

Nutritional assessment of olive fruit (*Olea europaea*) available at local market

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Abstract

Since last few years, olive fruits consumption has been increased to many folds in the country. Shelves of superstores have been occupied by olive fruit products from different commercial presentations. Eight olive fruit samples of different cultivars were analyzed for physicochemical characterizations to assess their nutritional values. Water content was found higher in Jordanian Cipressino, French Atton Manzanilla, and Spanish Reding samples and lower in Egyptian sample. Several factors such as variety, maturation level, growing environment, mode of irrigation, and processing method may be responsible for this variation. Calorific values ranged from 3.87-7.32 Cal/g contributing significantly in daily energy intake. Olives were found to be a good source of K, Na, Ca, Mg, and Fe. Traces of Mn, Co, Ni, Cu, Zn, Pb and Cd were also found. Significant differences were observed for K ($p=0.016$), Na ($p=0.032$) Cu ($p=0.049$), and Mn ($p=0.047$) in green, ripe and brine olives. French Atton Manzanilla and Labanon Nevadillo were identified as good varieties in terms of Na/K, and Ca/Mg ratios. Chemometric technique was used to discriminate the samples between green, ripe, and brine on the basis of K, Na, Ca, and Mn.

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Introduction

The total table olive growth reached 2,315 million tonnes in the year 2012, according to the data provided by the international olive oil council. Olive is cultivated in various parts of the world and the Mediterranean region is accounted for 98% of global cultivation (Ryan and Robards, 1998). The use of freshly harvested olive fruit is not common due to the presence of oleuropein, bitterness-causing chemicals (Soler-Rivas *et al.*, 2000; Vinhaa *et al.*, 2002). There are two major consumptions of olive (*Olea europaea*), namely olive oil and table olives. As the awareness about the benefits of olive fruit is increasing, its consumption has increased significantly in both ways (Vinhaa *et al.*, 2002). Table olives are classified into following types based on harvesting time. (i) Green olives, gather during ripening (ii) olives turning color, harvest before the stage of complete ripeness, have rose, wine-rose or brown color and (iii) black olives, collected after full ripeness (Codex Stan, 66-1981).

In Pakistan, about 44 million wild olives (*Olea Cuspitate W.* and *Olea Ferruginea R.*) are grown in natural forests in different parts of the country. Most of these trees are used as source of the wood for manufacturing handcrafts and as fire wood (Cima and Urbano, 1988). Just few years back, Olive consumers were limited in the country. Only rich persons were used olive oil in cooking purpose. Now

numbers of foreign pizza outlets have been opened in all the big cities of the country, using olive fruit in their recipes. Now its taste has been developed among large numbers of pizza lovers. Today's, shelves of superstores are occupied with different olive products.

In 2008, some serious effort has been taken at the government level for the cultivation of edible olive trees in Pakistan. A detailed Geographic Information System (GIS) based feasibility reports prepared by the Italian institute of agriculture for the selection of suitable areas for olive cultivation in Pakistan (Cima and Urbano, 2008). There are three major recipes, excepted globally, for the preparation of table olives: Spanish for green olives, the Californian for oxidized black olives, and naturally black olives in brine and dry salt (Sahan *et al.*, 2007). Currently, olives are available in all three recipes in the local market. Unlike other fruits, the composition of olive not only depends on cultivar, soil chemistry, irrigation, ripeness, agro climate factors and harvesting regime, but also its commercial presentation styles (Proietti and Antognozzi, 1996; Fernandez *et al.*, 1997; Nergiz and Engez, 2000)

Trace metals and some heavy metals are essential to maintain some important metabolic functions of the human body. Minerals are also play a vital role in biological phenomena. However, at higher

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concentrations, they can lead to poisoning by interfering in crucial biochemical processes such as the Krebs cycle and steroid synthesis (Reijnders and Brasseur, 1997). Heavy metals also increase the production of free radicals by attacking neutral molecules.

The aims of the present work were: (i) to determine the Na, K, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Pb and Cd in the most popular brands of table olive, available locally (ii) to analysis some selected physico-chemical parameters of the olive samples (iii) to discriminate local market samples with literature values using a chemometric technique (iv) to assess the obtained data on nutritional and non-essential bases for the health of the consumers.

Materials and Methods

Sampling

Eight samples of brined olives were purchased from local superstores of the Karachi city, Pakistan. The fruits were washed and air dried to remove the materials adherent at the surface of samples. The samples were stored in a freezer at -4°C for subsequent analysis. The dimensions (length and diameter) of the samples were measured using hand-held micrometer. The uniformity of size was ensured by selecting 100 olives of the same variety having approximately equal size. From the batch, one of the smallest and one of the largest horizontal diameters were removed. Horizontal diameters of the remaining 98 olives were measured. The difference between the horizontal diameters of the remaining olives should not exceed to 4 mm, if the international standard is to be met. The lengths of the samples were measured using the same method. The color of the samples was assessed subjectively.

Proximate composition

Water content, volatile matter, and ash content were determined sequentially by thermogravimetric analyzer (TGA-2000A, Las Navas-Spain) using ASTM method D 5142-90. Nitrogen gas was set to flow at a constant rate in the determination of moisture and volatile matter. Oxygen was set to flow for ash content. To measure the water content of the fruit, approximately 1.00 g of the sample was heated at 105°C in an inert environment until it reaches to a constant weight. Volatile matter was determined by heating the sample at 950°C for 7 minutes in the nitrogen environment. For the measurement of ash content, the sample was heated at 850°C in the oxygen environment till a constant weight is obtained.

Gross calorific value

The gross calorific value (GCV) of olive samples was determined by burning about 1 g of a sample in an oxygen rich environment using isoperibol bomb calorimeter (Parr 6300, USA). The calorimeter was calibrated against the pellets of standard benzoic acid (Parr Calorimeter).

Microwave assisted metal analysis

The samples were digested in a CEM MDS-200 microwave digester (CEM, USA). Each sample was thoroughly washed with distilled water, dried at 25°C for 24 hours, and then at 70°C for another 24 hours. The samples were de-pitted by hand, and the flesh was cut into small portions using plastic scalpel. The scalpel was already rinsed with 15% HNO_3 and deionized water. Stones were removed from the flesh and 0.5 g of this sample was blended with 4.0 ml of nitric acid (69.5%) and 2.0 ml hydrogen peroxide (30%) The blend underwent the microwave digestion at 300W for 15 min, 600W for 10 min, 1200W for 15 min, and finally at 300W for 5 min. Each sample was heated to a maximum temperature of 200°C . Samples were allowed to cool to room temperature, filtered in a 25 ml volumetric flask, and the volume was brought up to mark with 1% Nitric acid. 1% nitric acid was also used as the reagent blank for further analysis. The aliquot of each sample was packed in PVC decontaminated containers and stored at 4°C .

Metal ions in the samples were analyzed using Perkin Elmer atomic absorption spectrophotometer (PE-2380). Hollow cathode lamps of the respective elements were used for their analysis in flame atomization mode. For each olive sample duplicate solutions were prepared while each solution was measured thrice for its metal ion concentration.

Chemometric analysis

Chemometric techniques have been proven to be effective in characterization and classification of different food items on the basis of their physicochemical Characteristics (Lopez *et al.*, 2008; Khan *et al.*, 2011; 2010; 2008). In single factor analysis of variance (ANOVA), the differences were considered statistically significant when $p < 0.05$. Principal component analysis (PCA) and all statistical measurements were performed using the XLSTAT function of Microsoft Excel.

Results and Discussion

Different physicochemical aspects were considered to assess the quality of olive samples such as color, size, calorific value, and metal content

Table 1. Physicochemical properties of table olives (mean±standard deviation)

Sample code	S1	S2	S3	S4	S5	S6	S7	S8
Olives	Egyptian	French Atton	Greek Black	Greek Black	Jordanian	Labanese	Labanon	Spanish
Variety	-	Manzanilla	Sevillano	Sevillano	Cipressino	Manzanilla	Nevadillo	Reding
Color	Green	Black	Dark Brown	Black	Green	Dark Brown	Dark Brown	Black
Weight (g)	9.66±0.11	3.67±0.13	4.48±0.13	5.82±0.12	2.90±0.21	2.37±0.13	2.11±0.14	2.68±0.24
Length (cm)	8.15±0.12	5.30±0.15	5.70±0.14	5.80±0.14	4.53±0.16	4.40±0.18	4.90±0.17	4.90±0.15
Diameter (cm)	2.75±0.10	2.20±0.08	2.25±0.09	3.15±0.04	2.10±0.16	2.23±0.11	2.20±0.12	6.20±0.05
Moisture (%)	13.92±0.92	57.11±0.41	37.37±0.43	31.13±0.56	61.88±0.58	30.02±0.45	27.40±0.49	49.86±0.48
Volatile Matter (%)	81.29±1.41	34.10±1.05	60.32±1.13	63.50±1.45	30.82±1.89	65.20±1.46	66.47±1.84	46.08±0.84
Ash (%)	4.68±0.18	7.44±0.28	3.56±0.27	4.44±0.42	3.39±0.24	4.14±0.15	5.76±0.16	3.27±0.24
GCV (Cal/g)	3.87±0.12	6.98±0.11	7.22±0.12	6.29±0.11	7.01±0.16	5.54±0.12	5.79±0.13	7.32±0.14
Ca (mg/l)	2354.30±2.45	713.00±2.41	1015.70±3.14	1619.70±2.56	3796.10±1.59	4143.50±1.55	2356.10±2.08	7872.60±2.45
Cu (mg/l)	3.90±0.04	4.38±0.05	4.81±0.03	3.78±0.02	4.19±0.02	3.96±0.02	3.73±0.03	4.69±0.28
Fe (mg/l)	32.89±0.35	37.20±0.28	165.14±0.64	29.09±0.51	173.40±0.84	81.43±0.34	66.91±0.29	289.96±1.45
K (mg/l)	1266.40±4.18	3849.70±3.45	4132.30±3.25	4136.1±4.150	4248.90±2.58	2716.00±2.49	6036.10±3.45	821.50±4.09
Mg (mg/l)	404.10±0.98	405.41±0.87	272.30±1.08	606.90±1.13	486.50±0.83	1101.70±0.94	1495.80±0.73	961.70±1.13
Mn (mg/l)	4.22±0.03	5.12±0.05	3.88±0.04	4.33±0.06	9.15±0.05	4.31±0.04	5.25±0.05	4.34±0.08
Na (mg/l)	1973.60±9.45	1099.50±8.45	2525.70±8.37	16872.00±9.48	6696.20±8.45	4694.30±8.40	2963.10±9.24	1643.10±9.99
Zn (mg/l)	1.45±0.03	5.21±0.03	3.54±0.04	0.96±0.02	4.76±0.05	3.20±0.03	5.47±0.05	4.51±0.04
Co (mg/l)	7.04±0.12	6.05±0.16	6.80±0.16	8.67±0.15	7.22±0.14	7.18±0.13	5.25±0.15	6.76±0.14
Ni (mg/l)	37.59±0.67	17.71±0.54	28.17±0.21	33.74±0.49	32.75±0.57	51.25±0.24	53.82±0.24	48.38±0.43
Pb (mg/l)	3.77±0.17	2.15±0.14	2.15±0.12	4.04±0.13	1.49±0.16	0.01±0.00	4.69±0.01	1.98±0.02
Cd (mg/l)	0.52±0.01	0.29±0.03	0.24±0.01	0.53±0.02	0.63±0.02	0.53±0.01	0.48±0.02	0.39±0.01

(Table 1 and 2). Egyptian and Jordanian Cipressino were identified as a distinct olive green hue showing Spanish-style of green-ripe olives. French Atton Manzanilla, Greek Sevillano, and Spanish Reding with uniform black color, represents the Californian/Spanish-style of black-ripe olives. In this style, oxidation of polyphenols in the skin and flesh occurred. The remaining species show brownish black color. The dark color shows processing by soaking in water or brine or spontaneous fermentation of the olives. Water content of flesh varied from 13.92-61.88 % w/w. Jordanian Cipressino, French Atton Manzanilla, and Spanish Reding were found to have higher water content (61.88, 57.11, and 49.86 %, respectively); whereas, a lower amount of water (13.92%) has been observed in Egyptian sample. Several factors may be responsible for this variation in the prevalence; such as variety, maturation level, a growing environment, mode of irrigation, and processing method (Nergiz and Engez, 2000; Kailis and Harris, 2007; Guillen, 1993).

A major fraction of the edible portion of the fruit comprises volatile matter (30.82-81.29 %), which contributes mainly into oil and protein components of the flesh. The volatile compounds of the fruit chiefly contain esters, alcohols, aldehydes, ketones, lactones, and terpenoids. They are also responsible for the characteristic aroma of fresh fruit. The phenolic volatiles of olives are responsible for the bitter taste

perception (Garcia-Gonzalez *et al.*, 2010). These compounds are multifunctional antioxidants and can act as oxidative enzyme inhibitor, metal chelators, and free radical scavengers. Therefore, the varieties of high volatiles (Egyptians, Greek Black Sevillano, Labanese Manzanilla, and Labanon Nevadillo) may play a beneficial role in human health.

The gross calorific value of the samples ranged as 3.87 - 7.32 Cal/g with an average of 6.25 Cal/g. The energy content of the fruit in terms of calories is almost double than fig fruit (average: 3.50 Cal/g) as studied previously (Khan *et al.*, 2011). Between 75% and 85% of the calorific value of table olives is predominantly monounsaturated fatty acid (oleic acid) which help to reduce levels of low-density lipoproteins (LDL), or "bad cholesterol", preventing harmful arterial plaque forming on artery walls (Kailis and Harris, 2007). Therefore, the use of table olive in a daily diet not only provides a good source of energy, but it is also helpful to prevent coronary diseases.

Ash content in an olive flesh is an indication of the level of inorganics. It was varied from 3.27-7.44%. These values are comparable with those reported by other researchers (Sahan *et al.*, 2007; Lopez *et al.*, 2008). The levels of 12 metals, both macro-, and micro-, in processed olive flesh are presented in Table 1 and 2. The levels for each olive variety are of similar order except iron and calcium. The iron

Table 2. Metal contents (mg/l) of olive samples in reported data

Type	Sample code	Cu	Fe	Mn	Zn	Ca	Mg	Na	K	Co	Ni	Pb	Cd	Ref.
Brine	G	5.09	7.70	0.24	3.20	379.00	119.00	12675.00	1176.00	ND	ND	ND	ND	11
	M	5.22	5.51	0.554	1.98	447.00	143.40	11490.00	750.00	ND	ND	ND	ND	11
	H	3.99	7.62	0.92	2.90	337.00	56.10	13797.00	684.00	ND	ND	ND	ND	11
	Ar	10.93	4.09	0.29	1.55	613.00	67.70	13152.00	571.00	ND	ND	ND	ND	11
	Al	4.68	5.73	1.04	2.03	691.00	53.80	16600.00	902.00	ND	ND	ND	ND	11
	Vr	4.353	4.90	1.093	2.94	684.70	197.30	16020.00	766.00	ND	ND	ND	ND	11
Green	G	3.33	3.88	0.61	1.82	476.00	133.10	17221.00	538.80	ND	ND	ND	ND	11
	M	1.73	5.40	0.45	2.52	589.00	98.60	15057.00	333.00	ND	ND	ND	ND	11
	Cr	3.60	3.49	0.539	1.52	709.00	103.20	18144.00	393.00	ND	ND	ND	ND	11
	H	2.04	5.23	0.53	2.13	850.00	146.70	14378.00	444.00	ND	ND	ND	ND	11
	Gr.	0.78	7.08	ND	10.58	ND	37.64	ND	ND	ND	ND	ND	ND	6
Ripe	G	4.72	68.50	0.72	2.55	362.70	51.70	5706.00	81.70	ND	ND	ND	ND	11
	M	3.95	131.50	1.28	3.60	579.00	109.70	9386.00	190.00	ND	ND	ND	ND	11
	Cr	3.26	58.30	0.87	2.58	527.00	72.30	6750.00	176.00	ND	ND	ND	ND	11
	H	3.47	66.70	1.539	3.25	731.00	98.00	7194.00	223.00	ND	ND	ND	ND	11
	Cc	2.40	84.00	0.953	3.18	580.00	73.90	7964.00	168.80	ND	ND	ND	ND	11
	Ripe Bl.	1.48	12.65	ND	8.50	ND	79.28	ND	ND	ND	ND	ND	ND	6

ND = not detected

TS = this study

level in Spanish Reding was found markedly higher (289.96 mg/l) than others showing that the table olive is treated with ferrous gluconate or ferrous lactate to stabilize its black color. Such a higher level may contribute to daily iron requirements. It could be of particular benefit to women, vegetarians, and vegans when consumed. It was also noted that calcium, magnesium, and potassium levels were found to be higher in almost all the samples compared to the reported data (Sahan *et al.*, 2007; Kailis and Harris, 2007; Lopez *et al.*, 2008). The minerals of these metals seem to be leached from the soil to the olives. Potassium was identified as the most abundant among the elements quantified, in agreement with the values reported for other fruits (Khan *et al.*, 2008; 2010; 2011). Other metals were maintained at similar levels as reported in other studies (Sahan *et al.*, 2007; Lopez *et al.*, 2008). A large variation in sodium level (1099.5 - 18144.0 mg/l) was observed in the studied samples (Table 1) and the reported data (Table 2). It varied with the strength of sodium chloride used during processing. The maximum recommended daily intake (RDI) of sodium is around 2.3 g/25 g serve of olives. For a 25 g of commercially available table olives in Pakistan, the sodium content is of the following order of magnitude: Greek Sevillano gives 18.33% of the RDI; Jordanian Cipressino gives 7.28% of the RDI, Labanese Manzanilla give 5.10%

of the RDI, and the remaining olives give <5% of the RDI. Consumers that are sensitive for their salt intake should avoid eating large quantities of table olive products where there are more than 1000 mg of sodium per 100 g of an edible portion specified on the label. The levels for other trace metals (Co, Ni, Pb, and Cd) were of the same order of magnitude for all samples and were found within the permissible limits.

Statistical analysis

ANOVA-single factor analysis was applied on the results of metal content of our samples (Table 1) and the reported data (Table 2) to compare the significant differences in different varieties. Strong significant differences were observed for K ($p=0.016$), and Na ($p=0.032$) in green, ripe and brine olive samples. Weak significant differences were observed for Cu ($p=0.049$), and Mn ($p=0.047$). No significant differences were observed for Ca ($p=0.215$), Fe ($p=0.24$), Mg ($p=0.090$), and Zn ($p=0.267$) in green, ripe and brine samples.

Figure 1 represents PCA applied on the metal contents in the studied samples, and the metal contents present in the samples of reported data (Table 2). Eigen values of the three significant principal components (PC1, PC2, and PC3) were estimated as 4.36, 1.05, and 1.02 respectively (Figure 1(a)). Table

Table 3. Component matrix for metals

Variable	PC1	PC2	PC3
Cu	-0.020	0.159	0.968
Fe	0.366	0.538	-0.070
Mn	0.422	-0.240	0.050
Zn	0.367	0.024	-0.166
Ca	0.374	0.341	0.036
Mg	0.397	-0.254	0.049
Na	-0.369	-0.237	0.008
K	0.345	-0.625	0.157

3 shows that 54.49 % of the total variability for PC1 is predominantly a function of Fe, Mn, Zn, Ca, Mg, Na, and K. The second component (PC2) explained 13.07 % of the total variability and 39.05 % of its total is dependent on K. The third component (PC3) shows 12.74 % variation; 93.67% of which is dominated by Cu only. In Figure 1(b), distinct separations were observed for the samples of fresh green, ripe, and brine olives. It is important to note that the samples studied in the present work are placed in a wide area. Since these samples belong to the different regions of the world; therefore, a number of reasons may be proposed for this behavior. These factors are a type of seed, mode of cultivation, soil chemistry of the particular region, mode of preservation etc.

The pattern of each sample in the score plot was synchronized with the variable loadings. The loading plot showed enrichment of particular samples with particular metals. Na was identified as the dominant metal in brine and green samples. The values of Cu were found to similar in ripe samples. These samples also showed a low amount of K, Mg, and Mn. The loading plot also shows an inverse correlation of Cu with K, Mg, and Mn. Similarly Na was inversely correlated with Ca and Fe.

One way normal analysis of means (ANOM) was applied on the same data to identify the variables contributing to discrimination (Figure 2). It was found that Na contributes mainly in discrimination, while a significant contribution of Cu, Fe, Mg, Mn, and Zn were also observed in the same. No significant difference ($p < 0.05$) in the composition of olives was observed for Ca, and K. In the body, the ratio of Na (in the extracellular fluid) to K (in an intracellular fluid) is about 0.67 (Bender, 2009). A diet containing a low Na/K ratio may help to reduce high blood pressure and risk of stroke. It is important because an imbalance causes several chronic diseases, including hypertension and osteoporosis. In the studied samples, Na/K ratios were calculated as 1.55, 0.28, 0.61, 0.40, 1.57, 1.72, 0.49, and 2.00 for samples S1 to S8, respectively. Samples S1, S5, S6, and S8 are

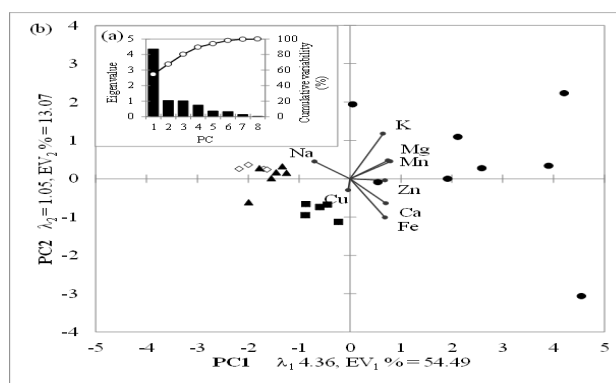


Figure1. Statistical analysis for Cu, Fe, Mn, Zn, Ca, Mg, Na, and K in olive samples

(a) Scree plot (b) Biplot of PCA ($\lambda_1, \lambda_2,$ and λ_3 are Eigenvalues and $EV_1, EV_2,$ and EV_3 explained variance %)
Olive samples: \diamond Green, \blacksquare Ripe, \blacktriangle Brine, \bullet Our samples

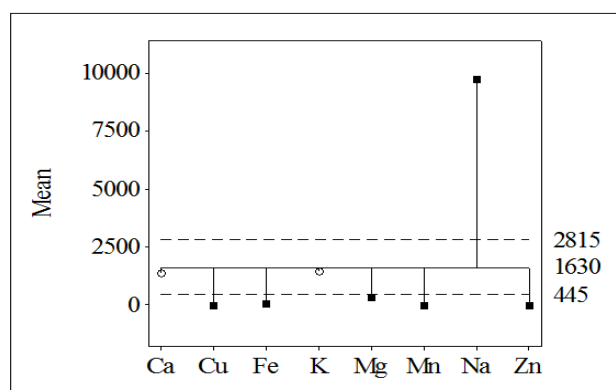


Figure 2. One-way ANOM plot for metals at $\alpha = 0.05$

rich in Na concentration may be due to the use of high strength brine during processing. If Na concentration increases in the body causes hypernatremia, while elevated K concentration causes hyperkalemia. Along with the Na/K ratio, the Ca/Mg ratio is another important ratio influence on human health. The Ca and Mg nutrients in balance adequacy are necessary for intercellular activities and physiological functions of the body (Rosanoff, 2010). The recommended total dietary Ca/Mg ratio is 2.0 (Durlach, 1989). It was estimated as 5.826, 1.758, 3.730, 2.668, 7.802, 3.743, 1.575, and 8.186 for samples S1 to S8, respectively. The lower Ca/Mg ratio in the samples of French Atton Manzanilla (S2) and Labanon Nevadillo (S7) may serve to protect against the development of type 2 diabetes mellitus (Resnick, 1992). The remaining samples (S1, S3, S4, S5, S6, and S8) are associated with a substantially higher risk of colorectal adenoma, the colon cancer ($Ca/Mg > 2.76$) (Dai *et al.*, 2007). Individuals using these varieties are suggested to use more vegetables, legumes, whole grains and nuts to raise their Mg adequacy and lower their dietary Ca/Mg ratio (Rosanoff, 2010).

Conclusion

Table olives, available in local market are a good source of energy and metal content especially potassium, iron and calcium. Sodium content of the fruit depends strongly on a mode of preservation and it may be detrimental, particularly in those who should be on a low-salt diet. The comparison between the compositions of green, ripe, and brine fruit shows significant differences between the content of K, Na, Cu, and Mn. French Atton Manzanilla and Labanon Nevadillo varieties were good in terms of Na/K, and Ca/Mg ratios.

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