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# Phenol content and β-glucosidase activity during the ripening period of olive fruits (Erkence cv.) from different locations

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**SUMMARY:** In the present study, Erkence olive fruits were examined during two harvest periods and in two orchards close to the sea at low altitude (A) and far from the sea at higher altitude (B). Olives from the sea-facing northeast (NE) and land-facing southwest (SW) orientations of the trees were analyzed in terms of maturity index, total phenol content, phenolic compounds, β-glucosidase enzyme activity, total protein content and specific enzyme activity. Climate data were collected from the trees and from the meteorology station. The maturity index, β-glucosidase enzyme activity, total protein content and specific enzyme activity of the olives increased during the ripening period. On the other hand, the total phenol content, oleuropein and other phenolic compound contents in the olives decreased. The altitude and location of the fruits on the tree affected the ripening process. Lower altitude and sea-facing side of the tree resulted in an increased enzyme activity, thus aiding in the ripening process.

***KEYWORDS:*** *Altitude; β-glucosidase; Climate; Enzyme; Olive; Phenol.*

**RESUMEN:** ***Contenido de fenoles y actividad de β-glucosidasa durante el periodo de maduración de frutos de olivo (Erkence cv.) presentes en diferentes localizaciones.*** En el presente estudio, los frutos de aceitunas Erkence se examinaron durante dos períodos de cosecha y se llevaron a cabo en dos huertos cerca del mar y de baja altitud (A) y lejos del mar y de mayor altitud (B). Se analizaron las aceitunas en las direcciones de los árboles orientadas al mar noreste (NE) y suroeste (SW) en términos de índice de madurez, contenido de fenoles totales, compuestos fenólicos, actividad de la enzima β-glucosidasa, contenido de proteína total y actividad enzimática específica. Los datos climáticos se recogieron en el árbol y en la estación meteorológica. El índice de madurez, la actividad de la enzima β-glucosidasa, el contenido de proteínas totales y la actividad enzimática específica de las aceitunas aumentaron durante el período de maduración. Por otro lado, el contenido de fenoles totales, oleuropeína y otros compuestos fenólicos de las aceitunas disminuyó. La altitud y la ubicación de los frutos en el árbol afectaron el proceso de maduración, las altitudes más bajas y el lado del árbol que mira al mar aumentaron la actividad enzimática, ayudando a la maduración.

***PALABRAS CLAVE:*** *Altitud; β-glucosidasa; Clima; Enzima; Fenol; Olivo.*

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## 1. INTRODUCTION

Olives are classified as sub-tropical fruits. In the daily life of people living in the Mediterranean Basin, it is a special fruit that has been grown for centuries. Due to its natural bitterness, fruits cannot be directly consumed without processing. Different green and black table olive processing methods are available around the world. These processing methods can be roughly divided into two, which consist of removing the bitterness by treating the fruit with a dilute solution of NaOH and another one which involves the direct brining of the fruit without any alkaline treatment (Ramírez *et al*., 2015). Raw olives to be used for this purpose may be affected by geographical location and growth conditions. This may affect olive processing conditions. It was reported that oleuropein concentration depends on the geographical origin of the fruit rather than growing conditions (Gutierrez-Rosales *et al*., 2012). During ripening, many changes occur in the composition of the olive fruit, including increase in oil content and a decreased phenolic content (Menz and Vriesekoop, 2010). Microclimates may cause differences in the fruit and the oil composition of olives. Chilling injury may occur in olives exposed to low temperatures. Parkin *et al*. (1989) drew attention to the lipid peroxidation that occurs during low temperature stress. Lower levels of secoiridoid phenolic compounds have been reported in olive oils obtained from fruits damaged by frost (García-Vico *et al*., 2017). The position of the fruit on the tree can affect the physicochemical structure of the fruit. Many factors such as the direction of the fruits towards the sun (Gómez-Del-Campo and García, 2012), the amount of light on the fruit (Tombesi *et al*., 1998), and the amount of fruit on the tree affect the structure of olive fruit and olive oil.

Türkiye has over ninety cultivars of olives and they are spread in coastal areas from north to south. The Mediterranean climate is generally dominant in these regions. Erkence is an important olive cultivar that is considered both for oil production and table consumption in and around İzmir. It develops quite vigorously under good maintenance conditions. It is considered a medium-yielding variety with a strong tendency to alternate bearing. In Türkiye, untreated olives which are edible straight from the tree are available. This phenomenon, seen in the peninsula of Karaburun and on cv. Erkence, is called 'natural de-bittering', and locally called "Hurma". When the olives are de-bittered, they become dark-brown and wrinkled. The Hurma olive is a valuable product for producers living in the peninsula (Susamci *et al.*, 2016). It was reported that the polymers formed during the enzymatic oxidation of oleuropein must be responsible for the dark-brown color and Hurma olives lose their bitterness during maturation on the tree due to the enzymatic oxidation of oleuropein (Susamci *et al*., 2017). Not only oleuropein, but also all the other phenolic compounds are affected in the natural de-bittering process (Aktas *et al*., 2014). Similar de-bittering phenomena have been reported for different regions in other countries, such as Greece and Tunisia (Rigane *et al*., 2011).

The Karaburun Peninsula, located in western Türkiye, is a region where old olive trees are present. The northern winds dominate this region. This study was planned to investigate the effect of climate and location on the phenolic composition and β-glucosidase activity of Erkence olives. For this purpose, the regular fruits of Erkence cultivar were observed during the ripening period, in two different orchards in the Karaburun Peninsula. Some structural properties of Erkence fruits were also examined. To research the local climate effect on ripening, various climate data in the canopy were measured.

## 2. MATERIALS AND METHODS

### 2.1. Olive samples

The study was conducted in seasons 2014/15 and 2015/16 at two orchards located in western Türkiye. Orchard A lies at sea level (100 m distance from the sea, latitude: 38º 32´ 56´´N, longitude: 26º 34´ 56´´E) and orchard B lies at an altitude of 174 m above sea level (1.4 km distance from the sea, latitude: 38º 33´ 42´´ N, longitude: 26º 33´ 5´´E). The canopy of the trees selected in September 2014 was determined by a compass and divided into two parts, namely northeast (NE, facing the sea) and southwestern (SW, inland). Erkence olives were picked manually from the NE and SW orientations of the trees during the maturation period from September to December in 2014/15 and from October to November in 2015/16. Three trees per orchard were selected as replicates. The fruit samples were transferred to the laboratory in PE bags, and the maturity

index (MI) of olive fruits was determined. After the removal of the stones, the olives were freeze-dried and stored at -20 ºC for further analyses.

### 2.2. Collection of climate data

Climate sensors were placed in the two orientations of the tree and the frequency of measurement was set at fifteen minutes. The data were evaluated with HOBOware Pro Graphing & Analysis Software (Version 3, Onset Computer Corporation, Bourne, MA) software. A HOBO Data Logger U12-013 Temp/RH/2 External was used for air temperature (AT) and relative humidity (RH) measurements. Daily rainfall and wind records were obtained from the automatic meteorological observation station of Karaburun (latitude: 38º 38′ 24′′ N, longitude: 26º 30′ 29′′ E) from the General Directorate of Meteorology. Since the northern winds are dominant in the region, the north-facing orientation was shown separately from the other orientations in the wind graphs.

### 2.3. Maturity index

One hundred olive fruits were selected randomly and classified according to their color (green, black, reddish-brown, etc.) and the number of fruits in each group was determined. The number of fruits per class was multiplied by the coefficient of that class and the maturity index (MI) was calculated according to the formula given by Morello *et al*. (2005).

### 2.4. Total phenolic compounds

Total phenolic compound content was determined by the method given by Hrncirik and Fritsche (2004). A calibration curve was obtained using a caffeic acid stock solution. One g of homogenized olive sample was mixed with 5 mL of a methanol:water (60:40, v/v) solution for 2 min. It was then centrifuged at 3500 rpm for 10 min. The supernatant was taken into a 10 mL sample tube and filtered through a coarse filter paper. The precipitate was washed with a methanol-water solution, centrifuged and filtered again. The filtered sample was made up to 10 mL with distilled water. Once the sample was well mixed, 0.1 mL was taken into a 50 mL volumetric flask with 5 mL of distilled water and 0.5 mL Folin-Ciocalteu reagent. The solution was left to stand for 3 min and 1 mL of a 36% (w/v) $Na_2CO_3$ solution was added. Then, the sample solution was made up to the volume with distilled water. After the solution was kept in the dark for 2 h, the absorbances were read at 725 nm. The concentration values that correspond to the absorbances were determined from the calibration curve for each sample and the results per fresh weight were calculated as mg CAE $100 \cdot g^{-1}$ considering the dilution factor.

### 2.5. Analysis of phenolic compounds

Phenolic compounds were determined by the method of Susamci *et al*. (2017) with some modifications. Aproximately 1.5 g of olives were mixed with 30 mL of dimethylsulfoxide (DMSO) in a measuring cylinder and homogenized at 11000 rpm with Ultra-turrax for several minutes and then left for 30 min in order for the phenolic compounds to dissolve. A small amount of the sample from the clear part of the solution was taken and centrifuged for 5 minutes at 9000 rpm and subsequently filtered into another tube through a 0.22 µm filter. 0.25 mL of the supernatant were diluted with 0.5 mL of DMSO and 0.25 mL of 0.2 mM syringic acid prepared in DMSO (internal standard). 20 µL of the mixture were injected into the High-Performance Liquid Chromatograph (HPLC). The HPLC system consisted of a 1260 vial sampler (Agilent Technologies), a 1100 (Quat. Pump) (Hewlett Packard), and a 1200 diode array detector (Agilent Technologies). A Phenomenex Luna 5 µm C18 100A 250 x 4.6 mm column was also used. Separation was achieved using an elution gradient with an initial composition of 90% (v/v) water (pH adjusted to 2.7 with phosphoric acid) and 10% (v/v) methanol. The concentration of the second solvent was increased to 30, 40, 50, 60, 70 and 100% (v/v) in 68 minutes. A flow rate of 1 mL/min and a temperature of 35 ºC were also used. The chromatograms were recorded at 280, 330, 444 nm for phenolic compounds. The results were calculated as $mg \cdot kg^{-1}$ fresh weight.

### 2.6. Extraction of β-glucosidase enzyme from olive samples

β-glucosidase was extracted from the olives using a newly modified version of existing methods (Lopez-Huertas and del Río, 2014). 25 mL of 100 mM, pH 9.0 borate buffer 6% (w/v) polyvinylpyrrolidone (PVP), 1% (v/v) β-mercapto ethanol and 1 mM phenyl methane sulfonic fluoride (PMSF) were

added to 4-5 g olive samples. They were then homogenized in an ice bath twice for 30 seconds with ultraturrax. The homogenate was mixed in an ice bath for 15-30 minutes using an orbital shaker (200 rpm). After that, it was centrifuged at 10.000 rpm for 30 minutes at 40 °C. The oil phase formed on top was carefully separated and discarded with a pasteur pipette. The pellet was also separated from the centrifugate and discarded. The aqueous supernatant phase was filtered through filter paper and the filtrate volume was recorded. The enzyme activity and protein content in the filtrate were determined spectrophotometrically. The enzymatic activity, protein content and specific activities for all samples were calculated.

### 2.7. Determination of β-glucosidase enzyme activity

β-glucosidase activity was determined using p-nitrophenyl-β-D-glucopyranoside (pNPG), as synthetic substrate of the enzyme (Hu *et al*., 2007). The procedure focuses on the determination of p-nitrophenol released from the alkali mixture.

The reaction mixture consisting of 520 µL of sodium citrate buffer (50 mM; pH 5.0), 40 µL of 10 mM pNPG (pH 5.0 and 50 mM prepared in citrate buffer) and 40 µL of enzyme solution was incubated at 50 °C for 20 minutes in a water shaking bath. After incubation, the reaction was stopped by the addition of 0.25 M sodium hydroxide. The amount of liberated p-nitrophenol was measured at 405 nm. A blank contained 40 µL sodium citrate buffer (50 mM, pH 5.0) instead of the enzyme solution. For the calculation of the enzyme activity, the standard graph prepared with free p-nitrophenol in the concentration range of 0.02-0.25 µmol was used. One unit (1 U) of enzyme activity was defined as the amount of enzyme which released 1 µmol of p-nitrophenol from pNPG per minute at pH 5.0 and 50 °C.

### 2.8. Determination of protein content

The protein concentrations in the enzyme extract were measured according to the Bradford method (Bradford, 1976) which uses the Coomassie Brilliant Blue G-250 as dye. Bovine serum albumin (BSA) was used as protein standard to draw the calibration graph. The reaction mixture contained 0.1 mL protein solution and 2 mL Bradford reagent. All tubes were kept at room temperature for 10 minutes and then the absorbances of each sample were read at 595 nm using a spectrometer. The enzyme protein contents were given as means of triplicate assays. Specific enzyme activity was expressed as $U \cdot mg^{-1}$ protein.

### 2.9. Statistics

Differences were determined by analysis of variance according to the split-split plot design with three replicates where week was the main plot, orchard was the sub plot, and orientation was the sub-sub plot. The Fisher's Least Significant Difference (LSD) test was used to determine the differences between the means ($p < 0.05$). Orchards, differences between orientations, were compared with the T-test.

## 3. RESULTS AND DISCUSSION

The region where the olive samples were taken is an area that overlooks the sea on the northeast side of the Karaburun Peninsula and is close to the sea. The climate in the region is generally defined as a typical Mediterranean climate, which occurs more commonly in Türkiye´s coastal areas. In this respect, it is a suitable region for olive cultivation, and it shows this with the existing tree presence and olive cultivation.

Daily air temperature (AT) and relative humidity (RH) values recorded for trees in orchards A and B for the 2014/15 and 2015/16 seasons are shown in Figure 1. It was observed that the AT changes in the 2014/15 season were greater than the 2015/16 season. The AT values in orchards A and B showed similar changes. There was no difference in AT between NE and SW orientations of the trees. After the temperature drops, the AT rose again within a few days, and this change created sudden temperature differences in short periods of one to two days. At this point, we can focus on the dew point formed by the joint effect of AT and RH. In this region, which is close to the sea and has high humidity, the level of humidity in the environment plays an important role in the formation of the dew point.

RH in the air increased in both seasons after September. RH varied between 40 and 97% in the 2014/15 season (Figures 1a and 1b) and between 50 and 96% in the 2015/16 season (Figures 1c and 1d). Although similar changes were observed in the RH values in the A and B orchards on the same dates in

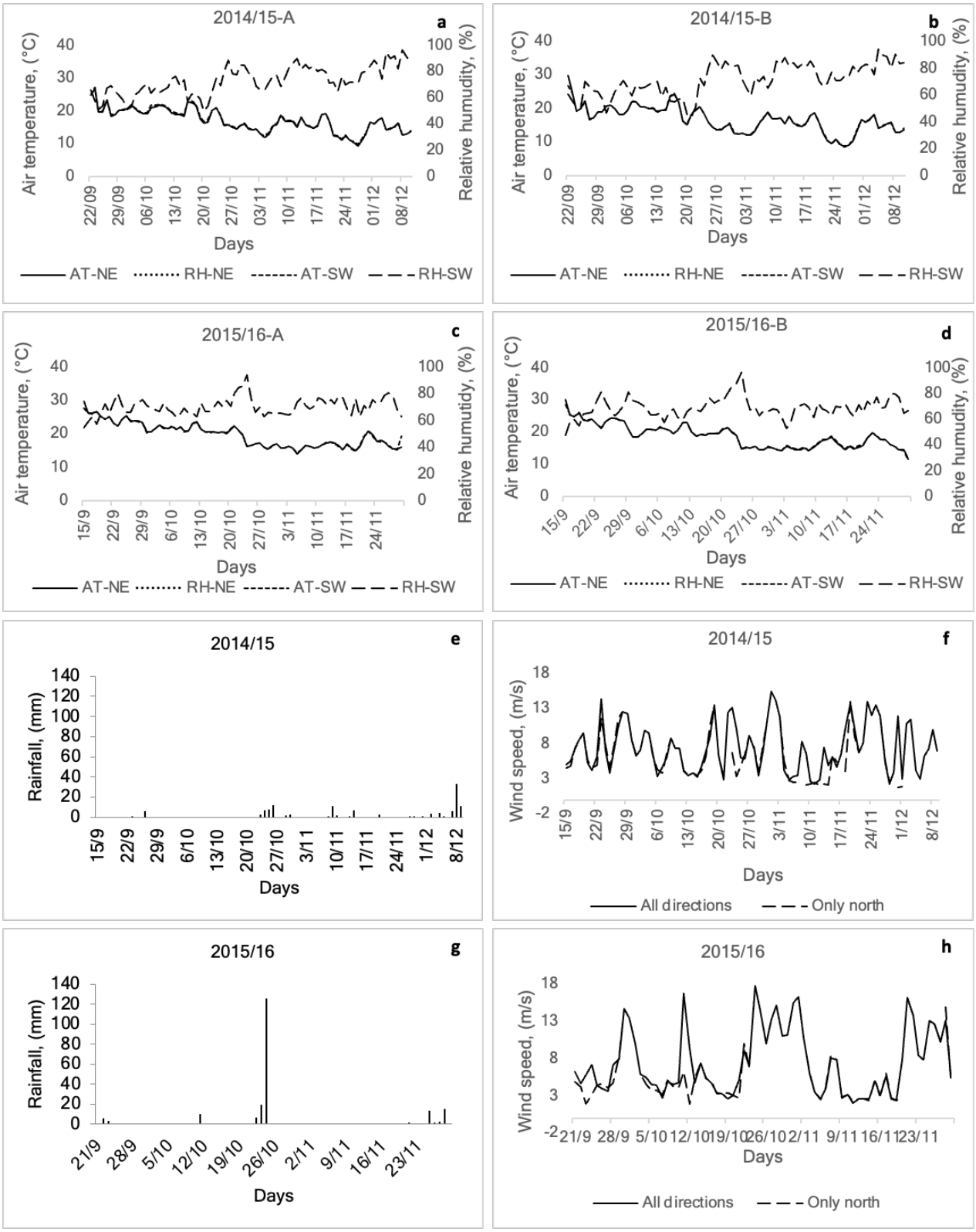


**Figure 1**. Climate data from both orchards during 2014/15 and 2015/16. Daily average air temperature (AT) and relative humidity (RH) recorded on the tree in different directions (NE: Northeast SW: Southwest) of the tree during the 2014/15 season (a) orchard A and (b) orchard B; and during the 2015/16 season (c) orchard A and (d) orchard B and (e) daily average rainfall and (f) wind speeds from the meteorological stations during the 2014/15 season; and (g) daily average rainfall and (h) wind speeds from the meteorological stations during the 2015/16 season.

both seasons, the RH in the B orchard were lower than in the A orchard on some dates. The fact that orchard B is farther from the sea may have caused this difference. There was no difference in RH between NE and SW orientations of the trees. It can be said that the 2015/16 season was rather dry compared to the 2014/15 season. The recorded daily maximum rainfall was around 30 mm in the 2014/15 season (Figure 1e) and 120 mm in 2015/16 season (Figure 1g). When compared, it can be seen that rainfall contributed significantly to the RH. The prevailing winds in the region were north winds. Wind speeds varied between 2 and 16 $m \cdot s^{-1}$ in the 2014/15 season (Figure 1f) and between 2 and 18 $m \cdot s^{-1}$ in 2015/16 season (Figure 1h). The wind was at storm level on some days. In general, the wind speeds in the 2015/16 season were lower than in the 2014/15 season.

No significant difference was found between the orientations of the trees in terms of climate parameters ($p > 0.05$). On the other hand, certain differences were detected between the orchards. Orchards overlooked the sea and the wind was blowing from sea to land. Here, it is useful to consider the direction of the wind and the dates when it was strong. Since the wind had blown from the sea towards the land, the effect of RH on the tree may have increased. These winds blowing from the north are cold winds for Türkiye. Olives exposed to cold at night get warmed by the sun the next day, so the changes that may occur in the fruit are a matter of discussion.

The olives had a maximum of 3.5 MI in the 2014/15 season and 4.4 MI in the 2015/16 season. (Tables 1 and 2). It was determined that the olives did not completely darken in the 2014/15 season. Fruit ripeness was different in both orientations of the trees in the 2014/15 season, and fruits from trees facing NE ripened a little later ($p < 0.05$). Although there was not much difference in terms of MI in both orchards, after October 27, the olives in orchard B had turned darker but remained stable. In the 2015/16 season, olives from the SW and NE orientations of the trees were the same in terms of MI. The darkening of the olives in orchard B was seen more easily. Olives in orchard A were harder to darken. Since Erkence is a cultivar that sheds easily after early ripening, the fruits taken from the tree may have remained at lower maturity. The amount of light is very important for the olive canopy. Beside this, the light also affects fruit development and yield. The fruits in the upper parts of the canopy ripen more and more quickly and the fruits become larger (Rallo *et al*., 2018). Erkence olives have been reported to have significant amounts of anthocyanins (Susamci *et al*., 2017).

Total phenol content (TPC) varied between 197 mg and 472 mg CAE·100 $g^{-1}$ in the 2014/15 season, and between 292 mg and 687 mg CAE·100 $g^{-1}$ in 2015/16 (Figures 2a and 2b). TPC decreased in both seasons. While the TPC in the SW and NE orientations in October were similar, as of November the olives from the NE orientation had a lower TPC than SW, especially in orchard A in the 2014/15 season. For this low content, the effect of the sea can be considered. In addition, TPC can vary according to the position of the fruit on the tree. It has been reported that oils obtained from fruits in the upper layer of the canopy have higher contents in phenolic compounds compared to the lower ones (Rallo *et al*., 2018). It has been stated that a more stable oil can be obtained from olives in this position, and the oils obtained from the olives in the North-South orien-

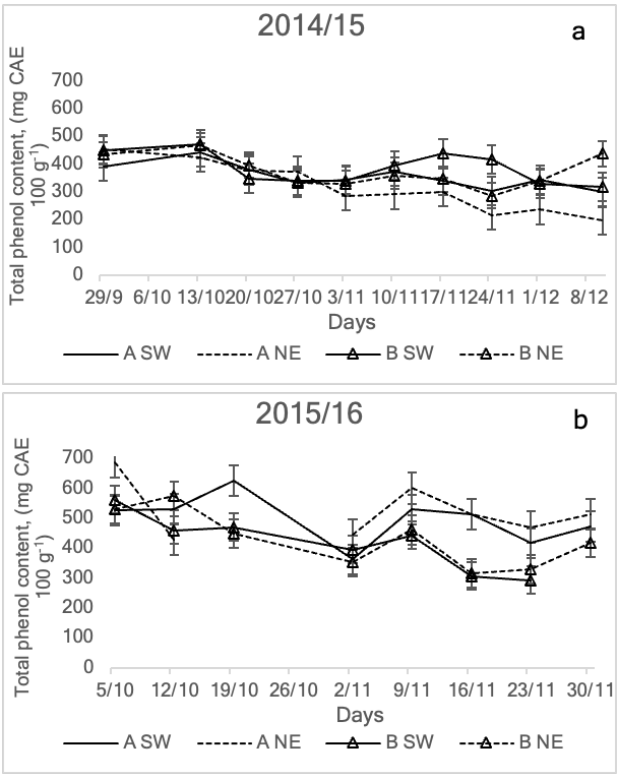


**Figure 2.** Total phenol content (mg CAE·100 $g^{-1}$) in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in orchards A and B during the 2014/15 (a) and 2015/16 (b) seasons. Each data point is the average of three replicates, and bars indicate the standard deviation.

Table 1. Three replicate averages of Maturity index (MI) and hydroxytyrosol (HTY), tyrosol (TY), verbascoside (VRB), rutin (RUT), apigenin (APIG), lutein (LUT) contents in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in orchards A and B during the 2014/15 season.

| | 2014/15 | | 29-Sep | 13-Oct | 20-Oct | 27-Oct | 3-Nov | 10-Nov | 17-Nov | 24-Nov | 1-Dec | 10-Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MI | A | SW | 1.8 | 2.4 | 2.7 | 2.7 | 2.8 | 2.7 | 2.9 | 2.7 | 3.2 | 2.8 |
| | | NE | 1.5 | 2.1 | 2.5 | 2.6 | 2.6 | 2.5 | 2.6 | 2.7 | 2.9 | 3.0 |
| | B | SW | 1.7 | 2.6 | 2.8 | 3.1 | 3.3 | 3.4 | 3.4 | 3.3 | 3.5 | 3.3 |
| | | NE | 1.6 | 2.2 | 2.6 | 2.9 | 3.2 | 3.2 | 3.3 | 3.3 | 3.3 | 3.3 |
| | | | LSD[b]drc[c]=1.52*[a] LSDwk[d]=0.17*** LSDwkxorc[e]=1.51** | | | | | | | | | |
| HTY | A | SW | 169.1 | 185.1 | 158.7 | 63.0 | 39.8 | 27.9 | 81.9 | 50.7 | 62.0 | 62.8 |
| | | NE | 111.8 | 117.5 | 131.6 | 46.4 | 44.4 | 22.5 | 80.2 | 36.0 | 53.0 | 36.2 |
| | B | SW | 131.1 | 135.3 | 95.3 | 56.3 | 49.7 | 55.2 | 121.6 | 43.8 | 78.2 | 98.5 |
| | | NE | 150.5 | 182.0 | 150.1 | 74.7 | 60.4 | 49.8 | 93.1 | 49.4 | 77.9 | 76.3 |
| | | | LSDwk=24.19*** LSDorcxdrc=152.66** | | | | | | | | | |
| TY | A | SW | 132.7 | 35.0 | 124.2 | 83.0 | 112.1 | 87.7 | 86.9 | 64.8 | 88.9 | 56.9 |
| | | NE | 148.9 | 18.7 | 99.4 | 71.9 | 83.2 | 86.9 | 59.9 | 48.8 | 74.0 | 51.9 |
| | B | SW | 68.0 | 120.9 | 144.9 | 94.8 | 127.3 | 80.0 | 101.0 | 80.7 | 119.6 | 55.3 |
| | | NE | 100.8 | 134.1 | 130.5 | 94.6 | 102.8 | 81.8 | 91.5 | 66.8 | 113.6 | 102.3 |
| | | | LSDwk=23.73*** LSDwkxorc=38.38*** | | | | | | | | | |
| VRB | A | SW | 38.3 | 35.8 | 26.2 | 35.3 | 0.0 | 12.2 | 25.1 | 34.4 | 62.6 | 31.0 |
| | | NE | 8.0 | 11.1 | 14.5 | 32.1 | 14.1 | 41.9 | 32.3 | 17.8 | 44.2 | 31.9 |
| | B | SW | 18.9 | 32.8 | 38.3 | 54.7 | 48.4 | 87.0 | 59.3 | 63.3 | 170.3 | 109.9 |
| | | NE | 25.0 | 31.8 | 45.6 | 66.3 | 77.3 | 61.3 | 50.9 | 53.7 | 115.0 | 135.9 |
| | | | LSDwk=25.43*** LSDwkxorc=87.82** | | | | | | | | | |
| RUT | A | SW | 431.6 | 370.6 | 394.7 | 292.9 | 376.8 | 406.8 | 247.8 | 156.2 | 285.9 | 259.8 |
| | | NE | 478.2 | 294.6 | 404.4 | 271.0 | 258.1 | 323.4 | 172.8 | 115.9 | 134.5 | 93.1 |
| | B | SW | 481.4 | 484.0 | 520.6 | 350.5 | 414.4 | 262.1 | 458.1 | 366.5 | 453.0 | 286.9 |
| | | NE | 488.9 | 405.6 | 345.1 | 311.9 | 314.5 | 311.6 | 311.0 | 240.0 | 266.5 | 355.5 |
| | | | LSDdrc=259.48** LSDwk=88.91*** | | | | | | | | | |
| APIG | A | SW | 13.1 | 14.6 | 14.2 | 15.4 | 14.5 | 13.1 | 11.9 | 12.8 | 12.1 | 11.0 |
| | | NE | 15.7 | 13.6 | 14.1 | 15.2 | 12.6 | 13.2 | 10.5 | 12.6 | 12.3 | 9.8 |
| | B | SW | 15.4 | 15.2 | 17.0 | 16.2 | 14.8 | 13.3 | 13.4 | 13.2 | 13.4 | 16.1 |
| | | NE | 15.0 | 16.6 | 16.9 | 16.4 | 14.7 | 14.1 | 13.2 | 13.2 | 13.6 | 12.8 |
| | | | LSDorc=0.98* LSDwk=1.56*** | | | | | | | | | |
| LUT | A | SW | 8.2 | 8.6 | 6.9 | 6.7 | 5.1 | 5.4 | 6.8 | 6.5 | 6.4 | 5.8 |
| | | NE | 12.9 | 9.5 | 9.8 | 6.7 | 5.3 | 5.1 | 6.4 | 5.7 | 6.3 | 4.6 |
| | B | SW | 12.1 | 9.6 | 6.2 | 4.8 | 3.8 | 7.4 | 6.5 | 6.5 | 6.1 | 7.6 |
| | | NE | 10.3 | 9.3 | 6.7 | 5.6 | 4.2 | 8.4 | 6.3 | 6.6 | 6.3 | 5.6 |
| | | | LSDwk=1.36*** | | | | | | | | | |

[a]*, ** and ***, the difference between means is statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, according to ANOVA.
[b]LSD, Fisher's Least Significant Difference Test, α=0.05. [c] drc=direction. [d] wk=week. [e] orc=orchard

tation may have high phenol content (Gómez-Del-Campo and García, 2012). As a result, the specific geographical location of the orchard significantly affects the composition of the olives. Besides general comparisons, each orchard should be evaluated according to its special situation. Olives from orchard A had lower TPC than olives from orchard B in the 2014/15 season. TPC in the NE orientation decreased steadily throughout October in the 2015/16 season. Olives from orchard B had a lower TPC than olives from orchard A, especially in November in the 2015/16 season. However, it was the opposite in the 2014/15 season. The reason for this situation can be the cold damage that occurred in orchard A in the spring of 2015/16. No statistical difference was found between the orchards or orientations in terms of TPC for both years ($p < 0.05$). In general, it has been reported that the TPC in olive fruit decreases with ripening (Uylaser, 2015). There are also many studies reporting the opposite. The TPC of Hurma olives, which are formed by the natural de-bittering of Erkence olives, has been reported in the range of

**Table 2.** Three replicate averages of Maturity index (MI) and hydroxytyrosol (HTY), tyrosol (TY), verbascoside (VRB), rutin (RUT), apigenin (APIG), lutein (LUT) contents in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in Orchards A and B during the 2015/16 season.

| | 2015/16 | | 5-Oct | 12-Oct | 19-Oct | 2-Nov | 9-Nov | 16-Nov | 23-Nov | 30-Nov |
|---|---|---|---|---|---|---|---|---|---|---|
| MI | A | SW | 2.4 | 2.7 | 2.7 | 2.8 | 2.8 | 3.2 | 3.0 | 3.2 |
| | | NE | 2.6 | 3.0 | 3.3 | 3.2 | 3.3 | 3.5 | 4.1 | 3.7 |
| | B | SW | 1.2 | 1.4 | 2.1 | 2.9 | 3.1 | 3.8 | 4.4 | |
| | | NE | 1.0 | 1.3 | 1.7 | 2.0 | 2.6 | 3.5 | 4.0 | 3.7 |
| | | | LSD[b]wk[d]=0.25*** LSDorcxdrc[c]=1.94*[a] LSDwkxorc[e]=0.97*** | | | | | | | |
| HTY | A | SW | 192.7 | 159.4 | 222.5 | 85.8 | 98.7 | 123.3 | 120.0 | 94.7 |
| | | NE | 183.1 | 112.0 | | 89.7 | 95.9 | 78.1 | 90.7 | 80.1 |
| | B | SW | 231.8 | 256.7 | 232.1 | 196.2 | 204.6 | 132.8 | 136.4 | |
| | | NE | 194.8 | 221.6 | 221.7 | 190.5 | 198.8 | 144.5 | 133.7 | 141.1 |
| | | | LSDwk=33.31*** | | | | | | | |
| TY | A | SW | 179.0 | 180.1 | 206.1 | 150.3 | 65.5 | 140.7 | 107.6 | 115.7 |
| | | NE | 323.1 | 185.0 | | 150.9 | 172.8 | 132.3 | 115.0 | 140.3 |
| | B | SW | 162.5 | 101.6 | 153.5 | 104.3 | 87.5 | 76.8 | 84.5 | |
| | | NE | 118.7 | 154.5 | 85.6 | 119.9 | 114.2 | 68.6 | 99.1 | 63.4 |
| | | | LSDwk=30.99*** LSDwkxorcxdrc=119.64* | | | | | | | |
| VRB | A | SW | 164.0 | 154.6 | 119.8 | 74.6 | 114.1 | 82.8 | 114.8 | 159.5 |
| | | NE | 60.6 | 61.7 | | 81.2 | 58.8 | 105.4 | 116.2 | 95.2 |
| | B | SW | 260.9 | 202.3 | 274.4 | 115.8 | 147.6 | 122.8 | 131.4 | |
| | | NE | 237.1 | 162.7 | 217.7 | 95.5 | 130.2 | 104.0 | 135.1 | 168.8 |
| | | | LSDwk=51.19* | | | | | | | |
| RUT | A | SW | 649.8 | 569.0 | 628.5 | 398.7 | 352.1 | 342.5 | 152.7 | 279.0 |
| | | NE | 660.2 | 727.8 | | 429.8 | 485.6 | 426.3 | 349.7 | 328.9 |
| | B | SW | 391.5 | 181.8 | 251.5 | 195.2 | 239.7 | 163.4 | 182.6 | |
| | | NE | 231.8 | 201.7 | 135.1 | 175.2 | 214.9 | 164.0 | 157.1 | 186.5 |
| | | | LSDwk=74.22*** LSDwkxorc=441.48** | | | | | | | |
| APIG | A | SW | 16.2 | 19.0 | 19.1 | 9.7 | 10.9 | 14.9 | 10.3 | 10.2 |
| | | NE | 18.6 | 16.7 | | 10.7 | 10.8 | 10.9 | 10.1 | 10.9 |
| | B | SW | 15.7 | 11.6 | 13.5 | 12.2 | 13.3 | 12.9 | 11.2 | |
| | | NE | 16.9 | 13.5 | 14.3 | 12.2 | 13.4 | 12.3 | 12.4 | 12.3 |
| | | | LSDwk=2.08*** LSDwkxorc=3.16* | | | | | | | |
| LUT | A | SW | 15.4 | 12.9 | 14.3 | 6.4 | 5.6 | 7.7 | 4.8 | 4.7 |
| | | NE | 15.5 | 10.3 | | 6.2 | 5.6 | 4.4 | 4.5 | 5.0 |
| | B | SW | 15.0 | 11.2 | 10.7 | 8.5 | 9.4 | 7.4 | 6.4 | |
| | | NE | 14.0 | 13.8 | 11.4 | 9.1 | 9.3 | 9.0 | 7.6 | 7.0 |
| | | | LSDwk=1.45*** LSDorcxdrc=2.98** LSDwkxorc=2.31* | | | | | | | |

[a]*, ** and ***, the difference between means is statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, according to ANOVA. [b]LSD, Fisher's Least Significant Difference Test, $\alpha=0.05$. [c] drc=direction. [d]wk=week. [e]orc=orchard

282.2-299.4 mg CAE·100 $g^{-1}$ and this low content may be the result of natural de-bittering (Susamci *et al*., 2016; Aktas *et al*., 2014).

During the ripening period of the olives, HPLC analysis showed that the main phenolic component was oleuropein, which was also reported in previous studies (Talhaoui *et al*., 2015). During the ripening of Erkence olives over two seasons, the oleuropein concentration decreased significantly as the change previously described for other olive varieties (Menz and Vriesekoop, 2010; Talhaoui *et al*., 2015), but this change was reported differently in one study (Aktas *et al*., 2014). In the 2014/15 season, the oleuropein content decreased from 16870 mg·$kg^{-1}$ downwards (Figure 3a). In some weeks, the oleuropein content in the olives from the NE orientation of trees was found to be lower than those from the SW orientation ($p<0.05$). The oleuropein content in the olives in orchard A was lower than those in orchard B. In 2015/16, oleuropein content in the olives decreased from 32970 mg·$kg^{-1}$ downwards (Figure 3b). The oleuropein content found in the olives from the

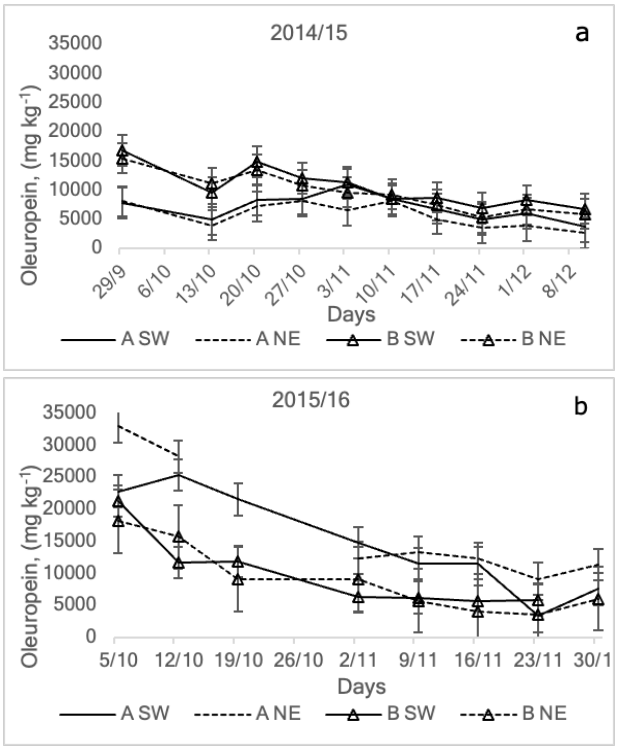


**Figure 3.** Oleuropein content (mg·kg$^{-1}$) in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in Orchards A and B during the 2014/15 (a) and 2015/16 (b) seasons. Each data point is the average of three replicates, and bars indicate the standard deviation.

2015/16 season was higher than from 2014/15. In 2015/16, the oleuropein content in the olives decreased in both orientations of trees. In this season, the oleuropein content in the olives from orchard B were lower than those in orchard A but showed a lesser decrease than in orchard A. It should be noted that previous studies reported a much lower concentration than the oleuropein content of Erkence olives we have detected (Aktas *et al*., 2014). Aktas *et al*. (2014) found that the content of apigenin in these olives was higher than that of oleuropein, whereas in our study the content of apigenin was quite low. Oleuropein is the main component of most varieties and decreases from green ripening to black ripe fruits, although large variations in phenolic compounds have been reported among varieties (Rallo *et al*., 2018). This decrease in oleuropein has also been reported for other phenolic compounds (Sousa *et al*., 2015). When the two seasons were evaluated together, a significant decrease was observed in the content of hydroxytyrosol, tyrosol, rutin, oleuropein and lutein during the maturation period. Verbascoside increased in the 2014/15 season, but decreased in the 2015/16 season. While there was no significant change in the apigenin content in the 2014/15 season, there was a decrease in the 2015/16 season. According to our results, it can be determined that all phenolic compounds decrease with maturation (Tables 1 and 2).

Although the phenolic compound contents did not differ significantly in terms of the orientation of the tree, it was determined that the contents of tyrosol, rutin and oleuropein were slightly lower in the olives from the NE orientation of the trees. In addition to the effect of orchard location, the position of the olives on the tree can also have an effect on phenolic compounds. Gómez-Del-Campo and García (2012) achieved higher phenol concentrations in olive oil extracted from the fruit located in the higher layers of olive trees than in the lower layers. Olive oils from sunny areas have been reported to contain a higher concentration of these compounds than those from shady areas (Romero *et al*., 2016). It should be noted that the synthesis of phenolic compounds is affected by solar radiation, the longer the exposure time of the fruit and leaves to sunlight, the higher the concentration of certain phenolic compounds in these materials (Morales *et al*., 2010).

The effect of orchard location on the content of phenolic compounds varied from season to season. The same effect was seen with hydroxytyrosol and verbascoside compounds in both seasons. The contents of hydroxytyrosol and verbascoside were lower in orchard A in both seasons. The content of phenolic compounds in orchard A were lower than in orchard B in the 2014/15 season (Table 1), while it was the opposite in the 2015/16 season (Table 2). This situation may have been caused by the cold damage experienced in orchard A in spring in the 2015/16 season. Another effect may be the differences in climatic conditions between the two seasons. The difference in altitude may have significantly affected the total phenol and individual phenol contents (Mousa *et al*., 1996). Di Vaio *et al*. (2013) stated that olive oils obtained from fruits grown at high altitudes have a higher content of phenolic compounds than those at low altitudes, Mousa *et al*. (1996) reported otherwise. However, many other factors such as soil, climate, and agronomic conditions affect the contents of phenolic compounds in plants. Positive correlations were reported between altitude and Coenzyme Q10 level, tocopherol, and phenolic compounds in

olive oils, while negative correlations with rainfall were reported (Borges *et al*., 2017).

β-Glucosidase activity varied from 0.006-0.0049 U·g$^{-1}$ in the 2014/15 season. Enzyme activity increased throughout October and early November. Significant increases were detected in the NE orientation of trees on November 3, and in the SW orientation on November 10 and 17. In November, the enzyme activity in the olives from orchard A remained constant, while the olives from orchard B had higher enzyme activity (Figure 4a). β-Glucosidase activity varied from 0.003-0.045 U·g$^{-1}$ in the 2015/16 season. Similar to the 2014/15 season, enzyme activity increased. In the 2015/16 season, the enzyme activity in the olives showed similar changes in the SW and NE orientations of the trees. In the 2015/16 season, the enzyme activity changes were similar in both orchards, although the enzyme activity in the olives from orchard A was higher than from orchard B, especially in the NE orientation (Figure 4b). Enzymes are in particularly close relationship with phenolic compounds. It has been reported that in the transformation of phenolic compounds (Velázquez-Palmero *et al*., 2017), they play an active role on the oleuropein molecule during the removal of bitterness (Ramírez *et al*., 2016) and β-glucosidase shows the highest activity against oleuropein (Romero-Segura *et al*., 2009).

Total protein content (TPR) in the olives varied from 0.06-0.78 mg·g$^{-1}$ in the 2014/15 season. It continued to be more stable after increasing at the end of October. The TPR in the NE orientation of the trees showed a greater increase in the last week of October and the first week of November than in the SW orientation ($p < 0.01$). Although there was an increase in TPR in both orchards in the last week of October, the increase in orchard A was found to be higher than in orchard B. The TPR in the olives from orchard A were determined higher than in those from orchard B (Figure 5a). The TPR of the olives in the 2015/16 season varied from 0.08-1.11 mg·g$^{-1}$. While it was observed that the TPR increased in the first week of November, it remained unchanged at the same level in other periods. In the 2015/16 sea-

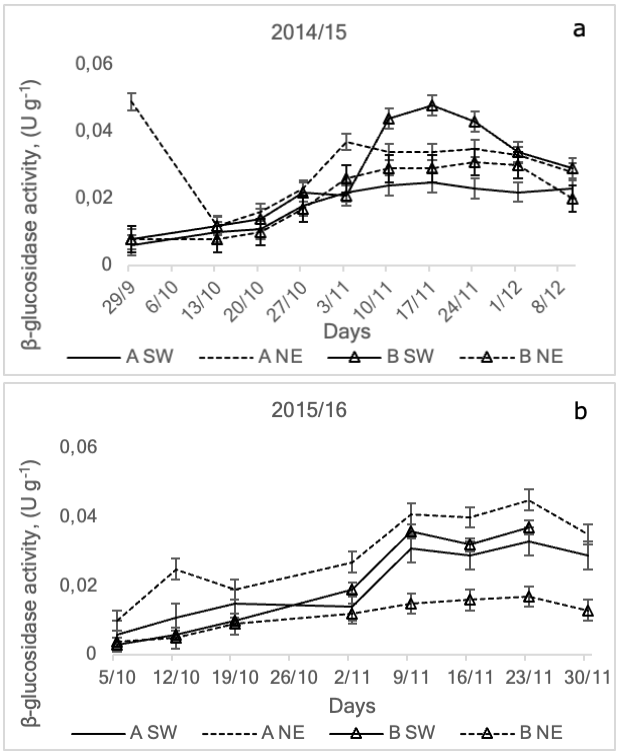


**Figure 4.** β-glucosidase activity (U·g$^{-1}$) in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in Orchards A and B during the 2014/15 (a) and 2015/16 (b) seasons. Each data point is the average of three replicates, and bars indicate the standard deviation.

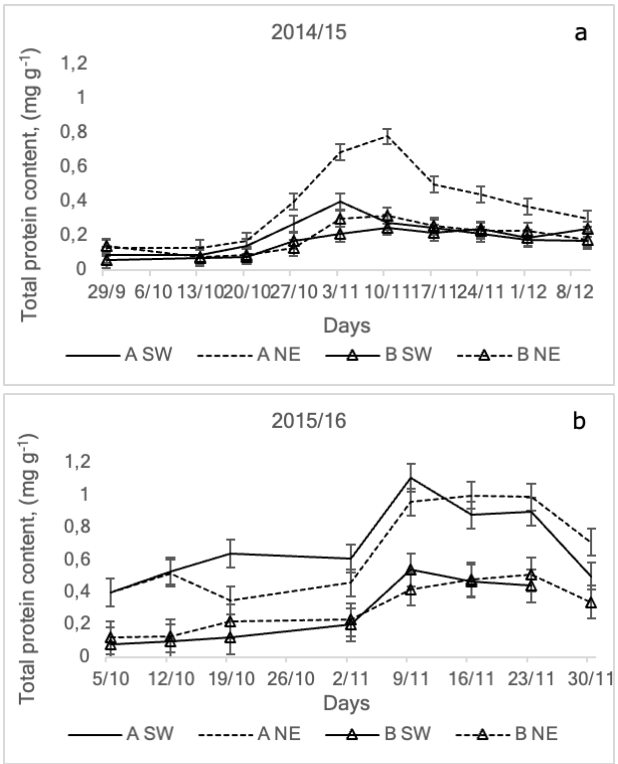


**Figure 5.** Total protein content (mg·g$^{-1}$) in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in Orchards A and B during the 2014/15 (a) and 2015/16 (b) seasons Each data point is the average of three replicates, and bars indicate the standard deviation.

son, the TPR in the olives showed similar variations in both orientations of the trees. Although there was an increase in TPR in both orchards in the first week of November, the TPR in orchard A was higher than in orchard B (Figure 5b).

The specific enzyme activity (SPA) in the olives ranged from 0.04-0.22 U·mg$^{-1}$ in the 2014/15 season. The SPA in the olives from the NE orientation of the trees was generally lower than those found in the SW orientation. There was a decrease at the end of October and an increase in the first weeks of November. The SPA showed the same trend in the olives from both orchards, but the differences were statistically significant ($p < 0.05$). However, the specific enzyme activity in the olives from orchard A was lower than those from orchard B (Figure 6a).

The SPA in olives ranged from 0.02-0.12 U·mg$^{-1}$ in the 2015/16 season. No statistically significant difference was found between simple effects and interactions. SPA increased in both orientations of the trees in October, while there was a decrease in the NE orientation olives and an increase in the SW orientation olives in November. SPA changes were similar in both orchards, except in the last week of November. As in the 2014/15 season, the SPA of the olives in orchard A was lower than in B (Figure 6b).

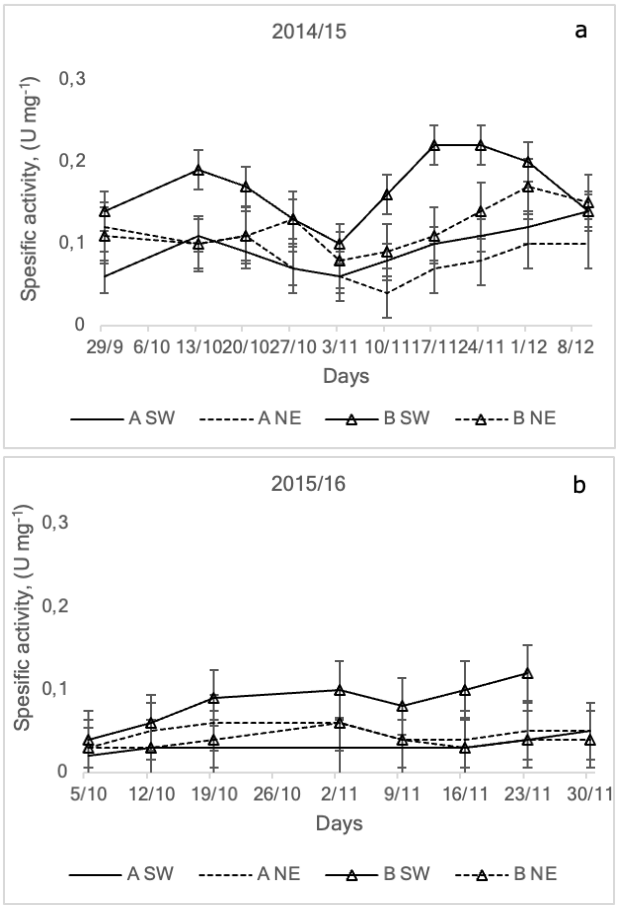


**Figure 6.** Spesific enzyme activity (U·mg$^{-1}$) in olives on the tree in different directions (NE: Northeast SW: Southwest) of the tree in Orchards A and B during the 2014/15 (a) and 2015/16 (b) seasons. Each data point is the average of three replicates, and bars indicate the standard deviation.

The β-glucosidase activity, TPR and SPA increased during the ripening period of Erkence olives. This increase was especially evident in late October and early November. The dates when de-bittering started should be noted. It has been reported that β-glucosidase enzyme activity increases with maturation, but the changes may differ depending on the variety. It was stated that the total soluble protein content increased in different periods of fruit ripening, decreased or remained constant after reaching the maximum level with full ripening (Ebrahimzadeh *et al*., 2003). It has been reported that β-glucosidase activity decreases with ripening in Picual and Arbequina olives (Romero-Segura *et al*., 2012). The climatic conditions and environmental effects of the region may affect the enzyme activity. The relationships between the enzyme activity in olives and phenolic compounds vary according to climatic conditions. Olive damage along with cold/frost damage affect the fruit's defense mechanism against some external effects. Low temperature and fruit injury caused an increase in LOX and HPL enzyme activities (Padilla *et al*., 2014). Reported as the endogenous pool of β-glucosidases, the key enzymatic system controls the hydrolysis of phenolic glycosides when olive tissues are damaged mechanically or by pathogen (Romero-Segura *et al*., 2009).

It can be concluded that enzyme activity in olives is increased by activating their defense system against the climatic conditions and damage, they are exposed to in Karaburun conditions, and this situation results in de-bittering. Olive leaves and fruit are rich in oleuropein, the bitter phenol glycoside, which inhibits the development of phytopathogens. In addition, after pathogen attack or mechanical damage, highly reactive molecules with antioxidant and antimicrobial activity are formed by the specific hydrolysis of oleuropein by β-glucosidases (Romero-Segura *et al*., 2009). In recent studies, the isolation and characterization of the β-glucosidase gene from olives (Koroneiki cv.) related to defense has been de-

scribed (Koudounas *et al*., 2015). It was stated that almost all reports of this enzyme converge at the point of physiological function of a defense mechanism that produces oleuropein-derived compounds with specific antimicrobial activities (Velázquez-Palmero *et al*., 2017). However, PPO activity and the effect of these enzymes on oleuropein and other phenolic compounds are an important part of the olive's defense mechanism, it has negative effects against herbivores, insects and other agents (Ortega-García *et al*., 2008).

Fruit location on the tree also had an effect on enzyme levels. It was stated that the position of the fruit in the canopy affects the β-glucosidase activity and this enzyme is sensitive to intense light (photosynthetic active radiation). It has been suggested that changes in the natural de-bittering of olives may be related to the activities of β-glucosidase and esterase enzymes and cause a decrease in phenolic compounds.

## 4. CONCLUSIONS

In conclusion, the all parameters examined in Erkence olives were affected by the ripening period. Olives took longer to darken in orchard A, near the sea. Although varying from year to year, the maturity index, β-glucosidase enzyme activity, total protein content and specific enzyme activity in the olives increased during the ripening period. On the other hand, the total phenol, oleuropein and other phenolic compound contents in the fruits decreased. The effect of orientation was especially clear on phenolic compounds and total phenol content. The effect of directionality was also evident on the total protein content and specific enzyme activity after de-bittering started. In particular, phenolic compounds and total phenol content showed the effect of the sea on the olives located in the NE orientation of the trees, which was the sea-facing side. While the total phenol content and phenolic compounds were found to be lower in orchard A, which is close to the sea and has a low altitude, β-glucosidase enzyme activity and total protein content were found to be high in the same orchard. This situation revealed the effect of altitude and sea on olives. The olives in the NE orientation of the trees in orchard A were more affected by the sea than the others. Although the sea's effect was observed, differences in age and position among fruits within the orchard led to varying reactions to climatic conditions. Further studies accounting for fruit age as a factor, could enhance the understanding of the impact of climatic conditions on Erkence olives.

## DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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## AUTHORSHIP CONTRIBUTION STATEMENT

E Susamcı: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing.

Ö Tuncay: Conceptualization, Investigation, Methodology, Writing – review & editing.

H Bayraktar: Formal analysis, Methodology, Writing – review & editing.

S Önal: Formal analysis, Methodology, Writing – review & editing.

## REFERENCES

Aktas AB, Ozen B, Tokatli F, Sen I. 2014. Phenolics profile of a naturally debittering olive in comparison to regular olive varieties. *J. Sci. Food Agric.* **94**, 691-698. https://doi.org/10.1002/jsfa.6308

Borges TH, Pereira JA, Cabrera-Vique C, Lara L, Oliveira AF, Seiquer I. 2017. Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. *Food Chem.* **215**, 454-462. https://doi.org/10.1016/j.foodchem.2016.07.162

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **72**, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Di Vaio C, Nocerino S, Paduano A, Sacchi R. 2013. Influence of some environmental factors on drupe maturation and olive oil composition.

*J. Sci. Food Agric.* **93**, 1134-1139. https://doi.org/10.1002/jsfa.5863

Ebrahimzadeh H, Motamed N, Rastgar-Jazii F, Montasser-Kouhsari S, Shokraii EH. 2003. Oxidative enzyme activities and soluble protein content in leaves and fruits of olives. *J. Food Biochem.* **27**, 181-196. https://doi.org/10.1111/j.1745-4514.2003.tb00276.x

García-Vico L, García-Rodríguez R, Sanz C, Pérez AG. 2017. Biochemical aspects of olive freezing-damage: Impact on the phenolic and volatile profiles of virgin olive oil. *LWT-Food Science and Technology*, **86**, 240-246. https://doi.org/10.1016/j.lwt.2017.07.052

Gómez-Del-Campo M, García JM. 2012. Canopy fruit location can affect olive oil quality in "Arbequina" hedgerow orchards. *J. Am. Oil Chem. Soc.* **89**, 123-133. https://doi.org/10.1007/s11746-011-1900-2

Gutiérrez-Rosales F, Romero MP, Casanovas M, Motilva MJ, Mínguez-Mosquera MI. 2012. β-Glucosidase involvement in the formation and transformation of oleuropein during the growth and development of olive fruits (*Olea Europaea L. Cv. Arbequina*) grown under different farming practices. *J. Agric. Food Chem.* **60** (17), 4348-4358. https://doi.org/10.1021/jf205209y

Hrncirik K, Fritsche S. 2004. Comparability and reliability of different techniques for the determination of phenolic compounds in virgin olive oil. *Eur. J. Lipid Sci. Technol.* **106**, 540-549. https://doi.org/10.1002/ejlt.200400942

Hu Y, Luan H, Hao D, Xiao H, Yang S, Yang L. 2007. Purification and characterization of a novel ginsenoside-hydrolyzing β-D-glucosidase from the China white jade snail (Achatina Fulica). *Enzyme Microb. Technol.* **40**, 1358-1366. https://doi.org/10.1016/j.enzmictec.2006.10.011

Koudounas K, Banilas G, Michaelidis C, Demoliou C, Rigas S, Hatzopoulos P. 2015. A defence-related Olea europaea β-glucosidase hydrolyses and activates oleuropein into a potent protein cross-linking agent. *J. Experiment. Bot.* **66**, 2093-2106. https://doi.org/10.1093/jxb/erv002

Lopez-Huertas E, del Río LA. 2014. Characterization of antioxidant enzymes and peroxisomes of olive (*Olea europaea L.*) fruits. *J. Plant Physiol.* **171**, 1463-1471. https://doi.org/10.1016/j.jplph.2014.06.014

Menz G, Vriesekoop F. 2010. Physical and chemical changes during the maturation of gordal sevillana olives (*Olea europaea L., cv. Gordal Sevillana*). *J. Agric. Food Chem.* **58**, 4934-4938. https://doi.org/10.1021/jf904311r

Morales LO, Tegelberg R, Brosché M, Keinänen M, Lindfors A, Aphalo PJ. 2010. Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in Betula pendula leaves. *Tree Physiol.* **30**, 923-934. https://doi.org/10.1093/treephys/tpq051

Morello JR, Vuorela S, Romero MP, Motilva MJ, Heinonen M. 2005. Antioxidant activity of olive pulp and olive oil phenolic compounds of the Arbequina cultivar. *J. Agric. Food Chem.* **53**, 2002-2008. http://dx.doi.org/10.1021/jf048386a

Mousa YM, Gerasopoulos D, Metzidakis I, Kiritsakis A. 1996. Effect of altitude on fruit and oil quality characteristics of "Mastoides" olives. *J. Sci. Food Agric*. **71**, 345-350. https://doi.org/10.1002/(SICI)1097-0010(199607)71:3<345::AID-JSFA590>3.0.CO;2-T

Ortega-García F, Blanco S, Peinado MÁ, Peragón J. 2008. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. "Picual" trees during fruit ripening. *Tree Physiol.* **28**, 45-54. https://doi.org/10.1093/treephys/28.1.45

Padilla MN, Hernández ML, Sanz C, Martínez-Rivas JM. 2014. Stress-dependent regulation of 13-lipoxygenases and 13-hydroperoxide lyase in olive fruit mesocarp. *Phytochem.* **102**, 80-88. https://doi.org/10.1016/j.phytochem.2014.01.024

Parkin KL, Marangoni, A, Jackman RL, Yada RY, Stanley DW. 1989. Chilling Injury, A Review of Possible Mechanisms. *J. Food Biochem.* **13** (2), 127-153. https://doi.org/10.1111/j.1745-4514.1989.tb00389.x

Rallo L, Díez CM, Morales-Sillero A, Miho H, Priego-Capotec F, Rallo P. 2018. Quality of olives: A focus on agricultural preharvest factors. *Sci. Horticult.* **233**, 491-509. https://doi.org/10.1016/j.scienta.2017.12.034

Ramírez E, Gandul-Rojas B, Romero C, Brenes M, Gallardo-Guerrero L. 2015. Composition of pigments and colour changes in green table olives related to processing type. *Food Chem*. **166**, 115-124. https://doi.org/10.1016/j.foodchem.2014.05.154

Ramírez E, Brenes M, García P, Medina E, Romero C. 2016. Oleuropein hydrolysis in natural green olives : Importance of the endogenous enzymes. *Food Chem.* **206**, 204-209. https://doi.org/10.1016/j.foodchem.2016.03.061

Rigane G, Salem RB, Sayadi S, Bouaziz M. 2011. Phenolic composition, isolation, and structure of a new deoxyloganic acid derivative from Dhokar and Gemri-Dhokar olive cultivars. *J. Food Sci.* **76**, C965-C973. https://doi.org/10.1111/j.1750-3841.2011.02290.x

Romero C, Ruiz-Méndez MV, Brenes M. 2016. Bioactive Compounds in Virgin Olive Oil of the PDO Montoro-Adamuz*, J. Am. Oil Chem. Soc.* **93**, 665-672. https://doi.org/10.1007/s11746-016-2803-z

Romero-Segura C, Sanz C, Perez AG. 2009. Purification and characterization of an olive fruit β-glucosidase involved in the biosynthesis of virgin olive oil phenolics. *J. Agric. Food Chem.* **57**, 7983-7988. https://doi.org/10.1021/jf901293c

Romero-Segura C, García-Rodríguez R, Sánchez-Ortiz A, Sanz C, Pérez AG. 2012. The role of olive β-glucosidase in shaping the phenolic profile of virgin olive oil. *Food Res. Internat.* **45**, 191-196. https://doi.org/10.1016/j.foodres.2011.10.024

Sousa A, Malheiro R, Casal S, Bento A, Pereira JA. 2015. Optimal harvesting period for cvs . Madural and Verdeal Transmontana, based on antioxidant potential and phenolic composition of olives. *LWT-Food Sci. Technol.* **62**, 1120-1126. https://doi.org/10.1016/j.lwt.2015.01.046

Susamci E, Ozturk Gungor F, Irmak S, Ataol Olmez H, Tusu G. 2016. A study on the nutritional value of Hurma olives (Erkence cv.) that lose the bitterness on the tree. *J. Agric. Sci.* **22** (4), 471-479. https://doi.org/10.1501/Tarimbil_0000001405

Susamci E, Romero C, Tuncay O, Brenes M. 2017. An explanation for the natural de-bittering of Hurma olives during ripening on the tree. *Grasas Aceites* **68**, 1-8. https://doi.org/10.3989/gya.1161162

Talhaoui N, Gómez-Caravaca AM, León L, De La Rosa R, Fernández-Gutiérrez A, Segura-Carretero A. 2015. Pattern of variation of fruit traits and phenol content in olive fruits from six different cultivars. *J. Agric. Food Chem.* **63**, 10466-10476. https://doi.org/10.1021/acs.jafc.5b04315

Tombesi A, Boco M, Pilli M. 1998. Fruit microclimate: effect of light on growth and on oil synthesis. *Riv. Frutticolt. Ortofloricolt.* **60** (7/8), 63-67.

Uylaser V. 2015. Changes in phenolic compounds during ripening in Gemlik variety olive fruits obtained from different locations. *CyTA J. Food* **13**, 167-173. https://doi.org/10.1080/19476337.2014.931331

Velázquez-Palmero D, Romero-Segura C, García-Rodríguez R, Hernández ML, Vaistij FE, Graham IA, Pérez AG, Martínez-Rivas JM. 2017. An oleuropein β-glucosidase from olive fruit is involved in determining the phenolic composition of virgin olive oil, *Front. Plant Sci.* **8**, 1-12. https://doi.org/10.3389/fpls.2017.01902