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Elemental Composition of Various Mulberry Species

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A comparative study on the elemental composition of various mulberry fruits and leaves was conducted with wavelength dispersive X-ray fluorescence (WDXRF). The element compositions of P, K, Ca, Mg, Na, Al, Si, S, Cl, Cr, Fe, Mn, Sr and Zn were determined in white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry leaves and fruits. Significant differences ($p < 0.05$ or $p < 0.01$) were observed in the mineral contents of mulberry leaves and fruits, depending on the species. The concentrations of elements in white, red and black mulberry leaves were 4.60, 4.53 and 4.61 %, respectively of the total mass and the quantities of the elements were 2.13, 2.0 and 2.23 % of the total mass. Among the various macronutrients in the mulberry species, K was present in the highest quantity for fruits samples, whereas Ca was predominant in the leaves. However, another result found with this study shows that the main advantage of this method is the non-modification of original sample, both during sample preparation and in the exciting X-ray beam. Therefore, this method might easily be used in the diagnosis of the nutrient status of fruit trees.

Key Words: Mulberry species, Elemental composition, WDXRF analyses.

INTRODUCTION

Although all plants require the same minerals to complete their life cycles, the quantities and balances necessary for optimum growth and production of high yields of quality produce vary greatly among species. For example, the nutrition of large woody trees, including fruit trees, differs in many ways from that of large herbaceous plants.

Three approaches are possible in the diagnosis of the nutrient status of fruit trees: (a) visual symptoms of deficiency or excess can be used to identify acute nutritional problems. However, symptoms vary from crop to crop and if more than one element is deficient, visual diagnosis may not work; (b) soil analysis can help to point out possible problem areas (related to pH and salinity), but it is not easy to use this method as a predictor for woody perennials; (c) plants analysis is a powerful diagnostic tool for agronomists, as it allows one to define the species of plants, their adherence type, state of growth, food value, nutrient deficiency and resistance to disease[1].

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Generally, elements in plants are determined by using methods such as AAS, ICP-MS and ICP-AES. These techniques have a prerequisite for the total destruction of the matrix by mineralization, which may lead to problems of contamination by reactants such as HCl and H_2SO_4 or disturbances of the measured concentrations by element losses due to incomplete solubilization. Moreover, the method of matrix destruction used strongly depends on the chemical composition of the sample and on the element to be determined[2].

The wavelength dispersive X-ray fluorescence (WDXRF) method offers several unique advantages over other analytical methods. Both the sample preparation and measurements are usually simple and fast. The sensitivity of this technique is good and compares to that provided by optical-based techniques, such as ICP-MS and ICP-AES. The sample preparation does not require any chemical treatment, drastically reducing any chance of contamination[3].

The mulberry belongs to the Urticales order, the Moraceae family and the genus *Morus*, with more than 30 species and 300 varieties. It comes from China and other species originate in other temperate countries and despite this, they can be considered "cosmopolitan" for their capacity of adaptation to different climates and altitudes[4]. There are three main species of mulberry: black (*M. nigra*), red (*M. rubra*) and white (*M. alba*).

In many mulberry-growing countries, in particular in India and China, mulberries are used for foliage, whereas in most European countries, including Turkey and Greece, mulberries are grown for fruit production[5]. Turkey is one of the major mulberry producers in the world with an approximate annual yield of 80,000 ton and most of this comes from the Erzincan region located in the eastern part of the country[6].

Mulberries are a good source of vitamins and minerals and contain an especially high amount of anthocyanin[7]. There have been studies on the flavor, dry matter, total sugar, total acidity, ash, ascorbic acid, pH and anthocyanin content of some mulberry species[8-10]. Recently, the mulberry has gained an important position in the local soft drink market, although its biological and pharmacological effects are still poorly defined. There exists only one reports on the mineral content of mulberry fruits[5]. Moreover, to our best of knowledge, there have been no comparative studies on the elemental composition of mulberry fruits and leaves, which stimulated this research. The aim of the present work is to investigate the relationship between the mineral contents of white, red and black mulberry fruits and leaves by using a sensitive method wavelength dispersive X-ray fluorescence (WDXRF).

EXPERIMENTAL

Collection and preparation of fruit and leaf samples: White (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits were collected at their optimum commercial maturity from the same region in the Uzumlu district, Erzincan. The mulberry trees in this region were subjected to chemical fertilizers or pesticides, therefore all of the produce was organic. Fruit samples were kept in cool bags for transport to the laboratory and washed in distilled water. For elemental composition measurements, *ca.* 50 g of the fruit was homogenized on ice using a blender. Whatman No. 1 filter paper was used to filter the sample. Mulberry leaf samples were taken from the middle of current season shoots of each tree and dried in the shade. They were then cut and ground with a blender mixer.

For the measurement of fruit samples, the cellulose paper discs (2 cm diameter; Rigaku) were impregnated with 100 µL of sample and dried at room temperature for 15 h. The powdered leaf samples were transferred onto a polyethylene mylar film. To obtain an XRF-mylar film, a small metallic sample holder made of aluminum with a diameter of about 3 cm was used. For each sample, the cell was filled with 300 ± 5 mg of leaf powder to allow for measurements under maximum vacuum conditions, which are important for light element analysis. This method of preparation is easy and rapid. Next, the samples were placed in a target holder inside the chamber for analysis. The measurements were performed in five replicates.

Determination of elemental composition: The measurements were carried out with a wavelength-dispersive spectrometer (Rigaku ZSX-100e with Rhodium target X-ray controlled by a software ZSX computer). The ZSX 100e, with tube above optics, offers the most flexibility and highest performance of any WDXRF spectrometer and has a 4 kW 70 kV and window X-ray tube, up to 5 primary beam filters, 10 analyzing crystals, 8 limiting area diagrams, optional secondary collimators and an automatic sample changer.

WDXRF spectrometry was used for the analysis of the semiqualitative method. The measurements were automatically corrected for all matrix effects, including line overlaps by the semiquantitative software package, SQX (Rigaku). SQX can also correct for the secondary excitation effect by photoelectrons, varying atmospheres, impurities and different sample sizes. Increased accuracy is achieved using the Matching Library and Perfect Scan analysis programs.

Statistical analyses: Statistical analyses were performed using SPSS version 13.0. To identify significant differences, the data were analyzed by bivariate correlation and analyses of variance using the general linear model, which permits the analysis of the sample with different numbers of replicates. The mean values were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Leaf analyses: The nutrients in plants and their roles are influenced by the intensity of cation exchange, growing conditions, moisture, pH value and other factors. Therefore soil analyses are not sufficient for a picture of the state of nutrition, whereas the leaves quickly provide information on the macro- and micro-elements.

The elemental composition and concentration of some mulberry leaves were evaluated by wavelength-dispersive X-ray fluorescence (WDXRF) analyses. Elements such as P, K, Ca, Mg, Na, Al, Si, S, Cl, Cr, Fe, Mn, Sr and Zn were determined in leaf samples. The limit of detection and the mean recovery of the elemental concentrations varied with the atomic number. The average concentrations found for different elements in the leaves are listed in Tables 1 and 2.

TABLE-1
MACRO ELEMENT CONTENTS OF MULBERRY LEAVES (ppm)†

Fruit species	P	K	Ca	Mg
White mulberry	2144.1 ± 40.2c	16018.7 ± 375.3a	20171.1 ± 338.1a	5020.4 ± 312.1b
Red mulberry	2276.7 ± 33.4b	15892.7 ± 354.2b	18530.7 ± 225.6c	5632.5 ± 298.7a
Black mulberry	2540.8 ± 39.3a	15902.1 ± 213.0b	19041.3 ± 309.3b	5192.9 ± 324.3b
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

*: Within columns, means followed by the same letters are not significantly different.
†: Results in units of per million (ppm).

TABLE-2
MICRO ELEMENTS AND HEAVY METAL CONTENTS OF LEAF SAMPLES (ppm)†

Fruit species	Na	Al	Si	S	Cl	Cr	Fe	Mn	Sr	Zn
White mulberry	205.4 ± 14.1a*	238.3 ± 13.3b	1090.2 ± 13.9b	513.3 ± 9.7b	515.7 ± 10.2c	1.2 ± 0.1	58.7 ± 5.7b	28.4 ± 3.3b	12.2 ± 1.9b	22.1 ± 6.6b
Red mulberry	158.3 ± 11.7b	247.5 ± 9.8a	1256.4 ± 15.7a	619.8 ± 9.5a	620.4 ± 9.1b	2.0 ± 0.2	72.5 ± 9.6a	32.1 ± 2.7a	18.3 ± 4.8a	27.9 ± 3.5a
Black mulberry	170.9 ± 7.9c	253.1 ± 11.2a	1362.2 ± 17.2a	704.5 ± 12.5a	780.6 ± 9.9a	2.5 ± 0.3	77.7 ± 6.7a	32.2 ± 1.7a	11.5 ± 2.6b	30.4 ± 5.7a
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p > 0.05$	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.01$

*: Within columns, means followed by the same letters are not significantly different.
†: Results, in units of per million (ppm).

The mean concentrations of elements; except Cr, in various mulberry leaves were significantly different ($p < 0.05$ or $p < 0.01$) depending on the species. The concentrations of elements in white, red and black mulberry leaves were 4.60, 4.53 and 4.61 of the total mass, respectively. According to the results shown in Table-1, the amount of the major elements Ca, K, Mg and P varied from 2144-20171 ppm, approximately. Indeed, it has been observed that these elements are present in large amounts in mulberry leaves. Potassium is necessary for the formation of sugars, starches, carbohydrates, protein synthesis and cell division in all parts of the plants. Calcium is a structural component of cell walls, activates enzymes, influences water movement in cell and is necessary for cell growth and division. Some plants also need Ca to take up nitrogen and other minerals[11].

The contents of K and Ca were high in white mulberry leaves, followed by black mulberry leaves. The highest Mg content in leaves was detected in red mulberry leaves (5632 ppm), whereas the highest P content was measured in black mulberry leaves (2540 ppm). Magnesium is a critical structural component of the chlorophyll molecule and is necessary for the functioning of plant enzymes in the production of carbohydrates, sugars and fats. Phosphorus is also necessary for photosynthesis, protein formation and almost all aspects of growth and metabolism in plants 11. In a previous study conducted on mulberry leaves, the highest content was Ca (2.98 %) followed by K (2.84 %), P (0.44 %) and Mg (0.43 %)[12].

The significant levels of Si, Al, Cl, S and Na were also observed in mulberry leaves ranging from 158-1362 ppm. The levels of Si, Al, Cl and S in black mulberry leaves measured by WDXRF were higher than in red and white mulberry leaves.

The concentration of other elements, such as Cr, Fe, Mn, Sr and Zn ranged from 1.2-77.7 ppm. The trace elements concentrations were found in lower levels than the other elements concentrations measured in this study. Although statistically significant differences were found in above mentioned element concentrations among the species the concentrations of these elements in the species were similar.

Fruit analyses: Tissue analysis is the most useful technique both for the diagnosis of specific nutritional problems and for monitoring the success of fertilizer programs. Leaves are the most commonly used tissue for analysis but because of the peculiarities of the distribution of certain elements within the plant, feeding roots, petioles, fruit or certain fruit parts may be used for some elements.

The mean concentration of each element present in mulberry fruits is given in Tables 3 and 4. Statistically significant differences among the mulberry fruits were observed for the mineral compositions, except Cr. The concentration of elements in white, red and black mulberry fruits were, 2.13, 2.0 and 2.23 % of the total mass, respectively. Potassium was dominant, followed by Ca, P, Mg, S, Si, Na, Cl, Al, Fe, Zn, Mn, Sr and Cr. According to the results shown in Table-3, K, Ca, P and Mg were present in the largest amounts in mulberry fruits. The Ca concentration of about 3510 ppm is higher than the results presented by Ercisli and Orhan[5]. The significant levels of S, Si, Cl, Al and Na range from 110-603 ppm whereas the concentrations of other elements, such as Cr, Fe, Mn, Sr and Zn, were between 1-44 ppm (Table-4).

TABLE-3
MACRO-ELEMENT CONTENTS OF MULBERRY FRUITS (PPM)†

Fruit species	P	K	Ca	Mg
White mulberry	1987.1 ± 20.2c	14205.7 ± 355.3a	3012.3 ± 98.1c	950.4 ± 82.1b
Red mulberry	2044.7 ± 25.4b	11806.7 ± 254.2c	3547.2 ± 50.6b	1105.5 ± 98.3a
Black mulberry	2292.8 ± 41.3a	13253.1 ± 313.0b	3970.6 ± 62.3a	1170.9 ± 99.3a
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

*: Within columns, means followed by the same letters are not significantly different.
†: Results, in units of per million (ppm).

The concentrations of the elements in fruits were evaluated considering the standard nutrient values for mulberry fruit reported by the USDA National Nutrient Database for Standard Reference[13]. The concentrations of Ca, K, P and Mg were higher levels in comparison to standard values. The concentrations of other elements in fruits were found to be within at normal ranges.

The recommended dietary allowance (RDA) values of the macro-elements Ca, K, Mg and P are 1200, 4700, 420 and 700 mg daily[13]. Mulberry fruits could nutritionally be important as a good source of these elements.

TABLE-4
MICRO ELEMENTS AND HEAVY METAL CONTENTS OF FRUIT SAMPLES (ppm)†

Fruit species	Na	Al	Si	S	Cl	Cr	Fe	Mn	Sr	Zn
White mulberry	198.8 ± 12.0a*	44.7 ± 6.3c	338.2 ± 13.9b	327.3 ± 14.7c	210.5 ± 9.2a	0.80 ± 0.10	34.7 ± 6.7b	17.4 ± 2.3b	8.5 ± 2.2b	18.1 ± 2.9b
Red mulberry	150.3 ± 9.5b	110.1 ± 8.8b	470.3 ± 11.7a	499.8 ± 9.1b	193.4 ± 7.1b	1.10 ± 0.40	41.7 ± 3.6a	22.4 ± 3.7a	17 ± 4.1a	32.2 ± 4.2a
Black mulberry	201.9 ± 2.0a	147.3 ± 10.2a	480.2 ± 9.2a	603.5 ± 14.5a	160.6 ± 8.9c	1.20 ± 0.50	44.5 ± 8.7a	26.0 ± 4.0a	9.2 ± 1.6b	36.3 ± 7.1a
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p > 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

*: Within columns, means followed by the same letters are not significantly different.
†: Results, in units of per million (ppm).

The trace elements Mn, Fe and Zn have RDA values of 2-5, 10-15 and 12-15 mg daily, respectively and were determined in the fruit to be 21.9, 40.3 and 28.8 ppm in fruits. The amounts of these elements, although higher than the RDA value, are well below the toxicity level. However, high amounts of certain minerals such as Co, Ni and Pb, which are toxic for most organisms, were found to be below the detection limits.

When the results of the current study were compared with the results of earlier works on strawberries[14] and grapes[15], mulberries appear to contain similar levels of the minerals studied, except Ca. A lower Ca content was found in mulberry fruits when compared to grapes and strawberries. The mineral content of the fruit is sufficient to provide much of the human daily requirements.

The distribution of the elements among the leaves and fruits is given in Table-5. The concentrations of all the elements determined in the leaves were remarkably higher than in the fruits, except Na, Sr and Cr. These differences also proved to be statistically significant, except Zn. Zinc concentration was higher in fruits than in leaves but did not largely differ.

The annual uptakes of Ca and K were similar and much greater than that of phosphorus. There was little of the total tree Ca in fruit (2 %), but more was found in the wood (44 %) and leaves (51 %). Potassium was almost equally divided between the fruit and leaves, with only 13 % in the wood[16].

In fact, according to a Pearson's correlation test, there is a statistically significant correlation (at least, $r = 0.78$) between the concentrations of elements in mulberry fruits and leaves in this study (Table-5).

Conclusion

In conclusion, many factors affect the composition of plants, including the variety, state of ripening, soil type and condition, irrigation and fertilization and other factors. The mulberry trees from which the sample fruits and leaves were collected have been naturally maintained and no fertilizer or any other agricultural support was used. In this respect, the results represent the natural environment of the eastern region of Turkey. Tissue analyses performed by WDXRF provided data on a wide spectrum of elements. The concentrations of 14 elements (P, K, Ca, Mg, Na, Al, Si, S, Cl, Cr, Fe, Mn, Sr and Zn) were determined in mulberry leaves and fruits. Among the various macronutrients estimated in mulberry plants, Ca was

TABLE-5
MINERAL ELEMENT CONCENTRATIONS OF
MULBERRY LEAVES AND FRUITS (ppm)

Species	P	K	Ca	Mg	Na	Al	Si
Fruit	2108.2	13088.5	3510.03	1075.6	183.6	100.7	429.5
Leaf	2320.5	15937.8	19247.70	5281.9	178.2	246.3	1236.2
P	p < 0.05	p < 0.01	p < 0.00	p < 0.00	p > 0.05	p < 0.00	p < 0.00
Correlation coefficients (r)	0.988**	0.842*	0.263	0.780*	0.642	0.959**	-0.418
Species	**S**	**Cl**	**Mn**	**Fe**	**Zn**	**Sr**	**Cr**
Fruit	476.8	188.1	21.9	40.3	28.8	11.56	1.03
Leaf	612.5	638.9	30.9	69.6	26.8	14.00	1.90
P	p < 0.00	p < 0.00	p < 0.01	p < 0.00	p > 0.05	p > 0.05	p > 0.05
Correlation coefficients (r)	0.990**	-0.920**	0.790*	0.992**	0.896*	0.979**	0.988**

**: Significant at 0.01, *: Significant at 0.05.

present in the highest quantity, followed by K, Mg and P. Micronutrients such as Fe, Zn, Mn and Cr in mulberry fruits were found below their toxicity level, which suggests that the mulberry is at safe fruit low in these toxic elements. Another result obtained in this study show that the proposed method is accurate and reproducible and presents several advantages for mineral elements analysis in fruit samples. It allows for the determination of some elements without any mineralization, which may lead to problems of contamination by reactants. In addition, only a small amount of plant parts are needed for preparing the sample and both the sample preparation and measurements are usually simple and fast. The methodology used in this study, can easily be used in the diagnosis of the nutrient status of fruit trees.

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