DETERMINATION AND COMPARISON OF MACROELEMENTS IN GREEN BEANS FROM THE REGIONS OF VELES AND BITOLA, R. N. MACEDONIA

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Abstract: Green beans (*Phaseolus vulgaris L.*) are a legume vegetable that represents a significant part of the human diet around the world. In addition to the high content of proteins, carbohydrates, and dietary fibers, green beans also have a high content of bioactive components, vitamins, and minerals. A large number of studies prove that green beans have antioxidant and anti-inflammatory effects, which have positive effects on human health. The purpose of this study is to determine the concentration of macroelements in green beans grown in two different regions of R. N. Macedonia because they play an important metabolic and physiological role in the human body. Insufficient intake of minerals through diet can cause various health problems and disorders. The concentration of macroelements (Ca, K, Mg, Na, P, and S) in green beans from six locations in two regions (Bitola and Veles) in R. N. Macedonia was determined. An average sample from each location was dried to a consistent mass and the percentages of water and dry matter were calculated. Quantitative analysis of macroelements in green beans was performed by inductively coupled plasma, optical emission spectroscopy. The obtained results are shown in the dry and fresh weight of the samples. The results were statistically processed with descriptive statistics and a t-test in order to determine the difference in the composition of macroelements in green beans according to the growing region. The content of dry matter in green beans ranges from 8.54 to 13.51%, and the water content is from 86.49 to 91.46%. Potassium and phosphorus have the highest concentrations of all macroelements determined in both dry and fresh green beans. The green beans from all locations have significant concentrations of calcium, magnesium, and sulfur, and sodium concentrations are the lowest. The obtained results show that the average concentration of the minerals calcium, potassium, phosphorus, and sulfur in green beans from the region of Bitola is higher than in green beans from the region of Veles. The green beans from the Veles region have a higher concentration of magnesium and sodium compared to green beans from the Bitola region. The statistical processing of the results with the t-test showed that there is no statistically significant difference between the concentration of minerals in green beans grown in the Bitola region and the Veles region. Green beans are a good source of macroelements, regardless of the location and region of cultivation. Due to the great importance of the intake of macroelements through food, green beans should be included in the human diet on a regular basis.
Keywords: determination, macroelements, minerals, green beans, dry matter.

1. INTRODUCTION

Green beans (*Phaseolus vulgaris* L.) are a member of Leguminosae, family *Phaseoleae*, which is a legume vegetable grown in different climates around the world (Sakumona et al., 2023; Chaurasia, 2020). Due to the high content of proteins, carbohydrates, and dietary fibers and the low content of fats, it represents a significant part of the human diet. Green beans are rich in vitamins A, C, and K, minerals, and bioactive compounds such as polyphenols, carotenoids, tocopherols, and flavonoids that have a high antioxidant potential (Hayat et al., 2015; Chaurasia & Saxena, 2014; Chaurasia, 2020). Due to their rich nutritional composition and antioxidant and anti-inflammatory effects, green beans have positive health effects, including improving the function of the digestive system (Kadyan et al., 2022), preventing cardiovascular diseases (Rodriguez et al., 2020), diabetes (Sokolova et al., 2022), cancer (Bernardi et al., 2024) and obesity (Thompson et al., 2017). Green beans are a good source of macroelements such as calcium, potassium, magnesium, sodium, phosphorus, and trace elements (Chaurasia et al., 2020; Lande et al., 2024).

Macroelements are essential for the human body to maintain its basic functions, including a wide range of metabolic and physiological processes, the proper functioning of organs, and good growth and development. Deficiency of macroelements can cause various health problems such as anemia, osteoporosis, muscle weakness, and various chronic diseases (Ali, 2023; Farag et al., 2023). The total amount of minerals in food is usually not the available amount of minerals in the human body through absorption, only a certain amount is bioavailable. The bioavailability of macro and microelements is defined as the fraction of the ingested mineral that is absorbed and subsequently used for normal physiological functions (Gharibzahedi & Jafari, 2017; Alegria-Toran et al., 2015). Knowledge of bioavailability is essential for estimating the amount of minerals that must be provided to meet daily intake requirements (Alegria-Toran et al., 2015). Mineral deficiencies in the human body occur due to low intake of minerals through diet, their loss during processing, or low absorption (Nayestani et al., 2020).

Calcium (Ca) is one of the most common electrolytes in the body, which is involved in many vital functions and which the body needs in significant quantities. It is essential for bone and tooth health and plays a key role in cell membrane function and intracellular signaling and communication. Also, calcium is essential for muscle function, transmission of nerve impulses, blood clotting, and secretion of various hormones and enzymes. It helps regulate heart rate, maintain healthy blood pressure and lower cholesterol levels, and maintain pH balance in the body (Zhang, 2023; Peacock, 2010; Farag et al., 2023).

Magnesium (Mg) is the fourth most abundant cation in the body and has important functions in the human body, including its role as a cofactor in more than 300 enzymatic reactions. It is essential for the regulation of various biochemical reactions such as energy production (ATP), oxidative phosphorylation, and glycolysis. It is important for the regulation of some basic functions such as muscle contraction, neuromuscular transmission, glycemic control, maintenance of heart rate, blood pressure, and immune functions. Magnesium has an important therapeutic and preventive role in several conditions such as diabetes, osteoporosis, bronchial asthma, migraine, and cardiovascular diseases (Al Alawi et al., 2018; Grober et al., 2015).

Potassium (K) is an important electrolyte that regulates fluid balance in the body. The balance of potassium and sodium (potassium-sodium pump) in the body is very important, especially the regulation of the potential of the cell membrane and thus the regulation of nerve and muscle activities. The effect of potassium on the human body is broad and includes all cells and tissues, as well as its significant role in the cardiovascular system. Adequate potassium intake can affect blood glucose control and reduce the risk of diabetes and kidney disease (D'Elia L., 2024; Stone et al., 2016; Weaver, 2013).

Sodium (Na) is the main cation in extracellular fluids, which regulates plasma volume and acid-base balance, is involved in maintaining the osmotic pressure of body fluids, and maintains membrane potentials. It has a key role in the normal functioning of nerves and muscles. It is related to metabolic and enzymatic processes as a cell activator, improving the function of the cell membrane and affecting blood clotting. Sodium is one of the most important electrolytes for maintaining an adequate level of hydration in the body. However, too much sodium can harm the kidneys and increase the risk of high blood pressure (Munteanu & Iliuta, 2011; Tremblay et al., 2024).

Phosphorus (P) is an essential mineral and is the sixth most common element in the human body. It is found extracellularly and intracellularly in the body and functions as a structural component of bones, teeth, and DNA, as well as enabling bipolarity of lipid membranes and circulating lipoproteins. Phosphorus is required for various processes such as ATP (adenosine triphosphate) synthesis, signal transduction, bone mineralization, and control of plasma and urine pH (Bird & Eskin, 2021; Serna & Bergwitz, 2020; Calvo & Lamberg -Allardt, 2015).

Sulfur (S) is the third most abundant mineral found in the human body, after calcium and phosphorus. It possesses considerable biological importance as it constitutes a component of several compounds, including amino acids, proteins, enzymes, and vitamins, and plays a crucial role in the formation of glutathione, a natural antioxidant in the body. Sulfur has important functions in the human body, such as cell signaling, free radical detoxification, regulation of gene expression, DNA methylation and repair, structural support, energy production, reducing inflammation, and improving immune system function (Hewlings & Kalman, 2019; Hansen & Venkatachalam, 2023; Farag et al., 2023).

Due to the significant value of macroelements and their presence in green beans, this research analyzed the macroelement content in green beans from the regions of Veles and Bitola in R. N. Macedonia.

2. MATERIALS AND METHODS

Sampling

Samples for analysis were collected from three locations near the city of Bitola: Novaci (coordinates 41°2′30″N 21°27′21″E), Gneotino (coordinates 40°58′59″N 21°28′59″E), and Porodin (coordinates 40°55′59″N 21°22′0″E), as well as three locations near the city of Veles: Bashino selo (coordinates 41°44′53″N 21°45′36″E), Rechani (coordinates 41°43'40.1"N 21°46'47.7"E), and Dolno Kalaslari (coordinates 41°41′18″N 21°50′15″E), R. N. Macedonia. One kilogram of green beans was collected from each location.

Sample preparation

The samples were washed with tap water, and all dust, soil residues and other impurities were removed. Edible parts were washed with distilled water and allowed to dry the surface water on filter paper at room temperature. Then, the green beans were chopped, and an average sample was taken from it. Each sample was weighed on an analytical balance (BRS-1000-C1, d=0.001 g, manufacturer MRC) and dried in a dryer (DRY-line manufactured by VWR) at 105°C to a constant mass. Then the samples are cooled in a desiccator and homogenized in a porcelain mortar. The water (W) and the dry matter percentage in green beans are calculated according to the following equations (Heghedus-Mindru et al., 2023):

$$\%W = \frac{(m1-m2)}{(m1-m3)} \cdot 100 \tag{1}$$

$$\%\ \text{dry matter} = 100 - \%W \tag{2}$$

Where:
m_1 is the mass of the sample before drying;
m_2 is the mass of the sample after drying;
m_3 is the mass of the drying vessel.

For the determination of macroelements, the samples were prepared by wet digestion with 65% nitric acid. After digestion, the samples are diluted with distilled water purified by Fisher Chemical (HPLC grade) to a final volume of 25 ml. The quantitative analysis of macroelements (Ca, K, Na, P, Mg and S) of all samples was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES, ARCOS FHE12, SPECTRO, Germany). Before ICP-OES analysis, samples were filtrated (0.45 µm). The analysis is performed on the dry weight of the sample. To obtain the concentration of elements in wet mass, the following equation is used (USDA, 2011):

$$C_{ww} = C_{dw}\left[\frac{100-W}{100}\right] \tag{3}$$

Where:
C_{ww} is wet-weight concentration;
C_{dw} is dry-weight concentration;
W is the percent water content.

Statistical analysis

The statistical analysis of the obtained results was performed with descriptive statistics and a t-test in Microsoft Excel in order to see if there is a statistically significant difference between the concentration of macroelements in green beans grown in different regions (Levine, 2020).

3. RESULTS AND DISCUSSIONS

The results of the concentration of macroelements in green beans grown in all six locations are shown in Table 1.

Table 1. Concentration of macroelements in green beans (mg/kg dry mass).

Region	**Bitola**			**Veles**		
Element	**Novaci**	**Gneotino**	**Porodin**	**Bashino selo**	**Rechani**	**Dolno Kalaslari**
Ca	3 086.24	2 113.40	3 901.20	2 496.762	3 920.30	2 155.23
K	20 197.95	28 884.64	20 063.86	16 647.07	19 609.44	21 780.51
Mg	1 175.02	1 368.92	1 436.93	1 594.01	1 738.44	1 585.02
Na	204.64	215.28	262.04	226.93	400.94	207.10
P	12 181.85	13 690.77	11 699.94	11 049.97	8 526.60	14 146.78
S	1 566.31	1 414.02	1 480.72	1 358.36	1 286.33	1 487.05

Source: Authors research

From Table 1 it can be seen that the concentration of potassium of all analyzed macroelements is the highest and ranges from 16 647.07 to 28 884.64 mg/kg dry mass in all samples of green beans. The second most abundant macroelement in green beans is phosphorus, with concentrations ranging from 8526.60 to 14146.78 mg/kg dry weight. Garcia-Caparros et al. (2021) reported high phosphorus concentrations of 1445.2–12651.7 mg/kg dry mass in green beans. Calcium is the third most abundant element in green beans, with a concentration of 2113.40 – 3920.30 mg/kg dry weight. It can be seen that there are no great differences in the concentration of magnesium and sulfur in all analyzed green bean samples (Mg: 1 175.02–1 738.44 mg/kg dry weight and S: 1 286.33–1 566.31 mg/kg dry weight). The concentration of sodium is the lowest of all analyzed macroelements (204.64 - 400.94 mg/kg dry mass). The percentage of water and dry matter in the samples was determined according to equations 1 and 2 and shown in Table 2.

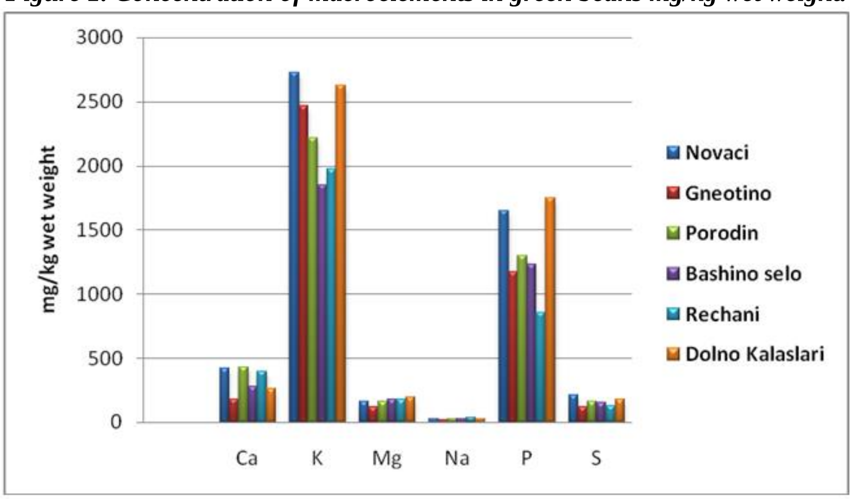
Table 2. Percentage of dry matter and water in green beans

Location	dry matter (%)	water (%)
Novaci	13.51	86.49
Gneotino	8.54	91.46
Porodin	11.06	88.94
Bashino selo	11.13	88.87
Rechani	10.06	89.94
Dolno Kalaslari	12.06	87.94

Source: Authors research

Green beans from Gneotino have the lowest dry matter content (8.54%), and green beans from Novaci have the highest dry matter content (13.51%). The percentage of water is in the interval 86.49-91.46%. The percentage of water in green beans is used to calculate the concentration of macroelements in the wet weight of the samples (equation 3). The concentration of macroelements in the wet weight of green beans is shown in Figure 1.

Figure 1. Concentration of macroelements in green beans mg/kg wet weight.



Source: Authors research

Figure 1 shows that potassium has the highest concentration of the minerals under analysis.The concentration of phosphorus is about 1000 mg/kg lower than the concentration of potassium in samples from both regions. The concentration of calcium, magnesium and sulfur is lower than the concentration of potassium and phosphorus, and sodium has the lowest concentration. Table 3 shows descriptive statistics of macroelements in green beans from both regions.

Table 3. Descriptive statistics of macroelements in green bean wet weight according to growing region.

	Bitola			Veles		
Element	**Max**	**Min**	**Mean + SD**	**Max**	**Min**	**Mean + SD**
Ca	431.46	180.55	342.95 ± 140.84	394.30	259.88	310.65 ± 73.10
K	2 728.14	2 218.66	2 471.47 ± 254.76	2 626.29	1 851.99	2 150.20 ± 416.68
Mg	158.90	116.95	144.85 ± 24.17	191.12	174.85	181.10 ± 8.77
Na	28.976	18.392	25.00 ± 5.76	40.33	24.972	30.18 ± 8.79
P	1 645.40	1 169.60	1 369.59 ± 246.79	1 750.82	857.61	1 279.24 ± 448.70
S	211.56	120.80	165.37 ± 45.40	179.31	129.38	153.27 ± 25.03

Max – maximum concentration, **Min** – minimum concentration, **Mean + SD** – mean value + standard deviation
Source: Authors research

Table 3 shows that the green beans from the Bitola region have a higher average concentration of the macroelements calcium, potassium, phosphorus and sulfur compared to the green beans from the Veles region. The average concentration of magnesium and sodium is higher in the samples from the Veles region compared to the samples from the Bitola region. The results obtained from our macroelement analysis for green beans are very similar to those of Chaurasia (2020), where the concentrations of calcium (370 mg/kg), potassium (2090 mg/kg), magnesium (250 mg/kg), sodium (60 mg/kg) and phosphorus (380 mg/kg) are reported. In our analyzed green beans, there are lower concentrations of magnesium (144.85-181.10 mg/kg) and sodium (25-30.18 mg/kg) and a significantly higher concentration of phosphorus (1 369.59-1 279.24 mg/kg).
Table 4 shows the results of the t test between the macroelements from the two regions in order to determine if there is a statistically significant difference in the mineral content of green beans according to the growing region.

Table 4. Student's t-test for the concentration of macroelements in green beans from different regions.

	Ca	**K**	**Mg**	**Na**	**P**	**S**
Value of t-test	0.352752018	1.139392043	-2.442281264	-0.853562359	0.305582676	0.404117734
Critical value for t	2.776445105					
Obtained p-value	0.742077678	0.318139524	0.071033505	0.441448283	0.775165046	0.706800181

Source: Authors research

From table 4, it can be seen that the value of the t-test is lower than the critical value of the t-test (2.776445105) in all macroelements between the two regions, which shows that there are no differences in the concentration of elements in green beans between the two regions. The obtained p-value for all macroelements is above the limit p-value (0.05), which means that there is no statistically significant difference between macroelements in green beans from both regions. According to the obtained p-value, the differences in the concentration of minerals from the two regions (Veles and Bitola) are in the following order: Mg>K>Na>S>Ca>P.

4. CONCLUSIONS

The concentration of calcium, potassium, magnesium, phosphorus, sodium and sulfur macroelements in green beans grown in six locations in two different regions in R. N. Macedonia has been determined. Among all samples of green beans analyzed, the concentration of potassium and phosphorus is the highest. Green beans from all locations have a significant amount of calcium, magnesium and sulfur and the lowest concentration of sodium. Green beans from the Bitola region have a higher average concentration of the minerals calcium (342.95 mg/kg), potassium (2471.47 mg/kg), phosphorus (1369.59 mg/kg) and sulfur (165.37 mg/kg) compared to green beans from the Veles region. The average concentration of magnesium (144.85 mg/kg) and sodium (181.10 mg/kg) is higher in the samples from the Veles region compared to the samples from the Bitola region. According to the results obtained from the t-test, the obtained p-value for all minerals is above the limit p-value (0.05), which means that there is no statistically significant difference between macroelements in green beans from both regions. It can be concluded that green beans are a good source of macro elements.

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