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Assessment of Mineral Profile in Edible Seeds by Microwave Induced Plasma Optical Emission Spectrometry (MIP OES): Ionomic Approach

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Abstract

Eighteen elements were quantified in sunflower, poppy and pumpkin seeds by microwave induced plasma optical emission spectrometry (MIP OES). In order to determine differences in the mineral profiles, as well as to get information from its mineral status, multivariate analysis of data matrix were performed. Toxic elements as Pb, Cd and as were not detected in analyzed species. 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. By multivariate analysis-performed by principal components analysis, PCA-it was possible to discriminate groups of seeds according to botanical origin. Furthermore, PCA allowed to infer about the seed quality concerning to presence of undesired elements as Al, Ba or Sr; the selectivity mechanisms of mineral uptake and filling seed; and the differential performance of seeds for initial plant growth (due Si and Ti content) of the three studied species. For these reasons, multivariate analysis turns an informative tool for ionomic and plant development studies.

Keywords: MIP OES; Elemental profile; Pumpkin; Poppy; Sunflower; PCA

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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 the most common choices, because they provide a wide range of nutrients such as amino acids, proteins, lipids, minerals, etc [1].

Emission atomic spectrometry is preferred for simultaneous multi-elemental analysis due to 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 [2]. From the point of view of human health, the multi-elemental analysis can provide important data about the nutritional quality of foods, because it can inform about major, trace and toxic elements, which can be present in seeds depending on the type of plant, geographical localization or environmental conditions [3].

Methods of chemometrics analysis have been successfully used to determine genotypical classification [4-6] as well as changes in biological systems [7-9]. Regarding to mineral profiles, chemometrics analysis achieves botanical classification of pumpkin oils and seeds [10], seeds of several Amaranth species [1], or species differentiation of edible seeds [11] by PCA, CA and LDA.

In the last years, many scientific works emphasize ionomic approaches for physiological functioning comprehension of plants regarding genetic and biochemic regulation of mineral composition in plants. Such studies allow identifying genes and gene networks including ion transporters, ion binding proteins and ion oxido-reductases that intervene in metabolism for uptake, transport, store and transformation of plant essential and non-essential minerals [12]. Furthermore, the study of plant mineral profile under influence of ionic stressors as non-essential heavy metal or exposure to high concentrations of micro nutrients has motived ionomic research for elucidation

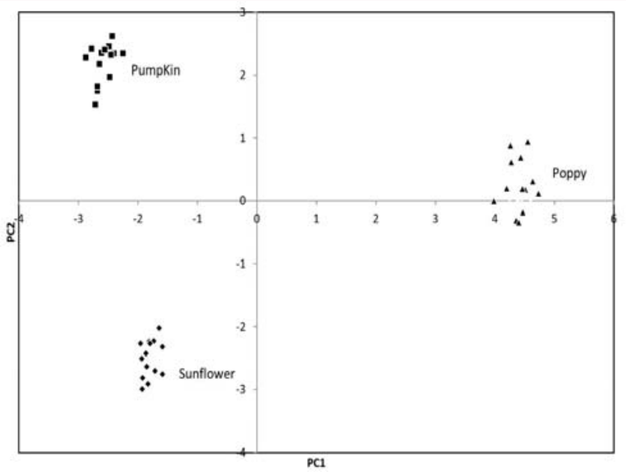

Figure 1: PCA scores plot showing botanical classification of seeds.

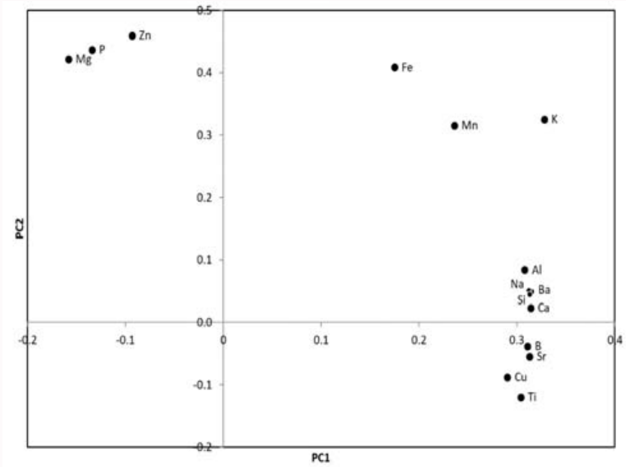

Figure 2: PCA loadings plot showing the influence of mineral content on grouping.

of ion homeostasis mechanisms in response to environmental stress [13-14].

Pumpkin (*Cucurbita sp.*), poppy (*Papaver somnifera*) and sunflower (*Helianthus annuss L.*) are usually consumed as whole seed, cooked seeds or flour providing high levels of mineral nutrients for human diet [15-19]. The quantity and quality of mineral content of seeds must be considered to determine the food quality regarding to environmental condition where they are cultivated, especially when they are domestic crops as in the case of the present work.

For the exposed, this work aims to determine the multi-elemental composition of pumpkin, poppy and sunflower seeds focusing on the quantification of 18 elements by microwave induced plasma optical 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 this way, our intention is to assess the chemometric analysis of seeds mineral profiles as a tool for botanical differentiation of species considering an ionomic approach. Based on the mineral content data, uni and multivariate statistical analysis were performed to achieve the botanical differentiation, and to make inferences about selectivity of minerals transport mechanisms in the filling seed process, which have direct consequences in physiological aspects of seeds as well as seed quality as food.

Materials and Methods

The elemental analysis was performed using an Agilent MP-AES 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 to 20Lmin^{-1} and the auxiliary gas flow to 1.5Lmin^{-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 Limits of Quantification (LOQ) for every element are shown in Table 1. LOQs were calculated as 10 times the standard deviation of blanks. The calibration straights presented a regression coefficient (r^2) ranged between 0.984 and 0.998 for all elements. Content of mineral components were expressed by μg g^{-1} of seed.

Ultra-pure HNO_3 and HCl (Merck, Darmstadt, Germany) were obtained by distillation into a Berghoff suboiling distiller system (Eningen, Germany). Deionized water -resistivity 18.2mΩ was produced in a Millipore Synergy water system (Billerica, MA, USA). For calibration step, multielement standards were used in a matrix of 5% HNO_3 (100μg mL^{-1}, Science Plasma Cal). Indium standard solution was prepared using Merck spectroscopic grade reagent (Darmstadt Germany).

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.

Two hundred grams of every seed were dried, milled and passed through a sieve of 120 meshes. Then, 2g of the obtained flours were accurately weighted and transferred to a hermetically sealed 100mL PTFE tube, and 5mL HNO_3 and 1mL 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 50mL volumetric flask and diluted with deionized water.

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

Results

Analytical validation

Two analytical validations were performed to assess the presence of systematic error during the mineralization step: 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 the case of indium, the recovery degree was evaluated in the whole mineralization step; while in the standard addition, the recoveries allowed to assess the quantified elements. The obtained performance for the recovery of in was 96±8% (n=42). Recovery ranges for studied elements in poppy (n=5), pumpkin (n=5) and sunflower (n=5) seeds, were from 90 to 112% and are showed in Table 2. These recovery degrees were similar to reported in previous works [11].

Elemental analysis

Table 1 shows the results obtained for 14 samples of each type

Table 1: Elemental analysis performed by MPAES in poppy, pumpkin and sunflower samples.

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

of seed, including LOQ's and selected wavelengths. Potassium and cooper presented similar concentrations in the three seeds; however, poppy had the highest content of all elements, except for Mg, P and Zn, whose maximum values were found in pumpkin. Elements such as As, Cd and Pb were not detected in none of the samples. Nevertheless, it was found high contain of nonessential nutrient as Al, Sr, Ba and Ti in poppy seeds.

Chemometric 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 achieved 98% with 4 PCs (final model) with a Root Square Mean of Error Prediction (RSMEP) of 0.45. Scores plot in Figure 1 shows three groups differentiating each type of seed according to botanical origin. Loading plot (Figure 2) show the influence of P, Mg and Zn on PC2 which is responsible for pumpkin samples grouping (Figure 1). On the other hand, loadings plot shows the influence of Al, B, Ba, Ca, Cu, Na, Si, Sr and Ti on PC1 responsible for poppy grouping. Sunflower performs a group away from the others due low content of minerals in comparison with poppy and pumpkin.

Discussion

The mineral profile found in poppy was similar to the found in literature [20]. Previous reports on pumpkin and sunflower have studied only a few elements in their seeds (Ca, Fe, Cu, K, Mg, Mn, Na and Zn) and the main differences between present work and literature were detected in pumpkin [21]; however, more similarities of pumpkin mineral profile were found with other authors [17,22-24]. 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 [21]; however, Fe, Cu, Mn and Zn were comparable with other authors [19,25-28]. Loadings plot shows that poppy contain higher levels of essential nutrient as Ca, Cu and B as well as non-essential nutrient as 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 the three species studied as mineral source. Nevertheless, the aforementioned bibliography places the three species as important contributors of minerals in the human diet.

Table 2: Recovery assay performed on seed samples.

Analyte	Recovery ranges[a]
Al	93- 105
As	94 - 103
B	96 - 104
Ba	93 - 106
Ca	98 - 105
Cd	97 - 104
Cu	98 - 103
Fe	99 - 110
K	96 - 112
Mg	90 - 101
Mn	94 - 105
Na	97 -108
P	99 -107
Pb	93 -101
Si	98 - 106
Sr	96 -103
Ti	96 - 109
Zn	95 - 106

[a] Ranges obtained for three seeds.

The observed capability of poppy to accumulate heavy metals imply that they arrive to the seeds by translocation of mineral nutrients across ionic transporters, which are the usual pathways for essential nutrient transport from root, shoots and leaves to the seed by symplastic, apoplastic and phloem ways [29]. Heavy metal transport can be explained by chemical analogy of elements and the affinity by same binding sites where the same ionic channels can co-transport non-essential elements across cell and plant compartments, which are known cases of Rb and K, Sr and Ca, Se and S, Co and Ni, Fe and Ti [12-13,30]. Furthermore, it is possible to consider that transport mechanism of mineral nutrients to seeds in pumpkin can be more selective than poppy to discriminate non-essential elements. In our work, we could considerate that analogy between Sr and Ca as a reason for Sr accumulation in poppy seeds coinciding also with the fact that Ca is present in higher quantities than 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 about how Ti is uptake in plants, correlation with Fe behavior suggest that Fe and Ti share the same ionic channels for reception and delivering in plants [30]. Loadings plot (Figure 2) shows that poppy and pumpkin seeds contain similar quantity of Fe but only poppy accumulate 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 transport selectivity mechanisms are different in the three species. Onion and maize can accumulate Al in different manners but the content of Al in roots is higher than aerial plants parts for both indicating selectivity trend in Al transport across the compartments of plants [31]. In addition, Al can be transported in different forms as ionic Al, Al $(Ox)^{+}$, Al-oxalate and Al-citrate complexes [32]. In the present work, the content of Al in poppy seeds is three-fold higher than pumpkin and sunflower, which indicate different mechanisms of Al transport to the poppy seed across plant compartments. That cumulus of evidence turns relevant the ionomic studies for channel transporters identification [13] that could explain high quantities presence of metals as Al, Ba, Sr and Ti in poppy seeds possibly by low selectivity of ionic transporters in poppy plants.

Poppy seeds demonstrated to have higher level of Si and Ti than pumpkin and sunflower. The beneficial effects of silicon and titanium on plants performance are known [30,33-34]. Although both elements are considered non-essential nutrients, Si improves the plant performance under stress condition reinforcing plant defense [35] while Ti is a positive effector for plant development [30]. Seed soaked with TiO_2 nanoparticles increase water/nutrient absorption and improve seed germination [36-37]. Then, Ti content in poppy seeds could be beneficial for further initial plant development, although it must be considered that Ti has 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. Although there are no specific studies considering the content of Si in seeds over physiological capabilities to improve plant development, there are reports about external Si application on seeds that improve germination under drought stress conditions [38].

Conclusion

The elemental analysis of sunflower, poppy and pumpkin seeds carried out by MIP OES showed in general, high concentrations of mineral elements and the absence of usual toxic elements as As, Cd or Pb, which confers to these seeds adequate properties for human nutrition. Nevertheless, it should be considered the accumulation of non-essential nutrients (*i.e.* Al, Ba, Sr and Ti) for assessing poppy seeds quality as food. Chemometrics combined with elemental analysis by MIP OES have been a simple method for evaluation of nutritional characteristics and botanical discrimination of pumpkin, poppy and sunflower seeds. Also, the present study permits an ionomic approach infering on transport selectivity of minerals across plant organs to seeds in the three species studied. Furthermore, the content of Ti and Si found in poppy seed flour could represent a major performance for initial development of plant but more studies are necessary in that issue.

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