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Evaluation of Total Concentration and Bioaccessible Fraction of Metals in Berry Fruits from Different Cultivars

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The objective of this work was to evaluate the total concentration and bioaccessible fractions of Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Pb, V and Zn in berry fruits and analyze the influence of different cultivars and growing conditions on the obtained results. The variations in bioaccessible concentrations were from 7.7-30, 17-29, 22-50, 1.8-96, 22-33 and 20-51% of the total concentration, for Ba, Cu, Fe, Mn, Pb and Zn, respectively. Copper showed greater bioaccessibility in blackberry and blueberry. For Fe, bioaccessibility was observed in all strawberries and blueberries and in some blackberries. Zinc presented higher bioaccessible fractions in strawberries. For V, only the sample Blackberry 124 showed bioaccessible concentration, (11% of the total concentration). Regarding the different forms of cultivation, the strawberries that received radiation from the red and white lamps presented a higher production of fruit compared to the other systems, however, in relation to the absorption of nutrients, a small variation was observed between the fruits.

Keywords: blackberry, strawberry, blueberry, cultivars, bioaccessibility, metals

Introduction

The consumption of fruits has increased since the population has choosing to consume healthier foods, which help the prevention of various diseases. The presence of macro and micronutrients in this type of food has contributed to the physiological needs of humans. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend the ingestion of at least 400 g of fruits and vegetables *per* day.[1,2]

Berry fruits are rich in phenolic compounds with antioxidant properties and are also a source of fibers and vitamins (B, C and K) and minerals, such as Ca, Cu, Fe, K, Mg, Mn, and Zn. Thus, the production and consumption of berry fruits, such as blackberry, blueberry, and strawberry, have been noteworthy in Brazil due to their nutritional importance, consumer acceptability, as well as the possibility of obtaining rapid economic return to the producer, mainly for family farming.[2-4] Additionally, a diet with berry fruits had demonstrated efficiently counteract obesity or obesity-associated complications.[5]

Genetic improvement studies allow the fruit to be boosted with properties that make it more attractive to the producer and to the consumer. In Brazil, the main berry fruit breeding program is in the Brazilian Agricultural Research Corporation (Embrapa) Clima Temperado, in Pelotas (Rio Grande do Sul, Brazil). Among their goals, they stand out for obtaining fruits with a sweeter taste, trees without thorns, larger size of fruit, ease harvesting and extensive harvest period.[6]

The blackberry (genus *Rubus*), a native plant of the United States of America, is the most commercially explored species in Brazil. Some cultivars available on the market are Ébano, Negrita, Guarani, Caingangue and Tupy, the latter being the most consumed due to its high productivity *per* m^2 and fruit quality.[6,7] More recently, the cultivars Xavante, BRS Xingu and BRS Cainguá also became available.

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The blueberry (genus *Vaccinium*) has less cultivated area in Brazil due to greater cultivation complexity. The varieties such as Bluebelle, Powderblue, Misty, O'Neal, Duke and Elliott are the most planted, as they are best suited to mild climates. For this reason, there is little availability of adapted cultivars, which requires more studies of genetic improvement for these species.[6]

Strawberries are the most representative fruit in the group of berry fruits, with higher production, wide commercialization, and greater acceptance by the consumer. The most used cultivars in Brazil are Oso Grande, Camarosa, Albion and San Andreas, coming from university improvement programs in the United States of America. Thus, it is necessary to develop national cultivars, which can adapt to climatic conditions and Brazilian soil.[8]

In addition to genetic improvement techniques, cultivation alternatives have been developed to expand marketing and facilitate production management. An alternative is cultivation off the ground, for example, for strawberries, also called semi-hydroponic cultivation, which has been widely used due to its advantages, such as production throughout the year, plant protection from climatic effects, reduction of diseases appearance and improvement of management conditions.[9] Also, due to the importance of light incidence to the growth and development of the fruit, new studies involving their exposure to lamps with different wavelengths for a given period of time are being developed.[10,11]

Genetic improvement and production programs are important to develop a climate-resilient plant, as well as to produce large, sweet and with superior quality fruits. However, these changes can directly influence the absorption of nutrients, thus increasing or decreasing the absorption of essential and toxic elements, for example, Cd, Cr and Pb, by the fruits. Therefore, knowledge of the elemental composition present in these new cultivars is extremely important, to guarantee greater food security for consumers.[12-14]

To evaluate the genetic influence and the role of fruit cultivation in the variation of elements present in the species, as well as determining the total concentration of such elements, it is important to estimate their bioaccessibility. These types of studies evaluate the concentrations of analytes that will be released from the fruit matrix into the human organism and that are soluble in the gastrointestinal tract, thus becoming available to be absorbed by the intestinal epithelium.[4,15,16]

With the increasing consumption of berry fruits, the need to determine the concentration of analytes released into the body is evident. This concentration may be at levels of essentiality, recommended daily intake, or at levels of toxicity, which may present risks to the health of the consumers. Based on this, the aim of the present work was to develop studies to evaluate the total concentration and the bioaccessible fraction of Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Pb, V and Zn in berry fruits (blackberry, blueberry and strawberry), evaluating the influence of different cultivars and growing conditions. All determinations were performed by microwave induced plasma optical emission spectrometry (MIP OES).

Experimental

Instrumentation

The measurements were carried out using a microwave-induced plasma optical emission (MIP OES) spectrometer, 4200 model, (Agilent Technologies, Melbourne, Australia) equipped with a OneNeb nebulizer. Nitrogen used to maintain plasma was extracted from atmospheric air with a compressor (model MSV12, Schulz, Joinville, SC, Brazil) and a nitrogen generator (model 4107, Agilent Technologies, Melbourne, Australia).

All measurements were performed in triplicate, with sequential detection mode, with an integration time of 3 s and peristaltic pump speed at 15 rpm (approximately 1.0 mL min^{-1}). The background signals were corrected automatically, by subtracting the spectra of a blank solution from that of the sample. The other operational parameters, such as wavelength, plasma viewing position and nebulization flow for each analyte, are shown in the Supplementary Information (SI) section, Table S1. All samples were weighed using an analytical balance model 2140 (Ohaus Adventurer, Barueri, SP, Brazil) and were homogenized in a 400 W power mixer (Philips Walita, Itapevi, SP, Brazil). For the sample preparation by acid decomposition, a digester block with reflux system was used, as described by Oreste *et al.*[17] For determination of bioaccessible fractions, a pH meter (pHS-3B model, PHtec, Curitiba, PR, Brazil), a Dubnoff bath with stirring and heating (model Q226M2, Quimis, Diadema, SP, Brazil) and a 10,000-rpm centrifuge (model 5804R, Eppendorf, Hamburg, Germany) were employed.

Reagents and standards

All reagents used were of analytical grade. The solutions were prepared with deionized water obtained from a glass distiller (model MA-075, Marconi, Piracicaba, SP, Brazil) followed by deionization through a column with cationic and anionic mixed resin (model CS1800, Permution, Curitiba, PR, Brazil). The standard solutions were prepared from a multielement standard solution 6 for inductively coupled plasma (ICP, Sigma-Aldrich, Buchs, Switzerland). Nitric acid (Synth, Diadema, SP, Brazil) and the HCl (Qhemis, Jundiaí, SP, Brazil) were purified by doubly subboiling distillation in a quartz system (Marconi, model MA-075, Piracicaba, SP, Brazil). Also, 30% (v/v) H_2O_2 (Synth, Diadema, SP, Brazil) was employed. The following reagents were used for bioaccessibility studies: alpha amylase from *Aspergillus oryzae* (PCode 101642338), pepsin from porcine gastric mucosa (PCode 101947953), bile extract porcine (PCode 1003443762) and pancreatine from porcine pancreas (PCode 1001987024) (Sigma-Aldrich, Saint Louis, Missouri, USA); $CaCl_2(H_2O)_2$, NaOH, KCl, NaCl, $MgCl_2(H_2O)_6$ and KH_2PO_4 (Synth, Diadema, SP, Brazil), $(NH_4)_2CO_3$ (Baker, San Bernardino County, USA), $NaHCO_3$ and HCl (Merck, Darmstadt, Germany).

Samples

Blackberry, blueberry, and strawberry samples were provided by Embrapa Clima Temperado (Pelotas, RS, Brazil), from the cultivars installed in the experimental field of the unit (approximately 1 kg of each sample) and produced in the same soil. For this study, different selections and cultivars of blackberry were used in the experimentation step: Black 05/96, Black 112, Black 118, Black 124, Black 128, Black 145, Black 178, Black 198, BRS Xingú, Guarani, Tupy and Xavante. For the blueberry samples, the O'Neal and Bluecrisp cultivars were used.

The strawberries (cultivar San Andreas) were cultivated in a controlled greenhouse with an off-soil production system (semi-hydroponic cultivation) and were employed as artificial lighting, lamps with 24 W of power with different spectral ranges: blue, red and white to accelerate the photoperiod of the plants. Also, we analyzed fruit samples without artificial radiation, i.e., produced only with natural light. For the analysis of the strawberry samples, any damaged leaves and/or parts were removed with the aid of a ceramic knife on a tempered glass "cutting board". All samples were washed with deionized water and then homogenized in a mixer. After that, the pulps of samples were stored in plastic containers and kept in a freezer (–15 ºC) until the moment of the sample preparation.

Procedures

Acid decomposition with reflux system

Masses of 3.3; 2.5 and 5.0 g of blackberry, blueberry, and strawberry pulps, respectively, were weighed directly into the glass digester tube. According to the moisture of the samples, these masses are equivalent to 0.5 g of dry mass. After, 5.0 mL of 65% (v/v) HNO_3 were added and then, the reflux system was coupled in the digester tube. The samples were heated at 200 °C during 2 h in the digester block. After this step, the solutions were cooled to room temperature, 1.0 mL of H_2O_2 was added and then they heated at 150 °C for another 1 h. At the end, the resulting solutions were transferred to polypropylene (PP) flasks and the final volume of 20 mL was filled with deionized water.

The accuracy of the method was evaluated by recovery tests at three concentration levels. Also, a comparative study was carried out between the proposed method (acid decomposition with reflux system) and another method (acid decomposition with closed system)[18] for the blackberry Tupy and strawberry (irradiated with red lamp) samples. In both methods, the pulp of samples were weighted (wet mass). For MIP OES analysis, the manufacturer of the spectrometer recommends a maximum acidity content of 5.0% (v/v) and dissolved solids of 3.0% (m/v), to avoid deposits and preserve the life of the torch, ensuring good plasma functioning during measurements. Therefore, the solutions that resulted from the preparation of the samples were diluted with deionized water.

Acid decomposition with closed system

Approximately 0.66 and 1.0 g of blackberry and strawberry samples were weighed directly into the polytetrafluoroethylene (PTFE) vessel and 2.5 mL of HNO_3 were added. These masses are equivalent to 0.1 g of dry mass. After that, the flasks were completely closed and sent to the digester block, heated at 140 ºC and remained there for 3 h. After that, the PTFE flasks were left for 4 h at room temperature to decrease the pressure. Finally, the flasks were opened, and the resulting solutions were transferred to PP flasks and the final volume of 20 mL was completed with deionized water. The resulting solutions were clear and without particles, being suitable to be introduced into the MIP OES spectrometer, without compromising its operation.

In vitro gastrointestinal digestion

For bioaccessibility study, the *in vitro* digestion method was applied, based on a model proposed by Minekus *et al.*,[15] which consists of simulating the human digestive system, considering three stages: mouth, stomach, and intestine, for which synthetic fluids are used. The fluid compositions are presented in Table S2 (SI section). For the procedure, approximately 5.0 g of each fruit were weighed in PP flasks and the methods described by Pereira *et al.*[4] were employed.

After the procedure, the supernatant was analyzed to determine the bioaccessible fraction and the solid part to determine the non bioaccessible fraction. For the determination of analytes in the supernatant, it was diluted 3 times, obtaining 3% (m/v) of total dissolved solids, following the recommendations of the equipment manufacturer. The obtained results were submitted to statistical paired *t*-test at 95% confidence level.

The non-bioaccessible fraction, corresponding to the solid part obtained by centrifugation, was subjected to acid decomposition to evaluate the accuracy of the bioaccessibility results through a mass balance.[16]

Results and Discussion

Figures of merit

The figures of merit for the determination of total concentrations and bioaccessible fraction of analytes by MIP OES are presented in Tables S3 and S4 (SI section), respectively.

According to the data in Table S3, calibration curves for all analytes presented coefficients of squared linear correlation ($R^2 > 0.998$). The same was observed for the calibration curves for bioaccessible fraction studies (Table S4, SI section), which presented values of $R^2 > 0.994$, although in this condition the solutions have elevated levels of salts. Therefore, the methodologies adopted with appropriate dilution provided adequate figures of parameters for the analysis by MIP OES, since the solutions to be introduced in the equipment presented low acidity and low levels of total solids, providing stability to the plasma functioning. The limits of detection and quantification were calculated considering three and ten times the standard deviation and the average of ten replicates of the blank solution from the calibration curve and, for the method limits, the sample mass was considered. The limits of detection (LOD) of the method were between 0.001 to 0.109 mg kg^{-1} for all investigated analytes, which allowed the determination of the total concentration for Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Pb, V and Zn and the bioaccessible fraction for Ba, Cu, Fe, Mn, Pb, V and Zn.

Analytical results for total concentration of metals in berry fruits

When this study was undertaken, a Certified Reference Material (CRM) similar to the investigated samples was not available in our laboratory and thus we used a comparison between methods and recovery tests. Also, mass balance was employed for bioaccessibility results, seeking all available possibilities to ensure the accuracy of the results. The accuracy was assessed through recovery tests and the obtained results are shown in Table S5 (SI section). The recovery ranges of the analytes varied from 80 to 120%, and the average relative standard deviations were < 5%, ensuring the repeatability of the results.

In addition to the recovery test, a comparative study using two methods was carried out: acid decomposition with reflux system *versus* acid decomposition in a closed system. This comparative study was performed with blackberry and strawberry (cultivated with red lamp) samples and the results are shown in Table S6 (SI section).

To verify if there were differences between the results obtained for both methods, Student's paired *t*-test with a 95% confidence limit was applied, which showed that there were no significant differences between the results found in the decomposition with reflux system method when compared with the decomposition in a closed system. In addition, observing the relative standard deviation (RSDs), the reflux system showed lower values than the closed system, proving that the former method showed better repeatability. Therefore, it was demonstrated that the acid decomposition with reflux system method for total determinations on berry fruits provides accurate results by MIP OES. When using acid decomposition methods on fruit samples, a high dilution of the analytes present may occur, since these samples have a large amount of water in their composition. Thus, using methods that allow a larger sample amount becomes advantageous. The cold surface of the "cold finger" allows the formation of a liquid film of the absorbing solution that is rich in water vapors and acids used during the mineralization process. Because of this, the analytes are quantitatively retained, avoiding losses by volatilization. Thus, using an adequate amount of HNO_3 in the digester tube, combined with a temperature enough to heat the digester block higher than its boiling temperature, cause the formation of the absorbing solution (azeotropic mixture) on the surface of the reflux flask, with high ionic strength, which allows the retention of analytes in the form of water-soluble nitrates and, after condensation and dripping, they return to the reaction medium in the digestion tube.

Thus, the acid decomposition method with reflux system was applied to different berry fruits (blackberry, blueberry, and strawberry) and the obtained total concentrations by MIP OES are shown in Tables 1 and 2. It should be noted that all the results obtained were calculated based on the wet mass of each sample.

According to the results presented in Table 1, it is possible to observe similar concentrations for some cultivars while for others there is a greater difference, even though these fruits come from the same soil and irrigation, which leads us to indicate that the genetic improvement does not interfere considerably in the concentration of the necessary

Table 1. Analytical results of total concentrations for Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Pb, V and Zn in blackberry samples from different cultivars obtained by MIP OES (n = 3)

Analyte	Concentration ± standard deviation / (mg kg^{-1})										
	Black 05/96	Black 112	Black 118	Black 124	Black 128	Black 145	Black 178	Black 198	BRS Xingú	Guarani	Xavante
Ba	2.84 ± 0.09	4.30 ± 0.17	3.12 ± 0.23	3.35 ± 0.15	2.16 ± 0.03	3.57 ± 0.05	1.77 ± 0.05	1.51 ± 0.14	3.78 ± 0.24	3.75 ± 0.07	1.89 ± 0.09
Ca	169 ± 3	189 ± 3	126 ± 3	145 ± 10	111 ± 6	215 ± 7	121 ± 10	106 ± 10	169 ± 3	336 ± 5	278 ± 15
Cd	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$
Cr	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$
Cu	1.11 ± 0.03	0.816 ± 0.039	0.899 ± 0.038	0.832 ± 0.024	0.618 ± 0.031	0.952 ± 0.006	1.03 ± 0.08	0.978 ± 0.066	1.01 ± 0.12	1.48 ± 0.04	1.30 ± 0.06
Fe	4.73 ± 0.17	2.97 ± 0.09	3.70 ± 0.26	3.60 ± 0.08	2.42 ± 0.17	3.52 ± 0.17	5.09 ± 0.17	4.54 ± 0.43	3.45 ± 0.26	3.86 ± 0.24	3.84 ± 0.19
K	1560 ± 89	1480 ± 41	1580 ± 7	1560 ± 41	1270 ± 38	1660 ± 51	1890 ± 10	1680 ± 58	1660 ± 17	2450 ± 110	1860 ± 140
Mg	184 ± 7	206 ± 3	191 ± 3	223 ± 7	177 ± 6	235 ± 3	240 ± 17	213 ± 14	211 ± 3	295 ± 6	273 ± 17
Mn	32.4 ± 0.1	37.6 ± 0.6	20.7 ± 1.2	34.3 ± 1.5	15.8 ± 0.6	28.5 ± 0.7	20.2 ± 0.6	15.4 ± 0.5	23.9 ± 1.5	8.37 ± 0.52	5.44 ± 0.15
Pb	0.20 ± 0.03	0.193 ± 0.005	0.162 ± 0.001	0.19 ± 0.02	0.149 ± 0.007	0.23 ± 0.02	0.212 ± 0.002	0.22 ± 0.02	0.21 ± 0.02	0.72 ± 0.08	0.71 ± 0.01
V	1.80 ± 0.01	1.79 ± 0.01	1.54 ± 0.01	1.91 ± 0.22	1.51 ± 0.17	2.16 ± 0.11	1.60 ± 0.01	1.76 ± 0.07	1.95 ± 0.10	11.2 ± 0.6	11.0 ± 0.3
Zn	2.12 ± 0.08	2.53 ± 0.08	2.30 ± 0.17	2.54 ± 0.34	1.64 ± 0.08	2.61 ± 0.08	2.73 ± 0.08	2.61 ± 0.08	1.80 ± 0.04	2.88 ± 0.18	3.03 ± 0.50

Mean ± standard deviation; method limit of detection (LOD$_{(m)}$) (mg kg^{-1}): Cd = 0.089; Cr = 0.004.

Table 2. Analytical results of total concentrations for Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Pb, V and Zn for blueberry Bluecrisp and strawberry San Andreas samples irradiated with lamps of different wavelengths and natural light (n = 3)

Analyte	Concentration ± standard deviation / (mg kg^{-1})				
	Blueberry Bluecrisp	Strawberry (blue lamp)	Strawberry (white lamp)	Strawberry (red lamp)	Strawberry (natural light)
Ba	0.360 ± 0.025 (6.9)	0.125 ± 0.001 (0.8)	0.109 ± 0.007 (6.4)	0.084 ± 0.001 (1.2)	0.101 ± 0.007 (6.9)
Ca	65 ± 2 (3.1)	117 ± 8 (6.8)	114 ± 1 (0.9)	72 ± 3 (4.2)	125 ± 4 (3.2)
Cd	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$
Cr	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$	< LOD$_{(m)}$
Cu	0.244 ± 0.005 (2.0)	0.376 ± 0.013 (3.4)	0.207 ± 0.009 (4.3)	0.311 ± 0.014 (4.5)	0.386 ± 0.017 (4.4)
Fe	2.88 ± 0.17 (5.9)	2.26 ± 0.03 (1.3)	1.80 ± 0.16 (8.9)	2.25 ± 0.21 (9.3)	2.84 ± 0.19 (6.7)
K	801 ± 21 (2.6)	1400 ± 28 (2.0)	1420 ± 60 (4.2)	1430 ± 60 (4.2)	1550 ± 70 (4.5)
Mg	53 ± 1 (1.9)	111 ± 5 (4.5)	100 ± 3 (3.0)	98 ± 5 (5.2)	123 ± 3 (2.4)
Mn	5.81 ± 0.07 (1.2)	3.72 ± 0.16 (4.3)	2.71 ± 0.17 (6.3)	2.63 ± 0.02 (0.8)	3.21 ± 0.08 (2.5)
Pb	0.342 ± 0.004 (1.2)	0.149 ± 0.006 (4.0)	< LOD$_{(m)}$	0.14 ± 0.01 (7.1)	< LOD$_{(m)}$
V	14.5 ± 1.0 (6.9)	2.35 ± 0.05 (2.1)	< LOD$_{(m)}$	1.39 ± 0.01 (0.7)	0.642 ± 0.039 (6.1)
Zn	2.46 ± 0.19 (7.7)	1.54 ± 0.05 (3.2)	1.39 ± 0.04 (2.9)	1.64 ± 0.10 (6.1)	1.80 ± 0.05 (2.8)

Mean ± standard deviation (relative standard deviation); method limit of detection (LOD$_{(m)}$) (mg kg^{-1}): Cd = 0.089; Cr = 0.004; Pb = 0.032; V = 0.109.

elements for its functioning. Note that blackberry, blueberry and strawberry samples were cultivated directly in soil and nutrient solutions were not employed. The strawberries (cultivar San Andreas) were cultivated with an off-soil production system and the use of lamps with different wavelengths. This similarity between the concentrations and the several types of blackberries evaluated, for instance, allows a permanent production, since some of these fruits can be grown and harvested out of season. This ensures that the consumer can consume blackberries all year round containing the essential nutrients. Together with the blackberries in Table 1, the concentration values for blackberry Tupy (Table S4, SI section) are relevant, due to its high productivity of fruit *per* m^2 and the quality of the fruits.[7,19]

In all the samples analyzed, the found concentrations for Cd and Cr were lower than the method limit of detection (LOD$_m$), presenting no risk to the consumer, since they can be considered toxic elements.[20,21]

For the sample Black 128 (blackberry), lower concentrations of Cu, Fe, K, Mg, Pb and Zn were found. For Ba and Ca, the lowest concentrations were in sample Black 198, and, for Mn, the lowest values were observed in blackberry Xavante. However, the highest concentrations of Ca, Cu, K, Mg, Pb and V were found in blackberry Guarani. For Ba and Mn analytes, the highest concentrations were for Black 112, and, for Fe, the highest values were for sample Black 178.

The values for total concentration found in this study were close to the concentrations provided by the United States Department of Agriculture (USDA)[22] for blackberries, which are 290; 6.2; 1620; 200 and 5.3 mg kg^{-1} for Ca, Fe, K, Mg and Zn, respectively. Furthermore, comparing the results obtained from the different cultivars with the ones described by Pereira *et al*.,[4] lower values were found only for Fe, as for the rest of the analytes, all of them presented higher concentration values, highlighted that the compared cultivars are produced in different conditions (soil, temperature, moisture, etc.). For blueberry samples, K, Pb, V and Zn concentrations in blueberry O'Neal (Table S4, SI section) were lower than blueberry Bluecrisp concentrations (Table 2). Concerning the other elements, the concentrations were higher for the first sample of blueberry studied. Comparing the results obtained with the USDA standard for blueberries, which are: 60; 2.8; 770; 60 and 1.6 mg kg^{-1} for Ca, Fe, K, Mg and Zn, respectively, the Ca values are higher than the concentration reported for the two analyzed samples of blueberries (USDA-ARS).

The values determined for Fe and Mg in blueberry O'Neal and for Fe, K and Zn in blueberry Bluecrisp were above the values reported by the USDA.[22] These differences between the concentrations obtained in our study and the reference values reported by the USDA, can be explained by the different types of cultivars, since the blueberries evaluated, in addition to the characteristic of soil where they were planted, were subjected to different climatic and technical conditions of post-harvest handling when compared to American soil cultivars.

The incidence of light is a principal factor for the growth and development of the fruit and the plant, which need at least 12 h of sunlight daily. In order to guarantee the production also during the winter, the fruits, during their growth, are exposed to lamps of different wavelengths and times.[10,11] So, aiming to evaluate the concentration of the elements in strawberry samples from an off-soil production system, the fruits were exposed to different types of lamps (red, blue, white) and natural light. Comparing the values (Tables 1, 2 and S5, SI section) for red, blue, and white lamps, and samples without the use of artificial light, the concentrations were close for most analytes. However, the lowest concentrations of Cu, Fe, Pb, V and Zn were found for strawberries grown under a white lamp.

Strawberries cultivated with red lamp presented lower concentrations of Ba, Ca, Mg and Mn, while strawberries cultivated with blue lamp showed lower values for K. Strawberries cultivated with blue lamp presented highest concentrations of Ba, Mn, Pb and V. However, strawberries cultivated without artificial light showed higher concentrations of Ca, Cu, Fe, K and Zn. Regarding productivity of fruit *per* m^2, strawberries that received radiation from red and white lamps showed a higher production compared to other systems.

The literature reports[23,24] that red and blue radiations are the main sources of energy for photosynthetic CO_2. As a result, these energy sources are considered the most important for plant development; however, as noted in the results obtained in this study, they do not directly influence the absorption of nutrients. In addition, for off-soil systems it is necessary to add nutrient solutions to meet the nutritional demands without causing deficiencies or toxicity in the plants. However, the results described by Pereira *et al*.[4] for strawberries grown in the soil showed higher concentration values for Ba, Ca, Fe, K, Mg and Mn, possibly due to the soil behaving like a natural buffer for these salts and, thus, retaining a greater amount of these analytes.[12]

Concerning the analytes concentration for strawberries, in comparison with the Brazilian Food Composition Table (TBCA),[25] which reports concentrations of 110; 0.6; 3; 1840; 100; 3.3 and 2 mg kg^{-1} for Ca, Cu, Fe, K, Mg, Mn and Zn, respectively, and the USDA, which presents the values of 160; 4.1; 1530; 130 and 1.4 mg kg^{-1} for Ca, Fe, K, Mg and Zn, respectively, the results we found for total concentrations are close for almost all elements, except for Mn in strawberries cultivated with blue lamp, where the concentration was higher than the value reported in the TBCA table.

Analytical results for determination of bioacessible fraction of metals in berry fruits

To validate the bioaccessibility study, after simulating the entire *in vitro* digestive process, the concentration of the analytes in the liquid fraction (bioaccessible concentration) and in the solid fraction (non-bioaccessible concentration) were determined. The sum of the concentration for both fractions must equal the value of the total concentration. Table 3 presents the obtained results for the samples of blackberry Tupy, blueberry O'Neal and for the strawberry (cultivated with red lamp).

According to the values presented in Table 3, it is possible to observe that the results found are reliable since the sum of the two fractions of the bioaccessibility study presented a recovery of 84 to 120% in relation to the total concentration. Thus, the method was applied to the other samples and the bioaccessibility values are shown in Table 4. The concentrations of Ca, K and Mg were not determined since these elements are found in large quantities in the reagents used to simulate the gastrointestinal digestion, making the determinations in the liquid phase unfeasible. For all samples, the total concentrations of Cd and Cr were lower than the LOD$_{m}$, and it was not possible to evaluate the bioaccessible concentrations for these analytes.

Regarding the results presented in Tables 3 and 4, it is possible to observe that there is a variation in the amount of elements released into the gastrointestinal tract, even for some samples that showed a close value for the total concentration determination. This variation is due to the presence and different concentrations of compounds, such as polyphenols, phytates and tannins, which can inhibit absorption of cations and decrease their bioaccessibility. Phytates are inhibitors of the intestinal absorption of minerals, as they contain phosphate groups that are rapidly ionized at the physiological pH of the human body, thus acting as a chelator of cations such as Fe and Zn.[26,27]

The analytes that showed bioaccessibility in almost all samples were Ba and Mn, as already observed in the study described by Pereira *et al.*,[4] demonstrating that the release of these analytes into the gastrointestinal tract is a characteristic of berry fruits. The analyte Cu showed higher bioaccessibility in blackberry and blueberry samples. In contrast, Zn presented bioaccessibility in strawberry samples, thus corroborating the fact that fruit matrices directly influence the release of analytes. For Fe, the release is observed in all strawberry and blueberry samples and in some blackberry samples, but the difference between the concentration of this analyte in the blackberry samples may be related to the chemical species in which the analyte is in solution, for example, Fe^{2+} is more easily absorbed than Fe^{3+}.[28,29] The analytes Pb and V showed low bioaccessibility for all investigated samples.

To assess the contribution of the essential and toxic elements, the concentrations found were compared to the recommended average according to the Dietary Reference Intake (DRI). Thus, the average value for adults (men and women) from different age groups for Cu, Fe, Mn and Zn is 0.7 to 0.9; 8 to 18; 1.6 to 2.3 and 8 to 11 mg kg^{-1} body mass *per* day, respectively. For V, there are no determined values due to the lack of data on its adverse effects.[30] For

Table 3. Results of total concentration (TC), bioaccessible fraction (BF) and non-bioaccessible fraction (NBF) in blackberry Tupy, blueberry O'Neal and strawberry (cultivated with red lamp) samples obtained by MIP OES (n = 3)

Analyte	Total concentration / (mg kg^{-1})	BF / (mg kg^{-1})	BF / %	NBF / (mg kg^{-1})	NBF / %
		Blackberry Tupy			
Ba	2.31 ± 0.06 (2.6)	0.262 ± 0.004 (1.5)	11	2.53 ± 0.10 (3.9)	109
Cu	0.99 ± 0.03 (3.0)	0.26 ± 0.01 (3.8)	26	0.78 ± 0.05 (6.4)	79
Fe	2.97 ± 0.26 (8.7)	< LOD$_{(m)}$	–	2.67 ± 0.14 (5.2)	90
Mn	19.0 ± 0.1 (0.5)	4.50 ± 0.25 (5.5)	24	14.2 ± 1.8 (12.7)	75
Pb	0.257 ± 0.007 (2.7)	< LOD$_{(m)}$	–	0.269 ± 0.018 (6.7)	105
V	1.40 ± 0.14 (10.0)	0.39 ± 0.03 (7.7)	26	0.98 ± 0.05 (5.1)	70
Zn	1.88 ± 0.09 (4.8)	< LOD$_{(m)}$	–	1.73 ± 0.13 (7.5)	92
		Blueberry O'Neal			
Ba	0.571 ± 0.001 (0.2)	< LOD$_{(m)}$	–	0.65 ± 0.05 (7.7)	114
Cu	0.466 ± 0.001 (0.2)	0.205 ± 0.013 (6.3)	44	0.231 ± 0.021 (9.1)	50
Fe	3.68 ± 0.23 (6.2)	0.737 ± 0.016 (2.2)	20	3.41 ± 0.14 (4.1)	93
Mn	26.1 ± 0.4 (1.5)	16.3 ± 0.3 (1.8)	62	8.68 ± 0.35 (4.0)	33
Pb	0.290 ± 0.005 (1.7)	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
V	0.56 ± 0.01 (1.8)	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Zn	0.656 ± 0.022 (3.3)	< LOD$_{(m)}$	–	0.657 ± 0.001 (0.1)	100
		Strawberry			
Ba	0.084 ± 0.001 (1.2)	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Cu	0.31 ± 0.01 (3.2)	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Fe	2.25 ± 0.21 (9.3)	0.57 ± 0.01 (1.7)	25	2.03 ± 0.14 (6.9)	90
Mn	2.63 ± 0.02 (0.8)	0.95 ± 0.07 (7.4)	36	1.26 ± 0.08 (6.3)	48
Pb	0.14 ± 0.01 (7.1)	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
V	1.39 ± 0.01 (0.7)	< LOD$_{(m)}$	–	1.23 ± 0.14 (11.4)	88
Zn	1.64 ± 0.10 (6.1)	0.44 ± 0.02 (4.5)	27	1.26 ± 0.05 (4.0)	77

Mean ± standard deviation (relative standard deviation). Method limit of detection (LOD$_{(m)}$) (mg kg^{-1}): Ba = 0.001; Cu = 0.019; Fe = 0.023; Pb = 0.047; V = 0.114; Zn = 0.050.

Table 4. Results of bioaccessible fraction (BF) in berry fruits by MIP OES (n = 3)

Analyte	Black 05/96 / (mg kg^{-1})	BF / %	Black 112 / (mg kg^{-1})	BF / %	Black 118 / (mg kg^{-1})	BF / %	Black 124 / (mg kg^{-1})	BF / %	Black 128 / (mg kg^{-1})	BF / %
Ba	0.310 ± 0.001 (0.3)	11	0.530 ± 0.015 (2.8)	12	0.239 ± 0.011 (4.6)	7.7	0.333 ± 0.011 (3.0)	9.9	0.281 ± 0.005 (1.8)	13
Cu	< LOD$_{(m)}$	–	0.163 ± 0.009 (5.5)	20	0.171 ± 0.008 (4.7)	19	0.207 ± 0.003 (1.4)	25	0.108 ± 0.008 (7.4)	17
Fe	1.11 ± 0.01 (0.9)	23	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Mn	8.02 ± 0.49 (6.1)	25	3.93 ± 0.10 (2.5)	10	5.75 ± 0.57 (9.9)	28	7.45 ± 0.90 (12.1)	22	< LOD$_{(m)}$	–
Pb	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
V	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	0.216 ± 0.022 (10.2)	11	< LOD$_{(m)}$	–
Zn	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Analyte	Black 145 / (mg kg^{-1})	BF / %	Black 178 / (mg kg^{-1})	BF / %	Black 198 / (mg kg^{-1})	BF / %	BRS Xingú / (mg kg^{-1})	BF / %	Guarani / (mg kg^{-1})	BF / %
Ba	0.357 ± 0.004 (1.1)	10	< LOD(m)	–	0.16 ± 0.01 (6.3)	11	0.367 ± 0.003 (0.8)	9.7	0.708 ± 0.008 (1.1)	19
Cu	0.187 ± 0.005 (2.7)	20	< LOD(m)	–	< LOD(m)	–	0.290 ± 0.021 (7.2)	29	< LOD(m)	–
Fe	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–
Mn	4.55 ± 0.21 (4.6)	16	1.50 ± 0.01 (0.7)	7.4	1.05 ± 0.01 (0.9)	6.8	0.42 ± 0.03 (7.1)	1.8	2.02 ± 0.26 (12.9)	24
Pb	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	0.154 ± 0.008 (5.2)	22
V	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–
Zn	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	< LOD(m)	–	1.06 ± 0.05 (4.7)	37
Analyte	Xavante / (mg kg^{-1})	BF / %	Blueberry Bluecrisp / (mg kg^{-1})	BF / %	Strawberry (blue lamp) / (mg kg^{-1})	BF / %	Strawberry (white lamp) / (mg kg^{-1})	BF / %	Strawberry (without lamp) / (mg kg^{-1})	BF / %
Ba	0.313 ± 0.006 (1.9)	17	0.109 ± 0.003 (2.7)	30	0.026 ± 0.002 (7.7)	21	0.026 ± 0.001 (3.8)	24	0.024 ± 0.001 (4.2)	24
Cu	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Fe	< LOD$_{(m)}$	–	1.43 ± 0.04 (2.8)	50	0.49 ± 0.01 (2.0)	22	0.76 ± 0.08 (10.5)	42	0.66 ± 0.01 (1.5)	23
Mn	0.62 ± 0.07 (11.2)	11	5.60 ± 0.04 (0.7)	96	1.06 ± 0.09 (8.5)	28	0.98 ± 0.06 (6.1)	36	1.01 ± 0.02 (2.0)	31
Pb	0.234 ± 0.005 (2.1)	33	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
V	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–	< LOD$_{(m)}$	–
Zn	0.620 ± 0.031 (4.8)	20	< LOD$_{(m)}$	–	0.37 ± 0.02 (5.4)	24	0.39 ± 0.04 (10.2)	29	0.92 ± 0.03 (3.2)	51

Mean ± standard deviation (relative standard deviation). Method limit of detection (LOD$_{(m)}$) (mg kg^{-1}): Cu = 0.019; Fe = 0.023; Pb = 0.047; V = 0.114; Zn = 0.050.

Ba and Pb, the maximum recommended daily intake is 7 and 0.0035 mg kg^{-1} body mass *per* day, respectively.[31] In addition, according to WHO and FAO,[1] it is recommended to eat at least 400 g of fruits and vegetables *per* day for the prevention of chronic diseases.

Despite the WHO and FAO[1] recommendations concerning these consumption values, in this work, a quantity of 100 g of fruit was considered, since the total recommended amount will be distributed among the consumption of other fruits and vegetables throughout the day. Converting all bioaccessible concentrations to mg *per* 100 g of ingested fruit, the values found are below the recommended daily intake, being above only for Mn in blueberry O'Neal and Bluecrisp, and in the following samples of blackberries:

05/96, 118 and 124. These results prove that fruits are essential to complement a healthy diet, since they present essential elements for humans. In addition, the need to consume a greater variety of fruits is clear, since some elements are more bioaccessible than others, depending on the fruit matrix, among other factors that may influence it.[32]

Conclusions

This work evaluated the total concentration and bioaccessibility of minerals and toxic elements present in blackberries, blueberries, and strawberries. The minerals K, Mn and Fe presented highest concentrations. It was possible to observe similar concentrations for some cultivars while for others there is a greater difference, even though the fruits come from the same soil and irrigation.

From the results of bioaccessibility, it was observed that the elements Ba, Cu, Fe, Mn, Pb and Zn are released in the gastrointestinal tract in different proportions. In blackberry and blueberry fruits, the Cu was the analyte more bioaccessible. Fe and Zn also presented higher values of bioaccessibility in strawberry, blueberry, and blackberry samples. The toxic elements, Pb and V presented low bioaccessibility for all investigated samples, but their control is important in fruit samples, since these analytes can become a health risk if their concentration exceeds the maximum permitted limit of daily consumption.

Regarding the different forms of cultivation of strawberries, it was observed that those irradiated with red and white lamps produced a higher amount of fruits. However, in relation to the absorption of nutrients, a small variation was observed between the fruits. Besides, the different cultivar modes did not significantly influence the total concentrations and bioaccessible fractions.

Considering the studies of genetic improvement and different cultivation methods, through this work, it was possible to observe the importance of investigating new cultivars and monitoring analyte concentrations on the production of fruits with a suitable quality for the consumer and to assess whether it is possible to harvest them throughout the year, containing the essential nutrients.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

C. C. Pereira was responsible for conceptualization, investigation, methodology, validation, formal analysis, investigation, writing original draft; A. O. Souza for investigation, formal analysis, writing original draft; D. H. Bonemann for investigation, formal analysis, writing original draft; E. Q. Oreste for formal analysis, writing original draft; L. E. C. Antunes for conceptualization, resources, review and editing; S. Cadore for resources, visualization, writing review and editing; A. S Ribeiro for conceptualization, validation, resources, writing review and editing, visualization, supervision, funding acquisition; M. A. Vieira for validation, resources, writing review and editing, visualization. All authors reviewed the manuscript.

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