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# Effect of Growing Regions on Discrimination of Turkish-Style Black Table Olives from Gemlik Cultivar

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**Abstract: Gemlik is a cultivar that grows in a distinct region of Turkiye and is ideal for brine fermentation of brine black table olives. Bursa Protected Designated Origin (PDO) and Izmir non-PDO Gemlik table olives have high levels of oleic acid (74%), total phenol (190 mg/kg), and dry matter (57%), while being low in linoleic acid (8%). The pH values and salt contents were observed to be in the range of 4.1 to 4.3 and 3.9% to 4.8%, respectively. During the fermentation of Gemlik table olives, a mass transfer occurred, resulting in a reduction in reducing sugar and total sugar contents as well as an increase in the salt content of the olives. Despite the reduction of phenolic content in both Gemlik PDO and non-PDO table olives, their antioxidant capacity remains high after fermentation. The oil content, antioxidant activity, phenolic contents, palmitic, palmitoleic, oleic, and linoleic acids were all found to be significant variables in distinguishing between Gemlik PDO and non-PDO table olives using PLS-DA analysis. There is a statistically significant correlation between the phenolic content and oleic (0.588) and linoleic (–0.659) acids ($p < 0.05$). Bursa PDO and Izmir non-PDO exhibit enhanced nutritional quality and antioxidant activity, unequivocally differentiating them from Hatay and Mersin non-PDO Gemlik table olives with 98% accuracy through discriminant analysis ($p < 0.05$). PLS-DA and DA can effectively identify variations in the quality of Turkish-style black table olives preserved in brine, originating from PDO and non-PDO growing areas.**

**Key words:** Black Gemlik table olives, PDO region, fatty acid composition, geographical origin, multivariate methods, PLS-DA, discriminant analysis

## 1 Introduction

Table olives are fermented foods and are rich sources of a wide range of essential micronutrients, including essential fatty acids and biologically active phytochemicals containing antioxidant compounds and phenolics, especially oleuropein and ligstroside derivatives[1]. Therefore, they have been added to the Healthy Eating Pyramid of the Mediterranean diet since 2010[2–4]. Eating 5-10 table olives per day could cover your daily polyphenol intake[3]. The global table olive production in the 2021–2022 season was approximately 2,847,000 tons, with Spain, Greece, Italy, and Portugal producing 31% and Turkiye producing 13.5% (International Olive Council, 2022). The annual olive production in Turkiye is 1,738,680 tons, with 558,833 tons of table olive production and 70,000 tons of export of green and black table olives[5]. Fresh olives are unpalatable, mainly because of their high oleuropein content (14% dry weight), glycosylated secoiridoids with a bitter taste. Various processes have been developed for bettering, and many are based on immersion in water, brine, alkali, or biological processes[1, 6]. Producing natural black olives in brine is a long-standing tradition in Turkiye. The region famous for black olive production is the Marmara region, especially the Bursa province. The Gemlik variety is the best Turkish variety for processing natural black olives in brine because of its unique organoleptic and sensorial characteristics, relatively high oil yield, early maturation, and low periodicity[5, 7–10]. Better quality olives command a better price on the market[11]. Olives that have been characterized as having PDO labels are required because of their higher prices in the market. Recently, the authentication of some PDO table olives[1, 4, 10–13] has been studied. The cultivation of the "Gemlik" variety has led to its cultivation outside its natural areas in Turkiye. Due to crop management, olive quality,

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and composition, planting Gemlik olives outside of their ecological niches may result in non-profitable production[5] as a result of climatic changes, erosion, and water stress. The European Union granted PDO status to Gemlik olives from Turkiye in June 2023. Gemlik olives are grown in Bursa as a PDO growing region and in non-PDO growing regions, where they may possess their own particular properties. To the best of our knowledge, there has been no research comparing Gemlik olives from various growing areas and harvest years. Therefore, the purpose of this study was to distinguish Gemlik table olives harvested in 2016 and 2017 from PDO (Bursa) and non-PDO growing areas (Hatay, Izmir, and Mersin).

## 2 Experimental Procedures

### 2.1 Materials

The purity of all standards was stated to be approximately 98%. Supelco (Bellefonte, PA) supplied the fatty acid methyl ester (FAME) mixture. The chemicals and solvents used in the study were HPLC-grade and were obtained from Merck (Darmstadt, Steinheim, Germany). In each crop season (2016 and 2017), 15 kg of Gemlik olives were hand harvested in December when the skin pigmentation was black (black ripe) and the maturity index was 4 to 6 from Bursa, Izmir, Hatay and Mersin.

### 2.2 Methods

#### 2.2.1 Table olive processing

Gemlik olives (15 kg) from each growing area Bursa (PDO), Izmir, Hatay, and Mersin (non-PDO) region in each crop season (2016 and 2017) were harvested by hand in December when they were black ripe with a maturity index of 4-6. The olives were sorted, cleaned, and graded before being placed in fiberglass containers with brine containing 14% salt. The olives were kept in brine by applying pressure (4 kg/m$^2$) to the olives in fiberglass containers for aerated spontaneous lactic acid fermentation. Salt concentrations in brine were monitored at regular intervals and adjusted to 10-11%, with corrections made as needed. Gemlik olives were fermented in brine at 18℃ for 150 days until the pH fell below 4.5 and most chemical parameters reached a steady state, at which point the Gemlik table olives were ready for consumption.

#### 2.2.2 Physical and chemical measurements

The olive maturation index and flesh/stone weight ratios were calculated using the official methods of the International Olive Council[14]. 100 olives were taken from 1 kg of olives and subjected to maturity index analysis. A digital compass was used to measure the width and height of the fruits and seeds (Mitutoyo, Japan). The dry matter and oil content of Gemlik olives were determined using the official IOC and Turkish Food Codex methods[14, 15]. A pH meter was used to measure the pH of the olive fruit pulp (Nel Model 890). The Mohr method was used to determine salt in olive samples according to method of TFC[15]. The total phenols and antioxidant activity of olives were determined using a spectrophotometer (Shimadzu 1200 UV/Vis, Japan) at wavelengths of 725 and 530 nm, respectively. Briefly, 10 g of freeze-dried olive pulp with ethanol/water (80:20 v/v) containing 2% Nametabisulphite was used. Subsequently, it was treated with hexane to remove pigments and lipids, and the phenolic compounds were extracted. The total polyphenol content of the olives was determined by a subsequent reaction with Folin–Ciocalteau reagent with 0.5 mL of phenolic extract of olive (diluted 1:25, v/v with distilled water). The mixture was incubated for 90 min in the dark at room temperature. The phenol content was determined at 725 nm, and the concentration was expressed as milligrams of caffeic acid equivalents per kg of olives[16, 17]. The radical-scavenging activity (RSA) of olives and olive oil was evaluated using the DPPH assay. Briefly, an aliquot 100 μL of methanol extract from the studied olives or olive oil ($3.6 \times 10^{-4}$ mol/L) was added to 2.9 mL of DPPH solution ($6 \times 10^{-5}$ mol/L in methanol), and the mixture was left in the dark at RT for 30 min. The absorbance of the mixture was measured at 515 nm against a blank solution. Triplicate measurements were made, and RSA was expressed as the inhibition percentage[16]. The reducing and total sugar content of olives were determined at 540 and 620 nm, respectively, using glucose solutions as standards, using a spectrophotometer (Shimadzu 1200 UV/Vis, Japan). The reducing sugar content of olives was determined according to the method of[18] and the amount of reducing sugar was calculated using a standard curve as g/100 g. Briefly, 5 g of the sample was diluted with distilled water to 50 mL in a volumetric flask, followed by 6 mL of dinitro salicylic acid solution (1 g dinitro salicylic acid, 20 mL 2 M NaOH, and 20 g K-Na tartarate per 100 mL distilled water), transferred in a tube. Then, 5 mL of Carrez I and Carrez II solutions were added, bleached using activated charcoal, and filtered. Then, 2 mL of this filtrate was added to the tube. The tube was placed in a boiling water bath for 5 min and cooled immediately and the absorbance was read against the blank at 540 nm. The total sugar content of olives was determined according to the method of[19]. Briefly, 0.1 g of olives was placed in a glass test tube. Sugars were extracted by suspending them in 5 mL 80% (v/v) ethanol in an 85℃ water bath for 1 h, after which ethanol was removed by repeating this procedure four times. The ethanolic solutions (approx. 20 mL) were combined and evaporated to dryness at 55℃. The extracted and dried sugars were dissolved in 1 mL of distilled water and stored at 80℃ until measurement. The absorbance was read against the blank at 620 nm. The results were calculated from standard curve of glucose solutions as standard and expressed as g/100 g.

2.2.3 Chromatographic conditions for fatty acid determination

The fatty acid composition was determined using the method described in International Olive Council T.20/Doc. No.332015[20]. Fatty acid methyl esters were analyzed using an HP 6890 gas chromatograph equipped with an ionization detector (FID). To prepare fatty acid methyl esters, 0.1 g of the oil sample was dissolved in 5 mL of $n$-hexane and 1 mL of potassium hydroxide in methanol. A split GC mode was used to inject approximately 1 L through a DB-23 capillary column (30 m × 0.25 mm i.d) with a 0.25 μm film thickness. The oven, injector, and FID temperatures varied from 170 to 210℃ in 2℃/min increments, with a 10-min hold at 210℃. The carrier gas was helium, which flowed at a rate of 1 mL/min. The Supelco FAME mix was used as a reference standard to identify the fatty acids in Gemlik table olives. The results were expressed as the relative area percentage of total fatty acid methyl esters determined by comparing their retention times with those of the reference compounds.

2.2.4 Statistical analysis

The results obtained from the study were given as the mean and standard deviation of triplicates of quality parameters including physical and chemical measurements and fatty acids of Turkish Gemlik style processed Black Table Olives. An ANOVA (two-way) test ($p < 0.05$) was used to determine the existence of significant pairwise differences between groups. PLS-DA and DA analysis were applied to 52 variables. PLS-DA is a discriminatory multivariate analysis designed to identify the significant variables affected by growing region and harvest date ($p < 0.05$). Pearson correlation test was also performed to determine the relationship between growing region, harvest year and significant discriminators. Geographic traceability was analysed through a statistical procedure, which was based on discriminant analysis (DA). The DA procedure was constructed to distinguish between Gemlik Table olives based on growing origin. The XLSTAT 2022.1.1.1251, Addinsoft, New York, NY, USA software package was employed for this purpose.

## 3 Results and Discussions

### 3.1 General properties of Gemlik fresh and table olives

Tables 1 and 2 shows the properties of fresh Gemlik olives and Gemlik table olives.

The maturity stage is well known to influence olive oil yield and quality[16, 21]. Maturity index values for Hatay and Mersin non-PDO fresh olives ranged between 4.7 and 5.59 for Hatay non-PDO and Bursa PDO Gemlik fresh olives and ranged between 5 to 5.35 in harvest years 2016 and 2017. Table 1 clearly shows that growing region and harvest years significantly affected maturity index values of Gemlik olives ($p < 0.05$). Ozdemir *et al.* (2020)[21], who found a maturity index of 5–6.1 for Egriburun, Karaman, Halhali, Saurani, and Hasebi olives and 0.34–6.9 for Gemlik olives[9, 22], agreed with our findings. Consumers prefer high-fruit-weight olives, and the price of olives rises in tandem with this value[21]. The pulp/seed ratio, which should be greater than 4, is an important quality parameter for table olives. The highest pulp/seed ratio (4.02) was found in Hatay non-PDO ($p < 0.05$) (Table 1). Our findings were consistent with those of researchers 14, 15, and 18 (who discovered a flesh/seed ratio of 3.34 to 7 for Gemlik olives)[7, 18, 21] and the results of[23] who found pulp/seed ratio as 2.1 to 4.45 for Koroneiki, Beleydi, and Mission olives. High-oil olive cultivars contain at least 45% oil[9]. Growing region had a significant effect on oil content of Gemlik olives ($p < 0.05$). Mersin and Izmir non-PDO and Bursa PDO Gemlik olives contained 47.7%–45.3% oil and can be considered high-oil-containing olive varieties (Table 1). Our findings were higher than the oil contents of Gemlik olives, which ranged between 13.27% and 45%, and similar oil contents were obtained

Table 1 Properties of Gemlik PDO and non-PDO fresh olives by growing regions and harvest years (two-way ANOVA).

| Gemlik Fresh Olives | Growing Region | | | | Significance | Harvest years | | Significance | Reported values in literature |
|---|---|---|---|---|---|---|---|---|---|
| | Bursa PDO | Izmir | Hatay | Mersin | | 2016 | 2017 | | |
| Maturity Index | 5.59[a] | 4.87[b] | 4.70[b] | 5.56[a] | ** | 5.01[b] | 5.35[a] | ** | 0.4-6.9 |
| Width | 15.23[a] | 15.71[a] | 16.77[a] | 15.85[a] | ns | 15.51[a] | 16.26[a] | ns | 14-21 |
| Length | 20.91[a] | 21.25[a] | 22.20[a] | 21.57[a] | ns | 21.34[a] | 21.63[a] | ns | 18-26 |
| Pulp/Seed | 3.69[b] | 3.81[b] | 4.02[a] | 3.42[b] | ** | 3.38[a] | 4.09[a] | ns | 3.5-7 |
| Dry Matter | 57.70[a] | 57.36[a] | 54.92[a] | 47.66[a] | ns | 54.86[a] | 53.96[a] | ns | 28-59 |
| Oil Content | 45.34[a] | 45.27[a] | 39.12[b] | 47.70[a] | ** | 45.75[a] | 42.96[a] | ns | 13-45 |
| Phenol Content | 232.55[a] | 213.65[a] | 140.75[a] | 86.70[b] | ** | 117.28[b] | 219.55[a] | ** | 64-544 |
| Antioxidant Activity | 88.23[a] | 86.46[a] | 89.65[a] | 89.40[a] | ns | 86.73[a] | 89.14[a] | ns | 67-84 |

Different letters in the same row denote a statistically significant difference ($p < 0.05$). **indicates significance ($p \leq 0.05$). *ns* indicates no significance ($p \geq 0.05$).

Table 2 Properties of Gemlik PDO and non-PDO table olives by growing regions and harvest years (two-way ANOVA).

| Gemlik Table Olives | Growing Region | | | | Significance | Harvest years | | Significance | Reported values in literature |
|---|---|---|---|---|---|---|---|---|---|
| | Bursa PDO | Izmir | Hatay | Mersin | | 2016 | 2017 | | |
| Dry Matter | 57.22[a] | 53.58[a] | 51.49[a] | 46.46[a] | ns | 58.81[a] | 45.56[b] | ** | 28-59 |
| Oil content | 35.41[b] | 34.46[b] | 35.11[b] | 41.77[a] | ** | 38.48[a] | 34.90[a] | ns | 32-40 |
| Phenol Content | 189.90[a] | 160.75[a] | 88.05[b] | 62.30[b] | ** | 176.08[a] | 174.43[a] | ns | 91-418 |
| Antioxidant Activity | 82.08[a] | 89.15[a] | 85.65[a] | 85.91[a] | ns | 83.55[a] | 87.84[a] | ns | 67-84 |
| Palmitic | 10.95[b] | 12.93[a] | 13.35[a] | 13.65[a] | ** | 13.33[a] | 12.11[b] | ** | 9-17 |
| Palmiteloic | 1.13[a] | 0.98[a] | 1.40[a] | 1.40[a] | ns | 1.29[a] | 1.16[a] | ns | 0.8-1.6 |
| Stearic | 3.06[a] | 3.40[a] | 3.35[a] | 3.26[a] | ns | 3.34[a] | 3.20[a] | ns | 1.3-4 |
| Oleic | 73.98[a] | 72.58[a] | 68.19[b] | 67.92[b] | ** | 70.58[a] | 70.76[a] | ns | 65-75 |
| Linoleic | 8.96[a] | 7.93[a] | 11.67[a] | 11.64[a] | ns | 9.56[a] | 10.54[a] | ns | 5.6-16 |
| Linolenic | 0.55[a] | 0.83[a] | 0.68[a] | 0.69[a] | ns | 0.67[a] | 0.70[a] | ns | 0.4-1.5 |

Different letters in the same row denote a statistically significant difference ($p < 0.05$). **indicates significance ($p \leq 0.05$). *ns* indicates no significance ($p \geq 0.05$).

for Kargaburun, Erkence, Halhali, Saurani, Ayvalik, Nizip, and Hasebi olive varieties reported by[22, 24].

Table 2 also shows other properties of Gemlik PDO and non-PDO table olives after fermentation.

Harvest year had a significant effect on dry matter contents ranged between 45.56% to 58.81% (Table 2). Several factors, including cultivar, agronomic conditions, geographic origin, environmental factors, fruit ripening level, and processing methods, influence the total phenolic content of olives[3, 25]. The total phenolic content of fresh and fermented Gemlik olives is presented in Tables 1 and 2. Growing region and harvest year had significant effect on phenolic contents of fresh and table olives ($p<0.05$). The total phenolic contents ranged from 86.7 to 232.55 mg/kg for fresh Gemlik olives and from 41.77 to 189.9 mg/kg for Gemlik table olives (Mersin non-PDO and Bursa PDO), respectively ($p<0.05$) (Tables 1 and 2). Fermentation during table olive processing result in a 18.5%–37.45% loss of total phenol compounds (Tables 1 and 2). The results clearly showed that the total phenol content was reduced by 25% for Izmir non-PDO and Bursa PDO Gemlik table olives after fermentation, compared with 37.45% for Hatay and Mersin non-PDO Gemlik table olives. This is caused by both hydrolysis and diffusion due to an increase in mass transfer, which leads to faster diffusion of solutes such as phenolic compounds from flesh to brine[26]. Our findings agree with those of[6, 26–30], who found that the phenol content of olives decreased gradually at the end of fermentation.

The antioxidant activity of Gemlik table olives ranged between 82.08 and 89.15% for Bursa PDO and Izmir non-PDO, respectively, antioxidant activity was not reduced significantly at the end of fermentation (Table 2) as compared to Gemlik fresh olives. Although the total phenolic content of Gemlik table olives decreased significantly after fermentation, the antioxidant activity remained unchanged. This result agrees with the studies of Uslu and Özcan[6], who found that the antioxidant activity of Gemlik and Ayvalik black fresh and table olives was the same but contradicts the findings of[21]. Significant reductions in antioxidant activity in green table olives after fermentation have been reported for green table olives by[6, 29] for Chetoui and Ayvalik olives. The changes in antioxidant activity in table olives could be due to differences in processing techniques such as black table olives (Turkish Gemlik style) and green table olives (Spanish style) and to the bioavailability of phenolic compounds that contribute to antioxidant activity in pickled Gemlik PDO and non-PDO table olives, which may contain primarily aglycons, which are known to have higher antioxidant activity. This supports the notion that olive phenolic compounds are bioavailable, as radical scavenging activity has increased in the plasma after olive administration by volunteers. The findings of this research support the fact that, while fermentation reduces phenolic compounds during table olive processing, table olives retain a high antioxidant capacity[2, 3, 30]. The olives with the highest dry matter content (Bursa PDO and Izmir non-PDO) have a slow diffusion of phenolic substances in brine, resulting in a higher phenolic content in table olives (Table 2). When olives are immersed in brine, their tissues function as semipermeable membranes for water transport. During the osmotic dehydration of olives, two primary countercurrent transfers occur concurrently. The osmotic solute (NaCl) flows from the solution into the olives, whereas water flows from the product into the osmotic solution (brine). As a result, the salt content of the table olives increases while the brine content decreases during processing. A third transfer process involves the diffusion of nutrients into the

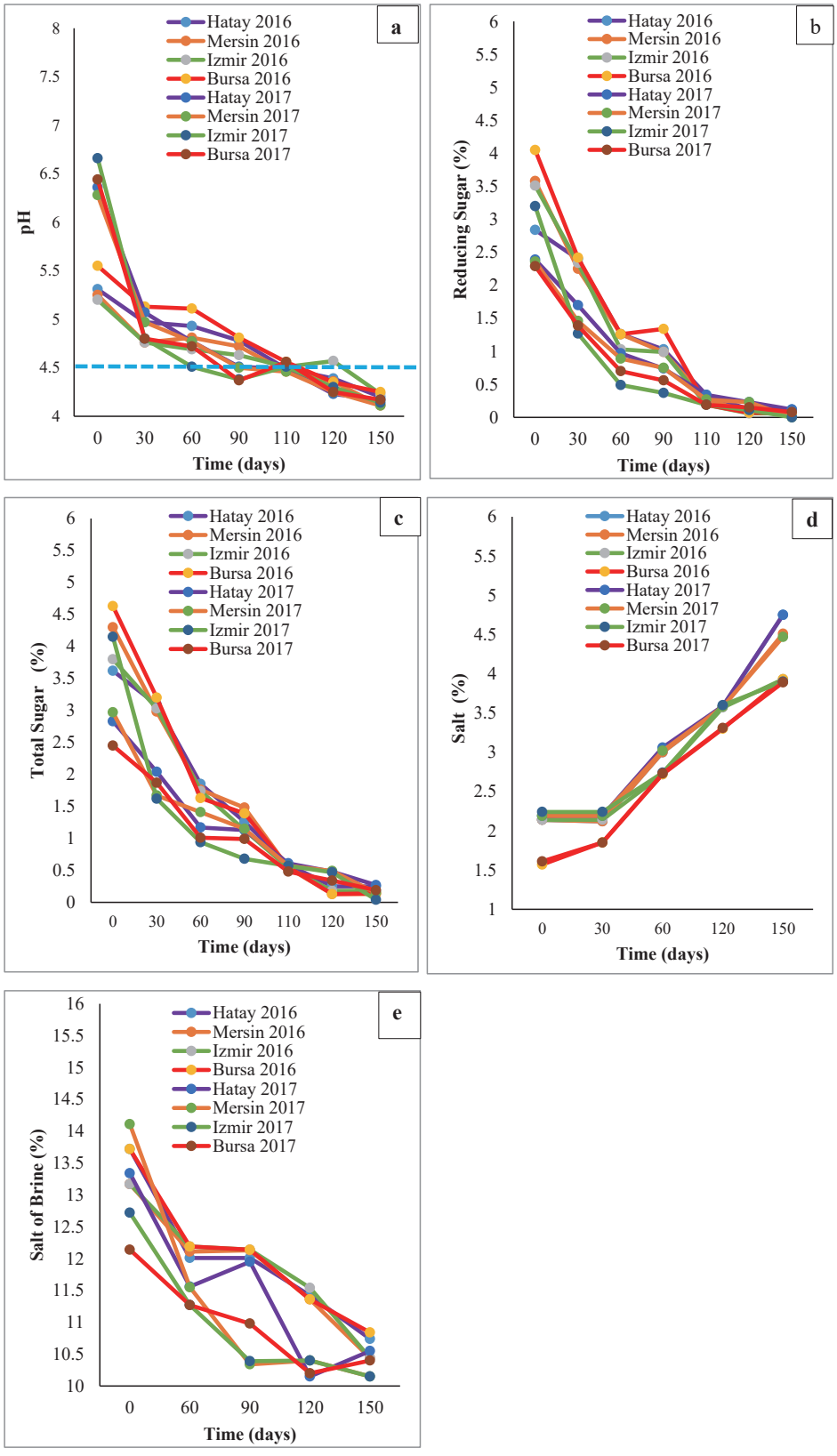


Fig. 1 (a) pH; (b) salt; (c) reducing sugar; (d) total sugar contents of Gemlik Bursa (PDO) and Izmir, Hatay, and Mersin (non-PDO) table olives in harvest years 2016 and 2017. (e) Salt contents of Gemlik Bursa (PDO) and Izmir, Hatay, and Mersin (non-PDO) table olive brines.

soaking medium and the leaching of product solutes (sugars, phenols, acids, minerals, and vitamins) into the solution (brine)[26]. This was clearly seen in this study, while reducing sugar, and total sugar contents decreasing, Gemlik table olive salt contents increased (Figs. 1b–1d).

### 3.2 Fatty acid compositions of Gemlik table olives

Olive oil is a rich source of essential fatty acids, and this property may contribute to the good nutritional value of this product in Mediterranean countries. The fatty acid composition (mainly oleic acid) of table olives is among the most important quality parameters[18]. Table 2 shows the fatty acid compositions of Gemlik table olives harvested in 2016 and 2017 from Bursa PDO and Hatay, Mersin, and Izmir non-PDO regions. Gemlik table olives from Bursa and Izmir regions have high oleic and low palmitic, palmitoleic, linoleic, and linolenic acid contents (Table 2). Growing region and harvest year have a significant effect on palmitic and oleic acid contents of Gemlik table olives ($p<0.05$), respectively. Bursa PDO and Izmir non-PDO had the highest oleic acid (73.98 and 72.58%) and the lower palmitic acid (10.95 and 12.93%) contents (Table 2). Linoleic acid content ranged from 7.93 to 11.67%, and linolenic acid content was less than 1% in Gemlik PDO and non-PDO table olives (Table 2). The oleic acid content of Gemlik PDO and non PDO table olives was consistent with the findings of several studies in which the oleic acid content ranged between 61% and 76% for Gemlik olives[16, 18, 25] and 67% and 76% for Kilis yaglik, Halhali, and Kargaburun olives determined by[22].

### 3.3 Properties of Gemlik table olives during fermentation

Figures 1a–1d shows the pH, reducing sugar, and total sugar and salt contents of Gemlik PDO and non-PDO table olives in the two harvest years.

Monitoring the pH and salt concentration of olives and brines is one of the most important parameters from a technological and safety point of view[1, 7]. During table olive fermentation, water-soluble compounds from the olives diffused to the brine and salt uptake occurred, and thus salt from brines entered the fruits' flesh until equilibrium was established that influence pH and acidity[31]. The pH of the brine falls below 4.5 throughout fermentation, preventing the germination of *C. botulinum* spores[32], and thus protecting the olives from spoilage, which is critical for the safety of table olives[33]. According to reports, the sodium chloride concentration and pH values around 4 at the end of the process help to extend shelf life, avoid spoilage, and maintain a safe product[32]. The initial pH values of Gemlik PDO and non-PDO black table olives ranged between 5 and 5.4, and dropped to 4.1 and 4.3 (Fig. 1a). The highest pH value was found in Bursa PDO table olives (4.3 and 4.2) in both harvest years, and the lowest pH value was found in Mersin non-PDO Gemlik table olives (4.1). Our findings agreed with those of Gemlik, Chétoui, Bella di Cerignola, and Oliva di Gaeta table olives reported by[1, 7, 18, 29]. Table olives must have a salt content of at least 6%, according to the IOC and Turkish table olive standards[15, 34]. In both harvest years, the lowest salt content was found in Bursa PDO and Izmir non-PDO (3.9%), and the highest salt content was found in Hatay non-PDO (4.8%) Gemlik table olives (Fig. 1b). At the end of 150 days of fermentation, the salt content and pH of Gemlik PDO and non-PDO table olives were approximately 4 (Figs. 1a and 1d). The results showed that the salt content of Gemlik PDO and non-PDO olives was less than the 6% limit specified by the IOC and TFC, but today's consumers prefer low-salt olives as part of a healthy diet[1, 31]. The results were similar to those of[21], who determined the salt content of table olives to be between 3.8% and 4.07%, but slightly lower than the results reported by[31] as 0.12% to 3.34%. The differences in the salt content of table olives could be attributed to the different olive varieties used as well as the brine's initial salt content, which ranges from 2% to 18% in the literature for table olive fermentation. During the fermentation process of table olives, sugars were the primary source of carbon for fermentative microorganisms to produce organic acids, which increased the acidity and decreased the pH[32]. Sugar diffusion from olive fruit to brine depends on many factors, such as skin permeability, fruit-to-brine ratio, salt concentration, and temperature[27]. Figures 1b and 1c show that the reducing and total sugars in the olives decreased throughout fermentation. At the start of fermentation, the reducing sugar content of Gemlik PDO and non-PDO table olives was found to be between 2.3% and 4%, and it decreased to 0.1% after 150 days (Fig. 1b). The highest total sugar content was found in Bursa PDO and Izmir non-PDO Gemlik table olives (Fig. 1c). This study found that reducing and total sugars in olives decreased throughout fermentation since microorganisms used fermentable substrates as soon as they diffused into the brine. Our findings were in line with the findings that the reduced sugar content of Gemlik olives decreased from 1.7% and 4.9% to 0.1 and 0.2% after 240 days reported by authors[7, 18, 35], but ref.[27] reported that the sugar content of the Picholine cultivar decreased from 6.7% to 1.1% after 66 days of fermentation. This distinction may be due to a quicker fermentation period, variations in cultivars, or variations in temperature. Figure 1e shows the salt contents brine used for fermentation of Gemlik PDO and non-PDO table olives.

The brine promotes microbial activity for fermentation and reduces the bitterness of the oleuropein (International Olive Council). The initial salt concentration of brines was adjusted to 14%, and the salt content of olive brines from Gemlik was controlled by fermentation for 150 days at a level between 10% and 11% (Fig. 1e). In the 2016 and 2017 harvest years, Izmir and Mersin non-PDO table olive brine had the lowest salt contents (10.44 and 10.15%).

### 3.4 Discrimination of Gemlik table olives according to the PDO region and harvest year

Multivariate analysis is appropriate for distinguishing fresh and processed table olives[8, 11, 12] to determine varietal origin as well as the effect of technological treatments, particularly the fermentation phase, on some table olive compounds[36]. PLS-DA and DA analyses were performed on 52 variables for Gemlik PDO and non PDO fresh and table olives. The results are shown in Figs. 2a and 2b.

PLS-DA with Jackknife cross validation and a fast algorithm is employed to find correlations between variables and factors and to obtain the greatest separation between the variables responsible for the separation. The factor axes that were extracted from the original explanatory

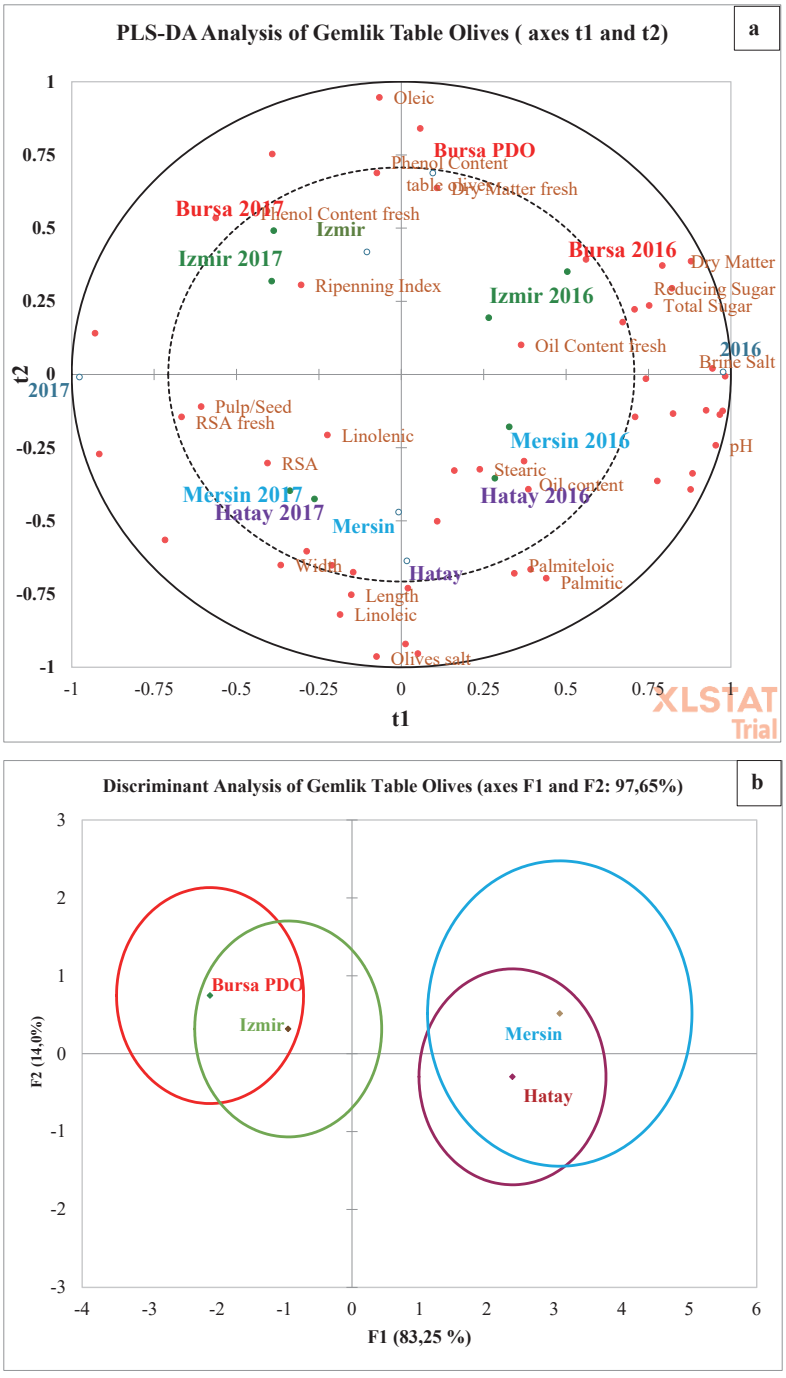


Fig. 2 (a) PLS-DA correlations of Gemlik Bursa (PDO) and Izmir, Hatay, and Mersin (non-PDO) fresh and table olives in harvest years 2016 and 2017. (b) Discriminant analysis (DA) of Gemlik Bursa (PDO) and Izmir, Hatay, and Mersin (non-PDO) fresh and table olives in harvest years 2016 and 2017.

variables are very effective in differentiating between Gemlik PDO and non-PDO olives, as shown in Fig. 2a. Six components make up the model, and Q4 is 0.61. Negative values [0.25 and 0.75] of the t1 component were obtained because Hatay and Mersin non-PDO Gemlik table olives were clearly distinguished from Bursa PDO and Izmir non-PDO Gemlik table olives. Clusters are created in the score plot by the placement of the variables (variables placed far from the origin have a significant impact on discrimination). The length, width, oil content and antioxidant activity of fresh Gemlik olives and dry matter and phenol contents, palmitic, palmitoleic, oleic, linoleic, linolenic acids as well as pH, reducing sugar, sugar and salt contents and brine salt contents were determined as significant discriminators in the PLS-DA analysis (Fig. 2a).

Table 3 displays the variable correlation matrix the most significant discriminators.

The correlation matrix in Table 3 effectively illustrates the relationship amongst salt, pH, fatty acids, reducing sugar, and total sugar in Gemlik table olives as they go through fermentation. This relationship was observed in brine derived from PDO and non-PDO growing areas and various harvest years. During the fermentation of Gemlik table olives, the dry matter content of the olives negatively correlated with the salt content of the olives ($-0.460$) and positively correlated with reducing the sugar (0.784) and the salt content of brine (0.846) (Table 3) due to mass transfer. Table 3 shows a correlation between oleic acid and palmitic acid ($-0.754$), linoleic acid ($-0.868$) and phenol content (0.588) in Gemlik table olives. Thus, a high oleic acid content leads to reduced palmitic acid and linoleic acid content, along with elevated total phenolic content in Bursa PDO and Izmir non-PDO Gemlik table olives (see Table 2). Consistent with our results[6, 16] have also highlighted a correlation between high oleic and low linoleic acid and high phenolic contents in table olives. Table 3 displays a negative correlation ($-0.837$) between salt content and pH values, as well as a positive correlation (0.560) between salt and total sugar contents. Furthermore, reducing sugar exhibited a negative correlation ($-0.813$) with total sugar, while showing a positive correlation with brine salt (0.704).

Figure 2b shows the discriminant analysis of Gemlik PDO and non-PDO olives.

According to Fig. 2b, discriminant analysis significantly identified the groups in the score plot related to both PDO and non-PDO growing regions and harvest years. Table olives from Hatay and Mersin non-PDO were clearly distinguished from those from Bursa PDO and Izmir non-PDO by the first two discriminant functions, which explained 97.65% of the total variance. In other words, a discriminant analysis with 98% accuracy could tell table olives from Bursa PDO and Izmir non-PDO apart from those from Hatay and Mersin-non-PDO Gemlik.

The maturity index and phenol contents of fresh Gemlik PDO and non-PDO olives are significantly influenced by the harvest year and growing region ($p<0.05$). Analysis using two-way ANOVA indicates that the oil, phenol, palmitic acid, and oleic acid levels were notably impacted by the growing region, while the oil and palmitic acid levels were significantly affected by the harvest year ($p<0.05$). The length, width, oil content, and antioxidative activity of fresh Gemlik olives, in addition to levels of dry matter, phenols, palmitic, palmitoleic, oleic, linoleic and linolenic acids, as well as pH, reducing and normal sugar, olive and brine salt concentrations of Gemlik table olives were revealed to be the statistically significant variables ($p<0.05$) according to PLS-DA analysis. Bursa PDO and Izmir non-PDO Gemlik table olives display elevated levels of oleic acid, total phenol, and dry matter, accompanied by reduced levels of palmitic acid and linoleic acid. These characteristics provide clear distinction from Hatay and Mersin non-PDO Gemlik table olives ($p<0.05$), as demonstrated by DA. This discrimination may be due to differences in altitude, temperature, and rainfall in these regions. Bursa PDO and Izmir non-PDO table olives might have higher phenol content and radical scavenging activity because of their comparable and lower altitudes (22 and 25 m), as compared to the Hatay non-PDO region (400 m). Although the average temperatures of growing regions fall between 10 and 25℃, they are comparable because they both meet the minimum temperature requirements for olive yield. The average temperature in Turkiye's growing regions ranges from 10 to 25℃, but the Bursa (Marmara) and Izmir (north Aegean) regions have better environmental conditions for the development of Gemlik olives than Hatay and Mersin (eastern Mediterranean) region. Our results from the discriminant analysis agreed with those of some other authors[8, 10–12] who distinguished between different varieties of table olives based on the morphological characteristics of the olives and stones associated with those from Spain, Greece, Italy, Portugal, and Turkiye. These authors estimated that these characteristics accounted for a total variance of 46%–82% among cultivars, harvest years, and geographical origins.

## 4 Conclusions

Olive cultivation can sequester carbon dioxide, with varying potential among different plantations. According to the International Olive Council, an average olive grove can neutralise the carbon emissions of one individual in the atmosphere per hectare. Hence, it is important to safeguard potential areas for olive cultivation. The present study shows that the fatty acids, physical and chemical indices of fresh and Gemlik-style processed black table olives can distinguish the Gemlik olives growing under both PDO and

Table 3 Pearson correlation matrix of Gemlik Bursa (PDO) and Izmir, Hatay, and Mersin (non-PDO) fresh and table Olives.

| | Growing Region | Harvest Year | Width | Length | Oil content | Antioxidant Activity | Dry Matter | Phenol Content | Palmitic | Palmiteloic | Oleic | Linoleic | Linolenic | pH | Reducing sugar | Total Sugar | Salt | Brine Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growing Region | 1 | 0,000 | 0,386 | 0,460 | 0,018 | 0,222 | -0,489 | -0,610 | 0,693 | 0,625 | -0,833* | 0,651 | 0,233 | -0,839* | -0,106 | 0,312 | 0,890* | -0,026 |
| Harvest Year | | 1 | 0,446 | 0,204 | -0,246 | 0,533 | -0,842* | -0,007 | -0,441 | -0,280 | 0,031 | 0,240 | 0,142 | 0,414 | -0,789 | 0,726 | 0,074 | -0,948* |
| Width | | | 1 | 0,879* | 0,052 | 0,218 | -0,447 | -0,252 | 0,351 | 0,141 | -0,697 | 0,712 | 0,460 | -0,198 | -0,471 | 0,839* | 0,611 | -0,214 |
| Length | | | | 1 | 0,070 | 0,290 | -0,321 | -0,278 | 0,528 | 0,192 | -0,835* | 0,805* | 0,513 | -0,368 | -0,440 | 0,737 | 0,619 | -0,030 |
| Oil content | | | | | 1 | -0,385 | 0,294 | 0,089 | 0,352 | -0,026 | -0,181 | 0,024 | 0,161 | 0,154 | 0,579 | -0,377 | -0,204 | 0,254 |
| Antioxidant Activity | | | | | | 1 | -0,652 | 0,359 | 0,048 | -0,397 | -0,103 | 0,080 | 0,630 | -0,132 | -0,610 | 0,533 | 0,336 | -0,644 |
| Dry Matter | | | | | | | 1 | 0,253 | 0,117 | 0,022 | 0,321 | -0,501 | -0,271 | 0,087 | 0,784* | -0,711 | -0,460 | 0,846 |
| Phenol Content | | | | | | | | 1 | -0,238 | -0,874* | 0,588 | -0,659 | 0,509 | 0,414 | 0,144 | -0,245 | -0,475 | -0,065 |
| Palmitic | | | | | | | | | 1 | 0,526 | -0,754 | 0,333 | 0,360 | -0,761 | 0,373 | 0,065 | 0,707 | 0,422 |
| Palmiteloic | | | | | | | | | | 1 | -0,554 | 0,389 | -0,509 | -0,567 | 0,205 | 0,073 | 0,557 | 0,293 |
| Oleic | | | | | | | | | | | 1 | -0,868 | -0,350 | 0,709 | 0,177 | -0,485 | -0,819 | -0,135 |
| Linoleic | | | | | | | | | | | | 1 | 0,229 | -0,439 | -0,510 | 0,608 | 0,607 | -0,076 |
| Linolenic | | | | | | | | | | | | | 1 | -0,269 | -0,210 | 0,313 | 0,351 | -0,111 |
| pH | | | | | | | | | | | | | | 1 | -0,104 | -0,085 | -0,837* | -0,388 |
| Reducing sugar | | | | | | | | | | | | | | | 1 | -0,813* | -0,228 | 0,704 |
| Total Sugar | | | | | | | | | | | | | | | | 1 | 0,560 | -0,575 |
| Salt | | | | | | | | | | | | | | | | | 1 | -0,029 |
| Brine Salt | | | | | | | | | | | | | | | | | | 1 |

* The results statistically significant ($p < 0.05$).

non-PDO regions. Bursa PDO and Izmir non-PDO Gemlik table olives, having high levels of oleic acid, total phenol, and dry matter, but low levels of palmitic and linoleic acid, were successfully differentiated from Hatay and Mersin non-PDO Gemlik table olives using discriminant analysis. The Marmara (Bursa PDO) and north Aegean (Izmir non-PDO) regions seem to possess more beneficial environmental conditions for the production of Gemlik olives than the eastern Mediterranean (Hatay and Mersin non-PDO regions). The use of chemometric approaches can effectively identify and differentiate the growing regions, protecting consumers from deceitful practices. These findings could serve as a valuable resource for global researchers studying Gemlik olives cultivated in both PDO and non-PDO areas of Turkiye.

## Author Contributions

Turkan Mutlu Keceli: Conceptualization, Validation, Methodology, Formal analysis, Resources, Supervision, Visualization, Writing-Original Draft, Writing-Review & Editing. Fulya Harp Celik: Conceptualization, Validation, Methodology, Formal analysis, Visualization, Writing-Original Draft, Writing-Review & Editing. Oya Koseoglu: Conceptualization, Formal analysis, Resources, Methodology, Visualization.

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## Conflicts of Interest/Competing Interests

The authors declare that there are no conflicts of interest.

## Availability of Data and Material

The data and material set supporting the materials and results of this study are included within the article.

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