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Principal Component Analysis for Identification of Mineral Content in Moroccan Lentils

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ABSTRACT

Background: Thirty-six samples of Moroccan lentils from the 2014 and 2015 harvests were selected in order to evaluate the nutritional characteristics, more precisely the micronutrient content Fe, Zn, Mn, Cu, Ca, Mg, P and K.

Methods: The mineral assay was performed using an ICP-AES atomic emission spectrometer. Analysis of the data was performed using the principal component analysis (PCA).

Result: The results showed that these samples are rich in P and K more than the other elements, which also have a good concentration. Analysis of the data using the principal component analysis (PCA) identified the sample with a right balance in the concentration of the elements studied. In addition, the study of the concentration with the aid of the husking seed made it possible to have an idea on which part of the seed the minerals are concentrated. We concluded from this second study that we could use the whole lentil seed as an additive to flour in order to enrich it with minerals and avoid the problems caused by mineral deficiency in the human body.

Key words: Atomic emission spectrometer, Food analysis, Food composition, Lentils, Micronutrient, PCA.

INTRODUCTION

Dietary mineral deficiencies impair the health of over three billion people globally and are responsible for illnesses and deaths during all stages of development from infancy through adulthood (Bailey *et al.,* 2015). It is estimated that two billion people globally suffer from iron deficiency (De Benoist *et al*., 2008). Iron deficiency during pregnancy causes increases in premature deliveries, low birth weights and maternal deaths, while deficiency during infancy and early childhood can reduce physical growth and development of cognitive functions (Bailey *et al*., 2015). Humans also suffer considerably from diseases caused by zinc deficiency. Adequate levels of zinc are essential to fetal development, healthy birth and subsequent physical growth (King, 2011). Approximately one billion people globally are estimated to be at risk of zinc deficiency (Wessells and Brown, 2012), primarily in sub-Saharan Africa and southeast Asia. In Bangladesh, over 100 million people are at risk of arsenicosis associated with dietary selenium deficiency (Combs, 2001). Diets deficient in selenium have been associated with a higher incidence of prostate cancer (Wu *et al*., 2015). Mineral deficiencies can often be treated by dietary consumption of mineral supplements or fortified foods (Miller and Welch, 2013). Unfortunately, in regions such as sub-Saharan Africa and southwest Asia that are strongly affected by mineral deficiencies (Yip and Ramakrishnan, 2002) various socioeconomic constraints can limit availability of mineral supplements and fortified foods.

In Morocco, lentil is produced mainly in low potential lands through the traditional farming system using landraces to insure home-consumption and livelihood security (Benbrahim *et al*., 2017). Seeds of lentil landraces constitute an economic dietary source of protein, essential minerals such as iron, zinc, calcium and magnesium and antioxidant compounds (Benbrahim *et al*., 2016). According to Moroccan Ministry of Health Surveys Data, (2000), the prevalence of malnutrition and micronutrient deficiency affects 37.2% of pregnant women, 31.5% of preschool aged children (6 months - 5 years old), 32.6% of women of childbearing age and 18% of men. The healthcare costs and productivity losses due to micronutrient deficiencies account for about 5% of national GDP (Aguenaou, 2007). Iron deficiency is among the most common micronutrient deficiency that affects about 50% of children aged between 7 and 9 old and 10% of women of childbearing age (Alaoui, 2005) (El Menchawy *et al*., 2015) (Petranovic *et al*., 2008). Iron deficiency and anemia lead to adverse consequences on human health and productivity as the decrease of work capacity and school performance (Alaoui, 2005) (Vieteri, 1994) (Petranovic *et al*., 2008) and costs two billion dirham's per year (Aguenaou, 2007). Current rates of deficiency

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and anemia are mostly related to insufficient iron daily intake (Alaoui, 1991) (EL Hioui *et al*., 2007). Plant uptake ability of iron from the soil is behind several genes expression (Mbasani-Mansi *et al*., 2019).

Another approach proposed to reduce the incidence of mineral deficiencies is biofortification, in which crop plants have higher concentrations of minerals in edible parts (White and Broadley, 2005). Biofortification may be accomplished by applying management practices that result in increased concentrations of minerals in edible parts, development of new cultivars with elevated mineral concentrations through plant breeding, or a combination of management and genetic approaches (Mbasani-Mansi *et al*., 2019). Several pulse crops, including lentils (*Lens culinaris* Medik.) (Ray *et al*., 2014) (Karaköy *et al*., 2012) have been proposed as targets for mineral biofortification. The development of new cultivars that stably express high concentrations of selected minerals require an understanding of the magnitude of genetics, environment and their interaction effects on mineral concentrations. Studies conducted in Morocco on lentils have shown that genotype, environment and their interaction effects were significant for seed concentrations of copper, magnesium, selenium and zinc (Mbasani-Mansi *et al*., 2019). Significant genotype effects on seed mineral concentrations were also observed for lentils grown in Turkey (Karaköy *et al*., 2012).

Our study focuses on Moroccan lentils to determine their micronutrient content because they are known for their richness in Fe, Ca, P, K (Guide Marocain de Nutrition, 2016). In addition, since a great deal of studies have not been done on Moroccan lentils, and given that annual lentils production in Morocco has dropped since the year 2000 (Table 1).

We were interested in studying the nutritional characteristics of Moroccan lentils, specifically the trace elements content. In the first place to know among the 36 samples which contain a better balance in the micronutrient composition, while using the analysis of the results given with PCA (principal component analysis) to encourage farmers to use these samples for future crops. Secondly, we conducted a study to find out in which part the whole seed cotyledon or the integument trace elements are concentrated more precisely. In addition, we wanted to know which sample contains a high concentration of Fe to use as a fortifier in the flours sold on the Moroccan market in order to increase their nutritional value and to avoid the population's health problems related to the lack of this element in the human body.

Table 1: Production of lentils in morocco (World Health Organization, 2016).

Year	Production (tons)
2014	30.671.00
2015	50.004.00
2016	2.004.00

MATERIALS AND METHODS

The work was carried out on 8 improved varieties noted V1 - V8 and 10 samples of local populations of the harvest of the years 2014 and 2015 (Table 2 and 3).

Decorticating lentils

Dehulling is to separate the seed from its envelopes and have the integuments that are considered, often as a by-product and the almond of economic and nutritional interests. The external abrasion of the seeds does it. Dehulling is crucial essential technological approach in the processing of cereals and legumes; it allows knowing the separability, the degree of adhesion of almonds to external envelopes.

Determination of mineral content

The mineral assay was performed using an ICP-AES atomic emission spectrometer at the CNRST (Centre National pour la Recherche Scientifique et Technique, Rabat, Maroc). In this study, the digestion of the lentils meal samples was performed by varying several conditions such as: the digestion time, the digestion temperature, the volume of the acid mixture as well as its nature and the mass of the sample to obtain the optimum conditions, as indicated in Table 4. The optimal conditions for digestion of the lentils samples are: the nitric acid has a volume of 1 ml, the digestion temperature is 120°C and the optimal time is 4 hours. Applying the optimized procedure, 0.05 g of homogenized lentil flour sample was put into a plastic vial. Then 1 ml of nitric acid was added and the mixture was digested in a hot

Table 2: Geographic localization of studied moroccan's landraces.

Site	ID	Latitude	Longitude	Altitude (m)
Zaer	P1	33°32′00″N	6°36′00″W	306
Tetouan	P2	35°34′42″N	5°22′06″W	121
Rhamna	P3	32°14′00″N	7°57′00″W	449
Larach	P4	35°11′35″N	6°09′20″W	38
Beni Mellal	P5	31°57′51″N	6°34′27″W	620
Mouley Bouselham	P6	34°52′43″N	6°17′36″W	12
Houceima	P7	35°14′57″N	3°55′58″W	80
Fes	P8	34°01′59″N	5°00′01″W	406
Taounate	P9	34°32′09″N	4°38′24″W	566
Taza	P10	34°13′26″N	4°00′24″W	510

plate at 120°C for 4 hours. Then, the digested solution was allowed to cool for 15 minutes. To the cooled solution, demineralized water was added and transferred to 14 ml tubes the flask was rinsed 3 times. Tests were conducted at three different temperatures in this study 120°C, 150°C and 200°C. The results obtained for the latter were similar. The results are analyzed using the Xlstat 2015 software.

Table 3: Nomenclature of improved varieties.

Variete-code	ID	Producer	Year registration
Bekria	V1	INRA	1989
Bichette (Ill 5562)	V2	INRA	2000
Chaouia (L8967-88)	V3	INRA	2004
Hamria (Flip 87-48l)	V4	INRA	2000
L 24	V5	INRA	1989
Abda (L 593153-16)	V6	INRA	2004
Chakkouf (Ill6001-81)	V7	INRA	2009
Zaria (L8635)	V8	INRA	2003

Table 4: The different volume of the acid mixture as well as its nature and the mass of the sample used to obtain the optimum conditions.

Acid composition	Volume (ml)	Mass (g)
$HNO_3/HCl/H_2O_2$	4/1/1	0.1
$HNO_3/HCl/H_2O_2$	2/0.5/0.5	0.1
HNO_3/H_2O_2	1/0.5	0.1
HNO_3	1	0.1
HNO_3/HCl	1/0.5	0.1
HNO_3	1	0.05
HNO_3/H_2O_2	1/0.5	0.05
HCl/H_2O_2	1/0.5	0.05
HNO_3/HCl	1/1	0.05
HCl	1	0.05

RESULTS AND DISCUSSION

Lentils have been recognized as relevant sources of several minerals in Moroccan diets. Table 5 shows the mineral composition of the 36 samples of the whole seeds of the crops of 18 of the year 2014 (Table 5a) and 18 of the year 2015 (Table 5b).

The seed samples from the 2014 harvest contained higher levels of potassium, phosphorus, magnesium and calcium compared to iron, manganese, copper and zinc. The high concentration of P and K can be explained by the use of fertilizers (PKN) in lentil cultures, while the concentration of Fe and Zn remains consistent with previous studies on lentils in Spain (Campos-Vega *et al*., 2010) (Cabrera *et al*., 2003) (Viadel *et al*., 2006) (Wang *et al*., 2006). Similarly, for the 2015 samples, we also recorded a higher rate of calcium, potassium, phosphorus, less zinc in Fe. In this study, all seed samples recorded a higher potassium level compared to the recommended dietary allowance (RDA) value for infants and children (MARTIN Ambroise, 2018). The high potassium content can be used in the diets of people who take diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid (Bernard and François, 2012). From these results, we can notice that the lentils of Moroccan origin are rich in trace elements.

Subsequently, we analyzed the results using the principal component analysis (PCA) statistical method in order to determine which samples have a better equilibrium of trace element concentrations and to know the correlation between trace element differences studied.

Table 5a: Micronutrient concentration in samples 2014 (mg/kg).

ID	Ca	Cu	Fe	K	Mg	Mn	P	Zn
P1	430.91	8.281	41.883	9947.96	944.128	13.31	3644.31	7.23
P2	849.163	8.012	33.837	10420.32	1083.80	16.475	3917.98	11.304
P3	636.514	12.681	56.1	9967.91	1124.14	8.297	5075.85	13.034
P4	826.39	10.871	39.261	8540.90	877.156	8.683	2696.21	4.184
P5	805.923	11.74	49.729	10759.65	1114.90	9.946	4882.79	16.455
P6	616.783	14.47	37.814	11013.20	1031.09	7.36	4381.10	7.296
P7	688.153	7.037	29.691	9323.38	879.835	4.855	3311.64	21.275
P8	584.107	9.676	37.091	9885.50	1115.42	16.98	4116.06	9.913
P9	809.661	10.276	48.231	11205.21	1122.56	14.711	4186.52	11.838
P10	530.022	12.816	39.663	9349.89	1106.54	7.55	4048.37	5.976
V1	477.54	16.58	14.83	11832.80	829.11	11.05	3859.77	24.33
V2	592.33	9.87	46.31	10546.79	1045.61	14.67	4606.34	13.96
V3	638.29	14.78	59.36	11823.93	1182.17	13.53	5703.79	13.90
V4	626.23	12.16	59.18	9927.75	1085.99	18.92	3904.75	11.63
V5	805.58	14.08	49.83	10693.14	1196.79	24.74	4816.47	20.21
V6	599.26	13.50	41.24	10389.95	1065.11	14.64	4589.74	8.20
V7	486.32	10.41	59.70	10224.83	1183.15	14.41	4983.13	9.45
V8	535.33	11.52	48.95	11381.11	1017.54	11.13	4456.65	6.28

Table 5b: Micronutrient concentration in samples 2015 (mg/kg).

ID	Ca	Cu	Fe	K	Mg	Mn	P	Zn
P1	863.207	14.226	12.72	10767.88	1144,90	8.536	3955.58	19.55
P2	1025.96	20.969	23.336	14686.09	1557.98	23.431	5698.33	14.62
P3	913.029	18.947	22.542	12758.60	1299.50	19.076	5079.07	13.61
P4	1456.75	19.613	24.396	13554.30	1581.10	17.499	5879.00	21.78
P5	942.993	14.148	124.377	10966.68	1086.78	15.811	3819.70	14.51
P6	1226.93	19.619	95.102	14087.88	1357.63	13.804	5105.94	33.67
P7	794.758	22.624	25.953	14609.25	1441.29	21.661	5641.47	56.71
P8	822.182	19.047	136.268	12512.08	1322.64	20.181	4567.11	41.72
P9	787.46	12.14	60.77	9880.66	1087.97	18.94	3861.86	21.42
P10	546.09	12.78	58.48	9992.55	988.72	18	3610.67	12.09
V1	724.73	12.28	74.45	10397.62	1111.22	19.62	4621.63	23.8
V2	505.55	11.05	44.56	11185.85	963.11	10.29	4624.57	15.61
V3	467.32	13.77	83.18	11016.92	1047.13	20.91	4036.42	13.01
V4	670.46	10.55	51.23	11324.21	1146.30	16.85	4356.54	15.11
V5	731.74	14.41	83.49	11125.90	1185.88	24.5	4068.94	20.93
V6	624.02	14.1	39.72	9981.72	995.65	14.43	3762.36	15.28
V7	579.81	11.93	68.23	10144.49	1206.91	19.45	4548.56	22.39
V8	738.119	17.403	78.013	11384.33	1199.10	19.229	4727.35	20.558

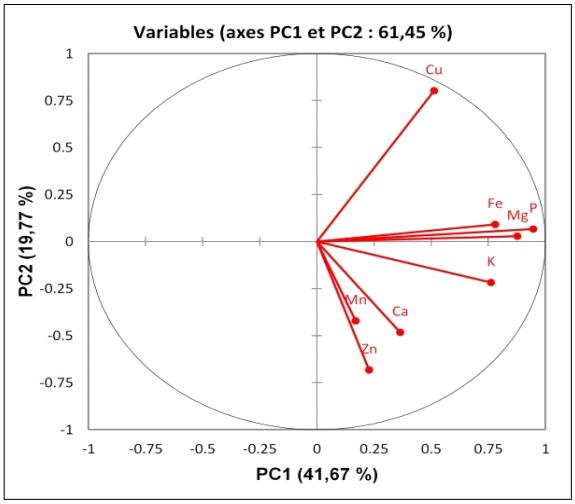

Fig 1: Presentation of variables.

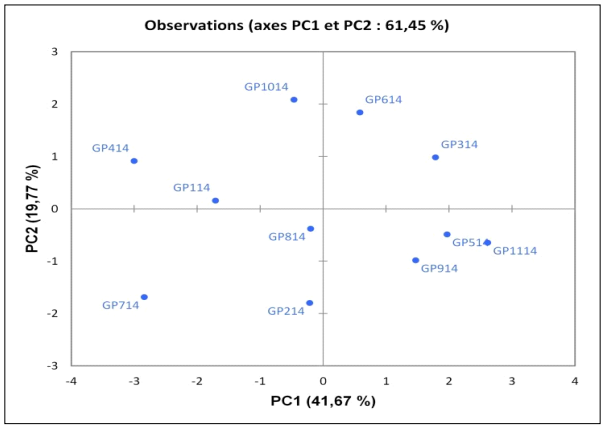

Fig 2: The representation of the samples.

Population analysis

Fig 1 shows the representation of the variables. We can conclude that the eight variables are strongly and positively correlated with the horizontal: the higher a sample is rich in metals, the higher it is on the horizontal axis. Reciprocally, the more a sample is "poor" in metals, the lower it is on this axis; the horizontal axis thus represents, in some ways, "the global concentration" in all 8 metals. Concerning the vertical axis, it opposes Cu Fe P and Mg (positive correlation) and K Zn Mn and Ca (negative correlation). The sample that has a right balance according to our analysis is the one that will have a positive value along the horizontal axis and a value almost zero along the vertical axis.

The representation of the samples is shown in Fig 2. Sample 3, 5, 9 and 11 have a near-zero value on axis PC2; *ie*. they have a homogeneous composition of metals. The highest sample on the graph, the one with the highest coordinate on axis PC2, is the 10 with the most conflicting results in favor of metals Cu Fe P and Mg. This is precisely the opposite for sample 2, whose results are the most contrasting in favor of metals Zn Mn and Ca. Simultaneous representation in Fig 3 confirms this conclusion.

Subsequently, the simultaneous representation for the other samples. For the P 2015 samples (Fig 4).

From Fig 4 we can conclude that 7 variables are positively correlated along the first axis the Fe negatively; so the samples that have a high coordinate along this axis are the richest in minerals except Fe. Regarding the axis PC2 vertical, it opposes, on the one hand, the Mn Cu KP and Mg (positive correlations with the axis PC2), on the other hand, Fe Zn and Ca (negative correlations with the axis PC2).

From Fig 4 the balanced samples are 3 and 8 but 5 is richer in Fe. From this analysis it can be concluded that the best balanced sample is 3.

Variety analysis

The simultaneous representation for V 2014 and V 2015 is given in Fig 5 and 6. From Fig 5, it can be concluded that 5 variables are positively correlated, along the first axis and three variables Cu K and Zn negatively. Therefore, the samples that have a high coordinate along this axis are the richest in minerals Fe Ca Mg P and Mn those with a low value along this negative coordinate axis, are rich in Cu K and Zn. About the vertical axis PC2, it opposes, on the one hand, the Cu Zn P Mn Mg K and Ca (positive correlations with the axis PC2), on the other hand, the Fe (negative correlations with the axis F2). From Fig 5 the stabilized samples are 3, 4 and 6.

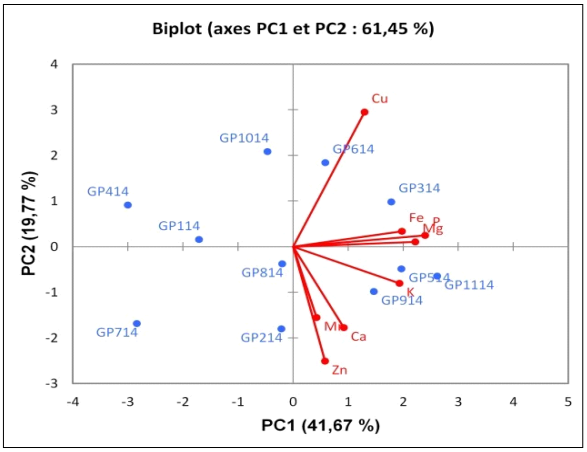

Fig 3: Simultaneous variable representation and sample.

From Fig 6 it can be concluded that the 8 variables are positively correlated along the first axis; so the samples that have a high coordinate along this axis are the richest in minerals. With regard to the vertical axis 2, the PC2 factor opposes, on the one hand, Cu Fe K and Mn (negative correlation with the axis PC2), on the other hand, Mg Ca P and Zn (correlations positively with the axis PC2).

From Fig 6 the balanced samples are 8 and 5. According to the analysis of the 2014 and 2015 samples of Moroccan lentil varieties, we did not have the same sample that has a right balance between the two years of harvest. This is most likely due to the depletion of the land minerals after the harvests of the year 2014.

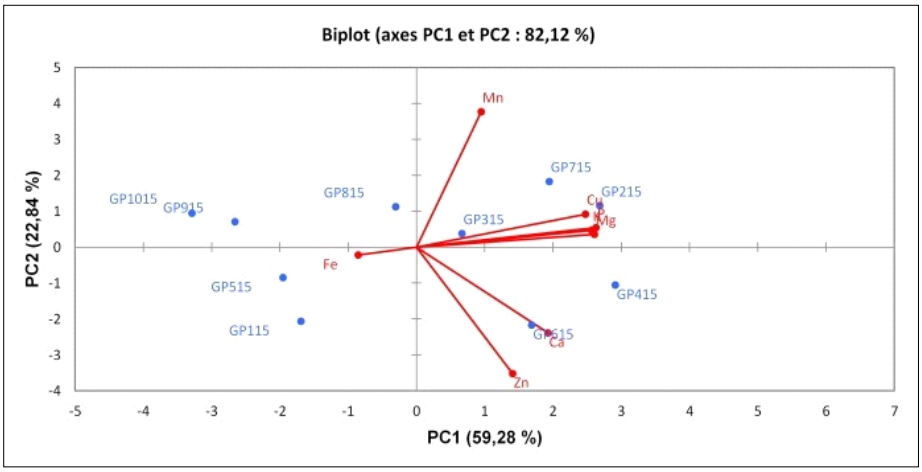

Fig 4: Simultaneous variable representation and sample P 2015.

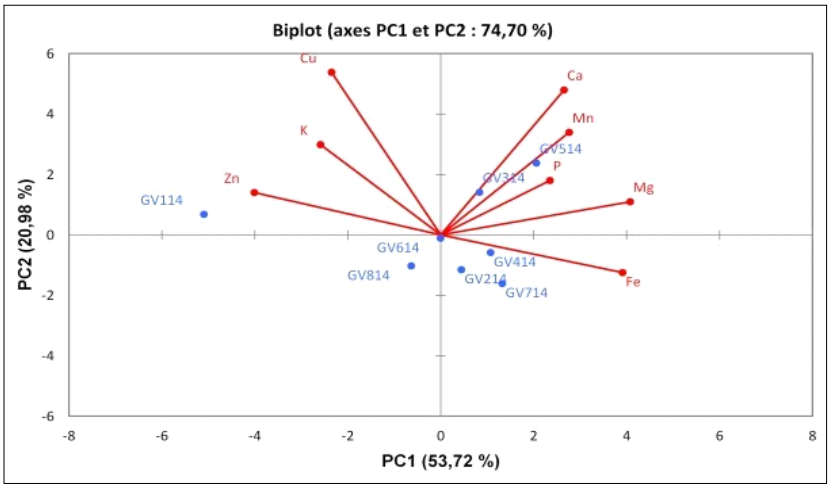

Fig 5: Variable simultaneous representation and sample V 2014.

In our second study of lentils this time we analyzed the results using the descriptive statistics. In this last one we drew graphs using a histogram to visualize the concentration of the metals in C. G. T. for all the samples of the year 2014 in Fig 7a, 7b and 7c. The year 2015 in Fig 8a, 8b and 8c. We have represented the Fe, Cu and P content as an example in the 2014 and 2015 samples.

For the samples of the year 2014 (Fig 7) note that the highest concentration of Fe is on the T except for samples 3 and 11. By cons the concentration of Cu is well distributed

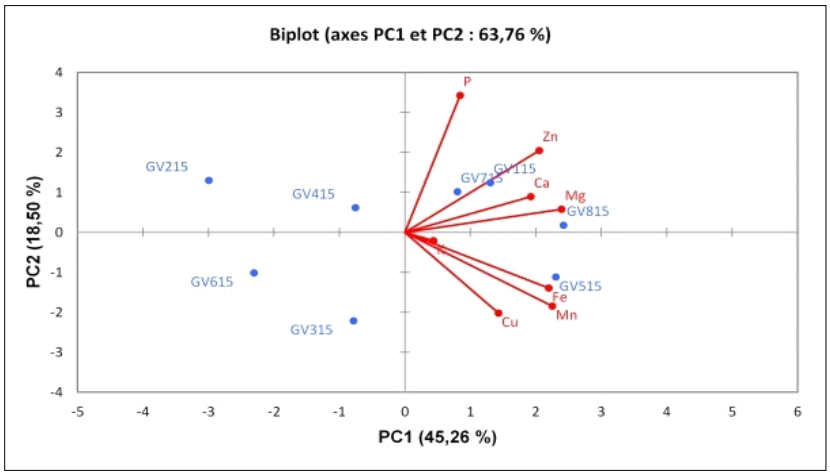

Fig 6: Variable simultaneous representation and sample V 2015.

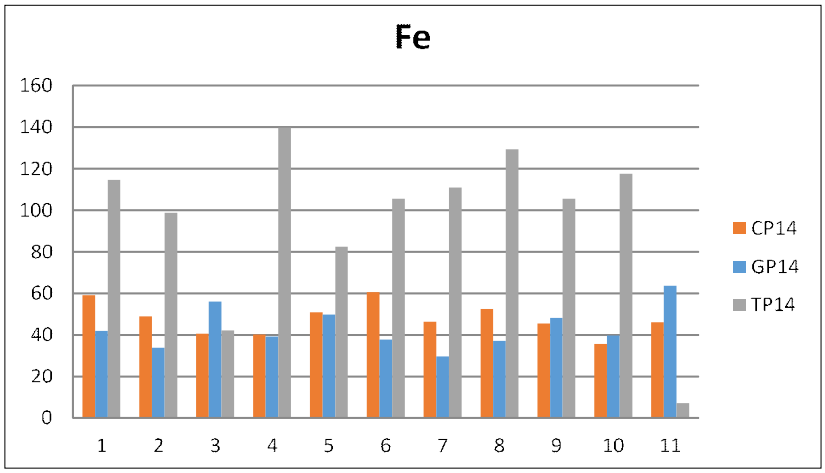

Fig 7a: The concentration of Fe in samples 2014.

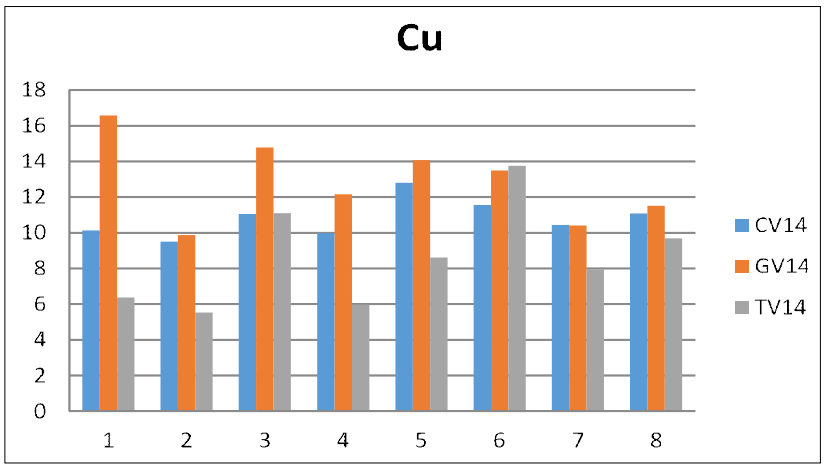

Fig 7b: The concentration of Cu in samples 2014.

over the three parts GTC (Fig 7b). For the concentration of P it is well distributed on C and G less in T (Fig 7c).

For samples P2015 (Fig 8a) note that the highest concentration of Fe is also on the T except for samples 3, 5 and 6.

For 2015 samples (Fig 8b) note that the Cu concentration is well distributed between G C and T for all samples.

For 2015 samples (Fig 8c) note that phosphorus is well distributed on the C and G more than T for all samples. In general, the concentration of micronutrients in the lentils is not homogeneous in the different parts of the seed that depends on the elements. So, in the example of the elements chosen we can notice that the highest concentration of Fe is on the T, Cu on all parts of the seed and P on the G and C.

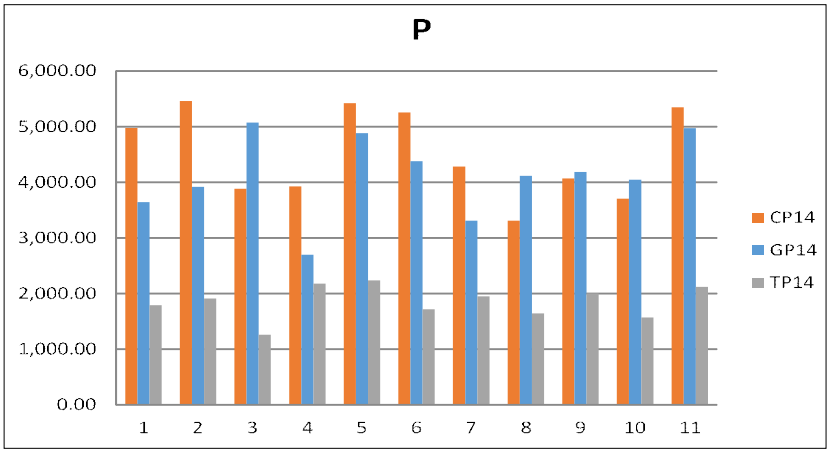

Fig 7c: The concentration of P in samples 2014.

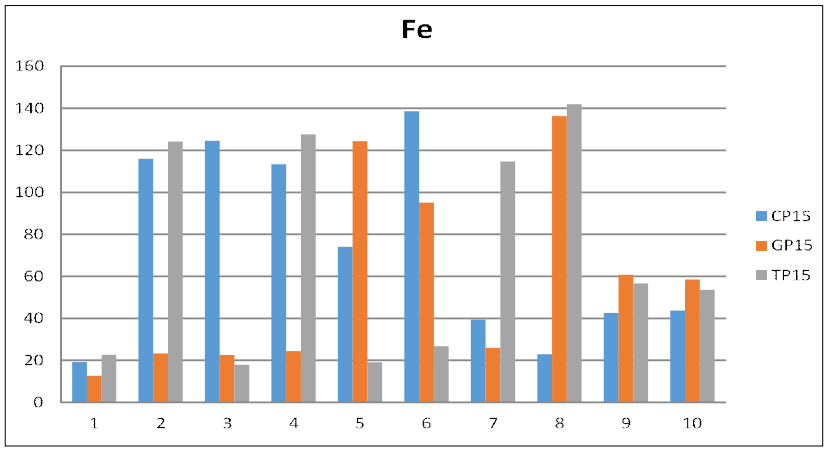

Fig 8a: The concentration of Fe in samples 2015.

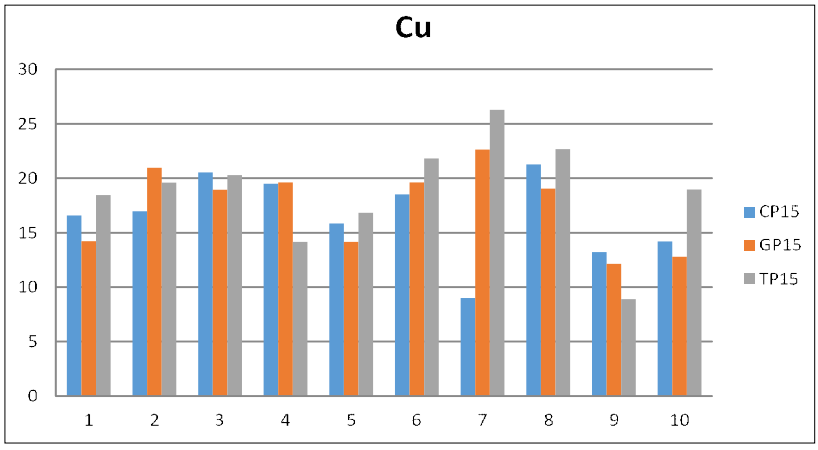

Fig 8b: The concentration of Cu in samples 2015.

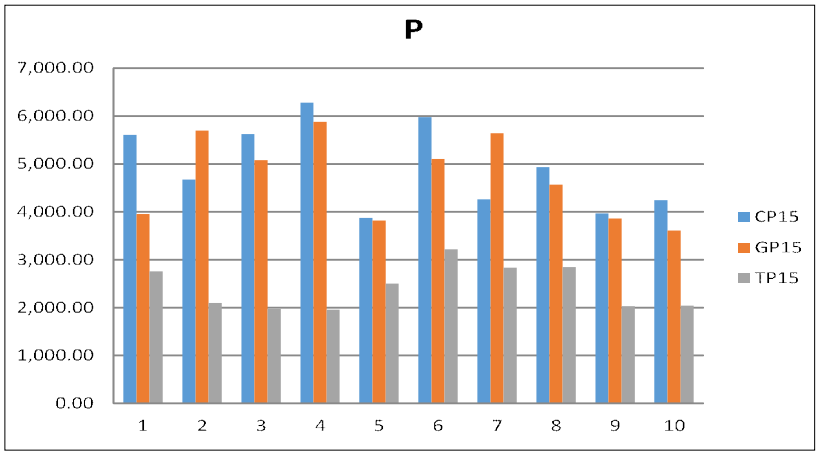

Fig 8c: The concentration of P in samples P 2015.

In a conclusion, if you want to use lentils as a fortifier in flours, it is more advisable to use the whole grain to take advantage of all trace elements.

CONCLUSION

Under the experimental conditions used, the mineral content of Moroccan lentils was high iron (5.03%-11.4%), calcium (6.81%-53.1%) and especially P K compared with lentil grown in Washington and Idaho (Vandemark *et al*., 2018), the results of the last two elements are not surprising knowing that Moroccan soils are very rich in P and K in addition to the use of fertilizers in crops.

Our study has also shown that we can use the whole lentil seed as an additive to flour to enrich it with minerals and avoid the problems caused by a mineral deficiency in the human body.

Our analysis of the data using PCA allowed us to identify among the lentil samples studied those with a better balance of concentration of trace elements so it can be recommended for future crops.

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Conflict of interest: None.

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