research on the nutritional contribution in terms of amino acid content in beans, it is necessary to promote the development of new studies that encourage the scientific community to assess different types of legumes, such as beans, since this will open better prospects for the development of national agriculture and ensure the bioavailability of protein in products of plant origin. The diffusion of new alternatives for the nutritional improvement of the population using legumes, especially in the country's impoverished urban and rural communities, will be reflected in an increase in the quality of Mexico's basic food basket.

The purpose of this study was to characterize the contribution of essential and non-essential amino acids, protein, iron (Fe), and zinc (Zn) concentration, and antioxidant activity of the main bean varieties produced and consumed in Mexico, which influence human health and diet. This not only leads to improved food security, but also improves the nutrition of the vulnerable communities in the urban and rural sectors of Mexico, as well as in the developing countries of the world.

## Materials and Methods

### *Plant material*

Twenty-three varieties of beans (*Phaseolus vulgaris* L.) were selected from different locations in Mexico (Table 1). The analyzed variables were: Raw protein (RP) content, antioxidant activity, Fe content, Zn content and amino acid profile: Aspartate (Asp), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine

(Thr), alanine (Ala), proline (Pro), cysteine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe).

**Table 1.** Bean varieties that were selected for the study

| Variety | Origin | Photography | Variety | Origin | Photography |
|---|---|---|---|---|---|
| Black-eyed (23) | San F. de Conchos, Chihuahua |  | Pinto (103) | Cuautla, Morelos |  |
| Black (46) | Conkal, Yucatán |  | Black (104) | Cuautla, Morelos |  |
| Navy (49) | Motul, Yucatán |  | Patol (105) | Canatlán, Durango |  |
| Black (81) | Mapastepec, Chiapas |  | Sangre de toro (127) | Villa Alta, Oaxaca |  |
| Wild (82) | Cuauhtémoc, Chihuahua |  | Michigan black (134) | Zoachila, Oaxaca |  |
| Guaca black (84) | Tuxtepec, Oaxaca |  | Pinto centauro (142) | Cusihuiriac hi, Chihuahua |  |
| Pinto Saltillo (97) | Namiquipa, Chihuahua |  | Bayo (146) | Calera, Zacatecas |  |
| Sangre de toro (98) | Tlayacapan, Morelos |  | Frijola (147) | Calera, Zacatecas |  |
| Black (99) | Cuautla, Morelos |  | Flor de Junio (150) | Calera, Zacatecas |  |
| Peruvian (100) | Cuautla, Morelos |  | Black (153) | Calera, Zacatecas |  |
| Flor de mayo (101) | Cuautla, Morelos |  | Peruvian (154) | Calera, Zacatecas |  |
| | | | Pinto Saltillo (155) | Calera, Zacatecas |  |

*Sample preparation*

One hundred seeds per variety of bean were taken, and then grounded in a blender. The resulting flour was placed in properly labelled plastic bags, which were then used in the interest analysis.

*Nutritional analysis of the beans*

The analyzed variables of the 23 varieties were the following: Protein concentration, antioxidant activity, Fe, Zn and amino acid profile. The soluble amino acids were determined by UPLC, with the AccQ-Fluor reagent kit and AccQ-Tag Eluent A solution, purchased from Waters (Milford, MA).

The Amino Acid Standard H was purchased from Thermo Scientific (Rockford, IL). All of the other reagents were of analytical grade.

The protein profile was determined using the Dumas method (Reussi-Calvo *et al*., 2008). The antioxidant activity was determined using the methodology of DPPH (Xu *et al*., 2007). The determination of Fe and Zn was made through an inductive Couple Plasma Optical Emission Spectrometer (Agillent Technologies 700 Series ICP-OES, California, USA), according to the method described by Karacan and Aslantas (2008).

*Determination of soluble amino acids though UPLC*

Bean varieties were subjected to an amino acid composition analysis using the ultra-performance liquid chromatograph (UPLC) according to the AccQ-Tag method (Astephen, 2018). Hydrolysis of the samples was performed in the presence of 6 mol/L HCl at 110 °C for 24 h under a nitrogen atmosphere. Amino acids were derivatized at 55 °C with AccQ-Fluor reagent kit. A 10 µL of derivatized sample was injected to the Waters AccQ Tag amino acid analysis column with 3.9 mm i.d. × 150 mm (Waters) set at 37 °C equipped with a UV detector (Waters Dual 2487 Absorbance Detector) set at 254 nm. Mobile phase was the gradient of 60% acetonitrile and AccQ Tag Eluent A solution (AccQ Tag Eluent A and Milli-Q water at a ratio of 1:10 v/v) with the flow rate of 1.0 mL/min. The identification and integration of peaks was performed by Empower software (Waters) using an external amino acid standard (Amino Acid Standard H, Thermo Scientific, IL) ranging from 10 to 200 pmol. The obtained aliquot was derivatized with o-phthalaldehyde reagent. The analysis was performed using UPLC (Amino Acid Analyzer, LC-6A, Shimadzu, Kyoto, Japan) with Shim-pack ISC-07/S 1504Na column equipped with a fluorescence detector (RF-20A/RF20A, Shimadzu) set at 55 °C. Mobile phase was isocratic of 0.2 mol/L boric acid in 0.6 mol/L sodium citrate at a flow rate of 0.4 mL/min. The identification and integration of the peak was carried out by comparing the retention time spectra of external tryptophan standard ranging from 0 to 200 pmol (AOAC, 1995).

*Determination of proteins*

The determination of protein through the Dumas method suggested by Reussi-Calvo *et al*. (2008) was used. First, a sample capsule with 3 µg of nickel was taken and 9 µg of vanadium pentoxide were added. Then, it was placed in the Flash 2000 equipment (Thermo Scientific ® Corporation, Cambridge, UK), and the protein concentration was expressed as a percentage.

*Determination of Antioxidant capacity*

The analysis was performed based on the methodology of Xu *et al.* (2008). The extract was obtained by macerating 1 g of dry sample in 5 mL of 80% methanol (v/v), centrifuged at 6000 rpm for 10 min at a temperature of 4 °C. 0.5 mL of extract was taken from the resulting supernatant, mixed with 2.5 mL of 0.1 mM DPPH solution, freshly prepared the mixture was incubated for 60 min in the dark and cold, the absorbance was measured by A517 spectrophotometry. The antioxidant capacity results were expressed in percent inhibition.

*Determination of iron and zinc*

Fe and Zn concentrations were determined by an inductive Couple Plasma Optical Emission Spectrometer (Agillent Technologies 700 Series ICP-OES, California, USA), according to the method described by Karacan and Aslantas (2008). The concentrations were expressed in ppm of dry weight.

*Statistical analysis*

The obtained data was subjected to an analysis of variance, a means separation test using the LSD test and a correlation analysis, using the statistical program SAS (SAS Institute, Inc., Cary, NC, EE.UU, 2002). Tukey's test (95%) was used to determine the difference between the means of the tested bean varieties. The significance levels of the evidence were expressed as follows: *p <0.05; **p <0.01; ***p <0.001 and NS, non-significant. The experiments were run in triplicate.

## Results and Discussion

*Amino acids profile*

The obtained results regarding the amino acid profile indicated that the 23 varieties of interest are high in both essential and nonessential amino acids (Table 2). A significant content was detected in each of the assessed samples; the collected values showed that the common bean (*Phaseolus vulgaris* L.) possesses 17 of the 20 amino acids that the human body needs to produce proteins (Table 3). Overall, the amino acid composition did not show a noticeable variation in the concentration ratio from one bean variety to another. Nevertheless, it is worth mentioning that the amino acids with the highest levels were: Glu, Asp, Lys, Ser and Leu (Table 2).

Glutamate concentrations were as high as 1.37 mg $g^{-1}$ d.w. in the cv. Black Guaca (84) implying glutamate is the essential amino acid with the highest concentration within the assessed profile. Comparing our data with similar research conducted by Miquilena and Moros (2012) we realize that our results are just as favourable as those reported by that author.

On the other hand, as for the content of Asp it was discovered that, it is the second amino acid with higher presence in the common bean and the most outstanding variety corresponded to Pinto Saltillo bean (97) with 1.36 mg $g^{-1}$ d.w. According to Rivas-Vega (2006), this amino acid was one of the highest values reported in its analysis. It is important to mention that Asp increases the intake and utilization of Zn through the intestinal mucous membrane, blood and cells (Table 3).

Regarding the content of Lysine, an amino acid classified as essential, it showed highly favourable concentrations, such is the case of the Black variety (99) with 0.50 mg $g^{-1}$ d.w. It was positioned within the analysis as the third amino acid with the highest concentration in terms of mg. Sathe (2002) mentioned in his research that one of the indicators involved in the nutritional quality of beans is their high Lys content, this leads us to infer that our concentrations are quite significant. Lys participates in the building of muscle mass, calcium intake and hormone production (Table 3).

According to the data obtained for Ser, values were found in a very similar ratio to those obtained in the determination of Lys, and these results ranged from 0.30-0.47 mg $g^{-1}$ d.w. In comparison to previous studies conducted by Miquilena and Moros (2012), our position agrees with that of the author. In addition, it is important to say that Lys is limited in cereals and therefore when combined with legumes, in this case with beans, a complete and high-quality protein is obtained (Maphosa and Jideani, 2017). This amino acid helps in the production of enzymes, and is a precursor of Gly and Cys (Table 3).

Regarding Leu concentrations, considerable values were found in a range of 0.21 to 0.49 mg $g^{-1}$ d.w., presenting its maximum values for Black-eyed (23) and Black (46) varieties. Previous studies, carried out by Miquilena and Moros (2012), who made an assessment of the amino acid profile of some bean flours, show that their results are consistent with those found in this research. This amino acid diminishes muscle destruction and provides energy to the body's organs (Table 3).

The five most outstanding varieties had from 5.87 to 6.48 mg $g^{-1}$ d.w. of total amino acids in the following order: 6.48 mg $g^{-1}$ d.w., 'Frijola' (147); 6.16 mg $g^{-1}$ d.w., 'Flor de Junio' (150); 6.04 mg $g^{-1}$ d.w., 'Guaca black bean' (84); 5.97 mg $g^{-1}$ d.w., 'Bayo' (146); 5.87 mg $g^{-1}$ d.w., 'Wild Bean' (82). Even the sulphur amino acids (Met and Cys), which can be classified as nutritionally limiting amino acids, presented good levels 0.05-0.15 mg $g^{-1}$ d.w. and 0.01-0.13 mg $g^{-1}$ d.w. respectively (Table 2).

It is important to mention that the analyzed samples contain eight of the nine amino acids classified and required as essential, according to the FAO/WHO. In addition, the sum of the total essential amino acids in the evaluated legumes varied from 2.16 to 2.73 mg $g^{-1}$ d.w., whose results are similar to those reported by other authors such as Miquilena and Moros (2021) and Mederos (2006). Therefore, most of the values indicated in this research are comparable to those of the above-mentioned works.

The comparison between the amino acid profile and the WHO/FAO/ONU reference values (FAO, 2013), shows that the Lys contents in the varieties under study are within the high values of the recommended ranges of amino acid requirements.

**Table 2.** Amino acid profile (mg $g^{-1}$ d.w.) in 23 bean varieties that are outstanding from a mineral point of view

| | Amino acids (mg $g^{-1}$ d.w.) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bean varieties** | **Asp** | **Ser** | **Glu** | **Gly** | **His** | **Arg** | **Thr** | **Ala** | **Pro** | **Cys** | **Tyr** | **Val** | **Met** | **Lys** | **Ile** | **Leu** | **Phe** |
| Black-eyed (23) | 0.60 | 0.47 | 1.27 | 0.24 | 0.11 | 0.24 | 0.26 | 0.26 | 0.26 | 0.11 | 0.23 | 0.34 | 0.11 | 0.30 | 0.29 | 0.41 | 0.30 |
| Black (46) | 0.75 | 0.36 | 1.13 | 0.25 | 0.19 | 0.39 | 0.24 | 0.25 | 0.33 | 0.09 | 0.17 | 0.31 | 0.08 | 0.41 | 0.25 | 0.41 | 0.30 |
| Navy bean (49) | 0.71 | 0.40 | 1.18 | 0.25 | 0.15 | 0.35 | 0.30 | 0.25 | 0.25 | 0.08 | 0.19 | 0.34 | 0.09 | 0.41 | 0.26 | 0.49 | 0.34 |
| Black (81) | 0.50 | 0.41 | 1.28 | 0.36 | 0.25 | 0.31 | 0.34 | 0.25 | 0.33 | 0.01 | 0.08 | 0.22 | 0.05 | 0.40 | 0.25 | 0.30 | 0.33 |
| Wild bean (82) | 0.55 | 0.46 | 1.32 | 0.34 | 0.29 | 0.32 | 0.31 | 0.21 | 0.33 | 0.03 | 0.08 | 0.25 | 0.05 | 0.46 | 0.27 | 0.29 | 0.31 |
| Guaca black (84) | 0.59 | 0.46 | 1.37 | 0.40 | 0.20 | 0.35 | 0.32 | 0.26 | 0.32 | 0.01 | 0.05 | 0.30 | 0.07 | 0.48 | 0.24 | 0.25 | 0.37 |
| Pinto Saltillo (97) | 0.91 | 0.31 | 1.32 | 0.26 | 0.14 | 0.31 | 0.25 | 0.26 | 0.32 | 0.10 | 0.12 | 0.32 | 0.10 | 0.41 | 0.26 | 0.32 | 0.30 |
| Sangre de toro (98) | 0.81 | 0.30 | 1.22 | 0.26 | 0.26 | 0.31 | 0.29 | 0.21 | 0.40 | 0.10 | 0.10 | 0.35 | 0.14 | 0.37 | 0.20 | 0.36 | 0.29 |
| Black (99) | 0.81 | 0.35 | 1.21 | 0.13 | 0.21 | 0.34 | 0.25 | 0.23 | 0.32 | 0.12 | 0.10 | 0.30 | 0.15 | 0.50 | 0.27 | 0.30 | 0.26 |
| Peruvian (100) | 0.89 | 0.38 | 1.36 | 0.20 | 0.24 | 0.32 | 0.30 | 0.23 | 0.35 | 0.13 | 0.18 | 0.23 | 0.09 | 0.34 | 0.21 | 0.36 | 0.24 |
| Flor de Mayo (101) | 0.85 | 0.32 | 1.27 | 0.24 | 0.22 | 0.31 | 0.29 | 0.30 | 0.35 | 0.12 | 0.16 | 0.26 | 0.11 | 0.39 | 0.26 | 0.33 | 0.26 |
| Pinto (103) | 0.70 | 0.34 | 1.10 | 0.22 | 0.15 | 0.36 | 0.25 | 0.23 | 0.24 | 0.10 | 0.16 | 0.30 | 0.08 | 0.39 | 0.21 | 0.41 | 0.27 |
| Black (104) | 0.78 | 0.33 | 1.14 | 0.23 | 0.11 | 0.35 | 0.23 | 0.25 | 0.25 | 0.12 | 0.15 | 0.30 | 0.05 | 0.38 | 0.21 | 0.38 | 0.28 |
| Patol (105) | 0.70 | 0.40 | 1.04 | 0.25 | 0.20 | 0.34 | 0.26 | 0.22 | 0.21 | 0.06 | 0.2 | 0.28 | 0.09 | 0.36 | 0.25 | 0.40 | 0.28 |
| Sangre de Toro (127) | 0.65 | 0.42 | 1.16 | 0.22 | 0.21 | 0.35 | 0.26 | 0.29 | 0.31 | 0.01 | 0.09 | 0.33 | 0.11 | 0.35 | 0.21 | 0.35 | 0.32 |
| Michigan black (134) | 0.77 | 0.37 | 1.27 | 0.22 | 0.17 | 0.34 | 0.36 | 0.26 | 0.23 | 0.12 | 0.19 | 0.25 | 0.10 | 0.40 | 0.26 | 0.35 | 0.34 |
| Pinto centauro (142) | 0.81 | 0.51 | 1.15 | 0.24 | 0.21 | 0.32 | 0.22 | 0.26 | 0.30 | 0.08 | 0.10 | 0.33 | 0.10 | 0.31 | 0.26 | 0.29 | 0.32 |
| Bayo (146) | 0.71 | 0.42 | 1.26 | 0.3 | 0.23 | 0.33 | 0.31 | 0.36 | 0.36 | 0.17 | 0.23 | 0.09 | 0.01 | 0.31 | 0.27 | 0.21 | 0.40 |
| Frijola (147) | 0.69 | 0.36 | 1.6 | 0.33 | 0.21 | 0.4 | 0.25 | 0.28 | 0.24 | 0.11 | 0.21 | 0.31 | 0.09 | 0.42 | 0.32 | 0.40 | 0.26 |
| Flor de Junio (150) | 0.69 | 0.41 | 1.38 | 0.33 | 0.12 | 0.35 | 0.28 | 0.25 | 0.47 | 0.11 | 0.15 | 0.30 | 0.08 | 0.31 | 0.28 | 0.41 | 0.24 |
| Black (153) | 0.68 | 0.31 | 1.05 | 0.33 | 0.24 | 0.41 | 0.30 | 0.31 | 0.4 | 0.13 | 0.11 | 0.21 | 0.07 | 0.31 | 0.20 | 0.30 | 0.26 |
| Peruvian (154) | 0.70 | 0.34 | 1.26 | 0.23 | 0.2 | 0.36 | 0.24 | 0.23 | 0.24 | 0.12 | 0.15 | 0.30 | 0.09 | 0.40 | 0.22 | 0.41 | 0.21 |
| Pinto Saltillo (155) | 0.78 | 0.45 | 1.17 | 0.3 | 0.24 | 0.35 | 0.26 | 0.22 | 1.20 | 0.14 | 0.11 | 0.25 | 0.05 | 0.30 | 0.25 | 0.31 | 0.25 |
| **Significance** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| **LSD** | 0.01 | 0.017 | 0.027 | 0.021 | 0.019 | 0.017 | 0.018 | 0.017 | 0.025 | 0.014 | 0.026 | 0.015 | 0.010 | 0.013 | 0.022 | 0.013 | 0.014 |

Significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS (non-significant). Aspartate (Asp), Serine (Ser), Glycine (Gly), Histidine (His), Arginine (Arg), Threonine (Thr), Alanine (Ala), Proline (Pro), Cysteine (Cys), Tyrosine (Tyr), Valine (Val), Methionine (Met), Lysine (Lys), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe).

Figure 1 shows the sum of total amino acids in the evaluated bean varieties. The variety that had the highest concentration was the 'Pinto Saltillo' bean (155) with 6.63 mg $g^{-1}$ d.w., while the lowest total concentration was observed in the 'Pinto' bean (103) with 5.51 mg $g^{-1}$ d.w. Figure 1 shows how the majority of the varieties showed similar behavior.

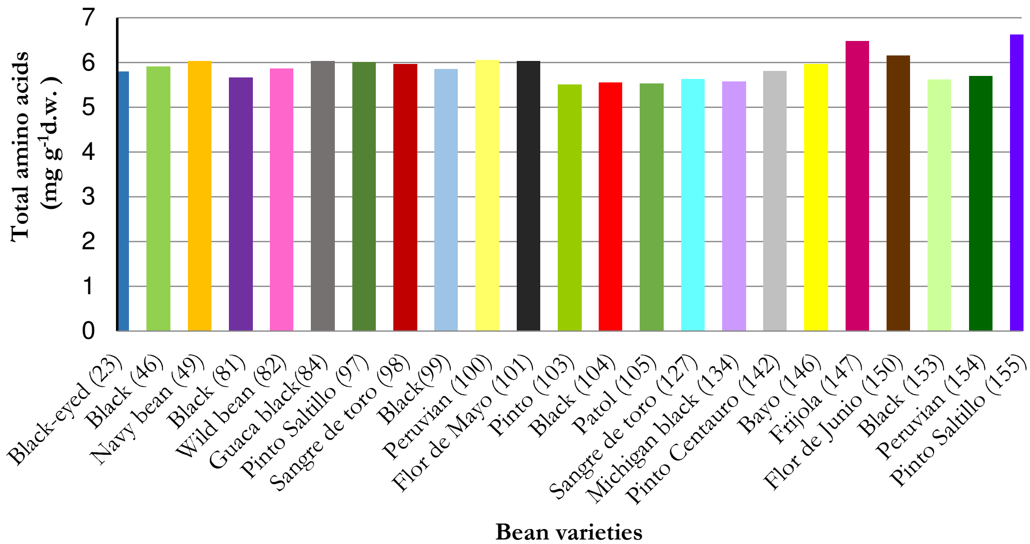



**Figure 1.** Total amino acid concentration expressed in mg $g^{-1}$ d.w. per variety

*Protein concentration*

The protein content of the 23 most outstanding bean varieties was determined from the mineral point of view (Figure 2). Although statistically all the varieties evaluated showed a very similar percentual concentration, they were classified in 2 groups: (1) High levels and (2) Medium levels of protein. The varieties: 'Pinto Saltillo' (97); 'Sangre de toro' (98); 'Pinto' (103); 'Patol' (105); 'Pinto centauro' (142); 'Flor de Junio' (150) and 'Black bean' (153) were group 2, with a protein range of 17-20%. While the remaining varieties were placed among the high levels, group 1 being: 'The Wild bean' (82) which is the most outstanding variety as it has 29.79% of protein.

Our results were similar to those reported by Acosta-Gallegos *et al.* (2016) with concentrations of 15-26.35%; however, in our study higher values were found (17.77-29.79%) (Figure 2). On the other hand, Mahajan *et al.* (2005) studied the protein content in 51 varieties of beans, finding a maximum value of 31.6%. So according to Celmeli *et al.* (2018) this indicates that the values of protein in beans are between a 15 to 25%, which shows us that the twenty-three varieties of beans have a good protein content when they are within the demonstrated ranges of these authors. The crude protein content in varieties reported by Golam-Mortuza and Tzen (2011) is comparable to other bean varieties whose contents range from 21 to 27%. It is noteworthy that although bean proteins are of a lower nutritional quality compared to animal proteins, dry beans contain 15 to 25% protein in dry weight (FAO, 2016), which places them as a plant product with a considerable protein concentration that contributes significantly to the protein intake of the human diet.

A comparison of the amino acid content of bean protein with the FAO/WHO reference protein indicates that beans are a good source of aromatic amino acids, Lys, Leu and Ile. However, it is deficient in sulfur amino acids (Met and Cys), Val, Trp and Thr, compared to the FAO/WHO reference standard Rivas-Vega (2006). Table 4 shows the correlation coefficients for protein and amino acids. There was a significant correlation with Ser and Asp (r=0.41* and r= -0.41*). The relationship with Ser was positive and significant (r=0.41*), while the relationship with Asp was significant and negative (r= -0.41*).

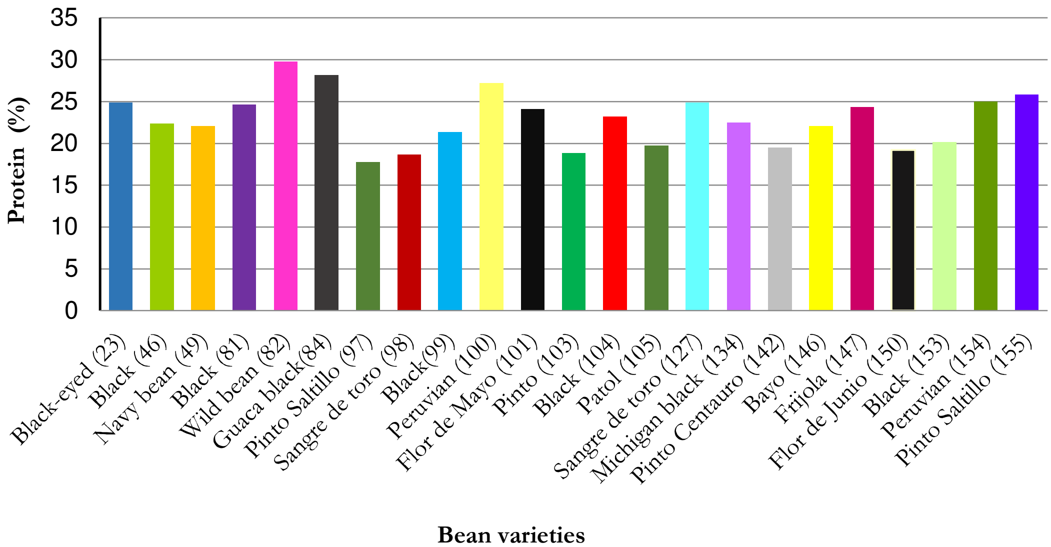


**Figure 2**. Protein concentration expressed in (%) per analysed bean variety

*Antioxidant capacity*

In Figure 3 the antioxidant capacity values are presented in 23 varieties of Mexican beans. The varieties were classified into three main groups: 1) High levels, 2) Medium levels, 3) Low levels of antioxidant capacity. In level 1 the bean varieties were found: 'Flor de Mayo' (101), 'Guaca black' (84), 'Sangre de toro' (98) and 'Navy bean' (49). However, within this group the most outstanding variety was: 'Flor de Mayo' presenting a 96.76% inhibition. At the medium level, varieties were found in a range of 63.26 to 89.81% inhibition, with the outstanding variety in this category being the 'Black' (46), reporting 89.81% inhibition. On the other hand, in the low level of antioxidant capacity, the varieties: 'Black eyed' (23) and 'Peruvian' (154) were found with 14.42 and 15.23% inhibition respectively.

The average value found for antioxidant capacity exceeds that reported by Boateng *et al.* (2008), who found an average value of 55.97% in varieties from the US. In turn, Garretson *et al.* (2018), reported as maximum value 94.3%, these studies show that in general terms, our varieties are within the range described for Antioxidant capacity, and even our maximum (96.76%) is above the data reported by Garretson *et al.* (2018). Table 4 presents the correlation coefficients regarding antioxidant capacity and amino acids. A significant negative correlation was found with Ser (r = -0.25 *), while a significant positive correlation was found with the Met (r = 0.30 *).

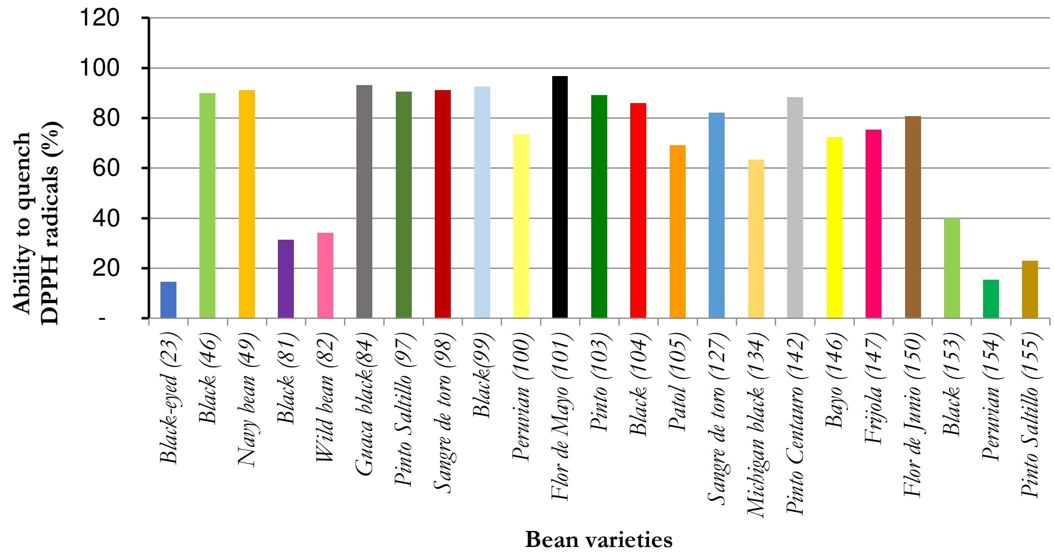



**Figure 3**. Antioxidant capacity (DPPH) of bean varieties. Data are shown as the mean ± standard error (n = 3). DPPH, 2-diphenyl-1 picryl hydrazyl

*Iron content (Fe)*

The results obtained in the micronutrient analysis showed significant differences in Fe concentration in the different bean varieties that were examined (Figure 4). The reported data ranged from 60.37-136.41 ppm, Pinto bean variety (103) being the most outstanding with a maximum of 136.41 ppm. According to Acosta-Gallegos *et al.* (2016), who carried out a study on Mexican bean varieties, it revealed that the content of Fe is in a range of 24.8-57.5 ppm. These values are an indication that our maximum values exceeded the concentrations presented by that author, even our average (84.82 ppm) was reported above its maximum, ie above 57.5 ppm. Table 4 shows the correlation coefficients for Fe and amino acids. It is worth mentioning that a negative and highly significant correlation was found for Phe (r=-0.48**), while Cys showed a positive and significant correlation (r= 0.42*).

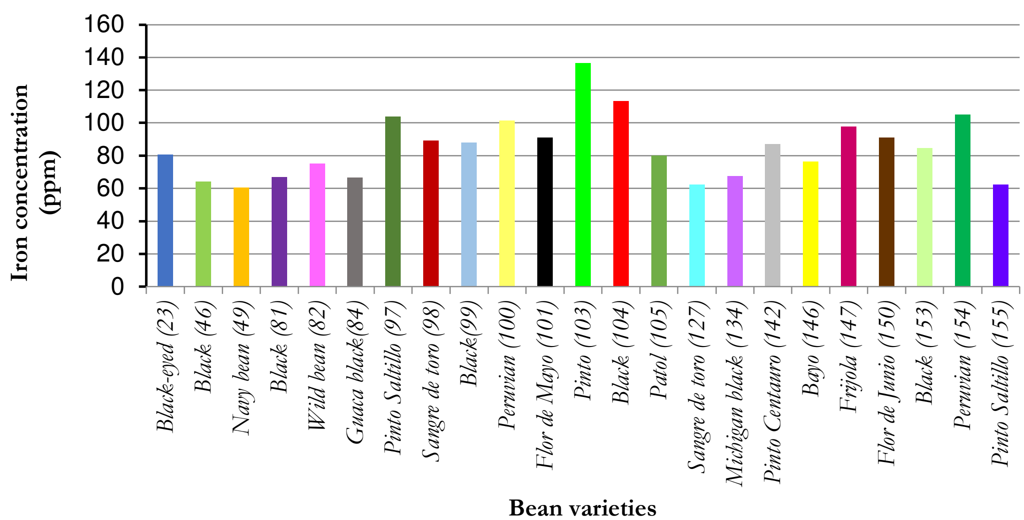



**Figure 4**. Iron concentration (ppm) in 23 varieties of common bean (*Phaseolus vulgaris* L)

*Zinc concentration (Zn)*

The collected values for Zn showed a maximum of 52.12 ppm for the Black-eyed bean variety (23) (Figure 5). According to Chávez-Mendoza and Sánchez (2017), the values of Zn in bean varieties from different regions in Mexico were found in concentrations of 30.83 ppm. This reported value is very similar to our average (29.98 ppm). Therefore, we can say that our results exceed those found by the already mentioned authors. The performed correlation analysis, which is shown in Table 4, shows the correlation coefficients regarding Zn and amino acids. Such analysis showed a negative and highly significant correlation of Arg (r= -0.73**). On the other hand, a positive and significant correlation was shown for the amino acid Phe (r= 0.42*).

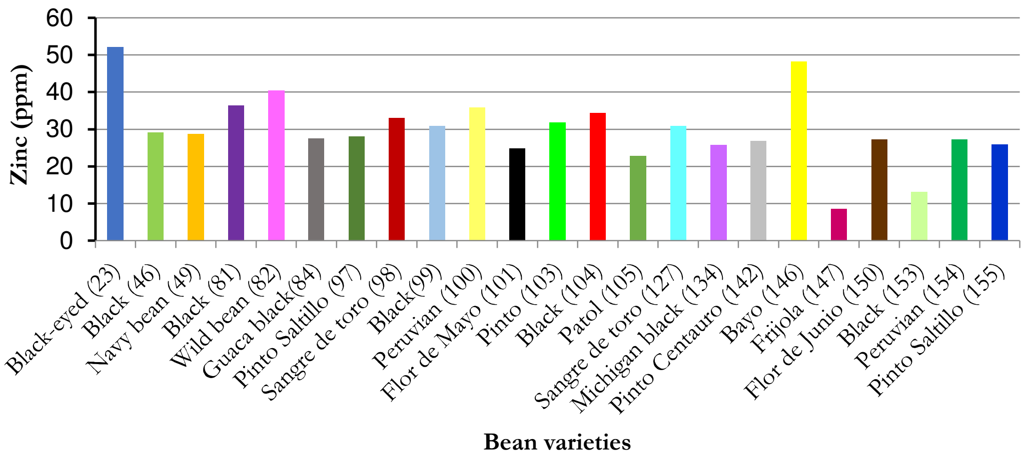
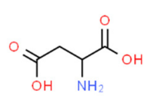
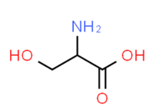
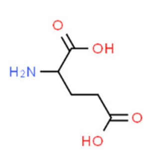



**Figure 5**. Zinc concentration (ppm) in 23 varieties of common bean (*Phaseolus vulgaris* L)

**Table 3.** Amino acids in beans and their body functions

| Amino acid | Type of amino acid | Role in human health |
|---|---|---|
|  Aspartate (Asp) | Nonessential | It increases the intake, circulation and utilization of zinc (Zn) through the intestinal mucous membrane, blood and cells. It promotes the synthesis of urea, the process of eliminating the liver's own and external toxins, especially ammonia (Wu, 2013, Wu, 2021). |
|  Serine (Ser) | Nonessential | Helps in the production of enzymes, brain cells and antibodies. Needed for the proper metabolism of fats, it reduces the levels of fatty acids. glycine and cysteine precursor. Strengthens the immune system. Works as a natural skin moisturizer (Wu, 2013, Wu, 2021). |
|  Glutamate (Glu) | Nonessential | Fundamental in the maintenance and growth of cells. Fights and eliminates free radicals. Nourishes the bloodstream. Repairs the intestinal mucous membrane and raises the intelligence quotient (IQ). Fights heart diseases, diabetes, high blood |

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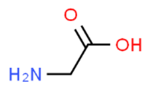
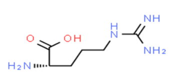
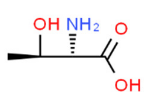
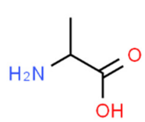
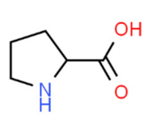
| | | pressure, and conditions such as impotence, arthritis, and allergies (Wu, 2013, Wu, 2021). |
|  Glycine (CGy) | Nonessential | Acts as an inhibitory neurotransmitter, on receptors in the brain stem and marrow. Intervenes actively in the development of the nervous system, brain plasticity and degenerative processes. Effective for preventing diseases such as: arthrosis, schizophrenia and osteoporosis (Wu, 2013, Wu, 2021). |
|  Histidine (His) | Essential | Helps to combat the negative effects of rheumatoid arthritis, to detoxify heavy metals and to treat impotence. It is essential for the production of red and white blood cells. Reduces blood pressure (Wu, 2013, Wu, 2021). |
|  Arginine (Arg) | Nonessential | It is part of the muscle growth. It is a direct generator of nitric oxide with various vasodilatory functions, thus improving blood circulation and increasing blood flow. It improves the healing of skin lesions. Important for the secretion of hormones such as glucagon, insulin and the growth hormone (Wu, 2013, Wu, 2021). |
|  Threonine (Thr) | Essential | It is important for muscle growth, synthesis of digestive enzymes and immune system proteins. It promotes better liver function by preventing fat accumulation. It is involved in phosphate transport, collagen, elastin and tooth enamel formation. Recommended for conditions such as poor digestion, liver congestion, arthritis, rheumatism and eczema (Wu, 2013, Wu, 2021). |
|  Alanine (Ala) | Nonessential | It is used as a source of energy for the muscles, brain and nervous system. Helps to metabolize organic acids and sugar. It's transformed into glucose. Effective treatment for arthrosis, hepatic processes, schizophrenia and prostate disorders (Wu, 2013, Wu, 2021). |
|  Proline (Pro) | Nonessential | It is essential to keep the joints, bones and skin in good condition and participates in the production of collagen. Helps with tissue regeneration, wounds and burns, |

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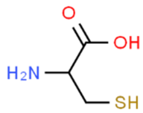
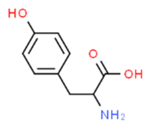
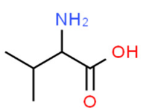
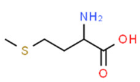
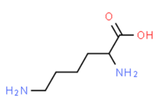
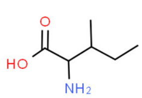
| | | stretch marks and tight skin (Wu, 2013, Wu, 2021). |
| <br>Cysteine (Cys) | Nonessential | It is involved in the improvement of immune functions. It is used to protect the liver and lymphatic system and helps to detoxify and protect the intestine. It prevents LDL cholesterol oxidation; controls blood glucose levels and reduces stroke damage (Wu, 2013, Wu, 2021). |
| <br>Tyrosine (Tyr) | Nonessential | Enhances memory, relieves depression, neutralizes free radicals, prevents various types of respiratory allergies, helps in the production of melanin pigment, participates in the formation of adrenaline, and promotes hair growth (Wu, 2013, Wu, 2021). |
| <br>Valine (Val) | Essential | It participates in the formation of muscle tissue, promotes a positive nitrogen balance, protects the nervous system, and helps to maintain balanced blood sugar levels (Wu, 2013, Wu, 2021). |
| <br>Methionine (Met) | Essential | It intervenes in the metabolism of lipids by reducing their accumulation in the liver and arteries. It participates in digestive and lymphatic function, decreases muscle weakness and promotes healthy hair, skin and nails (Wu, 2013, Wu, 2021). |
| <br>Lysine (Lys) | Essential | It participates in the building of muscle mass, recovery from injuries and wounds, production of hormones, enzymes and antibodies. Absorbs calcium and stimulates the release of the growth hormone. Its deficiency causes fatigue, anorexia, irritability, fertility alterations and restricts growth (Wu, 2013, Wu, 2021). |
| <br>Isoleucine (Ile) | Essential | Helps with the development of muscle tissue, promotes recovery after exercise, and it is necessary for hemoglobin formation. It helps to maintain balanced blood sugar levels. Liver injuries, bacterial infections, mental and nervous disorders (Wu, 2013, Wu, 2021). |

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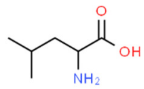
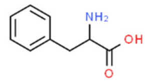
|  Leucine (Leu) | Essential | Mitigates muscle destruction. Provides energy to the muscles and body organs (Wu, 2013, Wu, 2021). |
|  Phenylalanine (Phe) | Essential | It is used in antidepressant treatments since it improves the feeling of well-being thanks to the increase in the endorphin levels. Contributes to reduce nervousness (Wu, 2013, Wu, 2021). |

**Table 4.** Pearson correlation coefficients of variables in common bean varieties

| Variable | Fe | Zn | Prot | Asp | Ser | Glu | Gly | His | Arg | Thr | Ala | Pro | Cys | Tyr | Val | Met | Lys | Ile | Leu | Phe | Antiox |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fe | 1 | | | | | | | | | | | | | | | | | | | | |
| Zn | -0.07 | 1 | | | | | | | | | | | | | | | | | | | |
| Protein | -0.34 | 0.23 | 1 | | | | | | | | | | | | | | | | | | |
| Asp | 0.38 | -0.19 | -0.41* | 1 | | | | | | | | | | | | | | | | | |
| Ser | -0.50* | 0.35 | 0.41* | -0.45* | 1 | | | | | | | | | | | | | | | | |
| Glu | 0.10 | -0.03 | 0.38 | -0.08 | 0.09 | 1 | | | | | | | | | | | | | | | |
| Gly | -0.31 | -0.14 | 0.28 | -0.58** | 0.29 | 0.38 | 1 | | | | | | | | | | | | | | |
| His | -0.30 | -0.08 | 0.36 | -0.11 | 0.07 | 0.07 | 0.25 | 1 | | | | | | | | | | | | | |
| Arg | 0.03 | -0.73** | -0.10 | -0.00 | -0.33 | -0.10 | 0.16 | 0.09 | 1 | | | | | | | | | | | | |
| Thr | -0.36 | 0.22 | 0.36 | -0.43* | 0.11 | 0.25 | 0.58** | 0.47* | -0.16 | 1 | | | | | | | | | | | |
| Ala | -0.13 | -0.00 | -0.07 | -0.03 | -0.01 | 0.08 | 0.20 | -0.02 | 0.13 | 0.18 | 1 | | | | | | | | | | |
| Pro | -0.28 | -0.06 | 0.14 | 0.15 | 0.19 | -0.04 | 0.21 | 0.26 | 0.05 | 0.06 | -0.13 | 1 | | | | | | | | | |
| Cys | 0.42* | 0.00 | -0.29 | 0.58** | -0.36 | 0.01 | -0.31 | -0.14 | 0.07 | -0.25 | 0.25 | 0.27 | 1 | | | | | | | | |
| Tyr | 0.19 | 0.14 | -0.15 | 0.15 | -0.09 | 0.07 | -0.26 | -0.40* | -0.06 | -0.20 | 0.27 | -0.23 | 0.54 | 1 | | | | | | | |
| Val | 0.09 | -0.21 | -0.19 | 0.12 | -0.05 | -0.01 | -0.30 | -0.45* | -0.08 | -0.52* | -0.51* | -0.21 | -0.32 | -0.14 | 1 | | | | | | |
| Met | 0.14 | -0.30 | -0.30 | 0.38 | -0.28 | -0.00 | -0.57** | -0.10 | -0.20 | -0.33 | -0.31 | -0.23 | -0.04 | -0.10 | 0.70** | 1 | | | | | |
| Lys | 0.02 | -0.12 | 0.25 | -0.11 | -0.21 | 0.26 | -0.00 | 0.12 | 0.15 | 0.10 | -0.30 | -0.34 | -0.39 | -0.35 | 0.23 | 0.22 | 1 | | | | |
| Ile | -0.18 | 0.02 | 0.09 | -0.16 | 0.38 | 0.58 | 0.23 | -0.12 | -0.18 | -0.00 | 0.16 | 0.00 | 0.08 | 0.34 | -0.00 | -0.02 | 0.13 | 1 | | | |
| Leu | 0.19 | -0.17 | -0.20 | 0.05 | -0.25 | -0.09 | -0.34 | -0.56** | 0.12 | -0.37 | -0.38 | -0.26 | 0.03 | 0.47 | 0.60 | 0.32 | -0.04 | -0.02 | 1 | | |
| Phe | -0.48** | 0.42* | 0.08 | -0.35 | 0.44* | -0.02 | 0.34 | 0.07 | -0.22 | 0.40* | 0.43* | -0.18 | -0.38 | -0.08 | -0.24 | -0.36 | 0.09 | 0.14 | -0.43* | 1 | |
| Antiox | 0.15 | -0.20 | -0.41 | 0.49 | -0.25* | -0.02 | -0.29 | -0.22 | 0.18 | -0.16 | 0.07 | -0.32 | -0.06 | 0.00 | 0.26 | 0.30* | 0.26 | -0.11 | 0.08 | 0.21 | 1 |

Prot= Proteine, Antiox= Antioxidant activity. *Significant correlation ($p<0.05$) **Highly correlation ($p<0.01$)

Among the 23 varieties of Mexican beans evaluated, the most outstanding in general terms were the following: 1) Black-eyed (23): Zn content 52.12 ppm; Fe content 80.56 ppm; protein content 24.86%; total amino acids concentration 5.8 mg $g^{-1}$ d.w. 2) 'Peruvian' (154): Zn content 27.2 ppm; Fe content 105.29 ppm; protein content 25.01%; total amino acids concentration 5.7 mg $g^{-1}$ d.w. 3) Flor de Junio (150): Zn content 27.23 ppm; Fe content 90.96 ppm; protein content 19.2%; total amino acids concentration 6.16 mg $g^{-1}$ d.w. 4) Pinto Saltillo (155): Zn content 25.96 ppm; Fe content 62.48 ppm; protein content 25.78%; total amino acids concentration 6.63 mg $g^{-1}$ d.w. Regarding the concentration of Antioxidant activity, Amino Acids and Protein, the most outstanding varieties were: 'Pinto Saltillo' (97) with 90.50% inhibition, 6.01 mg $g^{-1}$ d.w. and 17.77%; 'Frijola' (147) with 75.23% inhibition, 6.48 mg $g^{-1}$ d.w. and 24.29%; 'Flor de Junio' (150) with 80.62%

inhibition, 6.16 mg $g^{-1}$ d.w. and 19.2%. With these results it is concluded that the analysed parameters (Fe, Zn, protein, antioxidant activity and amino acids) presented highly favourable characteristics.

Beans are an excellent source of protein thanks to their high content of amino acids, as the present research found that they contain 17 of the 20 amino acids that the body requires to carry out its vital functions to the fullest. Contains good concentrations of antioxidant activity of up to 96.76% inhibition. In addition, they are a high source of minerals such as: Fe and Zn, which contribute to the proper functioning of the body. Given that the bioavailability of the bean's constituents determines its nutritional value. Therefore, the common bean (*Phaseolus vulgaris* L.) is an excellent vehicle for the biofortification of Fe, Zn, proteins and amino acids, that can contribute significantly to combat the global problems of undernutrition.

The effect that the consumption of this legume has on human health makes it a plant product with a high dietary quality (a high nutraceutical potential). It is important to continue the research on common beans, to evaluate their potential as an ingredient in the preparation of vegetable protein blends with a view to improving their nutritional value. Besides, it is recommended its use as a high-protein food supplement, high in minerals such as Fe and Zn. That is why the bioavailability of beans is a natural resource that must be safeguarded and exploited since it is a key element in tackling not only current, but also growing problems such as malnutrition, while at the same time improving food security on a national level and, why not, in the developing countries throughout the world.

## Conclusions

The analysed beans are an excellent source of essential amino acids (Histidine, threonine, valine, methionine, lysine, isoleucine, leucine and phenylalanine) and nonessential amino acids (aspartate, serine, glutamate, glycine, alanine, proline, cysteine, tyrosine and arginine). The most prominent amino acids in Mexican bean varieties were: Glutamate, proline, aspartate, serine, lysine and leucine. The bean varieties with the highest accumulation of antioxidant activity, amino acids, protein, iron and zinc, were: 1) 'Black-eyed' (23); 2) 'Peruvian' (154); 3) 'Flor de Junio' (150); 4) 'Pinto Saltillo' (97); 5) 'Frijola' (147). On the other hand, a significant and positive correlation was found between: Protein and serine ($r = 0.41^*$), iron and cysteine ($r= 0.42^*$), zinc and phenylalanine ($r= 0.42^*$), a significant and negative correlation between: Protein and aspartate ($r =-0.41^*$), Iron and phenylalanine ($r= -0.48^{**}$), zinc and arginine ($r= -0.73^{**}$). The coefficients regarding antioxidant capacity and amino acids and significant negative correlation was found with serine ($r = -0.25^*$), while a significant positive correlation was found with the amino acid methionine ($r = 0.30^*$).

Regarding antioxidant activity, high concentrations were reported in the varieties 'Flor de mayo' (101), 'Guaca black' (84), Black (99), 'Sangre de toro' (98) and 'Pinto Saltillo' (97). Finally, indicate that the common bean (*Phaseolus vulgaris* L.) is an excellent vehicle for the biofortification of iron, zinc, proteins, amino acids and antioxidant activity, which can contribute significantly to combat malnutrition problems and health of vulnerable communities in the urban and rural sectors of Mexico, as well as in the developing countries of the world.

## Authors' Contributions

Conceptualization: E.S. Methodology: I.M.H-H., C.A.R-E., and J.C.A-P., Formal analysis: S.P-A. Investigation, I.M.H-H., C.A.R-E., and J.C.A-P.; writing—original draft preparation: I.M.H-H., and E.S. Writing—review and editing: E.S. Funding acquisition: E.S. All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

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

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