Original Research Article

Assessment of pumpkin (*Cucurbita sp.*), poppy (*Papaver somniferum* L) and sunflower (*Heliantus annus* L) seeds based on their mineral profile

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ABSTRACT

A multivariate analysis was performed on sunflower, poppy and pumpkin seeds based on the analysis of 18 elements including Al, As, B, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, Si, Sr, Ti, and Zn which were quantified using microwave induced plasma optical emission spectrometry (MIP-OES). To avoid geographical effects, all samples were collected from the same region -San Luis province, Argentina- from 2016 to 2017. In order to determine differences in the mineral profiles as well as to get information from its mineral status, principal component analysis (PCA) was applied. In accordance with this study, toxic elements as pb, cd and as were not detected in the analyzed species, which is a remarkable point compared with other regions around the world. Furthermore, high levels of non-essential nutrients (Al, Ba and Sr) as well as those considered as beneficial non-essential nutrients (Si and Ti) were found in poppy. Applying multivariate analysis -performed by principal components analysis, PCA- it was possible to discriminate groups of seeds according to their botanical origins. Furthermore, PCA allowed inferring about the effect on seed quality due presence of Al, Ba or Sr, as well as the selectivity mechanisms of mineral uptake and filling seed and the differential performance of seeds for initial plant growth -due Si and Ti content- on the three studied species. For these reasons, multivariate analysis has been useful as an informative tool for ionomic and plant development studies.

1. Introduction

In recent years, natural products used as dietary supplements are gaining more attention due to their benefit for human nutrition and health. From these products, several seeds as well as their flours are among the most common choices, because they provide a wide range of nutrients as amino acids, proteins, lipids, minerals, etc (Aguilar et al., 2011). Pumpkin (*Cucurbita sp.*) is a plant widely distributed from North to South America, whose seeds have been used from ancient times as ingredients in foods. Nowadays, they are usually consumed as whole seed, cooked seeds or flour (Rezig et al., 2012). Seeds and flours present high levels of proteins, as well as

lipids with high content of unsaturated acids, which is beneficial to diminish the levels of cholesterol in risk populations (Fu et al., 2006; Joebstl et al., 2010). They also contained high levels of vitamin E, sterols and isoflavones (Murkovic et al., 2004). Regarding elemental analysis, pumpkin seed which is originated from Europe (Rodushkin et al., 2008) and Africa (Glew et al., 2006) was reported with high mineral content. On the other hand, poppy (*Papaver somniferum* L) which is originated from Europe can also be found around the world. Poppy seeds are normally used as condiment and in bakery products, while its petals are used in infusions, syrup and non-alcoholic drinks. These seeds are rich in essential nutrients, providing healthy fatty acids (omega-3 and omega-6), fiber,

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vitamins (Pushpangadan et al, 2012) -specially B1, B2, B3, B5, B6, B9, E and C- as well as minerals such as magnesium, calcium, manganese, potassium, phosphorous, iron, zinc and copper (Ozkutlu, 2008). Sunflower (*Helianthus annuss* L.) seeds are rich in antioxidants, fiber and considered as a healthy and natural food that provides cholesterol-free fats or non-saturated fatty acids. Furthermore, they contain proteins, high contents of some vitamins like vitamin E and minerals, such as potassium, phosphorous, magnesium, and calcium (Schneiter, 1997; Chaves et al., 2010). In previous works, chemometrics-based analyses have been performed for the geographical and botanical classification of pumpkin oils and seeds. (Joebstl et al. 2010) reported the geographical classification of pumpkin seed oils using the earth rare elements profile by ICP-MS in combination with discriminant analysis. Classification of seeds was done according to seven botanical origins by principal component analysis (PCA), clusters analysis (CA) and linear discriminant analysis (LDA) using elemental analysis by atomic absorption spectrometry (AAS) in roasted and raw seeds (Kafaoğlu et al., 2014). Regarding the analytical method, emission atomic spectrometry is preferred due to the possibility of simultaneous multi-elemental analysis, high sensitivity, wide range of linear response and low noise level compared to other methods, allowing the detection of a large number of elements, including a high number of metals and some non-metals (Ozbek and Akman, 2014). From the point of view of human health, the multi-elemental analysis can provide important data about the nutritional quality of foods, since it can inform about major, trace and toxic elements. These elements can be present in seeds depending on the type of plant, geographical localization or environmental conditions (Aguilar et al., 2015). The aim of present work is to determine the multi-elemental composition of the mentioned seeds focusing on the quantification of 18 elements by microwave plasma atomic emission spectrometry MIP-OES including Al, As, B, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, Si, Sr, Ti, and Zn in pumpkin, poppy and sunflower seeds from Argentina. Based on the mineral content data, uni and multivariate statistical analysis were performed to obtain the botanical classification, as well as to make inferences about selectivity of transport mechanisms of minerals in filling seeds process, which have direct consequences in the germination and initial development of seedlings.

2. Experimental

2.1. Apparatus

The elemental analysis was performed using an Agilent MP-AES MP 4100 (Santa Clara, USA) with a One Neb nebulizer, a glass cyclonic spray chamber and an auto-sampler system model SPS3 (Agilent). The nitrogen plasma gas flow was adjusted at 20 L min^{-1} and the auxiliary gas flow at 1.5 L min^{-1}; common settings were used for all analysis, including reads in triplicates. The viewing position and nebulizer pressure were previously optimized for each element. The mineralization step was performed using an Anton Paar MW 3000 microwave system (Graz, Austria). The wavelength used in the MIP-OES and the obtained quantification limits (LOQ) for every element are shown in Table 1. LOQ´s were calculated as 10 times of the standard deviation of blanks. The calibration straights presented a regression coefficient (r^2) range between 0.984 and 0.998 for all elements. Content of mineral components were expressed by µg g^{-1} of seed.

2.2. Reagents

Ultra-pure HNO$_3$ and HCl (Merck, Darmstadt, Germany) were obtained by distillation into a Berghoff suboiling distiller system (Eningen, Germany). Deionized water -resistivity 18.2 mΩ- was produced in a Millipore Synergy water system (Billerica, MA, USA). For calibration step, multi-element standards were used in a matrix of 5% HNO$_3$ (100 µg mL^{-1}, Science Plasma Cal). Indium standard solution was prepared using Merck spectroscopic grade reagent (Darmstadt Germany).

2.3. Sampling

Fourteen samples of each type of seed were collected from local farmers in San Luis province (Argentina) during the period November 2016 - March 2017. Samples were stored in plastic bags in a dark, dry environment until analysis.

2.4. Procedure

Two hundred gram portions of every seed were dried, milled and passed through a sieve of 120 meshes. Then, 2.0 g of each of the obtained flour was accurately weighed and transferred to a hermetically sealed 100 mL PTFE tube, and 5.0 mL of HNO$_3$ along with 1.0 mL of HCl were added. The mineralization was carried out at 235 °C for 30 minutes by triplicate in the microwave system. After that, all clear acid samples were transferred to a 50 mL volumetric flask and diluted with deionized water.

2.5. Chemometric data analysis

All data analysis and chemometric models were obtained by using Unscrambler X 10.3 software (CAMO AS, Trondheim, Norway).

3. Results and Discussion

3.1. Analytical validation

To assess the presence of systematic error during the mineralization step, two analytical validation approaches were performed: a) the addition of an internal standard of indium to all samples; and b) the addition of known concentration of all elements -Al, As, B, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, Si, Sr, Ti, and Zn- performed on 5 samples of each type of seed. In

Table 1
Elemental analysis performed by ICP-OES in poppy, pumpkin and sunflower samples (µg g^{-1}).

Element	LOQ	Poppy[a]	Pumpkin[a]	Sunflower[a]	Wavelength (nm)
Al	0.31	30.0 ± 2.0[a]	12.1 ± 0.8[b]	10.4 ± 0.5[c]	285.213
As	0.07	ND	ND	ND	189.043
B	0.10	23.9 ± 1.3[a]	10.7 ± 0.7[b]	13.2 ± 0.6[c]	249.681
Ba	0.04	5.6 ± 0.4	<LOQ	<LOQ	233.527
Ca	0.10	1363 ± 122[a]	368 ±18[b]	1174 ± 102[c]	317.933
Cd	0.02	ND	ND	ND	226.505
Cu	0.11	15.7 ± 0.9[a]	11.3 ± 0.8[b]	12.6 ± 0.6[c]	327.393
Fe	0.05	106.9 ± 6.3[a]	94.1 ± 6.4[b]	36.4 ± 1.4[c]	238.204
K	0.01	6982 ± 444[a]	6924 ± 352[a]	6310 ± 350[b]	766.490
Mg	0.02	3816 ± 172[b]	6105 ± 300[a]	3571 ± 182[c]	285.213
Mn	0.02	65.2 ± 5.2[a]	49.9 ± 2.7[b]	30.3 ± 2.1[c]	257.61
Na	0.76	9.8 ± 0.9	<LOQ	<LOQ	589.592
P	0.84	8330 ± 758[b]	12416 ± 727[a]	7426 ± 360[c]	213.617
Pb	0.46	ND	ND	ND	220.353
Si	0.10	844 ± 68	<LOQ	<LOQ	251.611
Sr	0.22	38.9 ± 1.5[a]	2.3 ± 0.4[c]	10.3 ± 0.8[b]	407.771
Ti	0.77	2.8 ± 0.4	<LOQ	0.9 ± 0.1	283.244
Zn	0.01	49.7 ± 2.8[b]	70.4 ± 4.4[a]	40.2 ± 1.8[c]	206.207

[a] Interval confidence mean ± SD (n= 14). Concentrations expressed in µg g^{-1}. Letters indicate significant differences by Tukey means test (P<0.05) Decimals in nm are informed according to the resolution ability of detector.

the case of indium, the recovery degree was evaluated in the whole mineralization step; while in the standard addition method, the recoveries allowed to assess the quantified elements. The obtained performance for the recovery of In was 96 ± 8% (n=42). The averages of individual recoveries for studied elements (n=15) ranged from 93-109% and are shown in Table 2. These recovery degrees indicate a correct performance in the quantification of all elements by MIP-OES and low errors in the total analysis and were similar to those reported in previous works (Gulfen and Özdemir, 2016).

3.2.   Elemental analysis

In Table 1, the results obtained for 14 samples of each type of seed, including LOQ's and selected wavelengths have been shown. Toxic elements such as As, Cd and Pb were not detected in none of the samples. Potassium

and copper presented similar concentrations (values) in the three seeds, although they have significant differences. On the other hand, poppy had the highest content of all elements, except for Mg, P and Zn, whose maximum values were found in pumpkin. Nevertheless, the highest concentration of non-essential nutrients as Al, Sr, Ba and Ti was found in poppy seeds. Seeds can contain chemical substances in their composition which are produced by the plant and are considered as anti-nutritional agents, because they reduce the use of proteins, vitamins and minerals and subsequently decrease its nutritional value (Maradini et al., 2015). It has been shown that anti-nutrients such as phytic acid, oxalates, lectins, tannins, saponins and enzyme inhibitors reduce the availability of nutrients causing a growth-decreasing impact. However, when anti-nutrients are found in low concentrations, they can produce beneficial effects on health, since they

can reduce the glucose and insulin responses in blood, as well as cholesterol and triglycerides in plasma (Gemede and Ratta, 2014). Although this work does not prioritize the quantification of these compounds, it is important to consider that anti-nutrients can affect the bioavailability of minerals, i.e. phytic acid can form insoluble complexes with different minerals, such as Ca, Cu, Fe, Mg, Mn and Zn, making them less bioavailable (Martinez et al. 1996). However, as mentioned earlier, it must also be taken into consideration that the intake of these antinutrients at low concentrations can be beneficial for health if they are incorporated into the diet.

3.2. Multivariate analysis

Based on the elemental profile, chemometric classification models for poppy, pumpkin and sunflower were obtained. The variables used to obtain the model were the concentration of Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Si, Sr, Ti and Zn. With this PCA model, a 93% of explained variance was obtained with first 2 PCs and achieves 98% with 4 PCs (final model) with a root square mean of error prediction (RSMEP) of 0.45. Scores plot in Fig. 1 shows three groups differentiating each type of seed according to botanical origin. Regarding PC1, poppy presented highlighted differences in comparison to pumpkin and sunflower; also, it can be seen that sunflower and pumpkin are separated between them mainly by PC2. According to the loading plot (Fig. 2), there are several elements that have important influence on PC1, as Al, Na, Si, Ca, Ba, B, Cu, Ti, but low influence on PC2, except to K, Mn and Fe which had also influence on PC2. These last elements were in part responsible of poppy grouping on the right side of figure. On the other hand, P, Mg and Zn had strong influence on PC2 but moderate in PC1, reason for which was also responsible of separation between poppy from the others groups (Fig. 1); that means, poppy had high concentration in all elements excepting to P, Mg and Zn, whereas pumkin had high concentration of P, Mg Zn, as well as K, Mn and Zn. Finally, sunflower had the lowest concentrations of elements in all groups. In general, the mineral profile found in poppy at present work was similar to a previously reported study (Ozcan, 2004). However, previous studies only reported few elements (Ca, Fe, Cu, K, Mg, Mn, Na and Zn) in pumpkin and sunflower seeds. About pumpkin, the mineral content reported in this work, was similar to found with other authors (Juranovic et al., 2003; Soylak et al., 2006). In the case of sunflower, the concentration of Ca, K, Mg and Na found in this work were higher than those previously reported by (Gulfen and Özdemir 2016); however, Fe, Cu, Mn and Zn were comparable with other authors (Cabrera et al., 2003; Özcan 2006; Kirbaşlar et al., 2012; Albuquerque et al., 2013). Loadings plot shows that poppy contains higher levels of essential nutrient as Ca, Cu and B as well as non-essential nutrients like Al, Ba, Na, Si, Sr and Ti, while pumpkin accumulate essential elements as Mg, P, and Zn, which are more valued as mineral nutrients for food quality; sunflower would be the minor contributor of three studied species as mineral

source. Nevertheless, the aforementioned bibliography places the three species as important contributors of minerals in the human diet. Table 3 summarizes the comparative results of this work with previous reports. The observed capability of poppy to accumulate heavy metals imply that they arrive to the seeds by translocation of mineral nutrients across ionic channels that they are the usual pathways for essential nutrient transport from root, shoots and leaves to seed by symplastic, apoplastic and phloem ways (Khan et al., 2014). That can be explained by chemical analogy of elements and affinity by same binding sites where the same ionic channels can co-transport non-essential elements across cell and plant compartments. These are explained by known cases related to the element pairs Rb-K, Sr-Ca, Se-S, Co-Ni and Fe-Ti (Baxter, 2009; Huang et al., 2016; Lyu et al., 2017). Furthermore, it is possible to considerate that transport mechanism of mineral nutrients to seeds in pumpkin can be more selective than poppy to discriminate non-essential elements. This fact can be observed in this work, due high Sr concentrations founded in poppy seeds, in comparison to those found in pumpkin and sunflower seeds. More interesting is the Ti case because Ti^{4+} shares physical and chemical properties with Fe^{3+} and they share thermodynamic preference for similar binding sites. Although there are not previous reports on how Ti is uptaked in plants, correlation with Fe behavior suggest that Fe and Ti shares the same ionic channels for reception and delivering in plants (Lyu et al., 2017). Loadings plot (Fig. 2) shows that poppy and pumpkin seeds contain similar quantity of Fe but only poppy accumulates Ti. Therefore, this could indicate that Fe and Ti have different acquisition and delivering ways for both metals in the three species, or at least Fe-Ti selectivity of transports mechanisms are different in the three species. Micronutrients from tissues, as well as xylem sap can translocate minerals to phloem vessels by specific transporters, which can be discharged into the seeds. In annual plants, seed maturation is accompanied by the gradual senescence of plants. Optimal remobilization of micronutrients requires the coordination of the senescence process from source tissues (leaves, shoots, roots) with the target tissues during seed filling (Pottier 2014). In recent years, it has been suggested that micronutrients are mobilized from different tissues to seeds by autophagy. In senescent tissues at the final stages of annual plants (or under stress conditions), autophagy catabolizes cytoplasmatic components facilitating the recycling of cellular material. Autophagy implies the formation of membranes vesicles (autophagosomes) that engulf and sequester proteins, lipids and any kind of micronutrients contained in degraded or unfunctional macromolecules. Vesicles drive the translocation of nutrients to phloem and subsequently transport to seed (Pottier, 2014; Masclaux-Daubresse, 2017). In the present work, the content of Al in poppy seeds is three-fold higher than pumpkin and sunflower, which indicates differential mechanisms for transport of Al to the poppy seed across plant compartments. That

cumulus of evidence turns relevant the ionomic studies for identification of channel transporters that could explain the presence of high quantities of metals as Al, Ba, Ti, and Sr in poppy seeds possibly by low selectivity of ionic transporters in poppy plants (Baxter, 2009). Poppy seeds have been shown to have higher levels of Si and Ti than pumpkin and sunflower. The beneficial effects of silicon and titanium on plants performance are known (Epstein, 2009; Moldes et al., 2016). Although both elements are considered as non-essential nutrients, Si improves the plant performance under stress condition reinforcing plant defense (Adrees, et a., 2015), while Ti is a positive effector for plant development (Lyu, 2017). Seed soaked with TiO_2 nanoparticules increased water/ nutrient absorption and improved seed germination (Hatami et al., 2014; Cox et al, 2016). Then, Ti content in poppy seeds could be beneficial for further initial plant development, although it must be considered that Ti have hormetic behavior, so toxic levels are possible from certain Ti levels in seeds that could be investigated for present species. On the other hand, Si content found in poppy seeds may be beneficial for germination and initial development of poppy seedlings under stress conditions in comparison with pumpkin and sunflower.

Table 2
Recovery assay performed on seed samples.

Analyte	Recovery (%)[a]
Al	107± 7
As	94± 9
B	96± 9
Ba	104± 6
Ca	102± 5
Cd	97± 4
Cu	98± 3
Fe	108± 9
K	98± 5
Mg	109±9
Mn	106± 8
Na	97± 4
P	107± 8
Pb	93± 7
Si	98± 3
Sr	103± 6
Ti	96± 7
Zn	101± 2

[a] Average ± SD (n=15).

Although there is no specific studies about these physiological capabilities of silicon accumulators to improve performance in germination or initial plant development under stress conditions, considering the content of Si in seeds, there are reports about external Si application on seeds that improves germination under drought stress conditions (Rizwan et al., 2015).
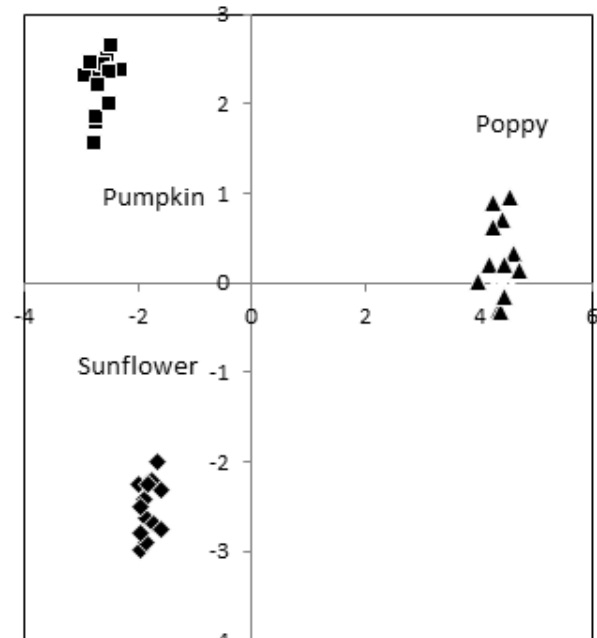

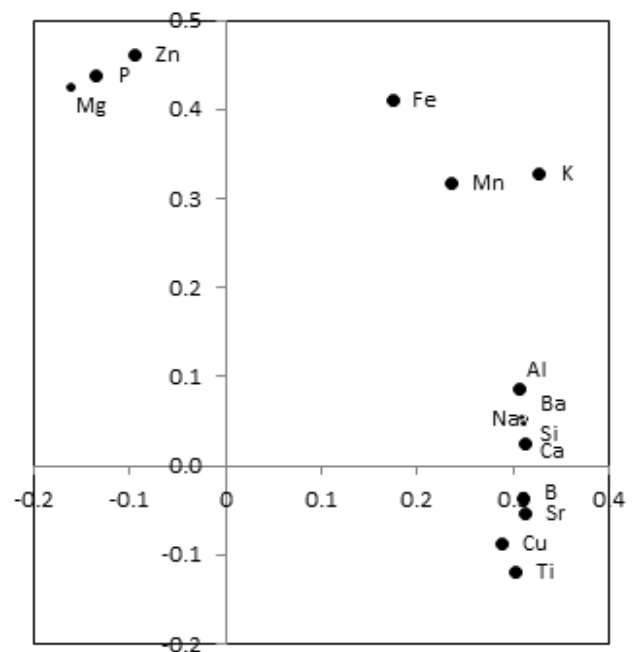**Fig. 1.** PCA scores plot showing botanical classification of seeds.

Fig. 2. PCA loadings plot showing the influence of mineral content on grouping.

Table 3
Comparison between the concentrations obtained by other authors and this work.

	Al	B	Ba	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	Sr	Ti	Zn	References
Poppy																
	19.6	30.31	118	10583	14.4	91.1	5906	4256	56.1	4.63	5795		70.6	-	42.5	(Ozcan, 2004)
	29.98	23.9	5.6	13663	15.7	106.9	6982	3816	65.2	9.8	8330	844.8	38.9	2.83	49.7	This work
Pumpkin																
	-	-	-	-	24.9	113.4	-	-	77.8	-	-	-	-	-	-	Soylak et al., 2006
	-	-	-	131	6	47.3	3261	289	18.1	226.58	-	-	-	-	34.2	Gulfen, 2016
	-	-	-	271.9	-	-	-	-	-	-	-	-	-	-	-	Rezig et el., 2012
	-	-	-	-	-	-	7100	5600	-	57	-	-	-	-	-	Rodushkin et al., 2008
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	59.64	Juranovic et al., 2003
	12.12	10.7	ND	368	11.3	94.1	6924	6105	49.9	ND	12416	ND	2.3	ND	70.4	This work
Sunflower																
	-	-	-	133.4	-	-	-	-	-	-	-	-	-	-	-	Albuquerque et al., 2013
	-	-	-	490	15.2	49	3716	323	15.4	336	-	-	-	-	32.4	Gulfen, 2016
	-	-	-	-	18.5	-	-	-	-	-	-	-	-	-	-	Özcan, 2006
	-	-	-	-	-	66	-	3500	24.31	25.44	-	-	-	-	-	Chaves et al., 2010
	-	-	-	-	-	-	3094	-	-	-	-	-	-	-	-	Kisbaslar et al., 2012
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	69	Cabrera et al., 2003
	10.44	13.2	ND	1174	12.6	36.4	6310	3571	30.3	ND	7426	ND	10.3	0.95	40.2	This work

Concentrations expressed in $\mu g\ g^{-1}$.

4. Concluding remarks

The elemental analysis of sunflower, poppy and pumpkin seeds carried out by MIP-OES showed high concentrations of mineral elements, as well as the absence of toxic elements, reason for which they can be advisable for human nutrition. However, the presence of antinutrients as fitic acids, phenolic compounds and tannins could affect the bioavailability of mineral elements. Also, these seeds contribute highly to the relative daily value (RDV) which is in agreement with previous reports. On the other hand, the absence of toxic elements (As, Cd, Pb) in all samples is remarkable since these elements are present in seeds in several regions around the world. Chemometrics combined with elemental analysis by MIP-OES could be considered as a simple method for evaluation of nutritional and botanical quality of pumpkin, poppy and sunflower seeds. In addition, the present study permits to infer about differential selectivity of the three studied species in the mineral transport across plant organs to seeds. Furthermore, the content of Ti and Si found in poppy seed flour can represent a major performance for initial development of plant. However, more studies are still necessary to clarify this case.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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