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Original Article

Multidimensional scaling (MDS) to visual representation of proximities for quality and phytochemical characteristics in *Vitis vinifera* L. cv. 'Ercis'

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Summary. This study was conducted to determine quality and phytochemical characteristics of 'Ercis' cultivar that is indigenous to Van province for wine and must as well as table grapes due to specific aroma. In this framework, physical (color, cluster weight (g), berry length (mm), berry width (mm) and berry weight (g)), chemical (pH, titratable acidity (TA%), total soluble solids (TSS%), maturation index (MI), sugars, organic acids, total antioxidant activity, macro and micro elements) and phytochemical characteristics (phenolic compounds) of the cultivar were examined. Paired sample t test was performed to determine differences between years. In addition, multidimensional scaling was utilized to indicate visual representation of proximities for the characteristics. Differences between years for cluster weight, berry length, berry width and berry weight were found statistically significant, however, there is no significant differences for other characteristics. In addition, some negative and positive correlations were observed among the physical, chemical and phytochemical characteristics.

Key words: multidimensional scaling (MDS), quality characteristics, phytochemical characteristics

Introduction

Substances which are existed naturally in plant foods, vegetables, fruits, cereals and legumes are called phytochemicals. The basic of a healthy diet is to convenient vitamins, minerals, phytochemicals and fiber consumption. Except from macronutrients called carbohydrates, fats and proteins and 13 vitamins as well as 17 minerals which are necessary for health, the importance of phytochemicals has been realized recently. Unlike vitamins and minerals, phytochemicals cannot to be accepted as food. Today, it has been known that phytochemicals have antioxidant properties for being innocuous of molecules which are called free radicals and attack to cells. These compounds are effective for preventing of cancer, heart diseases, arteriosclerosis, diabetes and weakening of the immune system as well as potential difficulties and problems. Onions, garlic, leeks, cabbage, cauliflower, broccoli, soybeans, tomatoes, grapes, citrus fruits, carrots, nuts, grains, green tea, olives, beans, peas and cherry are the main source of phytochemicals. As compared to other plant species, grapes come forward due to extensive using as fresh fruit or processed products.

Some physical and chemical changes occur in internal and external structure of the grape berries from verasion period. In addition to these changes, the total soluble solids content increases and titratable acidity ratio decreases during berry maturating period (1).

Basically, grape consists of organic acids, sugars, anthocyanins, tannins, flavoring agents, pectic substances, nitrogenous substances, minerals, enzymes and vitamins.

'Ercis' grape cultivar is local varieties of Van province and has grown the most widely in eastern regions of Turkey about 3000 years. Historical records indi-

cated that 'Ercis' grape cultivar had been grown in Medes, Persians, Romans, Byzantines, Armenians, Arabs, Seljuks, Karakoyunlus and especially, the ancient civilization of Urartu. The purpose of this study is to determine quality and phytochemical characteristics of 'Ercis' grape cultivar and point out to this cultivar in terms of health. In addition, this study also aims to use multidimensional scaling for visual representation of proximities among the characteristics.

Materials and Methods

Plant material

'Ercis' grape cultivar was used as materials of this study. Grape samples with 5 replications were collected at the full maturity period. Ripening of the grapes in the vineyard was determined with amount of soluble solids by measuring the digital refractometer. Twenty grapevines which can represent the vineyard were identified and clusters were taken from these grapevines. Each cluster was sampled from different heights of grapevines by changing of direction and considering of randomization. Cluster samples were separated from berries at room temperature, placed in polyethylene bags and stored at -20°C until related analysis.

Chemicals

In this study, chemicals with analytical purity were used. Phenolics standards (Denise Folin solution, gallic acid, catechin, caffeic acid, chlorogenic acid, *p*-coumaric acid, vanillic acid and rutin), organic acid standards (tartaric acid, citric acid, malic acid, succinic acid and fumaric acid), and sugar standards (glucose and fructose) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Physical analysis

In the laboratory, the following variables of 20 randomly sampled clusters were analyzed: cluster weight, berry length, width and weight. The color of the berries was measured with a colorimeter (CR-400, Minolta Inc., Ramsey, NJ, USA). The color values were recorded as L*, a*, b*, C* and h° (2).

Chemical analysis

The samples were analyzed according to Office International de la Vigne et du Vin (3) procedures for pH, titratable acidity (TA%), and total soluble solids (TSS%). The maturation index (MI) was obtained from the TSS/TA ratio.

Determination of fructose and glucose levels

Sugars were determined by modified methods of Torije *et al*. (4) and Karkacier *et al*. (5). Whole berries were crushed and ground with a hand blender and made into a mesh. 3 g grape samples were ground into mortar and pestle with 25 mL of methanol (80%). The mixture was homogenized in an Ultra Tissue Lysis (Ultrasonic Processor, Jenway Ltd. UK) and incubated in magnetic stirrer at 65°C for 30 min. Then, it was centrifuged at 2000 rpm for 15 min. Methanol was removed by rotary evaporator and the residue was dissolved in 5 mL double distilled water. Extracts were passed through Sep-Pack C18 cartridge. Samples were injected directly into High performance Liquid Chromatography (HPLC). Sugars were determined as three replications by using an HPLC (Hewlett Packard Series 1525, Binary HPLC Pump, Hewlett Packard Gmbh, Waldbronn, Germany) system. Detector: Hewlett Packard refractive index 2414 detector (HP 2414, Tokyo, Japan); Column: 5 μm NH_2 carbohydrate analysis column (Waters; 4.6 x 250 mm Catalog PSS831115); Mobile phase: 83% Acetonitrile. The column was calibrated by fructose and glucose standards.

Extraction and determination of organic acids

Organic acids extraction, the method by Bevilacqua and Califano (6) was modified. About 200 g of samples was taken and 5 g from each sample was transferred to centrifuge tubes. The 10 ml of 0.009 N H_2SO_4 was added to the samples and the samples were homogenized with Heidolph Silent Crusher M, Germany. Then, the samples were mixed for an hour with a shaker (Heidolph Unimax 1010, Germany) and centrifuged at 15.000 × *g* for 15 min. The supernatant were passed through coarse filter paper and then through 0.45 mm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) twice, and finally through the Sep-Pak C_{18} cartridge. The concentration

of organic acids was determined by HPLC using an Aminex column (HPX-87H, 300 mm × 7.8 mm, Bio-Rad) fitted on an Agilent 1100 series HPLC G 1322 A, Germany). Organic acids were detected at 214 and 280 nm wavelengths. As the mobile phase, 0.009 N H_2SO_4 was passed through 0.45 µm filter membrane.

Extraction and determination of total antioxidant activity

For the standard trolox equivalent antioxidant capacity (TEAC) assay, TEAC extract was prepared: ABTS was dissolved in acetate buffer and prepared with potassium per sulfate, as described by Rice-Evans *et al*. (7) and Ozgen *et al*. (8). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (8). For the spectrophotometric assay, 3 ml of the $ABTS^+$ solution and 20 µl of fruit extract were mixed and incubated for 10 min and the absorbance was determined at 734 nm determined after 6 min from mixing.

Nutrient analysis

Nitrogen (N) and Phosphorous (P) values were determined by Kjheldahl and Iodophenol blue method, respectively. Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn) and Copper (Cu) contents were determined with atomic absorption spectrophotometer (9).

Phytochemical analysis

Determination of tannins in the grape extracts Cemeroglu (10) which is made by the spectrophotometer method. Denise Folin solution was performed using 750 nm in length and worth reading g/kg was calculated.

Extraction and determination of phenolic compounds

The phenolic compounds were determined using the HPLC separation method described by Rodriguez-Delgado *et al*. (11). About 100 g of samples were taken and 3 g from each sample was transferred to centrifuge tubes. The samples were homogenized then diluted 1:1 with distilled water and centrifuged at 15.000 × *g* for 15 min. The supernatant was passed through 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), then injected into HPLC system (gradient). The chromatographic separation in Agilent 1100 series HPLC took place in DAD detector (Agilent, USA) with 250 mm × 4.6 mm, 4 µm ODS column (HiChrom, USA). The following solvents in water with a flow rate of 1 ml/min and 20 µl injection volume were used for spectral measurements at 254, and 280 nm: as mobile phase solvent A: methanol-acetic acid-water (10:2:88) and Solvent B: methanol-acetic acid-water (90:2:8)

Statistical analysis

Descriptive statistics for the studied variables (characteristics) were presented as mean. Paired sample t test was used to compare years. In addition, multidimensional scaling was performed to indicate visual representation of proximities for the characteristics. In order to facilitate and clarity to comprehensive, the characteristics were classified into three groups: physical, chemical and phytochemical. Then multidimensional scaling was performed. In multidimensional scaling, ALSCAL algorithm was utilized and Euclidean distance was computed for distance measurement. Convergence value was fixed as 0.001 for the calculation of stress value. Statistical significance levels were considered as 5% and SPSS (ver: 13) statistical program was used for all statistical computations.

Results and Discussion

The results for physical characteristics of 'Ercis' grape cultivar are presented in Table 1. For cluster weight (g), berry width (mm) berry length (mm) and berry weight (g), differences between years were found statistically significant, however there was no significant differences for color identification components: L*, a*, b*, C* and h° values.

Cluster weight of 'Ercis' grape cultivar ranged from 241.20 to 296.30 g. Similarly, for berry width, length, and weight changed from 13.72 to 15.09 mm, 11.86 to 14.28 mm and 2.75 to 3.72 g, respectively.

L* value which indicates darkness and lightness coordinates was found 24.85-24.35. a* and b* values which indicate density of color and these values were found 3.60-4.20 and -0.40 and -0.39, respectively. C* value representing the degree of color saturation

ranged from 3.60 to 4.30. Similarly, h° value also changed from 355.40 to 355.50. According to these findings, 'Ercis' grape cultivar can be classified into blue and black color interval.

The results for chemical characteristics of 'Ercis' grape cultivar are displayed Table 2. As seen from Table 2, there was no statistically significant difference between years for pH, TA (%), TSS (%) and MI (%) in 'Ercis' grapes. TSS (%) varied from 16.50 to 17.0. Also, pH ranged from 3.80 to 3.90. For TA (%) and MI (%), the values were found 0.51% - 0.54% and 31.41-32.35, respectively.

Mean values of mineral content are presented in Table 2. As seen in Table 2, there were no statistically significant changes between years. The minerals found in grapes are taken from soil by vine and transported to the berry. Although the amount of minerals in grapes is within particular limits, this amount is likely to change with degree of ripeness, soil type, fertilization and climatic conditions (12). The main minerals existed in grapes are potassium, calcium, phosphorus, sodium, iron and magnesium. In grapes, about 2-3% of the skin and 1-2% of flesh consist of minerals (13). It can be stated that mineral values of 'Ercis' grape cultivar are relatively low due to not fertilization of vineyards.

Five organic acids that are citric, tartaric, malic, succinic and fumaric were included into study. For all organic acids, there was no statistically significant difference between years (Table 2). In the previous studies (14, 15), it was reported that tartaric and malic acids have been main acids in the grape and these two acids constitute 90% of total acidity. Similarly, Winkler *et al*. (16) noted that other considerable organic acid is citric acid and this acid consists of 5-10% of total acidity in grapes. In harvest period, antioxidant capacity of 'Ercis' grape cultivar was recorded 10.95 μmol TE/g FW (fresh weigh) for 2011 and 11.50 μmol TE/g FW for 2012. Keskin (17) reported that antioxidant properties of grapes are more than 50 times of vitamin E and 30 times of vitamin C.

Table 1. Comparison results of years for the physical characteristics

Physical characteristics	2011	2012	p
Cluster weight (g)	296.30	241.20	0.012
Berry width (mm)	13.72	15.09	0.031
Berry length (mm)	11.86	14.28	0.043
Berry weight (g)	2.75	3.72	0.048
L*	24.85	24.35	0.161
a*	3.60	4.20	0.101
b*	-0.40	-0.39	0.874
C*	3.60	4.30	0.120
h°	355.40	356.50	0.541

Phytochemical characteristics of 'Ercis' grape cultivar are given in Table 3. Tannin values for 2011 and 2012 were found 3.04% and 3.12%, respectively. The difference between years was not statistically significant. Tannins are complex esters of phenolic acids and sugars. These compounds are existed in the skin and seed of grape. In maturity period, the tannin amount in the skin increases with the same ratio of color (12). Similarly, Navarro *et al*. (18) reported that total tannin has increased with maturation.

Margagyan *et al*. (19) reported that genetic, agronomic or environmental factors play crucial role in phenolic composition and concentration. It is well known that the composition of phenols in grapevines depends on variety, species, season and environmental and management factors such as soil conditions, climate and crop load.

The phenolic compounds of 'Ercis' grape cultivar are presented as mean values in Table 3. As seen in Table 3, there was no significant difference between years. In general, multivariate statistical analysis, such as principal component, multidimensional scaling and factor analyses, enable us to interpret the results in reduced dimensions. Multidimensional scaling (MDS) has become more and more popular as a technique for both multivariate and exploratory data analysis. MDS analysis aims to find measure of the global proximities (similarity or dissimilarity) of the characteristics or objects. In this study, multidimensional scaling was performed to determine proximities among the characteristics. According to the multidimensional scaling results, coordinate values of the characteristics are presented in Table 4 for the two dimensions. As shown in Table 4, all characteristics, except from cluster weight and h°, have negative values for the first dimension. Similarly, berry width, length, and weight have also

Table 2. Comparison results of years for the chemical characteristics

Chemical characteristics	2011 year	2012 year	p
pH	3.90	3.80	0.541
TA (%)	0.51	0.54	0.471
TSS (%)	16.5	17.0	0.814
MI (%)	32.35	31.41	0.714
Glucose (g/l)	105.52	109.45	0.451
Fructose (g/l)	110.0	115.40	0.214
Glucose/Fructose	0.96	0.95	0.358
Tartaric acid (g/l)	15.12	15.14	0.621
Malic acid (g/l)	10.33	10.82	0.632
Citric acid (g/l)	1.63	1.65	0.184
Succinic acid (g/l)	0.40	0.42	0.781
Fumaric acid (g/l)	0.15	0.16	0.819
TEAC (μmol TE/g FW)	10.95	11.50	0.791
N (%)	0.52	0.50	0.754
P (ppm)	250	254	0.999
K (ppm)	1150	1140	0.897
Ca (ppm)	85.28	86.53	0.754
Mg (ppm)	140	141.5	0.915
Fe (ppm)	5.40	5.32	0.684
Mn (ppm)	0.21	0.19	0.621
Zn (ppm)	0.27	0.29	0.701
Cu (ppm)	0.38	0.40	0.597

Table 3. Means and comparison results of phytochemical characteristics

Phytochemical analysis	2011	2012	p
Tannin (%)	3.04	3.12	0.568
Gallic acid (mg/l)	1.23	1.50	0.214
Clorogenic acid (mg/l)	3.22	3.14	0.758
Catechin (mg/l)	2.32	2.36	0.032
Caffeic acid (mg/l)	0.82	0.80	0.531
p-Coumaric acid (mg/l)	0.05	0.05	0.471
Vanilic acid (mg/l)	0.32	0.34	0.999
Rutin (mg/l)	5.11	5.50	0.789

Table 4. Coordinate values of the physical characteristics in 2 dimensions

	Dimension 1	Dimension 2
Cluster weight	1.9248	0.0980
Berry width	-0.6037	-0.3538
Berry length	-0.6433	-0.3685
Berry weight	-0.8043	-0.3650
L*	-0.4593	0.2160
a*	-0.8253	0.2377
b*	-0.9061	0.1529
C*	-0.8244	0.2358
h°	3.1416	0.1468
Stress = 0.10575	RSQ = 0.98038	

negative values for the second dimension, while other characteristics have positive values. With correspond to Table 4, view of the characteristics on two-dimensional space is illustrated in Figure 1.

As shown in Figure 1, only cluster weight and h° value were located in the same region that is positive region for the both dimensions. These characteristics were negatively correlated with others in terms of first dimension. Similarly, according to second dimension, berry width, length, and weight were also negatively correlated with other characteristics. a*, b*, C*, and L* values were found positively correlated with each other. Likewise, correlation between cluster weight and h° value was positive.

For the chemical and phytochemical characteristics, results of multidimensional scaling are given in Table 5.

With correspond to Table 5; views of the characteristics on two-dimensional space are displayed in Figure 2 and 3. Figure 2 showed that there were considerable similarities among the some chemical characteristics. Also, Figure 3 presented that some chemical and phytochemical characteristics have some similarities in terms of both dimensions, especially for dimension 1.

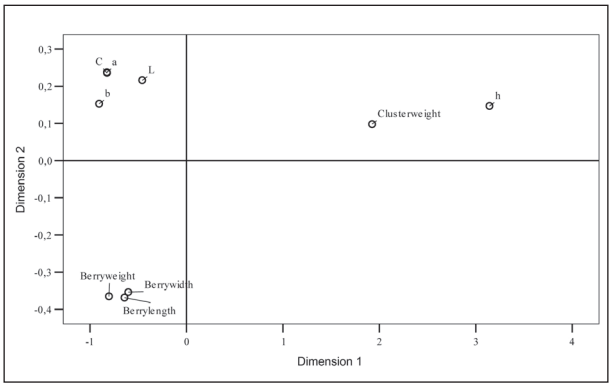

Figure 1. Configuration map of the physical characteristics

Conclusion

With related to healthy nutrition, attentions to the species of fruit that contain rich phytochemical content has increased in the world recently. Grape is one of the richest fruit species for phytochemical content. This study determined physical and chemical characteristics as well as phytochemical content of 'Ercis' grape cultivar which is local to Van province for wine and must while consumed for table grapes due to specific aroma. It can be considered that 'Ercis' grape cultivar has been partially rich for phytochemical content. Therefore, results of this study indicated that 'Ercis' grape cultivar has great potential for healthy nutrition, especially, due to having considerable phytochemical content. In addition, according to results of this study, using of multidimensional scaling method can be proposed to determine similarity or dissimilarity among the quality and phytochemical characteristics.

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Table 5. Coordinate values of chemical and phytochemical characteristics in 2 dimensions

	Dimension 1	**Dimension 2**		**Dimension 1**	**Dimension 2**
pH	0.5941	-0.0031	Tannin	0.0897	-0.3758
TA	0.6113	-0.0028	Gallic acid	0.7065	-0.5508
TSS	0.5276	-0.0012	Clorogenic acid	0.0530	0.2219
MI	0.4494	-0.0047	Catechin	0.3238	0.5003
Glucose	0.0669	0.0017	Caffeic acid	0.9448	-0.4991
Fructose	0.0323	0.0134	p-Coumaric acid	1.2990	0.0127
Glucose/Fructose	0.6091	-0.0029	Vanilic acid	1.1668	-0.3287
N	0.6114	-0.0029	Rutin	-0.4604	-0.0323
P	-0.6869	0.0127	Tartaric acid	3.4701	-0.0751
K	-5.2971	-0.0047	Malic acid	1.6795	-0.0793
Ca	0.1706	0.0021	Citric acid	0.5726	0.5787
Mg	-0.1126	0.0039	Succinic acid	1.1107	0.4143
Fe	0.5864	-0.0030	Fumaric acid	1.2351	0.2787
Mn	0.6130	-0.0029	TEAC	-1.8920	-0.0655
Zn	0.6126	-0.0028			
Cu	0.6120	-0.0028			
Stress = 0.0001 RSQ = 0.9999			Stress = 0.13970 RSQ = 0.95165		

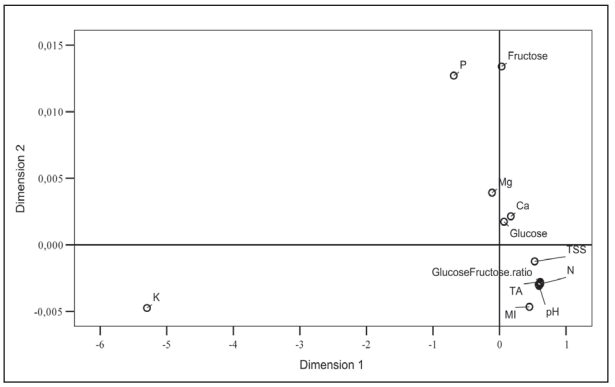

Figure 2. Configuration map of the some chemical characteristics

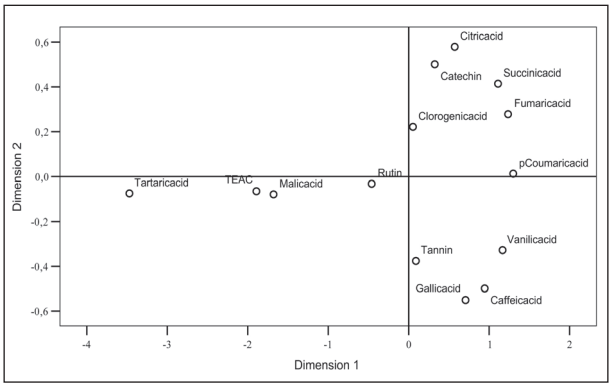

Figure 3. Configuration map of the some chemical and phytochemical characteristics

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