ANOM approach for the statistical evaluation of organic acid contents of clones of the grape variety 'Kalecik Karası'

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Organic acids have an important effect on flavor, aroma and color in berries and wines of grapes and they also play an important role for the microbiological and biochemical stability of the wine. The variety 'Kalecik Karası' is one of the exclusive red grape varieties of Turkey. The aim of this study is to evaluate and visualize the differences between the 23 clones of 'Kalecik Karası' with respect to organic acids. As organic acids were considered: tartaric, malic, citric, succinic and fumaric acids. The berries of the vintages 2016 and 2017 were harvested with 23 % soluble solids and HPLC (High Performance Liquid Chromatography) was used to identify organic acid content. Using Analysis of Means (ANOM) method the mean of each group was compared to the overall or grand mean to identify statistically significant differences. Thus the differences between the 'Kalecik Karası' clones in terms of organic acids were compared and visualized by this method. According to the results, for all organic acids, except for tartaric and fumaric acid, differences between means of clones and overall mean are statistically significant ($p<0.05$). Both ANOVA and ANOM can be appropriate for determining differences between the groups. However, ANOM provides a simple graphical representation for group means. Therefore, it can be concluded that this method can be suggested to visualize differences between the groups for easy interpretation.

Keywords; analysis of means, organic acid, *Vitis vinifera*, clone, 'Kalecik Karası'

Mittelwertanalyse zur statistischen Auswertung des Gehalts an organischen Säuren von Klonen der Rebsorte 'Kalecik Karası'. Organische Säuren haben einen wichtigen Einfluss auf Geschmack, Aroma und Farbe in Beeren und Weinen von Trauben und spielen auch eine wichtige Rolle für die mikrobiologische und biochemische Stabilität des Weins. Die Rebsorte 'Kalecik Karası' ist eine der exklusiven roten Rebsorten der Türkei. Ziel dieser Studie ist es, die Unterschiede zwischen 23 Klonen der Sorte 'Kalecik Karası' in Bezug auf organische Säuren zu bewerten und zu visualisieren. Als organische Säuren werden angesehen: Weinsäure, Äpfelsäure, Citronensäure, Bernsteinsäure und Fumarsäure. Beeren der Jahrgänge 2016 und 2017 wurden mit einem Gehalt von 23 % löslichen Feststoffen geerntet, und mittels HPLC wurde der Gehalt an organischen Säuren ermittelt. Unter Verwendung der Mittelwertanalyse (Analysis of Means; ANOM) wurde der Mittelwert jeder Gruppe mit dem Gesamtmittelwert verglichen, um statistisch signifikante Unterschiede zu identifizieren. So wurden die Unterschiede zwischen den 'Kalecik Karası'-Klonen in Bezug auf organische Säuren verglichen und

sichtbar gemacht. Gemäß den Ergebnissen sind für alle organischen Säuren mit Ausnahme von Weinsäure und Fumarsäure die Unterschiede zwischen den Mittelwerten der Klone und dem Gesamtmittelwert statistisch signifikant ($p<0,05$). Sowohl ANOVA als auch ANOM können zur Bestimmung von Unterschieden zwischen den Gruppen geeignet sein. Die Mittelwertanalyse bietet jedoch eine einfache grafische Darstellung der Gruppenmittelwerte. Daher kann diese Methode vorgeschlagen werden, um Unterschiede zwischen den Gruppen für eine einfache Interpretation zu visualisieren.
Schlüsselwörter; Analyse der Mittelwerte, organische Säure, Vitis vinifera, Klon, 'Kalecik Karası'

Schlagwörter: Mittelwertanalyse (Analysis of means; ANOM), organische Säuren, Klone, 'Kalecik Karası'

Grapevine, which has an important position among horticultural crops, is one of the fruit species that is grown the most in the world, with approximately 75 million tons produced each year (Fennell, 2004; Kaya, 2020). It is also one of the most commonly consumed fruits: while almost 50 % of the grapes are utilized to make wine, one third is consumed as fresh fruit and the rest is dried, consumed as grape juice or stored in the form of grape musts (FAOSTAT, 2018). Based on TUIK data (2019), 4.1 million tons of grapes were produced in Turkey, and 2.1 million tons (50 %) are consumed as fresh fruit, 1.6 million tons (39 %) are dried and 451 thousand tons (11 %) are used for wine, must and grape juice. Turkey also is one of the leading world grape producers and grape exporting countries, and it has important table and wine grape varieties. These varieties can be grown in many regions of Turkey, but some grape varieties come to the fore with the regions where they are grown, and they have their own aroma, taste and flavor compared to other regions. 'Kalecik Karası', a native grape variety of *Vitis vinifera* grown in Turkey (Central Anatolia region), is one of the best quality red wine grape varieties (Çelik, 2006).

'Kalecik Karası' is a medium-sized thick-skinned and round grape. Alcohol content of the wine produced from the grapes is 12 to 14 % and its acid contents range from 4 to 7 grams per liter. Wine produced from this grape have a rich and well-balanced structure. Especially fruit flavors are encountered in this grape. Flavors such as vanilla and cocoa are other typical flavors (Çelik et al., 2019). The structure of this wine is suitable for aging. It is stated by the producers that it can be aged between 5 to 10 years and it continues its development and maturation during this period. 'Kalecik Karası' has actually long been known in Turkey as a wine grape, but once it was threatened with abandonment. In the 1980s, this grape variety almost disappeared. With the important studies of Ankara University Faculty of Agriculture in Kalecik this grape variety has been brought back to Turkish winemaking since 1990. In this context, clonal selection studies have been initiated for the variety (Fidan et al., 1988 and 1991). As is known, the goal of clonal selection is to select superior individuals of a grape variety within the original grape populations considering the yield and quality features (Keskin et al., 2020). A detailed clonal selection study was conducted by Çelik et al. (2019), and 23 clones of 'Kalecik Karası' were re-eveluated. Although the agronomic properties and *t*-resveratrol potential of 23 clones have been determined in previous studies (Çelik et al., 2019; Keskin et al., 2020), our knowledge of clonal organic acid content is still limited. Therefore, investigating the organic acid accumulation in clones can contribute to a better understanding of clonal genetic performance. On the other hand, in order to analyze events and processes in nature, it is examined whether there is a relationship between variables or there is a difference between treatments. In this regard, analysis of variance (ANOVA) is widely used to determine whether more than two treatments affect the response variable when the assumptions are provided. Additionally, another analysis method, Analysis of Means (in short ANOM), can be considered as an alternative method for ANOVA. It is one of the statistical methods that graphically determines the difference of any treatment from the overall mean. It presents graphed results, thus the results can be easily understood by non-

statisticians. This method is actually a multiple comparison procedure. Furthermore, ANOM requires two assumptions; the first one is that the samples are approximately normally distributed, and a second one is that the populations from which each of the samples of size were selected do not differ with respect to variability (Elamir, 2016). ANOM was firstly introduced by Ott (1967) as a statistical quality control tool. In the 1990, it started to be used in industry, healthcare practices and quality improvement (Ryan, 2011). Thus, ANOM has become popular and has been widely used in recent years. However, as noted by Ryan (2011), few researchers have been active in publishing research articles about ANOM. In general, ANOM is the graphical method to specify treatment or group means that significantly differ from the overall mean. Any ANOM decision chart consists of three lines. Overall mean is located in the center of the chart. In addition to the centerline, ANOM chart has upper and lower decision lines. The individual group means are shown by dots in the chart. Being from outside the decision limits for any individual group mean indicates that this mean is significantly different from the overall mean. Assuming different amounts of organic acids in grape varieties or clones, it is inevitable to determine these amounts. Furthermore, it is important to identify and select the clones that are superior in terms of the concerned characteristics by considering mainly qualitative differences in a variety. In the present study, we addressed the following questions: (i) are there differences between 23 clones with respect to the organic acids present in grape must of 'Kalecik Karası' clones? (ii) can the ANOM approach be used to explain the differences between 'Kalecik Karası' clones with respect to concentrations of tartaric, malic, citric, succinic and fumaric acid? (iii) is there a relationship between the 'Kalecik Karası' clones and organic acids by using Nonlinear Principal Component analysis (NLPCA)?

Material and Methods

Chemicals

In this study, chemicals with analytical purity were used. Standards (tartaric, malic, citric, succinic and fumaric acids) were obtained from Sigma-Aldrich (St. Louis, USA).

Samples

The material of the study are clones of the 'Kalecik Karası' grape variety. These clones were grown in the vineyards of the Research Station for Viticulture (University of Ankara, Faculty of Agriculture). This vineyard was established in 1999 in Kalecik, 70 km northeast of Ankara. 23 clones with the same age of 'Kalecik Karası' (released from the clonal selection breeding program) were used. Clones were grafted on 41B clone 172, with 2 × 3 m planting density, trained as bilateral Guyot on a trunk of 80 cm. The experiment was set up in a randomized complete block design with four replicates (four vines per rep.). Berry specimens were taken from one vineyard location for each 'Kalecik Karası' clone. The berries of the clones were harvested at around 23 % soluble solids in the vintages 2016 and 2017. Berry sampling was conducted on 16 vines for each clone. We followed a defined protocol for berry collection to avoid bias in sampling. Berry sampling in clones first defined four zones on each grape cluster to be sampled; the left and right shoulders, the tail and middle section. We alternated from each of these cluster zones for berry collected as we moved from cluster to cluster throughout the vine. We also alternated from the rear of the cluster to the front of the cluster as we picked the berries as described above. For example, if there were 16 clusters, the first four berries would be collected from the outer-facing or front side of the first eight clusters (one berry from each of the four zones), and the second four berries would come from the rear-facing side of the remaining eight clusters (in sequence with the four cluster zones). In total, 16 clusters from 16 vines were taken for each clone and eight berries were sampled from each cluster (128 berries in total for each clone). After harvest one subsample of 128 berries for each harvested clone was directly stored in a -20 °C freezer and then transferred to a -80 °C freezer for long-term storage up to analysis.

Extraction and determination of organic acids

The method of Bevilacqua and Califano (1989) was used to extract organic acids. Mixtures containing 5 ml grape must and 20 ml 0.009 NH_2SO_4 were homogenized. The mixtures were blended by a shaker for 1 h and then were centrifuged for 15 min at 15.000 rpm. The supernatants were filtered first through filter paper and then twice through a 0.45 µm membrane filter before being passed through a SEP-PAK C18 cartridge. An Aminex column (HPX-87 H, 300 mm × 7.8 mm) was used in the HPLC system, and the instrument was controlled by a PC with Agilent software. The DAD detector in the system (Agilent, USA) was set at wavelengths of 214 and 280 nm. The mobile phase was 0.009 NH_2SO_4 that had been filtered through a 0.45 µm membrane filter.

Statistical analysis

In our study, the data obtained from the 23 clones were evaluated using the ANOM method. As in confidence interval computation, upper and lower decision lines (UDL and LDL) were calculated by the method. Before calculation of upper and lower decision values, overall or grand mean ($\bar{\bar{X}}$) was computed as follows:

$$\bar{\bar{X}} = (\bar{X}_1 + \bar{X}_2 + \cdots + \bar{X}_g)/g \quad (1)$$

Then UDL and LDL is obtained from the following equation:

$$\text{UDL, LDL} = \bar{\bar{X}} \mp h_{g,n_j}\sqrt{\frac{S_p^2\ (g-1)}{n}} \quad (\text{j = 1, 2,..., g}) \quad (2)$$

Where

g: number of groups in the study

n_j; sample size for group j

n; total number of observation where all of the n_j sample size equal

S_p^2; pooled variance estimation from g group variances $S_p^2 = (S_1^2 + S_2^2 + \cdots + S_g^2)$/g

h_{g,n_j}; critical values of Nelson's h statistic with g groups and n*j* equal observation per group obtained

from the table of the h statistics

Thus the following equation can be written for UDL and LDL:

$$\text{UDL} = \bar{\bar{X}} + h_{g,n_j}\sqrt{\frac{S_p^2\ (g-1)}{n}} \text{ ve LDL} = \bar{\bar{X}} - h_{g,n_j}\sqrt{\frac{S_p^2\ (g-1)}{n}}$$

For performing ANOM, two assumptions or requirements must be met as for ANOVA:
(1) Homogeneity of variances; there should be no or only slightly difference between variances of all compared groups (Nelson et al., 2005).
(2) Normality of the data

Principal component analysis (PCA) is a dimension reduction method that can explain the variation between original variables as much as possible and translate them into new variables called principal components. Assumptions of normality and linearity are required for this widely used method. However many studies include categorical or ordinal variables as well as continuous variables. In this case,

normality or linearity assumption is violated and PCA cannot be applied. Thus, Nonlinear Principal Component Analysis (NLPCA) stands out as a more convenient method. NLPCA includes continuous categorical and ordered variables and examines the non-linear relationships as well as the linear ones. Additionally, this method uses an optimal scaling approach known as optimal quantification or optimal scoring. In this study,

therefore, NLPCA was carried out to evaluate the relationship between the 'Kalecik Karası' clones and organic acids. MINITAB (v. 14) and SPSS (v. 20) for Windows programs were used for statistical computations in the study.

Results and Discussion

Descriptive statistics for the organic acids, which are tartaric, malic, citric, succinic and fumaric acid, are presented in Table 1. As seen in Table 1, means of the clones for tartaric acid varied between 3.528 g/l (clone 4) and 3.928 g/l (clone 2). Furthermore, overall mean was found 3.800 g/l. Similarly, the means of the clones for malic acid ranged from 1.704 g/l (clone 13) to 1.983 g/l (clone 16) with 1.833 g/l overall mean. For citric acid, the lowest value was obtained from clone 2 with 0.032 g/l, while the highest value (0.085 g/l) from clone 16. Overall mean of 23 clones was found 0.045 g/l. By 0.647 g/l overall mean, succinic acid values of the clones ranged from 0.550 g/l (clone 11) to 0.786 g/l (clone 23). For fumaric acid, while the values of the clones ranged from 0.339 g/l (clone 12) to 0.390 g/l (clone 23), overall mean was observed as 0.364 g/l. The important organic acids in grapes are tartaric and malic acid, and they make up more than 90 % of the total acidity (Salunkhe and Kadam, 1995). Citric acid is the

third-most common organic acid in grapes and it constitutes 5 to 10 % of the total acidity (Winkler et al., 1997). Apart from these acids, there are organic acids such as oxalic and fumaric acid in grapes. However, their presence is not as important as tartaric, malic, and, to some extent, citric acid (Çelik et al., 1998). Considering the previous study results, the present study results are consistent with these findings.

On the other hand, the mean of each group was compared to the overall mean to determine statistically significant differences from the overall mean. Not only group means were compared with the data, but also organic acid contents and variances. As seen in Figure 1, the overall mean of tartaric acid was 3.800 g/l and this value is located in the center of the chart. Upper and lower decision lines were found at 4.113 g/l and 3.486 g/l, respectively. The ANOM chart showed that means of all clones were located within upper and lower decision limits. Thus, the differences of the all clones' means from the overall mean were not statistically significant. As is known, titratable acidity is the measurement of total acid in grape juice and it is expressed as the content of tartaric acid (Cox, 1999). Additionally, tartaric acid is the most abundant organic acid in grapes and constitutes 40 to 80 % of the total acidity in grapes (Ribéreau-Gayon et al., 2006). Although it is reported that it varies between 2 and 15 g/l depending on the varieties, ripening time, temperature and region, it is generally found to be around 6 g/l (Palma and Barrosa, 2002). Indeed, it was determined that the tartaric acid content in fresh grape juices of 11 different white grape varieties varied between 4.98 and 7.48 g/l (Soyer et al., 2003). In our study, the tartaric acid values obtained from the clones were determined between the values reported in literature.

Table 1: Descriptive statistics for the organic acids obtained from 23 grape clones

Clones	Tartaric acid (g/l) Mean ± SEM	Malic acid (g/l) Mean ± SEM	Citric acid (g/l) Mean ± SEM	Succinic acid (g/l) Mean ± SEM	Fumaric acid (g/l) Mean ± SEM
1	3.666 ± 0.027	1.817 ± 0.004	0.055 ± 0.004	0.555 ± 0.018	0.361 ± 0.014
2	3.928 ± 0.004	1.933 ± 0.018	0.032 ± 0.001	0.728 ± 0.005	0.355 ± 0.018
3	3.733 ± 0.018	1.855 ± 0.009	0.038 ± 0.002	0.617 ± 0.005	0.355 ± 0.009
4	3.528 ± 0.004	1.788 ± 0.005	0.034 ± 0.003	0.761 ± 0.023	0.339 ± 0.012
5	3.772 ± 0.022	1.828 ± 0.023	0.035 ± 0.000	0.571 ± 0.022	0.350 ± 0.022
6	3.605 ± 0.315	1.750 ± 0.022	0.066 ± 0.002	0.561 ± 0.031	0.366 ± 0.018
7	3.806 ± 0.004	1.944 ± 0.018	0.055 ± 0.005	0.583 ± 0.005	0.355 ± 0.027
8	3.866 ± 0.009	1.761 ± 0.051	0.036 ± 0.001	0.611 ± 0.009	0.361 ± 0.003
9	3.715 ± 0.225	1.833 ± 0.027	0.058 ± 0.001	0.666 ± 0.009	0.353 ± 0.005
10	3.850 ± 0.022	1.769 ± 0.002	0.035 ± 0.001	0.728 ± 0.023	0.380 ± 0.016
11	3.839 ± 0.013	1.777 ± 0.009	0.054 ± 0.001	0.550 ± 0.031	0.378 ± 0.006
12	3.772 ± 0.022	1.772 ± 0.022	0.038 ± 0.001	0.733 ± 0.027	0.339 ± 0.002
13	3.866 ± 0.018	1.704 ± 0.002	0.038 ± 0.001	0.575 ± 0.005	0.362 ± 0.010
14	3.866 ± 0.009	1.839 ± 0.004	0.047 ± 0.001	0.655 ± 0.009	0.377 ± 0.009
15	3.771 ± 0.004	1.717 ± 0.013	0.049 ± 0.001	0.577 ± 0.014	0.344 ± 0.007
16	3.883 ± 0.013	1.983 ± 0.005	0.085 ± 0.005	0.666 ± 0.018	0.372 ± 0.005
17	3.904 ± 0.004	1.882 ± 0.006	0.037 ± 0.001	0.617 ± 0.011	0.385 ± 0.004
18	3.896 ± 0.005	1.917 ± 0.003	0.047 ± 0.001	0.661 ± 0.014	0.360 ± 0.008
19	3.891 ± 0.044	1.861 ± 0.001	0.058 ± 0.001	0.777 ± 0.017	0.382 ± 0.001
20	3.737 ± 0.147	1.861 ± 0.014	0.048 ± 0.001	0.644 ± 0.009	0.361 ± 0.003
21	3.760 ± 0.161	1.955 ± 0.009	0.042 ± 0.001	0.580 ± 0.010	0.383 ± 0.005
22	3.875 ± 0.036	1.825 ± 0.001	0.037 ± 0.001	0.683 ± 0.004	0.366 ± 0.007
23	3.878 ± 0.015	1.857 ± 0.002	0.035 ± 0.001	0.786 ± 0.005	0.390 ± 0.005
p value	0.349	0.001	0.001	0.001	0.189
Overall	3.800 ± 0.021	1.833 ± 0.016	0.045 ± 0.003	0.647 ± 0.016	0.364 ± 0.003

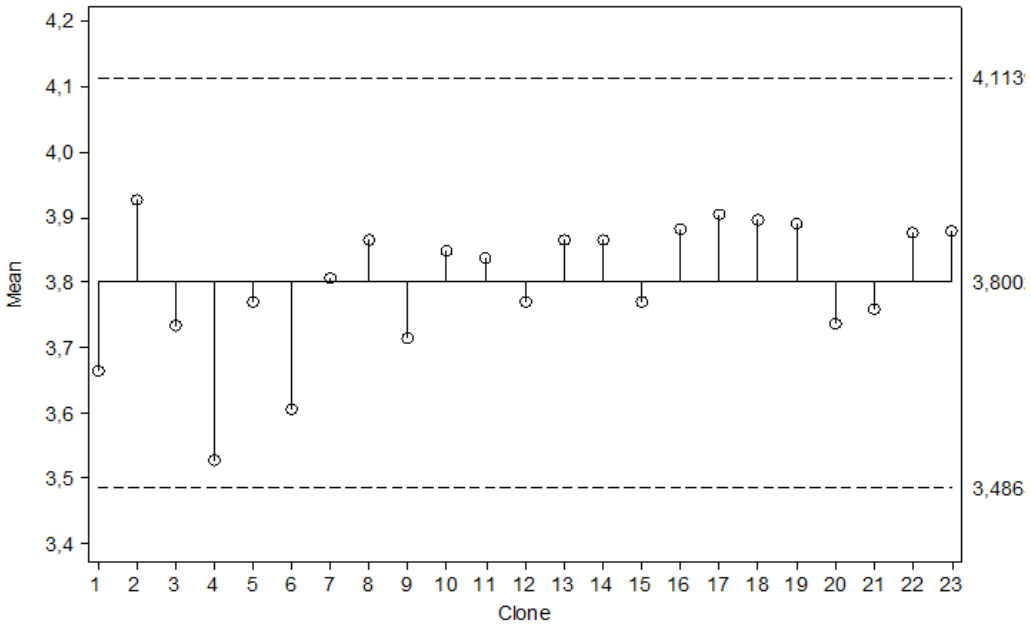

Fig. 1: ANOM chart for tartaric acid obtained from 23 grape clones

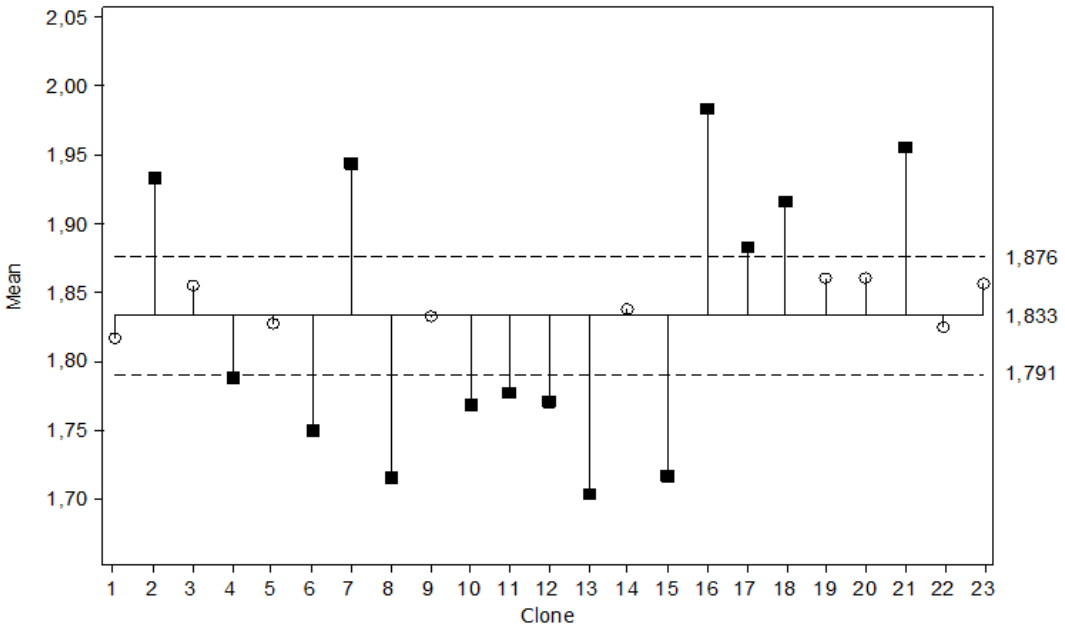

Fig. 2: ANOM chart for malic acid obtained from 23 grape clones

As shown in Figure 2, the overall mean of malic acid was found at 1.833 g/l. Upper decision line for this mean was 1.876 g/l while lower decision line was 1.791 g/l. Only 9 of the 23 clones were located within the upper and lower decision lines. Clones 2, 7, 16, 17, 18, and 21 were above the upper decision line and these clones were found to be significantly higher in malic acid than the overall mean. Similarly, clones 4, 6, 8, 10, 11, 12, 13 and 15 were located below the lower decision line. The differences of these clones' means from the overall mean were also found statistically significant. It was determined that after the tartaric acid in grapes, malic acid is the most common organic acids and that malic acid in grapes is between 1 and 3 g/l (Palma and Barrosa, 2002). It has also been found that malic acid varies between 1.43 and 3.40 g/l in different grape varieties (Escobal et al., 1998; Soyer et al., 2003). In our results, which are consistent with the previous study results, the malic acid value of 23 clones was found to be higher than the minimum level (1 g/l) reported in literature.

In our study, the overall mean for citric acid was 0.045 g/l, with 0.051 g/l upper decision line and 0.039 g/l lower decision line (Fig. 3). It was observed that only 5 of the 23 clones were located within upper and lower decision lines. Clones 1, 6, 7, 9, 11, 16, and 19 were found to be significantly ($p<0.05$) higher than the overall mean. In addition, clones 2, 3, 4, 5, 8, 10, 12, 13, 17, 22, and 23 were located below the lower decision line and these clones were statistically significant lower than the overall mean. Citric acid is the third-most abundant organic acid in grape berries and it constitutes 5 to 10 % of total acidity (Winkler et al., 1997). It can also be found in various concentrations depending on variety and environmental conditions (Garcia et al., 2003). This result may explain the citric acid differences between clones. On the other hand, citric acid has been reported to be between 0.2 and 3.0 g/l in unfermented grapes (Amerine and Ough, 1980). In our findings, the highest citric acid content of clones was lower than the maximum in literature.

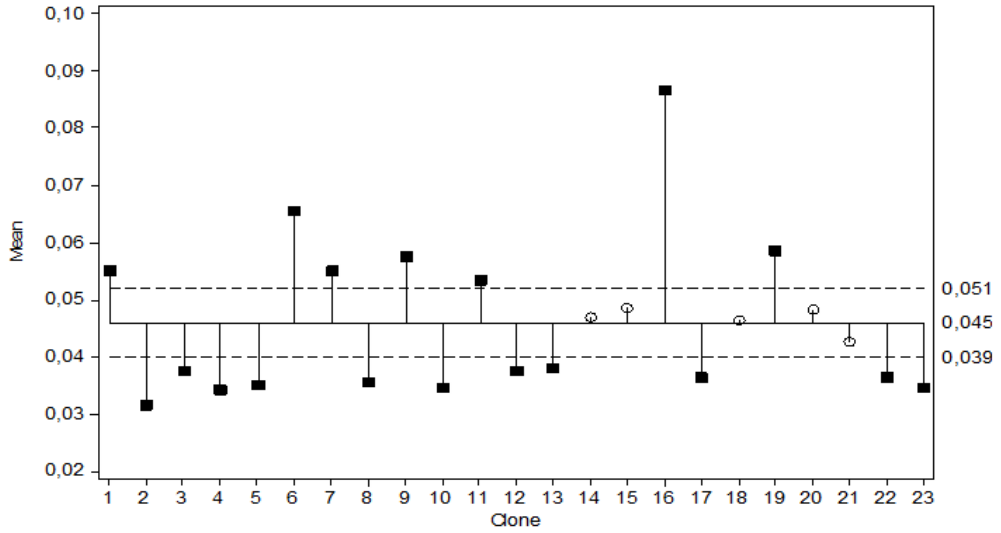

Fig. 3: ANOM chart for citric acid obtained from 23 grape clones

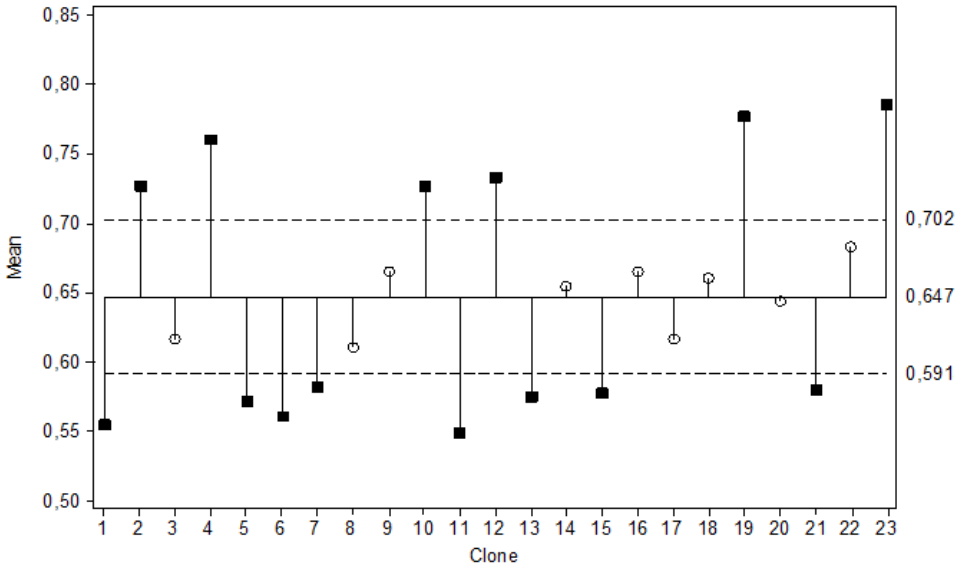

Fig. 4: ANOM chart for succinic acid obtained from 23 grape clones

Overall mean for succinic acid was found at 0.647 g/l while upper and lower decision lines were 0.702 g/l and 0.591 g/l, respectively (Fig. 4). Accordingly, means of 9 clones fall inside UDL and LDL. Clones 2, 4, 10, 12, 19 and 23 were located above the upper decision line. Thus, the means of these clones were significantly higher than the overall mean. Similarly, clones 1, 5, 6, 7, 11, 13, 15, and 21 fall below the lower decision line. This indicated that means of these clones were significantly lower than the overall mean. Succinic acid, if at all, is found only in trace amounts (<0.1mg/kg) in the berries of *Vitis vinifera* varieties (Soyer et al., 2003; Coulter, 2004). It is also known as a normal by-product of alcoholic fermentations (Lamikanra et al., 1995). Succinic acid has been suggested to be the main non-volatile carboxylic acid produced by yeast during the fermentation of grape juice (Radler, 1993). Other researchers have reported that succinic acid is formed during the stationary phase and throughout fermentation. However, some studies did not quantitatively determine the succinic acid content of grape and grape juices (Soyer et al., 2003). On the other hand, it has been reported that the level of succinic acid in red wines ranged from 0.1 to 2.6 g/l, with a mean of 1.2 g/l, while the level of succinic acid in white wines ranged from 0.1 to 1.6 g/l, with a mean of 0.6 g/l. The succinic acid content obtained from clones in our study is compatible with the literature.

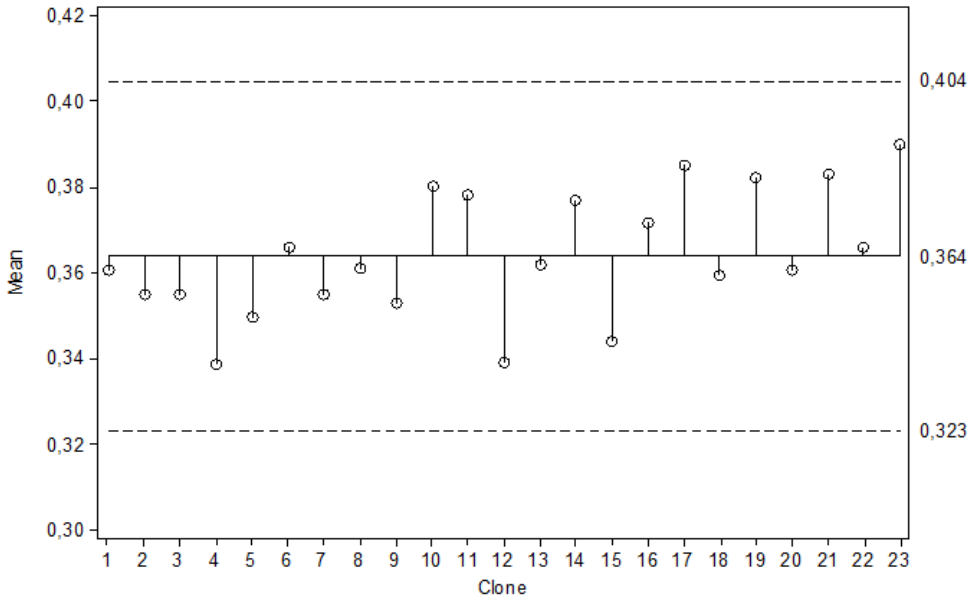

Fig. 5: ANOM chart for fumaric acid obtained from 23 grape clones

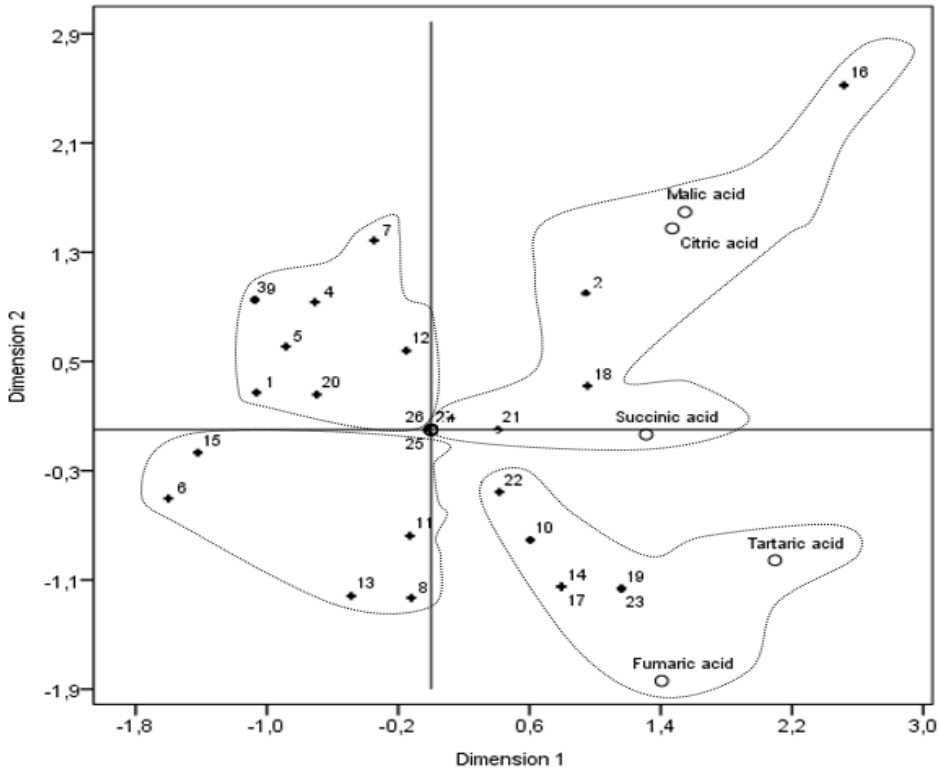

Fig. 6: Configuration of the clones and organic acids on the two-dimensional map

For fumaric acid value of clones, ANOM results are presented in Figure 5. Overall mean was 0.364 g/l while upper and lower decision lines were 0.404 g/l and 0.323 g/l, respectively. From Figure 5, it can be observed that all clones fall inside the UDL and the LDL. The differences of clones from the overall mean were not statistically significant (*p=0.189*). Fumaric acid is found in trace amounts in grape berries (Ribéreau-

Gayon, 1999), and it has indeed been determined that the fumaric acid content obtained from some grape varieties is in a very narrow range (from 0.0011 to 0.0046 g/l) (Keskin et al., 2013). In our results, the fumaric acid content of the clones was significantly higher than those reported by previous authors. On the other hand, ANOM presents visual results for the statistically significant differences without any multiple comparison. ANOM charts can denote both statistical and practical significance of the differences. In ANOVA, a multiple comparison test is required for determination of different treatment means after rejection of null hypothesis. However, ANOM can determine these differences simultaneously in a single step and present the results graphically. Thus, as mentioned by Ryan (2011), ANOM can be used either alone or as a supplement to ANOVA. Nelson and Dudewicz, (2002) emphasized that ANOM has the advantages that it identifies any treatment means that differ from the overall mean, and provides a graphical display that aids in assessing practical significance. Furthermore, Nelson et al. (2005) stated that if any of the treatments are statistically different, ANOM indicates exactly which ones are different. In addition, ANOM provides graphical presentation which allows to easily evaluate both the statistical and practical significance of the differences. Pallman and Hothorn (2015) reported that ANOM and ANOVA can be applied to similar testing problems, and therefore we may consider them as competitors. However, it is important to notice that ANOM and ANOVA provide substantially different information. In the same way, Balakrishnan (2013) stated that ANOM method is very similar to ANOVA in concept however more useful for visualization of the results by depiction via control charts with decision lines. Balakrishnan, (2013) also indicated that when studying main effects, ANOM is more advantageous than ANOVA: (1) if any of the treatments are statistically different, ANOM indicates exactly which ones are different; and (2) ANOM can be presented in a graphical form, which allows one to easily evaluate both the statistical and the practical significance of the differences. Furthermore, Kalanka et al (2018) emphasized that ANOM procedure is convenient to use, however it has not been as widely used as ANOVA due to more complexity of the mathematical base of ANOM than that of ANOVA. In addition to ANOM, Non-Linear Principal Component Analysis (NLPCA) was performed to indicate relationships between the clones and organic acids. According to the result of NLPCA, the first dimension accounted for 34 % of the total variance, while 24 % of the variance was accounted by the second dimension. Two dimensions accounted for 58 % of the variance together. Configuration of the clones and organic acids of the two-dimensional map is presented in Figure 6. Results indicated that 23 clones and 5 organic acids were classified into four groups on the two-dimensional configuration. All organic acids were located in the positive region of the first dimension. Figure 6 showed that tartaric and fumaric acids were positively correlated with the 10, 14, 17, 19, 22, and 23 clones. Similarly, malic, succinic and citric acids were positively related with 2, 16, 18, and 21 clones. There were negative relationships between the other remaining clones and organic acids for the first dimension. Based on these results, we can state that the clones 10, 14, 17, 19, 22, and 23 are different in terms of fumaric acid content compared to other clones. Besides, the 2, 16, 18, and 21 clones differed in malic, succinic and citric acid contents compared to the other clones. These results show that significant differences occurred between the clones selected from the 'Kalecik Karası' varieties. Therefore, clones were divided into four groups in terms of organic acid content and differences occurred in their malic, fumaric, succinic and citric acid contents. In previous reports supporting our findings, it has been reported that the organic acid content of grape varieties varies depending on the varieties. As a matter of fact, for the 'Muscat of Alexandria', 'Italia', 'Isabella', 'Muscat of Hamburg' and 'Alphonse Lavellee' grape varieties, Sabir et al. (2010) reported malic acid (3.0, 3.1, 3.4, 2.8 and 3.6 g/l), tartaric acid (5.0, 4.8, 5.2, 4.2 and 3.8 g/l), and citric acid contents (0.3, 0.2, 0.4, 0.3 and 0.3 g/l), respectively.

Conclusion

In this study, organic acid content data obtained from 23 clones of 'Kalecik Karası' grape were evaluated using the ANOM method. In ANOM method the mean of each group was compared to the overall or grand mean to identify statistically significant differences. The results showed that there are significant differences in organic acid composition between the clones of 'Kalecik Karası' grape grown in the Ankara province of Central Anatolia region, Turkey. To sum up, in the development of the wine grape industry of the Ankara province the present study will provide industrialists at first and then breeders to get baseline information for the selection of grape clones for cultivation or breeding programs, which can gain great importance with the support of more comprehensive studies in the following years.

Authors' contributions

Nurhan Keskin, Birhan Kunter and Hasan Çelik made a significant contribution to experimental design, acquisition of data, analysis and drafting of the manuscript. Özkan Kaya and Sıddık Keskin have made a substantial contribution to interpretation of data, drafting and carefully revising the manuscript for intellectual content. All authors read and approved the final manuscript.

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