



## Characterization of Some Olive Varieties Consumed without Pre-Treatment: Naturally Debittered Olives in Türkiye

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### ABSTRACT

In table olive production it is necessary to remove oleuropein by brine or dry salting method because it gives bitterness. However, some olive varieties such as Throuba Thassos-Greece, Djemali-Tunisia can be consumed without any de-bittering process. Some olives in Türkiye are also consumed without any pre-treatment and these are Kilis Yağlık, Butko, Hurma (*Erkence cv.*), and Nizip Yağlık olives. These naturally de-bittered olives were aimed to be characterized in this research. The lowest moisture content was determined for Kilis Yağlık (6.84%) and the highest moisture content was determined for Butko (50.01%). The oil and protein content of the samples was between, 16.66-68.46% and 0.19-18.13%, respectively. Total phenolic content (mg GAE/100g) of Kilis Yağlık, Butko, Hurma and Nizip Yağlık olive varieties were determined as 458.87, 152.09, 109.73, 234.33, respectively. The lowest antioxidant capacity was determined for Butko and the highest value was determined for Kilis Yağlık. The hardness values of the olives were found between 677.44-3688.06 (g). The  $L^*$ ,  $a^*$ ,  $b^*$  values of olive samples were found between 26.14-32.05, 2.02-4.78, 2.37- 7.18, respectively. Highest oleic was determined for Hurma, highest linoleic acid was determined for Butko whereas the highest linolenic acid was determined for Nizip Yağlık. Volatile component analysis results of olives showed that 24, 23, 16 and 17 volatile components were detected in Kilis Yağlık, Butko, Hurma and Nizip Yağlık olives, respectively.

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## Introduction

The homeland of the olive tree is the Mediterranean, Asia and Africa and characterized as the symbol of goodness, wisdom, nobility, perseverance, and peace (Ünsal, 2019). The majority of the olives are used to obtain oil and the rest are processed as table olives (Kayahan and Tekin, 2006). The olive color varies from green to yellow-green, brown, red-violet and to black during ripening, depending on the variety. The olive fruit flesh contains 10-25% oil and the rest are water (Guo et al. 2018). Carbohydrate (19%), cellulose (5.8%), protein (1.6%), and minerals (1.5%) were determined in olive flesh. The other important components of olive are determined as organic acids, pectin, phenol glycosides and pigments (Boskou, 2006).

The phenolics of the olive are between 2-3%. Oleuropein, hydroxytyrosol, tyrosol, luteolin 7-oglycoside, hydroxytyrosol-4- $\beta$ -D-glycoside, verbacoside, and rutin are the major phenolics. Oleuropein is the major phenolic in olives which prevents the direct consumption of olive fruit by giving the bitter taste (Kailis and Harris, 2007). Fermentation or brine process is used to decrease the bitterness of olive fruit with the removal of the oleuropein (Susamcı et al 2017). Hydrolytic decomposition of

oleuropein happens in brine waiting and oleuropein turns to hydroxytyrosol, oleuropein aglycone and elenoic acid glycoside which reduces bitterness. *Lactobacillus pentosus* (L. pentosus) and *Lactobacillus plantarum* (L. plantarum) are used for the industrial fermentation of table olives, whereas most of the fermentation process starts spontaneously (Guo et al. 2018). Also, enzymatic reactions can be used for the hydrolyzation of the oleuropein compound. It is known that  $\beta$ -glucosidase and esterase enzymes in the fruit degrade oleuropein during the ripening and storage stages of olive (Guggenheim et al. 2018; Ramirez et al. 2014). In the first step, hydrolyzation of oleuropein to aglycone takes place by oleuropein  $\beta$ -glucosidase enzyme, in the next step, hydrolyzation to hydroxytyrosol and elenoic acid by oleuropein aglycone esterase enzyme takes place (Marsilio and Lanza, 1998).

As seen from literature review, the bitterness of some olive varieties reduces naturally. These olive varieties can be consumed without applying any process. The known olive varieties that de-bittered naturally are Throuba Thassos in Greece, Hurma (*Erkence cv.*) in Türkiye, and

Dhokar in Tunisia (Aktas et al. 2014; Bouaziz et al. 2004; Jemai et al. 2009; Zoidou et al. 2010). Limited studies on these olive varieties were conducted. Jemai et al. (2009) determined the phenolics, total flavonoids, antioxidant activity, enzyme activity and total sugar of Dhokar olive variety in 5 maturation stages. Zoidou et al. (2010) determined the hydroxytyrosol and oleuropein content of some olive varieties from Greek markets including Throuba Thassos. Aktas et al. (2014) stated that the Erkence variety olive grown in İzmir/Karaburun peninsula is naturally de-bittered during the ripening phase and is ready for consumption as it is harvested from the tree. The Erkence type consumed in this way is called Hurma. Aktas et al. (2014) determined the organic acid, fatty acid and sugar composition of Erkence, Gemlik and Hurma olives harvested at different maturation stages in two different seasons. In another study Aktas et al. (2014), compared Gemlik, Erkence and Hurma olive varieties collected at different harvest times for total phenolic content and phenolic components. In two different studies Hurma olives harvested from the Karaburun peninsula were evaluated for the nutritional characteristics (Sahan et al. 2013; Susamcı et al. 2017) According to these studies, the amount of oleuropein decreased and turned to hydroxytyrosol during the ripening stage of these olives. Since the consumption of de-bittered olives is healthy in terms of phenolic substances, it is important to investigate these olives. It was determined that there are 3 more olive varieties besides Hurma olive, edible without any pre-treatment, namely, Kilis Yağlık (Kilis Y) (Attun), Butko and Nizip Yağlık (Nizip Y) olives. Quality characteristics of table olives were determined in several studies. However, studies on naturally debittered olives are limited and the growing place and growing season may be effective in the quality characterization of these olives. The aim of this study is to interpret the quality attributes of naturally debittered Kilis Y, Butko, Hurma and Nizip Y olives, native to Türkiye, edible without any pre-treatment.

## Materials and Methods

Kilis Y, Butko, Hurma and Nizip Y olives were used as material. The harvest time and region of the olives are given in Table 1. The olives samples were kept in sealed plastic bags at -18°C throughout the analyses.

Pitted olives were used for the analyses, separation of the kernels from the flesh were performed manually. The moisture, oil by Soxhlet method, protein by Dumas method and reduced sugar content by Luff-Schoorl method were determined according to Susamcı et al. (2017).

Total phenolic content of the olives was determined at 750 nm by the spectrophotometer (Agilent Technologies, Cary 60, USA) (Sahan et al 2013). The total phenolic content of the olives was determined as gallic acid equivalent (mg GAE/100g olive).

Antioxidant activity of the olives was determined by DPPH and ABTS methods. The same extraction method suggested by Jemai et al.(2009) was used for both analyses. Homogenized olive paste (100g) was extracted by 80% methanol water mixture (250ml). After filtering, 100 ml of hexane was added. The extract was stored at 0°C, in dark after the evaporation, until the analysis. DPPH analysis was conducted using 0.1 ml of the extract and the results were

evaluated as % inhibition value (Sahan et al. 2013). ABTS analysis was implemented as recommended by Re et al. (1999) and Jemai et al. (2009) at 732 nm using the spectrophotometer (Agilent Technologies, Cary 60, USA). Trolox solution was used to obtain the calibration curve and the results were given as Trolox equivalent (mM Trolox/g olive).

Texture analysis of olive samples was performed using TA.XT.Plus Texture Analyzer (Stable Microsystems Ltd., UK). The flesh of 10 samples from each olive type were used and analysis was performed using a cylinder probe (2 mm diameter). The speed of the probe was 0.5 mm/s (Romeo et al. 2009).

Color of the olives were determined by Konica Minolta (CR-400, Japan) colorimeter. The color of 10 samples of each olive type measured from 3 different surfaces and  $L^*$ ,  $a^*$ ,  $b^*$  values were determined. The results were given as the average of 30 measurements (Mastralexi et al. 2019).

The method suggested by Cano-Lamadrid et al. (2015) was used for determining the fatty acid composition of the samples. The pitted olives (2g) were extracted by cyclohexane (3ml) at 4500 rpm for 30 minutes and 0.2g of this oil was used. After the addition of KOH solution, hexane and methanol was added and, 1µl from the upper phase was injected to the GC-FID (Agilent Technologies-7820A) (Garcia-Gonzalez et al. 2014). SP-2380 column (60 meters long, 0.2 µm film thickness) and Helium as the carrier gas (1ml/min flow rate) was used. The injector and detector temperatures were both set at 250°C. The column temperature was programed as follows: 170°C for 10 minutes; 1.5°C min<sup>-1</sup> to 200°C; per 8 min at 200°C.

The volatile component analysis of the samples was conducted using SPME/GC-MS. Hewlett Packard 6980 GC/ Hewlett Packard 5973 MS (Agilent Technologies, USA). After homogenizing 100 grams of olive sample, 2.5 grams of sample were taken and 300g/l 7.5 ml NaCl solution and internal standard of 100 µL 3-octanol (2 ppm) was added. Mixing at 600 rpm and heating to 60°C was applied. Extraction was performed in the block heater at 60°C for 60 minutes with SPME using DVB/CAR/PDMS fiber (Sanchez et al. 2018). DB-WAX (60 m x 0.25 mm x 0.50 µm) column was used. Carrier gas was helium at 1.0 ml/min flow rate. Injection temperature was 265°C and injection mode was splitless. The temperature program was applied as; 40°C (5 min), 3°C min<sup>-1</sup> to 195°C, 10°C min<sup>-1</sup> to 240 ° C (15 min). The electron energy was 70 eV and mass range were m/z 30-400. Volatile compounds of the olives were determined using WILEY and NIST MS libraries. The results were evaluated as µg/kg using the equation given below.

$$C_i = (A_i/A_{st}) \times C_{st}$$

$C_i$ =concentration of the volatile compound

$A_i$ =peak area of the volatile compound

$A_{st}$ =peak area of the internal standard

$C_{st}$ =concentration of the internal standard

One-Way ANOVA Post-hoc Tukey's test ( $\alpha=0.05$ ) was conducted by IBM SPSS 25 statistical program to evaluate the obtained data. Principal Component Analysis (PCA) and Cluster analysis was applied for the fatty acid composition and volatile components of olive samples to determine the differences between olive types using XLSTAT 2023 trial version.

Table 1. Olive varieties and region where olives are grown

|              | Kilis Y  | Butko    | Hurma     | Nizip Y   |
|--------------|----------|----------|-----------|-----------|
| Harvest time | December | November | November  | December  |
| City         | Kilis    | Artvin   | İzmir     | Gaziantep |
| District     | Gokdeniz | Yusufeli | Karaburun | Nizip     |

Table 2. Moisture, oil, protein, reduced sugar, total phenolic content, antioxidant activity, texture (hardness, chewiness, gumminess) and color values ( $L^*$ ,  $a^*$ ,  $b^*$ ) of naturally de-bittered olives

|  | Kilis Y                     | Butko                       | Hurma                      | Nizip Y                    |
|--|-----------------------------|-----------------------------|----------------------------|----------------------------|
| Moisture (%)                                 | 6.84±0.10 <sup>a</sup>      | 50.01±0.17 <sup>d</sup>     | 38.62±0.41 <sup>c</sup>    | 27.93±0.11 <sup>b</sup>    |
| Oil (%)                                      | 68.46±0.10 <sup>c</sup>     | 16.66±1.31 <sup>a</sup>     | 36.29±3.46 <sup>b</sup>    | 34.88±3.39 <sup>b</sup>    |
| Protein (%)                                  | 18.13±2.08 <sup>c</sup>     | 3.36±0.89 <sup>ab</sup>     | 0.19±0.08 <sup>a</sup>     | 4.46±1.04 <sup>b</sup>     |
| Reduced Sugar (%)                            | 1.01±0.04 <sup>a</sup>      | 0.91±0.03 <sup>a</sup>      | 1.00±0.04 <sup>a</sup>     | 1.08±0.04 <sup>b</sup>     |
| Total Phenolic Content (mg GAE/ 100 g olive) | 458.87±44.11 <sup>c</sup>   | 152.09±7.46 <sup>a</sup>    | 109.73±6.81 <sup>a</sup>   | 234.33±2.39 <sup>b</sup>   |
| DPPH (%inhibition)                           | 87.83±1.34 <sup>c</sup>     | 77.56±0.40 <sup>a</sup>     | 79.87±1.21 <sup>a</sup>    | 83.46±0.52 <sup>b</sup>    |
| ABTS (mM Trolox/g olive)                     | 77.67±1.91 <sup>d</sup>     | 26.03±0.58 <sup>a</sup>     | 34.9±0.51 <sup>b</sup>     | 51.62±1.14 <sup>c</sup>    |
| Hardness (g)                                 | 3668.06±808.23 <sup>b</sup> | 1464.74±662.16 <sup>a</sup> | 677.44±405.62 <sup>a</sup> | 836.66±197.48 <sup>a</sup> |
| Chewiness (gs)                               | 16.41±6.98 <sup>b</sup>     | 9.84±5.22 <sup>ab</sup>     | 5.82±2.93 <sup>a</sup>     | 3.12±1.00 <sup>a</sup>     |
| Gumminess (g)                                | 235.90±84.42 <sup>b</sup>   | 115.85±54.63 <sup>a</sup>   | 63.01±28.74 <sup>a</sup>   | 48.92±13.41 <sup>a</sup>   |
| $L^*$  | 26.75±4.31 <sup>a</sup>     | 26.14±2.43 <sup>a</sup>     | 30.69±3.76 <sup>a</sup>    | 32.05±5.62 <sup>a</sup>    |
| $a^*$  | 4.78±1.31 <sup>b</sup>      | 2.96±0.54 <sup>ab</sup>     | 5.16±1.61 <sup>b</sup>     | 2.02±0.79 <sup>a</sup>     |
| $b^*$  | 4.86±0.41 <sup>b</sup>      | 2.37±0.30 <sup>a</sup>      | 7.18±1.26 <sup>c</sup>     | 4.12±0.91 <sup>b</sup>     |

Table 3. Fatty acid composition of olive varieties (%)

| Fatty Acid Composition   | Kilis Y                   | Butko                     | Hurma                     | Nizip Y                   |
|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Myristic acid (C14:0)    | 0.015±0.004 <sup>bc</sup> | 0.009±0.001 <sup>ab</sup> | 0.008±0.001 <sup>a</sup>  | 0.017±0.001 <sup>c</sup>  |
| Palmitic acid (C16:0)    | 15.630±0.105 <sup>c</sup> | 14.243±0.046 <sup>b</sup> | 12.819±0.158 <sup>a</sup> | 16.804±0.087 <sup>d</sup> |
| Palmitoleic acid (C16:1) | 1.305±0.013 <sup>b</sup>  | 2.202±0.017 <sup>c</sup>  | 0.713±0.011 <sup>a</sup>  | 1.292±0.007 <sup>b</sup>  |
| Margaric acid (C17:0)    | 0.092±0.004 <sup>b</sup>  | 0.070±0.006 <sup>a</sup>  | 0.088±0.003 <sup>b</sup>  | 0.119±0.002 <sup>c</sup>  |
| Margoleic acid (C17:1)   | 0.140±0.007 <sup>a</sup>  | 0.156±0.003 <sup>bc</sup> | 0.153±0.131 <sup>b</sup>  | 0.166±0.004 <sup>c</sup>  |
| Stearic acid (C18:0)     | 3.480±0.006 <sup>d</sup>  | 2.880±0.018 <sup>b</sup>  | 2.582±0.013 <sup>a</sup>  | 3.386±0.003 <sup>c</sup>  |
| Oleic acid (C18:1)       | 67.097±0.086 <sup>c</sup> | 60.028±0.056 <sup>a</sup> | 69.216±0.092 <sup>d</sup> | 64.480±0.058 <sup>b</sup> |
| Linoleic acid (C18:2)    | 10.636±0.020 <sup>a</sup> | 19.152±0.027 <sup>d</sup> | 13.197±0.090 <sup>c</sup> | 12.036±0.026 <sup>b</sup> |
| Linolenic acid (C18:3)   | 0.602±0.006 <sup>c</sup>  | 0.594±0.003 <sup>a</sup>  | 0.479±0.005 <sup>b</sup>  | 0.683±0.004 <sup>d</sup>  |
| Arachidic acid (C20:0)   | 0.531±0.003 <sup>c</sup>  | 0.352±0.001 <sup>a</sup>  | 0.365±0.003 <sup>b</sup>  | 0.544±0.007 <sup>d</sup>  |
| Gadoleic acid (C20:1)    | 0.252±0.003 <sup>b</sup>  | 0.194±0.002 <sup>a</sup>  | 0.251±0.006 <sup>b</sup>  | 0.244±0.003 <sup>b</sup>  |
| Behenic acid (C22:0)     | 0.135±0.003 <sup>c</sup>  | 0.082±0.001 <sup>a</sup>  | 0.090±0.001 <sup>b</sup>  | 0.136±0.003 <sup>c</sup>  |
| Lignoceric acid (C24:0)  | 0.086±0.001 <sup>c</sup>  | 0.039±0.002 <sup>a</sup>  | 0.047±0.002 <sup>b</sup>  | 0.095±0.002 <sup>d</sup>  |

## Results and Discussion

Moisture, oil, protein, reduced sugar, total phenolic content and antioxidant activity, texture and color values of the olive samples were given in Table 2. Fatty acid composition and volatile compounds of the olive samples were given in Table 3 and 4, respectively.

The difference among the moisture of the samples was determined to be significant ( $p<0.05$ ). The difference seen in the moisture content of olives may be due to the de-bittering conditions of the samples; Butko and Hurma olives de-bittered on the tree, whereas Kilis Y and Nizip Yağlık olives de-bittered on the ground. It was also observed that the surface of Kilis Y and Nizip Y olives are not smooth, but the surface of the other samples is plumpy and smooth. The oil contents of Kilis Y and Butko olives were determined to be statistically different from Hurma and Nizip Y samples ( $p<0.05$ ). The oil content of Kilis Y was the highest with 68.46% and the oil content of Butko olive was the lowest with 16.66%. No statistical difference was determined for protein content of Butko, Hurma and Nizip Y, but Kilis Y olive (18.13±2.08%) was determined

to have the highest protein content. The reason for the oil and protein content of Kilis Y to be higher than the other olives may be that the analysis results were not given on dry basis and the dry matter of Kilis Y olive was higher than the other three olive varieties. No statistically significant difference was determined among the reducing sugar contents of 4 olive samples ( $p<0.05$ ). Susamci et al. (2017) reported in their study that the oil content of Hurma olives varies between 33.83-42.26% depending on the region where they are collected. Aktas et al. (2014) found the oil content of Hurma olives between 14.57-61.52%. Gavriilidou and Boskou (1993) stated in their study that unprocessed olive grains had an average protein content of 1.3%, while Susamci et al. (2017) found the protein content of Hurma olives between 0.89-1.45%. When the results were compared with the literature, the Hurma olive determined to have a lower protein content while the Kilis Y olive was determined to have a higher protein content than the literature. This difference can be due to climatic change or regional differences.

Table 4. Volatile compounds of olive varieties ( $\mu\text{g}/\text{kg}$ )

| Volatile Compounds                | Kilis Y                             | Butko                               | Hurma                               | Nizip Y                             |
|-----------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| 1-Hexanol                         | 9026.63 $\pm$ 1047.10 <sup>b</sup>  | 20741.12 $\pm$ 1013.38 <sup>c</sup> | nd                                  | 6697.26 $\pm$ 650.98 <sup>a</sup>   |
| 1-Heptanol                        | 1205.489 $\pm$ 84.87 <sup>a</sup>   | 2400.79 $\pm$ 34.57 <sup>b</sup>    | nd                                  | 2060.56 $\pm$ 405.72 <sup>b</sup>   |
| 1-Octanol                         | 3777.24 $\pm$ 313.11 <sup>a</sup>   | nd                                  | nd                                  | 6504.38 $\pm$ 1299.66 <sup>b</sup>  |
| 1,6-Octadiene-3-ol, 3,7-dimethyl- | nd                                  | 26703.42 $\pm$ 467.21 <sup>a</sup>  | nd                                  | nd                                  |
| 1-Butanol, 2-methyl-              | nd                                  | nd                                  | nd                                  | 3722.20 $\pm$ 369.30 <sup>a</sup>   |
| 1-Butanol, 3-methyl-              | nd                                  | nd                                  | 3455.31 $\pm$ 183.23 <sup>a</sup>   | nd                                  |
| 1-Octenol                         | nd                                  | nd                                  | 2735.23 $\pm$ 97.94 <sup>a</sup>    | nd                                  |
| 1-Pentanol                        | nd                                  | 9421.85 $\pm$ 359.38 <sup>a</sup>   | nd                                  | nd                                  |
| 1-octen-3-ol                      | 6513.92 $\pm$ 398.51 <sup>a</sup>   | nd                                  | 6271.51 $\pm$ 184.21 <sup>a</sup>   | 2174.06 $\pm$ 423.10 <sup>a</sup>   |
| 2-Heptenal, (Z)                   | 16197.26 $\pm$ 1703.06 <sup>b</sup> | 6814.12 $\pm$ 443.92 <sup>a</sup>   | 14533.96 $\pm$ 712.44 <sup>b</sup>  | 8855.36 $\pm$ 2047.98 <sup>a</sup>  |
| Acetic acid                       | 5004.50 $\pm$ 235.75 <sup>a</sup>   | 10186.75 $\pm$ 345.39 <sup>a</sup>  | 33273.01 $\pm$ 5282.90 <sup>b</sup> | 7426.13 $\pm$ 268.88 <sup>a</sup>   |
| Benzaldehyde                      | 8018.20 $\pm$ 499.68 <sup>a</sup>   | 16193.77 $\pm$ 255.19 <sup>b</sup>  | 57926.45 $\pm$ 3698.47 <sup>c</sup> | 8782.28 $\pm$ 1802.70 <sup>a</sup>  |
| D-Limonene                        | 80974.51 $\pm$ 6989.55 <sup>b</sup> | 25991.01 $\pm$ 791.81 <sup>a</sup>  | 74018.82 $\pm$ 1459.91 <sup>b</sup> | 18782.06 $\pm$ 3287.37 <sup>a</sup> |
| Hexanal                           | 26219.82 $\pm$ 3045.57 <sup>b</sup> | 36917.22 $\pm$ 986.70 <sup>c</sup>  | 22916.06 $\pm$ 337.32 <sup>b</sup>  | 15527.97 $\pm$ 1416.64 <sup>a</sup> |
| Hexanoic acid                     | 14194.06 $\pm$ 1586.92 <sup>b</sup> | nd                                  | 14810.33 $\pm$ 2282.80 <sup>b</sup> | 5792.00 $\pm$ 543.69 <sup>b</sup>   |
| Nonanal                           | 73293.07 $\pm$ 4722.21 <sup>c</sup> | 7596.572 $\pm$ 146.27 <sup>a</sup>  | 18649.65 $\pm$ 1409.32 <sup>b</sup> | 14392.34 $\pm$ 1816.09 <sup>b</sup> |
| Octanoic acid                     | 3023.48 $\pm$ 291.22 <sup>a</sup>   | 11852.27 $\pm$ 209.92 <sup>c</sup>  | 4236.205 $\pm$ 90.68 <sup>b</sup>   | nd                                  |
| Phenol                            | 1660.34 $\pm$ 162.42 <sup>a</sup>   | 3368.354 $\pm$ 31.08 <sup>b</sup>   | 5920.741 $\pm$ 135.76 <sup>c</sup>  | nd                                  |
| Phenylethyl Alcohol               | 7088.10 $\pm$ 654.29 <sup>a</sup>   | 21646.98 $\pm$ 330.19 <sup>b</sup>  | 83491.61 $\pm$ 7849.33 <sup>d</sup> | 40152.39 $\pm$ 5436.55 <sup>c</sup> |
| $\delta$ -Octalactone             | 1674.07 $\pm$ 164.61 <sup>a</sup>   | 3523.56 $\pm$ 85.63 <sup>b</sup>    | nd                                  | 9861.74 $\pm$ 1185.09 <sup>c</sup>  |
| 2-Nonenal, (E)-                   | nd                                  | 5671.83 $\pm$ 440.17 <sup>a</sup>   | nd                                  | nd                                  |
| 2-Octenal, (E)-                   | 3404.81 $\pm$ 274.41 <sup>a</sup>   | 8485.99 $\pm$ 204.80 <sup>b</sup>   | nd                                  | nd                                  |
| 3-Octanone                        | 1697.31 $\pm$ 111.6 <sup>a</sup>    | nd                                  | nd                                  | nd                                  |
| 5-Hepten-2-one, 6-methyl-         | 15552.95 $\pm$ 1070.20 <sup>a</sup> | 34096.21 $\pm$ 627.49 <sup>b</sup>  | nd                                  | nd                                  |
| 6-Hepten-1-ol, 2-methyl-          | 1165.10 $\pm$ 99.10 <sup>a</sup>    | nd                                  | nd                                  | nd                                  |
| Benzene acetaldehyde              | nd                                  | nd                                  | 9395.73 $\pm$ 342.24 <sup>a</sup>   | nd                                  |
| Benzyl alcohol                    | nd                                  | nd                                  | 19718.01 $\pm$ 3304.84 <sup>a</sup> | nd                                  |
| Butanal, 3-methyl-                | nd                                  | 33133.65 $\pm$ 825.50 <sup>a</sup>  | nd                                  | nd                                  |
| Butanoic acid, 3-methyl-          | nd                                  | nd                                  | nd                                  | 10130.94 $\pm$ 1128.32 <sup>a</sup> |
| Cymene                            | 12414.83 $\pm$ 1059.95 <sup>a</sup> | nd                                  | nd                                  | nd                                  |
| Furan, 2-pentyl-                  | nd                                  | 5414.20 $\pm$ 497.47 <sup>a</sup>   | 6136.873 $\pm$ 271.87 <sup>b</sup>  | nd                                  |
| Heptanal                          | nd                                  | 2905.02 $\pm$ 298.48 <sup>b</sup>   | nd                                  | nd                                  |
| Nonanoic acid                     | 3060.66 $\pm$ 192.08 <sup>a</sup>   | nd                                  | nd                                  | nd                                  |
| Octanal                           | 10380.23 $\pm$ 811.09 <sup>b</sup>  | nd                                  | nd                                  | 4234.70 $\pm$ 1059.29 <sup>a</sup>  |
| Octanoic acid, methyl ester       | nd                                  | 5275.51 $\pm$ 10.65 <sup>a</sup>    | nd                                  | nd                                  |
| p-Cresol                          | nd                                  | nd                                  | nd                                  | 2020.91 $\pm$ 56.28 <sup>b</sup>    |
| Pentanoic acid                    | 2762.42 $\pm$ 238.37 <sup>a</sup>   | nd                                  | nd                                  | nd                                  |
| $\gamma$ -Nonalactone             | nd                                  | 7848.18 $\pm$ 593.80 <sup>a</sup>   | nd                                  | nd                                  |
| $\gamma$ -Octalactone             | 2526.97 $\pm$ 207.64 <sup>a</sup>   | nd                                  | nd                                  | nd                                  |

The highest total phenolic content was determined for Kilis Y olive (458.87 $\pm$ 44.11 mg GAE/100g olive), the lowest phenolic content (109.73 $\pm$ 6.81 mg GAE/100g olive) was determined for the Hurma sample. The highest inhibition was obtained for Kilis Y olive (87%), and the lowest inhibition was obtained for Butko (77.56%) using DPPH method. Similar results were obtained with ABTS method; Kilis Y was determined to have the highest value and Butko the lowest value, 77.67 mM Trolox/g olive and 26.03 mM Trolox/g olive, respectively. However, according to the results of the ABTS method statistical differences were determined for the olive samples, but according to the DPPH method significant difference was not detected between Butko and Hurma olives ( $p < 0.05$ ). Aktas et al. (2014) determined the total phenolic content and phenolic components of the Hurma olives harvest in two different seasons. As a result of the study, the total phenolic content of Hurma olives in the first season was determined as 337.68-649.64 mg GAE/100g olives, while the total phenolic content of Hurma olives collected in the

second harvest period was determined as 29.21-344.34 mg GAE/100g olives. Jemai et al. (2009) found that the total phenolic content of Dhokar olives, which grow in Tunisia and can be consumed without pre-treatment, was between 508 - 768 mg GAE/100 g olives in different harvest periods. Sahan et al. (2013) reduced the bitterness of Gemlik olives by using California type, Spanish type, dry salting method and soaking in brine. The highest antioxidant capacity was detected in black olives processed with brine (744.27  $\mu\text{mol}$  Trolox/g), and the green olives processed using Spanish type demonstrated the lowest antioxidant capacity (735.68  $\mu\text{mol}$  Trolox/g) by DPPH method. The antioxidant capacity of Dhokar olives was determined by the ABTS method according to harvest time by Jemai et al. (2009) and the lowest antioxidant capacity of olives was determined as 0.83 mM Trolox/g and the highest as 1.65 mM Trolox/g.

Kilis Y olive was determined to have the highest hardness (3668.06 g) and gumminess (235.90 g) values ( $p < 0.05$ ). Kilis Y olive had the highest chewiness value and

Nizip Y olive had the lowest value, 16.41 (gs) and 3.12 (gs), respectively. No significant difference was determined between Hurma and Nizip Y olive samples, while Kilis Y olives differed from these two olive cultivars statistically ( $p < 0.05$ ). Garcia-Serrano et al. (2020) reduced the bitterness of Manzanilla and Hojiblanca olive varieties by treating them with NaOH and KOH solutions. In Manzanilla olive variety, hardness was determined as 5.3 when NaOH was used and 5.2 when KOH was used. The hardness of the Hojiblanca olive variety was found to be the same (7.9) for both NaOH and KOH solution.

No statistical difference was determined among the  $L^*$  values of the olive samples ( $p < 0.05$ ). The highest  $a^*$  value was 5.16 for Hurma olive, and the lowest value was 2.02 for Nizip Y olive ( $p < 0.05$ ). Considering the  $b^*$  values of the olive samples, no statistical difference was determined between Kilis Y and Nizip Y olives, but Butko and Hurma olives differed statistically ( $p < 0.05$ ). Diarte et. Al (2021) determined the  $L^*$ ,  $a^*$  and  $b^*$  values of nine local olive varieties specific to Spain according to their degree of ripening. The  $L^*$  values of the Arbequina, Argudell, Empeltre, Farga, Manzanilla, Marfil, Morrut, Picual olives were determined between 16.35-66.39. The  $a^*$  and  $b^*$  values were determined between -3.27-11.01 and -1.85-20.79, respectively.

Considering the fatty acid composition, a significant difference was determined among the olive varieties for palmitic, stearic, oleic, linoleic, arachidic and lignoceric acid content ( $p < 0.05$ ). Oleic acid was determined to be highest in Hurma, linoleic acid was determined to be highest in Butko samples. Kilis Y, Hurma and Nizip Y olives were statistically similar in terms of gadoleic acid content, Kilis Y and Nizip Y olives were statistically similar in terms of palmitoleic acid and behenic acid content, Kilis Y and Hurma olives were statistically similar in terms of margaric acid content, Butko and Hurma olives were statistically similar in terms of margoleic acid content ( $p < 0.05$ ). Sahan et al. (2013) processed Gemlik type olive varieties by Spanish Type, California Type, and dry salt. No statistical difference was determined for oleic acid content the four olive varieties depending on the process. Aktas et al. (2014) examined the transformation of the Erkence olive variety into the Hurma olive for two different seasons. In Hurma olives collected in the 2011/2012 and 2012/2013 seasons, stearic, oleic and

linoleic acid were determined between 1.67-5.22%, 60.30-72%, and 11.78-19.97%, respectively.

Cluster analysis was applied using the fatty acid composition of the olive samples to evaluate the relationship between the olive samples. Two main groups were determined; Kilis Y, Nizip Y and Hurma formed first cluster, Butko formed the second cluster, and a subgroup was formed under the first group (Figure 1).

Principal Component Analysis (PCA) was applied to specify the fatty acids contributing to the clusters. Two main components were obtained, F1 (60.49%) and F2 (29.77%) to explain the total variation (Figure 2). The biplot diagram showed that linoleic acid and palmitoleic acid were effective in discriminating Butko olive. Linolenic, palmitic, stearic, myristic, arachidic, lignoceric and behenic acid were determined to be the differentiating fatty acids for Kilis Y and Nizip Y forming the subgroup (Figure 2).

Considering the volatile compounds of the olive samples, 24 volatiles in Kilis Y, 22 volatiles in Butko, 16 volatiles in Hurma and 17 volatiles in Nizip Y were determined (Table 4). The common volatile compounds for the olive samples were as follows; 2-Heptenal (Z), acetic acid, benzaldehyde, d-limonene, hexanal, nonanal and phenylethyl alcohol.

Mikrou et al. (2021) investigated the effects of olive processing technologies, olive variety and the region where olives grow on the volatile compounds of olives. It was stated that a total of 120 types of volatiles were determined, and the detected volatiles differed according to the analyzed olive varieties and growing regions as a result of the PCA. In addition, while all olive samples have higher contents of esters, alcohols and acids, volatile phenol compounds were predominantly detected only in Halkidiki green olives processed with the Spanish type processing technology. Sanchez et al. (2020) in their study, processed Manzanilla and Hojiblanca olives, collected from Spain, with Greek type processing technology, and the volatile components of the olive varieties ready for consumption were determined by the HS-SPME method. It was reported that 74 types of volatile compounds were detected. As the result of PCA no significant difference was detected in terms of olive variety and samples of the same olive variety collected from different regions.

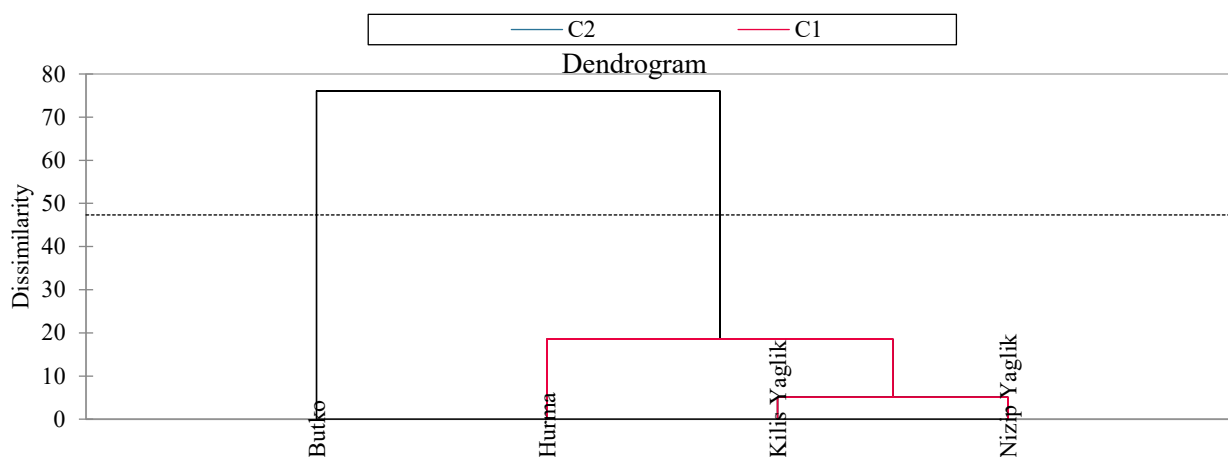


Figure 1. Cluster diagram for the fatty acid composition

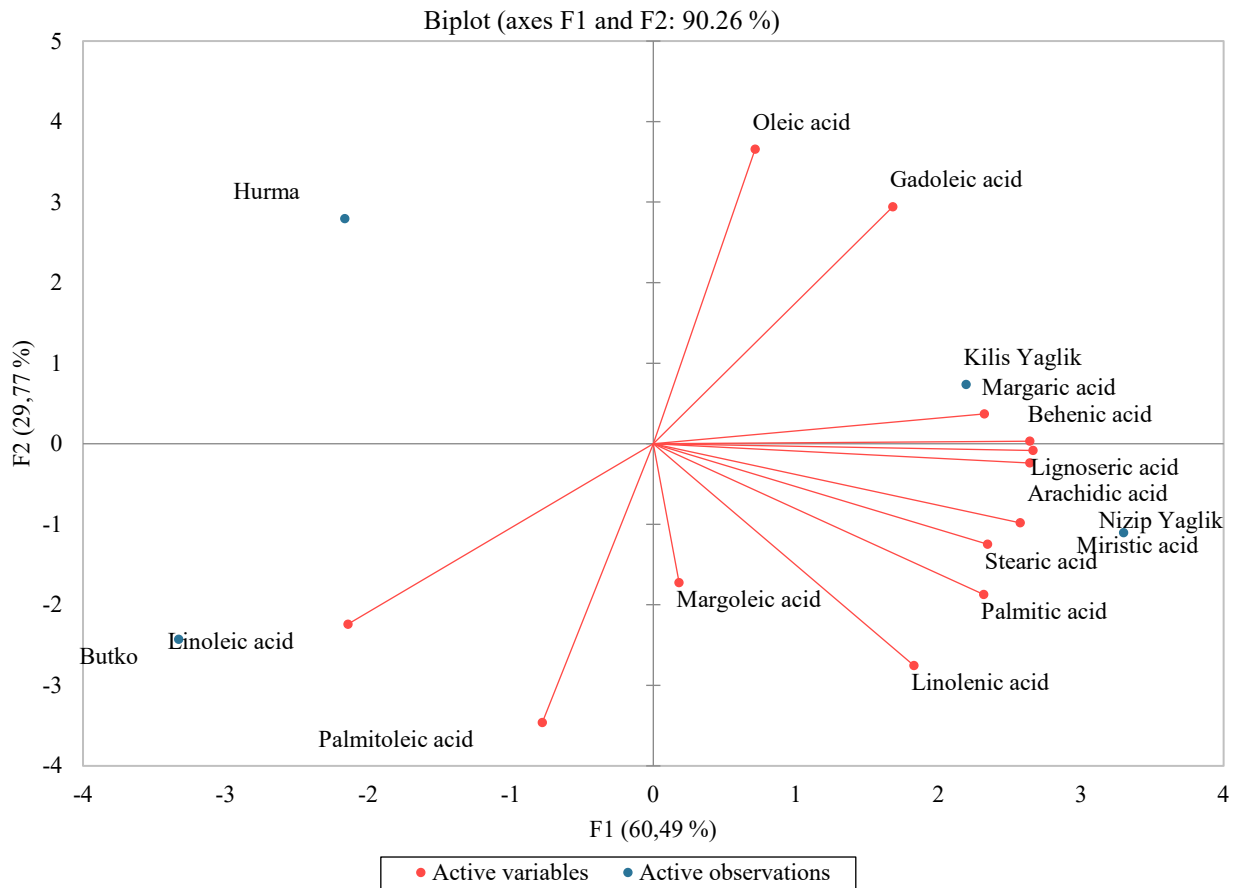


Figure 2. PCA for the fatty acid composition

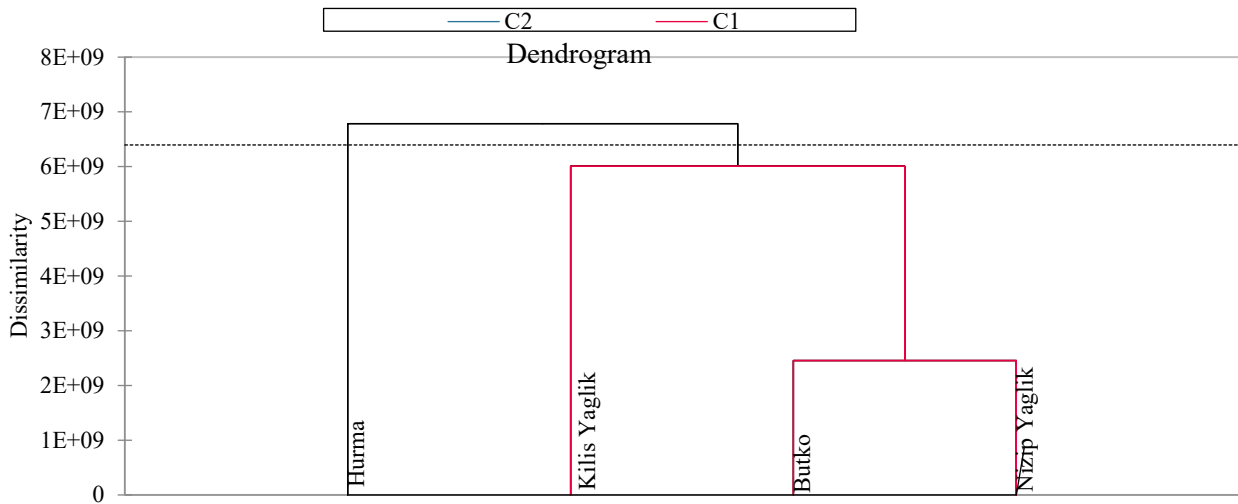


Figure 3. Cluster diagram for the volatile compounds

Cluster analysis was applied using the volatile compounds of the olives to evaluate the relationship between the olive samples. Two main groups were determined; Kilis Y, Butko, Nizip Y formed one cluster and Hurma formed the second cluster, and a subgroup was formed under the first group (Figure 3).

PCA was applied to specify the volatiles contributing to the clusters. Two main components was obtained, F1 (41.94%) and F2 (31.94%) to explain the total variation (Figure 4). 1-butanol -3 methyl, 1-octenol, acetic acid, benzaldehyde, phenol, phenylethyl alcohol, benzene acetaldehyde and benzyl alcohol were determined as

discriminating volatiles for Hurma olive. 1-octanol, nonanal, 3-octanone, 6-heptene 1-ol,2-methyl, cymene, nonanoic acid, octanal, pentaic acid and  $\gamma$ -octalactone were determined as the characteristic volatiles for Kilis Y olives. The subgroup formed by Butko and Nizip Y olives were characterized by 1-heptanol, 2-heptenal, (Z) and D-limonene.

Naturally de-bittered olives are not widely known and consumed all over the world. In this study four naturally debittered olives were evaluated in terms of some important characteristics.



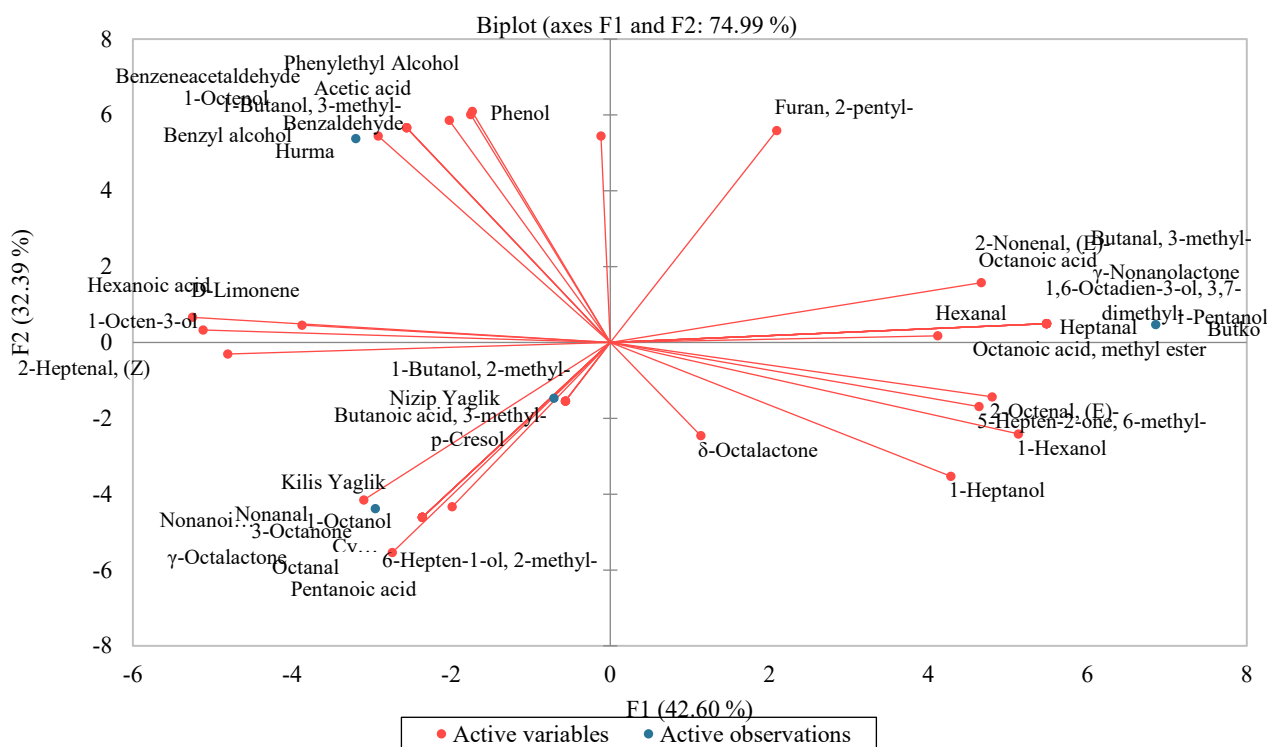


Figure 4. PCA for the volatile compounds

Hurma, the softest olive, which is the most known among the olive varieties consumed without any pre-treatment were low in protein content and total phenolic content followed by Butko olive. Kilis Y olive, followed by Nizip Y were determined to have the highest total phenolic content and the highest antioxidant capacity. Oleic acid content of Hurma and the linoleic acid content of Butko were distinguishing fatty acids. Kilis Y olive was rich in volatile compounds, followed by Butko, Nizip Y and Hurma. As stated, before these naturally debittered olives were different from the processed olives in terms of taste, texture and bitterness and the consumer is not accustomed to this difference. So, it is important to evaluate these olives by sensory evaluation and determine consumer preferences in future studies.

## Declarations

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### Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by YMB. The manuscript was drafted by YMB and edited and commented by YE. All authors read and approved the final manuscript.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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