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Determination by Atomic Absorption Spectrometry of mineral content from maize (*Zea mays*) hybrids and their carotenoidic extracts

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Abstract

The paper presents the determination, by atomic absorption spectrometry (AAS), of some minerals concentration (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni) in different maize (Zea mays) hybrids and also in the purified carotenoidic extracts obtained from this raw materials. It was used an atomic absorption spectrometer Varian Spectra AA 110 with four lamps. The main macroelement both in the maize flour and in the carotenoidic extracts was potassium (between maximum 4029 ppm and minimum 3663 ppm maize flours, respectively between maximum 3006,00 ppm and minimum 970 ppm in maize flour carotenoidic extracts). The macroelements: K, Na, Ca and Mg, from the analyzed carotenoidic extracts are in the lower concentration that in the raw materials, this minerals being removed to a great extent during the extraction process. The heavy metals concentrations was under maximum lawful limit, except Cu from the Laureat maize hybrid flour. All the obtained data were statistical processed by Principal Component Analysis (PCA) method. It was obtained a good cases classification by this multivariate analysis; the main elements responsible for the first classification were magnesium and potassium.he paper abstract will be written with Times New Roman 10 pt., justify. It will contains maximum 150 words.

Keywords: atomic absorption spectrometry, mineral content, maize, carotenoidic extract.

1. Introduction

Maize (Zea mays) is grown primarily for its kernel, which is largely refined into pro-ducts used in a wide range of food, medical and industrial goods. Maize kernel contains: sugars (66-76%) from which about 80% are represented by the starch, proteins (7-14%), water (9.5-12.3%), fatty acids (4.5%), dietary fibers (0.8-2.9%), minerals (1.3%), carote-noids (lutein, zeaxanthin, cryptoxanthin, β-carotene, and others), vitamins: E, B1, B2, B3 [1-3]. Corn grains are rich in some mine-rals such as: potassium (3248±339 ppm), phosphorus (2996±578 ppm), magnesium (1079±94 ppm) and, as the most cereal grains, is low in calcium content (483±123 ppm) and also low in heavy metals. The maize germ contains most (78%) of the minerals (ash) found in the kernel [2,3]. Minerals are very important food compo-nents. They are of nutritional, functional and, in some case, toxic importance [4]. In maize grains, as in other cereals, the mineral content is affected by several factors including environment pollution, soil and agricultural practice [5-7].

The carotenoidic extracts are known for their remarkable role in some important biochemical processes for the animal and human life: the eyesight process, growth and reproduction; they have anticance-

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rigenic effect, antioxidant effect, skin epithelization, cicatrisation and photoprotection effect [8-19]. We used the maize carotenoidic extract to obtain a dermatological ointment, that was clinically tested and presents a very good efficiency in some skin diseases such as: basocellular epithelium, erythematos lupus, impetigo, psoriasis, photodermatosis, chronic eczema and others [20].

Along the carotenoids extraction process, a part of the minerals are removed from the extracts and others are kept in a grater or less measure by the carotenoids molecules. In this study we are accomplished a comparison between the minerals content from the maize hybrids flours and the minerals concentration in the carotenoidic extracts obtained from this, using the atomic absorption spectrometry method. [21,22].

2. Materials and Method

Reagents and materials

For minerals content determination was used six maize flour hybrids: *Lovrin 400, NSSK444, NSSK640, NSSK420, Florența* and *Laureat,* cultivated at the Didactic Station of the Agricultural Science and Veterinary Medicine Banat's University Timisoara and the purified carotenoidic extracts obtained from this. For the Laureat hybrid maize flour was analyzed both the final purified carotenoidic extract and the unsaponified carotenoidic extract. The extraction solvents: ethanol (96%), petroleum ether and acetone (analitical purity) was purchased from Chimopar, Bucharest. Butylhydroxytoluene (BHT) used for carotenoids oxidative degradation prevention was from Merck company like the potassium hydroxide used for saponification process. The calibration curves for each determined mineral was achieved using standard solutions (concentration of 1000±2 mg/l) from Merck compa-ny, like the nitric acid and hydrochloric acid used in the sample preparation.

Aparatus

- The laboratory apparatus used was:
- rotative evaporator model RV-05 basic 1-B (Shimadzu, Japan);
- centrifuge model Universal 32 R (Hettich, Germany);
- atomic absorption spectrometer Varian Spectra AA 110 (Mulgrave, Australia);
- water bath Precisterm (Selecta, Spain);
- analytical digital balance AW 320 (Shimadzu, Japan);
- calcination oven Labortherm LHT 04/18 (Nabertherm, Germany);
- drying stove EC 50 (SC Caloris Group SA, Bucharest).

Obtaining of the carotenoidic extracts

For the carotenoidic extracts obtaining, the dried maize grains were grinded to a fine powder. The maize flour (table 1.) were introduced in an 1000 ml vessel, moisten with distilled water, then treated with ethanol 96% and let at rest 50 minutes- for starch hydrolysis and advanced carotenoids liberation from cells [20]. For carotenoids oxidative degradation prevention was aded BHT 0,1% (reported at the raw material). Carotenoidic pigments were extracted with a solvents mixture of: petroleum ether: acetone: ethanol 96% (6:3:1, v:v:v) [23,24]. Extraction was repeated for few times with new solvent portions until colourless. The etheric extracts were concentrated under vacuum, at 35ºC, in an rotative evaporator, until a soft extract was obtained.

The soft extract was treated with 40 ml petroleum ether and let at rest over night (16 hours) at -10˚C, for sterols precipitation. Precipitated sterols were removed by centrifugation for 10 minutes at 2000 rpm. The supernatant was then concentrated under vacuum, at 35ºC, to complete removing of the solvent.

The remaining extract, after sterols removing, was submitted to saponification with 40 ml KOH 20% ethanolic solution, for 16 hours at room temperature and in darkness. Carotenoids were then reextracted with petroleum ether in a separation funnel, washed for several times with NaCl saturated solution and then with distilled water until the complete removing of soaps and alkalies. The etheric extracts were treated with anhydrous Na_2SO_4 for dehydration, filtered and then concentrated under vacuum, at 35ºC, for complete removing of the ether. Samples were redissolved in petroleum ether and keeped at -20ºC. From every maize hybrid were achieved three samples in the same conditions.

Raw material and final extracts quantities (average values) is presented in table 1.

Samples mineralization

In porcelain capsules, previously brought at constant weight by drying at 105^{o}C, was weighing 1,000g maize flour, respectively 0,200g carotenoidic extract. The capsules with samples were introduced into drying stove at 50-60^{o}C for almost 8 hours. Then the temperature was increased at 105^{o}C for 5-6 hours. After this time, the sample capsules were took out from drying stove and introduced into calcinations oven, at cold. It was increased progressively the temperature at 200-250ºC and it was maintained to complete calcinations of the samples [25].

Then the temperature was increased to 500ºC and the samples were calcined for 6-8 hours to a white ash was obtained. The samples that were incomple-tely calcined were treated with 1ml concen-trated HNO_3, drying on the sand bath and then calcined at 500ºC for others 2 hours.

After cooling, the ash was treated with 0,5 ml bidistilled water and 1 ml hydrochloric acid 6N and was evaporated to dry, on the sand bath; the operation was repeated for two times. The residue was dissolved in small portions of 5 ml HCl 0,5N, passed quantitatively into a 50 ml glass balloon and completed to exactly 50 ml with HCl 0,5N solution.

The balloon content was finally filtered in a perfectly dry flask. For each sample set was achieved a control sample [25].

Minerals determination by atomic absorption spectrometry

The minerals (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni) was measured from the obtained hydrochloric solution by pulverization in the air-acetylene flame and measurement of the absorbance (for Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni), respectively emission (for K and Na) at the characteristic wavelength for each analyzed element. It was used an atomic absorption spectrophotometer Varian Spectra AA 110, with four lamps, controlled by PC. For the spectrophotometer calibration were prepared sets of etalons of different concentrations in HCl 0,5N solution for each analyzed element, starting to the concentrated standard solutions. The concentration (C) for each determined element was calculated with the following formula: C (mg/kg or ppm) = a x f /m, where:

- f - dilution factor;
- element content indicated by apparatus (mg/l);
- m - sample initial weight.

Principal component analysis (PCA)

All the experimental data concerning the each mineral concentration both in the maize flours and in the carotenoidic extracts were statistical processed by Principal Component Analysis (PCA) method [26-29], in the aim to estabilish the alike samples groups and the variables with significantly importance in the studied cases discrimination.

The PCA analysis for the mineral elements from the maize flours and the carotenoidic extracts obtained from this was achieved in the following conditions:

- total variables number for all maize flours and carotenoidic extracts samples: 13;
- total number of cases for all maize flours and carotenoidic extracts samples: 12.

3. Results and discussions

The results obtained by atomic absorption spectrometry determination of minerals content in the maize analyzed hybrids and in their carotenoidic extracts are presented in the table 1 (figures 1-6).

Table 1. - Minerals content of maize hybrids flours and in the carotenoidic extracts obtained from this

No.	Sample	Code	K	Na	Ca	Mg	Fe	Mn	Cu	Zn	Pb	Co	Cr	Ni
1.	*Lovrin 400* maize extract	ExP_L	3006	131	275	225	49	0	0.35	0.59	0.33	0	0.06	0.12
2.	*Lovrin 400* maize flour	FP_L	3841	1570	320	2858	117	0	3	2	0.38	0	0.09	0.16
3.	*NSSK444* maize flour	FP_444	3833	1587	267	2999	100	0	0.40	2	0.35	0	0.12	0.17
4.	*NSSK444* maize extract	ExP_444	1734	49	167	0	142	0	0.20	1	0.18	0	0.09	0.16
5.	*NSSK640* maize flour	FP_640	3698	176	309	785	52	0	0.09	0.27	0.28	0	0.10	0.14
6.	*NSSK640* maize extract	ExFP_640	970	24	121	13	41	0	0.27	0.85	0.10	0	0.30	0.08
7.	*NSSK420* maize extract	ExP420	1315	31	130	0	87	0	0.02	0.84	0.11	0	0.07	0.07
8.	*NSSK420* maize flour	FP_420	3908	1111	229	3317	142	0	3	3	0.34	0	0.09	0.24
9.	*Florența* maize flour	FP_F	4029	1394	241	3454	117	0	0.35	2	0.33	0	0.08	0.18
10.	*Florența* maize extract	ExP_F	973	27	97	0	81	0	0.07	0.70	0.07	0	0.05	0.07
11.	*Laureat* maize flour	FP-La	3663	1285	303	2267	140	0	6.53	3	0.53	0	0.10	0.27
12.	*Laureat* maize extract	ExP - La	1028	28	102	0	93	0	0.19	0.87	0.12	0	0.04	0.12
13.	*Laureat* maize unsaponified extract	ExP_Nesap	3242	101	412	132	22	0	0.01	0.17	0.23	0	0.003	0.10

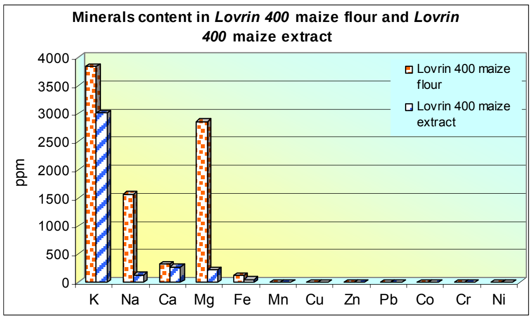

Figure 1.-Minerals content in the *Lovrin 400* maize hybrid flour and in the carotenoidic extract obtained from this.

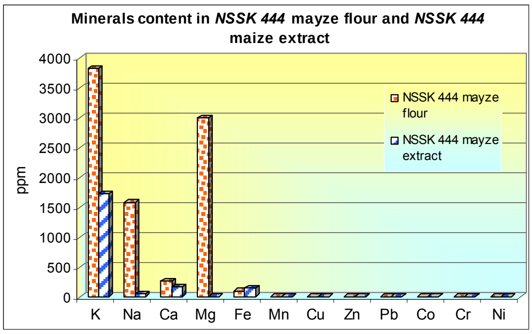

Figure 2.-Minerals content in the *NSSK 444* maize hybrid flour and in the carotenoidic extract obtained from this.

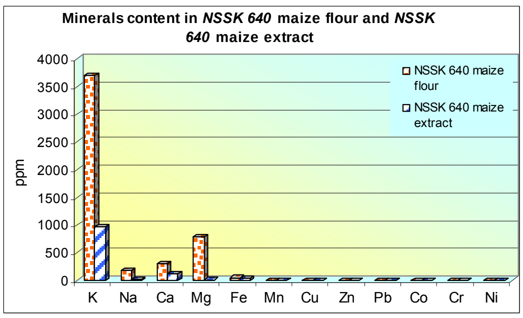

Figure 3.-Minerals content in the *NSSK 640* maize hybrid flour and in the carotenoidic extract obtained from this.

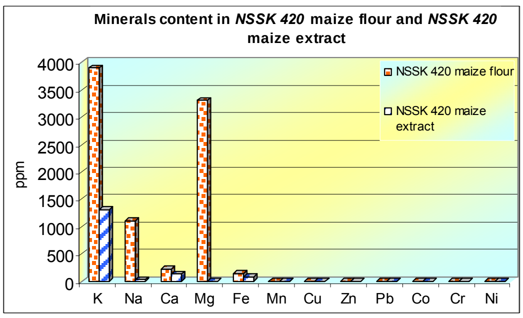

Figure 4.-Minerals content in the *NSSK420* maize hybrid flour and in the carotenoidic extract obtained from this.

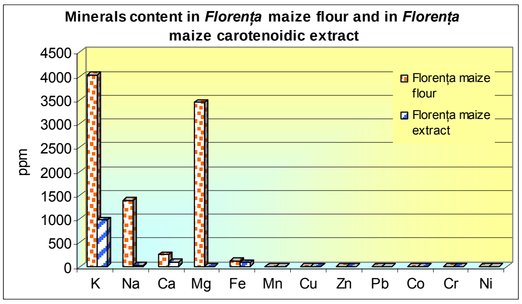

Figure 5.-Minerals content in the *Florența* maize hybrid flour and in the carotenoidic extra.

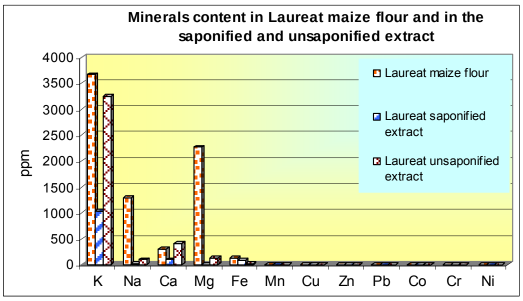

Figure 6.-Minerals content in the *Laureat* maize hybrid flour and in the carotenoidic extracts saponified and unsaponified obtained from this.

From the experimental data it was observed that the heavy metals with toxic potential are not greater concentrations that the maximum lawful limits [30,31], neither for the maize hybrids flours nor for the extracts, with a single exception: in the *Laureat* maize hybrid flour, the Cu content is of 6,53 ppm, the maximum lawful limit are of 5,00 ppm. The content of the others minerals in the maize hybrids flour are in the ranges indicated in the literature data [2,3].

Potassium is the best represented macro-element both in the maize flours and in the carotenoidic extracts. The K content in the analysed maize hybrids flours vary beetwen 4029 ppm – for the *Florența* hybrid and 3663 ppm – for the *Laureat* hybrid. In the carotenoidic extracts, potassium content vary between 3006 ppm – for *Lovrin 400* hybrid extract and 970 ppm – for *NSSK640* hybrid extract. In the case of the unsaponified extract from *Laureat* hybrid, potassium concentration (3242 ppm) is less than that in flour (3663 ppm), but greater than that in the saponified final extract (1028 ppm).

Sodium are found in a more less quantities than the potassium, both in the maize flour and in the extracts. In the maize flour, the sodium content vary between 176 ppm- in the case of *NSSK640* hybrid and 1587 ppm- for the *NSSK444* hybrid. The sodium content from the finals carotenoidic extracts are much less than that in the maize hybrids flours: between 24 ppm - in the case of *NSSK640* maize carotenoidic extract and 131 ppm - for *Lovrin 400* maize carotenoidic extract. In the unsaponified extract from *Laureat* maize hybrid, the sodium content (101 ppm) are much less than that in the flour (1285 ppm), but greater than that in the final saponified extract (28 ppm), the sodium salts being, to a great extend, removed from this.

Calcium, very important macroelement for human body, are also present in the maize flours and in the carotenoidic extracts. The calcium content vary for maize flour between 229 ppm- in *NSSK420* hybrid and 320 ppm- in *Lovrin 400* hybrid, and for carotenoidic extracts between 97 ppm- in *Florența* hybrid extract and 275 ppm- in *Lovrin 400* hybrid extract. In the *Laureat* hybrid unsaponified extract, the calcium content is greater than that in the saponified extract.

Magnesium are present in the maize flour in greater quantities that calcium, varying between 3454 ppm- in *Florența* hybrid and 783 ppm- in *NSSK640* hybrid. In the carotenoidic extracts calcium in are much less quantities, this element being even absent in the *NSSK444, NSSK420, Florența* and *Laureat* hybrids carotenoidic extracts. The magnesium content in the *Lovrin 400* and *NSSK640* hybrids carotenoidic extracts is of 225 ppm, respectively 13 ppm, and in the *Laureat* hybrid unsaponified extract the content is of 132 ppm- greater than in the saponified extract in which magnesium are not identified. Although in the maize flour magnesium is present in greater quantities than calcium, magnesium are not present in much of the carotenoidic extracts, being completely removed in the extraction process, while calcium are present in all the extracts, because the carotenoidic compounds keep a part of this macroelement.

Iron is well represented in the maize flour, varying between 52 ppm- in *NSSK640* hybrid and 142 ppm - in *NSSK420* hybrid. In the carotenoidic extracts, iron concentration is only a little less than in the maize flours, varying between 41 ppm- in *NSSK640* hybrid and 142 ppm - in *NSSK444* hybrid. The *NSSK444* maize extract is more rich in iron than *NSSK444* maize flour, this extract having the same iron concentration as the *NSSK640* maize flour - that have the greatest iron concentration. The iron are not remove by the saponification process: iron concentration in the unsaponified *Laureat* maize extract being less (22 ppm) than iron concentration in the final saponified extract (93 ppm).

Manganese was not identified in any maize hybrids or in the carotenoidic maize extracts.

Copper is present in small quantities in maize flour, except the *Laureat* hybrid that contain with 1.53 ppm more copper than the lawful maximum limit (5.00 ppm [30]). In the carotenoidic extracts the copper concentration are less than in the maize flours, except the *NSSK640* maize extract, where copper quantities (0.26 ppm) is a little greater than in the raw material (0.09 ppm). Copper are not remove from extracts through saponification process, his concentration in the unsaponified extract being less (0.01 ppm) than in the saponified one (0.19 ppm).

Zinc content in the analysed maize flours vary between 0.26 ppm - for *NSSK640* hybrid and 3 ppm - for *Laureat* and *NSSK420* hybrids, values that are under lawful maximum limit (15 ppm [30]). In the extracts, zinc content is less than in the raw materials, except the *NSSK640* hybrid extract where zinc is in greater concentration (0.85 ppm) than in the flour (0.26 ppm). Like copper, zinc are not remove along the saponification process, his concentration in the saponified extract being greater (0.87 ppm) than in the unsaponified one (0.17 ppm – for *Laureat* hybrid).

Lead, heavy metal with a marked toxic character, vary, in the analyzed maize flour, between 0.28 ppm- for *NSSK640* hybrid and 0.53 ppm - for *Laureat* hybrid (values that are less than the lawful maximum limit of 1.00 ppm [30]). In the carotenoidic extracts, lead is present in less concentrations than in flours (between 0.07 ppm- for *Florența* hybrid extract and 0.33 ppm- for *Lovrin 400* hybrid extract). This heavy metal are remove from extracts along their saponification process, the lead content being greater in the unsaponified *Laureat* hybrid extract (0.23 ppm), than in the saponified one (0.12 ppm).

Cobalt was not identified in any maize hybrids or in the carotenoidic maize extracts.

Chromium are present in very small quantities in the maize flour (between 0.08 ppm – for *Florența* hybrid and 0.12 ppm – for *NSSK444* hybrid) and in the maize flour carotenoidic extracts (between 0.05 ppm - for *Florența* and *Laureat* hybrids extracts, 0.29 ppm- for *NSSK640* hybrid extract). In the case of *NSSK640* hybrid, chromium concentration is greater in the carotenoidic extract (0.29 ppm) than in the flour (0.10 ppm). This metal are not remove through saponification process, chromium content in the saponified extract being greater (0.05 ppm) than in the unsaponified one (0.003 ppm).

Nickel concentrations in the maize flours are small (between 0.14 ppm- for *NSSK640* hybrid and 0.27 ppm – for *Laureat* hybrid).

Carotenoidic extracts contain very small quantities of Ni: between 0.07 ppm - for *NSSK420 and Florența* hybrids extracts, 0.16 ppm - for *NSSK444* hybrid extract. This metal are not remove along the carotenoidic extracts saponification, his content in the saponified *Laureat* hybrid extract being greater (0.12 ppm) than in the unsaponified one (0.10 ppm).

Principal Component Analysis (PCA) of the obtained data show that all results concer-ning the carotenoidic extracts samples are grouped on the left part of graphic and those concerning the maize flours are grouped in the right part (figure 7.). The principal elements responsible for first classification are magnesium and potassium (figure 8). From figure 7 it could be observed that the *Laureat* hybrid unsaponified extract and *Lovrin 400* hybrid saponified extract are the richest in the mineral elements than the others analyzed maize carotenoidic extracts.

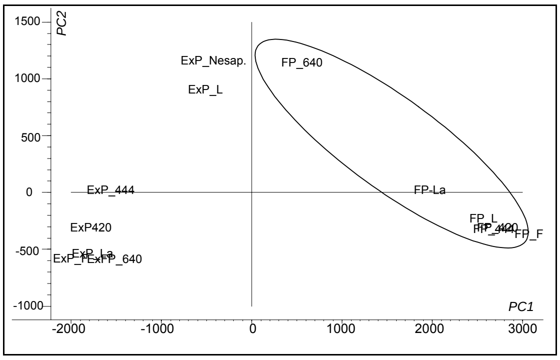

Figure 7.- Scores *PC2 vs PC1* from the PCA analysis of data for maize hybrids flour and carotenoidic extracts.

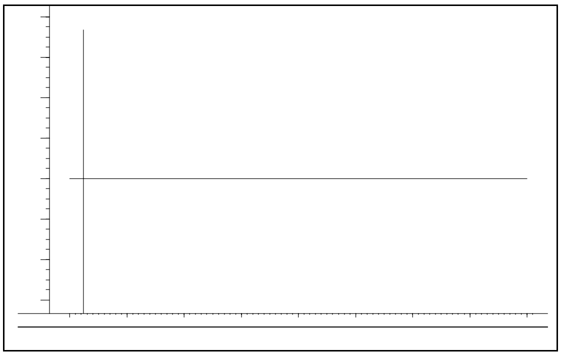

Figure 8.- Loadings *PC2 vs PC1* from the PCA analysis of data for maize hybrids flour and carotenoidic extracts

4. Conclusions

As a result of minerals content determination in different maize flour hybrids and in their carotenoidic extracts by atomic absorbtion spectrometry it could take out the following conclusions:

- All the macroelements from the analyzed maize flours hybrids are in concentrations that correspond with the literature data. The concentrations of potassium, sodium, calcium and magnesium in the maize carotenoidic extracts are much less than in the corresponding maize flours, this macroelements being removed in a great extent along the extraction process.
- The best represented macroelement both in the maize flours and in the carotenoidic extracts are potassium (between maximum 4029 ppm and minimum 3663 ppm in the case of maize flour, respectively between maximum 3006 ppm and minimum 970 ppm in the case of maize flour carotenoidic extracts).
- Iron concentration in the maize hybrids extracts is only a little less than in the corresponding maize flours, except the *NSSK444* hybrid extract that are a greater iron concentration than the *NSSK444* hybrid flour.
- All the heavy metals determined was under maximum lawful limit, both in the maize flours and in the carotenoidic extracts, except Cu from the *Laureat* maize hybrid flour that was found in a 6,53 ppm concentration, 13% more than the maximum lawful limit.
- In the carotenoidic maize extracts, the heavy metals concentrations are much less than in the maize flours.
- Manganese and cobalt was not identified in any maize hybrids or in the carotenoidic maize extracts.
- Among the heavy metals, lead are removed along the carotenoidic extracts saponification process, but copper, zinc, chromium and nickel are not.
- Statistical processing of the experimental data by Principal Component Analysis (PCA) method show a good cases classification; the main elements responsible for the first classification are magnesium and potassium.

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